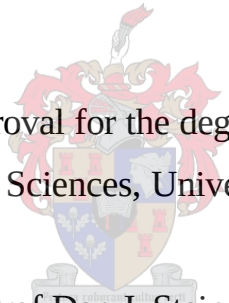
# Investigating the Molecular Aetiology of Obsessive-compulsive disorder (OCD) and Clinically-defined Subsets of OCD

**S.M.J. Hemmings**



Dissertation presented for approval for the degree of Doctor of Philosophy at the Faculty of Health Sciences, University of Stellenbosch.

Promoter: Prof Dan J. Stein

Co-promoters: Prof Johanna C. Moolman-Smook

Prof Valerie A. Corfield

April 2006

# DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature ……

Date ……

## ABSTRACT

Obsessive-compulsive disorder (OCD), a debilitating psychiatric disorder, affects 2-3% of the general population, and represents a global health problem. Evidence from family studies suggests that genetic factors play a role in mediating disease development. However, the pattern of inheritance is not consistent with monogenic disorders, but is "genetically complex".

Case-control association analysis, which facilitates dissection of the genetic aetiology of complex disorders, has yielded many inconsistent results in OCD studies, making identification of predisposing alleles difficult. These discrepant findings can largely be attributed to inappropriate statistical methodology and the lack of OCD phenotypic resolution. Although classified as a single clinical entity according to structured algorithms, OCD probably represents a final common outcome of multiple underlying aetiologies. Thus, numerous clinical subtypes of the disorder have been proposed; these "intermediate" phenotypes may be more closely related to a particular genetic substrate than the higher order construct of OCD.

Furthermore, although genes encoding serotonergic (5-HT) and dopaminergic components are most commonly investigated, it is likely that the behavioural manifestations of OCD are mediated by a broader network of interconnected neurotransmitter and signalling pathways.

Consequently, the aim of the present study was two-fold: to address the factors that may have confounded previous genetic case-control association studies and to investigate the genetic aetiology of OCD phenotypes while accounting for these factors.

Case and control individuals were drawn from the reportedly genetically homogeneous Afrikaner population. However, as no empirical evidence existed to support the absence of genetic substructure, which would confound genetic association studies, a Bayesian model-based clustering algorithm (*Structure*), that groups individuals on the basis of observed genotype data, was employed to assess population stratification in both case and control Afrikaner subjects.

OCD patients were clinically stratified by gender, symptom severity, age at onset, the presence of selected co-morbid disorders and the presence of selected symptom dimensions, to facilitate the identification of susceptibility genes more closely related with these subtypes. Candidate genes included those coding for components of the 5-HT (5-HT receptors 1Dβ, 2A, 2C and 6), dopaminergic (dopamine receptors 1, 2, 3 and 4, dopamine transporter and catechol-O-methyltransferase [COMT]), glutamatergic (glutamate receptor subunit 2B [GRIN2B]) and neurodevelopmental pathways (brain-derived neurotrophic factor [BDNF] and homeobox 8 [HoxB8]), as well as previously uninvestigated genes (angiotensin-converting enzyme I, inositol-trisphosphate, phospholipase-C-gamma 1 and estrogen receptor alpha). The relationship between variants in these genes and OCD (or OCD subtypes) was investigated in a single locus and a haplotype context, while meta-analyses using published population-based case-control association data were also conducted.

Significant associations noted between distinct *COMT* variants and OCD implicated *COMT* in the development of a genetically discrete, gender-dependant, early-onset, tic-related phenotype in males. Furthermore, investigations of variations in *BDNF* and *GRIN2B* point towards a genetically distinct, neurodevelopmental subtype of the disorder, mediated, in males at least, primarily by dysfunctions in *BDNF*. The striking gender dimorphism noted in these associations indicates the possibility of an epigenetic hormonal influence. Moreover, the significant association of polymorphisms within *GRIN2B*, in both a single locus and haplotype context, suggests the involvement of this gene in mediating a phenotypic subtype characterised by an early-onset, more severe form of the disorder.

The present investigation forms part of ongoing research to elucidate genetic components involved in the aetiopathology of OCD and OCD-related subtypes. Such studies may pave the way towards more efficacious pharmacotherapeutic strategies, which will ease the suffering of individuals who are afflicted with this incapacitating condition.

## OPSOMMING

Obsessiewe-kompulsiewe steuring (OKS) is ’n aftakelende psigiatriese siektetoestand wat 2-3% van die algemene bevolking affekteer en ’n globale gesondheidsprobleem verteenwoordig. Familiestudies dui daarop dat genetiese faktore ’n rol in die ontwikkeling van hierdie siekte speel. Die patroon van oorerwing is egter nie verenigbaar met dié van monogeniese siektes nie, maar is geneties “kompleks”.

Geval-kontrole assosiasie-ontleding, wat die disseksie van die genetiese etiologie van komplekse siektes fasiliteer, het teenstrydige resultate in OKS gelewer en dit bemoeilik die identifikasie van predisponerende allele. Die teenstrydige bevindings kan grootliks aan ontoepaslike statistiese metodiek en die gebrek aan fenotipiese differensiasie in OKS toegeskryf word. Alhoewel dit volgens gestruktureer algoritmes as ’n enkele kliniese entiteit geklassifiseer word, verteenwoordig OKS waarskynlik die eindresultaat van veelvoudige onderliggende oorsake. Baie kliniese subtipes van die toestand is al voorgestel en dié “intermediêre’ fenotipes mag nader verwant aan ’n spesifieke genetiese substraat as die hoër orde konsep van OKS wees.

Verder, alhoewel die gene wat die serotonergiese (5-HT) en dopaminergiese komponente kodeer meestal ondersoek word, is dit waarskynlik dat die gedragsmanifestasies van OKS deur ’n breër netwerk van intergekonnekteerde neuro-oordragstof- en seinoordragpaaie meegebring word

Gevolglik was die doel van die huidige studie tweevoudig: om faktore wat vorige genetiese geval-kontrole assossiasie-studies verwar het aan te spreek en om die genetiese etiologie van OKS-fenotipes te ondersoek met in ag neming van hierdie faktore.

Geval- en kontrole-individue is gekies uit die Afrikaner-bevolking wat as geneties homogeen beskryf kan word. Daar was geen empiriese bewyse vir die afwesigheid van ’n genetiese substruktuur (wat genetiese assossiasie-studies sou verwar),nie. Daarom is ’n Bayesiese model-gebaseerde groeperings-algoritme (*Structure*), wat individue op grond van waargenome genotipiese data groepeer, gebruik om die populasie-stratifikasie is beide geval- en kontrole- Afrikaner-individue te bepaal.

OKS-pasiënte is klinies gestratifiseer volgens geslag, ernstigheid van simptome, ouderdom by aanvang van simptome, die teenwoordigheid van geselekteerde komorbiede siektetoestande en die teenwoordigheid van geselekteerde simptoomdimensies of -groepe, om die identifikasie van moontlike vatbaarheidsgene wat nader verwant is aan die verskillende subtipes te fasiliteer/vergemaklik. Kandidaatgene het ingesluit: dié wat kodeer vir komponente van die 5-HT-(5-HT reseptore 1Dß, 2A, 2C and 6), dopaminergiese (dopamien-reseptore 1, 2, 3 and 4, dopamien-transporter and katesjol-O-metieltransferase [*COMT*]), glutamatergiese (glutamaat-reseptor subeenheid 2B [*GRIN2B*]) and neuro-ontwikkelingspaaie (brein-gederiveerde neurotrofiese faktor [*BDNF*] en homeobox 8 [*HoxB8*]), sowel as die gene wat nie voorheen ondersoek is nie (angiotensien-omsettingsensiem I, inositol-trisfosfaat, fosfolipase-C-gamma 1 en estrogeen-reseptor alpha). Die verhouding tussen variante in hierdie gene en OKS (of OKS-subtipes) is ondersoek in ’n enkel-lokus en haplotipe konteks, en meta-analises, wat gepubliseerde bevolkings-gebaseerde geval-kontrole ontledingsdata gebruik het, is ook gedoen.

Beduidende assosiasies gevind tussen spesifieke *COMT*-variante en OKS in mans, het daarop gedui dat *COMT* in die ontwikkeling van geneties-diskrete, vroeë-aanvang, senutrekking ("tics") -verwante fenotipe in mans betrokke is. Verder het ondersoeke van variasies in *BDNF* en *GRIN2B* daarop gedui dat ’n geneties-afsonderlike, neuro-ontwikkelings-subtipe van.OKS wat, ten minste in mans, primêr deur wanfunksie van *BDNF* meegebring word. Die opvallende geslags verskil wat in hierdie assosiasies gesien word, dui op die moontlikheid van ’n epigenetiese hormonale invloed. Bowendien, die beduidende assosiasie van polimorfismes in *GRIN2B* in beide die enkel-lokus en haplotipe konteks, dui op die betrokkenheid van hierdie geen in die meebring van ’n fenotipiese subtipe wat deur ’n vroeë aanvang, en meer ernstige vorm van die siekte gekenmerk word.

Die huidige ondersoek vorm deel van voortgesette navorsing om die genetiese komponente wat betrokke is by die etiopatologie van OKS en OKS-subtipes, bloot te lê. Sodanige studies kan die weg baan na meer doeltreffende farmakoterapeutiese strategieë wat die lyding van indi vidue wat deur hierdie aftakelende toestand geraak word, kan verlig.

**INDEX** **PAGE**

## ACKNOWLEDGMENTS

I would like to express my sincere gratitude to the following people who have assisted me during the course of this degree:

My promoter, Prof. Dan Stein, and co-promoters, Prof. Hanlie Moolman-Smook and Prof. Valerie Corfield for their time, effort and invaluable input and guidance, without which this study would not have been possible.

Christine Lochner for recruiting and interviewing the patients, and Lize van der Merwe, for her statistical expertise.

Craig Kinnear, not only for allowing me the use of some of his genotyping data for use in the "Structure" analyses, but for being a really good friend, and for understanding my mood swings (and putting up with them!) these past few months. The members of the "Magic Lab", for their support and assistance, their intellectually stimulating discussions, and for providing me with much-needed comic relief!

My family and friends: Dad, Kate, Gethin, Dennis and Graham for supporting me throughout the course of this study, and for always being there to lend support. My "in-laws" Rose and Ken Chaplin, for their support, and for helping out where they could. Samantha, for her unwavering support and encouragement

Finally, Matthew, my pillar of strength. Without your love, help, support and understanding, this dissertation would not have been possible. For the many hours you spent helping out with the final touches, and for the many hours looking after Megan whilst I was at work. Megan, my little monster, for reminding me that there is so much more to life! Lastly, Mom, my guardian angel, who, when she was alive, was always there for me and believed in me every step of the way.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| $\chi^2$ | :chi-squared |
| $\lambda$ | : relative risk |
| **˚C** | : degrees celcius |
| **5-HIAA** | : 5-hydroxyindoleacetic acid |
| **5-HT** | : serotonin |
| **5-HT$_{1A}$** | : serotonin receptor type 1A |
| **5-HT$_{1D\beta}$** | : serotonin receptor type 1Dβ |
| **5-HT$_{2A}$** | : serotonin receptor type 2A |
| **5-HT$_{2C}$** | : serotonin receptor type 2C |
| **5-HT$_6$** | : serotonin receptor type 6 |
| **5-HTT** | : serotonin transporter |
| **5-HTTLPR** | : serotonin transporter promoter-linked polymorphism |
| **6-FAM** | : 6-carboxyfluorescein |
| **ACE** | : angiotensin-converting enzyme |
| **ADRA1C** | :adrenergic receptor type 1C |
| **AMPA** | :alpha-amino-5-hydroxy-5-methyl-4-isoxalzolepropionic acid |
| **AngII** | **:**angiotensin II |
| **APA** | **:**American Psychiatric Association |
| **APO** | **:**apomorphine |
| **ASREA** | **:**Allele-specific restriction enzyme analysis |
| **BDD** | **:**Body dysmorphic disorder |
| **BDNF** | **:**Brain-derived neurotrophic factor |
| **BLD** | **:**Background linkage disequilibrium |
| **Ca$^{2+}$** | **:**calcium |
| **cAMP** | **:**cyclic adenosine monophosphate |
| **CD/CV** | **:**common disease/common variant |
| **CGN** | **:**cortical-limbic-glutamatergic-neuron |
| **CI** | **:**confidence interval |
| **CMI** | **:**clomipramine |
| **CMT** | **:**chronic motor tics |
| **CNS** | **:**central nervous system |
| **CNTNAP2** | **:**contactin-associated protein 2 |
| **COMT** | **:**catechol-O-methyltransferase |

**CD/RA** **:**common disease/rare allele hypothesis
**CSA** **:**complex segregation analysis
**CSF** **:**cerebrospinal fluid
**CSTC** **:**cortico-striatal-thalamocortical system
**DAF** **:**disease allele frequency
**DAG** **:**diacylglycerol
**dATP** **:**deoxy-adenosine triphosphate
**DAT** **:**dopamine transporter
**DBH** **:**dopa-beta hydroxylase
**dCTP** **:**deoxy-cytosine triphosphate
**ddNTP** **:**di-deoxy nucleotide triphosphate
**dGTP** **:**deoxy-guanosine triphosphate
**DLX-6** **:**distal-less like homeobox 6
**DMSO** **:**dimethylsulfoxide
**DNA** **:**deoxyribonucleic acid
**DRD1** **:**dopamine receptor 1
**DRD2** **:**dopamine receptor 2
**DRD3** **:**dopamine receptor 3
**DRD4** **:**dopamine receptor 4
**DSM-IV** **:**Diagnostic and Statistical Manual, 4th ed.
**dTTP** **:**deoxy-thymidine triphosphate
**DY-BOCS** **:**Dimensional Y-BOCS
**DZ** **:**dizygotic
**ECA** **:**epidemiologic catchment area
**EDTA** **:**ethylene-diamine-tetra-acetic acid
**EM** **:**expectation-maximisation
**EO** **:**early-onset
**ERE** **:**estrogen response element
**ES** **:**effect size
**ESRα** **:**estrogen receptor alpha
**EtBr** :ethidium bromide
**FWER** **:**family-wise error rate
**FXIIIB** **:**Factor 13B
**GAD** **:**generalised anxiety disorder
**GNAS** **:**guanine nucleotide-binding α subunit of G
**GRIN2B** **:**glutamate receptor subunit 2B
**GRR** **:**genotype relative risk

**$H_1$** **:**alternate hypothesis

**$H_0$** **:**null hypothesis

**$^1$H-MRS** **:**proton magnetic resonance spectroscopy

**HOX** **:**homeobox

**HOXB8** **:**homoebox 8

**HPA** **:**hypothalamo-pituatary-adrenal axis

**HRR** **:**haplotype relative risk

**HVA** **:**homovanillic acid

**IBD** **:**indentical by descent

**IED** **:**intermittent explosive disorder

**IMMPL2** **:**inner mitochondrial membrane peptidase 2 like

**IMPASE** **:**inositol monophosphatase

**INPP-1** **:**inositol-polyphosphatase-1 (gene)

**INS/DEL** **:**insertion/deletion polymorphism

**$IP_3$** **:**inositol trisphosphate

**$IP_2$** **:**inositol bisphosphate

**$IP_1$** **:**inositol monophosphate

**IPPase** **:**inositol-polyphosphatase-1

**LCA** **:**latent class analysis

**LD** **:**linkage disequilibrium

**LO** **:**late onset

**LOD** **:**logarithm of odds

**MAF** **:**marker allele frequency

**MAO-A** **:**monoamine oxidase A

**mCPP** **:**Meta-chlorpiperazine

**MDD** **:**major depressive disorder

**MHIC** **:**Mental Health Information Centre

**MHPG** **:**metabolite 3-methoxy-4-hydroxyphenylethyleneglycol

**MRC** **:**Medical Research Council

**MRCA** **:**most recent common ancestor

**MRI** **:**magnetic resonance imaging

**MZ** **:**monozygotic

**NAA** **:**N-acetyl-aspartate

**NCBI** **:**National Centre for Biotechnology Information

**NK-1** **:**Neurokinin-1

**NMDA** **:**N-methyl-D-aspartate

**NMDAR** **:**N-methyl-D-aspartate receptor

**NPL** **:**nonparametric linkage signal

**OCD** **:**obsessive-compulsive disorder

**OCS** **:**obsessive-compulsive symptoms

**OCSD** **:**obsessive-compulsive spectrum disorder

**OR** **:**odds ratio

**p** **:**p-value

**PA** **:**phosphatidic acid

**PD** **:**panic disorder

**PFC** **:**prefrontal cortex

**PI** **:**phosphoinositide

**PIP2** **:**phosphatidyl-4,5-biphosphate

**PLC** **:**phospholipase C

**PLC-γ1** **:**Phospholipase C gamma-1

**PPI** **:**prepulse inhibition

**PV92** **:**predicted variant *Alu* insertion repeat

**RNA** **:**ribonucleic acid

**SA** **:**South African

**SADS-L** **:**Structured Clinical Interview for Affective Disorders and Schizophrenia-lifetime version

**SCID-1/P** **:**Structured Clinical Interview for DSM-IV Axis I Disorders, patient version

**SIB** **:**self-injurious behaviour

**SINE** **:**short interspersed repetitive element

**SNAP-25** **:**Synaptosomal-associated protein 25kDa

**SNAP-29** **:**Synaptosomal-associated protein 29kDa

**SNP** **:**single nucleotide polymorphism

**SP** **:**substance P

**SRI** **:**serotonin reuptake inhibitor

**SSRI** **:**selective serotonin reuptake inhibitor

**TE** :Tris-EDTA

**TBE** **:**Tris, boric and EDTA buffer

**TDT** **:**Transmission disequilibrium test

**TPA25** **:**Tissue plasminogen activator

**TPH** **:**tryptophan hydroxylase

**TrkB** **:**tyrosine kinase B

**TS** **:**Tourette Syndrome

**TTM** :trichotillomania

**U** **:**unit

**UTR** **:**untranslated region

**VMAT2** **:**vesicular monoamine transporter type-2

**VNTR** **:**variable number of tandem repeats

**YaNBC182** :Ya subfamily Alu insertion sequence NBC182

**YaNBC241** **:**Ya subfamily Alu insertion sequence NBC241

**Y-BOCS** **:**Yale-Brown obsessive-compulsive Scale

**YBOCS-CL** **:**Yale-Brown Obsessive-Compulsive Symptom Checklist

**YGTSS** **:**Yale Global Tic Severity Scale

# LIST OF FIGURES

**FIGURE** **PAGE**

# LIST OF TABLES

# CHAPTER I

## INTRODUCTION

**INDEX** **PAGE**

# CHAPTER I: INTRODUCTION

## I.1. A BRIEF INTRODUCTION TO PSYCHIATRIC GENETICS

*"Of all the hereditary diseases, madness is supposed to be the most constant and persevering, for even if one generation escape, the taint is presumed to cling to the succeeding branches…"*

*John Johnstone (1786-1863)*

It is clear that, from the quote by John Johnstone, a genetic viewpoint on the complex genetic inheritance of psychiatric disorders has been appreciated for centuries. Indeed, one of the founders of modern-day psychiatry, Emil Kraepelin, believed that a psychiatric disorder constituted a "heredity taint" (Barondes, 1998).

However, during the early 20$^{th}$ century, psychiatry underwent a long period in which it was not considered as belonging to any particular category of medical science (Freimer and Sabatti, 2004). Nowadays, with the modern expedience of bioinformatics and biotechnology, interest in the field of psychiatric genetics has escalated to phenomenal proportions. More recently, there has been a sense of urgency to dissect the aetiology of these disorders, given their staggering burden on society (Uhl and Grow, 2004; Murray and Lopez, 1997).

Psychiatric disorders are complex, multifactorial disorders comprising a range of environmental and genetic contributions, that can be amalgamated to form an observed, normally distributed variable termed liability (Falconer, 1981). Psychiatric disorders may therefore be regarded as dichotomous entities by simple virtue of the fact that the underlying liability exceeds some threshold (Rannala, 2001). Indeed, multiple thresholds may also exist, with individuals who possess liability scores between the threshold values representing the mild phenotypic or so-called spectrum cases.

Delineating the contribution of genetics to the development of psychiatric disorders has not been easy. Although psychiatric disorders aggregate within families, they do not segregate within these families, intimating their complex transmission patterns and genetic aetiology. Different genetic mechanisms and interactions, including epistasis, locus heterogeneity, allelic

heterogeneity, incomplete penetrance and genetic imprinting, may be implicated in bestowing increased susceptibility to a disorder on an individual (Nothen et al, 1993; Souery et al., 2001; Stoltenberg and Burmeister, 2000).

Besides the genetic complexity of psychiatric disorders, non-genetic factors also confound the detection of genes implicated in these conditions. Genetic factors may be necessary, but are not sufficient, to precipitate the clinical phenotype of the disorder. Most complex disorders require the simultaneous input from non-genetic (environmental) factors. Possible substrates acting as environmental aetiological factors include those of a psychosocial, immunological, developmental, nutritional and infectious nature. Despite a myriad confounding factors, research designs have been employed to analyse the cause of individual differences within the normal range of behavioural variation, and the aetiologies of various psychopathologies and mental illnesses; subsequently, heritability estimates have been identified for a number of psychiatric conditions (Owen and Cardno, 1999). In a recent meta-analysis of the epidemiology of anxiety disorders (pertinent to the present dissertation since it included obsessive-compulsive disorder [OCD]), it was estimated that the heritabilities across the disorders were in the range of 30% to 40%, although the authors acknowledge that this may represent an underestimation (Hettema et al., 2001).

Heritability of a disorder refers to the ratio of genetic variance to the overall phenotypic variance. It is worthwhile to note that these values are based on a specific situation involving a particular phenotype in a population, and may well differ between populations. Heritability must thus be viewed as a descriptive statistic of a trait pertaining to a particular population at a specific time, and the heritability estimates should be viewed as just that – estimates (Sherman et al., 1997). Estimates of heritability are based on a process of biometrical model fitting, which allows the determination of whether, and to what extent, genetic and environmental factors contribute to the liability to a psychiatric disorder (Owen et al., 2000; Sherman et al., 1997). It is important to note, however, that simply estimating the degree of heritability does not give one an indication of the mode of inheritance of a disorder.

The present dissertation investigates the genetic contribution that may play a role in the heritability of OCD, the main focus being the identification of the genetic substrates comprising the disorder. However, in order to understand the underlying genetic basis of the

disorder, it is necessary to briefly review the results of studies providing evidence for the role of genetics in the pathology of the disorder.

## I.2. OBSESSIVE-COMPULSIVE DISORDER (OCD)

### I.2.1. Phenotypic Characteristics of OCD

"*Having OCD is like being allergic to life – every waking moment is spent in a state of mental hypersensitivity"*

*Anonymous*

Obsessive-compulsive disorder was described as far back as the 19th century by Esquirol (1838), Falret (1850) and Westphal (1878). The German writer, Westphal, formulated the modern definition of the syndrome, which was considered to be psychological in origin and was classified amongst the group of neuroses. With Freud's psychoanalysis of the "Rat Man" (1909/1955), OCD was hypothesized as being the result of unconscious conflicts and the isolation of thoughts and behaviours from their emotional antecedents (Jenike, 2001).

Nowadays, OCD is looked upon as a severe and debilitating condition that is classified as an anxiety disorder in the Diagnostic and Statistical Manual ($4^{th}$ Edition) (DSM-IV, 1994). The disorder is characterised by pathognomonic features of recurrent obsessions (persistent, intrusive thoughts) and/or compulsions (physical or mental rituals or acts), which the individual feels compelled to perform so as to reduce distress brought on by the obsessions, or to prevent some feared situation. Clinical diagnosis of OCD, according to the DSM-IV, requires that the obsessions and compulsions cause significant distress to the patient and consume more than one hour a day of their time, ultimately interfering with normal home, work and social routine. In addition, the patient should recognise that the obsessions and compulsions are excessive and unreasonable.

Around 1938, Westphal described obsessional thoughts as "ideas that in an otherwise intact intelligence, and without being caused by an emotional or affect-like state, against the will of the person…come into the foreground of the consciousness". Obsessions include recurrent or persistent ego-dystonic ideas, thoughts, images or impulses which the individual attempts to suppress or ignore because he finds them morally reprehensible and repugnant. Most patients are secretive regarding their obsessions, and consequently experience constant inner turmoil

because they are aware that, although their actions are unreasonable, they are powerless to resist them. OCD patients commonly endorse obsessions involving contamination, doubt, fear of aggression towards others or acting on sexual impulses, disgust with bodily function, and a need for symmetry and order (Rasmussen and Eisen, 1988, DSM-IV, 1994) (Table I.1). Obsessions are not excessive worries about real-life problems (DSM-IV, 1994).

Compulsions embody the physical corollary to obsessions - they represent the uncontrollable urge to repeatedly enact stereotypic behaviours or mental rituals in an attempt to neutralise or prevent discomfort brought on by obsessions (DSM-IV, 1994). Compulsions are normally amplified beyond utility and usually possess no realistic connection with the obsession they are designed to neutralise. Performing compulsions may become a major lifetime activity, leading to marital, occupational or social disability. If interrupted whilst performing the compulsions, the patient believes that they should be started again in order to be effective. Common compulsions include checking, washing, cleaning, counting, querying behaviours (asking or confessing), and arranging or hoarding objects (Rasmussen and Eisen, 1988; DSM-IV, 1994) (Table I.1).

***Table I.1.*** *Typical OCD symptoms*

| Common obsessions | Frequency (%) | Common compulsions | Frequency (%) |
|---|---|---|---|
| Contamination fears | 45 | Checking rituals | 63 |
| Repetitive doubts | 42 | Washing/cleaning rituals | 50 |
| Somatic obsessions | 36 | Need to confess | 36 |
| Need for symmetry | 31 | Covert counting | 36 |
| Aggressive impulses | 28 | Ordering/symmetry | 31 |
| Repeated sexual imagery | 26 | Hoarding | 18 |
| Multiple obsessions | 60 | Multiple compulsions | 48 |

Adapted from Rasmussen and Eisen, 1990.

Most patients with the disorder suffer from both multiple obsessions and compulsions, particularly now that the DSM-IV has redefined compulsions to include mental rituals. A remarkable feature of OCD is the “relatively restricted repertoire” of symptom type experienced by individuals with the disorder (Samuels and Nestadt, 1997) - the clinical manifestations of the condition have been found to remain consistent across populations and

cultures, only a limited number of obsessions and compulsions have been described (Robins et al, 1984; Nelson and Rice, 1997).

### I.2.2. The Epidemiology of OCD

OCD was originally thought to have a relatively low prevalence of roughly 0.05% in the general population, and to be fairly unresponsive to pharmacological forms of treatment (Woodruff and Pitts, 1964). This finding was probably due to the clinicians' relative unfamiliarity with the disorder until the last decade. Moreover, patients' secretiveness about their symptoms and the fact that the average wait before seeking psychiatric help was 7.5 years could have contributed to this finding (Rasmussen and Tsuang, 1986).

It is thus only since the mid-1980s that the disorder has become recognised as one of the most common psychiatric disorders, with a significant impact on health and the economy (Du Pont et al., 1995). OCD is presently classified as being amongst the most disabling medical conditions in the world (Murray and Lopez, 1997). According to well-characterised, replicated studies in the US carried out in the Epidemiological Catchment Area (ECA), the disorder has a lifetime prevalence of between 1% and 3% (Karno et al., 1988; Robins et al., 1984; Samuels and Nestadt, 1997; Nestadt et al., 2000[a]; Maina et al., 1999; Weissman et al., 1994), and affects approximately 50 million individuals worldwide (Fineberg and Roberts, 2002).

OCD generally pursues a chronic course, marked by episodes of illness with periods of incomplete remission (Jenike, 2001). The disorder presents with a bimodal age at onset, peaking first in the early teens (early-onset [EO]), and subsequently in the early 20s (late-onset [LO]). The mean age at onset of OCD is between the ages of 20 and 24 years – more than 80% of patients develop symptoms before they reach the age of 35 years (Minichiello et al., 1990; Fineberg and Roberts, 2002). Overall, the disorder is slightly more common in females than males, with an overall gender ratio of 1.5:1 (Bebbington, 1998; Sasson et al., 1997; Angst et al., 2004; Fineberg and Roberts, 2002). However, subtle gender differences have been found to exist with regard to the age at onset (Antony et al., 1998). EO OCD seems to affect more males than females, whereas LO OCD is found to affect more females than males. In addition, the mean age of LO OCD in males (21 years) tends to be earlier than that for females with LO OCD (24 years).

It is possible that the genes thought to contribute to OCD may reflect these gender differences, and may perhaps account for a part of the phenotypic variability observed between the two sexes. Indeed, Karayiorgou et al. (1997) reported on a sexually dimorphic relationship between a candidate gene, catechol-O-methyltransferase (*COMT*) and OCD. These results were subsequently replicated by the same group (Karayiorgou et al., 1999), who also observed a sexually dimorphic association between monoamine oxidase A (*MAO-A*) and the disorder.

The heterogeneous nature of OCD in terms of its symptomatology (**section I.4.2.2**), as well as its age at onset, makes the elucidation of its genetic aetiology a rather formidable and challenging task. This is because individuals with different symptoms, that may comprise different genetic substrates are, by convention, diagnosed with the same general disorder. Enormous advances have, however, been made over the course of the last century in an effort to disclose the possible psychobiological basis of the disorder and, in doing so, have created a solid platform on which many molecular studies can be based.

## I.3. AETIOLOGICAL MODELS OF OCD

### I.3.1. The Biological Basis of OCD

OCD is proposed to be a multifactorial disorder, with numerous factors acting together in an additive manner to result in the expression of the clinical OCD phenotype. The aetiology of the disorder is thought to comprise neurobiological, genetic, behavioural and immunological components. Since the focus of the present dissertation is on the genetics of OCD and OCD-related subtypes, the neurobiological component will briefly be discussed in the context of biologic plausibility of the candidate genes that have been selected for investigation in this particular study. Of course, as already mentioned, OCD comprises numerous behavioural components, and is thought to comprise an immunological component as well (Swedo et al., 1998); however, a detailed discussion of these components is beyond the scope of this dissertation, and, for the sake of brevity, are mentioned briefly in only pertinent sections of the thesis.

### I.3.2. The Genetic Basis of OCD

#### I.3.2.1. Family studies in OCD

Familial aggregation of individuals afflicted with a specific psychiatric disorder at a higher rate than is prevalent in the general population indicates the possibility that genetic factors are involved in the development or course of the disease. Family risk studies are designed to determine the extent to which the disorder runs in families because, strictly speaking, all genetic disorders should have increased rates of expression amongst relatives. It should be noted, however, that familial clustering itself is not foolproof evidence of genetic involvement: other factors, such as family environment, culture or infectious agents, may also be transmitted within the family unit.

Over the past 60 years, researchers have proposed that OCD is a familial disorder (Kringlen, 1965; Rasmussen and Tsuang, 1986; Pauls et al., 1995; Nestadt, 2000[a]; Lenane et al., 1990; Swedo et al., 1989[a]; Riddle et al., 1990). Direct interview family studies have reported higher morbid risks of OCD between first degree relatives of OCD probands compared to relatives of psychiatrically normal controls (10% vs. 1.9%, respectively) (Black et al., 1992; Pauls et al., 1995). In fact, Pauls et al. (1995) conducted five separate studies, all of which confirmed a degree of familiality in OCD. Similar studies on children and adolescents reported increased rates (ranging between 20% and 25%) of developing the disorder in family members of OCD sufferers (Swedo et al., 1989[a]; Lenane et al., 1990; Rasmussen and Eisen, 1990; Riddle et al., 1990). Moreover, by employing multivariate analysis, Clifford et al. (1984) estimated the heritability of obsessive-compulsive symptoms to be approximately 40%, and the National Society of Genetic Counselors has estimated the heritability of OCD to lie between 40 and 50%.

A recent study conducted by Nestadt et al. (2000[a]) was designed to extend knowledge regarding the familial nature of OCD by providing as rigorous a test of the hypothesis as was possible. It was reported in this study that a 4 to 15-fold higher lifetime prevalence of OCD occurs amongst first-degree relatives of OCD probands. These results replicated those of an earlier study (Pauls et al., 1995).

It has also been observed that OCD morbidity rates are significantly higher amongst relatives of OCD probands whose age at onset is below 14 years (8.8% vs. 3.4%) (Bellodi et al., 1992).

These findings are consistent with those by Nestadt et al. (2000[a]), who found that the age at onset of obsessive-compulsive symptoms in probands was strongly related to familiality. In this study, no cases were reported in relatives of probands where the age at onset exceeded 17 years. The differences in rates amongst relatives of EO and LO probands was also reported by Pauls et al. (1995). It has also been reported that the risk of subclinical OCD (where substantial obsessions and compulsions are experienced, but are not considered severe enough to meet full OCD criteria [Lenane et al., 1990]) is greater in relatives of OCD probands with EO (<19 years) compared to those with LO (9.4% vs. 2.2%) of the disorder. This suggests that an earlier age at onset is likely to be valuable in characterising a familial subtype of the disorder, as will be discussed in **section I.4.2.2.4** .

Hettema et al. (2001) performed a meta-analysis to estimate summary statistics associated with aggregate familial risk and heritability for a number of anxiety disorders, including OCD. Information from the five studies included in the meta-analysis (McKeon and Murray, 1987; Black et al., 1992; Pauls et al., 1995; Nestadt et al., 2000[a]) indicated an odds ratio (OR) of 4.0 (95% CI:2.2-7.1) for OCD, and that there was substantial evidence for familial aggregation of the disorder. In a more recent two-year follow-up study, it was found that the offspring of OCD probands were at a greater risk of developing a lifetime overanxious disorder, separation anxiety disorder or OCD (Black et al., 2003). In addition, 23% of the offspring of OCD probands developed full-blown OCD, and 30% developed subclinical OCD. The investigators concluded that female gender, family dysfunction and high symptom levels for OCD were all predictive of broadly-defined OCD (broadly-defined OCD refers to clinical and subclinical OCD).

Familial transmission has been found to extend beyond the domain of OCD, and into that of disorders occurring co-morbidly with OCD (please refer to **section I.4.2.2.1** for a detailed discussion on OCD and comorbid disorders), indicating the likelihood of a common physiological, genetic and psychological aetiology for these disorders. Indeed, evidence from family studies indicates a putative common genetic basis for OCD and Tourette's syndrome (TS) (Pauls et al., 1986; Pitman et al., 1987; Grad et al., 1987) and certain obsessive-compulsive spectrum disorders (OCSD). It has also been reported that subclinical forms of OCD may be transmitted within families, and that they may represent a less severe form of OCD, thereby forming a fundamental part of the OCD spectrum (Black et al., 2003). Lenane et al. (1990) reported that, of 46 children with OCD participating in their study, the

prevalence for subclinical OCD was found to be 13% in the parents of these children, and 4% in sibs of the OCD sufferers. In a controlled study, Black et al. (1992) found the morbid risk for broadly defined OCD to be 20% amongst primary relatives of first-degree relatives of probands. In addition, parents of obsessional probands presented with an increased risk for broadly defined OCD compared to parents of the controls (15.6% vs 3.0%, respectively) ($p=0.034$). The same group also investigated OCSDs and found that the risk for developing one of these disorders was greater in relatives of probands with OCD.

**I.3.2.2. Twin studies in OCD**

Since the 1920s, psychiatric geneticists have cited twin studies as providing evidence of the role of genetic factors in the aetiology of mental disorders (Wilson, 1934). The primary method of analysis used in this case is the "classical twin method" (Joseph, 2000), which compares the concordance (or co-incidence) rates of reared-together identical twins (monozygotic or MZ twins) to the concordance rates of reared-together, same-sex non-identical twins (dizygotic or DZ twins). A pair of twins is said to be concordant for a specific condition if both members of the pair express the condition, and discordant if only one member of the pair expresses the condition. If only the rearing environment of the twins has contributed to the development of the disorder in one twin, then the co-twin, regardless of whether MZ or DZ, should also be at risk and the rates for both MZ and DZ twins should be elevated compared to the general population rate, and should be equal. However, if genes play a role in predisposition to the disorder, the concordance rate for MZ twins will be greater than that for DZ twins. It follows that MZ twins will be concordant for any genetically determined characteristic, regardless of the mode of inheritance or the number of genes involved.

Twin studies pertaining to OCD are capable of delimiting the genetic and environmental influences on the variation in liability to the disorder, although they have been somewhat limited by the paucity of the subjects. Nonetheless, the few twin studies that have been conducted suggest the involvement of hereditary components, with MZ concordance rates of between 53% and 87% and DZ concordance rates between 22% and 47% (Inouye, 1965; Rasmussen and Tsuang, 1986; Pauls et al., 1995; Carey and Gottesman, 1981).

In a larger study of twin samples, a higher rate of concordance for OCD and subclinical OCD was found in MZ twins when compared to DZ twins (Inouye, 1965; Skre et al., 1993), and the specific nature of the symptoms and response to therapeutic agents has been found to be more

similar for MZ than DZ twins (Kim et al., 1990; McGuffin and Mawson, 1980). It has also been shown that MZ twins show a higher concordance for OCSD than DZ twins (Clifford et al., 1984), with heritability recently calculated at between 26% and 33% (Jonnal et al., 2000). Although all the results implicate higher concordance rates for MZ, compared to DZ twins, the transmission in MZ twins has been shown to be incomplete; hence the hypothesis that environmental factors such as birth complications and other physiologic vulnerabilities may have significance in the development of OCD (Petronis, 2001).

The cumulative evidence from family and twin studies suggest that some forms of OCD are indeed familial, with the possibility of genetic factors playing a role in the phenotypic manifestation of these familial forms. Early age at onset seems to indicate a higher degree of familial loading, and may therefore be important when characterising familial subtypes (Nestadt et al., 2000[b]). However, in the only study of its kind thus far conducted, Albert et al. (2002) observed no differences in clinical features between familial and non-familial OCD (including age at onset as criterion). Nonetheless, they did observe a lower threshold for precipitating events amongst OCD patients with the familial form of OCD, implying that it may be this characteristic that sets the familial and non-familial forms apart.

**I.3.2.3. Complex segregation analysis of OCD**

Once sufficient evidence for the familial transmission of OCD has been attained, the next step is to determine whether genetic factors are responsible for the observed familiality, and, if so, what the mode of genetic transmission is. Complex segregation analysis (CSA) is a method designed to evaluate the transmission of a trait within a pedigree, to determine whether the segregation of a major gene occurs in the presence of familial resemblance of the disorder. The analysis also tests the magnitude of the genetic sources of variation in the trait. Parameters that are usually estimated in the CSA include transmission probabilities, allele frequencies, penetrances for each genotype (for qualitative traits) and genotype means (quantitative traits), variance within genotypes and residual correlations not explained by Mendelian inheritance (Jarvik, 1998).

The success of a genetic association study depends critically upon the allelic architecture of the disease. Allelic architecture refers to the number of alleles involved in a disorder, and their respective penetrances (Pritchard and Cox, 2002). It follows that, in order to detect a significantly increased frequency in disease alleles in a group of affected individuals, the

allelic spectrum should be simple – in other words, a few predominant alleles should account for the phenotype (Reich and Lander, 2001). Assumptions regarding the allelic spectrum provide insight into the most efficient strategy to employ when searching for genetic contributions to complex disorders.

In order to understand the allelic architecture of a disorder, it is important to comprehend how the disease causing alleles are (and have been) affected by factors such as population growth, mutation rate, genetic drift, and to appreciate the role of selection against disease alleles (Reich and Lander, 2001; Pritchard and Cox, 2002). The common disease/common variant (CD/CV) hypothesis maintains that the genetic variation underlying complex disorders arose within the founding population of contemporary humans; hence the disease alleles are common, usually with frequencies in excess of 1% in the general population (Collins et al., 1997; Lander, 1996; Risch and Merikangas, 1996). The disease allele remains within the population at a relatively moderate frequency due to their selective neutrality (Wright and Hastie, 2001). If the CD/CV hypothesis is assumed, it is possible for the disease-causing alleles to be sought using indirect methods, such as linkage disequilibrium (LD) mapping.

The alternative hypothesis is known as the common disease/rare allele (CD/RA), or genetic heterogeneity, model. This model posits that rare alleles at numerous loci, each with a large number of alleles, can comprise the genetic contribution to the disorder. In contrast to the CD/CV hypothesis, the CD/RA hypothesis suggests that disease susceptibility alleles arose independently, in various geographically distinct, dispersed populations (Smith and Lusis, 2002), with the result that a disease allele in one population may not be evident in another. If one adopts this hypothesis, it is important to note that, although the susceptibility alleles comprise a large proportion of the genetic risk for the disease, they will not be conducive to indirect genetic association methods.

Clearly, it is in the investigator's best interest to identify the disorder's underlying allelic architecture; indeed, numerous segregation analyses have been conducted in an attempt to elucidate the mode of genetic transmission of OCD (Table I.2). In the first published segregation analysis, Nicolini et al. (1991) investigated the mode of transmission in OCD probands. Their segregation analysis included all affected individuals with a diagnosis of OCD, chronic motor tics (CMT) or TS. The investigators found that the autosomal dominant model was most compatible with the observed levels of segregation, although, due to the

small numbers of probands included in the study, neither the autosomal dominant nor autosomal recessive models could be rejected, indicating that OCD cannot be explained by a simple mode of transmission.

Cavallini et al. (1999) confirmed the presence of a major locus, with Mendelian properties accounting for most of the liability to OCD. They could not, however, exclude the possibility of the existence of potential heterogeneity in their model when the phenotypic boundaries were widened to include OCD, TS and CMT. They also noted differential penetrance values for OCD phenotypes between males and females, with females exhibiting slightly higher penetrance values.

In an attempt to limit the phenotypic heterogeneity of OCD, Alsobrook et al. (1999) categorised families in the sample according to four factor analytic symptom dimensions of OCD (**section I.4.2.2.5**). Segregation analysis of 96 families allowed only rejection of the no-transmission model, providing evidence that OCD is indeed genetically transmitted, although no specific mode of transmission could be specified. In the most recent, and only controlled, segregation analyses conducted, Nestadt et al. (2000[b]) found that neither Mendelian dominant nor codominant models could be rejected, indicating the presence of a major locus. However, unexplained familial factors were also observed to be important in the expression of OCD: for example, significant heterogeneity on the basis of gender of the proband was detected. This prompted separate segregation analyses of families with male and female probands. The transmission of OCD in female proband families was compatible with the Mendelian major locus (either dominant or codominant) model. In the male proband families, although a Mendelian mode of transmission was found to be the most compatible, the details of this model were found to be less evident than for the female group.

Collective evidence from the segregation analyses indicates that the familial transmission of OCD is indeed due, in part at least, to genetic factors, and that this mode of inheritance is not simple. The genetic contribution to the disorder is more than likely complex, representing a mixed mode of transmission, involving genes of major effect with appreciable impact, operating against a milieu of polygenic inheritance.

Once it has been proven that genetic transmission accounts for at least some of the familiality of a disorder, the next logical step is to locate the susceptibility gene(s). This can be achieved

using a number of molecular genetic methodologies that are broadly categorised into parametric (model-based) and non-parametric (non-model-based) strategies.

***Table I.2:*** *Complex segregation analyses in OCD*

| Reference | No. proband families | No. control families | Mode of transmission most compatible (i.e models that were not rejected) |
|---|---|---|---|
| Nicolini *et al*. (1991) | 24[a] | 0 | Autosomal dominant or recessive model |
| Cavallini *et al*. (1999) | 107 (*418 1st degree relatives; 1121 2nd degree relatives*) | 0 | Autosomal dominant model, with possibility of polygenic inheritance |
| Alsbrook *et al*. (1999) | 100 (*466 1st degree relatives*) | 0 | All models except mixed model were rejected for whole sample;<br>Symmetry and ordering symptom subset: major locus model |
| Nestadt *et al*. (2000[b]) | 80 (*340 1st degree relatives*) | 70 *(303 1st degree relatives*) | Mendelian major locus (dominant or codominant) model, with familial residual effects for whole sample.<br>*Females*: Mendelian dominant or codominant model;<br>*Males*: Mendelian model |

[a]No additional information available

[b]The dominant model was found to be more parsimonious than the co-dominant model

### I.3.2.4. Genetic linkage studies

Genetic linkage analysis provides a powerful approach with which to elucidate the underlying genetic factors in inherited disorders. Linkage analysis serves to demonstrate the existence of a major locus to clarify the observed pattern of inheritance of a particular trait within a family (Alsobrook and Pauls, 1998). The objective is to establish the co-segregation of polymorphic genetic markers of known chromosomal location with the disease phenotype within a family unit or pedigree (i.e., the non-random sharing of marker alleles between affected members of each family).

After initial localisation of a putative genetic marker for a disease susceptibility locus by means of linkage analysis, it is possible to type additional polymorphic markers in order to generate a high resolution map of the relevant genomic region surrounding the disease-causing gene (Alsobrook and Pauls, 1998). Thereafter, the location of the causative genetic variant(s) may be inferred by means of, for example, fine mapping procedures.

Unfortunately, linkage analysis has had limited success in detecting genes involved in psychiatric disorders due to their complex, multifactorial nature. Nowadays, searching for linkage between candidate loci and complex psychiatric disorders involves conducting a whole genome screen (Risch and Merikangas, 1996; Collins et al., 1997). This strategy involves screening the entire genome using evenly spaced genetic markers, to allow identification of regions of potential linkage. The regions are recognised by calculating an appropriate linkage statistic at each position along the genome, and identifying those regions in which the statistic indicates a significant deviation from what would be expected under the rule of independent assortment (Visscher and Haley, 2001). A peak is produced where the test statistic exceeds a predetermined significance threshold caused by one or more loci that may influence the trait. A second stage of mapping may then be applied to fine-map the linked chromosomal region.

In genome-wide linkage studies, either sib-pairs or families can be used. This method has met with much success in identifying susceptibility loci for psychiatric disorders, including social phobia (Gelernter et al., 2004); simple phobia (Gelernter et al., 2003); schizophrenia (Stefansson et al., 2002); irritable bowel syndrome (Hugot et al., 2001; Rioux et al., 2001; Stoll et al., 2004); bipolar disorder (Middleton et al., 2004), panic disorder (Knowles et al., 1998) and panic syndrome (Hamilton et al., 2003).

To date, one OCD genome-wide linkage study has been published (Hanna et al., 2002). This study was conducted using seven probands (who ranged in age from six to 17 years), whose age at onset was between three and 14 years. Fifty-six individuals from the seven families were initially genotyped with 349 microsatellite markers spaced at any average of 11.3 centiMorgan (cM). A region on the telomere of chromosome 9p met with the criterion for suggestive linkage (using the dominant model, the logarithm of odds score [LOD score] was equal to 2.25). The investigators also observed weak evidence for linkage between OCD and regions on chromosomes 2q, 6q, 16q, 17q and 19q.

Hanna et al. (2002) then followed up their initial findings on chromosomes 2q, 9p and 16q (i.e., those regions with dominant LOD scores of greater than 1) by genotyping 24 additional markers at an average spacing of 1.6 cM in the original 56 subjects, as well as in 10 additional family members from one of the families. They found the region that displayed the strongest evidence for linkage (with a LOD score of 1.97) was 9p24. Interestingly, there has been a

report of 9p monosomy in a patient with TS (Taylor et al., 1991) and a region on chromosome 6p has also been found to be associated with TS in an Afrikaner subject sample (Simonic et al, 1998), providing further support for the proposed genetic link between the two disorders.

Recently, the evidence for linkage on 9p24 has been replicated (Willour et al, 2004) using 50 pedigrees (consisting of 193 subjects) from the John Hopkins OCD family study (Nestadt et al., 2000[a]). Here, genomic DNA samples from all individuals were genotyped for 13 microsatellite markers spanning a distance of 19 cM across 9p24, with an average inter-marker spacing of 15 cM. The narrow phenotype model for OCD, combined with dominant parameters and penetrance of 0.5, produced the strongest findings in the study (LOD score = 2.26), with the strongest nonparametric findings also observed under a narrow phenotype model (nonparametric linkage signal [NPL] = 2.52; p = 0.006).

It is notable that the original LOD score for chromosome 9p24 (LOD = 2.25) obtained by Hanna et al. (2002) resembled the LOD score in the study by Willour et al. (2004) very closely. The NPL peak observed by Willour et al. (2004) was situated at D9S1813, which lies only 350kb away from that observed by Hanna et al. (2002) at D9S288, suggesting the location of a susceptibility locus in that region. The 9p24 chromosomal region spans approximately 75 megabases (Mb) and thus contains numerous potential candidate genes that could be investigated for the role they may play in the development of OCD. Veenstra-vanderWeele et al. (2001) conducted a mutation screen of the gene encoding the neuronal and epithelial glutamate transporter (situated approximately 350cM centromeric to the NPL peak identified by Willour et al. [2004]). They reported eight exonic synonymous single nucleotide polymorphisms (SNPs) that did not appear to alter the functioning of the gene. Using a family-based association study, the investigators observed no statistically significant association between two of the intronic polymorphisms and OCD.

Nonetheless, the results from both the genome scans are noteworthy, and a collaborative effort is underway to collect almost 300 sib-pair and multiplex families, in an attempt to improve the power of the previous studies, and replicate the results (Willour et al., 2004).

***Limitations of using pedigree linkage analyses in complex psychiatric disorders***

In OCD, as for all psychiatric disorders, the underlying mechanisms, degree of penetrance and mode of inheritance remain unknown (Crowe, 1993). The environmental contribution to the aetiology of the disorder, diagnostic misclassification, and the epistatic and additive interactions between genes conferring susceptibility to mental illness may also limit the success of identifying susceptibility genes utilising linkage strategy (Crowe, 1993; Nothen et al., 1993; Souery et al., 2001; Stoltenberg and Burmeister, 2000; Ghosh and Schork, 1996).

Moreover, identifying families with multiple affected individuals poses a problem – there still seems to be an amount of stigma attached to being diagnosed with OCD; consequently a large proportion of affected individuals prefer to keep their affliction hidden from family members. The nature of the susceptibility allele further weakens the power of linkage analysis for complex disorders, since the susceptibility allele is neither necessary nor sufficient to produce the clinical phenotype (Greenberg, 1993; Hodge, 1993), but may simply increase the chances that the allele-carrier will develop the disorder. Therefore, a proportion of the patients will not possess the associated allele, in which case the "susceptibility" allele may not necessarily co-segregate with the disorder (Nothen et al., 1993; Propping et al., 1993).

Overall, it has been found that association designs have a greater power to detect disease alleles in disorders with mild risk factors (with a genotypic risk ratio [GRR] < 4) than in comparably sized linkage analyses (parametric and non-parametric). This is because for diseases with a GRR <2, unrealistic family sample sizes (in excess of 2500) are required to achieve the same power as for association studies (Risch and Merikangas, 1996; Risch and Botstein, 1996).

### I.3.2.5. Genetic association analyses

Association studies offer an alternative strategy to study genetic factors involved in complex psychiatric disorders. Historically, genetic association analyses have been conducted in a population-based setting (Silverman and Palmer, 2000), where the aim of the association study has been to demonstrate a significantly different distribution of allelic variants in affected (case) and unaffected (control) individuals. The basic unit of analysis in association studies is the individual, who can be included regardless of the status of their other family members.

Association studies can be conducted using one of two strategies, both reliant on the CD/CV hypothesis (**section I.3.2.3**). Candidate gene association analyses investigate variants within a particular genomic region, based on physiological, biochemical or pharmacological evidence. These investigations take advantage of the increased power of association studies to detect genes of moderate effect, whilst capturing an account of the current biological understanding of the tissues, proteins and genes likely to play a role in the pathogenesis of OCD. An alternative to candidate-gene based analyses, known as genome-wide association analyses, involves screening the entire genome for causal genetic variants. No prior assumptions are made regarding the location of the susceptibility variants, implying that the procedure represents an unbiased, systematic approach to identifying the causal variants (Hirschhorn and Daly, 2005). Genetic association studies pertaining directly to OCD will be discussed in **section I.6**.

In both of these association methods, the usefulness of the selected marker depends on its ability to identify the susceptibility allele. This is achieved by exploiting the preferential association between the marker and susceptibility loci, due to a characteristic known as linkage disequilibrium.

#### *I.3.2.5.1. Linkage Disequilibrium (LD)*

LD refers to the non-random statistical association of sequence variants along an individual chromosome that results in an increased tendency for the alleles of closely linked loci to co-segregate with an increased frequency across a population. This represents a powerful tool for investigating population history, human evolution and the genetic aetiology of complex disorders (Jorde et al., 1995; Kidd et al., 1998).

In LD mapping, a group of affected individuals, descended from a single founder, form part of a large multigenerational pedigree of which all initial generations, except the current few, are missing. Numerous meiotic and recombination events have therefore occurred, narrowing the region of DNA that possesses the susceptibility allele. The ability to identify genetic components of complex phenotypic variation depends to a large extent on our knowledge of how different parts of the genome are correlated. Focussing on LD and haplotype analyses (discussed further on) will afford a unique insight into these processes.

### *i. Measures of LD*

The majority of LD measurements represent the pairwise association between markers (Pritchard and Przeworski, 2001), with the most widely used being the absolute values of the normalised disequilibrium coefficient (|D'|) (Hedrick, 1987; Lewontin, 1964) and the absolute value of the correlation coefficient, $r^2$ (Hill and Robertson, 1968). Both of these measurements are derived from the LD pairwise coefficient, D, but have slightly different interpretations (Wall and Pritchard, 2003).

The value of |D'| = 1 indicates the lack of recombination between the two loci under investigation (complete LD), whereas |D'|<1 represents a disruption in LD sometime in the past. However, since D' is not dependent on allele frequencies, |D'| = 1 if three out of the four possible haplotypes are present (i.e. the alleles in LD with one another do not have to possess the same allele frequencies) (Weiss and Clark, 2002). Although |D'| does not depend on allele frequencies *per se*, it does depend on the size of the sample under study - values of |D'| have been found to be inflated in small samples, even when the loci are in linkage equilibrium (Gabriel et al., 2002). Moreover, the intermediate values of |D'| are difficult to interpret, and have been found to vary in simulations for pairs of sites at a given distance (Wall and Pritchard, 2003).

The value of the correlation coefficient, $r^2$ represents the statistical correlation between two sites. The value of $r^2 = 1$ if, and only if, *no* historical recombination has occurred, and the markers have the same allele frequencies (i.e. only two out the four possible haplotypes are observed in the sample). The value of $r^2$ is useful in that it is indicative of the power of the LD study – the inverse of $r^2$ represents the factor by which the sample size should be increased to detect statistically significant association between the marker locus and disease, providing a rough guide as to the usefulness of a given level of LD (Ardlie et al., 2002; Weiss and Clark, 2002). Higher values of $r^2$ are indicative of a greater ability of one SNP to predict the behaviour of the SNP in LD with it.

Another useful advantage to using the $r^2$ value is that it is related to the average recombination fraction in the population which summarises LD over a particular genomic region, not just between pairs of markers (Pritchard and Przeworski, 2001). A further advantage of using $r^2$ to measure LD is that it is comparable across studies. However, due to its sensitivity to allele frequency, it may mean that two markers that are adjacent to one another may yield different

$r^2$ values with a third marker (Ardlie et al., 2002). It is therefore at the discretion of the investigator to decide on the most appropriate measurement of LD to use in the study conducted. In the present study, both D' and $r^2$ values are represented, in order to allow the reader a comprehensive view of pairwise LD between markers investigated.

### *ii. LD in genetic association studies*

LD patterns are useful in association studies because they impart knowledge regarding the genetic distance over which signals of causation may be generated in case-control studies. Furthermore, they facilitate the identification of the susceptibility allele by identifying the neighbourhood surrounding the variant (Risch and Merikangas, 1996). However, it should be noted that there are various factors that disturb the relationship between LD and distance, both evolutionary (e.g. population dynamics and natural selection [Nordborg et al., 2002; Reich and Lander, 2001; Kruglyak, 1999[a]; Pritchard and Przeworski, 2001]) and genetic (recombination, inversion [Pritchard and Przeworski, 2001] and conversion polymorphisms [Langley et al., 2000; Ardlie et al., 2002; Frisse et al., 2001], genetic drift and mutation rate [Terwilliger et al., 1998; Ardlie et al., 2002]) . Indeed, markers that are closely linked have been found to exhibit low levels of LD, or none at all (Clark, 1998; Moffat et al., 2000; Ardlie et al., 2002; Kidd et al., 2000; Rieder et al., 1999; Templeton et al., 2000), whilst relatively high levels of LD have been observed between markers that are comparatively far apart from one another (Collins et al., 1999; Abecasis et al., 2001; Reich et al., 2001; Stephens et al., 2001; Gordon et al., 2000).

### *iii. The importance of demographic history in LD association studies*

It is clear that patterns of LD in the human genome are strongly shaped by evolutionary history; in turn, each disease has its own genetic architecture, shaped by aspects of population dynamics and history. The demographics of any population is complex, with each population experiencing differential degrees of isolation, migration, admixture, expansion and bottlenecks (Ardlie et al., 2002), aspects of which will inevitably remain unknown. This underscores the critical importance of characterising the LD landscape in the region of interest in the population under investigation.

Significantly higher levels of LD have been noted in "younger", more recently founded populations (Jorde et al., 2000; Peltonen et al., 2000; Puffenberger et al., 1994; Hall et al., 2002), implying a large degree of LD over longer stretches of the genome. For example, LD

has been found to extend over much longer regions in younger, non-African populations, probably reflecting the loss of genetic variation caused by the bottleneck that occurred when modern humans migrated out of Africa (Weiss and Clark, 2002; Frisse et al., 2001; Reich et al., 2001; Tishkoff et al., 1996; Wall, 2001). Such populations may be useful in the coarse-mapping of disease susceptibility alleles (i.e. identifying the region in which the allele may be situated), but will not be amenable to fine-mapping procedures. Older populations, on the other hand, exhibit less LD and larger amounts of recombination over shorter genomic regions, thus facilitating fine-mapping procedures (Jorde et al., 2000; Wilson and Goldstein, 2000).

***I.3.2.5.2. Haplotype association analysis***

Single marker investigations may provide little information regarding the association, and, although they may be situated in the candidate gene, it is possible that the markers may not be in LD with the susceptibility allele. A haplotype refers to a specific combination of alleles that co-occur on an individual chromosome, and therefore share a common evolutionary history. Single nucleotide polymorphisms (SNPs), genotyped sequentially over the length of a chromosome, can be ordered into haplotypes. Such haplotype scanning may provide more information regarding variation within specific genomic fragments and interrelationships between polymorphisms in the surrounding regions, thereby imparting a greater amount of power to the study (Akey et al., 2001). This is because the historical crossover points can be analysed with greater accuracy from preserved and non-preserved portions of the mutation-bearing chromosome, which will, in turn facilitate localisation of the disease allele.

It is also possible that alleles at several SNPs jointly influence susceptibility to a disease by influencing regulation and/or functioning of the susceptibility variants, or that the alleles may act in combination with one another (much like a “super-allele”) to precipitate the phenotype, or certain aspects of the phenotype. Indeed, haplotype association studies have allowed the successful localisation of susceptibility genes in Hirschsprung disease (Puffenberger et al., 2004) and Crohn’s disease (Hugot et al., 2001; Rioux et al., 2001), and in locating candidate susceptibility regions in schizophrenia (Shifman et al., 2002) and cerebral malaria (Burgner et al., 2003). In addition, the merits of haplotype analyses in association studies have been illustrated using known associations between the apolipoprotein E locus and Alzheimer’s disease (Fallin et al., 2001), and adenine phosphoribosyltransferase gene and adenine phosphoribosyltransferase deficiency (Kuno et al., 2004).

### *i. Haplotype inference*

The investigation and subsequent analysis of haplotype data rests on the assumption that the haplotypic phase information pertaining to the individuals in the study is available. Ambiguous haplotypes can be resolved using data from relatives or genealogical information, allowing one to infer ancestral haplotype compositions. However, these methods are often costly (due to extra genotyping efforts) and impractical in population-based case-control association studies, where there is usually limited access to any kind of family genetic data. An alternative would be to employ laboratory-based molecular haplotyping methodology, such as chromosomal localisation, single-molecule dilution or allele-specific polymerase techniques (Ruano and Kidd, 1989; Clark et al., 1998; Ruano et al., 1990; Michalatos-Beloin et al., 1996), which are also expensive and technologically demanding (Niu et al., 2002).

The solution therefore seems to be to predict the haplotype phase of unrelated, diploid individuals probabilistically, based on estimated allele frequencies from the population. Several assumption- and likelihood-based methods have been created, these can be roughly divided into three major categories, based on the algorithm employed: Clark's algorithm (Clark, 1990); the expectation-maximisation (EM) algorithm (Dempster et al., 1977; Hawley and Kidd, 1995; Long et al., 1995; Excoffier and Slatkin, 1995) and the coalescent-based algorithm (implemented in the program "Phase" [Stephens et al., 2001]). Recently several other methods, mostly based on the three aforementioned ones, have been created and successfully used to infer haplotype phase in genetic association studies (Zollner and Pritchard, 2005; Niu et al., 2002; Qin et al., 2002; Gusfield, 2001).

Clark's algorithm assigns haplotypes to phase-unambiguous (i.e. homozygotes or single-site heterozygotes) individuals first. For each unresolved, ambiguous haplotype, the aim is to determine whether the known haplotype can be formed from some combination of the ambiguous sites (hence the "subtraction method" as the alternative name for this method). Each time a haplotype is inferred in this way, it is viewed as another potential unambiguous haplotype from which the ambiguous haplotypes can be inferred. This chain of inference continues until all haplotypes have been recovered, or until one identifies a sequence that cannot be derived from any of the known haplotypes (Clark, 1990; Clark et al., 1998).

The EM algorithm obtains maximum likelihood estimates of haplotype frequencies within the sample, and uses the initial set of frequencies to calculate conditional distributions for

haplotype pairs that an individual carries (the expectation step). In the maximisation step, the haplotype frequencies are updated based on haplotypes inferred in the previous step. The EM algorithm iterates between the two steps until the frequency estimates converge. This method may, however, not be viable when analysing a large number of markers, due to the computational burden involved (Fallin and Schork, 2000).

The Phase algorithm uses a combination of the coalescent-based ancestry model and Bayesian-based algorithms to assign phases to the linked loci and estimate haplotype frequencies accordingly (Stephens et al., 2001). The method regards unknown haplotypes as random quantities and aims to evaluate their distribution, given the genotype data. The program also confronts certain population genetics features of haplotype inference by incorporating prior knowledge that the unresolved haplotypes will be more similar to commonly observed, resolved haplotypes. Phase can be applied to both SNP and multiallelic data.

In studies comparing the EM and Phase methods, Zhang et al. (2001) observed no major differences in accuracy between the two methods. Xu et al. (2002) incorporated levels of LD between markers into their study and found that, when LD between the markers was maintained, all three methods performed equally well. However, if LD between the markers was not maintained, Clark's algorithm did not perform as well as Phase or the EM algorithms. In a more recent study, Adkins (2004) compared the efficacies of leading computational methods in haplotype inference (including Phase and EM algorithms) and found that all performed with high accuracy, even when identifying rare haplotypes. They also observed that haplotype assignment remained accurate among subjects for up to five sites. It is thus clear that there is no agreement as to which algorithm may be best in estimating haplotype frequencies in population-based association studies; perhaps it would be more conducive to the investigators to focus on parameters that decrease the estimation error in computational inference of haplotypes.

According to Fallin and Schork (2000), error in haplotype estimation can be reduced by following a few pointers. Firstly, they advocate using the appropriate set of markers, taking note of LD between them. Some algorithms may not be able to handle the large number of haplotypes if the markers in the study are in linkage equilibrium (Zhao et al., 2003). They also suggest increasing the sample size and decreasing the haplotype ambiguity (i.e. fewer

individuals with haplotypes that cannot be resolved) where possible. Finally, increasing the dispersion of haplotype frequency values in a sample can also result in fewer haplotype estimation errors: as haplotype values become less uniform, the difference between the most and least common haplotypes becomes more extreme. The null-frequency haplotypes can thus be accurately predicted as zero, since there will be little evidence from the data for their non-zero frequencies, resulting in more accurate estimation of the commoner haplotypes.

### *ii. Haplotype blocks and recombination hotspots*

Recently, studies investigating several genomic regions have indicated the presence of long chromosomal tracts in which the markers exhibit strong LD and limited haplotype diversity (known as haplotype blocks), separated by areas in which the recombination rate is relatively high (recombination hotspots) (Daly et al., 2001; Johnson et al., 2001; Patil et al., 2001; Gabriel et al., 2002). Due to the limited haplotype diversity within the haplotype blocks, a small number of haplotypes represent the varation in most of the chromosomes within the population. Furthermore, high levels of LD within the blocks signify that some of the markers contain redundant information, allowing the variation within the haplotype blocks to be distinguished by one, or a few, SNPs. Therefore, theoretically, a disease susceptibility gene could be mapped to one of the haplotype blocks using a so-called tag SNP, which would improve the chances of detecting association when only a fraction of the markers are genotyped, saving significantly on time and money (Gabriel et al., 2002; Patil et al., 2001; Johnson et al., 2001).

Efforts are presently underway by the United States National Human Genome Research Institute, in the form of an international intiative, the HapMap project, which aims to delineate the structure and boundaries of common haplotype and LD blocks in the genome, using populations from Africa, Asia and Europe (The International HapMap Consortium, 2003). However, although the idea of haplotype blocks and recombination hotspots affords an insight into the distribution of LD within the human genome, even they seem to have an erratic distribution (Stephens et al., 2001; Pritchard and Przeworski, 2001), underscoring the importance of investigating the LD landscape for each region of interest, rather than applying a general LD value for that particular region.

## I.4. DETERMINING THE VALIDITY OF AN ASSOCIATION: STATISTICAL INFERENCE

*"Our belief in a hypothesis can have no stronger basis than our repeated unsuccessful attempts to refute it."*

*Karl Popper*

Numerous association studies (both family- and population-based) have been conducted in order to delineate the genetic contribution to OCD over the past decade. Unfortunately, these have been met with very little concrete evidence as to the involvement of any one particular gene or genetic variant, since they have yielded mostly inconsistent results, as will be evident further on in the dissertation. The discrepancies have resulted in a large amount of scepticism regarding the usefulness of candidate-gene based association studies. Doubts have also arisen as to the generalisability of these results across certain populations and ethnic groups – it seems that there is no guarantee that loci that appear to be associated with OCD in one population will have an effect in another. Numerous aspects of the causes and consequences of the aforementioned inconsistencies will be dealt with in this section and the next.

A statistically significant association, although initially exciting, cannot (and, indeed, *should* not) be viewed as a necessary causal association. The first step in assessing the validity of the result of a case-control association study involves statistical inference. The test of association evaluates the evidence for two competing hypotheses: the null hypothesis ($H_0$) states that the alleles (or genotypes) under investigation occur at equal frequencies in the case and control populations, whilst the alternative hypothesis ($H_1$) states that a significant difference exists in the allele and/or genotype frequencies between cases and controls. For categorical variables, it is assumed that the test of association follows the known probability distribution of a chi-square ($\chi^2$) variable under the null hypothesis (Lalouel and Rohrwasser, 2001).

Favouring one hypothesis over the other always entails the risk of error. In statistical terms, these are known as the Type I and Type II errors (Neyman and Pearson, 1933). The Type I error ($\alpha$) refers to the probability of rejecting the null hypothesis when it is in fact true (i.e. a false-positive result), whilst the Type II error ($\beta$) refers to the probability of accepting the null hypothesis when it should have been rejected (i.e. a false negative result). These probabilities are usually predetermined and set at 5% and 20%, respectively (Neyman and Pearson, 1933).

The p-value (p) of a statistical hypothesis refers to the possibility of obtaining a value of the test statistic (in this case a $\chi^2$ value) as extreme as, or more extreme that, that observed by chance alone, if $H_0$ is true (Dawson and Trapp, 2004). It is equal to the significance level (α) of the test for which one would only just reject the null hypothesis, but, unlike α, is calculated *after* the statistical test has been performed. The p-value is compared with α, and if it is smaller, the result obtained is significant. Small p-values indicate that the null hypothesis is unlikely to be true - the smaller the p-value, the more convincing the rejection of the null hypothesis becomes.

The p-value of significance presents one with a dichotomous, qualitative index of the strength of evidence against the null hypothesis, and to most researchers, the resultant values simply indicate whether the results are either significant or not; they do not give one any indication of the *magnitude* of the effect, and cannot incorporate additional evidence (Lilford and Braunholtz, 1996; Rothman, 1986). Consequently, the strength of evidence in one study cannot be related to, or combined with, the strength of evidence from another study. Hence, p-values are often misleading (and misunderstood) – investigators may become excited about a "highly significant" result (i.e. a low p-value) without taking supplemental evidence or factors into account. A common misconception is that, if $p< 0.05$ (i.e. the null hypothesis is rejected), the positive predictive value (Vecchio, 1966) of the test is 95%, where in actual fact it may be quite low, due to insufficient power (Sterne and Davey-Smith, 2001).

It thus is important to remember that the validity of the p-value is ultimately determined by the quality of the input data and subsequent analyses, and should be interpreted in conjunction with supplemental evidence (e.g. from OR [the effect size (ES) of the study and confidence intervals (CIs)] in order to make the appropriate scientific judgement and to inform research decisions. The ES of a variant is the name given to a family of indices that measure the magnitude of the experimental effect (in other words, it represents the difference between the null and alternate hypotheses.

In candidate gene LD-based analyses, where a marker allele is normally used as a proxy for the disease susceptibility allele, the term "apparent ES" refers to the ES of the marker locus (Zondervan and Cardon, 2004). Therefore, ES in population-based association analyses depends on the agreement between the marker and disease allele frequencies (hereafter referred to as MAF and DAF, respectively), and the extent of LD between the marker and

disease alleles. When LD is not complete, and the MAF is equal to the DAF, the apparent ES will be approximately proportional to the true ES, multiplied by the value of D'. When LD is complete, and the MAF is greater than the DAF, the apparent ES will differ from the true ES by a scale factor of the allele frequency ratios. However, when MAF is less than DAF, even in complete LD, apparent ES will decay rapidly with increasing discrepancy between MAF and DAF, since not even complete LD will be able to compensate for the fact that the disease allele may occur on haplotypes other than the marker haplotype.

In case-control association studies, investigators normally adopt a method of inductive inference to determine whether sufficient support exists for a causal association between the candidate gene and the phenotypic trait. This involves investigating the likelihood that other factors (namely, confounding, chance and bias) could have resulted in the statistically significant observations. Once these factors have been eliminated as agents delivering the positive association, one can move onto the process of causal inference for association (Campbell and Rudan, 2002).

### I.4.1. CONFOUNDING

In epidemiology, confounding is defined as a "situation in which a measure of the effect of an exposure on risk is distorted because of the association of allele exposure with other factors that influence the outcome of the study" (Last, 2000). When referring to confounding in genetic association studies, the "exposure" would refer to the allele or genotype under investigation; hence the definition of confounding for our purposes would be: a situation in which the measure of the effect of a genetic variant on OCD is distorted because of the association of the genetic variant with other factors that influence the outcome of the study. Confounding is thus a problem of comparison, where there is an imbalance in extraneous risk factors, measured or unmeasured, between case and control individuals. For a genetic variable to be a confounder, it requires two associations: first, the confounder must be associated with the disease, and second, it must be associated with the genetic variable in the underlying population from which the sample is drawn. A variable will not be considered to be a confounding factor if it merely represents an intermediate risk in the causal chain between the genetic variant and OCD (Schoenbach and Rosamond, 2000).

Restricting the analysis to a group of participants with only certain characteristics or stratifying the disorder within categories of potential confounders whilst holding all other

factors constant, will eliminate the influence that potential confounders may have on the outcome. One of the most serious potential confounders in population-based case-control association analyses is population stratification (Risch, 2000). Population stratification or substructure results from non-random mating between subgroups within a population, and may confound the results of an association study, depending on the extent of the genetic differences between the groups. This confounder will be discussed in more detail in the following section.

#### I.4.1.1. Population stratification

> *"Human populations differ from one another almost entirely in the varying proportions of the allelic genes of the various sets of hereditary factors, and not in the kinds of genes they contain. The extreme positions held by those who on the one hand maintain that there are no significant genetic differences between human races, and those who on the other hand hold that certain races are 'superior' and others 'inferior', require drastic modification in the light of the accumulated data on the gene frequency dynamics of human populations."*
> *Laurence Snyder, 1951*

The definition of race is usually subjective and based on proxies such as skin colour, language, physical properties and geographical location (Bamshad et al., 2003; Pritchard et al., 2000), with little insight into the genetic differences or similarities between populations (Foster and Sharp, 2002; Witzig, 1996; Goodman, 2000). Indeed, genetically similar groups may be labelled differently due to cultural differences or geographical location. Likewise, genetically dissimilar groups may be classified as a single population.

Comparative studies of within-group versus between-group genetic diversity indicates that approximately 90% of genetic variation occurs within human populations. Consequently, only 10% of genetic variation attributed to between-group differences (Lewontin, 1972; Cavalli-Sforza and Piazza, 1975; Jorde et al., 2001; Barbujani et al., 1997), which influences average differences in physical characteristics, disease susceptibility and treatment outcome amongst populations. In all the major population groups, there seems to be some degree of cryptic population substructure, which generally follows ethnic lines (Ziv and Burchard, 2003).

In population stratification, the observed association between a genetic susceptibility variant (G) and disease (D) is biased, due to the fact that G is associated with some true risk factor that varies with ethnicity. Therefore, if the population under investigation comprises cryptic subpopulations in which allele frequencies for the candidate gene *and* baseline risks of disease differ, it may result in spurious association between a genetic variant and the disease under investigation. This is because *any* allele that has a higher frequency in the subpopulation possessing a greater disease risk will appear to be associated with the disease. Likewise, it is also possible that population stratification may result in a Type II error – if a disease is more prevalent amongst a subgroup possessing a lower frequency of the disease-causing allele, the association with the disease will be masked (Deng, 2001).

Population stratification normally arises when the genetic background of the source populations differ between cases and controls (Cardon and Palmer, 2003), although it can also occur as a result of "cryptic relatedness" within a population considered to represent a sample of independent cases and controls. The hypothesis is that, if the disorder (in this case OCD), has a genetic aetiology, the affected individuals in the study are likely to be more genetically similar than case-control pairs, because they share a common genetic disorder that has, in essence, a common genetic basis. Thus, under the initial assumption of an independent sample and no genetic association with the disease, the false-positive rate may be increased due to cryptic relatedness within the case subjects (Bacanu et al., 2000; Devlin and Roeder, 1999).

In most epidemiological and disease risk studies, self-reported ancestry can serve as a suitable proxy for genetic clustering, with the obvious exception of recently admixed populations (Thomas and Witte, 2002). However, in case-control genetic association studies that require the identification of loci with very small effects, even the slightest difference in genetic ancestry between cases and controls may result in false positive results. Therefore, in such studies, if one is uncertain about the presence of cryptic subpopulations or the degree of admixture in the population from which the sample is drawn, methods that are capable of detecting, and correcting for, such stratification should be employed. The goal when correcting for population stratification is to determine whether cases and controls differ in ancestry to such an extent that an excess number of markers will, by chance, be associated with disease status. One can account and correct for stratification by using better measures of populations, making use of family members as controls and by means of genomic adjustment (Thomas and Witte, 2002).

#### *I.4.1.1.1. Better measures of populations*

As has already been mentioned, broad conventional population categories usually result in confounding due to population stratification. It has therefore been proposed that more specific, detailed information regarding a subject's ethnic origin be obtained when conducting association studies, so that individuals can be allocated to the finest ethnic origins that can be determined. For example, in mixed ethnic families, it may be more valuable to obtain information regarding the origins of an individual's parents and grandparents. This allows one to construct a covariate for each stratum in the analysis (rather than allocating the individual to a single stratum), noting the proportion of ancestors derived from each stratum, subsequently adjusting for these covariates using a multiple logistic regression model (Thomas and Witte, 2002).

#### *I.4.1.1.2. Using family members as controls*

Two major family-based association tests presently make use of parents and siblings as family-based controls: the transmission disequilibrium test (TDT) and the haplotype relative risk (HRR). Briefly, the TDT compares the frequency of a marker allele at a given locus in a sample of probands with the frequency of the parental non-transmitted alleles ("controls"). If transmission of the marker allele from heterozygous parents to affected individuals exceeds that expected by chance alone, it is assumed to be associated with the disorder in some way (Spielman et al., 1993).

The haplotype relative risk test looks at an affected individual and both his/her parents (Falk and Rubenstein, 1987). All three individuals are typed for a genetic marker that is hypothesised to be associated with susceptibility to the disorder. The genotypic frequencies in affected children are calculated and compared to the genotypic frequencies formed by merging the parental alleles that are not transmitted to the affected child. In effect, this creates a "pseudo-control" genotype from the alleles that are not transmitted to the affected offspring (Terwilliger and Ott, 1992). The marker allele frequencies are then compared between the case and pseudo-control group, and the resulting odds ratio is known as the HRR (Falk and Rubenstein, 1987; Schaid, 1998; Schulze and McMahon, 2002).

However, utilising family members in genetic studies may not always be the most feasible option, since the studies have been found to possess limitations. Not every case has a sibling, and Teng and Risch (1999) found that using unaffected siblings as controls resulted in a

substantial decrease in power when compared to studies using unrelated controls. When using parents as controls, at least one parent has to be readily available, and the possibility exists that some of the parent-case trios will be discarded because they are uninformative. Moreover, TDT-related methods yield approximately two-thirds of the genotyping efficiency of the case-control design, because for every case-control pair, genotype information is required from *two* parents *and* the proband. Finally, it is especially difficult to obtain large collections of family members for psychiatric disorders, since there seems to be a certain amount of stigma attached to being diagnosed with a psychiatric disorder.

Therefore, population-based case-control association analyses offer numerous advantages over the family-based association methods, including easier and cheaper recruitment of subjects; greater power to detect associations where the GRR is low; the inclusion of a more representative sample of subjects than in family-based designs, and the ability to explore environmental co-actions.

#### *I.4.1.1.3. Genomic Adjustment*

If population substructure affects candidate gene allele frequencies, then, theoretically, there should also be systematic differences in the allele frequencies at other genes (Devlin and Roeder, 1999; Pritchard and Rosenberg, 1999). It is these differences that are exploited when using genomic adjustment to detect and control for population stratification. These methods can be divided into two broad categories: first, model-based or structured association (SA) methods, which assume that the heterogeneous sample population is composed of genetically homogeneous subpopulations. Programs implementing this design are *Structure* (Pritchard et al., 2000) and latent class analysis (LCA) programs (such as L-POP) (Sham and Purcell, 2002). Second, non model-based, or genomic control methods, which correct for population stratification by accounting for overdispersion of statistics generated by population substructure can also be implemented. Genomic Control (GC) (Devlin and Roeder, 1999) is an example of a program implementing non model-based methodology. Both categories utilise a panel of polymorphic markers that may or may not be linked to the candidate locus.

##### *i. Model-based methods*

Model-based methods attempt to detect the underlying population substructure and adjust the association accordingly. They are conducive to association studies since they allow the identification of situations resulting in false positive and negative findings, and the choice of

markers should not bias the subsequent correction in any way. *Structure* (Pritchard et al., 2000) is a Bayesian model-based algorithm that assigns individuals probabilistically to one or more subpopulations based on allelic frequencies at each locus studied. It involves genotyping random markers (in linkage equilibrium with each other) in order to reflect the baseline genetic differences between cases and controls. The procedure places individuals into 'K' number of clusters. 'K' is chosen in advance, but can be varied across independent runs of the *Structure* algorithm. It is possible for individuals to have membership in multiple clusters; in this case, the program will indicate an estimate of the fraction of the individual's genome that originated from each of the 'K' subpopulations, providing a means for capturing the degree of admixture.

The major drawback of this method is that, although it allows the detection of population structure, the algorithm itself offers no means of adjusting the significance value if the stratification is found to influence the validity of the association. However, a program called "*strat*" has been designed (Pritchard, 2000) in order to correct for confounding due to stratification. S*tructure* is a presently widely-used program, and has been successfully implemented in numerous studies attempting to delineate human population structure (Rosenberg et al., 2002; Bamshad et al., 2003), the genetic structure of certain dog breeds (Parker et al., 2004) and it has even been used to distinguish between selected breeds of chickens (Rosenberg et al., 2001).

A slightly modified approach to the methods implemented in *Structure* is represented by the latent class analysis (LCA) of population substructure (Satten et al., 2001; Purcell and Sham, 2004). This implementation involves the simultaneous estimation of population membership *and* the effect of the disease-susceptibility variant in case subjects in the respective subpopulations, thus bypassing the 2-stage procedure required by *Structure*.

***ii. Non-model-based methods***

Genomic Control (GC) methods utilise unlinked markers that are usually independent of disease to calculate a correction factor to control for the inflated $\chi^2$ value that is a consequence of population stratification (Devlin et al., 2001; Devlin and Roeder, 1999; Bacanu et al., 2000). In other words, the method involves re-calibrating the $\chi^2$ value for association according to how many of the control markers (the null loci) are found to be associated with the disease. It is therefore important to choose the control markers so that they are randomly

distributed and thus provide a true reflection of the overall differences between case and controls; any marker that assumes a higher degree of differentiation between cases and controls will result in an overly conservative adjustment of the $\chi^2$ values.

A drawback of this method is that the number of markers required can be prohibitive – for a reliable and valid correction for the presence of population substructure, 50 or more control markers may be required (Devlin et al., 2001). Moreover, the method is limited only to SNPs (Bacanu et al., 2000; Devlin and Roeder, 1999). It has also been found that GC methods do not control against a Type I error if the difference in candidate allele frequencies between populations is small (Redden and Allison, 2003).

***Accuracy to detect population substructure using genomic adjustment***

The resolution at which population substructure can be detected depends largely on a combination of the characteristics of the genetic data utilised in the study, including expected heterozygosity or number of alleles at a locus (Shriver et al., 1997; Bamshad et al., 2003) and maximal difference in allele frequencies between the populations under investigation (Rosenberg et al., 2001). The more informative a marker, the greater the power with which to accept or reject the null hypothesis of no genome-wide differences in allele frequencies between the case and control populations will be. For biallelic markers, informativity will be maximised if one allele is absent in one of the subgroups or populations under investigation, and is only limited to one of the populations (Bamshad et al., 2003).

The accuracy with which subpopulations are characterised will also depend on the level of genetic variance within and between the subpopulations. A positive Fst value (a measure that determines overall genetic differentiation between subpopulations) indicates that individuals from the same subpopulation are more genetically similar than those from different subpopulations. One also has to take note of the variance *within* subpopulations – if variances within subpopulations are high, it becomes more difficult to assign individuals to a particular population (Bamshad et al., 2003). Interestingly, Bamshad et al. (2003) found that *Alu* insertion/deletion markers possessed higher Fst values than microsatellite markers, and that these values were similar to those obtained for diallelic markers, and could be attributed to the high mutation rate of these polymorphisms. These polymorphisms have been successfully utilised to infer population structure, and have been found to possess comparable power to detect structure and assign origin – Bamshad et al. (2003) found that a minimum of 60 *Alu*

markers were required to assign individuals to the correct continent of origin with a mean accuracy of 90%.

The number of markers required depends on a combination of the number of alleles, heterozygosity and Fst values. Obviously, a population that exhibits a fine substructure requires a larger number of markers (and larger sample size) to resolve this substructure, although, in such a case, the degree to which the genetic association is confounded would be lower (Pritchard and Rosenberg, 1999).

#### I.4.1.1.4. The value of isolated populations in genetic association studies, and a brief overview of the genetic history of the South African Afrikaner

The statistical power to detect a true association depends, to a large extent, on the amount of background noise within the population from which the subjects are sampled. This "noise" comprises a number of genetic and environmental aspects, which may vary amongst populations. Association studies in heterogeneous populations present with varying degrees of background noise; consequently, large samples are required to attain sufficient statistical power. In homogeneous populations, however, environmental and genetic variation is limited, improving the signal-to-noise-ratio, and the statistical power of the study.

By definition, all isolated populations originate from a few founders. Most of these populations experience bottlenecks, after which periods of rapid population growth (due to increased reproduction, not immigration) occur. During the bottleneck, the population experiences inbreeding and random genetic drift, ultimately limiting the genetic diversity and the number of new mutations occurring within the current population. Since recessive and neutral alleles are both subject to genetic drift in a population isolate, each population usually has its own set of recessive diseases that occur at relatively high frequency. Genetic drift will have much the same effect on rare marker alleles and haplotypes as it does on recessive and neutral alleles; common marker alleles and haplotypes will, on the other hand, not be affected to any large extent by drift, unless the number of initial founders is very small.

It is highly probable that, for complex diseases, the underlying susceptibility alleles are relatively common (**section I.3.2.3**), and experience very little selection pressure (Lander, 1996; Collins et al., 1997; Risch and Merikangas, 1996). Consequently, these variants predate the "Out-of-Africa" expansion, and the genome segments on which they are located have

experienced recombination over a large number of years (approximately 100 000 years), erasing much of the LD around the susceptibility locus. A high marker density will thus be required in order to detect these susceptibility variants using LD mapping strategies (Kruglyak, 1999[b]).

Exploiting the genomic structure of populations that exhibit an increased level of LD will thus be conducive to the detection of the underlying susceptibility alleles. Decay of LD is related to the number of recombination events and the effective population size (Hartl and Clark, 1997); therefore, one of the major demographic features influencing LD mapping studies is the number of generations to the most recent common ancestor (MRCA) (Wright et al., 1999). Recombination events serve to equilibrate linked alleles – for disease alleles that are relatively ancient, the number of recombination events that would have occurred is high, therefore, the surrounding genomic regions that are identical-by-descent (IBD) will be relatively small. The rate of decay of LD is reduced by other factors that reduce the effect size of the population, such as inbreeding.

It is thus evident that isolated populations, with relatively less average time to the MRCA, and a high occurrence of inbreeding, exhibit higher degrees of LD over longer genomic distances compared to more stable, outbred populations (Graham and Thompson, 1998; Shifman and Darvasi, 2001). One will recall from the section on LD and haplotype mapping, that the value of $r^2$ is directly proportional to the required increase in sample size when testing the association between a single SNP and complex disorder (Ardlie et al., 2002; Shifman and Darvasi, 2001; Laan and Paabo, 1997). Consequently, due to the extended levels of LD, isolated populations require a smaller increase in sample size to identify the specific disease gene, and are therefore more conducive to the genetic association mapping procedure. Ultimately, the genetic heterogeneity will also be reduced, resulting in a significant increase in GRR, making the association between variant and disease easier to detect (Shifman and Darvasi, 2001).

Genealogical records form the mainstay of any genetic investigation in isolated populations, since they allow for the identification of large pedigrees. These pedigrees will probably comprise multiple affected individuals, and, by utilising the genealogical records, one will be able to delineate the number of meiotic steps separating affected individuals, which will facilitate the identification of IBD segments (Kruglyak, 1999[b]; Heutink and Oostra, 2002).

Genealogical records also allow the identification of genetic "incomers", facilitating the more accurate estimation of genetic variability (Angius et al., 2001).

***The South African Afrikaner population: a brief overview of their history and suitability in localising disease-susceptibility genes by means of association studies***

The Afrikaans-speaking Caucasian sub-population in South Africa, often referred to as Afrikaners, are of predominantly Dutch, German and French descent (Dunning et al., 2000; Jenkins, 1990; Botha and Beighton, 1983). Their history over the past 350 years has contributed to their geographic and cultural isolation, and their relative genetic homogeneity.

The Dutch were the first Caucasians to settle in the Cape in 1652, and their numbers were subsequently boosted by French Hugenot and German immigrants. By 1687, the founding Afrikaner population consisted of only about 90 families (Theal, 1964). From this time on, the settler population expanded rapidly with large families of 10 or more children (Botha and Beighton, 1983), and marriage between family members was common in early generations. Over a period of about 300 years, the Afrikaner population underwent a 2500-fold increase, compared to Britain's population increase at the same period, which was only six-fold (Jenkins, 1990). This population growth was almost entirely due to reproduction, as the immigration following the founding event in 1652 was minimal (Jenkins, 1990).

The Afrikaner immigrants spread inland from about 1838, forming small, geographically isolated communities. Their language and religion (most Afrikaners were members of the Dutch Reformed Church) contributed further to their isolation and social cohesion. This cultural identity has, for the most part, been maintained, largely due to intermarriage (Botha and Pritchard, 1972).

The bottleneck caused by the original founding event resulted in increased genetic drift, with a substantial loss in genetic diversity, and a consequent increase in the frequency of previously rare alleles. Indeed, genetic drift by a founder event has been suggested for the high rate of certain monogenic diseases in Afrikaners, such as familial hypercholesterolemia (Defesche et al., 1996), keratolytic winter erythema (Starfield et al., 1997), hypertrophic cardiomyopathy (Moolman-Smook et al., 1999), myotonic dystrophy (Goldman et al., 1996), Fanconi anemia (Rosendorff et al., 1987; Tipping et al., 2001), variegate porphyria (Groenewald et al., 1998), long QT syndrome (de Jager et al., 1996) and progressive familial

heart block type I (PFHB I) (Brink et al., 1995). In addition, a large number of conserved haplotypes have been found to be situated around these rare disease genes (Pronk et al., 1995; Groenewald et al., 1998).

Gordon et al. (2000) performed a background linkage disequilibrium (BLD) study in the Afrikaner population and found that significant evidence for BLD (up to distances of about 5cM) existed. These results were in line with those obtained by Hall et al. (2002), who performed a comparative BLD study between Afrikaners and two other inbred populations (from Finland and Sardinia) and two outbred populations (from Britain and North America). The results indicated consistently higher mean levels of LD in Afrikaners for distances over 3cM compared to the other populations. In fact, in some regions on chromosome 18, they found that LD was detectable at a distance of approximately 6cM in Afrikaners. However, it should be noted that in a previous study by Dunning et al (2000), who compared LD between markers on chromosomes 13q12-13, 19q13.2 and 22q13.3-ter in two inbred populations (Afrikaners and Ahskenazim) and one outbred population (East Anglian British), no significant differences in allele frequencies or LD were detected. These disparate results may be due to the recent finding that LD is not uniform throughout the genome, and further emphasises the importance of measuring LD between markers within the region of interest, for the population used in the study.

In summary, the ethnically and relatively genetically homogeneous Afrikaner population presents with unique characteristics advocated for the successful elucidation of genetic risk factors in OCD. Firstly, the original Afrikaner group was a small number of founders. In addition, population expansion in the Afrikaner subpopulation occurred as a result of population growth, not immigration. Furthermore, at present, the Afrikaner population represents a large population (about 3 million) whose genealogy can be examined using well-preserved death certificates, birth notices, ship records of immigration and records from the original Dutch trading company that settled in the Cape in 1652. Finally, Afrikaners are a very religious group, and the Dutch Reformed Church has kept very good records over the last few hundred years that further facilitates genealogical investigation.

The Afrikaner subpopulation has already been used successfully in a comparative genome scan of patient and control groups to identify a number of markers associated with TS (Simonic et al., 1998; Simonic et al., 2001), a disorder thought to share a common genetic

aetiology with OCD. Moreover, from a sample of 98 Afrikaner schizophrenia probands, 87 have been genealogically traced to a single couple who immigrated to South Africa approximately 12.5 generations ago (Karayiorgou et al., 2004), suggesting that the affected individuals share a small number of disease alleles.

Mention should, however, be made of the drawbacks of using isolated populations in the search for complex genes. Firstly, the related genetic findings may not be valid, especially in larger, more outbred populations, particularly if the isolated populations possess new mutations that are associated with OCD, or if old mutations remain in the isolated population, whilst they become extinct in older populations (Heutink and Oostra, 2002). Secondly, epistatic interactions may account for the variation in allelic associations between populations. Thus, in the genetic context of an isolated population, it may be that associations are produced that are not detectable elsewhere in the world.

However, for Afrikaners, it is likely that their Northern European origin will enable any findings within this population to be extrapolated to a number of other Western populations. In addition, even if only a subset of susceptibility variants segregated with the original founders, the knowledge gained from the identification of these will be important in imparting insight into the molecular and cellular mechanisms of OCD.

### I.4.2. HETEROGENEITY OF OCD

The second major confounding factor presenting a major obstacle in delineating the genetic contribution to OCD is the genotypic and phenotypic heterogeneity of the disorder.

#### I.4.2.1. Genetic heterogeneity

Genotypic heterogeneity refers to the production of the same or similar phenotypes by different genetic mechanisms. Genetic heterogeneity is divided into two categories – allelic heterogeneity, where different alleles at a locus can produce variable expression of a condition, and locus heterogeneity, the phenomenon whereby mutations at different loci are able to produce the same phenotype. However, if the CD/CV hypothesis holds true for common disorders, it is unlikely that the allelic architecture of OCD will be very complex, since population theory predicts that the half-life of the ancestral allelic spectrum will be very long – in the order of 1.5 million years (Reich and Lander, 2001), and is therefore unlikely to contribute to any large extent to between-study heterogeneity. If the disease-causing allele

frequencies are found to differ between populations, it is most likely due to their rare occurrence, or the fact that they have been subjected to strong selection pressure, resulting in a relatively short period of time to the MRCA (Colhoun et al., 2003).

### I.4.2.2. Phenotypic heterogeneity

It is likely that the disease phenotype characterised by OCD represents a final common pathway of multiple aetiologies. The disorder is currently defined and diagnosed according to structured algorithms (such as is presented in the DSM-IV [APA, 1994]). The phenotype as described by these structured algorithms provides a relatively homogeneous view of the disorder, which facilitates treatment. However, it is unknown how much bearing this diagnosis has on the underlying genetic aetiology of OCD, since this characterisation represents a combination of related subtypes, which may present with distinct genetic aetiologies. Classifying OCD according to these subtypes should be more informative and statistically powerful (Silverman and Palmer, 2000), since the traits are are probably controlled by fewer loci and environmental factors. Such proximal phenotypes may further strengthen the genetic signal by increasing the effective population size that is informative for mapping, since even individuals who do not present with the clinical phenotype of OCD may present with the proximal phenotype under consideration.

Subtypes of OCD that have been investigated as proximal phenotypes include those based on obsessive and compulsive symptom dimensions (Baer, 1994; Leckman et al., 1997; Mataix-Cols et al., 1999; Summerfeldt et al., 1999; Calamari et al., 1999); co-morbidity with related psychiatric disorders (Mataix-Cols et al., 2000; Sobin et al., 2000; Leckman et al., 2003; Keuthen et al., 1996; Stanley et al., 1997), particularly tic disorders (Holzer et al., 1994; Leckman et al., 1995; Zohar et al., 1997); demographic and clinical features, namely, age at onset, severity (Rasmussen and Tsuang, 1986; Minichiello et al., 1990; Noshirvani et al., 1991) and gender (Lensi et al., 1996). It is important to keep in mind that, as one increases the number of subgroup analyses using the same sample, the chance of spurious association increases. Therefore, although subgroup analyses represent a valid means of generating hypotheses (Cardon and Bell, 2001), these should ideally be conducted using different patient samples. The following section deals with those subtypes, in particular, that are investigated in the present study.

***I.4.2.2.1. Identification of subtypes based on comorbidity with related disorders***

A wide range of DSM Axis I and II disorders are found to occur co-morbidly with OCD, and vice versa (Perugi et al., 1997; Fireman et al., 2001; Tukel et al., 2002) (Table I.3). In fact, it has recently been reported that less than one-third of OCD patients will be diagnosed *without* a lifetime history of a co-morbid pathology, probably indicating the poor discriminant validity of the present OCD diagnosis (Denys et al., 2004[a]).

***Table I.3.*** *Co-morbid disorders and associated current and lifetime frequencies in OCD patients*

| Diagnosis | Current | Lifetime |
|---|---|---|
| Major depression (MDD) | 31 | 67 |
| Specific phobia | 7 | 22 |
| Social Phobia | 11 | 18 |
| Eating Disorder | 8 | 17 |
| Alcohol abuse | 8 | 14 |
| Panic Disorder (PD) | 6 | 12 |
| Tourette's syndrome (TS) | 5 | 17 |

Current and lifetime frequencies are given as percentages

Adapted from Rasmussen and Eisen, 1998.

These disorders are associated with OCD due to common phenomenology and treatment responses (Stein, 2000). Many of the comorbid disorders have also been found to occur more frequently in relatives of OCD probands (Nestadt et al., 2003). However, the presence of co-morbid disorders in OCD cannot be easily explained – it is not known whether the relationship between the disorders represents a common genetic diathesis or some sort of psychodynamic or developmental commonality (Stoltenberg and Burmeister, 2000).

Presently, there appears to be a large degree of variability in the reported rates of OCD co-morbidity, probably due to the differing diagnostic methodologies employed by various researchers (Denys et al., 2004[b]). However, the majority of studies report that the most frequently associated co-morbid disorder occurring secondarily to OCD is major depressive disorder (MDD) (Goodwin et al., 1969; Weissman et al., 1994; Rasmussen and Eisen, 1994; Rasmussen and Eisen, 1992, Jenike, 2001, Denys et al., 2004[b]), indicating that the two disorders may share some neuropathological aspects (Insel et al., 1982; Mancini et al., 2002).

In order to determine whether there are underlying clinical constructs that distinguish OCD-related subgroups, Nestadt et al. (2003) performed latent class analyses on 450 OCD patients and observed the existence of two major OCD subgroups – one exhibiting co-morbid panic disorder (PD), tic disorders and agoraphobia, and the second characterised by the presence of co-morbid generalized anxiety disorder (GAD), recurrent major depressive disorder (MDD) and OCSD. Familial loading was evident in both groups. The authors suggested that these two groups represent aetiologically distinct subgroups, each comprising a certain degree of familial liability. In the group characterised by GAD, MDD and OCSD, however, the familiality was graded, with a better likelihood for the relatives to develop OCD with an increase in OCD-related co-morbid diagnosis in the proband. Furthermore, age at onset was observed to be later for the PD/tic disorder/agoraphobia group, whereas for the other group, earlier age at onset was associated with increase in OCD-related co-morbidity (Nestadt et al., 2003). Overall, it was concluded that the second group possessed a multifactorial aetiology, with genetic and environmental interaction necessary for the expression of the phenotype.

The reduced familial loading and later age at onset for the group comprising tic disorders contradicts what has been found in literature with regard to OCD presenting with comorbid tic disorders (Grados et al., 2001; Rosario-Campos et al., 2001; Pauls et al., 1995). This may be due to the exclusion of co-morbid TS in OCD patients in the study. Perhaps if TS had been included, tic disorders would have fallen into the group comprising OCSDs and would have been associated with early age at onset and higher familial loading, as has been found in other studies (Chacon et al., 2004).

#### *I.4.2.2.2. Obsessive-compulsive spectrum disorders (OCSDs)*

OCSDs refer to those psychiatric syndromes which may be related to OCD with respect to phenomenology, and other associated features such as clinical course (age at onset, chronicity of course), co-morbidity, familial transmission and response to behavioural and pharmacologic treatment (Hollander and Wong, 1995; Hollander and Benzaquen, 1997; Ravindran, 1999; Jenike, 1989); consequently, they may serve as marker traits with potential predictive value. OCSDs are so named because OCD is considered to represent the prototype for the group of disorders enveloped by this conceptual scheme. Many of these so-called spectrum disorders appear highly comorbid with OCD, and it is therefore important to address their relationship to OCD, with emphasis on the possible shared genetic aetiology between them.

Conceptualisations of OCSDs differ between researchers, resulting in a fair amount of discrepancy as to which disorders can be included in the scheme. One outlook proposes that OCSDs may be viewed along a continuum in which compulsive, ego-dystonic disorders, characterised by good insight, excessive harm avoidance and risk aversion, are situated at one pole, whilst impulsive, ego-syntonic disorders, characterised by a lack of insight, and risk-seeking behaviour, are situated at the other end (Figure I.1) (Stein and Hollander, 1993; McElroy et al., 1994; Hollander and Cohen, 1996; Hollander, 1998). Both groups of disorders are proposed to share several common features, including symptom profile (repetitive thoughts and behaviours), associated features (such as age at onset), aetiology (biologic and neurologic factors) and response to pharmacotherapeutic interventions (Hollander, 1998; Stein, 2000).

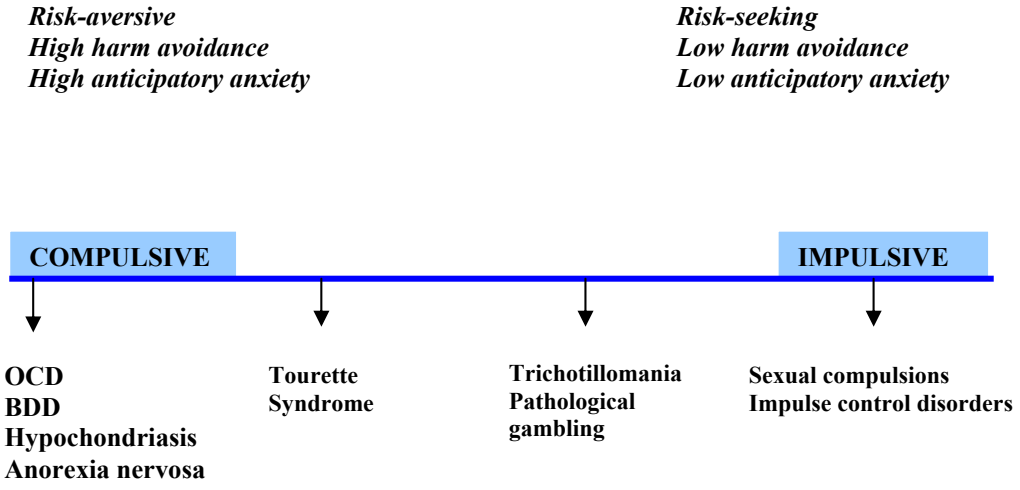



***Figure I.1. The hypothetical axis of the spectrum of obsessive-compulsive disorders****. The compulsivity/impulsivity dimension, with predominantly compulsive components on one end, and predominantly impulsive components on the other.*

**Abbreviations**: **OCD**: obsessive-compulsive disorder; **BDD**: body dysmorphic disorder.

OCSDs are now recognised as distinct diagnostic entities related to OCD and are found to affect up to 10% of the American population (Hollander et al., 1996). The range of disorders include grooming disorders (e.g. trichotillomania [TTM] - hair pulling) (Swedo et al., 1989[b]; Swedo and Leonard., 1992; Christenson et al., 1991; O'Sullivan et al., 1997), impulse control disorders, such as repetitive self-mutilation (Favazza, 1992) and compulsive buying (McElroy et al., 1991; 1994), somatoform disorders (such as body dysmorphic

disorder (BDD) and hypochondriasis) (Hollander et al., 1989; Ravindran, 1999), eating disorders (anorexia and bulimia nervosa) (Rubenstein et al., 1992; Ravindran, 1999; Bellodi et al., 2001), and tic disorders (e.g. TS) (Stein and Hollander, 1995, Miguel et al., 1997).

***I.4.2.2.3. Subtyping according to the presence or absence of tics***

Tic-related versus non-tic-related OCD is presently thought to be one of the clearest distinctions that can be made amongst OCD subtypes (Leckman et al., 2000). Evidence for the distinction stems from differential phenomenology and symptomatology (Leckman et al., 2000; McKay et al., 2004), demography (males and EO OCD present with co-morbid tics more often [Grados et al., 2001]), and neurology (Hanna et al., 1991), between OCD patients presenting with co-morbid tic disorders (OCD+tics) and OCD without tics (OCD-tics).

Of particular interest is the proposed neurobiological, genetical and clinical relationship between TS and OCD (Leckman, 1993; Pauls et al., 1991; Robertson et al., 1988; Comings and Comings, 1987; Frankel et al., 1986; Lees et al., 1984; Nee et al., 1980). TS is a developmental neuropsychiatric disorder characterised by motor and vocal tics (DSM-IV, 1994; Leonard et al., 1992). TS and OCD have been found to share certain clinical features, such as waxing and waning of symptoms, early age at onset and egodystonic behaviour (Eapen et al., 1997[a]).

Tic disorders are hypothesised to represent part of a familial OCD phenotype (Grados et al., 2001; Eapen et al., 1997[a]). Indeed, evidence from family studies indicates the putative common genetic basis for OCD and TS (Pauls et al., 1986; Pitman et al., 1987; Grad et al., 1989). Rates of OCD amongst first-degree relatives of TS probands have been reported to range from 6%-26% (Pauls et al., 1986; Pitman et al., 1987; Eapen et al., 1993), greater than for the control samples in the respective studies. Moreover, Grados et al. (2001) found that tic disorders were twice as common amongst relatives of OCD probands compared to relatives of controls. These results were consistent with those attained by Pauls et al. (1995), who observed tics in 4.6% of OCD case relatives, compared to 1% in control relatives. In addition, Riddle et al. (1990) observed that, of 21 children and adolescents with OCD, more than half had parents with motor tics.

An earlier age at onset amongst first-degree relatives with OCD+tics, compared to OCD without tics has also been observed (Grados et al., 2001), suggesting the likelihood of an

association between the age at onset of OCD and the expression of tics. Moreover, it has been found that, when comparing obsessive-compulsive symptoms in individuals with OCD and TS, the OCD probands who shared a common symptom profile with TS patients all had a family history of OCD (Eapen et al., 1997[a]).

Although family studies have thus far provided the most convincing evidence for an overlap between tic disorders and OCD, a wide range of brain imaging studies have implicated the basal ganglia and related cortical thalamic structures in the aetiopathology of tic disorders, compatible with a serotonergic (5-HT)-dopaminergic dysfunction in OCD-related TS (Eapen et al., 1997[a]). However, recent biochemical data suggest that 5-HT dysregulations in OCD + tics and OCD-tics patients may be distinct, indicating subtle differences in their underlying neuropathologies (Cath et al., 2001). Pharmacotherapeutic evidence has also provided evidence for the partial involvement of the 5-HT system in the pathophysiology of OCD-related tic disorders. George et al. (1993) observed a synergistic effect of a combination of neuroleptic and selective serotonin reuptake inhibitor (SSRI) therapy in the treatment of TS patients who presented with obsessive-compulsive symptoms, although the SSRI itself caused worsening of the TS symptomatology. Moreover, McDougle et al. (1994) observed the efficacy of a combined neuroleptic and SSRI therapy in OCD patients presenting with comorbid tic disorders, and that OCD patients who presented with a family history of tic disorders were less responsive to SSRI monotherapy, suggesting the involvement of other neurotransmitter systems in the pathology of OCD + tics.

A dysfunction in dopaminergic neurotransmission has been implicated in the pathology of TS (Segawa et al., 2003), based mainly on parallels between tics, vocalisations and obsessive-compulsive behaviours seen in some patients with encephalitis lethargica (Devinsky, 1983), which is thought to be dopaminergically mediated. OCD has also been found to be prevalent amongst individuals suffering from tic disorders (Steingard and Dillon-Stout, 1992): lifetime prevalences of OCD in TS have been found to range from 50% to 62% (Pitman et al., 1987; Pauls et al., 1986). Moreover, in children diagnosed with TS, it was found that significantly more met diagnostic criteria for OCD, in comparison to control children (Apter et al., 1993; Grad et al., 1987). Interestingly, it has also been suggested that some forms of EO OCD may represent genetic variants of TS (Eichstedt and Arnold, 2001).

Cytogenetic abnormalities associated with the OCD/TS/tic disorder phenotype have been reported. An individual with an abnormality in chromosome 7q31, resulting in the disruption of the inner mitochondrial membrane peptidase 2 like (*IMMPL2)* gene (a human homologue of the yeast mitochondrial inner membrane subunit 2) was observed by Petek et al. (2001). Moreover, a gene encoding an axonal membrane protein, contactin-associated protein 2 (*CNTNAP2*), has been found to be interrupted in a family presenting with OCD/TS phenotype (Verkerk et al., 2003). Finally, rearrangements in chromosome 18q22 have been associated with the OCD/TS/tic disorder phenotype in 3 separate instances (Boghosian-Sell et al., 1996; State et al., 2003; Cuker et al., 2004).

Given the observations from family and cytogenetic studies, it has been hypothesised that the association between some forms of OCD and TS may be a result of the common underlying genetic factors predisposing the individual to develop tic disorders.

***I.4.2.2.4. Subtyping according to age at onset***

Once believed to be rare in children, OCD is now recognized to be as prevalent in children as in adults (Flament et al., 1988; Valleni-Basile et al., 1994). Distinct peaks for the age at onset of OCD exist; for EO OCD, a mean age of onset of 10 years has been observed (Geller et al., 1996; Hanna, 1995; Riddle et al., 1990; Swedo et al., 1989[a]; Thomsen, 1993), whilst forLO OCD, the mean age at onset has been observed as 21 years (Karno et al., 1988; Minichiello et al., 1990; Pauls et al., 1995; Rasmussen and Eisen, 1992; Thyer et al., 1985).

It has been suggested that EO OCD represents a developmental subtype of OCD (Geller et al., 2001; Rosenberg and Keshavan, 1998). Indeed, EO OCD has been found to exhibit distinct patterns of neuropathology (Busatto et al., 2001) and phenotypic expression (reviewed by Geller et al., 1998) compared to LO OCD. With regard to the latter, EO OCD has been associated with male preponderance (Geller et al., 1998; Millet et al., 2004; Fontenelle et al., 2003), although this has not been consistently observed (Riddle et al., 1990; Rosario-Campos et al., 2001; Flament et al., 1988). Moreover, EO OCD presents with a higher rate of TS and comorbid tic and disruptive disorders when compared to LO OCD (Geller et al., 1996; Rosario-Campos et al., 2001; Millet et al., 2004). The correlation between tics and EO OCD is indeed striking: it was reported in one study that 20-59% of children with OCD present with tics (Leonard et al., 1992), whilst in another, 48% of EO OCD patients presented with tics (Rosario-Campos et al., 2001).

In addition, a higher number of obsessions and compulsions have been demonstrated in EO OCD patients (Millet et al., 2004; Sobin et al., 2000; Fontenelle et al., 2003) compared to LO OCD patients. EO OCD patients seem to experience more somatic fears, symmetry and superstitious obsessions and more cleaning, repeating, counting and tapping/rubbing compulsions (Sobin et al., 1999; 2000; Fontenelle et al., 2003; March et al., 1996). The obsessions and compulsions have also been found to be more severe in EO OCD, as evidenced by increased Y-BOCS scores (Rosario-Campos et al., 2001; Fontenelle et al., 2003). Moreover, Sobin et al. (2000) found that LO OCD patients reported a relatively large delay between the appearance of clinically important symptoms and full syndromal OCD compared to EO OCD patients, suggesting a less aggressive form of the disease in LO.

It has also been hypothesized that 5-HT may play less of a role in the development of this subtype (Eichstedt and Arnold, 2001), based on evidence from pharmacological studies, where it has been found that EO OCD is associated with a poor response to SSRI monotherapy (Rosario-Campos et al., 2001; Ackerman et al., 1994; Ravizza et al., 1995). Given the clear association between tic disorders and EO OCD, it may be hypothesised that dopamine is involved in the pathophysiology of EO OCD. However, the possibility that other neurotransmitter systems may be involved in the pathology of EO OCD cannot be ruled out. For example, a large amount of support for the glutamatergic hypothesis of EO OCD has recently been obtained (**section I. 6.1.3** ).

Support for the genetic contribution to EO OCD stems from the results of two genome-wide linkage studies, based on EO OCD probands, where evidence for suggestive linkage was found on chromosome 9p24 (Hanna et al., 2002; Willour et al., 2004) (please refer to **section I.3.2.4** for a more detailed discussion). Moreover, it has recently been found that diagnosis of OCD (according to the obsessive-compulsive scale found in the Child Behavioural Checklist [CBCL]) was influenced by genetic (55%) and environmental (45%) factors in a younger OCD sample (<12 years). In the older sample (>12 years), only common environmental factors were found to contribute to the development of the disorder (Hudziak et al., 2004).

Further support for the genetic contribution to EO OCD stems from a recent observation that EO OCD patients experienced a more gradual appearance of symptoms, whilst LO OCD patients exhibited a sudden symptom onset usually succeeding some kind of environmental trigger or depressive episode. The latter is, in turn, indicative of the environmental nature of

LO OCD compared to EO OCD (Millet et al., 2004). However, it is important to note that some forms of childhood-onset OCD are environmentally triggered. Indeed, more than 60% of children with Sydenham's chorea (a neurological manifestation of rheumatic fever) have been found to exhibit the onset of obsessive-compulsive symptoms (Swedo et al., 1989[c]). Furthermore, for a subset of children with OCD, symptom exacerbation has been found to occur after group A β-hemolytic-streptococcal infections (Leonard and Swedo, 2001; Trifiletti and Packard, 1999).

An inverse relationship between age at onset and familial loading has also been observed. Morbidity risks for obsessive-compulsive symptoms have been found to be twice as high in family members of EO OCD probands compared to relatives of LO OCD probands (Pauls et al., 1995). In line with these findings, Nestadt et al. (2000[b]) reported no cases of OCD in relatives of LO OCD patients. Likewise, observed increased rates of tics, OCD and TS have been observed amongst the relatives of EO OCD probands (Leckman et al.,2003; Pauls et al., 1995; Grados et al., 2001).

Taken together, these results suggest that EO OCD exhibits a stronger familial component than LO OCD, with increased rates of OCD and tic disorders found amongst relatives of EO OCD probands. It is, however, not yet clear whether the differences between EO OCD and LO OCD represent a specific neurobehavioural subtype of OCD, or a developmentally variable manifestation of the same disorder.

***I.4.2.2.5. Identification of subtypes based on obsessive and compulsive symptom dimensions***

According to diagnostic nosology, the definition of OCD is based upon the presence of obsessive and compulsive subgroups of symptoms that have been found to be relatively stable over time (Mataix-Cols et al., 2002; Rufer et al., 2005). Factor analyses have yielded remarkably consistent results in demonstrating the presence of three to five symptom dimensions, or subtypes (Leckman et al., 1997; 2001; Baer, 1994; Mataix-Cols et al., 1999; 2002; Summerfeldt et al., 1999; Cavallini et al., 2002; Calamari et al., 2004), which may be present in varying combinations and degrees within each patient (Mataix-Cols et al., 2002). These symptom dimensions are indicated in Table I.4. Please note that symptom dimensions and symptom subtypes are used interchangeably through the rest of this dissertation.

***Table I.4***. *Symptom dimensions in OCD*

| Symptom Dimension | Obsession | Compulsion |
|---|---|---|
| **Hoarding** | Hoarding | Hoarding |
| **Symmetry/ordering** | Symmetry | Repeating, counting, ordering |
| **Contamination** | Contamination | Washing |
| **Sexual/religious** | Sexual/religious | Checking, counting, repeating |
| **Aggressive** | Aggressive | Checking |

Some of these symptom dimensions have been shown to comprise differential patterns of genetic inheritance (Leckman et al., 2003; Alsobrook et al., 1999), co-morbidity (Samuels et al., 2002), pharmacotherapeutic response (Black et al., 1998; Winsberg et al., 1999; Mataix-Cols et al., 1999; 2002) and neurological substrates (Saxena et al., 2004; Phillips et al., 2000; Mataix-Cols et al., 2004), indicating the possibility of distinct biological underpinnings for specific obsessive and/or compulsive contents. Indeed, a neuro-imaging study has indicated that some of the symptom dimensions may comprise different neural substrates. Here, the severity of aggressive/sexual/religious symptoms were found to correlate with regional cerebral blood flow in the striatum, whereas contamination/washing symptom severity correlated with blood flow in mainly cortical regions (Rauch et al., 1998). In a more recent study, Mataix-Cols et al. (2004) observed that OCD patients experiencing checking compulsions exhibited activation of brain areas involved in motor and attentional functions, but when the same OCD patients experienced washing compulsions, brain regions involved in the processing of emotions (particularly disgust) were found to be activated. Moreover, neurochemical dysfunctions involving the dopaminergic system have recently been implicated in the pathology of checking compulsions, given the recent findings from an animal model (discussed in more detail in **section I.6.1.2**) (Schetzman et al., 1998; 2001).

Evidence for a genetic contribution to the symptom dimensions stems from two recent complex segregation analyses (Alsobrook et al., 1999; Leckman et al., 2003). Alsobrook et al. (1999) stratified their OCD patient sample according to four symptom factors, and found that the relative risk of OCD was higher in relatives who obtained high factor scores on the aggression/sexual and symmetry/ordering symptom dimensions, indicating that these subtypes may possess a familial component. Segregation analysis of their entire dataset (consisting of 96 probands [experiencing hoarding, contamination, symmetry/ordering and

aggression/sexual symptoms] and 453 first degree relatives) allowed rejection of only the no-transmission model, consistent with the hypothesis of a genetically heterogeneous aetiology for OCD.

When their analyses were limited to only families with probands who had high symmetry/ordering scores, only the no-transmission and polygenic inheritance models were rejected, indicating the possibility of the involvement of a major locus. On the other hand, limiting the analyses to only those families with OCD probands with high aggression/sexual symptom scores did not produce any significant results, with the polygenic model of inheritance reaching only a borderline rejection ($p=0.06$). This indicates the possibility that, whilst some OCD subtypes may be more genetically mediated than others, OCD as a whole appears to be genetically modulated in a heterogeneous model.

These results have been corroborated in a recent study by Hanna et al. (2005), who found that ordering compulsions were significantly more common in familial OCD probands. Leckman et al. (2003), on the other hand, investigated the transmission of the aforementioned symptom dimensions in families with TS. They found evidence for genetic transmission in all four factors, with dominant inheritance the most likely mode of transmission for aggressive, sexual and religious type symptoms, and the symmetry/ordering obsessive-compulsive symptoms, and recessive inheritance the most parsimonious solution for the transmission of contamination and hoarding symptoms. However, whether these results can be extrapolated to OCD patients without TS remains to be seen.

#### *I.4.2.2.5.1. Hoarding as a genetically distinct symptom dimension*

Hoarding, in particular, has been studied as a distinct subtype of OCD. Hoarding is probably an evolutionarily conserved trait, associated with survival in times of adversity; however, extreme forms of hoarding may contribute to the symptomatology of OCD and/or related disorders. Frost and Gross (1993) describe compulsive hoarding as the acquisition of and failure to discard possessions of little use or value. Although hoarding obsessions and compulsions are exhibited most often by OCD patients (Frost et al., 1996), they have also been observed in other neuropsychiatric disorders including anorexia (Frankenburg, 1984), organic mental disorders (Greenberg, 1990), psychotic disorders (Luchins et al., 1992), TS (Zhang et al., 2002) and Attention Deficit/Hyperactivity disorder (Moll et al., 2000). Hoarding has also been found to be associated with the presence of personality disorders including

obsessive-compulsive and avoidant personality disorders (Mataix-Cols et al., 1999; Samuels et al., 2002).

Compared to the other symptom dimensions, a wider range of studies have been conducted in order to determine the underlying pathology relating to hoarding, probably due to its involvement in multiple neuropsychiatric disorders. Animal studies have indicated that the phenotype is mediated by the ventromedial striatum, globus pallidus and dorsal thalamus (Mogenson et al., 1988); which have all previously been implicated in OCD. Food hoarding in rodents is thought to be mediated via the anterior cingulate gyrus (de Brabander et al., 1991), the hypothalamus (Blundell et al., 1973), and the hippocampus and the septum (Kolb, 1974).

Animal studies have also implicated dopamine and 5-HT dysfunctions in the aetiology of hoarding (Blundell et al., 1973; Kalsbeek et al., 1988; Kelley and Stinus, 1985; Fantino et al., 1988), and it has also been proposed that gonadal steroids, benzodiazepines and opiates may modulate hoarding behaviour (Coling and Herberg, 1982; Kavaliers and Hirst, 1985). However, it remains debatable whether animal hoarding behaviours can be equated to those in humans (Saxena et al., 2004).

Nonetheless, pharmacological and imaging studies conducted using OCD patients have highlighted neurobiological distinctions between OCD hoarders and non-hoarders. Indeed, the presence of hoarding obsessions appears to presage a poorer response to SSRI monotherapy, in comparison to OCD nonhoarders (Mataix-Cols et al., 1999; 2002; Black et al., 1998; Abramowitz et al., 2003; Baer, 1994; Saxena et al., 2002; Winsberg et al., 1999). In addition, lower glucose metabolism in the anterior and posterior parts of the cingulate cortex, which modulates activity in some of the brain regions implicated in OCD, has been observed in hoarders (Saxena et al., 2004).

Hoarding behaviours have also been found to exhibit distinct genetic underpinnings. A recent genome scan of the hoarding phenotype in TS patients revealed significant allele-sharing for both the quantitative and qualitative forms of the trait on 4q35-35 ($p=0.007$), 5q35.2-35.3 ($p=2x10^{-5}$) and 17q25 ($p=2x10^{-4}$) (Zhang et al., 2002). Interestingly, 17q25 has recently been postulated to be involved in the aetiology of TS (Paschou et al., 2004), indicative of a putative common genetic aetiological link between TS and the hoarding phenotype. The results may,

however, simply corroborate previously conducted studies investigating the genetic aetiology of TS. To this end, the finding on chromosome 5q is interesting, since chromosome five has never been implicated in the pathophysiology of TS, and may as such represent a distinct hoarding locus.

Further evidence indicating that hoarding represents a distinct clinical subgroup stems from a study by Samuels et al. (2002), where OCD probands with hoarding were compared to OCD probands who did not exhibit the hoarding phenotype. The investigators found that OCD patients presenting with hoarding symptoms possessed an earlier age at onset of the disorder, increased severity of OCD symptoms, and a higher prevalence of social phobia and pathological grooming behaviours, including nail-biting, skin picking and TTM.

### I.4.3. EFFECT MODIFICATIONS

Effect modification, a term often used in epidemiological studies, refers to the variation in the relationship between exposure (i.e. the genotype in the present study) and the disorder (OCD) due to some modifying factor, which is known as the effect modifier (Schoenbach and Rosamond, 2000). Effect modification is thus not concerned with whether an association between a genetic variant and disease exists, but more with the specifics of the association.

Effect modifiers are usually regarded as part of the background, and are assumed to be uniformly distributed; consequently, they are normally disregarded in association studies. However, it is important to identify those modifying factors that do not exhibit uniform distribution across cases and controls, or populations, since they have the potential to result in inconsistencies across association studies. More importantly, such modifications may be involved in disease aetiology.

#### I.4.3.1. Epistasis

Epistasis refers to the non-additive interactions that occur between loci or genes, and can thus impact the expression of a trait or phenotype quite substantially (Frankel and Schork, 1996). In genetic association studies involving complex traits, investigating loci in isolation undermines the complex, multigenic nature of such traits, and affords one a representation of only the marginal effect that the locus may have on disease expression. The power of the study will also be reduced if the effect of one gene is masked or altered by that at another locus (Cordell, 2002). Indeed, evidence of for such genetic interactions has been reported in

complex diseases such as Alzheimer's disease (Kamboh et al., 1995), sporadic breast cancer (Ritchie et al., 2001), and type 2 diabetes (Cho et al., 2004; Hu et al., 2004).

The core issue in analysing epistatic interactions is the choice of regions to investigate. The ideal would obviously be to perform genome scans on the disorder, from which the choice of regions to include in such analyses would, theoretically, become clearer. However, for OCD, the findings from such scans have not been clear-cut (**section I.3.2.4.**). This, together with the relatively small amount of data from presently available literature indicating which regions may be suitable for inclusion in epistatic analyses, results, at best, in estimates of regions that interact with one another (Sullivan et al., 2004). Moreover, the issue of multiple testing becomes relevant, because if one investigates K loci, the number of possible pairwise comparisons is K(K-1)/2. Therefore, investigating the significance of each of these marker pairs will require stringent means to control the possibility of false positive results (Hoh et al., 2001).

It is also imperative to remember that a statistical interaction need not imply a biological interaction (Cordell, 2002). In statistical terms, epistasis refers to a departure from independence whereby the joint effect of two genes is greater than their individual effects (Cordell, 2002). Statistical tests for epistasis are thus restricted to testing specific hypotheses pertaining to defined quantities within mathematical models, which do not always correspond to biological models of epistasis (Cordell et al., 2001; Cordell, 2002). It is only if a prior biological model has been postulated in a fair amount of detail that statistical modeling will provide insight into underlying biological mechanism of OCD. Unfortunately, in OCD, the present knowledge regarding the underlying biological models is incomplete; hence one is not able to specify prior biological models upon which to base such analyses.

Nonetheless, considering the different modes of interaction between putative susceptibility loci has been found to improve power with which to detect modest genetic effects (Cordell et al., 1995; 2000; Cox et al., 1999). Furthermore, even identifying the most parsimonious statistical model for the joint effects of alleles at particular loci will improve our understanding of OCD, and facilitate the identification of further genetic loci which may a role in development of the disorder (Cordell et al., 2001).

### I.4.3.2. Epigenetics

Epigenetic effects refer to changes in the genetic material that alters gene expression in a manner that is heritable, but that are non-mutational and are thus fully reversible (Tycko and Ashkenas, 2000) epigenetic effects (or epigenetic "marks") usually occur as a result of DNA methylation and/or histone modification, (Bird et al., 2002; Hmadcha et al., 1999; Jenuwein and Allis, 2001), and can result in, amongst others, genomic imprinting and X- chromosome inactivation (Bestor et al., 1994).

An epigenetic framework has been proposed to explain the role of environmental mechanisms in complex disorders (Bjornsson et al., 2004); epigenetic mechanisms, by providing a transitional fine-tuning of the genome, allow for the preservation of information on environmental exposures. Indeed, epigenetics is also important in neural development – the mature adult nervous system requires genetic and environmental factors and interactions to allow organization and maturation; sensory deprivation results in aberrant responses and ultimately disease (Abdolmaleky et al., 2004).

Epigenetic marks have been found to differ across individuals, producing what is known as the epigenetic "polymorphism", comprising 2 "epialleles". Epigenetics may thus, at least partially, explain the inconsistent results often found in genetic association studies. If epigenetic phenomena are responsible for the association between a disorder and a genetic variable, the higher the epigenetic difference between cases and controls, the stronger the association will be, and vice versa. Epigenetics may also (once again, at least partially) explain how seemingly non-functional polymorphisms may be associated with a particular disorder (although one can not discount the possibility that the non-functional variant may be in LD with a functional variant, which may precipitate the clinical manifestation of the disorder).

Epigenetics has been hypothesised to play a role in the development of mood disorders and schizophrenia, since the chromosomal locations of the putative susceptibility loci for these disorders are compatible with those of genes that contribute to DNA methylation (Abdolmalesky et al., 2004; Asherson et al., 1994; Ohara et al., 1997). TS has also been proposed to possess possible parent-of-origin (epigenetic) effects: a greater motor-tic complexity and earlier age at onset has been found to occur when the disorder is maternally inherited, and a greater vocal-tic complexity has been observed when the disorder is

paternally inherited (Eapen et al., 1997[b]). This indicates the possibility for some forms of OCD (especially those with co-morbid tic disorders) to possess epigenetic parent-of-origin effects.

### I.4.4. CHANCE

Chance is always a theoretically alternative explanation for any association (Rothman, 1986). Although it may be that chance is sometimes too readily accepted as a means of validating or explaining complex results, one should never attempt to dismiss it as an explanation before examining the phenomena which may have resulted in it being responsible for the final observation namely, lack of adjustment for multiple comparisons, insufficient power and measurement error.

#### I.4.4.1. Multiple comparisons

Psychiatric disorders are genetically complex, necessitating the investigation of numerous candidate genes for the role they may play in the development of the disorder of interest. When one performs multiple, independent tests on the same dataset, with each test possessing a Type I error risk equivalent to the predefined level of statistical significance, the overall probability of making a Type I error increases.

The most common form of multiple testing occurs where numerous markers, located in independent genomic regions, are investigated for association to a disorder. Other forms of multiple testing include subgroup analyses (such as stratifying the dataset according to age, gender or phenotypic variables), and pooling alleles from multi-allelic polymorphisms (Nyholt et al., 2001). Pooling alleles seems to be common practice in genetic association studies, and it is imperative to remember to correct for multiple testing if the statistical grounds for grouping alleles together was created after examining the original dataset.

There is presently no consensus as to the most appropriate means to correct for multiple testing. The family-wise error rate (FWER) seems to be the most often controlled quantity, and represents the probability of obtaining one or more false positive results out of all the hypotheses tested. The most often cited correction for the FWER is the Bonferroni correction, which posits that, if there are $m$ statistical tests, each test is controlled so that the probability of obtaining a false positive result is less than, or equal to, $\alpha/m$.

However, when performing the Bonferroni correction, one assumes that the prior probabilities for each candidate gene or variant tested are all equal; therefore, the test does not control for dependence that may exist between variants in LD with one another. Investigating markers that are in LD with each other does not represent independent tests as such, since each new test does not provide a completely independent opportunity for a Type I error to occur (Ott, 1999). Consequently, it is not necessary to apply conservative adjustments for multiple testing (such as the Bonferroni correction).

It should be mentioned at this point that that it is not possible to control for a Type I error without increasing the probability of a Type II error (Rothman, 1990). As the risk of the Type II error increases, the sensitivity of the test decreases, increasing the chances of overlooking a potentially important association. Methods that control for multiple testing therefore do so at the cost of decreasing the sensitivity of the investigation – the more conservative the test, the greater the probability of making a Type II error. It is therefore becoming increasingly more popular for investigators to employ less stringent means of correcting for multiple testing.

Indeed, alternative means of correcting for multiple testing have also been created, in an effort to eliminate the increase in Type II errors caused by the multiple correction tests. For example, Murray (1991) proposed a method for adjusting for multiple comparisons that preserves the sensitivity of the investigation: he proposed that an *a priori* hierarchy of comparisons, in which there is a single comparison of primary interest, be described at the outset of the investigation. The results from these associations should be accepted at face value, and not corrected for. Thereafter, he suggests that a limited number of secondary investigations be performed, the results of which carry a lighter weight, although any significance should not be too lightly dismissed (a kind of hypothesis-based test). Any comparison made after the primary and secondary comparisons should be purely exploratory to generate hypotheses for future studies. Significance in this case should be awarded very little weight until the results have been replicated in independent studies, and these studies should be clearly labeled as exploratory (Bender and Lange, 1999).

In summary, it is evident that if one is testing multiple independent hypotheses, using the same dataset, some measure of adjustment for, or in the very least acknowledgment of, multiple testing is required. This becomes particularly important in confirmatory studies,

where the significance tests are used as tools for statistical evaluation and not simply to generate hypotheses.

### I.4.4.2. Inadequate power

The power of a study is defined as the probability of rejecting the null hypothesis when it is appropriate to do so. Mathematically, power = 1- the probability of a Type II error (β).
In other words, it represents the probability of reaching the right conclusion; consequently, it is imperative that the power of an association study be as high as possible, so that one can put the results of a study into perspective. A low statistical power results in the experiment possessing poor predictive value (Vecchio, 1966; Pfeiffer and Gail, 2003).

The power of case-control association studies is determined by numerous factors, which are not necessarily mutually exclusive. These include: sample size; the frequency of the allele and/or genotype under investigation (which determines the maximal LD between them); the desired p-value; and the ES of the gene (or variant) (i.e. the penetrance of the allele) (Pfeiffer and Gail, 2003; Schork, 2002; Hwang et al., 1994; Berry et al., 1998; Long and Langley, 1999; Cox and Bell, 1989). Most of these variables will be unknown in an association study of a complex disorder (such as OCD), although tenable estimates of power can be obtained by making reasonable assumptions regarding most of the susceptibility loci.

Determining adequate and efficient sample size is often critical in designing worthy case-control association experiments. The larger the sample size, the greater the power of the study (Long and Langley, 1999). In determining the sample size required to attain a particular statistical power for case-control association analyses, it is necessary to know the extent of LD between the marker and susceptibility loci. This is because the required sample size increases with decreasing LD by a factor of $1/r^2$, where $r^2$ represents the correlation coefficient, a measure of LD between two markers (**section I.3.2.5.1**) (Kruglyak, 1999[a]). The significance level and ES will also influence the number of subjects that need to be recruited for the study – the sample size is inversely proportional to both.

### I.4.5. BIAS

*"Garbage in, garbage out"*

Bias, or systematic error, refers to a type of differential error in which the measurements in a sample are skewed in one direction, due to imperfect sampling or classification procedures, and results in the dataset misrepresenting the true sample (Vineis and McMichael, 1998). It is thus important to avoid, or at least minimise, the inclusion of factors not related to the aetiology of OCD in the study, so as not to mask any modest differences (and therefore association) thought to exist between OCD patients and healthy controls. Sources of bias include selection bias, interviewer bias, information bias, interpretation bias and publication bias (Vineis and McMichael, 1998; Schoenbach and Rosamond, 2000; Kaptchuk, 2003).

Publication bias arises when statistically significant results are more likely to be published than research presenting null, or non-significant, results (Easterbrook et al., 1991). It has been found that non-significant associations are rarely submitted for publication and if they are, there is relatively little chance of them being published. This skews any review of published results in favour of positive association results, and consequently compromises meta-analytical studies (discussed in the forthcoming section). However, a graphical method of detecting this bias has been developed, in which the effect sizes of the individual studies are plotted on the vertical axis, and some measure of precision (sample size or standard error) is plotted on the horizontal axis. In the absence of publication bias, this plot should take on the shape of an inverted funnel, the assymmetry of which can be detected visually, or statistically (using Begg's [Begg and Mazumdar, 1994] or Egger's [Egger et al., 1997] tests).

One explanation for publication bias is the misconception that non-significant association studies are of poorer quality than those yielding significant results. In fact, Easterbrook et al. (1991) tested this hypothesis, and found no association between the quality of design and the likelihood of publication. There was also no evidence suggesting that studies reporting positive associations were of any higher quality that those with non-significant results. Interestingly, one of the factors they did find increased the likelihood of publication was an investigator's enthusiasm for his/her study, gauged by the rating on the importance of study findings. To this end, a positive finding is usually regarded as more important than a negative finding, largely due to the erroneous belief amongst researchers that a negative result (i.e. $p>0.05$) equates to the non-existence of an association. In reality, before any association

between a genetic variant and disorder can be excluded, the statistical power of the study needs to be calculated, to ensure that no associations were "missed" due to insufficient power. Fortunately, the confirmation of negative results is becoming increasingly popular amongst research scientists, since well-designed association studies producing non-significant results may highlight the presence of previously reported (and published) false associations.

One way of overcoming publication bias would be to encourage scientists to publish brief reports of their negative association studies in some kind of web-based format on the internet (Little et al., 2002). A step towards achieving this goal has been realised by the "Genetic Association Database" (http://www.geneticassociationdb.nih.gov/), an archive of published human genetic association studies in complex diseases and disorders, presented in a web-based format.

### I.4.5.1. Meta-analysis

As discussed in the previous section, the investigator needs to assess the overall contribution of the particular hypothesis being tested as multiple studies and tests accumulate. Numerous statistical methods exist to perform such evaluations, with meta-analysis gaining increasing popularity (Egger et al., 2002). Meta-analysis employs statistical methodologies that combine the results from a number of studies to detect a common main effect, whilst controlling for variations brought about by study-dependent factors (Glass, 1976; Fisher, 1925).

Meta-analyses have the potential to increase the precision and statistical power of association studies, thereby highlighting areas in research where there is a lack of evidence, and identify where further studies are required (Egger et al., 2002; Munafo and Flint, 2004). It is, however, important to realise that meta-analyses do not represent a panaceae for the case-control genetic association study. Indeed, it has been found that performing meta-analyses can introduce further bias and risk for confounding into an investigation, largely due to between-study heterogeneity and publication bias (as discussed in the aforegoing section) (Gambaro et al., 2000).

Critics of the method have voiced concern over how much heterogeneity (genotypic, phenotypic and methodological) can be included in the meta-analysis for the results to still be acceptable. If between-study heterogeneity is expected, it may be conducive to employ a form

of weighted pair (Woolf, 1955), or random variable analysis (Naylor, 1996) in order to control for confounding caused by such heterogeneity.

Meta-analyses thus represent a potentially powerful tool for genetic association studies. However, one must be cautioned that it cannot be considered a substitute for adequately powered genetic studies, but rather an adjunct to well-conducted studies, facilitating the identification of further avenues of research potential.

## I.5. DETERMINING THE VALIDITY OF AN ASSOCIATION: CAUSAL INFERENCE

*"The world is richer in association than in meanings, and it is the part of wisdom to differentiate the two"*
*John Barth, novelist*

Once confounding, chance and bias have all been eliminated as possible contributory factors to an observed association, the validity thereof can be assessed by employing a set of inductive epidemiological criteria, originally proposed by Hill in 1965 (Hill, 1965). These criteria, some of which are applicable to genetic association studies, include consistency (or replication) and biologic plausibility.

### I.5.1. REPLICATION, REPLICATION, REPLICATION

In the absence of a more extensive understanding of the effects at the molecular level that may contribute to the aetiology of OCD, replication (preferably in multiple independent studies) may be the most powerful evidence in favour of causality (Campbell and Rudan, 2002). In fact, it has been suggested that statistically significant associations should be replicated before any declaration for a susceptibility gene is accepted (Colhoun et al., 2003; Little et al., 2002). In population-based genetic association studies conducted on OCD, a large degree of inconsistencies in results has been observed; however, the problem of non-replication is not limited to OCD investigations, but seems to be plague most association studies investigating the genetic aetiology of complex disorders.

At least three studies investigating how wide-spread the problem of non-replication of population-based genetic association studies in complex disorders is, have been conducted.

Hirschhorn et al. (2002) observed that only the results of six studies (out of 166 "positive" genetic association studies) were consistently reproduced. Similarly, Ioannidis et al. (2003) performed 55 meta-analyses (comprising 579 study comparisons), and found that only 16% of the genetic associations identified were replicated without the influence of heterogeneity or bias. On the other hand, in a recent study Lohmueller et al. (2003) concluded that approximately a quarter of previously published associations are true, with false negative associations (usually due to underpowered studies) accounting for a large proportion of the inconsistent results. The authors subsequently advocate testing previously reported associations, replicated at least once, in large samples to identify the true genetic risk factors.

It could, of course, be argued that the inconsistencies and inability to replicate statistically significant findings may represent true variations of underlying associations between populations (Colhoun et al., 2003), as co-factors associated with the disease may be represented variably in different populations. Factors such as different degrees of LD between marker and susceptibility alleles, allele and haplotype frequency differences, environmental modifiers and patient ascertainment strategies may all contribute towards discrepant genetic association results between populations (Vieland, 2001; Stephens et al., 2001; Glatt et al., 2001).

As already mentioned, the degree of LD between the marker and disease-susceptibility alleles may vary between populations. For this reason, LD should always be examined within the context of the study population, rather than simply assuming LD between two variants based on the results from another study involving a different population. Moreover, association studies may be difficult to replicate if the variant under investigation possesses a low ES, variable penetrance and/or allele frequencies across populations. In particular, significant associations with rare alleles (with a frequency of below 5%) are more likely to be population-specific, and thus less likely to be replicated (Campbell and Rudan, 2002; Pritchard, 2001; Wright and Hastie, 2001).

To circumvent the potential lack of replication, it may be conducive to examine alternative, more common (where possible, functional) variants in the same gene for association with the disorder. It has also been suggested that an "internal check" for association be conducted in the same population as the original positive study (Campbell and Rudan, 2002). Ideally, replication should be conducted in both family- and population-based association studies,

demonstrating both linkage and association of the variant with the disorder, further reinforcing evidence for the association (Owen et al., 1997).

In order to interpret an association that has not been consistently replicated, it is necessary to distinguish which of the factors are most relevant to discrepancies between the studies that results in non-replication, and to control for them. It is also important to note that a lack of replication does not negate the causal relationship between the variant and the disorder; instead, it may indicate the need for further studies in certain populations, or a more detailed analysis of the gene containing the variant under consideration (Tabor et al., 2002).

### I.5.2. BIOLOGICAL PLAUSIBILITY OF THE CANDIDATE GENES AND POLYMORPHISMS IN GENETIC ASSOCIATION STUDIES

A candidate gene is one that, on the basis of prior physiological, genetic or biochemical characterisation, is suspected to contribute to the aetiology of the disorder under investigation. Candidate genes can be categorised into either positional candidates, chosen on the basis of a genomic location that has previously been found to be associated with the disorder, or hypothesis-driven candidates, chosen because of their (hypothesised) role in the aetiology of the disorder.

Obviously, any investigation into the causality of an observed association needs to account for the biological validity of the candidate gene, which depends on the prior probability that the candidate gene (and the variant under investigation) are involved in (in this case) OCD pathology. Therefore, it follows that a low prior probability of candidature will result in an increased risk of attaining false positive results.

In theory, the idea of prior probability would enable one to quantify biologic plausibility on a probability scale, and to incorporate it into subsequent statistical analyses; in reality, however, the prior probability that a candidate gene is involved in the development of OCD is difficult to determine exactly, due to the presently incomplete knowledge regarding the biological mechanisms of pathology. Prior probability can therefore, at best, be estimated, and is thus largely subjective and hypothesis-driven (Freimer and Sabatti, 2004).

On the basis of such estimation, the prior probability that a candidate gene is biologically plausible is increased if the gene has been found to be associated with existing familial forms

of the disorder and/or the same disease in a population of different ethnicity; if the gene variant is found to be involved in molecular mechanisms of the disorder; if the gene possesses a high mRNA copy in tissues thought to be affected by the pathological process of the disorder; and if sufficiently valid experimental evidence (for example, animal studies, gene knock-out models) exists to support the role of the candidate gene (or variant) in the disorder (or related disorders).

The prior probability that the variant under investigation is involved in the disorder (or is in LD with a susceptibility allele) cannot be dismissed when assessing the biologic plausibity of candidate genes. Given the wide array of variants that one can choose from in an association study, available data needs to be sifted through to prioritise and select which polymorphisms will be most conducive to detecting association. The most likely polymorphisms to be associated with disease are those that affect the function of the candidate gene and its associated protein (Tabor et al., 2002). Therefore, at first glance, it would seem that an expedient approach would be to identify variants within the coding regions of candidate genes for use as markers. However, even non-coding variants have been found to influence gene function, especially those contained in regulatory regions (Horikawa et al., 2000). Consequently, searches restricted to only coding variants may bypass important information contained within the non-coding regions. Unfortunately, present knowledge pertaining to the characterisation of regulatory regions and their effect on level of gene or phenotype expression is still in its infancy. In spite of this, it is known that the functional effects of polymorphisms within candidate genes are normally complex, and that, at a molecular level, the combinatorial nature of alleles should be taken into account. Therefore, it would be more conducive to the study that the genetic variants under investigation be considered in their haplotypic context, rather than in isolation.

## I.6. THE PRESENT STUDY

The aims of the present study were two-fold: firstly, to investigate selected candidate genes for the role that they may play in the development of OCD, taking into account the biological and statistical intricacies that encompass such studies (many of which have been mentioned in the previous sections), and secondly, to determine whether any type of cryptic population substructure, which would possibly confound the results of the association studies, exists within the Afrikaner population. The following sections expand on these two facets of the present analyses.

The genetic diathesis provided by family and twin studies in OCD (**sections I.3.2.1 and I.3.2.2**) provides a solid foundation for conducting population-based case-control association studies, which forms the basis of the present dissertation. An extensive literature search was conducted in order to select candidate genes and variants within candidate genes for investigation in the present genetic association studies. The candidate genes were prioritised according to biological validity as follows: first, genes encoding products that have been shown to play a role in the aetiology of OCD or OCD subtypes (based on animal, pharmacological and previous genetic studies) were selected. Second, genes coding for protein products that have been shown to be involved the pathophysiology of disorders closely related in aetiology to OCD, or disorders occurring co-morbidly with OCD (for example MDD), were chosen for investigation.

The choice of polymorphisms within selected candidate genes is also important in association studies (**section I.5.2**); in the present study variants were prioritised according to the following criteria: those known to affect the function of the relevant gene were given the highest priority. Polymorphisms in plausible candidate genes with minor allele frequencies of greater than 5% (according to published or validated data) were also prioritised, given the present CD/CV hypothesis regarding the genetic aetiology of many complex disorders (**section I.3.2.3**). It should be mentioned that various concerns have been raised regarding the reliability and validity of SNP data deposited in public databases, with only 50 to 60% of SNPs in the database representing *bona fide* sequence variations (Marth et al., 2001). However, SNPs that are reported to occur in multiple human subpopulations have been found to be particularly well-validated (Marth et al., 2001). Therefore, where possible, SNPs that have been validated in more than one population were preferred over those that had been validated in only one population (particularly if it had been validated using a small number of individuals, or in a population geographically unrelated to the Afrikaners).

### I.6.1. Factors influencing the selection of OCD candidate genes

As mentioned in the previous section, the probability that a candidate gene is involved in the aetiology of OCD is increased if it is found to be transcribed at fairly high levels in the tissue affected by the pathological process. It is thus imperative to take the neurobiological and neurochemical aetiology of OCD into account when choosing candidates for investigation in genetic association studies.

Presently, a fairly large body of evidence exists to support the neurobiological basis of OCD, including the observation that 90% of OCD patients exhibit neurological "soft signs" consistent with some neurological disorder; for example, the emergence of obsessive-compulsive symptoms as part of postencephalitic Parkinson's syndrome (Graybiel and Rauch, 2000) and the onset of OCD following head trauma (McKeon et al., 1984).

Initial proposals focused on a role for basal ganglia abnormalities associated with OCD since several other disorders involving basal ganglia pathology have been found to either occur co-morbidly with OCD, or to exhibit obsessive and/or compulsive symptoms (Cummings and Frankel, 1985; LaPlane et al., 1989). Such disorders include von Economo's encephalitis (Schilder, 1938), Sydenham's chorea (Swedo et al., 1989[c]) and TS (Cummings and Frankel, 1985; Luxenberg et al., 1988). Indeed, results from cumulative functional and structural neuroimaging studies have converged to form a relatively cohesive picture implicating a dysfunction in the cortico-striato-thalamo-cortical (CSTC) network (a neuronal loop linking the basal ganglia and frontal association areas [Alexander et al., 1986]) in the pathology of OCD (Rauch, 2003; Luxenberg et al., 1988; Robinson et al., 1995; Saxena et al., 1998; Rosenberg et al., 1997; 2000; Rauch et al., 1994; 1997; Lacerda et al., 2003; Pujol et al., 2004; Swedo et al., 1989 (a); Szeszko et al., 2004; Baxter et al., 1987; 1988). Furthermore, evidence collected from a constellation of functional and structural neuroimaging studies has also implicated the prefrontal cortex (PFC) in the aetiology of the disorder (Baxter et al., 1987; 1988; Saxena et al., 1998; Otto, 1990; Rauch and Baxter et al., 1998).

Thus, taken together, neuroimaging studies implicate the orbitofrontal-subcortical circuits in the pathophysiology of OCD. A working hypothesis explaining the involvement of the CSTC pathway in the development of OCD is that the disorder is associated with a failure to inhibit subsets of CSTC "mini-circuits", resulting in hyperactivity of the CSTC circuit (Modell et al., 1989; Rapoport, 1991; Rapoport and Wise, 1988; Baxter et al., 1992; 1994; Insel, 1992). Obsessive-compulsive symptoms arise when processing of cortical input to the basal ganglia is defective, resulting in an excitatory drive and excessive cortical activity, which further exacerbates the abnormal basal ganglia function (Greenberg et al., 2000). .

Obviously the main interest, from a genetics point of view, would be to identify the neurotransmitter systems that play a role in the proposed CSTC dysfunction in OCD. The CSTC circuitry is innervated by a variety of neurotransmitter pathways – 5-HT and dopamine

serve to modulate the activity of efferents from the basal ganglia, whilst glutamate has been found to moderate excitatory inputs to the network (Baxter et al., 1996). These neurotransmitter systems thus play a pivotal role in maintaining both physiological and psychological processes in the brain.

This section therefore provides a review of current literature pertaining to neurochemical hypotheses of OCD, followed by a brief summary, in the form of a table, of the genetic components within each of the pathways that have been investigated in genetic association studies (either family- or population-based). Thereafter, the presently investigated candidate genes, and variants therein, within each of the pathways are discussed.

### I.6.1.1. The serotonergic hypothesis of OCD

5-HT is a monoamine neurotransmitter which is synthesized from the essential amino acid tryptophan in a two-step process. Tryptophan hydroxylase (TpH) catalyzes the rate-limiting step in the synthesis of 5-HT by converting tryptophan (Trp) to 5-L-hydroxytryptophan. L-amino acid decarboxylase subsequently converts 5-L-hydroxytryptophan to 5-HT. The vesicular monoamine transporter type-2 (VMAT2) transports 5-HT into presynaptic vesicles. These vesicles then release 5-HT extraneuronally, where 5-HT interacts with postsynaptic receptors including 5-$HT_{2A}$ and 5-$HT_{2C}$. 5-HT also binds to somatodendritic (5-$HT_{1A}$) and terminal (5-$HT_{1B}$) autoreceptors. The serotonin transporter (5-HTT) transports 5-HT from the extraneuronal space back into the presynaptic neuron. MAO-A breaks down 5-HT within the presynaptic neuron.

Serotonergic releasing neurons have their cell bodies located in brainstem raphe nuclei and provide highly collateralised axonal innervation to almost all areas of the central nervous system (CNS) by virtue of bifurcation of the neurons. Prominent forebrain serotonergic terminal regions include the hypothalamus, cortex, hippocampus, amygdala and striatum, with particularly dense innervation in the cerebral cortex, basal ganglia and limbic structures (Figure I.2). It is important to note that serotonergic innervation does not function homogeneously because the neuronal projections have a high degree of anatomical, morphological and pharmacological specialisation.

The synaptic effects of serotonin neurotransmission are mediated by a number of pre- and postsynaptic serotonergic receptors. Of all the CNS neurotransmitters, 5-HT represents the

most perplexing array of receptor interactions. 5-HT receptors are presently divided into seven classes (5-$HT_1$ to 5-$HT_7$) and these classes comprise a total of 14 structurally and pharmacologically distinct mammalian receptor subtypes (Hoyer et al., 1994; Hoyer and Martin, 1996). All 5-HT receptors (with the exception of 5-$HT_3$) belong to the superfamily of G-protein coupled receptors, all containing the predicted 7-transmembrane domain structure.

Amongst others, 5-HT play as important role in controlling self-esteem, stabilising mood, facilitating co-operative and competent social behaviour and allowing for the suppression of aggression; behavioural paradigms which are all characteristically disturbed in psychiatric disorders (Jacobs, 1991; Golden et al, 1991; Chopin and Briley, 1987). The study of peripheral markers of 5-HT function is based on the assumption that, in OCD, peripheral abnormalities may reflect abnormal 5-HT function in the CNS. Peripheral markers in these studies include 5-HT content of whole blood and platelets, platelet imipramine-binding capacity and the concentration of the major end metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF). Reports indicate that CSF 5-HIAA concentrations tend to decrease in certain subgroups of OCD patients, particularly those responding to pharmacotherapy (Insel et al., 1985; Thoren et al., 1980; Flament et al., 1987), although these findings have proven inconsistent and difficult to replicate (Hanna et al., 1991; Leckman et al., 1995; Lopez-Ibor, 1988).

More recently, studies have been undertaken to determine the availability of 5-HTT in OCD patients. This is a protein that is critical to the regulation of 5-HT, and represents the initial site of SSRI action. As such, 5-HTT may be useful as a marker of 5-HT function in OCD. Indeed, the availability of the 5-HTT has been found to be significantly reduced in the midbrain and upper brainstem of OCD patients (Stengler-Wenzke et al., 2004). These results were not, however, consistent with those from two previous studies, in which no difference (Simpson et al., 2003), and an increase (Pogarell et al., 2003) in 5-HTT availability were noted when OCD patients were compared to controls. The most likely explanation for the aforementioned discrepant results is difference in study designs (Stengler-Wenzke et al., 2004); nonetheless, the particular area of study seems promising and requires further attention.

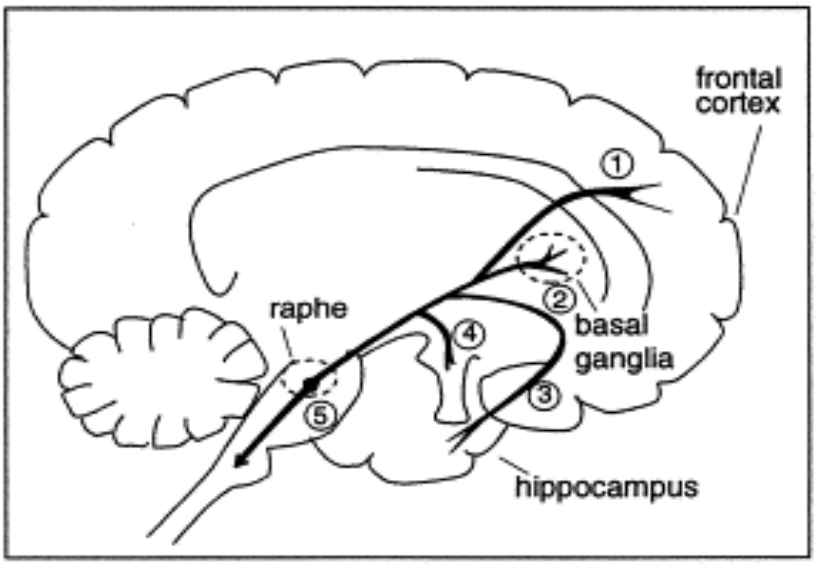



***Figure I.2. Schematic representation of serotonin pathways in the central nervous system***. *Five pathways are indicated, including projections from midbrain raphe to prefrontal cortex (1); basal ganglia (2); hippocampus (3), hypothalamus (4), and spinal cord (5) (After Stahl, 1996).*

The acute administration of a pharmacological agent that affects the 5-HT system allows the functional integrity of the system to be assessed. Meta-chloropiperazine (*m*-CPP) is a potent, relatively non-selective, 5-HT receptor agonist, which is utilised in studies to assess central 5-HT receptor sensitivity in OCD. This 5-HT agonist has complex effects on brain systems in that it binds potently to 5-HT receptors 2C (5-$HT_{2C}$) and 2A (5-$HT_{2A}$), and with weaker affinity to 5-HT receptor 1A (5-$HT_{1A}$) (Kahn and Wetzler, 1991), and has been shown to exacerbate OC symptoms in patients (Zohar et al, 1987; Hollander et al., 1991; 1992[a]; Broocks et al., 1998). Many hypotheses abound regarding the exacerbation of OCD symptoms after *m*-CPP administration, one being that it exacerbates an underlying 5-HT hyperfunction, and in doing so, increases OCD symptomatology (Hollander and Stein, 1997). However, it is important to note that these results were not consistent in other investigations of *m*-CPP challenge (Charney et al., 1988; Goodman et al., 1995; Khanna et al., 2001). Administration of other 5-HT probes have also been used in pharmacologic challenge research, for example, the selective 5-HT receptor 1D (5-$HT_{1D}$) agonist, sumatriptan, has been used to explore the role of, in particular, the 5-$HT_{1D\beta/1D\alpha}$ receptors in OCD (refer to **section I.6.1.1.1[i]** for a more detailed discussion on the role of sumatriptan challenges in OCD). Here, as with *m*-CPP investigations, inconsistent results have been found, possibly indicating

the heterogeneous neurobiological aetiology of the disorder (Zohar and Insel, 1987; Charney et al., 1988; Zohar et al., 1987; Barr et al., 1993).

The neurobiological actions of medication commonly used in the treatment of OCD and related disorders has also shed some light on the neurochemical pathology of such disorders. Clomipramine (CMI), a tricyclic antidepressant, was first introduced in 1966, but it was not until the mid-1980s that the full potential of this potent 5-HT reuptake inhibitor (SRI) in the treatment of OCD was realised (Greist and Jefferson,1998). CMI has been found to be superior in treating OCD when compared to other non-5-HT antidepressants (Thoren et al., 1980; Ananth et al., 1981). It was thus proposed that it was the ability of CMI to inhibit 5-HT reuptake that was responsible for its anti-OCD properties. Subsequently, alternatives to CMI treatment, namely, SSRIs, have been formulated, each with effective anti-OCD properties. At present, SSRIs represent first-line pharmacotherapy in the treatment of OCD (Greist and Jefferson, 1998; Rapoport and Inoff-Germain, 2000; Zohar and Insel, 1987; Leonard et al., 1989; Greist et al., 1995; Piccinelli et al., 1995). These agents also tend to be effective in a variety of disorders that share phenomenological characteristics with OCD, such as TTM and BDD (Stein, 2000; McElroy et al., 1994).

Understanding the mechanism of action of SSRIs in the treatment of OCD has enabled the identification of many of the 5-HT components that may be involved in mediating the disorder. 5-HT released from the raphe neurons into the synapse can act on a variety of postsynaptic 5-HT receptors, as well as at the $5\text{-HT}_{1A}$ and $5\text{-HT}_{1B/D}$ presynaptic auto-receptors, which normally act to decrease 5-HT neuronal firing rates, thereby maintaining effective levels of 5-HT in the synaptic sites (Gothert, 1990). After administration, SSRIs block the reuptake of 5-HT into the presynaptic nerve terminal almost immediately, increasing the concentration of 5-HT present in the synapse. This results in an increased activation of the presynaptic inhibitory receptors, which causes a reduction in the firing rate of the 5-HT neurons.

However, after a lag period of approximately 2 to 3 weeks, downregulation and subsequent desensitisation of the autoreceptors causes a disinhibition of 5-HT release at the axon terminals. This results in an increased 5-HT firing rate, which ultimately increases the 5-HT concentrations in the cortex to therapeutic levels. It is proposed that it is the desensitisation of the presynaptic receptors and subsequent increase in synaptic 5-HT that brings about the

desired effect of the SSRIs, and acts as a likely explanation as to why the delay exists in achieving full therapeutic effects when employing an SSRI treatment regimen in OCD (Nutt et al., 1999; Blier and Montigny, 1998; El Mansari et al., 1995; Blier and Bouchard, 1994).

It should, however, be noted that a significant proportion of OCD patients do not respond to SSRI monotherapy – the mean response rate to SSRIs has been reported to be approximately 50% (Greist et al., 1995; Stein et al., 1995). In addition, pharmacological challenge studies in drug-free OCD patients have, as of yet, identified no consistent abnormality in 5-HT function (Barr et al., 1992; Goodman et al., 1995). This could be explained in terms of the involvement of other neurotransmitter systems in the pathology of the disorder. In fact, it has been proposed that if there is any possibility of an isolated dysfunction of the 5-HT system, it is probably only present in a small group of OCD patients who respond well to SSRI monotherapy (Khanna et al., 2001). Paradigms have therefore been developed to evaluate the role that other neurotransmitter or neuropeptide systems may play in the neuropathology of the disorder.

Based on the aforementioned information, a number of genetic association studies (both case-control and family-based) have been conducted, in various populations, in order to investigate the contribution that genes encoding components within the 5-HT system may have in the aetiology of OCD and related subtypes (Table I.5).

#### *I.6.1.1.1. Serotonergic candidate genes investigated in the present study*

**i. The 5-HT Receptor 1Dβ gene *($5HT_{1D\beta}$)***

In the brain, 5-$HT_{1D\beta}$ pre- and postsynaptic receptors are concentrated in the basal ganglia, striatum and frontal cortex, with the highest receptor densities found in the basal ganglia (Moret and Briley, 2000; Graeff, 1997), and may be situated either pre- or post-synaptically relative to 5-HT neurons. 5-$HT_{1D\beta}$ auto-receptors are found primarily at the sites of 5-HT release (i.e. synaptic terminals or axonal varicosities) (Gothert, 1990), and their primary function is to inhibit the release of 5-HT by modulating the firing rates of the neurotransmitter from the neurons (Gothert and Schlicker, 1987).

The 5-$HT_{1D\beta}$ receptor is not only found on 5-HT neurons, but may also be situated on non-5-HT neurons (Uphouse, 1997), where it acts as a heteroreceptor, controlling the release of

neurotransmitters other than 5-HT. Such neurotransmitters include acetylcholine, glutamate, noradrenaline and γ-aminobutyric acid (Iyer and Bradberry, 1996; Gothert, 1990).

The receptor has been clearly implicated in basic behavioural activities in animals: 5-$HT_{1D\beta}$ auto-receptor knockout mice exhibit an increase in aggression and impulsivity, as well as increased alcohol consumption (Huang et al., 1999; Montgomery and Fineberg, 1989). Pre-clinical evidence of the role that the 5-$HT_{1D\beta}$ autoreceptor may play in OCD has been obtained from animal studies, where it was found that, in the orbito-frontal cortex, the enhanced release of 5-HT brought about by SSRIs was attributable to the desensitisation of the 5-$HT_{1D\beta}$ auto-receptor (el Mansari et al., 1995).

The putative role that the 5-$HT_{1D\beta}$ auto-receptor may play in the mediation of OCD in humans has been investigated by means of pharmacological challenge with 5-HT probes, namely *m*-CPP and sumatriptan. Zohar and Kindler (1992) observed that orally administered *m*-CPP aggravated OCD symptoms, whereas the administration of MK-212 (another 5-HT agonist) did not. Taking into account the sites of action of the two receptor agonists (*m*-CPP stimulates 5-$HT_{1D\beta}$, but MK-212 does not), it was proposed that the 5-$HT_{1D\beta}$ receptor may be implicated in OCD (Koran et al., 2001). Similarly, the administration of a more selective 5-$HT_{1D\beta}$ auto-receptor agonist, sumatriptan, to untreated OCD patients, was also found to produce a transient worsening of obsessive-compulsive symptoms (Stern et al., 1998; Gross-Isseroff et al., 2004; Koran et al., 2001) (although again, this effect was not observed in all studies [Ho Pian et al., 1998]).

Moreover, in a functional brain imaging study combined with symptom provocation with sumatriptan in OCD patients, Stein et al. (1999) reported a heterogeneous behavioural response, with some patients showing acute symptom exacerbation, while others demonstrated a decrease in symptoms. It was also reported that those patients exhibiting an increase in OCD symptomatology after acute sumatriptan challenge had a poorer response to SSRI therapy. Interestingly, anecdotal reports have suggested that the chronic administration of sumatriptan has therapeutic effects on depressive, and perhaps on obsessive-compulsive, symptoms in OCD patients who are highly resistant to conventional pharmacotherapy (Stern et al., 1998; Pathak et al., 2003).

***Table I.5**: Published population and family-based genetic association studies in OCD: serotonergic candidate genes*

| Gene | Variant(s) | Population | Study design | Phenotype investigated | Sample number affected (case) | Sample number affected (families) | Sample number control | Result (p-values, and implicated risk allele) | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***5-HTT*** | | | | | | | | | |
| | | Not specified | CC | OCD | 72 | | 72 | NS | 1 |
| | | European-American | FB | OCD | | 35 | | p<0.03 ($L^a$-allele) | 2 |
| | | Caucasian American | CC | OCD | 75 | | 397 | p=0.023 (*LL*-genotype) | 3 |
| | | Afrikaner | CC | OCD | 54 | | 82 | NS | 4 |
| | | Jewish (Ashkenazi [A]and non-A) | CC | OCD | 75 (39A) | | 172 (112A) | NS | 5 |
| | ***5-HTTLPR*** | ***META-ANALYSIS[b]*** | CC | OCD | 129 | | 479 | NS | 4 |
| | | Mexican | CC/ FB | OCD | 115 | 43 | 136 | NS | 6 |
| | | Italian | CC | OCD | 180 | | 112 | NS | 7 |
| | | German | FB | OCD | | 63 | | NS | 8 |
| | | Brazilian | CC | OCD | 79 | | 202 | NS | 9 |
| | | French/German | CC/ FB | OCD | 106 | 86 | 171 | NS | 10 |
| | 17bp VNTR in intron 2 | Japanese | CC | OCD | 15 | | 106 | p=0.033 for 12-repeat allele OR=10.2 (95% CI: 1.34-77.4) | 11 |
| ***5-HT$_{2A}$*** | | | | | | | | | |
| | *T102C* | Mexican | CC | OCD | 67 | | 54 | NS | 12 |
| | | Jewish (Ashkenazi [A]and non-A) | CC | OCD | 75 (39A) | | 172 (112A) | NS | 5 |
| | | Afrikaners | CC | OCD | 71 | | 129 | NS | 13 |
| | | SA Caucasians, stratified into Afrikaner (Afr) | CC | EO[c]OCD vs. LO OCD | n(EO)=95[45 Afr]; n(LO) = 85 [35 Afr] | | | NS | 14 |
| | ***1438G/A*** | German | CC | OCD | 55 | | 223 | p=0.046 (genotype) *A*-allele | 15 |
| | | N. American Caucasian | CC | OCD | 101 | | 138 | p=0.015 and p=0.023 (allele and genotype); *A*-allele in females | 16 |
| | | N. American Caucasian | CC | | 62 | | 144 | *A*-allele increased in OCD patients p<0.05 | 17 |
| | ***T102C and -1438 A/G*** | Turkish | CC | OCD | 58 | | 83 | NS | 18 |
| | ***C516T*** | Brazilian | CC | OCD | 79 | | 202 | p=2x10$^{-4}$ (genotype); p=7x10$^{-5}$ ; *C*-allele) | 9 |
| ***5-HT$_{2C}$*** | | | | | | | | | |
| | ***ser23cys*** | Italian | CC | OCD | 109 | | 107 | NS | 19 |
| | | | | OCD+tics vs. OCD-tics | n(OCD+tics)=23 | | | NS | |
| | | Jewish (Ashkenazi [A]and non-A) | CC | OCD | 75 (39A) | | 172 (112A) | NS | 5 |
| ***5-HT$_{1Dβ}$*** | | | | | | | | | |
| | ***G861C*** | Italian | FB | OCD | | 32 | | p<0.006 / OR=5.26 (1.92-13.10) *G*-allele | 20 |
| | | Italian | FB | OCD | | 48 | | NS | 21 |
| | | Italian | FB | OCD | | 121 | | p=0.02 (*G*-allele) | 22 |
| | | Afrikaners | CC | OCD | 71 | | 129 | NS | 13 |
| | | Mexican | FB | OCD | | 72 | | Higher Y-BOCS obsession scores for males carrying the *G*-allele (p=0.034) | 23 |
| | | German | FB | OCD | | 64 | | NS | 8 |
| | | SA Caucasians, stratified into Afrikaner (Afr) | CC | EO[c]OCD vs. LO OCD | n(EO)=95[45 Afr]; n(LO) = 85 [35 Afr] | | | NS | 14 |
| ***TPH*** | | | | | | | | | |
| | rs1800532 (intron 7) | Jewish (Ashkenazi [A]and non-Ashkenazi) | CC | OCD | 75 (39A) | | 172 (112A) | NS | 5 |
| | | German | FB | OCD | | 59 | | NS | 8 |
| | ***T1095C*** (exon10) | American Indians & Caucasians[d] | | OCD | 88 | | 142 | NS | 24 |

[a]*L* refers to the "long" allele; [c]Studies included in meta-analysis are: references 3and 4;[c] EO OCD ≤ 15 years;; [d]Caucasians were of US, Italian and Finnish origin

**Abbreviations*:* OCD**: Obsessive-compulsive disorder; **CC**: population-based case-control association; **FB**: family-based association **OR**: Odds ratio**; CI**: confidence interval; **NS**: non-significant finding (p>0.05); **VNTR**: variable number of tandem repeats; **SA**: South African; **EO:** early-onset OCD**; LO:** late-onset OCD**;; Afr:** Afrikaner; ***5-HTT:*** serotonin transporter; ***5-HTTLPR***: variable number of tandem repeat polymorphism in the promoter region of 5-HTT, producing either long (*L*) or short (*S*) alleles; ***5-HT$_{2A}$***: serotonin receptor 2A; ***5-HT$_{2C}$***: serotonin receptor 2C; ***TPH***: Tryptophan hdroxylase;

**References:1:** Billet et al. (1997)**; 2:** McDougle et al. (1998)**; 3:** Bengel et al. (1999)**; 4:** Kinnear et al. (2000); **5:** Frisch et al. (2000);**6:** Camarena et al. (2001); **7:** Cavallini et al. (2002); **8:** Walitza et al. (2004); **9:** Meira-Lima et al. (2004); **10**: Chabane et al. (2004); **11:** Ohara et al. (1998)**; 12:** Nicolini et al. (1996); **13:** Hemmings et al. (2003); **14:** Hemmings et al. (2004); **15:** Walitza et al. (2002); **16:** Enoch et al. (2001)**;17:** Enoch et al. (1998); **18:** Tot et al. (2003); **19:** Cavallini et al. (1998); **20**: Mundo et al. (2000); **21:** Di Bella et al. (2002); **22:** Mundo et al. (2002); **23:** Camarena et al. (2004)**; 24:** Han et al. (1999).

Finally, it has been proposed that the 5-$HT_{1D\beta}$ auto-receptor may be involved in the efficacy of SSRIs (el Mansari et al., 1995; Dolberg et al., 1996). As stated before,the initial effect of the SSRI is to increase 5-HT concentration, the pharmacotherapeutic effect of these agents seems to be associated with the subsequent adaptive down-regulation of 5-$HT_{1D\beta}$ auto-receptors. Hence the importance of investigating the probable role that the gene encoding the 5-$HT_{1D\beta}$ auto-receptor may play in mediating susceptibility to OCD.

*5-$HT_{1D\beta}$* maps to chromosomal location 6q13 (Hamblin et al., 1992; Demchyshyn et al., 1992) and is intronless, with a size of 1179 bp (Lappalainen et al., 1995[a]). The polymorphic variant analysed in the present study is characterised by a SNP that can be detected by means of *Hinc*II restriction enzyme. The SNP is created by a silent *G-C* substitution at nucleotide position 861 of the coding region (*G861C*) (Sidenberg et al., 1993; Lappalainen et al., 1995[a]). The functional significance of this polymorphism has not yet been established, although, individuals homozygous for the *G861* allele have been found to possess higher $B_{max}$ values for 5-$HT_{1D\beta}$ binding in the PFC (Huang et al., 1999).

Preliminary evidence from two genetic studies utilising TDT analysis have implicated the possible involvement of the 5-$HT_{1D\beta}$ auto-receptor in the aetiology of OCD (Mundo et al., 2000; 2002) (Table I.5). In these studies, a significant association was observed between the distribution of the polymorphic *G861C* allelic variants of the *5-$HT_{1D\beta}$* and OCD, with preferential transmission of the *G*-allele to the affected subjects. These results have, however, not been replicated in subsequent family (Di Bella et al., 2002; Camarena et al., 2004; Walitza et al., 2004) and population-based (Hemmings et al., 2003; 2004) association studies (Table I.5).

The variant has also been investigated for its role in early-onset OCD in both family-based (Walitza et al., 2004) and population-based (Hemmings et al., 2004) studies, with neither study yielding significant results. It should, however, be mentioned that, although Camarena et al. (2004) observed no significant association between *G861C* and the DSM-IV diagnosis of OCD, they did observe that subjects with a preferential transmission of *G861* exhibited higher Y-BOCS Obsession scores than those with the *C861* alelle, implicating the gene in the severity of obsessions.

The present study builds upon data presented in two previously published articles (Hemmings et al., 2003; Hemmings et al., 2004), investigating the role that the *G861C* polymorphism may play in OCD and related subtypes.

**ii. The Serotonin Receptor 2 genes (*5-HT$_{2A}$* and *5-HT$_{2C}$*)**

5-HT$_2$ receptors are widely studied in psychiatric disorders and have been implicated in a broad range of behavioural and physiological processes and cellular development. They also represent the sites of action of hallucinogens and certain psychotherapeutic drugs (Morilak et al., 1994; Kapur and Remington, 1996). These receptors are widely distributed, both peripherally and in CNS tissue, occuring in high densities in the cerebral cortex and regions of the amygdala and hypothalamus (Uphouse, 1997). To date, genes encoding three human 5-HT$_2$ receptor subtypes have been cloned, namely the gene encoding the 5-HT$_{2A}$ receptor (*5-HT$_{2A}$*) (Chen et al., 1992), the 5-HT$_{2B}$ receptor (*5-HT$_{2B}$*) (Choi et al., 1994) and the 5-HT$_{2C}$ receptor (*5-HT$_{2C}$*) (Xie et al., 1996).

***a. The Serotonin Receptor subtype 2A gene* (5-HT$_{2A}$)**

There is presently a wide range of pharmacological and genetic evidence implicating the 5-HT$_{2A}$ receptor in the pathophysiology of OCD. Recent case reports indicate that the chronic use of hallucinogenic drugs, which are potent stimulators of the 5-HT$_{2A}$ receptor, may have beneficial effects in individuals with OCD and related OCSDs (Moreno and Delgado, 1997; Leonard and Rapoport, 1987; Hanes, 1996) suggesting a beneficial role of 5-HT$_{2A}$ receptor activation. Supporting this notion is the increasing number of reports that describe the tendency of clozapine, a 5-HT$_{2A}$ receptor antagonist, to unmask obsessive-compulsive symptoms in schizophrenic subjects (Patil, 1992; Baker et al., 1992; Poyurovsky et al., 1996). In addition, it has been shown that risperidone, a highly selective, potent 5-HT$_{2A}$/DRD2 receptor antagonist, results in the exacerbation of obsessive-compulsive symptoms in psychosis (Dodt et al., 1997; Andrade, 1998).

However, confusing the issue somewhat are reports that risperidone has been found to enhance therapeutic responses when administered to SSRI-refractory OCD patients (McDougle et al., 1995; 1997; 2000; Jacobsen, 1995; Stein et al., 1997; Marek et al., 2003). The therapeutic efficacy of augmentation of risperidone to SSRI monotherapy in OCD has been hypothesised to be due to a possible synergistic action between the blockade of the 5-

$HT_{2A}$ receptor (due to risperidone) and the activation of non-5-$HT_{2A}$ receptors (due to 5-HTT blockade caused by SSRI) (Marek et al., 2003).

The gene encoding 5-$HT_{2A}$ receptor, located on chromosome 13q14-21 (Sparkes et al., 1991), has been one of the most widely investigated for its potential role in the aetiology of OCD. The gene comprises two alternative promoters, with a silencer element located downstream of the second promoter (Zhu et al., 1995). Two variants in particular, one occurring near the promoter region (*-1438 A/G*; dbSNP rs6311), and a silent variation reported to be in LD with it, in exon 1 (*T102*C: dbSNP rs6313), have been found to be associated with numerous psychiatric disorders, including anorexia nervosa (Collier et al., 1997; Sorbi et al., 1998; Enoch et al., 1998), bipolar disorder (Bonnier et al., 2002; Chee et al., 2001), seasonal affective disorder (Enoch et al., 1999) and schizophrenia (Williams et al., 1997; Arranz et al., 1998).

The function of the *T102C* polymorphism is presently under debate. Polesskaya and Sokolov (2002) investigated the relationship of the *C102* and *T102* alleles and expression of *5-$HT_{2A}$* in post-mortem brain tissue of normal and schizophrenic heterozygous individuals, and found that the expression level of *C102* was significantly decreased in relation to that of the *T102* allele. Similarly, Khait et al. (2005) assayed platelet 5-$HT_{2A}$ binding kinetics, and observed that the *T102T* genotype was associated with increased 5-$HT_{2A}$ receptor density (and thus increased expression). However, Bray et al. (2004), using a quantitative allele-specific primer extension assay, observed no differences in expression between the *C102* and *T102* alleles in several adult cortical regions in a post-mortem assay, and concluded that neither allele affected mRNA expression.

On the other hand, it is possible that the -1438 *A/G* promoter polymorphism may affect expression by altering promoter function. Spurlock et al. (1998) investigated this possibility using a reporter gene assay in heterologous cells and found no significant differences in levels of expression between the two alleles. However, in a more recent study, Parsons et al. (2004) made use of two different reporter gene assays and found that the presence of the *-1438 A* allele increased promoter activity. However, this was only observed in those cell lines expressing endogeneous 5-$HT_{2A}$, indicating that transcriptional factors may be required to elicit this increase.

Interestingly, OCD has been found to be associated with the *A* allele of the *-1438 G/A* promoter polymorphism (Enoch et al., 1998; 2001) (Table I.5) although the association was only observed in female subjects, indicative, perhaps, of the differential 5-HT effects operating in men and women with OCD. These results were replicated in a study by Walitza et al. (2002), who found that the *A*-allele was associated with OCD in German children and adolescents.

A number of studies have also investigated the *T102C* polymorphism for association with OCD, although none have yielded significant findings in Afrikaner (Hemmings et al., 2003), Turkish (Tot et al., 2003), Brazilian (Meira-Lima et al., 2004) or Jewish (Frisch et al., 2000) populations (Table I.5). However, a more recent study using a Turkish population, Tot et al. (2003) found that, although neither the *T102C* or the *-1438A/G* polymorphisms were associated with OCD *per se*, the *T102T* genotype from the *T102C* variant and the *AA* genotype from the *-1438A/G* variant were found to be associated with increased severity of OCD,as measured by Y-BOCS score.

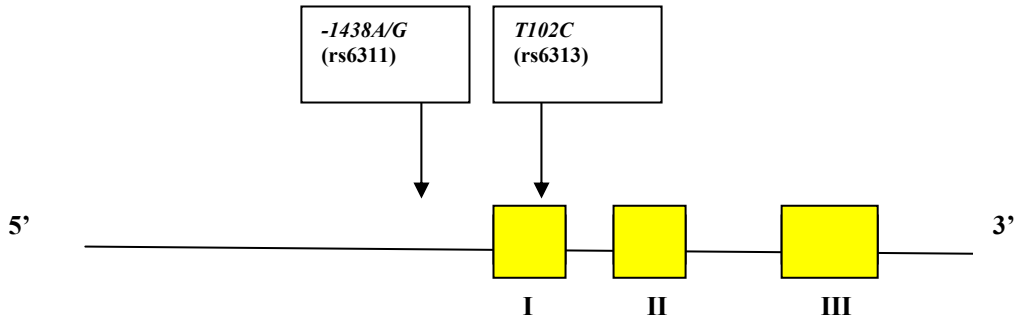



***Figure I.3. Schematic representation of the* 5-HT$_{2A}$ *locus and flanking region***. *The location of SNPs investigated in the present study are indicated by arrows. Exons are represented by yellow blocks, and introns are represented as horizontal lines between the exons (not drawn to scale). The distance between the two SNPs is 1.5kb.*

One would assume that, if the two loci are in complete LD with one another, that association at one would infer association at the other. It may, however, be that, in some populations, LD between the two polymorphisms is not complete, in which case an association between OCD and one variant may be detected, while it may not be detectable with the other. Neither of the aforementioned studies investigating the role that either of the polymorphisms plays in the

development of OCD conducted haplotype analysis. Given the strong potential for the involvement of *5-HT$_{2A}$* in the aetiology of OCD, the present study investigates the possible involvement of two *5-HT$_{2A}$* polymorphisms in the aetiology of OCD and OCD- related subtypes: the promoter region -*1438 A/G* SNP, and the exon 1 *T102C* SNP (Figure I.3), using both single locus and haplotype analyses.

***b. The Serotonin Receptor subtype 2C gene* (5-HT$_{2C}$)**

Numerous animal and pharmacological studies have suggested the possible involvement of 5-HT$_{2C}$ in the aetiology of OCD and/or OCD related disorders. Mice lacking the gene encoding 5-HT$_{2C}$ (*5-HT$_{2C}$*) exhibit seizure syndromes, manifested by spontaneous tonic-clonic seizures and an increase in grooming behaviour (Heisler and Tecott, 2000). In addition, Chou-Green et al. (2003) observed that *5-HT$_{2C}$* knockout mice exhibited compulsive-like syndromes, demonstrated by several defined, highly organized behaviours such as increased chewing of non-nutritive clay and the chewing of a plastic mesh screen in a neat pattern.

Pharmacological studies have indicated an acute worsening of obsessive-compulsive symptoms after the administration of the selective 5-HT$_{2C}$/5-HT$_{1A}$/5-HT$_{1D}$ agonist, *m*-CPP, and a recent study using an animal model of OCD suggests that the 5-HT$_{2C}$ receptors are most likely involved in the exacerbation of OC symptoms after m-CPP administration (Graf et al., 2003; Tsaltas et al., 2005). In addition, it has been suggested that chronic SSRI treatment could result in the reduction of mesocorticolimbic dopaminergically-mediated transmission via 5-HT$_{2C}$ activation, representing an important step in therapeutic efficacy of SSRIs (Prisco and Esposito, 1995; Di Matteo et al., 2001).

The gene encoding 5-HT$_{2C}$ has been mapped to Xq24, consists of 6 exons, and stretches over approximately 230kb (Milatovich et al., 1992). A structural variant in the extracellular N-terminal region of the receptor, resulting in a cysteine to serine amino acid substitution at position 23 (*cys23ser*), has been described (Lappalainen et al., 1995[b]). The function of this polymorphism is presently not clear: first, Lappalainen et al. (1999) reported a higher cerebrospinal fluid concentrations of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) in males possessing the *ser23* allele, compared to *5-HT$_{2C}$ cys23cys* homozygotes, although no changes were noted in CSF concentrations of either dopaminergic or 5-HT metabolites. These results were, however, not replicated in a more recent study by Jönsson et al. (2004), although it is possible that the selection of subjects

may be responsible for the variation in results: Lappalainen et al. (1999) included 73% alcohol-violent offenders, with only 27% healthy controls, whilst Jönsson et al. (2004) included only healthy controls in their study. On the other hand, results from recent functional analyses, comparing the expression of the two alleles, indicated that the 5-$HT_{2C}$ receptor expressing the *ser23*-allele showed reduced high affinity binding to m-CPP and 5-HT, compared to that expressing the *cys23*-allele (Okada et al., 2004).

In a case-control genetic association study to assess the relationship between *5-$HT_{2C}$* and OCD, Cavallini et al. (1998) found no significant association between the *5-$HT_{2C}$ cys23ser* (dbSNP rs6318) variant and OCD in an Italian population, or between the variant and a subgroup of OCD subjects stratified according to the presence or absence of tics (Table I.5). Likewise, Frisch et al. (2000) observed no statistically significant differences in genotypic or allelic distribution between OCD patients and controls drawn from both an Ashkenazi Jewish population and a non-Ashkenazi Jewish population. However, the sample sizes in the latter study are relatively small (Table I.5), implicating that their results may be inconclusive.
Therefore, given results from recent animal models, it would be valuable to investigate the role that this gene may play in mediating the development of OCD symptoms. The *cys23ser* variant was thus investigated in the present study.

**iii. The Serotonin Receptor subtype 6 gene (*5-$HT_6$*)**

The gene encoding 5-$HT_6$ has, as of yet, not been investigated for the role that it may play in the aetiology of OCD and OCD-related disorders. However, it represents an interesting and plausible OCD-susceptibility candidate gene for a number of reasons. First, the mRNA of 5-$HT_6$ has been found to be particularly abundant in the nucleus accumbens, caudate-putamen, olfactory tubercle and the striatum (Ruat et al., 1993; Ward et al., 1995; Gerard et al., 1996; 1997; Branchek and Blackburn, 2000), brain areas that have been implicated in OCD. Secondly, dopaminergic neurons are found to occur in large numbers in these regions, and it has been proposed that dopaminergic neurotransmission may be modulated by 5-HT via 5-$HT_6$ in these brain regions (Matsumoto et al., 1999), although no direct evidence exists for the involvement of 5-$HT_6$ as yet (Roberts et al., 2002).

Perhaps more relevant to the study of OCD and OCD-related disorders is the observation that, in the rat brain, ontogenic studies have revealed that 5-$HT_6$ expression correlates with the appearance of the first 5-HT cell bodies (Grimaldi et al., 1998). This suggests that 5-$HT_6$ may

play a role in mediating certain aspects of 5-HT growth factor properties; consequently, a dysfunction in 5-$HT_6$ may mediate the development of OCD, or related disorders, in accordance with the combined 5-HT/neurodevelopmental hypothesis of OCD. Animal studies have also indicated that 5-$HT_6$ receptors may play a role in the aetiology of anxiety responses (Otano et al., 1999; Pouzet et al., 2002).

A distinctive feature of the receptor is that it exhibits high affinity for antispsychotic compounds, such as risperidone and clozapine, which have been found to be efficacious in treating OCD patients presenting with comorbid tic disorders (Hollander et al., 2003[a]) and for various tricyclic antidepressants, namely CMI, amitryptyline and amoxipine (Monsma et al., 1993; Roth et al., 1994; Boess et al., 1997).

The human gene encoding 5-$HT_6$ has been mapped to chromosome 1p35-36, and comprises three exons (Kohen et al., 1996). To date, the most widely studied polymorphism in the gene occurs in the first exon, and is characterised by a synonymous *T* to *C* transversion at nucleotide 267 (*T267C*; dbSNP rs1805054). This variant has been investigated for the role that it plays in various neuropsychiatric disorders, including bipolar disorder, schizophrenia and Parkinson's disease (Vogt et al., 2000; Tsai et al., 1999; Messina et al., 2002). The variant has also been implicated in patients' response to antipsychotic agents: the *T267T* genotype has been associated with superior response for both clozapine and risperidone treatment in treating the anxiety and depressive characteristics experienced by schizophrenic patients (Lane et al., 2004; Yu et al., 1999).

To the author's knowledge, the present study is the first to investigate *5-$HT_6$* as a candidate in a population-based OCD genetic association study.

### I.6.1.2. The dopaminergic hypothesis of OCD

Dopamine is a catecholaminergic neurotransmitter that is synthesised from the amino acid tyrosine by means of the enzyme tyrosine hydroxylase. This reaction constitutes the rate-limiting step in which 3,2-dihydroxyphenylalanine (L-DOPA) is formed. L-DOPA is subsequently decarboxylated by dopa decarboxylase (DDC) to form dopamine.

Once released into the synaptic space, dopamine acts on various types of postsynaptic dopaminergic receptors, which allows the transduction of the dopaminergic signals across the

post-synaptic neuronal membrane. Excess released dopamine is recaptured via an active re-uptake mechanism and is inactivated in the pre-synaptic neuron by MAO-A and COMT. Dopaminergic neurotransmitters are clustered in the midbrain and branch into four major pathways - the nigro-striatal, the mesolimbic, the mesocortical, and the tuberoinfindibular pathways (Figure I.4). The neurotransmitter modulates a variety of functions, including neuro-endocrine, spatial and memory functioning, cognitive and emotional functioning, and reward and motivational behaviour (Jaber et al., 1997; Giros et al., 1992; Pani et al., 2000).

Dopaminergic postsynaptic effects are mediated by at least five physiologically and pharmacologically distinct receptors (named DRD1 to DRD5). These five dopaminergic receptors can, in turn, be divided into two subfamilies whose properties resemble either those of the DRD1 or DRD2 receptors, which were originally defined (Jarvie and Caron, 1993). The two subfamilies are termed DRD1-like (which includes the DRD1 and DRD5 receptors) and DRD2-like (consisting of the DRD2, DRD3 and DRD4 receptors). Dopaminergic neurotransmitter action is terminated by the dopamine transporter (DAT), which is thus important in maintaining transmitter homeostasis. DAT can function in either direction, transporting dopamine into or out of the neuron, depending on the existing gradient.

Significant homologies have been found to exist amongst the dopaminergic receptor subtypes. The receptors belong to the super-family of G-protein coupled receptors, all containing the predicted 7-transmembrane domain structure with membrane spanning helices linked by intracellular and extracellular loop. The DRD1-like receptors often exert their physiological influences by means of stimulating adenylate cyclase via a stimulatory G-protein ($G_s$), whilst DRD2-like receptors exert their influence by inhibiting adenylate cyclase via an inhibitory G-protein (Gi).

Dopaminergic dysregulation is thought to contribute to the pathophysiology of OCD based on neurobiological and pharmacological data obtained from humans and animals. In animal models, agents that increase synaptic dopamine levels, such as amphetamine, methylphenidate and L-DOPA, produce stereotypies and repetitive behaviour that resemble some of the symptomatology manifested by individuals with OCD (Goodman et al., 1990; Fog, 1972; Wallach, 1974; Creese and Iversen, 1974). Acute administration of dopaminergic agonists has been found to induce perseverative behaviours in rodents, whilst chronic administration causes an increased rigidity in perseverative behaviour (Eilam et al., 1989).

Rats treated chronically with the DRD2/DRD3 dopaminergic agonist, quinpirole, meet the ethological criteria of compulsive checking in OCD (Einat and Sczechtman, 1995; Schetzman et al., 1998; 2001), which was found to be partially attenuated by CMI. A more recent study has indicated that juvenile male rats are more sensitive to the perseverative actions of quinpirole than adult male rats, and that CMI prevents the drug-induced reaction in adult, but is less effective in juvenile, male rats (Ulloa et al., 2004). These findings may indicate a lower sensitivity in 5-HTT (Ulloa et al., 2004) in juvenile rats, analogous with findings that EO OCD is a predictor of poor response to treatment (Rosario-Campos et al., 2001; Ackerman et al., 1994; Ravizza et al., 1995) (please refer to **section I.4.2.2.4** for a more detailed discussion on the clinical features of EO OCD).

In addition, a novel transgenic mouse model of cortical and limbic neural stimulation has recently been characterised. These mice express a neuropotentiating transgene in a subset of DRD1-expressing neurons in regions of the amygdala and cortical areas that project to the orbitofrontal cortex and striatum. Chronic potentiation of these neurons (known to induce efferent glutamatergic neurotransmission to the striatum) resulted in the transgenic mice exhibiting TS/OCD-like behaviours, including repeated leaping, OCD-like persistent grooming, non-aggressive OCD-like repeated biting of cagemates, episodes of repetition or perseverance of normal behaviours and repeated climbing or leaping and tics (Nordstrom and Burton, 2002, Campbell et al., 1999[a]; 1999[b]; McGrath et al., 2000). These results not only indicate the possibility that DRD1 may be involved in such OCD-like behaviour, but also that this behaviour may be mediated through glutamatergic activity (**section I. 6.1.3**).

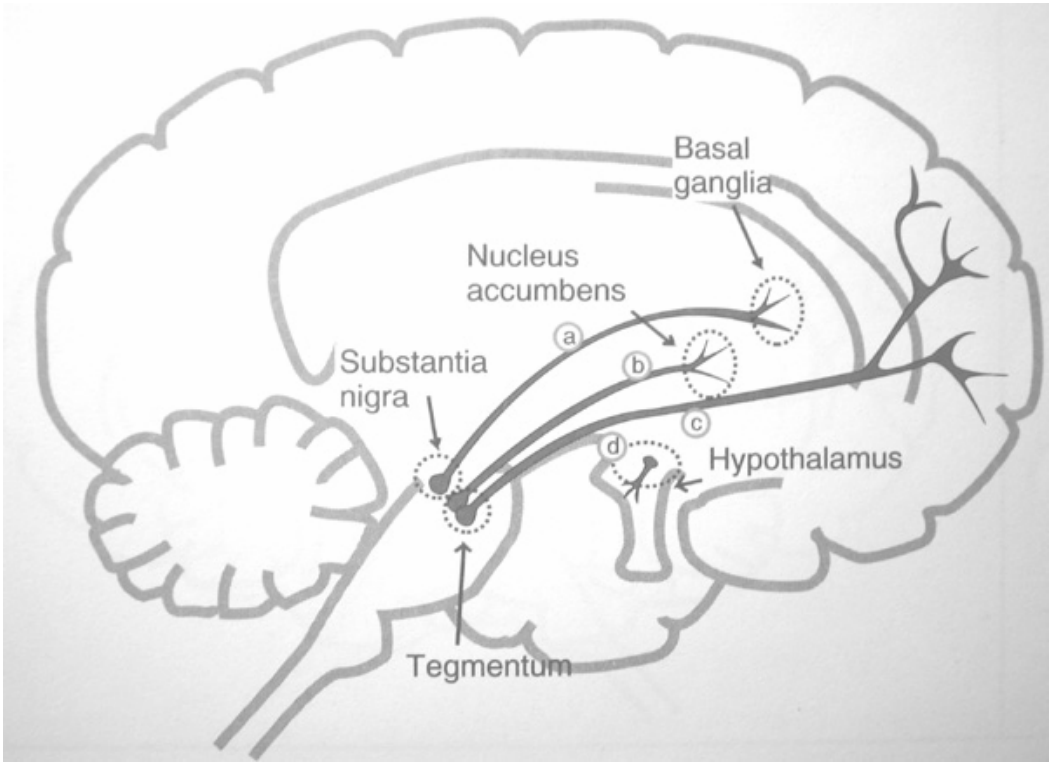



***Figure I.4. Four dopamine pathways in the brain****: (**a**) the nigrostriatal dopamine pathway projects from the substantia nigra to the basal ganglia, and is thought to control movements. (**b**) The mesolimbic dopamine pathway projects from the midbrain ventral tegmental area to the nucleus accumbens. (**c**) The mesocortical pathway projects from the midbrain ventral tegmental area to the limbic cortex. (**d**) The tuberoinfundibular pathway extends from the hypothalamus to the anterior pituitary gland (after Stahl, 2000).*

Joel and Doljansky (2003) created a rat model of OCD that simulates a deficiency in response feedback mechanism that has been hypothesised to underlie obsessions and compulsions. They found that post-signal attenuation leads to a response involving excessive lever pressing, akin to the excessive, unreasonable behaviour noted in OCD. Administration of a DRD1 antagonist reduced the number of compulsive lever presses and, based on further electrophysiological data, it was proposed that the reduction in lever pressing depended on a phasic increase in the stimulation of DRD1. In addition, results from a recent DAT-knockdown mouse model indicate that hyperdopaminergic mice exhibit excessively strong and rigid manifestations of a complex fixed action pattern (thought to characterise a number of human disorders involving the basal ganglia, including OCD and TS), in comparison to wild-type mice (Berridge et al., 2005). It is, however, not presently known whether the sequential stereotypic behaviours of the DAT knockdown mice depend on DRD1 circuit activation (as observed by, for example, Campbell et al. [1999(a)]) or not. Nonetheless, results from the aforementioned investigations suggest a role for dopaminergic receptors, DRD1, DRD2, DRD3 and DAT in the aetiology of OCD.

Functional neuroimaging studies have also implicated the role of the dopamine system in OCD: DRD2 binding in the left caudate has been found to be significantly lower in OCD patients compared to controls (Denys et al., 2004[c]), and van der Wee et al. (2001) reported an increased dopamine transporter (DAT) binding ratio in the left basal ganglia of OCD patients. Likewise, Kim et al. (2003) reported an increased DAT binding ratio in the right basal ganglia, and a tendency towards increased DAT binding in the left basal ganglia, in OCD patients. Conversely, Pogarell et al. (2003) did not report any difference in DAT binding ratios between OCD and control subjects. On the whole, these studies suggest the probable involvement of the dopamine system (and in particular, DRD2 down-regulation and an increased concentration of DAT) in the pathophysiology of OCD.

Baseline concentrations of dopamine and its metabolite, homovanillic acid (HVA) have also indicated a role for dopamine in the pathology of OCD (Hollander et al., 1992[b]; Zahn et al., 1996), although, once again, not all studies have yielded consistent results (Thoren et al., 1980; Benkelfat et al., 1991). Moreover, Marazziti et al. (1992) reported an increase in dopamine neurotransmission in patients with the disorder, based on increased platelet levels of sulfotransferase (an enzyme involved in dopamine catabolism).

In pharmacological challenge studies, Brambilla et al. (1997; 2000) examined the function of the dopaminergic system in a group of individuals with OCD by measuring growth hormone (GH) (Brambilla et al., 1997) and cortisol (Brambilla et al., 2000) responses to stimulation with apomorphine (APO), a short acting dopamine agonist. Results of these experiments are thought to be good indicators of postsynaptic dopaminergic function in the hypothalamus and hypothalamo-pituatary-adrenal (HPA) axis, respectively. However, inconsistent results were found in the two studies: in the first investigation, GH responses to APO were found to be blunted in OCD patients, indicative of a dysregulation in the dopaminergic system in these individuals, but in the second, cortisol responses to APO were found to be equivalent in OCD patients and controls. These findings suggest heterogeneity in the dopaminergic system functions in OCD, with dopamine neurotransmission being impaired in brain regions related to OC pathology, and normal in other brain regions (Brambilla et al., 2000).

Finally, a significant improvement has been noted in SSRI non-responders with OCD when neuroleptics, which are primarily dopamine antagonists, are added to the ongoing SSRI monotherapy (McDougle et al., 1994, 2000; Stein et al., 1997; Shapira et al., 2004). In particular, it has been suggested that particularly OCD patients who exhibit tics benefit from the combined SSRI/ dopamine antagonist treatment, suggesting that both the 5-HT and dopamine systems may be involved in the clinical manifestation of specific subtypes of OCD.

#### I.6.1.2.1. Dopaminergic candidate genes investigated in the present study

**i. The Dopamine Receptor 4 gene *(DRD4)***

DRD4 belongs to the group of DRD2-like dopamine receptors, and represent G-protein coupled receptors in that the α-helices are organised into seven relatively hydrophobic transmembrane spanning segments joined by three less hydrophobic intracellular peptide segments. The third intracellular loop, situated between transmembrane segments 5 and 6, provides the link to G-proteins, facilitating interaction with other downstream molecular elements, which represents the initiation of dopaminergically-mediated neurotransmission (Oak et al., 2000; Van Tol et al., 1991). It is interesting to note that both norepinephrine and epinephrine bind to DRD4 with high affinity, although not as efficiently as dopamine, suggesting the involvement of DRD4 in a wider range of signal transduction pathways than originally thought (Lanau et al., 1997).

***Table I.6**: Published population and family-based genetic association studies in OCD: dopaminergic candidate genes*

| Gene | Variant(s) | Population | Study design | Phenotype investigated | Sample number: affected: case | Sample number: affected: families | Sample number: control | Results (p-value and allele implicated) | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***DRD4*** | | | | | | | | | |
| | **13bp deletion** | | CC | OCD | 157 | | 162 | NS | 1 |
| | **48bp VNTR** | Mexican | CC | OCD+tics vs. OCD-tics | 61[n (OCD+tics) =12] | | | p=0.018 | 2 |
| | | N. American Caucasian | CC | OCD | 118 | | 118 | p=0.021; NS for genotype (overall allele frequency) | 3 |
| | | Jewish (Ashkenazi [A]and non-A) | CC | OCD | 75 (39A) | | 172 (112A) | non-Ashkenazi: *A7*[a] less frequent in OCD (p=0.04) | 4 |
| | | French | CC/FB | OCD | 49 | 34 | 63 | CC: $p<1x10^{-4}$ (genotype); $p=1x10^{-4}$ (allele); FB: p=0.03 for genotype and allele analyses Both implicated *A2*[b] as *protective* allele | 5 |
| | | | | OCD+tics vs. OCD-tics | n(OCD+tics) =16 | | | NS | |
| | | Afrikaner | CC | OCD | 71 | | 129 | NS | 6 |
| | | SA Caucasians, stratified into Afrikaner (Afr) | CC | EO** OCD vs. LO OCD | n(EO)=95[45 Afr]; n(LO) = 85 [35Afr] | | | SA Caucasians: p=0.0128 (overall allele frequency); NS for Afrikaners | 7 |
| ***DRD2*** | | | | | | | | | |
| | ***Screened exons 4, 5, 6*** | | CC | OCD | 45 | | 26 | NS | 8 |
| | ***Taq*1A** | Mexican | CC | OCD | 67 | | 54 | NS | 9 |
| | | | | OCD+tics (n=12) vs. control | | | | p=0.014 (*CC*-genotype implicated as risk factor) | |
| | ***ser311cys*** | N.American Caucasian | CC | OCD | 110 | | 110 | NS | 3 |
| ***DRD3*** | | | | | | | | | |
| | ***ser9gly*** | Italian | CC | OCD | 97 | | 97 | NS | 10 |
| | | Mexican | CC | OCD | 67 | | 54 | NS | 9 |
| | | N.American Caucasian | CC | OCD | 103 | | 103 | NS | 3 |
| *DAT*[k] | | | | | | | | | |
| | **3' UTR 40bp VNTR** | N.American Caucasian | CC | OCD | 103 | | 103 | NS | 3 |
| | | Jewish (Ashkenazi [A]and non-A) | CC | OCD | 75 (39A) | | 172(112A) | NS | 4 |
| | | Afrikaners | CC | OCD | 71 | | 129 | NS | 6 |
| | | SA Caucasians, stratified into Afrikaner | CC | EO[c]OCD vs. LO OCD | n(EO)=95[45 Afr]; n(LO) = 85 [35Afr] | | | NS | 7 |
| | **rs100532** | German | FB | OCD | | 64 | | NS | 11 |
| ***MAO-A*** | | | | | | | | | |
| | **Exon 8 *T-G*** | N. American | FB | OCD | | 110 | | Males: p=0.0186 (TDT ) , p=0.0129 (HHRR) for *G*-allele as risk factor; OR=2.9 (1.23-6.81) | 12 |
| | | | | OCD+MDD (males only) | | 25 | | p=0.0004 *(G*-allele as risk factor) | |
| | **Exon 14 *T1046C*** | Mexican | CC / FB | OCD | 122 | 51 (19 females) | 124 | CC: p=0.0053 (*C*-allele in males); FB: p=0.022 (*T*-allele as risk factor in OCD females compared to OCD males) | 13 |
| | | Afrikaner | CC | OCD | 71 | | 129 | NS | 6 |
| ***COMT*** | | | | | | | | | |
| | ***val158met*** | N.American Caucasian | CC | OCD | 73 | | 148 | p=2x10-4 (*LL*[d]genotype and *L*[d]-allele as risk factor in males);OR=8.40 (2.44-28.91) | ***14*** |
| | | Japanese | | OCD | 17 | | 35 | NS | 15 |
| | | N.American Caucasian | FB | OCD | | 110 (54 male) | | Males ( for *L*[d]-allele as risk factor): p=0.0079 (one-tailed TDT); p=0.0146 (one-tailed HHRR); OR=2.58 (0.92-5.87) | 12 |
| | | Not specified | FB | OCD | | 67 | | Homozygosity for *H*[e] or *L*[d] allele as risk factors p=0.006[f] | 16 |
| | | Afrikaner | CC | OCD | 54 | | 54 | p=0.0017 (*HL*[e] genotype as risk factor) | 17 |
| | | Probands collected from Israel, France and USA | FB | | | 56 | | p=0.048 (*L*-allele as risk factor in females) NS in males | 18 |
| | | Turkish | CC | OCD | 59 | | 114 | NS | 19 |
| | | META-ANALYSIS[g] | | OCD | 144 | | 337 | NS | 20 |
| | | Brazilian | CC | OCD | 79 | | 202 | NS | 21 |
| | ***C/T* in ERE** | Afrikaner | CC | OCD | 48 | | 48 | NS | 22 |

[a]*A7* refers to the *DRD4* 48bp VNTR 7-repeat allele; [b]*A2* refers to the *DRD4* 48bp VNTR 2-repeat allele [c] EO OCD ≤ 15 years; [d]*L* refers to the *COMT val158met* low-activity allele (*met158*, or *A*); [e]H refers to the *COMT val158met* high activity allele (*val158*, or *G*); [f]non-informative matings excluded; [g]Meta-analysis included investigations by references 15; 14; 16, 12, 18; **Abbreviations: OCD:** obsessive-compulsive disorder; **CC**: case-control; **FB**: family-based **OR**: odds ratio; **CI:** confidence interval;; **EO:** early-onset OCD; **LO**: late-onset OCD; **NS**: non-significant (p>0.05); **VNTR**: variable number of tandem repeats polymorphism; **SA**: South African; **Afr**: Afrikaner; **TDT**: Transmission disequilibrium test; **HHRR**: haplotype-based haplotype relative risk analysis; ***DRD4***: dopamine receptor 4; ); ***DRD2***: dopamine receptor 2; ***DRD3***: dopamine receptor 3; ***DAT***: dopamine transporter; ***MAO-A*:** monoamine oxidase A; ***COMT***: catechol-O-methyltransferase; **SA**: South African.; **ERE**: estrogen response element.

**References**: **1**: Di Bella et al. (1996); **2**: Cruz et al. (1997); **3:** Billet et al. (1998); **4**: Frisch et al. (2000); **5**: Millet et al. (2002); **6**: Hemmings et al. (2003); **7:** Hemmings et al. (2004); **8**: Novelli et al. (1994); **9**: Nicolini et al. (1996); **10**: Catalano et al. (1994); **11**: Walitza et al. (2004); **12**: Karayiourgou et al. (1999); **13**: Camarena et al. (2002); **14**: Karayiourgou et al. (1997); **15**: Ohara et al. (1998); **16**: Schindler et al. (2000); **17**: Niehaus et al. (2001); **18**: Alsobrook et al. (2002); **19**: Erdal et al. (2003); **20**: Azzam and Matthews (2003); **21**: Meira-Lima et al. (2004); **22**: Kinnear et al. (2001).

The DRD4 protein is expressed in a number of brain regions, with the highest expression noted in the PFC (Ariano et al., 1997). Although expression has also been established in the amygdala, hippocampus and hypothalamus (Cohen et al., 1992; O'Malley et al., 1992; Meador-Woodruff et al., 1994; 1996; Lidow et al., 1998), recent investigations suggest that the participation of DRD4 in the PFC is more critical than in other areas (Falzone et al., 2002). Selective lesions and pharmacological challenge studies have indicated that modified dopaminergic neurotransmission in the PFC impairs alertness, behavioural reactions to stress and waking memory performance (Espejo, 1997; Simon et al., 1980). The receptors are thought to act as inhibitors of neuronal firing, particularly in the PFC, given that DRD4 knockout mice exhibit supersensitivity to cocaine, methamphetamine and ethanol, and also exhibit a reduction in exploration of novel stimuli (Oak et al., 2000; Rubinstein et al., 1997; 2001).

It has been found that, in rats, and thus possibly humans, DRD4 is located on the terminals of corticostriatal glutamatergic projections, thus implicating the receptor in the control of glutamate release into the basal ganglia (Tarazi and Baldessarini, 1999; Tarazi et al., 1997; 1998). If this can indeed be extrapolated to humans, it may have important consequences for the support of the involvement of dopamine and glutamate in the development of OCD, as the basal ganglia is one of the main areas of pathology for this disorder (**section I.6.1**). It could be hypothesised that the DRD4 and certain glutamatergic genes are interacting with each other epistatically and in doing so, contribute to the pathology of OCD.

Both atypical and typical antipsychotics exhibit relatively high affinities for DRD4, indicating that the receptor may contribute to their antipsychotic effects, and subsequently the pathologies of disorders treated by such drugs (Tarazi et al., 1997[a]; 1998; Florijn et al., 1997; Tarazi et al., 1997[b]; Murray et al., 1995; Tarazi and Baldessarini, 1999). Indeed, the behavioural effects of a number of DRD4-selective dopaminergic agonists and antagonists were found to be indicative of antipsychotic activity – these effects included inhibition of conditioned avoidance responses and the reversal of deficits in prepulse inhibition (PPI) of acoustic startle responses (see Tarazi and Baldessarini, 1999 for review). These results are interesting, as deficiencies in PPI in OCD patients have been reported (Schall et al., 1996; Swerdlow et al., 1997).

The gene encoding DRD4 (*DRD4*) consists of four exons, which code for 419 amino acids (Van Tol et al., 1991), with the transcription site situated approximately 400bp to 500bp upstream from the translational initiation codon (Oak et al., 2000). The gene has been mapped to chromosome 11p15.5 (Gelernter et al., 1992) and lies adjacent to the Harvey-Ras oncogene and tyrosine hydroxylase gene (Gelernter et al., 1992; Kennedy et al., 1991; Petronis et al., 1993).

A variable number of tandem repeats (VNTR) polymorphism, occurring within the third cytoplasmic loop (situated in the third exon), has been of great interest in psychiatric genetic investigations (van Tol et al., 1991; Lichter et al., 1993; Li et al., 1997; Kotler et al., 1997; La Hoste et al., 1996; Swanson et al., 1998; Eisenberg et al., 2000; Rowe et al., 1998; Smalley et al., 1998; Faraone et al., 1999; Ebstein et al., 1997; Benjamin et al., 1996). This polymorphism consists of a variable number of imperfect 48bp motifs that may be repeated between 2 and 10 times (van Tol et al., 1991; Lichter et al., 1993; Asghari et al., 1994). To date, twenty different receptor protein variants have been found for this polymorphism (van Tol et al., 1991; Lichter et al., 1993; Asghari et al., 1994).

The 4-repeat allele (*A4*) has been reported to be the most common allele, occurring with a global frequency of approximately 64%, whilst the 2- and 7-repeat alleles (*A2* and *A7*, respectively) are the next most common alleles (Ding et al., 2002; Lichter et al., 1993; Chang et al., 1996). *A7* exhibits a high degree of variability worldwide with frequencies ranging from 17% to 39% (Adamson et al., 1995; Chang et al., 1996; Ding et al., 2002). These three alleles (*A2*, *A4* and *A7*) account for more than 90% of the observed allelic diversity in most populations.

Presently, no major functional consequences of the polymorphism have been elucidated, although small effects on signaling efficiency have been reported. Studies have indicated that *A7* possesses the ability to blunt the receptor's response for the reduction of cyclic adenosine monophosphate (cAMP), requiring at least three-fold more dopamine to induce responses similar in magnitude to receptors containing *A4* alleles (Asghari et al., 1995; Wang et al., 2004). Moreover, *A2* alleles have been found to produce DRD4 receptors that have a cAMP response that is somewhat blunted, and lies midway between responses for *A4* and *A7* alleles (Wang et al., 2004).

The results of functional studies thus indicate the lack of a linear relationship between the length of the polymorphism (i.e. number of repeats) and the functional receptor pharmacology. Therefore, genetic studies employing strategies that pool short and long alleles may result in Type I or II errors in these association studies (Wang et al., 2004; Oak et al., 2000). Subsequently, it has been suggested that characterising individuals according to the presence of *A4* allele, *versus* absence thereof, may be more conducive for testing associations between the gene and certain disorders (Grady et al., 2003; Wang et al., 2004).

*DRD4* is of interest in molecular studies of psychiatric disorders, due to its involvement in higher brain functions and the modulatory role it plays in dopamine synthesis and turnover in the brain (Rubinstein et al., 1997*)*. Indeed, it has been widely investigated to determine the role it may play in the development of OCD (Table I.6). One of the earliest published studies reported on the significantly higher frequency of *A7* amongst Mexican OCD individuals who presented with co-morbid tics, compared to OCD patients who did not possess co-morbid tics (Cruz et al., 1997). Moreover, Billet et al. (1998) observed statistically significant differences in overall allele distribution between Canadian OCD and control subjects, although this association did not remain significant after correcting for multiple testing.

On the other hand, Frisch et al. (2000) observed a reduced number of *A7* alleles amongst OCD patients compared with healthy controls, although the authors do concede that this significant finding may be as a result of a Type I error due to multiple testing. This result was not replicated in a larger French study, which utilised both family- and population-based designs (Millet et al., 2003). This same investigation did, however, report a significantly lower frequency of *A2* amongst OCD patients compared to a control cohort, replicating this result in a subsequent family study. Their results remain significant even after correction for multiple testing. However, these results were not replicated in a study published by the author (Hemmings et al., 2003), where no statistically significant differences in either allele or genotype distribution were observed between Afrikaner OCD and control individuals. Interestingly, however, in a more recent study, when a South African Caucasian OCD patient group was stratified categorically according to age at onset of the disorder, a statistically significant difference was noted in the allelic distribution between early and late-onset OCD, although no significant differences were noted in the genotypic distribution (Hemmings et al., 2004).

The conflicting results in the aforementioned studies may be due to inconsistency in grouping individuals according to genotype. None of the studies characterised subjects according to the presence or absence of *A4*, as suggested by Wang et al. (2004). This could result in false positive or false negative results. Discrepancies between the results may also be due to variations within the sequence of repeats, and the order in which they appear in different populations (Lichter et al., 1993). A certain haplotype may be responsible for an association, but this haplotype may in turn be associated with a different repeat length allele in different populations. Finally, the variant which has been found to be associated with OCD may in fact not be causal, but may simply be in LD with the causal variant situated in *DRD4* or an adjacent gene. Thus, in an effort to determine the molecular basis of reported positive associations in the present investigation, three DRD4 variants were examined for the role they may play in increasing susceptibility to OCD or related subtypes.

In the present study, the *DRD4* 48bp VNTR investigation, reported on by the author in 2003 and 2004 (Hemmings et al., 2003, 2004), was extended to include more OCD and control subjects. Moreover, a *C* to *T* transition, occurring in the region 5' to the transcription start site at position -521 (*-521C/T* dbSNP rs1800955) (Okuyama et al., 1999) was also investigated (Figure I.5). This polymorphism is thought to affect transcriptional activity of *DRD4*, since the activity of the *T*-allele was reported to be 40% lower than that of the *C*-allele (Okuyama et al., 1999). To the author's knowledge, the latter polymorphism has not yet been investigated for the role that it may play in increasing susceptibility to OCD.

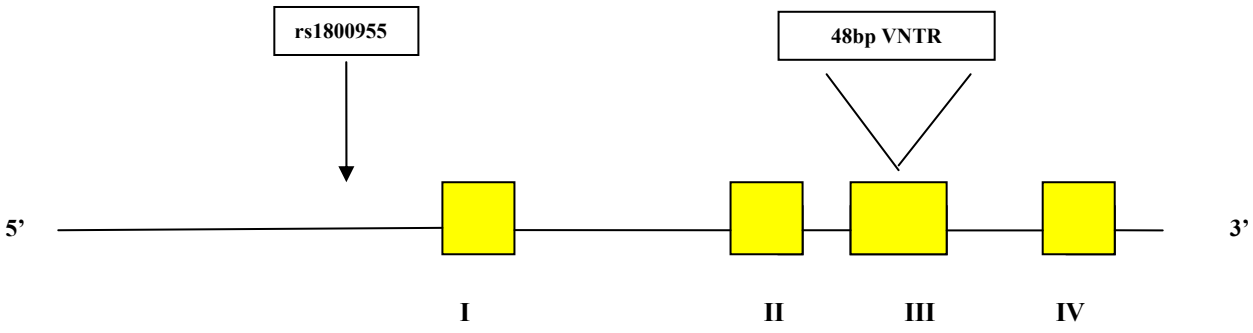



***Figure I.5. Schematic representation of the DRD4 locus.*** *The location of the polymorphisms investigated in the present study indicated by arrows. Exons are represented by yellow blocks, and introns are represented as horizontal lines between the exons (not drawn to scale). The distance between the two variants is approximately 3kb.*

**ii. The Dopamine Receptor 1 gene *(DRD1)***

Dopamine receptor 1 (DRD1) represents the most abundant dopaminergic receptor in the CNS. It serves to modulate DRD2 activity, regulate neuron growth and differentiation, and mediate certain behavioural responses (Clark and White, 1987; Grandy et al., 1990). Furthermore, the receptor is expressed at high levels in the dorsolateral PFC, and therefore plays an important role in modulating mesocorticolimbic circuitry, and consequently, cognitive functioning (Basile et al., 2002). DRD1 in the PFC and associated regions has been found to be down-regulated during chronic treatment with neuroleptics, namely, clozapine, haloperidol and remoxipride (Lidow and Goldman Rakic, 1994) which have been found to be useful as adjuncts to first line SSRI pharmacotherapy in some treatment refractory OCD patients (McDougle et al., 1994; 1997).

The DRD1 gene (*DRD1*) has been mapped to chromosome 5q35.1 (Grandy et al., 1990), and codes for approximately 44 amino acids (Sunahara et al., 1990). An *A* to *G* transition polymorphism, which is situated in the 5'untranslated region (UTR) (Cichon et al., 1994; Liu et al., 1995) and that is of unknown functional significance, was investigated in the present study. *DRD1* has been widely investigated for the role it may play in dopamine-related disorders, such as schizophrenia (Liu et al., 1995; Cichon et al., 1996; Kojima et al., 1999); TS and addictive behaviours (Comings et al., 1997); alcoholism (Heinz et al., 1996); essential hypertension (Sato et al., 2000) and aggression and psychosis in Alzheimer's disease (Holmes et al., 2001; Sweet et al., 1998). Brain metabolic and clinical response to clozapine have also been thought to be related to variations in the DRD1 gene (Potkin et al., 2003).

Recent animal studies have supported a role for DRD1 in OCD and associated behaviours (**section I.6.1.2**) (Joel and Avisar, 2001; Joel and Doljansky, 2003). Interestingly, CNS *DRD1* activation has been found to induce modulated grooming behaviour in rodents (Molloy and Waddington, 1987; Wachtel et al., 1992). This grooming behaviour is enhanced upon administration of DRD1 agonist, SKF38393, and by the removal of 5-$HT_{2A}$ receptors (Lucki and Kucharik, 1988). Similarly, the behaviour is attenuated following chronic infusion with *5-$HT_{2A}$* antisense oligonucleotide (that upregulates 5-$HT_{2A}$), implicating 5-$HT_{2A}$ in the modulation of DRD1-mediated grooming behaviour (Scalzitti et al., 1999). Given these findings, it is possible that firstly, if DRD1 mediates certain aspects of grooming behaviors, then the gene encoding DRD1 that functions "below par" could result in abnormalities in grooming behaviours (e.g. TTM). Secondly, it is plausible that the interaction between the

two receptors on a neuronal level implies an interaction on the genetic level; warranting an investigation of epistatic effects between the *DRD1* and *5-HT$_{2A}$* alleles and the role that this may play in the development of OCD and/or TTM.

Despite evidence from animal and pharmacological models implicating DRD1 in at least some aspects of OCD, relatively little genetic evidence exists to support these findings. In fact, to date, only one case-control association study investigating the role that *DRD1* may play in OCD has been published. In this study, Thompson et al. (1998) screened the coding region of the gene to determine whether any association existed between *DRD1* variants and TS patients, and TS patients presenting with co-morbid OCD. No association was detected, although it should be noted that the study was very small – only 30 cases and 50 controls were included. It is thus possible that the results obtained by Thompson et al. (1998) represent false negative results due to lack of power. Furthermore, they did not investigate OCD as a single phenotype, but rather in conjunction with the occurrence of TS, which may have "diluted" the effect that DRD1 has in OCD. Further investigation into the role that *DRD1* may play in OCD and subtypes of OCD is thus warranted; this was addressed in the present study.

**iii. The Dopamine Receptor 2 gene *(DRD2)***

Dopamine receptor 2 (DRD2) is expressed throughout the brain, but is found at high levels in the basal ganglia, especially in the neostriatum and pars compacta of the substantia nigra (Missale et al., 1998). Interestingly, DRD2-like binding potentials in brain regions outside the striatum have indicated gender differences, which may reflect either a difference in DRD2 density and/or affinity (Kaasinen et al., 2002). Indeed, a generally lower DRD2 binding affinity was noted in females (Pohjalainen et al., 1998), but these differences have been observed only in older age groups, reflecting the possible influence that sex steroid changes can have on the dopamine-binding characteristics in the brain (Farde et al., 1995; Pohjalainen et al., 1998).

*DRD2* represents an interesting gene to study with regard to the role that it may play in mediating the development of OCD, given recent evidence obtained from structural, functional, clinical and genetic studies. The CSTC circuit is modulated by DRD1 and DRD2 that are innervated by the substantia nigra (Harvey et al., 2001; Insel et al., 1992), thus any imbalance in efficacy of these receptors could result in an imbalance of the circuit, causing deficits that may well lead to the development of OCD.

As already discussed in **section I.6.1.2**, pharmacological studies have indicated that the DRD2/DRD3 antagonist, quinpirole, may constitute an animal model of OCD checking (Szechtman et al., 1998; 2001). Furthermore, chronic administration of inositol to rats has been found to result in a significant decrease in DRD2 density (Harvey et al., 2001), which is interesting in light of observations that inositol has been found to be effective in the treatment of OCD (Fux et al., 1996) (**section 1.6.1.5.2**). These findings suggesting that a state of hyperresponsiveness of DRD2 may contribute to the pathology of the disorder (Harvey et al., 2001; Brambilla et al., 1997). Additional support for the role that DRD2 may play in OCD has been obtained from a recent functional study in which lower left caudate DRD2 binding ratios were observed in OCD patients compared to controls. The binding ratios in the left caudate were also found to be lower than those in the right caudate in the same OCD patients (Denys et al., 2004[c]).

The gene encoding DRD2 (*DRD2*) has been mapped to chromosome 11q22-23 (Grandy et al., 1989; Eubanks et al., 1992). The gene consists of 8 exons, with an intron of over 250 kb separating the putative first exon from those encoding the receptor protein (Grandy et al., 1989). The *DRD2 Taq*1A polymorphism is situated in the 3' UTR, approximately 9.5kb downstream from the gene (Grandy et al., 1989) and results in a *T* to *C* transition representing the *A1* (*T*) and *A2* (*C*) alleles, respectively. Although this polymorphism does not represent a fuctional variant *per se*, it is thought to be in LD with a variant or gene that affects DRD2 density within the brain, given that DRD2 density has been found to be 30%-40% lower in the caudate nucleus and striatum in *A1*-carriers (Thompson et al., 1997; Pohjalainen et al., 1998). It has also been proposed that the *A1*-allele may be in LD with a gene or variant that modifies the expression of DAT, since alcoholic carriers of the *A1*-DRD2 allele exhibited a higher density of DAT compared with those homozygous for *A2* (Laine et al., 2001). Finally, OCD patients with compulsive hoarding syndrome exhibited lower glucose metabolism compared to nonhoarding patients (Saxena et al., 2004), suggesting that the gene, and particularly the *A1* allele, may play a role in the aetiology of this OCD symptom dimension.

Interestingly, the *Taq1A* polymorphism has recently been mapped to the last ankyrin repeat of the newly discovered ankyrin repeat and kinase domain containing 1 (*ANKK1*) gene, where it represents a potentially functional nonsynonymous SNP (Neville et al., 2004). However, the interaction between the *ANKK1* and *DRD2* genes has yet to be elucidated, and further work is required to determine whether *ANKK1* is expressed in the brain (Neville et al., 2004).

The perceived functionality of the *Taq1A* polymorphism may be due to a functional, synonymous polymorphism, *C957T*, that has been found to be in LD with both of the aforementioned variants (Duan et al., 2003). The *957T* variant has been shown to be associated with a decrease in *DRD2* mRNA translation, stability and diminished dopamine-induced upregulation of *DRD2*, which may explain the association of the synonymous, non-functional variants with various neuropsychiatric disorders (Duan et al., 2003; Hirvonen et al., 2004).

Genetic association studies investigating the involvement of the *DRD2* in OCD have yielded inconsistent results (Table I.6). In one of the first investigations, Novelli et al. (1994) screened the 8 exons of the gene for mutations in a group of OCD patients with and without tics. No structural changes were observed, prompting the suggestion that the gene was not involved in the aetiology of the disorder. On the other hand, a later case-control study conducted in a Mexican sample demonstrated an association between the homozygous *A2* genotype of the *Taq*IA polymorphism and OCD patients with comorbid tic disorder (Nicolini et al., 1998): 58% of those patients presenting with co-morbid tic disorder possessed the *A2/A2* genotype, whilst only 27% of the OCD patients without tic disorder carried the genotype (Table I.6). However, it should be noted that the investigators did not observe any significant association of the polymorphism when the genotype and allele distribution of the polymorphism was compared between the unstratified sample of OCD patients and control subjects. In a population-based case-control study by Billet et al. (1998), observed no statistical significance was observed between either the *DRD2 TaqI*A polymorphism, or a serine to cysteine missense mutation ocuring in the seventh exon, and OCD. The conflicting results may be due to the fact that Nicolini et al. (1998) were investigating the genetic aetiology of a subtype of OCD (tics), whereas Billet et al. (1998) did not stratify their OCD patients according to the presence or absence of tics.

It may thus be possible that *DRD2* plays an important role in the development of this OCD subtype (i.e. OCD + tics), but that this effect may be masked when the disorder is examined as a single entity. Secondly, the conflicting results may be due to the small sample sizes used in both the studies, but particularly by Nicolini et al. (1998), where the number of OCD patients with tics amounted to only 12, versus 54 without tics. As mentioned in **section I.4.4.2**, small sample sizes may lead to lack of power of the study and subsequent false positive results.

In order to clarify the role that the receptor may play in mediating the development of OCD, the relationship between OCD and haplotypes within *DRD2* needs to be investigated in an independent population stratifying patients for OCD symptoms and co-morbidity, particularly tics. The present study thus investigates the role that the *DRD2 Taq*IA (rs1800497) polymorphism may play in mediating the development of OCD (or clinically-defined subsets thereof).

**iv. The Dopamine receptor 3 gene *(DRD3)***

Dopamine receptor 3 (DRD3) is a D2-like receptor that is expressed almost exclusively in the limbic structure of the brain (Sokoloff et al., 1990; Suzuki et al., 1998), with the highest concentrations noted in the ventral striatum, islands of Cajella and nucleus accumbens (Landwehrmeyer et al., 1993; Sokoloff et al., 1990). The receptor is thus thought to play a role controlling motor behaviour, and regulating certain facets of motivation and emotion (Landwehrmeyer et al., 1993; Sokoloff et al., 1990). Indeed, DRD3 knockout mice display increased motor activity and exploratory behaviour, which may indicate a reduction in anxiety (Accili et al., 1996). Furthermore, the administration of DRD3 antagonist, nafatopride, also results in increased spontaneous locomotor activity in rats (Sautel et al., 1995), whilst DRD3 agonists have been found to decrease locomotor activity in rats (Svensson et al., 1994).

The regulatory mechanism controlling DRD3 differs from that of other dopaminergic receptors, in that brain-derived neurotrophic factor (BDNF), not dopamine, controls and maintains DRD3 expression during development and adulthood (Guillin et al., 2003). Indeed, the expression of both BDNF and DRD3 have been found to be down-regulated during periods of stress and depression, and these effects are reversed after the administration of SSRIs that targets the mesolimbic dopaminergic system (Lammers et al., 2000; Nibuya et al., 1995). It is thought that the effect of the antidepressant on DRD3 is mediated by its primary action on BDNF, implicating a putative interaction between BDNF and DRD3 in mesolimbic dopaminergic pathways.

Pharmacological evidence supports the role of DRD3 in anxiety: dopaminergic antagonists acting at the receptor have been found to exhibit anxiolytic properties, and the receptor has been found to possess similar affinities for typical neuroleptics as DRD2 (Sokoloff et al., 1990; 1992), although it has also been found to exhibit a high affinity for atypical neuroleptics (Guo et al., 1995). Moreover, it has been suggested that DRD3 expression and function are

down-regulated during stress and depression, and that chronic treatment with noradrenergic and SRIs result in the increase of the DRD3 mRNA, thereby reversing the initial effect of stress (Lammers et al., 2000).

The DRD3 gene consists of seven exons and has been mapped to chromosome 13p13.3 (Le Coniat et al., 1991; Sokoloff et al., 1992). The most widely studied SNP is characterised by an *A* to *G* transition occurring in the N-terminal extracellular domain in the first exon. The transition is non-synonymous, representing a *gly* to *ser* amino acid substitution at codon position 9 (*ser9gly*) (Lannfelt et al., 1992). Although the polymorphism results in a modified protein sequence, the functional consequences have yet to be elucidated. However, the variant is thought to affect the pharmacological properties of DRD3, since the presence of the *gly9* allele in homozygous form displayed a higher affinity for dopamine than either the *ser9*-homozygotes or heterozygotes did. Moreover, the *gly9gly*-homozygotes and *ser9gly*-heterozygotes exhibited a significantly higher affinity for the selective DRD3 ligand, GR99841 (Lundstrom and Turpin, 1996).

It is of interest to note that it has been demonstrated that DRD2 and DRD3 form functional heterodimers, capable of inhibiting the downstream DRD2 effector, adenylyl cyclase, more efficiently than DRD2 alone (Scarselli et al., 2001). In light of the unresponsiveness of *ser9*-positive schizophrenic patients to antipsychotic medication, and a more pronounced prefrontal executive dysfunction in *ser9*-positive individuals (Rybakowski et al., 2001), it has been hypothesised that the *ser9*-variant may exhibit a reduced capacity to form DRD2-DRD3 heterodimers, resulting in less efficient signal transduction, although this necessitates further investigation.

The earliest study conducted in order to determine whether any association exists between the *ser9gly* variant and OCD, OCD with or without tics, or a family history of OCD and tics was by Catalano et al. (1994) (Table I.6). No statistically significant differences were observed in genotype or allele frequencies between the OCD and control subjects, or between the various OCD sample subsets. It should be noted (as the authors do) that this investigation should be viewed as preliminary, since the power of the study to detect a small effect was comparatively low (0.30), probably due to the small sample size. However, neither Billet et al. (1998) nor Nicolini et al. (1996) observed any association between the *ser9gly* variant and OCD However, a more recent study implicated the *gly9gly* genotype in the development of

obsessive-compulsive personality traits and disorders (Joyce et al., 2003). It could thus be hypothesised that, although the receptor may not play a role in the higher order construct of OCD, it may well be involved in certain aspects of the symptomatology of the disorder, warranting the investigation in the present study.

**v. The Dopamine Transporter gene (*DAT*)**

DAT (or SLC6A3) is a transmembranous protein that is a member of a highly conserved group of $Na^+/Cl^-$ dependent transporters (Kilty et al., 1991; Shimada et al., 1991). DAT plays a pivotal role in the removal of dopamine from the synapse in the midbrain; the reuptake and diffusion of dopamine by DAT alters the magnitude, duration and spatial domain of transmitter induced receptor activation, thereby modifying dopaminergic neurotransmission (Giros et al., 1996; Frazer et al., 1999).

DAT is found in high concentrations in the nucleus accumbens, striatum and olfactory tubercle (Frazer et al., 1999; Ciliax et al., 1995) and acts exclusively on the plasma membrane of the presynaptic dopaminergic neurons (Inada et al., 1996; Kouzmenko et al., 1997; Sano et al., 1993; Kelsoe et al., 1996; Souery et al., 1996). Cocaine has been found to bind to DAT in the CNS, inhibiting dopaminergic reuptake accordingly (Gawin and Ellinwood, 1988). Cocaine exacerbates obsessive-compulsive behaviour in OCD patients, and has been found to induce OCD-like behaviour in individuals with a family history of the disorder (Satel and McDougle, 1991). Thus, DAT may constitute an important component in the aetiology of OCD. Indeed, as already discussed (**section I.6.1.2)**, DAT-knockdown mice have been shown to exhibit excessive sequential stereotypy of behavioural patterns, characteristic of disorders of the basal ganglia, like OCD and TS (Berridge et al., 2005).

The DAT gene (*DAT*) is expressed only in the CNS, and within a small subset of neurons (Ciliax et al., 1995). The expression of *DAT* is therefore more restricted than the expression of genes encoding other dopaminergic biosynthetic enzymes, and the control of dopamine signaling by DAT has been found to be largely region-specific, depending on its density, activity and regulation (Madras et al., 2005). *DAT* represents a single gene product that has been mapped to chromosome 5p15.3 (Giros et al., 1992; Vandenbergh et al., 1992). The protein encoded by *DAT* contains 620 amino acids with 12 putative transmembrane domains (Kilty et al., 1991). A polymorphic VNTR has been found in the genomic sequence encoding the 3' untranslated region (3'UTR) (Sano et al., 1993; Vandenbergh et al., 1992, 2000).

The number of repeats in the 3'UTR VNTR may vary from 3 to 11, although it has been found that more than 90% of Caucasian and African-Americans display either the 9 repeat (*A9*) or the 10 repeat (*A10*) alleles (i.e., 440bp and 480bp alleles respectively) (Vandenbergh et al., 1992; Persico et al., 1995; Doucette-Stamm et al., 1995). In fact, in most population surveys on *DAT* allele frequencies at the 3'UTR VNTR, it has been found that *A10* occurs more frequently, with some regional variation (Doucette-Stamm et al., 1995; Nakatome et al., 1995, 1996; Kang et al., 1999).

The function of the polymorphism is presently unclear: investigations have yielded contradictory results. Heinz et al. (2000) found that the *A10/A10*-genotype resulted in a higher density of DAT, whilst Jacobsen et al. (2000) found that the *A10/A10* genotype yielded a lower DAT density than the A9/A10 repeat. On the other hand, a study by Martinez et al. (2001) suggested that the polymorphism did not play a role in affecting the density of DAT.

The association between the *DAT* 40bp VNTR and increased susceptibility to OCD has previously been investigated, in three separate studies, all of which yielded negative results (Billet et al., 1998; Frisch et al., 2000; Hemmings et al., 2003). Although the polymorphism has previously been investigated in the Afrikaner population for the role it may play in mediating the development of EO OCD (Hemmings et al., 2004), with no significant association, the age at onset grouping was categorical, resulting in small sample sizes for each. The present investigation involves the investigating the possible association of *DAT* with selected OCD subtypes, including age at onset, which is investigated as a quantitative variable, which may impart more power to the study. To the author's knowledge this is the first time that the *DAT* 40bp VNTR has been investigated for the role that it plays in the development of OCD symptom subtypes examined in the present dissertation.

**vi. The Catechol-O-methyltransferase gene (*COMT*)**

COMT (EC 2.1.1.6) is an $Mg^{2+}$-dependent enzyme that is involved in the inactivation of catecholamines (norepinephrine, epinephrine and dopamine) (Axelrod and Tomchick, 1958). The gene encoding COMT (*COMT*), situated on chromosome 22q11.1-11.2 (Grossman et al., 1992; Winqvist et al., 1992), encodes two distinct forms of the enzyme – soluble COMT (s-COMT) that predominates in most tissues, and membrane-bound COMT (MB-COMT), most abundant in the CNS (Rivett et al., 1983), where it is responsible for approximately 60% of the dopaminergic degradation (Lundstrom et al., 1995; Bertocci et al., 1991). S-COMT is 50

amino acids shorter than MB-COMT, and its transcription begins near exon 3 (Tenhunen et al., 1994). The transcription of each form of the enzyme is driven by different promoters that are both located in exon 3: the P1 promoter drives s-COMT transcription, whilst the P2-promoter drives MB-COMT transcription. Interestingly, both P1 and P2 promoters have been found to contain estrogen response elements (ERE) (Xie et al., 1999), and evidence exists to suggest that human *COMT* transcription is downregulated by estrogen, mediated via estrogen receptor α (ESRα) and EREs (Jiang et al., 2003).

A widely studied polymorphism in *COMT* is a biallelic SNP, involving a valine to methionine substitution at codon 158 (*val158met* [rs4680]). This polymorphism has been found to be co-dominantly associated with either the thermolabile (low activity; represented by the *met158* [*A*] allele) or thermostable (high activity; represented by the *val158* [*G*] allele) (Grossman et al., 1992; Karayiorgou et al., 1998; Lachman et al., 1996; Lotta et al., 1995) form of the enzyme. It has recently been shown that the low activity of the enzyme is due to the lower expression of *COMT* (resulting in lower protein levels) in *met158* individuals (Doyle et al., 2004), and that the high activity form of the enzyme is associated with abnormal prefrontal cortical function (Egan et al., 2001; Bilder et al., 2002; Goldberg et al., 2003; Malhotra et al., 2002; Mattay et al., 2003; Gallinat et al., 2003). This may be as a result of the enzyme performing a critical role in maintaining dopaminergic flux in this region (Chen et al., 2004).

*COMT* represents an attractive candidate for OCD genetic studies (Table I.6). Individuals with 22q11 microdeletions encompassing COMT have been found to manifest a number of psychiatric symptoms, including OCD and increased anxiety (Karayiorgou et al., 1995; Pulver et al., 1994; Papolos et al., 1996). Numerous association studies, both family- and population–based, have investigated the role that the *val158met* polymorphism may play in the development of OCD. Initial reports were exciting, in that Karayiorgou et al. (1997) observed a significant association between the *met158* (low activity) allele and *met158/met158* genotype and OCD in a North American male sample, although no association was observed for the female sample in the same study. Karayiorgou et al. (1999) replicated these findings in a family-based sample, providing further impetus for the role of *COMT* in the disorder. Interestingly, in an attempt to replicate the findings by Karayiourgou et al. (1997; 1999), Alsobrook et al (2002) observed an association between the *met158/met158* genotype and the OCD in *females*, but not in males. Furthermore, although Schindler et al (2000) observed an association between homozygosity (either *met158/met158* or *val158/val158* genotypes) at the

*val158met* locus and OCD, their findings were not gender-specific. In contrast, our group, Niehaus et al. (2001), investigated the relationship between the *val158met* polymorphism and OCD in an Afrikaner population, and detected a non-gender-based positive association between the *heterozygous* (*val158/met158*) genotype and susceptibility to OCD.

On the other hand, no association between the polymorphism and OCD was detected in studies by Ohara et al. (1998), Erdal et al. (2003) or Meira-Lima et al. (2004), or in a meta-analysis which included three case-control (Niehaus et al., 2001; Ohara et al., 1998; Karayiorgou et al., 1997) and three family-based (Schindler et al., 2000; Karayiorgou et al., 1999; Alsobrook et al., 2002) studies, and one unpublished study (Veenstra-Vanderweele, see Azzam et al., 2003).

The results from aforementioned studies are, as is the case for many association studies, contradictory. However, *COMT* remains an intriguing gene to investigate in the genetics of OCD, given the large role that it plays in the inactivation of dopamine, which has been proposed to be involved in the aetiopathology of at least some forms of OCD. The present study therefore aims to re-evaluate the role of *COMT* in OCD, by extending the Afrikaner sample genotyped by Niehaus et al. (2001), and by stratifying the OCD sample so there are less phenotypic variables to consider when analysing the potential role of the genetic variables.

Furthermore, the original study has been extended to include haplotype analysis. The *val158met* has, thus far, been the most widely studied for its role in OCD. However, most of the earlier studies testing the activity and stability of *COMT* were conducted using S-COMT, not MB-COMT, which is most relevant to dopaminergic functioning in the CNS, and therefore to OCD (DeMille et al., 2002). Consequently, less common variants that contribute to the aetiology of the disorder may have been bypassed. Keeping this in mind, two additional polymorphisms were genotyped to determine the role they possess in the development of the disorder: a SNP occurring at position -1217 (rs2097603), situated within the estrogen sensitive region of P2, and a single base insertion/deletion polymorphism, situated 3' to the stop codon of exon 6 (rs362204) (Chen et al., 1996; Karayiorgou et al., 1998) (Figure I.6).

These variants, together with the *val158met* variant, create a gene-encompassing haplotype spanning 28kb, and will impart more power to detect genetic associations between *COMT* and OCD than single-SNP studies.

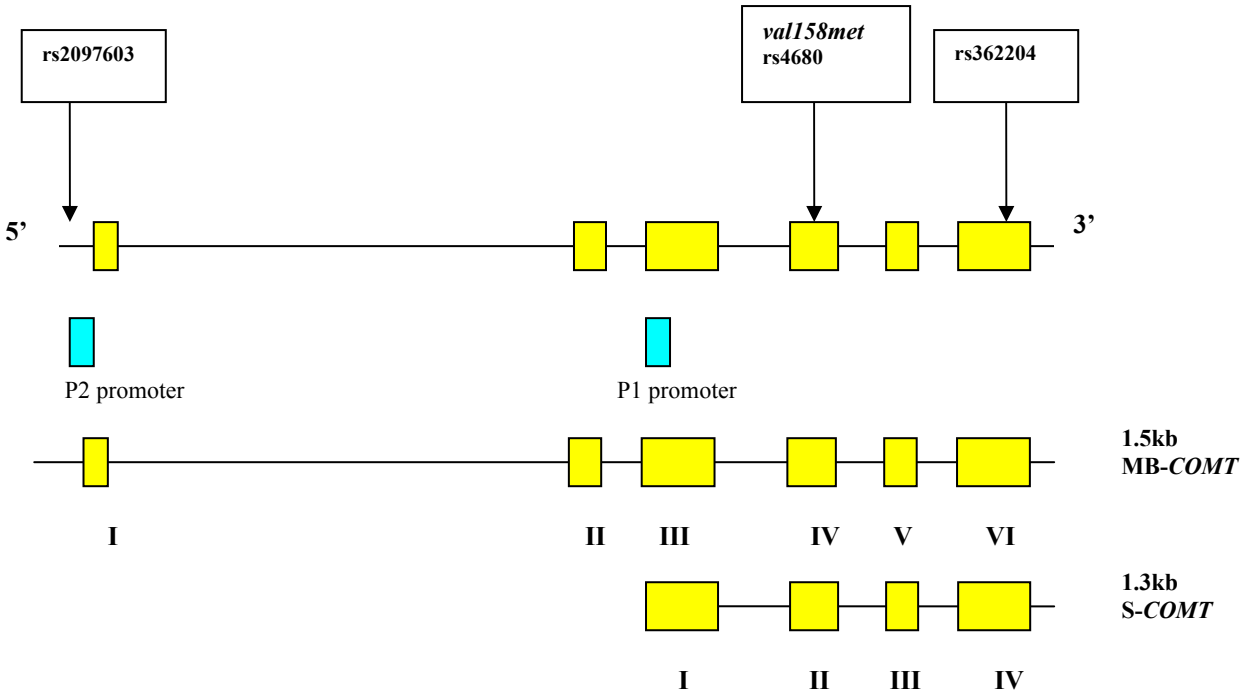



***Figure I.6. Schematic representation of the* COMT *locus.*** *The location of polymorphisms investigated in the present study are indicated by arrows. Exons are represented by yellow blocks, and introns are represented by horizontal lines between exons. The diagram also indicates the positions of the two promoters, P1 and P2 (blue boxes), and the corresponding transcripts (MB-COMT and S-COMT, respectively) (not drawn to scale). The distance between rs2097603 and rs4680 is approximately 23.1kb, whilst that between rs4680 and rs362204 is approximately 4.9kb.*

### I.6.1.3. The Glutamate system and OCD

Glutamatergic pathways, namely the major corticospinal neurons that project from the PFC to the anterior striatum, nucleus accumbens and substantia nigra (Taber and Fibiger, 1993; Kalivas et al., 1989), represent those that may be relevant to the pathophysiology of OCD (**section I.6.1**). Recent animal and pharmacological data have also suggested that glutamate may play a role in mediating certain aspects of the development of OCD. As previously

mentioned in **section I.6.1.2,** Campbell et al. (1999) created an animal model of cortical and limbic neuropotentiation - these transgenic mice express a neuropotentiating gene in the D1 neurons in cortical (known to glutamatergically activate orbitofrontal and corticostriatal glutamate output) and amygdala (known to indirectly induce amygdalar glutamate output) regions (Campbell et al., 1999; McGrath et al., 2000). Chronic potentiation of these neurons resulted in transgenic mice exhibiting OCD and TS-like behaviours – namely, episodes of perseverance and repetition, non-aggressive biting of cagemates, and skin picking during grooming.

The investigators also observed repeated climbing and leaping behaviours, and tics, reminiscent of TS symptomatology. Based on these findings, it has been suggested that tics and primary compulsions or obsessions are induced by the glutamatergic output from the cortical limbic neurons to striatal efferent targets, resulting in the "cortical-limbic-glutamatergic-neuron" (CGN) neuronal circuit model which has recently been proposed by Nordstrom and Burton (2002).

Brain imaging data also provides support for the role of glutamate in OCD. Using proton magnetic resonance spectroscopy ($^{1}$H-MRS) and a direct, non-invasive *in vivo* measurement of N-acetyl-aspartate (NAA), (a reliable marker of neuronal viability and glutamate compounds [Birken and Oldendorf, 1989; Tsai and Coyle, 1995]), a glutamatergically-mediated thalamocortical-striatal dysfunction was observed in OCD patients (Rosenberg et al., 2000; Bolton et al., 2001). These NAA abnormalities may be as a result of increased metabolic activity in these circuits (Rosenberg et al., 2000). Fitzgerald et al. (2000) also noted a decrease in NAA levels in the thalamus that may be associated with the clinical presentation of EO OCD. The decrease in NAA levels indicate either reduced neuronal responsivity to tonic striatal inhibition, or an excess of glutamatergic activity from the ventral prefrontal striatal circuit neurons that project to the thalamus.

It has also been hypothesised that abnormalities in the glutamate-5-HT interactions may underlie the aetiology of OCD (Rosenberg and Keshavan, 1998; Moore et al., 1998; Rosenberg et al., 2000). Indeed, a reversible, glutamatergically-mediated thalamocortical-striatal dysfunction has been proposed to represent a biological marker for EO OCD (Rosenberg and Hanna, 2000; Bolton et al., 2001; Rosenberg et al., 2004). Furthermore, pharmacological investigations suggest that the effects of the SSRI treatment may be the

result of a 5-HT-mediated reduction in glutamate concentration in the caudate, cortex and ventral striatum (Saxena et al., 1998; Rosenberg et al., 2000).

#### I.6.1.3.1. Glutamate receptor subunit 2B (*GRIN2B*)

The action of glutamate is mediated by two general types of receptors – ionotropic (ligand-gated) and metabotropic (G-protein coupled) receptors. The present study investigated the role that glutamate ionotropic receptor subunit 2B (GRIN2B) may play in OCD, therefore this section will focus only on ionotropic receptors, with emphasis on GRIN2B. Ionotropic receptors can be further classified into three groups, according to their affinities for glutamate analogues: N-methyl-D-aspartate (NMDA), kainic acid (KA) or alpha-amino-5-hydroxy-5-methyl-4-isoxalzolepropionic acid (AMPA) (Nakanishi et al., 1998). Ionotropic receptors have a large extracellular N-terminal domain and four hydrophobic membrane segments, and an intracellular C-terminal domain (Petralia and Wenthold, 1992; Tingley et al., 1993). NMDA receptors (NMDAR) usually co-exist with AMPA and KA receptors, and are generally distributed throughout the brain, the highest concentrations being in the limbic and cortical regions, with subsequent roles in cognition, mood and perception (Krystal et al., 1999). NMDAR also mediate numerous other functions, including excitatory neurotransmission, synaptic plasticity and efficacy, memory formation and pain perception (Mayer and Westbrook, 1987; Bliss and Collingridge, 1993).

Functional NMDAR are assembled from two ubiquitous NMDAR subunit type 1 (GRIN1) components, and two of a family of 4 type 2 subunits (GRIN2A-D) that are differentially expressed in the brain (Monyer et al., 1992; Ishii et al., 1993). GRIN1 subunits are capable of forming homomeric channels that are responsive either to L-glutamate or glycine, whereas the GRIN2 subunits can only function in the heterotrimeric state. These subunits determine the pharmacological properties of the receptor, and in doing so, modulate the function of the receptor (Monyer et al., 1992; 1994; Ishii et al., 1993).

A defining characteristic of GRIN2 subunits is their long intracellular C-terminal tails, required for channel functioning (Sprengel et al., 1998). It is suggested that GRIN2B plays an important role in mediating certain aspects of cellular signal transduction, given that, firstly, it is found to be the major tyrosine phosphorylating protein in the postsynaptic density fraction (Moon et al., 1994). Secondly, alpha-actinin-2, an actin-binding protein that assists in regulating receptor localisation and function in the brain, binds to the cytoplasmic tail of

GRIN2B (Wyszynski et al., 1997). Finally, a phosphorylation site has been located in the carboxyl end domain of this receptor subunit (Omkumar et al., 1996).

Regional specification of specific NMDAR is common in the basal ganglia, with GRIN2B abundantly expressed in the striatum (Loftis and Janowsky, 2003; Schito et al., 1997; Standaert et al., 1994), and highly expressed in the frontal cortex (Rudolf et al., 1996), areas which are believed to be involved in OCD pathology (**section I.6.1**). Interestingly, in rats, GRIN2B was found to be ubiquitously expressed at birth, but during the first three postnatal weeks, the subunit became confined to the anterior forebrain structures (Wenthold et al., 2003). Therefore, a dysregulation of a similar expression pattern in the human brain may be consistent with the neurodevelopmental theory of OCD (Rosenberg and Keshavan, 1998).

The gene encoding GRIN2B (*GRIN2B*) is situated on chromosome 12p12 (Mandich et al., 1994) and contains 13 exons, with the coding sequences represented by exons 2 to 13 (Ohtsuki et al., 2001). Studies to identify functional variants within *GRIN2B* have thus far yielded no obvious results (Ohtsuki et al., 2001), suggesting selective pressure to maintain a conserved sequence, stressing the crucial role of the subunit in numerous physiological processes.

Genetic evidence for the role of glutamate in OCD stems mainly from a recent genome scan, based on EO OCD probands, although the investigation of a haplotype containing two SNPs within the the gene encoding the glutamate transporter (*SLC1A1*) revealed no statistically significant linkage with the disorder (Veenstra-VanderWeele et al., 2001). In spite of this, the possibility that the gene plays a role in the development of OCD cannot be ruled out, since regulatory regions of *SLC1A1* remain to be sequenced, and there are many more genes involved in glutamate neurotransmission. Indeed, in a recent report on the involvement of a kainite glutamate receptors in OCD, a positive association between the gene coding for kainite receptor type 2 (*GRIK2*) and OCD was observed, thereby supporting the role that glutamate may play in the disorder (Delorme et al., 2004).

Support for *GRIN2B,* in particular, as a candidate gene in OCD stems from a family-based association study in which Arnold et al. (2004) observed significant genotypic and haplotypic associations between *GRIN2B* variations and increased susceptibility to develop OCD. The genotypic association, based on a non-additive model of inheritance, observed an association

between the *5072T/G* (rs890) variant in the 3'UTR of *GRIN2B* and OCD. The haplotypic association, based on a recessive model of inheritance, indicated that the presence of two copies of *5072T-5988T* variants (also in the 3'UTR) increased susceptibility to OCD. However, the authors could not exclude the possibility that the *5072G-5988C* haplotype may confer a protective role against developing OCD.

The functionality of neither *5072TC* nor *5988TC* has been elucidated, making it difficult to determine the role that these variants may play in OCD. However it is possible that the polymorphisms affect transcriptional regulation in some way, or that they are in LD with functional variants within *GRIN2B* that have yet to be identified. Moreover, other variants may form haplotypes with those mentioned above, and may thus even strengthen the role that the haplotype plays.

In the present study, two polymorphisms within *GRIN2B* were investigated: the aforementioned SNP found by Arnold et al. (2004) to be associated with OCD (*5072TC*, or rs890) (Figure I.7) and an *A* to *G* transversion at nucleotide 3743 in exon 13 (rs1806191).

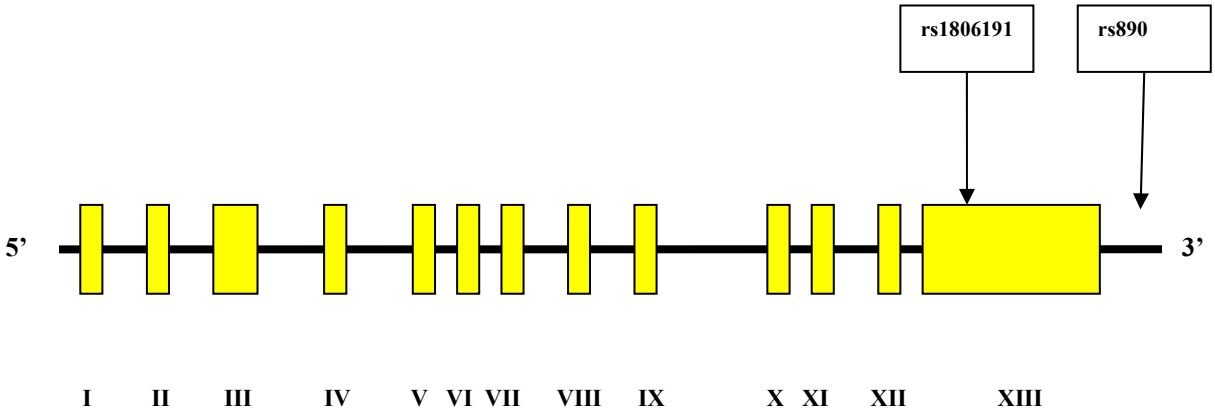



***Figure I.7. Schematic representation of the* GRIN2B *locus.*** *The location of SNPs investigated in the present study indicated by arrows. Exons are represented by yellow blocks, and introns are represented by horizontal lines between the exons (not drawn to scale). The distance between the two SNPs is approximately 1.3kb.*

### I.6.1.4. The neurodevelopmental theory of OCD

The notion that OCD may comprise a developmental subtype has been alluded to in a previous section (**section I.4.2.2.4**). To recap, this developmental subtype is thought to be characterised by male preponderance, an earlier age at onset and a more pronounced basal ganglia dysfunction (Rosenberg and Keshavan, 1998). Moreover, the observation that neurological soft sign abnormalities reported in paediatric patients with OCD do not deteriorate with illness progression lends further support to the neurodevelopmental hypothesis of the disorder (Rosenberg and Keshavan, 1998).

In addition, it has been found that the PFC undergoes a substantial amount of re-organisation during childhood and adolescence (Rosenberg and Keshavan, 1998; Huttenlocher et al., 1979; Jernigan and Tallal, 1990). Indeed, in a magnetic resonance imaging (MRI) study by Rosenberg and Keshavan (1998), in which treatment-naïve paediatric OCD patients were used, abnormalities in the ventral-PFC striatal anatomy were reported. It was proposed that these abnormalities represented an early pathological marker related to abberant neurodevelopment. Of course, these changes may be related to alterations in any of the abovementioned neurotransmitter systems, but may also be as a result of mutations in genes more directly involved in neurodevelopment.

#### *I.6.1.4.1. Developmental candidate genes*

**i. Brain-derived neurotrophic factor (BDNF)**

The exact role of BDNF in OCD has yet to be elucidated, but a closer look at the function of the protein may aid in determining its possible contribution to the aetiology of the OCD. BDNF is the most abundant neurotrophin in the brain – it is expressed throughout the CNS, with the highest level of expression in the hippocampus and cerebral cortex (Murer et al., 2001). The protein is an activity-dependent endogenous neurotrophin involved in neurodevelopment, neuronal survival, morphology and differentiation (Hoglinger et al., 1998; Lindholm et al., 1996). It also plays a pivotal role in modulating synaptic plasticity (Ying et al., 2002; Huang et al. 1999) and efficiency (Huang et al., 1999; Lohof et al., 1993). Moreover, it is involved in regulating protein activity at presynaptic terminals, allowing BDNF to modulate short and long term synaptic transmission (Popoli et al., 2002).

It has also been suggested that BDNF may possess a role as an extracellular transmitter, given that it is anterogradely transported (Altar et al., 1997; von Bartheld et al., 1996) and is released upon neuronal depolarization, triggering rapid intracellular signals via the transmembrane receptor, tyrosine kinase B (TrkB) (Altar and DiStefano, 1998). Indeed, it has been found that mice lacking the *BDNF*/*TrkB* genes have decreased synaptic inervation and lower levels of synaptic vesicle protein (Pozzo-Miller et al., 1999), indicating the involvement of BDNF in normal neuronal signaling.

It is particularly significant to research into the aetiology of OCD that BDNF has been reported to promote and augment the function and growth of 5-HT neurons in the brain (Mamounas et al., 1995) and induce the sprouting of 5-HT nerve terminals (Siuciak et al., 1994; 1996). In addition, knockout mice heterozygous for the BDNF gene show behavioural abnormalities that are in line with 5-HT dysfunction, such as an increase in impulsivity, aggressiveness and appetite. Interestingly, these effects were reversed using antidepressant (including SSRI) therapy (Lyons et al., 1999). BDNF has also been found to modulate 5-HTT function (Mossner et al., 2000), which plays a pivotal role in bringing about the therapeutic action of SSRIs. Consistent with these findings is the possibility that BDNF may represent a downstream target in antidepressant treatment (Nibuya et al., 1995; Hashimoto et al., 2004).

BDNF may also affect the expression of dopamine: the two have been found to reciprocally potentiate each other, particularly in the striatum (Kuppers and Beyer, 2001). Certainly, evidence exists suggesting that the expression of *DRD3* is positively controlled by BDNF (Takahashi et al., 2000), and that BDNF modulates the synaptic plasticity of the DRD3-secreting neurons in the striatum of the brain (Guillin et al., 2003). Chronic stress causes a decrease in the expression of *BDNF* and *DRD3*, and chronic antidepressant (SSRI) treatment increases the expression of both of these genes. This points to, and reinforces the notion of, the possibility that antidepressants may act primarily on BDNF, subsequently altering dopaminergic neurotransmission, probably by means of DRD3.

Furthermore, recent reports have indicated that an increase in BDNF expression in the striatum is associated with the prevention of stereotyped behaviour (Turner and Lewis, 2003). To this end, dopaminergic antagonists have been found to attenuate stereotyped behaviours in mice (Turner et al., 2001), providing further evidence for a possible link between striatal concentrations of, and interactions between, dopamine and BDNF, and stereotyped behaviour

(Turner and Lewis, 2003). Therefore, modulation of dopaminergic responsiveness by BDNF may contribute to the aetiology of OCD or OCD-related disorders.

The gene encoding BDNF is primarily a developmental gene that is a member of a family of a group of proteins known as neurotrophic growth factors that includes neurotrophic factors 3, 4 and 5 (Hallbook et al., 1991; Rosenthal et al., 1990; Ernfors et al., 1990). These proteins all possess similar coding sequences and only slight differences in functionality, and may therefore represent part of a larger gene family of growth factors (Okazama et al., 1992). Neurotrophins are all synthesised in a precursor form (the pro-protein), and are cleaved intracellularly to produce the mature protein. However, the pro-protein forms are also thought to possess functional biological activity, since it has been found that they may be secreted and cleaved extracellularly (Lee et al., 2001). In particular, cross-species conservation of the precursor portion of pro-BDNF has been reported, indicative of possible functional importance (Green and Craddock, 2003).

*BDNF* has been mapped to chromosomal position 11p14.1 (Leibrock et al., 1989), and has recently been found to comprise at least six 5' exons, each with its own putative promoter region. These exons are differentially spliced to a single 3' terminal exon, that contains the only functional splice acceptor site for the splicing of different pre-mRNAs. This terminal exon also contains the entire coding sequence of mature BDNF (Jiang et al., 2005; Liu et al., 2005; Marini et al., 2004).

Recently, a common SNP occurring at nucleotide 196 (*196G/A*; rs6265) in the terminal exon of the proBDNF sequence, resulting in an amino acid substitution (*val66met*), was found to be associated with OCD in a family-based case-control association study (Hall et al., 2003) in single and multiple loci analyses. Here, a haplotype containing the rare *met66* allele was identified as possibly imparting a protective effect in OCD.

The *val66met* variant is located in the 5'pro-BDNF sequence, and although it is unlikely to affect biological activity of the mature protein, the results of *in vitro* transfection studies suggest that the *met66* allele may affect the intracellular trafficking and activity-dependant secretion of BDNF (Egan et al., 2003). These results, however, remain to be replicated *in vivo*. Moreover, the *met66*-allele has recently been found to be more abundant in individuals

with both anxiety and depression, and was found to be associated with increased levels of Harm Avoidance, a personality dimension often associated with OCD (Jiang et al., 2005).

On the other hand, it has been hypothesised that the *met66* allele allows for more *efficient* processing of the BDNF protein (Mowla et al., 2001; Sen et al., 2003), although this hypothesis seems to stem mainly from the observation that the *met66*-allele may confer protection against developing bipolar depression in Caucasians (Sklar et al., 2002;Green and Craddock, 2003; Sen et al., 2003). The *met66*-allele has also been found to confer protection against development of EO OCD (Hall et al., 2003). However, the *met66*-allele has also had its share of associations where it has been implicated as the risk allele: it has been found to play a role in memory disturbance and poor hippocampal functioning (Egan et al., 2003; Dempster et al., 2005), and has been found to be associated with the restrictive subtype of anorexia nervosa (Ribases et al., 2003), which has features in common with OCD. One reason for the discrepant findings may, of course, be that the *val66met* variant is not functional, but is in LD with a functional variant in the gene, or one nearby.

Thus, despite the controversy regarding the allele implicated in the abovementioned disorders, *BDNF* presents an attractive candidate to investigate for its involvement in OCD and related disorders and subtypes in the context of population-based case-control association studies. Three polymorphisms were chosen for investigation in the present study, the most terminal being the aforementioned *val66met* polymorphism (dbSNP rs6265), and two intronic SNPs, rs2049046 and rs988748 (Figure I.8) These two intronic polymorphisms are located immediately upstream of exon 3, the mRNA transcript of which was found to be the major $Ca^{2+}$/activity-inducible transcript in cortical neurons (Tao et al., 2002). These two polymorphisms were also found to be associated with OCD in the recent study by Hall et al. (2003).

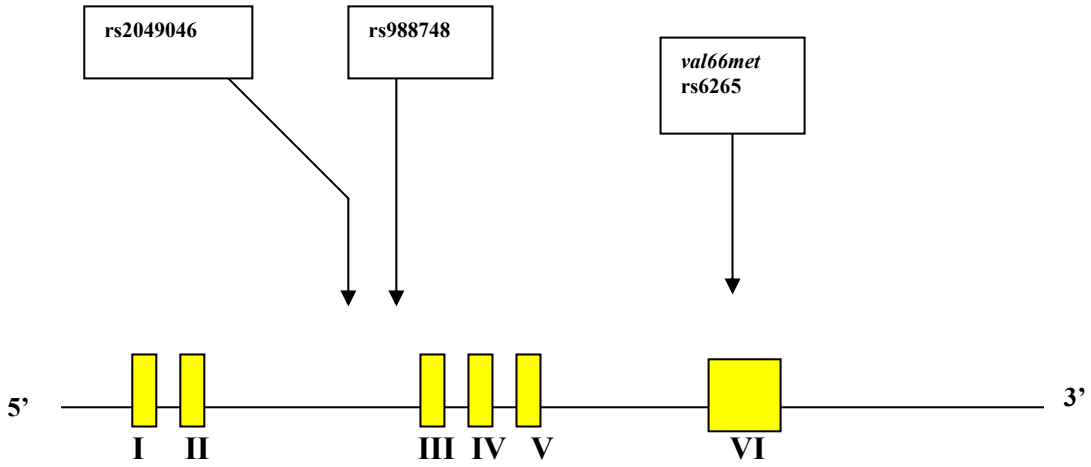



***Figure I.8. Schematic representation of the* BDNF *locus****. The location of SNPs investigated in the present study indicated by arrows. Exons are represented by yellow blocks and introns are represented by horizontal lines between the exons. (not drawn to scale). The distance between rs2049046 and rs988748 is approximately 970bp, whilst that between rs988748 and rs6265 is approximately 43.9kb.*

**ii. The HoxB8 gene (*HoxB8*)**

HoxB8 is a member of the highly conserved mammalian Hox (homeobox-containing) complex that controls early development by supplying positional information along the antero-posterior axis (Capecchi, 1997). Numerous Hox genes (including *HoxB8*) have been found to be expressed in the CNS: in a recent study Greer and Capecchi (2002) detected *HoxB8* expression within the adult mouse olfactory lobe, basal ganglia, hippocampus, cortex, cerebellum, brainstem, orbitofrontal cortex, anterior cingulate cortex and caudate-putam, some of which comprise the OCD circuit, reinforcing a role for the gene in the development of these brain regions.

In the same study, Greer and Capecchi (2002) demonstrated that mice homozygous for a loss-of-function *HoxB8* mutation exhibited pathological grooming behaviours, including excessive hair removal, self-inflicted wounds at the overgroomed sites, an excessive amount of time spent grooming and excessive grooming of control littermates. By means of histological and behavioural analyses, skin and peripheral abnormalities were excluded as reasons for the excessive grroming behaviours, leaving them with the conclusion that the pathological behaviour was a result of the CNS *HoxB8* abnormalities.

The grooming behaviours observed in the *HoxB8* knockout mice were reminiscent of those exhibited by patients suffering from TTM, suggesting that a dysfunction in a genetically controlled neural network may contribute to the aetiology of the disorder. Since TTM is considered by some to be part of the spectrum of obsessive-compulsive disorders, investigating the gene as a candidate in association studies may provide clues as to the proposed neurodevelopmental basis of both disorders.

The human *HoxB8* is located on chromosome 17q21-q22 and comprises two small exons. Very few genetic association studies using the gene have been conducted, and very few of the proposed SNPs therein have been formally validated. To the author's knowledge, there exists no previously published data that investigates the proposed relationship between genetic variants within *HoxB8* and increased susceptibitly to OCD and/or TTM. Therefore, the present study entails genotyping a polymorphism within *HoxB8*, to elucidate the potential role the gene may play in the development of OCD.

#### I.6.1.5. "Novel" candidate genes

Based on extensive literature searches, the final candidate genes have been chosen by the author due to their potential involvement in pathways related to those presumed to be dysfunctional in the pathophysiology of OCD and/or related disorders and subtypes. These genes are placed under the heading "novel candidate genes" largely due to the fact that there is not quite as much evidence for their involvement in OCD as there exists for the aforementioned candidates. However, the evidence which is available is worthy of, at least, some exploratory investigation.

##### I.6.1.5.1. The Estrogen Receptor type alpha gene (*ESRα*)

*ESRα* encodes a transcription factor that regulates numerous neurotransmitter pathways, including 5-HT, in the brain (McEwen et al., 1997). The gene has been mapped to 6q25.1, and comprises 8 exons. The gene has recently become a popular candidate amongst researchers investigating the genetic aetiology of anxiety disorders. A 5' microsatellite polymorphism was observed to be associated with increased susceptibility to anxiety (Comings et al., 1999), and more recently it was found that two intronic variants within the gene account for between 1.6% and 2.8% of the total variance for anxiety experienced by a large cohort of adolescents (Prichard et al., 2002).

*ESRα* thus represents an interesting candidate gene to investigate for the role it may play in increasing susceptibility to OCD. A point of contention would, however, be that the male: female ratio in OCD in the general population is roughly equal; consequently, the role of any estrogenic component in the aetiology of the disorder would be questionable. However, gender differences have been observed in certain aspects of OCD, the most notable being the differences in obsessions and compulsions, comorbidity and age at onset experienced by either sex (Tukel et al., 2004; Noshirvani et al., 1991; Lensi et al., 1996; Bogetto et al., 1999; Matsunaga et al., 2000; Castle et al., 1995; Fahy et al., 1993; Lochner et al., 2004). In a recent study, our group observed that female patients experienced changes in obsessive-compulsive symptomatology during the pre- or postmenstrual period, as well as during or after pregnancy and menopause (Lochner et al., 2004). These results are consistent with those from an earlier study indicating that the postpartum period represents a greater risk factor for OCD in susceptible individuals (Maina et al., 1999). The exacerbation of obsessive-compulsive symptomatology during these periods may be due to the role that hormones (including estrogen and progesterone) play in altering the 5-HT neurotransmission, thereby triggering OCD in vulnerable subjects.

Further evidence for a putative role of *ESRα* in the aetiology of OCD stems from the finding that the promoter regions of *COMT* (P1 and P2) contain EREs (**section I.6.1.2.1** [**vi**]). The expression of the gene is therefore modulated by estrogen and estrogenic transcription factors; consequently, any dysfunction within the estrogenic modulatory system may result in the sub-optimal functioning of *COMT*. This, in combination with numerous other genetic and environmental factors, may ultimately precipitate the OCD phenotype.

The present study investigated the role that two SNPs (rs2234693 and rs9340799), situated in intron 1(Figure I.9) may play in the development of OCD.

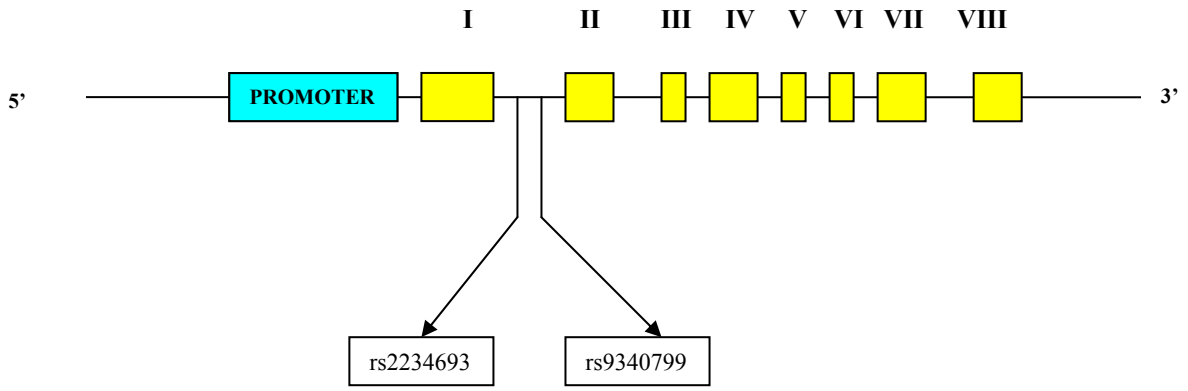



***Figure I.9. Schematic representation of the* ESRα *locus.*** *The location of SNPs investigated in the present study indicated by arrows. Exons are represented by yellow blocks and introns are represented by horizontal lines between the exons (not drawn to scale). The distiance between the two SNPs is approximately 46bp.*

**I.6.1.5.2. The Inositol Polyphosphatase-1 gene (INPP-1)**

Inositol, a glucose isomer, is a key metabolic precursor in the phosphatidylinositol (PI) second messenger system (Kofman and Belmaker, 1993; Michell, 1997). PI has been found to be the second messenger for numerous hormones and neuotransmitters, including 5-HT (Barabian et al., 1989). Exogeneous administration of myo-inositol itself has been found to demonstrate clinical efficacy in a number of neuropsychiatric disorders, including OCD (Levine 1997; Fux et al., 1996), panic disorder (Benjamin et al.,1995), depression (Levine et al., 1993; 1995; Kaplan et al., 1996) and compulsive skin-picking (Seedat et al., 2001). Interestingly, the administration of exogenous inositol has been found to exacerbate ADHD symptoms (Levine et al., 1995), and to be ineffective in treating schizophrenia, Alzheimer's disease and autism (Levine, 1997).

Inositol and phosphoinositides are important for numerous cellular processes, including neuronal survival, differentiation, neuroprotection, and signal transduction from growth factors, hormones and neurotransmitters including 5-HT, dopamine and glutamate (Fisher et al., 1992; Harvey et al., 2002; Delmas et al., 2002; Berridge, 1993). However, in order to understand the mechanism of action of inositol in attenuating OCD symptomatology, it is necessary to briefly consider the major components of the PI signalling pathway (Figure I.10).

The PI pathway involves the hydrolysis of membrane phosphatidyl-4,5-biphosphate ($PIP_2$), by means of the phosphorylation of phoshpholipase C (PLC), to inositoltrisphosphate ($IP_3$) and diacylglycerol (DAG) (Berridge and Irvine, 1984; 1989; Berridge, 1993). $IP_3$ and DAG therefore represent the second messengers: DAG activates DAG-regulated protein kinase C (PKC) that is responsible for protein phosphorylation, and has also been found to be involved in 5-HT release (Harvey et al., 1997; Berridge, 1993; Berridge and Irvine, 1989). $IP_3$ promotes the mobilisation of calcium from internal stores, thereby activating $Ca^{2+}$-dependent enzymes, including $Ca^{2+}$-regulated isoforms of PKC and $Ca^{2+}$-calmodulin-regulated protein kinases. $IP_3$ can be dephosphorylated by means of inositol-polyphosphatase-1 (IPPase) and inositol monophosphatase (IMPase) to yield inositol. Phosphoinositol can be formed by the combination of DAG and inositol, in a reaction catalysed by PI-synthase (Majerus et al., 1988). PI is subsequently phosphorylated, in two separate steps, to yield $PIP_2$, thereby replenishing the supply of membrane $PIP_2$, and completing the cycle (Figure I.10).

The precise mechanism of clinical action of inositol in OCD, or any of the other aforementioned disorders, has not yet been elucidated. However, it is interesting to note that the spectrum of activity of inositol appears to be very similar to that of SSRIs in humans and animals: both inositol and SSRIs require chronic administration of relatively high doses over a number of weeks to be effective (presumably due to selective changes in gene expression that appear only after a lag period of a few weeks), and the co-administration of SSRIs and inositol does not result in the augmentation of their therapeutic efficacy (Fux et al., 1999; Levine et al., 1999; Levine, 1997; Einat et al., 1999).

It may therefore be that inositol and SSRIs share a common mechanism of action in the treatment of OCD. In fact, it has recently been found that the administration of SSRIs to previously untreated OCD patients results in a decreased number of $5\text{-}HT_{2A}$ binding sites, and a decrease in platelet $IP_3$ concentrations (Delorme et al., 2004). This proposed involvement of the phosphoinositide signalling system in SRI treatment supports the finding by Marazziti et al. (2000) who observed an increase in PKC activity (that was most likely due to an increase in activity within the PI pathway) in drug-free OCD patients.

These findings suggest that the fundamental defect of the disorders responsive to SSRI and inositol therapy (including OCD) may lie not with neurotransmitter synthesis or metabolism, but rather, with the regulation of the underlying signalling cascade. It would thus be pertinent

to investigate selected components of these signalling cascade(s) as candidate genes for the development of OCD, as has been done in the present study.

If one refers to the figure representing the PI signal transduction system (Figure I.10), it is clear that IPPase plays an important role in replenishing the levels of membrane $PIP_2$, by catalysing the removal of a phosphate group from the inositol ring on $IP_2$, which can then be converted by inositol monophoshatase to myo-inositol. It may therefore be that a dysfunctionality in the genes encoding IMPase and/or IPPase may play a role in the development of OCD, or at least certain neuropathological aspects of the disorder. This hypothesis is indeed in line with the previously reported finding by Marazziti et al. (2000), in which the increased activity of downstream components of the PI-PLC pathway was noted in OCD patients.

IPPase has been suggested as a target for the mood-stabilising effects of lithium (Inhorn et al., 1988; York et al., 1993), commonly used in the treatment of bipolar disorder, which is found to co-occur with numerous anxiety disorders, including OCD (reviewed in Freeman et al., 2002). Lithium has been found to uncompetitively inhibit IPPase and IMPase (Berridge and Irvine, 1989), and it is this characteristic that forms the grounding for the "inositol-depletion" hypothesis: by the inhibition of IMPase or IPPase, the level of inositol would be reduced, subsequently reducing the synthesis of PI and DAG. Although the effect of lithium has not been widely studied in anxiety disorders, Golden et al. (1988) and Stern and Jenike (1983) have reported on the possible efficacy of lithium treatment in OCD patients presenting with bipolar or epileptiform features. Indeed, a SNP, in the gene encoding IPPase (*INPP-1*), characterised by an *A* to *C* transversion at nucleotide position 973, has been shown to be associated with lithium response: the *973C* variant has been found to be more frequent amongst lithium responders, compared to non-responders (Steen et al., 1998; Lovlie et al., 1999).

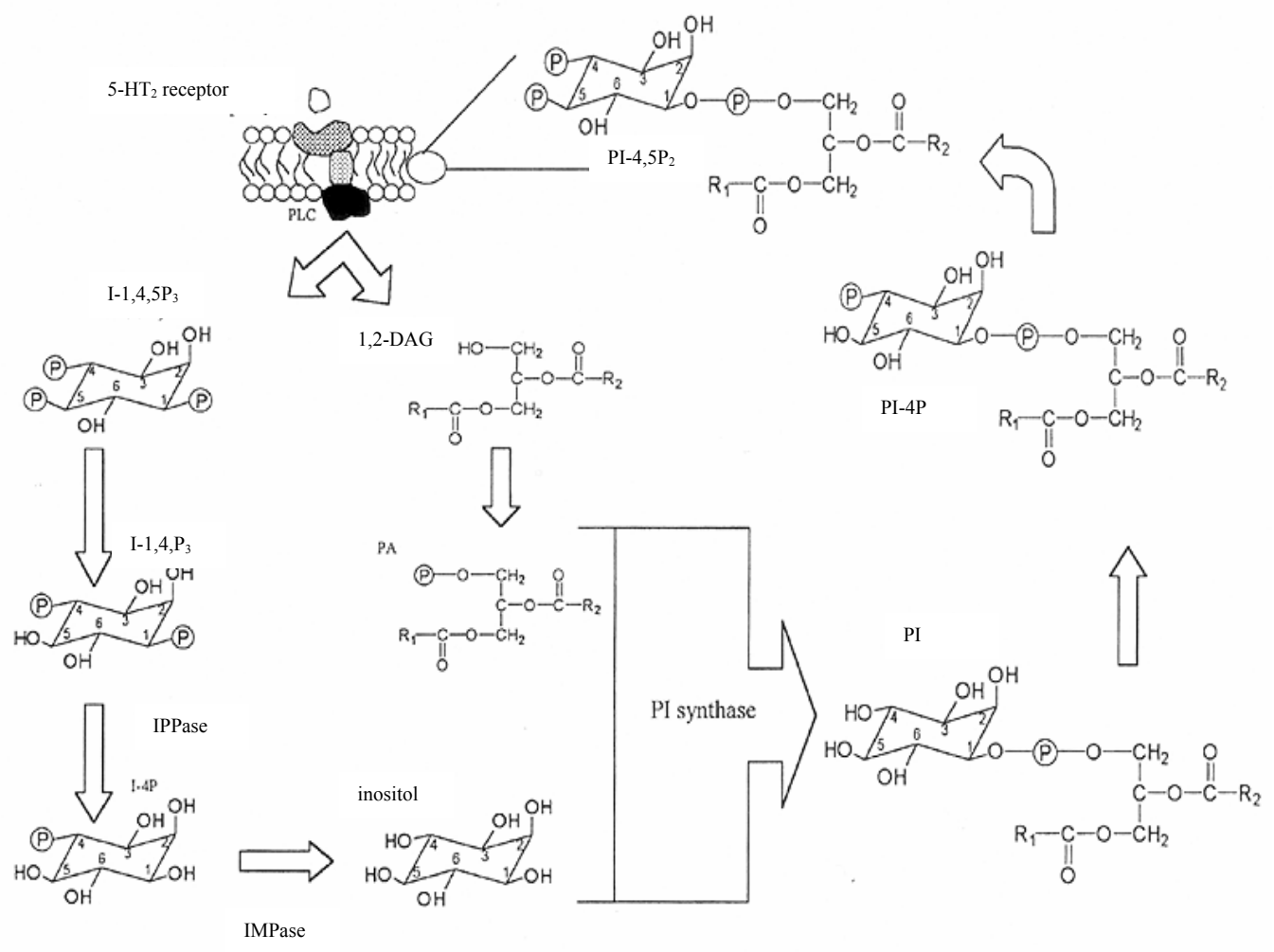



***Figure I.10***. ***Pathways of phosphoinositide degradation and re-synthesis***. *Receptor-mediated hydrolysis of $PIP_2$(PI-4,5P2) via Phosphoinositide-specific PLC (PI-PLC) results in the formation of $IP_3$ ($I\text{-}1,4,5P_3$) and DAG. $IP_3$ binds to intracellular receptors to release sequestered $Ca^{2+}$ & is sequentiall depohosphorylated to form $IP_2$ ($I\text{-}4,5P_2$), $IP_1$ (I-4P) and finally inositol, which can be reutilised by PI synthase to form PI. DAG contributes to the activation of DAG-regulated PKC proteins, and also joins up with inositol to form PI (adapted from Harvey et al., 2002).*

***Abbreviations:*** *5-$HT_2$: serotonin receptor 2; PLC: phospholipase C; $PIP_2$ (PI-4,5P2): phosphatidylinositolbisphosphate; $IP_3$ ($I\text{-}1,4,5P3$): inositol trisphosphate; $IP_2$ ($I\text{-}4,5P2$): inositol bisphosphate; $IP_1$ (I-4P): inositol monophoshate; IMPase: inositol-1-monophosphatase; IPPase: inositol-1-polyphosphatase (INPP-1); 1,2DAG: 1,2 diacylglycerol;PA: phosphatidic acid; PI-4P: phosphatidyl-inositol 4-phosphate (PIP).*

*INPP-1* maps to chromosomal location 2q32, and is comprised of six exons. Polymorphisms within the gene have been linked not only to lithium response in patients with bipolar disorder (Steen et al., 1998; Lovlie et al., 1999), but also to autistic disorder, which is thought to share some aspects of genetic lability with OCD, given that motor tics, OCD and affective disorders were found to be more prevalent amongst first degree relatives of autistic probands (Bolton et al., 1998). Indeed, one characteristic of the autism diagnosis is marked resistance to change, shown by rigid and repetitive behaviours and restricted interests, representing OC-like symptom dimensions which may represent the link between the two disorders. Moreover, Hollander et al. (2003[b]) reported, in a recent preliminary investigation, that autistic children presenting with more repetitive behaviours were significantly more likely to have parents with OC traits or OCD.

Given the proposed involvement of inositol in OCD, the *C937A* SNP in *INPP-1* was investigated as a novel candidate gene for the role that it may play in mediating the development of OCD.

**I.6.1.5.3. The Phospholipase-C gamma-1 gene (*PLCγ-1*)**

At least eleven PLC isoforms, grouped into four subfamilies, namely β, γ, ε and δ, are found in the brain, each representing the product of a separate gene (Rhee et al., 1989; 2001). Although all four groups of isozymes serve to activate the inositol phopholipid signalling pathway, their mechanisms of activation differ. The mechanisms of activation for the PLC-β and PLC-γ subgroups appear to be the most well-founded: PLC-β is activated by G-protein coupled receptors (including thromboxan-A2, histamine, vasopressin and endothelin receptors [Rhee, 2001]), whilst the PLC-γ class is activated by the tyrosine kinase class of receptors. $Ca^{2+}$ and a recently discovered type of GTP-binding protein appears to play a role in the activation of PLC- δ, although this remains to be further investigated (Feng et al., 1996; Rhee, 2001), and the PLC-ε isozyme is though to be an exchange factor for, and effector of, Ras-GTP (Lopez et al., 2001; Song et al., 2001 [cited in Rhee, 2001]).

Of particular interest in the present study was the PLC-γ1 isozyme, given its fundamental role in the intracellular signalling cascade brought about by numerous growth factors, including neural growth factor (NGF) and BDNF (Yuen and Mobley, 1999). As previously mentioned, neurotrophic factors play an important role in regulating the survival and differentiation of specific neuronal populations during development, and maintain specific neuronal functions

during adulthood (Lewin and Barde, 1996). All neurotrophins bind to one or more receptors belonging to the tropomyosin receptor kinase (Trk) family. These Trk receptors (Trk A, B and C) represent a subgroup of a larger family of proteins with tyrosine kinase enzymatic activity (reviewed in Patapoutian and Reichardt, 2001). The binding of the neurotrophin causes dimerisation of the relevant Trk receptor, resulting in the phosphorylation of specific tyrosine residues on the receptor. These phosphorylated residues provide an ideal docking site for the PLC-γ1 isozyme, catalysing the subsequent phosphorylation of the bound enzyme. This interaction activates PLC-γ1, and facilitates its interaction with the substrate molecule, $PIP_2$ (Figure I.10), and subsequent activation of the PI signalling pathway. It has been found that signaling pathways activated in neuronal cells by Trk-mediated activation of PLC-γ1 extend to the nucleus, implicating its involvement in the regulation of certain transcritional events (Toledo-Aral et al., 1995). Moreover, it has recently been found that the activation of PLC-γ is required for the regulation of neurotrophin secretion (Canossa et al., 2001).

In an attempt to delineate the physiological fuctioning of the Trk-mediated PLC-γ1 signalling pathways, Minichiello et al. (2002) mutated the TrkB PLC-γ1 recruitment site (Y816) in mice. Mice homozygous for the mutation (*trkB*$^{PLC-/PLC-}$) were found to be more hyperactive than control littermates, and possessed significant deficiencies in the induction of early and late phases of hippocampal long-term potentiation (LTP) via cAMP response element binding protein (CREB) activation (Minichiello et al., 2002). Since BDNF couples to the TrkB receptor, and has previously been found to be involved in EO OCD (Hall et al., 2003), it is possible that an abnormality in the activation of nuclear transcription factors, via PLC-γ1 activation, may underlie certain aspects of OCD pathology. Indeed, the need for chronic administration of both SSRIs and inositol to achieve clinical efficacy in OCD patients is indicative of the effect of these agents on secondary gene expression, which would allow for such a change in response over a period of chronic exposure.

Given the aforementioned role that PLC-γ1 plays in the PI signalling pathway, and its activation by neurotrophins, which have been implicated in the pathophysiology of OCD (**section I.6.1.4.1[i]**), the gene encoding PLC-γ1 represents a plausible candidate gene to investigate for the role it may play in increasing susceptibilty to OCD and related subtypes. The gene encoding PLC-γ1 is situated on chromosome 20q12-13 (Bristol et al., 1988), and comprises 32 exons. A SNP situated in exon 9, resulting in a *ser* to *gly* amino acid substitution at position 279 (dbSNP rs8192707), was recently described by Ftouhi-Paquin et

al. (2001). Since this SNP occurs in the coding region of the gene, it may possess functional consequences (although these have not yet been fully elucidated), and was therefore investigated in the present study.

**I.6.1.5.4. The Angiotensin Converting Enzyme gene (*ACE*)**

Angiotensin converting enzyme (ACE) plays an important role in the renin-angiotensin system, converting inactive angiotensin I to the active peptide angiotensin II (Ang II) (Johnston, 1990). ACE is also expressed in the CNS, with high concentrations in the striatum, choroid plexus and periventricular nucleus of the hypothalamus (Bardelay et al., 1989), where it functions additionally to catalyse the degradation of substance P (SP) (Skidgel and Erdos, 1987), a neuropeptide that has recently been of considerable interest in the field of anxiety (Griebel et al., 1999; Hasenörhl et al., 2000). It has also been proposed that ACE might hydrolyse numerous other neuropeptides, such as Met and Leu-enkephalin, dynorphin and neurotensin (Skidgel et al., 1984).

Ang II interacts with its specific angiotensin receptors, $AT_1$ and $AT_2$, and has been found to co-localise with dopaminergic neurons in the striatum and substantia nigra (Jenkins et al., 1995; Hasenörhl et al., 2000). As such, Ang II may regulate the dopaminergic content of certain regions of the brain. In rats treated with the ACE inhibitor, perindopril (which has been found to cross the blood-brain barrier), striatal dopamine synthesis and release was noted (Reardon et al., 2000). Moreover, chronic treatment with the dopamine antagonist, haloperidol, has been found to result in elevated DRD2-receptor densities, accompanied by an increase in Ang II receptor, $AT_1$, in the nucleus accumbens (Jenkins et al., 1995). Therefore, by controlling the level of Ang II in the brain, ACE in turn, could play an important role in regulating dopaminergic content.

The function that ACE plays in the degradation of SP is also relevant to the present hypothesis that it may be involved in the pathophysiology of at least some forms of OCD. Substance P is a member of the tachykinin family, representing the preferential agonist of the neurokinin-1 (NK1) receptor. The neuropeptide is widely distributed in the CNS, including those areas involved in the regulation of affective behaviour and neurochemical responses to stress, including the striatum, nucleus accumbens, hippocampus and the lateral nucleus of the hypothalamus (Quartara and Maggi, 1998; Shults et al., 1984; Hasenörhl et al., 2000). Another characteristic of SP is that it co-localises in the same neuron with other

neurotransmitters, including 5-HT, dopamine, and glutamate (Hasenörhl et al., 2000), thereby regulating their release and/or enhancing their effects.

Numerous animal studies have indicated the role that a disruption in SP levels play in anxiety: administration of NK1 receptor antagonists was found to yield anxiolytic activity (File et al., 1997; Santarelli et al., 2001). Conversely, NK1 receptor agonists have resulted in anxiogenic behaviour (Aguiar and Brandao, 1994; Duarte et al., 2004). In addition, and importantly, it has been found that NK1 receptor antagonists function as efficiently as SSRIs in the treatment of major depression (Kramer et al., 1998). The exact mechanism of therapeutic efficacy of NK1 receptor antagonists (and thereby the way in which SP may influence the development of such disorders) is presently unknown. However, recent studies indicate that the genetic deletion of NK1 receptors results in the desensitization of 5-$HT_{1A}$ receptors (Froger et al., 2001; Santarelli et al., 2001; 2002), representing a similar mechanism of action to many of the classical antidepressants, including SSRIs (Blier and Montigny, 1994; Chaput et al., 1991).

The gene encoding ACE is located on chromosome 17q23 and consists of 16 exons spaced over approximately 24kb (Rieder et al., 1999). A well-characterised intron 16 A*lu* insertion/deletion (ins/del) polymorphism has been investigated in numerous somatic and psychiatric disorders. This variant has been found to account for 27% to 49% of the total variance of serum levels of ACE (Rigat et al., 1990), with the *D*-allele (i.e. the deletion of the 287bp *Alu* sequence) associated with higher levels of circulating ACE (Rigat et al., 1990; McKenzie et al., 1995; Villard et al., 1996; Keavney et al., 1998; Tiret et al., 1992; Cambien et al., 1988). The polymorphism may also influence interindividual variability in SP levels in some parts of the brain, especially those in which SP levels are highly expressed (such as the caudate, the putamen, the substantia nigra and certain regions of the hypothalamus) (Arinami et al., 1996). In fact, the *D/D* genotype has been associated with increased susceptibility to affective disorders (Arinami et al., 1996), and depression in females (Baghai et al., 2004). Moreover, patients with major depression who carried at least one *D*-allele were found to respond better to pharmacotherapy with different antidepressants than those patients who were homozygous for the *I*-allele (Baghai et al., 2001; Bondy et al., 2005).

It is thus possible, that by altering the levels of ACE, the insertion/deletion polymorphism may influence the levels of Ang II and/or SP in the brain, and as such, may play an important role in the development of OCD and/or related disorders. Indeed, in light of the presented

evidence, it would be interesting to compare OCD patients with co-morbid MDD and those without, with regard to the presence or absence of such a polymorphism.

### I.6.2. ANALYSIS OF AFRIKANER POPULATION STRUCTURE

As already discussed (**section I.4.1.1.4**.), the South African Afrikaner population represents a potentially important population in which to investigate with regard to the inheritance of multifactorial disorders, given its high levels of genetic homogeneity. It is, however interesting to note that, to date, no formal analysis has been conducted into whether the Afrikaner population represents a unified, genetically homogeneous population, or whether certain "sub-populations" exist. This is particularly relevant when conducting case-control analyses, since the cases may be more related than the controls due to a recent ancestor who possessed a disease-predisposing allele. In such an event, the cases' contribution to the analysis will be correlated, whilst the statistics used assume that they are independent of one another. This may result in inflated effective sample sizes, and overoptimistic p-values (Devlin and Roeder, 1999).

To determine whether any cryptic sub-populations exist within the Afrikaner sample used in the present study, a Bayesian model-based algorithm, *Structure* (Pritchard et al., 2000), was implemented (**section I.4.1.1.3[i]**). *Structure* assigns individuals probabilistically to one or more sub-populations based on allelic frequencies at each locus. It involves genotyping random markers (preferably in linkage equilibrium with each other) in order to reflect the baseline genetic differences between cases and controls. The procedure places individuals into 'K' number of clusters. 'K' is chosen in advance, but can be varied across independent runs of the *Structure* algorithm. It is possible for individuals to have membership in multiple clusters; in this case, the program will indicate an estimate of the fraction of the individual's genome that originated from each of the 'K' subpopulations, providing a means for capturing the degree of admixture.

The major drawback of this method is that, although it allows the detection of population structure, the algorithm itself offers no means of adjusting the significance value if the stratification is found to influence the validity of the association. However, a program called "*strat*" has been designed (Pritchard, 2000) in order to correct for confounding due to stratification. S*tructure* is a presently widely-used program, and has been successfully implemented in numerous studies attempting to delineate human population structure

(Rosenberg et al., 2002; Bamshad et al., 2003), the genetic structure of certain dog breeds (Parker et al., 2004) and it has even been used to distinguish between selected breeds of chickens (Rosenberg et al., 2001).

The polymorphisms included in the population *Structure* analysis comprised those that were genotyped for concurrent psychiatric genetics studies in our laboratory, including some of those in the present study. In addition, five *Alu* insertion polymorphisms were used. These autosomal polymorphisms are short interspersed repetitive elements (SINEs), with each *Alu* element represented by a dimeric ~300bp retroposon that is homologous to, and ancestrally derived from, the 7SL RNA component of the signal recognition particle (Ullu and Tschudi et al., 1984). These polymorphisms are commonly found in introns, intergenic genomic regions, and 3'UTRs of genes (Maklolowski et al., 1994).

The *Alu* insertion polymorphisms were chosen on the basis that they are widely used in human evolutionary studies, since these insertion events are widely distributed, infrequent and irreversible (Batzer et al., 1994; 1996; Stoneking et al., 1997; Novick et al., 1998). Indeed, the Y, Ya and Yb subfamilies of *Alu* insertion polymorphisms are still active, and produce new *Alu* insertions that are polymorphic in most human populations (Roy et al., 2000; Batzer et al., 1990; Deininger and Batzer, 1999; Arcot et al., 1995). In addition, the ancestral allele is represented by the absence of an insertion; therefore individuals with an *Alu* insertion at a given locus share a chromosomal region that is identical by descent, facilitating investigation of population genetic structure and history.

# CHAPTER II

## METHODS AND MATERIALS

**INDEX** **PAGE**

# CHAPTER II: METHODS AND MATERIALS

## II.1. STUDY SUBJECTS

The protocol was approved by the Ethics Committee of the University of Stellenbosch, and all subjects (case and control) provided written informed consent, after being presented with a complete description of the study (**Appendix III**).

All case and control subjects participating in the present study were of Afrikaner descent - ancestry was determined based on information provided by each subject regarding his or her parents and grandparents. For the purpose of this investigation, subjects were classified as Afrikaners if at least three of their four grandparents were of Afrikaner descent.

### II.1.1. Control Subjects

Control subjects were recruited throughout South Africa by trained clinical psychologists and via media advertisements. All controls were required to complete a questionnaire pertaining to his/her personal demographic data and present state of physical health.

### II.1.2. Case Subjects

#### II.1.2.1. Clinical diagnosis

One hundred and thirty-two unrelated OCD patients, between 9 and 65 years of age, were recruited through physician referral, media advertisements, the Mental Health Information Centre (MHIC) and the OCD Association of South Africa (OCDSA) over a period of approximately 8 years. To be eligible for inclusion in the study, patients had to meet the DSM-IV criteria (APA, 1994) for a primary diagnosis of OCD on the Structured Clinical Interview for Axis I disorders – Patient Version (SCID-I/P) (First et al., 1998). All diagnoses were made by trained clinical psychologists at the MRC Unit on Anxiety and Stress Disorders.

The SCID I/P, and selected sections of the Structured Clinical Interview for Axis II Disorders-Patient Version (SCID-II/P) (including obsessive-compulsive, avoidant, schizotypal and borderline personality disorders) (First et al., 1998) were used to assess co-morbidity. Both of these structured diagnostic instruments are used frequently in psychiatric

research. In addition, the relatively newly developed structured clinical interview for the diagnosis of putative obsessive-compulsive spectrum disorders (SCID-OCSD) (du Toit et al., 2001) was administered to assess OCD-related conditions not covered by the SCID-I.

The Yale-Brown Obsessive-Compulsive Symptom Checklist (YBOCS-CL) and severity scale (YBOCS-SS) (Goodman et al., 1989) were used for the assessment of the typology and severity of obsessive-compulsive symptoms, respectively (Goodman et al., 1989). Y-BOCS is a 10-item balanced scale that is designed to rate the severity and type of symptoms experienced by patients with OCD. The scale measures symptoms, on a scale of 0-4, without being influenced by the type of obsessions or compulsions, with the total Y-BOCS score indicating the range of severity for patients who exhibit both obsessions. The dimensional Y-BOCS (DY-BOCS) interview was conducted in the present study to allow for assessment of the typology and severity of OCD symptoms.

The YBOCS-CL was developed in 1986 and has been frequently used in research and clinical settings since, and is generally assumed to have good validity and reliability (although data on the YBOCS-SS is relatively limited). Patients' level of insight into the senselessness or excessiveness of their OC symptoms was assessed using the relevant YBOCS-SS item (Goodman et al., 1989). The presence/absence of tics (current and past) and the nature thereof (e.g. motor and/or vocal) was assessed with the SCID-OCSD and the Yale Global Tic Severity Scale (YGTSS), respectively (Leckman et al., 1989). In the genetic analyses, both the categorical phenotype of OCD diagnosis and the quantitative phenotype of total Y-BOCS score were considered.

A miscellaneous medical questionnaire, which included questions pertaining to head injury, current medication, medical screening, developmental history, the presence of tics (current or past) and family history was also answered by the patients. OCD patients with a significant history of neurological disease, schizophrenia, schizo-affective disorder, other psychotic conditions or a history of substance dependence, as determined from the interviews or previous medical records, were excluded from the study.

## II.2. CANDIDATE GENE ASSOCIATION ANALYSES

### II.2.1. Blood Collection

Blood samples were drawn from OCD subjects and controls by means of venous puncture and collected into 5ml ethylene-diamine-tetra-acetic acid (EDTA) tubes. If blood was drawn from patients or controls at the MRC Unit on Anxiety and Stress Disorders, it was collected immediately and brought to the research laboratory at the MRC Centre for Molecular and Cellular Biology. Blood that was drawn from participants around South Africa was couriered to the research laboratory within 24 hours of sampling.

### II.2.2. DNA Extraction

#### II.2.2.1. Extracting nuclei from whole blood

Blood from three 5ml EDTA tubes per patient was transferred into a 50ml Falcon tube. The tube was then filled to 20ml with ice-cold lysis buffer (**Appendix I**). After gently inverting the tubes a few times, the sample was incubated on ice for 5-10 minutes. The sample was then centrifuged at 2500-3000 rpm at room temperature in a Beckman model TJ-6 centrifuge (Scotland, UK). The supernatant was discarded and the pellet was re-suspended in 20ml ice-cold lysis buffer, followed by another round of incubation and centrifugation. The supernatant was then discarded and the pellet re-suspended in 900µl sodium-EDTA (**Appendix I**) and 100µl 10% sodium dodecyl sulphate (SDS) (**Appendix I**). The nuclei were then either immediately used for DNA extraction or stored at -70˚C until the DNA was required.

#### II.2.2.2. Extracting DNA from nuclei.

To the freshly prepared or thawed nuclei, 100µl of proteinase K (10µg/ml) was added and the mixture was incubated overnight at 37˚C. After this step, 2ml distilled water, 500µl 3M sodium-acetate (**Appendix I**) and 25µl phenol/chloroform (**Appendix I**) were added to the sample. The tubes were subsequently inverted and mixed gently for 10 minutes on a Voss rotator (Voss of Maldon, England) at 4˚C. The mixture was then transferred to a glass Corex tube so that the aqueous phase could subsequently be clearly distinguished from the organic phase, followed by centrifugation in a Sorvall RC-5B refrigerated super-speed centrifuge (rotor SS 34, Dupont Instruments) at 8000 rpm for 10 minutes at 4˚C.

The upper aqueous phase containing the DNA was transferred to a clean Corex tube using a sterile plastic Pasteur pipette, taking care not to disturb the interface or the organic phase. Approximately 25ml chloroform/octanol (**Appendix I**) was added to the aqueous phase, after which the tube was closed with a polypropylene stopper and gently inverted for 10 minutes. This mixture was then centrifuged at 4˚C, followed by the removal of the upper aqueous phase as described earlier. The DNA was then ethanol-precipitated by adding two volumes of ice-cold 96% ethanol and inverting gently until strands appeared as a white precipitate.

These DNA strands were removed using a yellow-tipped Gilson-pipette and placed in a clean, 1.5ml Eppendorf microfuge tube. One millilitre 70% ethanol was then added to the DNA and the mixture centrifuged in a Beckman microfuge for 3 minutes at 13 000 rpm. The ethanol was carefully decanted and the 70% ethanol wash repeated one more time in order to remove any excess salts. After careful removal of most of the ethanol, the DNA pellet was air-dried for 30 to 60 minutes at room temperature by inverting the Eppendorf microfuge tube on Carlton paper. Two hundred microlitres Tris-EDTA (TE) (**Appendix I**) buffer was added and the DNA was resuspended initially by stationary incubation at 37˚C overnight and subsequently by gentle mixing in a Voss rotator (Voss of Maldon, England) at 4˚C for a further 3 days. This was followed by stationary incubation at 4˚C for one to three weeks until the DNA had been completely re-suspended.

Thereafter, the optical density (OD) of the DNA was determined in a Milton Roy series 120i spectrophotometer (USA) at 260nm ($OD_{260}$). The DNA concentration was determined by diluting 10µl of DNA in 500µl of TE and multiplying the measured $OD_{260}$ by a factor of 2.5. This gave the DNA concentration in µg/µl. The purity of the DNA was determined by calculating the ratio of the $OD_{260}$ and the $OD_{280}$, which should be approximately 1.8 for pure DNA.

### II.3. POLYMORPHISM SELECTION

Candidate genes were chosen based on an extensive literature search, using the PubMed literature database (www.ncbi.nih.gov/PubMed). Variants within the candidate genes were selected from those deposited in the National Centre for Biotechnology Information (NCBI) database of genetic variation (dbSNP). Variants with minor allele frequencies of greater than 5% were selected using SNPper, a web-based application developed by The Children's Hospital Informatics Program (CHIP) (http://snpper.chip.org).

## II.4. POLYMERASE CHAIN REACTION (PCR)

### II.4.1. Primer Design

#### II.4.1.1. External amplification primers

Where possible, both forward and reverse external amplification primer sequences were obtained from published data. If no published data were available, primers were designed using nucleotide sequences deposited in the Genbank database (http://www.ncbi.nlm.nih.gov/Entrez), and the Primer3 program (Whitehead Institute for Biomedical Research, Cambridge MA, USA; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Primer selection parameters (melting temperature ($T_m$) and percentage *GC* content) were left on the Primer3 default settings.

Each set of amplification primers was screened for melting temperature compatibility, self-complementarity and primer-primer complementarity using DNAman 4 (Lynnon Biosoft, Quebec, Canada) and the Autodimer program (Vallone and Butler, 2004). (http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutodimerProgramHomepage.htm).

#### II.4.1.2. Internal interrogation primers for SNaPshot genotype analysis

Internal interrogation primers used in the single nucleotide di-deoxy nucleotide triphosphate (ddNTP) primer extension method (SNaPshot analysis [Perkin Elmer Applied Biosystems, Warrington, UK]) for genotyping (**section II.5.3**) were also synthesised using Primer3 software. These primers were designed to terminate immediately 5' to the nucleotide base of the SNP under investigation (Figure II.1).

To allow for multiplex reactions, interrogation probes with lengths differing by at least 4 to 6 bases were required, to avoid overlap of peaks after capillary electrophoresis (**section II.5.3**). Using the Autodimer and DNAman software described in the previous section, the internal probes were investigated for, and rejected if, they were predicted to undergo self- or cross-dimerisation, or form 3' hairpin structures.

### II.4.2. Primer Synthesis

All oligonucleotide primers were synthesised on a 50nm scale according to standard phosphoramidite methodology at the Department of Biochemistry, University of Cape Town (UCT), UCT Medical School, Cape Town, South Africa. *DRD4* 48bp VNTR primers that were labelled with a fluorophore, 6-carboxyfluorescein (6-FAM) were synthesised on a 100μM scale by Inqaba Biotech, South Africa. External amplification primers used in the present study are listed in Tables II.1 (a) and (b).

### II.4.3. PCR conditions

Each locus was amplified individually. DNA amplification was performed in a 50μl reaction containing 100ng template DNA, 200μM of each deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dTTP), deoxycytidine triphosphate (dCTP) and deoxyguanosine triphosphate (dGTP) (Promega Corp, Madison Wisconsin USA), 5μl of 10X *Taq* DNA polymerase buffer (Bioline UK Ltd, London UK), 1.5mM $MgCl_2$ (Bioline UK Ltd, London, UK), 0.5 units (U) Taq DNA polymerase and 0.5μM of each primer, with bi-distilled water ($ddH_20$) making the mixture up to a final volume of 50μl.

Thermal cycling was performed in a GeneAmp® PCR system 9700 (Perkin Elmer Biosystems, Foster City, CA, USA) for 30 cycles. In brief, an initial denaturation step (the pre-denaturation step) was performed at 94˚C for approximately 3 minutes. Thereafter, a denaturation step was performed at 94˚C for 30 seconds (s), followed by the primer annealing step, at temperatures of between 55.5˚C and 70˚C (depending on the composition and length of the primer sets) for 30s, and an elongation step, performed at 72˚C for 45s. A final elongation step, at 72˚C for 7 minutes, was performed. The annealing temperatures and length of the PCR amplified product for each set of oligonucleotide primers are presented in Tables II.2(a) and (b).

Where the *GC* content of the product was very high, as for the amplification of the 48bp VNTR in *DRD4*, half of the dGTP in the reaction mixture was substituted with 7-deaza-dGTP (Roche Applied Science, Basel, Switzerland) (i.e. 100 μM dGTP, 100 μM 7-deaza-dGTP) in order to decrease the melting temperature of the PCR product. Furthermore, the addition of either 5 % dimethyl sulfoxide (DMSO) or 5 % formamide was required in order to increase the specificity and /or yield of some of the PCR reactions (Tables II.2[a] and [b]).

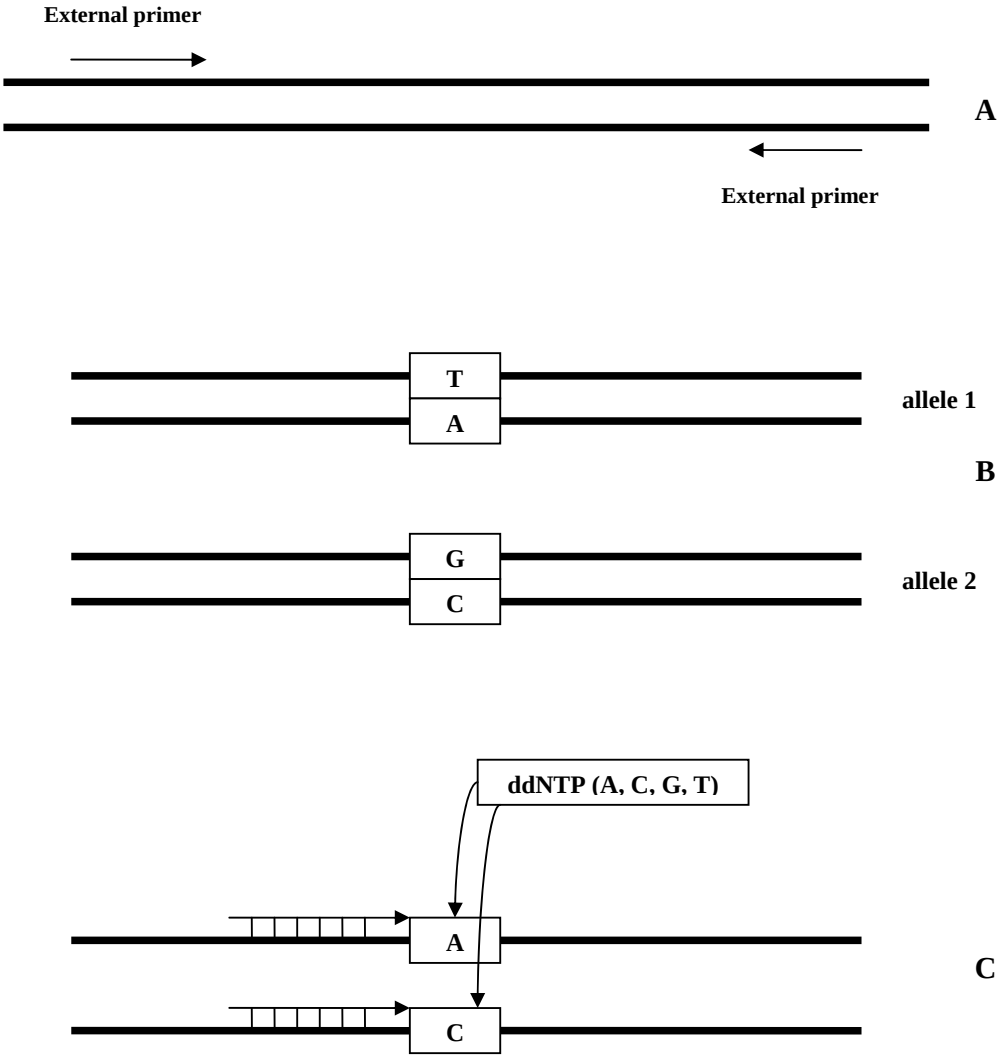



***Figure II.1.*** *Overview of the SNaPshot procedure, indicating the positions of the external and internal interrogation primers.* ***A****: the initial PCR reaction, as described in section 4.3.* ***B****: The PCR-amplified products, indicating the SNP of interest (in this case, an* A *to* C *transition).* ***C****: the PCR-amplified products are denatured, the interrogation primers, which bind immediately adjacent to the SNP site, are added and complementary fluorescently-labelled ddNTPs are incorporated. The fluorescently-labelled extension primers can then be visualised by capillary electrophoresis (**section I.5.3**) (adapted from Turner et al., 2002).*

**Abbreviations: ddNTP:** di-deoxy nucleotide triphosphate

Following amplification, 5µl of each amplified sample was electrophoresed on 2% agarose gels (**section I.6.1**) to verify that a fragment of the expected size had been amplified. In the interest of quality control, a negative control (water blank) was included in each batch of amplifications performed.

## II.5. GENOTYPING

Depending on the type of polymorphism, four methods of genotyping were employed in the present study: agarose gel electrophoresis, allele-specific restriction endonuclease analysis (ASREA), single nucleotide dideoxy nucleotidetriphosphate (ddNTP) primer extension method (SNaPshot analysis [Applied Biosystems, California, USA]) and separation by capillary electrophoresis. Assessment of genotypes was conducted blind to diagnosis, and by two independent investigators. Genotypes were only used if the genotyping results obtained by the two independent investigators concurred.

### II.5.1. Genotyping the VNTR and *Alu* insertion polymorphisms by agarose gel electrophoresis

Detection of the *DRD4* (48bp VNTR), *DAT* (40bp VNTR) and the *Alu* insertion polymorphisms involved size fractionation of the relevant PCR amplified products on an agarose gel of appropriate concentration) (**section II.6.1**). Table II.3 lists the percentage (w/v) of agarose gels used for genotyping these polymorphisms, and the sizes of the alleles that could be detected.

### II.5.2. Allele-specific Restriction Enzyme Analysis (ASREA)

The majority of polymorphisms investigated in this study involve the substitution of single base pairs, which creates or abolishes a restriction endonuclease recognition site. Genotyping variants by exploiting this knowledge is referred to as allele-specific restriction enzyme analysis (ASREA).

Briefly, 5µl of the pertinent PCR amplified product was added to a cocktail containing 1-3U of the relevant restriction enzyme (Tables II.4[a] and [b]) in the appropriate buffer supplied by the manufacturer, with the final volume being 10µl. These reactions were then incubated for 2 to 3 hours at the optimal temperature for the restriction enzyme.

***Table II.1 (a).*** *Description of the candidate polymorphisms used in the genetic association analyses, indicating the chromosomal location of the variants and sequences of the external amplification primers.*

| Gene | Chromosomal Location | Variant identification | DNA variant | Domain | Forward Primer (5'-3') | Reverse Primer (5'-3') | Ref. |
|---|---|---|---|---|---|---|---|
| ***5-HT$_{2A}$*** | **13q14-21** | rs6311 (*-1438A/G*)[#] | SNP*[A→G]* | Promoter | *GGTAGCCTACTGTGGCCTTG* | *TGGGCTTTCCATGCAACTAT* | 1 |
| | | rs6313 (*T102C*) | SNP [*T→C*] | Exon 1 | *CGCCCGCCGCGCCCCGCGCCCGTCCCCGCC GTCTGCTACAAGTTCTGGCTT* | *CTGCAGCTTTTTCTCTAGGG* | 2 |
| ***5-HT$_{1Dβ}$*** | **6q13** | rs6296 (*G861C*)[#] | SNP [*C→G*] | After Stop Codon | *TCGTCGGACATCACTTGTTG* | *GTGGAACCAGCAGGCATCTT* | 3 |
| ***5-HT$_{6}$*** | **1p35-36** | rs1805054[#] | SNP[ *C→T*] | Exon 1 | *CTGCAGCGTCTCCGAGGCCTGACTG* | *TGCTGATGCCGCTCATCTGCACTCA* | 4, 5 |
| ***5-HT$_{2C}$*** | **Xq24** | rs6318 (*cys23ser*) | SNP [*G→C*][a] | Exon 4 | *GGCCTATTGGTTTGGCCAT* | *CTGCCATGATCACAAGGATG* | 6, 7 |
| ***DRD4*** | **11p15.5** | rs1800955[#] | SNP [*C-T*] | Promoter | *TCAACTGTGCAACGGGTG* | *GAGAAACCGACAAGGATGGA* | 8 |
| | | N/A | 48bp VNTR | Exon 3 | *GCGACTACGTGGTCTACTCG* | *AGGACCCTCATGGCCTTG* | 9 |
| ***DRD2*** | **11q23.2** | rs1800497 (*Taq1A*)[#] | SNP[*C→T*] | 3'UTR | *CCGTCGACCCTTCCTGAGTGTCATCA* | *CCGTCGACGGCTGGCCAAGTTGTCTA* | 10 |
| ***COMT*** | **22q11.2** | rs2097603 | SNP [*A→G*] | Promoter | *CTCTGGCGGAAAGGAAT* | *TCGGCATCAAAAGGAGGAAAAAG* | 11 |
| | | rs4680 (*val158met*)[#] | SNP [*G→A*][b] | Exon 4 | *TCACCATCGAGATCAACCCC* | *ACAACGGGTCAGGCATGCA* | 12 |
| | | rs362204 | *C+-C-*[c] | 3'UTR | *TGCGGAAGGGGACAGTGCTAC* | *CCGGAGCCGCAGAAGGTCA* | 11 |
| ***DAT*** | **5p15.3** | N/A | 40bp VNTR[#] | 3' UTR | *TGTGGTGTAGGGAACGGCCTGAG* | *CTTCCTGGAGGTCACGGCTCAAGG* | 13 |
| ***DRD3*** | **3q13.3** | rs6280 (*ser9gly*)[#] | SNP [*A→G*][d] | Exon 1 | *GCTCTATCTCCAACTCTCACA* | *AAGTCTACTCACCTCCAGGTA* | 14 |
| ***DRD1*** | **5q35.1** | *A-48G*[#] | SNP [*A→G*] | Promoter | *GGCTTTCTGGTGCCCAAGACAGTG* | *AGCACAGACCAGCGTGTTCCCCA* | 15 |

[a]*G*-allele corresponds to the *cys23*-allele; [b]*G*-allele corresponds to the *val158* allele; [c] *C+* refers to the insertion of the *C*-allele*, C-* refers to the deletion of the *C*-allele; [d]*A*-allele corresponds to the *ser9*-allele.
[#]Variants used in structure analysis as well. **Abbreviations: *5-HT$_{2A}$*:** serotonin receptor 2A; ***5-HT$_{1Dβ}$***: serotonin receptor 1Dβ; ***5-HT$_{6}$***: serotonin receptor 6; ***5-HT$_{2C}$***: serotonin receptor 2C; ***DRD4:*** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: catechol-O-methyltransferase;, ***DAT*:** dopamine transporter; ***DRD3***: dopamine receptor 3; ***DRD1*:** dopamine receptor 1; **VNTR**: variable number of tandem repeats polymorphism.**References:**1: Arranz et al., 1998; 2: Warren et al; 1993; 3: Present Study; 4: Kohen et al., 1996; 5: Masellis et al., 2001; 6: Ebstein et al., 1997; 7: Rietschel et al., 1997; 8: Okuyama et al., 1999; 9: Lichter et al., 1993; 10: Arinami et al., 1997; 11: De Mille et al., 2002; 12: Karayiorgou et al., 1997; 13: Vandenbergh et al., 2000; 14: Crocq et al., 1992; 15: Cichon et al., 1996.

***Table II.1 (a) (continued).*** *Description of the candidate polymorphisms used in the genetic association analyses, indicating the chromosomal location of the variants and sequences of the external amplification primers.*

| Gene | Chromosomal Location | Variant identification | DNA variant | Domain | Forward Primer (5'-3') | Reverse Primer (5'-3') | Ref. |
|---|---|---|---|---|---|---|---|
| ***GRIN2B*** | **12p12.3** | rs1806191 | SNP [*A→G*] | Exon 13 | *GCAGTGTGCTAAATGGTCTCA* | *AGGAGCGGAGTGATGACTTTA* | 1 |
| | | rs890 | SNP [*A→C*] | 3' UTR | *GCAGTGTGCTAAATGGTCTCA* | *AGGAGCGGAGTGATGACTTTA* | |
| ***BDNF*** | **11p14.1** | rs6265 (*val66met*) | SNP [*G→A*][a] | Exon 6[c] | *AAAGAAGCAAACATCCGAGGACAA* | *ATTCCTCCAGCAGAAAGAGAAGAGG* | 2 |
| | | rs2049046 | SNP [*A→T*] | Intron 5[c] | *CTCTGTCAACCGTCTACCTGTG* | *CTGCATTCGAATTGCTTGTG* | 1 |
| | | rs988748 | SNP [*C→G*] | Intron 5[c] | *TTGGAGTAGGGTTCCTCCAGT* | *AGAGGGCATGAAGCTGGATA* | 1 |
| ***HOXB8*** | **17q21.32** | rs2303486 | SNP [*A→T*] | Promoter | *AAAACAGCCCTCAGACTGTCA* | *GGTGGGAGGTGGGGAGTA* | 1 |
| ***ESRα*** | **6q25.1** | rs9340799[#] | SNP [*A→G*] | Intron 1 | *CTGCCACCCTATCTGTATCTTTTCCTATTCTCC* | *TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA* | 3 |
| | | rs2234693 | SNP [*C→T*] | Intron 1 | *CTGCCACCCTATCTGTATCTTTTCCTATTCTCC* | *TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA* | |
| ***PLC-γ1*** | **20q12** | rs8192707 (*ser279gly*) | SNP [*G→A*][b] | Exon 9 | *GAGCTTTGCCGAGTGTCC* | *ATCGGGCTCACCTCATCCAGG* | 4 |
| ***ACE*** | **17q23** | N/A | *Alu ins/del* | Intron 16 | *CTGGAGACCACTCCCATCCTTTCT* | *GATGTGGCCATCACATTCGTCGTCAGA* | 5 |
| ***INPP-1*** | **2q32** | rs1882891[#] | SNP [*C→A*] | Exon 6 | *TAACCAGCAACAGGACAAAG* | *CTAGAAGAAACGGCAGTGAAAC* | 6 |

[a]*G*-allele corresponds to the *val66*-allele; [b]*A*-allele corresponds to the *ser279*-allele; [c]the polymorphism occurs in pro-*BDNF*; [#]Variants used in structure analysis as well.
**Abbreviations:** ***GRIN2B*****:** glutamate receptor subunit 2B; ***BDNF:*** brain-derived neurotrophic factor; ***HoxB8*****:** homeobox gene B8; ***ESRα*****:** estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme; **3'UTR**: 3' untranslated region. **References:** 1: Present study; 2: Sen et al., 2003; 3: Yaich et al., 1992; 4: Ftouhi-Paquin et al., 2001; 5: Rigat et al., 1992; 6: Steen et al., 1998.

***Table II.1 (b).*** *Description of the variants used in "*Structure*" analysis, indicating the chromosomal location and the sequences of external amplification primers.*

| Gene | Chromosomal Location | Variant identification | Type of variant | Forward Primer (5'-3') | Reverse Primer (5'-3') | Reference |
|---|---|---|---|---|---|---|
| ***FXIIIB*** | 1q31-32 | N/A | *Alu* insertion | *TCAACTCCATGAGATTTTCAGAAGT* | *CTGGAAAAAATGTATTCAGGTGAGT* | 1 |
| ***YaNBC182*** | 7 | N/A | *Alu* insertion | *GAAGGACTATGTAGTTGCAGAAGC* | *AACCCAGTGGAAACAGAAGATG* | 2 |
| ***DLX*** | 7q21.3 | *DLX int1C/T* | SNP [ *C→T* ] | *TGGTGCAGCTTCCTTTACCT* | *TGGTGCAGCTTCCTTTACCT* | 3 |
| ***TPA25*** | 8p12 | rs4646972 | *Alu* insertion | *GTGAAAAGCAAGGTCTACCAG* | *GACACCGAGTTCATCTTGAC* | 4 |
| ***ADRA1C*** | 8p21 | *cys492arg* | SNP [ *C→T* ] | *ATGCTCCAGCCAAGAGTTCA* | *TCCAAGAAGAGCTGGCCTTC* | 5 |
| ***YaNBC241*** | 15 | N/A | *Alu* insertion | *GGTTCCAATAGAGAGCAACAGAA* | *ACCTTAAGCTTTCCCCCAGA* | 7 |
| ***PV92*** | 16q24 | N/A | *Alu* insertion | *AACTGGGAAAATTTGAAGAGAAAGT* | *TGAGTTCTCAACTCCTGTGTGTTAG* | 8 |
| ***5-HTT*** | 17q11 | N/A | VNTR | *ATGCCAGCACCTAACCCCTAATGT* | *CGACCGCAAGGTGGGCGGGA* | 9 |
| ***SNAP25*** | 20p12 | *SNAP25 MnlI* | SNP [ *G→T* ] | *TTCTCCTCCAAATGCTGTCG* | *CCACCGAGGAGAGAAAAT* | 10 |
| ***GNAS*** | 20q13.2 | rs7121 | SNP [*A→G*] | *CTCCTAACTGACATGGTGCAA* | *TAAGGCCACACAAGTCGGGGT* | 11 |
| ***SNAP29*** | 22q11.21 | *C56T* | SNP [ *C→T* ] | *GGAAGGAGTTCGCGCGACGA* | *GCGAGTCCACACCAGCCCTG* | 12 |

**Abbreviations***:* ***FXIIIB***: Factor 13B; ***YaNBC182****:*Ya subfamily *Alu* insetion sequence ***NBC182; DLX6***: Distal-less like homeobox 6; ***TPA25***: Tissue plasminogen activator *Alu* insertion; ADRA1C: Adrenergic receptor α1C; DBH: Dopamine-β hydroxylase; ***YaNBC241***: Ya subfamily of *Alu* insertion repeats; ***PV92***: predicted variant *Alu* insertion repeat; ***5-HTT***: serotonin transporter; ***SNAP25***: Synaptosomal-associated protein 25kDa; ***GNAS***: guanine nucleotide-binding α subunit of G, ***SNAP29***: Synaptosomal-associated protein 29kDa; **VNTR**: variable number of tandem repeats.

**References:** 1: Batzer et al., 1996; 2: Carroll et al., 2001; 3: Nabi et al., 2003; 4: Tishkoff et al., 1996; 5: Barr et al., 2001; 6: Nabi et al., 2003; 7: Watkins et al., 2001; 8: Comas et al., 2001; 9: Gelernter et al., 1997; 10: Barr et al., 2000; 11: Jia et al., 1999; 12: Saito et al., 2001.

***Table II.2 (a).*** *PCR conditions for the genetic variants investigated, indicating optimal primer annealing temperatures, additives and size (in bp) of the resultant product.*

| Gene | Variant identification | $T_A$ (ºC) | Additive | Amplimer (bp) |
|---|---|---|---|---|
| ***5-HT$_{2A}$*** | rs6311 (-1438*A/G*)# | 63 | None | 468 |
| | rs6313 (*T102C*) | 60 | None | 372 |
| ***5-HT$_{1Dβ}$*** | rs6296 (*G861C*)# | 60 | None | 662 |
| ***5-HT$_{6}$*** | rs1805054# | 63.5 | DMSO | 578 |
| ***5-HT$_{2C}$*** | rs6318 (*cys23ser*) | 60 | None | 184 |
| ***DRD4*** | rs1800955# | 59 | Formamide | 380 |
| | 48bp VNTR | 60 | 7-deaza-GTP | 475[b] |
| ***DRD2*** | rs1800497(*Taq1A*)# | 60 | None | 310 |
| ***COMT*** | rs2097603 | 55.5 | DMSO | 306 |
| | rs4680(*val158met*) | 60 | None | 95 |
| | rs362204 | 66 | None | 277 |
| ***DAT*** | 40bp VNTR# | 70 | None | 480[c] |
| ***DRD3*** | rs6280 (*ser9gly*)# | 55 | DMSO | 462 |
| ***DRD1*** | rs4532# | 68 | DMSO | 405 |
| ***GRIN2B*** | rs1806191 | 60 | None | 1456 |
| | rs890 | 60 | None | 1456 |
| ***BDNF*** | rs6265 (*val66met*) | 63 | None | 274 |
| | rs2049046 | 60 | DMSO | 270 |
| | rs988748 | 60 | None | 178 |
| ***HOXB8*** | rs2303486 | 60 | None | 420 |
| ***ESRα*** | rs9340799# | 61 | DMSO | 1300 |
| | rs2234693 | 61 | DMSO | 1300 |
| ***INPP-1*** | rs1882891 | 59 | DMSO | 584 |
| ***PLCγ1*** | rs8192707 | 64 | DMSO | 282 |
| ***ACE*** | ACE Alu ins/del# | 63.5 | None | 534[a] |

# Variants used in "*Structure*" analyses as well; [a]size of the insertion allele indicated; [b]size of the 4-repeat allele indicated; [c]size of the 10-repeat allele indicated.

**Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A; ***5-HT$_{1Dβ}$***: serotonin receptor 1Dβ; ***5-HT$_{6}$***: serotonin receptor 6; ***5-HT$_{2C}$***: serotonin receptor 2C; ***DRD4:*** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: catechol-O-methyltransferase;, ***DAT*****:** dopamine transporter; ***DRD3***: dopamine receptor 3; ***DRD1*****:** dopamine receptor 1; **VNTR**: variable number of tandem repeats polymorphism. ***GRIN2B*****:** glutamate receptor subunit 2B; ***BDNF:*** brain-derived neurotrophic factor; ***HoxB8*****:** homeobox gene B8; ***ESRα*****:** estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme; **DMSO**: dimethylsulphoxide.

*[$MgCl_2$] of 1.5mM was used in all reactions.*

***Table II.2 (b).*** *PCR conditions used in the amplification of the polymorphic sites in the genes utilised in* "Structure" *analyses, indicating optimal primer annealing temperatures, additives and size (in bp) of the resultant product.*

| Gene | Variant identification | $T_A$ (ºC) | Additive | Amplimer (bp) |
|---|---|---|---|---|
| ***FXIIIB*** | N/A | 51 | None | 795[a] |
| ***YaNBC182*** | N/A | 60 | Formamide | 563[a] |
| ***DLX*** | *DLX int1C/T* | 60 | None | 203 |
| ***TPA25*** | N/A | 53 | Glycerol | 570[a] |
| ***ADRA1C*** | *cys492arg* | 58 | None | 501 |
| ***YaNBC241*** | N/A | 55.5 | None | 392[a] |
| ***PV92*** | N/A | 55.5 | Glycerol | 450[a] |
| ***5-HTT*** | 44bp VNTR | 66 | None | 419[a] |
| ***SNAP25*** | *SNAP25 MnlI* | 60 | None | 261 |
| ***GNAS*** | rs7121 | 59 | None | 345 |
| ***SNAP29*** | *C56T* | 68 | None | 377 |

[a]size of the allele containing the insertion indicated**.**
**Abbreviations:** ***FXIIIB*****:** : Factor 13B Alu insertion polymorphism; ***YaNBC182***: Ya subfamily *Alu* insertion sequence ***NBC182; DLX6:*** Distal-less like homeobox 6; ***TPA25*****:** Tissue plasminogen activator *Alu* insertion; ***ADRA1C:*** adrenergic receptor α 1C; ***DBH***: dopamine beta –hydroxylase; ***YaNBC241*****:** Ya subfamily *Alu* insetion sequence ***NBC241; PV92***: predicted variant *Alu* insertion repeat; ***5-HTT***: serotonin transporter; ***SNAP-25***: synaptosomal-associated protein of 25 kDa; ***GNAS***: guanine nucleotide-binding α subunit of G; ***SNAP-29:*** synaptosomal-associated protein of 29 kDa; **DMSO**: dimethylsulfoxide.
*[$MgCl_2$] of 1.5mM was used in all reactions.*

For the subsequent genotyping of the polymorphisms, the digested products were electrophoresed through either agarose (**section II.6.1**), or 12% non-denaturing polyacrylamide (**section II.6.2**) gels, depending on the size of the resultant fragments. As a control for incomplete digestion, an aliquot of undigested PCR amplified sample was co-electrophoresed alongside samples to be genotyped. Moreover, the *5-$HT_{2C}$*, *COMT (val158met)*, *DRD3*, *DRD1*, *BDNF (val66met), INPP-1, SNAP-25* and *SNAP-29* amplified products contained internal controls for restriction digestion in the form of additional, invariant, restriction enzyme recognition site that resulted in constitutive fragments produced during restriction digestion (Tables II.4[a] and [b]).

***Table II.3.*** *Expected size fragments produced by the* DRD4 *48bp VNTR,* DAT *40bp VNTR,* Alu *insertion polymorphisms and* 5-HTT *44bp VNTR, with the % (w/v) agarose gel used to resolve the fragments for genotyping.*

| Gene | Polymorphism | Allele | Fragment size | % (w/v) agarose gel |
|---|---|---|---|---|
| ***DRD4*** | 48bp VNTR | *A2* | 379 | 3 |
| | | *A3* | 427 | |
| | | *A4* | 475 | |
| | | *A5* | 523 | |
| | | *A6* | 571 | |
| | | *A7* | 619 | |
| | | *A8* | 667 | |
| ***DAT*** | 40 bp VNTR | *A2* | 160 | 2 |
| | | *A6* | 320 | |
| | | *A7* | 360 | |
| | | *A8* | 400 | |
| | | *A9* | 440 | |
| | | *A10* | 480 | |
| | | *A11* | 520 | |
| ***FXIIIB*** | *Alu* ins/del | *I*[c] | 795 | 2 |
| | | *D*[d] | 501 | |
| ***YaNBC182*** | *Alu* ins/del | *I*[c] | 563 | 2 |
| | | *D*[d] | 287 | |
| ***TPA25*** | *Alu* ins/del | *I*[c] | 570 | 2 |
| | | *D*[d] | 260 | |
| ***YaNBC241*** | *Alu* ins/del | *I*[c] | 392 | 3 |
| | | *D*[d] | 66 | |
| ***PV92*** | *Alu* ins/del | *I*[c] | 450 | 2.5 |
| | | *D*[d] | 130 | |
| ***5-HTT*** | 44bp VNTR | *L*[a] | 419 | 2 |
| | | *S*[b] | 375 | |
| ***ACE*** | *Alu* ins/del | *I*[c] | 534 | 2 |
| | | *D*[d] | 243 | |

[a]L: "long" allele; [b]S: "short" allele [c]I: allele formed by the insertion of an *Alu* element; [d]D: allele formed by the deletion of an *Alu* element;. **Abbreviations:** ***DRD4:***Dopamine receptor 4; ***DAT***: Dopamine Transporter; ***FXIIIB*****:** : Factor 13B; ***YaNBC182***: Ya subfamily *Alu* insertion sequence NBC182; ***TPA25*****:** Tissue plasminogen activator *Alu* insertion; ***YaNBC241*****:** Ya subfamily *Alu* insertion sequence ***NBC241; PV92***: predicted variant *Alu* insertion repeat; ***ACE***: Angiotensin-converting enzyme; **VNTR**: variable number of tandem repeats.

***Table II.4(a).*** *Genotyping details for SNPs in candidate genes that were genotyped using ASREA.*

| Gene | Polymorphism | Restriction enzyme | Allele | Fragment size (bp) | Allele detection |
|---|---|---|---|---|---|
| ***5HT$_{2A}$*** | rs6311 | *Msp*I | *A* | 468 | 2.5% agarose |
| | | | *G* | 244 + 224 | |
| | rs6313 | *Hpa*II[1] | *T* | 372 | 2.5% agarose |
| | | | *C* | 156+216 | |
| ***5-HT$_{1DB}$*** | rs6296 | *Hinc*II[1] | *G* | 662 | 2% agarose |
| | | | *C* | 482+180 | |
| ***5-HT$_{6}$*** | rs1805054 | *Rsa*I[1] | *T* | 578 | 2.5% agarose |
| | | | *C* | 449+129 | |
| ***5-HT$_{2C}$*** | rs6318 | *Nla*III[2] | *C(ser23)* | 150+**30+4*** | 3% agarose |
| | | | *G(cys23)* | 130+**30**+20+**4*** | |
| ***DRD4*** | rs1800955 | *Fsp*I[2] | *C* | 380 | 2.5% agarose |
| | | | *T* | 228 +152 | |
| ***DRD2*** | rs1800497 | T*aq*I[1] | *T* | 310 | 2.5% agarose |
| | | | *C* | 130+180 | |
| ***COMT*** | rs2097603 | *Hind*III[1] | *A* | 306 | 2.5% agarose |
| | | | *G* | 271 + 75 | |
| | rs4680 | *Nla*III[2] | *G(val158)* | 82 + **13*** | 12% PAGE |
| | | | *A(met158)* | 68 + 14 + **13*** | |
| | rs362204 | *Bgl*I[1] | *C deletion* | 278 | 2.5% agarose |
| | | | *C insertion* | 196 + 82 | |
| ***DRD3*** | rs6280 | *Msc*I[3] | *A (ser9)* | 304+**111+47*** | 3% agarose |
| | | | *G (gly9)* | 206+98+**111+47*** | |
| ***DRD1*** | A-48G | *Dde*I[3] | G | 259+**146*** | 2.5% agarose |
| | | | A | **146***+42+217 | |
| ***BDNF*** | rs6265 | *Nla*III[2] | *G(val66)* | 217+**57*** | 3% agarose |
| | | | *A(met66)* | 140+77+**57*** | |
| ***ESRα*** | rs9340799 | *Xba*I[1] | *G* | 1300 | 1.5% agarose |
| | | | *A* | 910+390 | |
| | rs2234693 | *Pvu*II[1] | *C* | 1300 | 1.5% agarose |
| | | | *T* | 850+450 | |
| ***INPP-1*** | rs1882891 | *Dde*I[3] | *C* | 256+**143**+**142**+**43*** | 2.5% agarose |
| | | | *A* | 215+41+**143**+**142**+**43*** | |
| ***PLC-γ1*** | rs8192707 | *Blp*I[2] | *G(gly279)* | 282 | 2.5% agarose |
| | | | *A(ser279)* | 203+79 | |

*invariant restriction enzyme recognition sites. Only the underlined ones were visible on the gels used in the present study, and could thus serve as a control for complete digestion.

[1] Promega Corp., Madison, WI. USA; [2] New England Biolabs, Beverly, MA., USA; [3] Roche Applied Science, Basel, Switzerland.

**Abbreviations**: ***5-HT$_{2A}$*****:** serotonin receptor 2A; ***5-HT$_{1Dβ}$***: serotonin receptor 1Dβ; ***5-HT$_{6}$***: serotonin receptor 6; ***5-HT$_{2C}$***: serotonin receptor 2C; ***DRD4:*** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: catechol-O-methyltransferase;, ***DRD3***: dopamine receptor 3; ***DRD1*****:** dopamine receptor 1; ***GRIN2B*****:** glutamate receptor subunit 2B; ***BDNF:*** brain-derived neurotrophic factor; ***HoxB8*****:** homeobox gene B8; ***ESRα*****:** estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; **12% PAGE**: 12% non-denaturing polyacrylamide gel electrophoresis

***Table II.4(b).*** *Genotyping details for SNPs genotyped using ASREA that were included in "*Structure*" analyses.*

| Gene | Polymorphism | Restriction enzyme | Allele | Fragment size | Allele detection |
|---|---|---|---|---|---|
| ***DLX*** | *int1C/T* | *Apo*I[2] | *C*<br>*T* | 203<br>176+27 | 3% Agarose |
| ***ADRA1C*** | *cys492arg* | *Pst*I[1] | *C(cys492)*<br>*T(arg492)* | 502<br>477+25 | 3% Agarose |
| ***SNAP25*** | *SNAP25 Mnl*I | *Mnl*I[2] | *T*<br>*G* | 256+**6***<br>211+44+**6*** | 2% Agarose |
| ***GNAS*** | rs7121 | *Fok*I[2] | *T*<br>*C* | 345<br>263+82 | 2.5% Agarose |
| ***ABCG1*** | *G2457A* | *Hha*I [1] | *A*<br>*G* | 425<br>321+104 | 2% Agarose |
| ***SNAP29*** | *C56T* | *Dde*I[3] | *C*<br>*T* | 269+**108***<br>177+**108***+92 | 12% PAGE |

*invariant restriction enzyme recognition sites. Only the underlined ones were visible on the gels used in the present study, and could thus serve as a control for complete digestion.

[1] Promega Corp., Madison, WI. USA; [2] New England Biolabs, Beverly, MA., USA; [3] Roche Applied Science, Basel, Switzerland.

**Abbreviations:** ***DLX6:*** Distal-less like homeobox 6; ***ADRA1C:*** adrenergic receptor α 1C; ***SNAP-25***: synaptosomal-associated protein of 25 kDa; ***GNAS***: guanine nucleotide-binding α subunit of G; SNAP-29: synaptosomal-associated protein of 29 kDa; **12% PAGE**: 12% non-denaturing polyacrylamide gel electrophoresis.

### II.5.3. Genotyping by Single Nucleotide ddNTP Primer Extension (SNaPshot) Analysis

The SNaPshot genotyping method (Applied Biosystems, Foster City, California, USA) involves the extension of an oligonucleotide probe (that terminates immediately 5' to the SNP of interest) by one of four fluorescently-labelled dideoxynucleotides complementary to the base sequence at the SNP site of interest (Figure II.1). Please refer to **section II.4.1** and Table II.5 for the details on design and sequences of the interrogation primers, respectively.

***Table II.5.*** *Sequences of the internal interrogation primers (5'-3') used in the SNaPshot genotyping procedure.*

| Gene | dbSNP[a] | Internal interrogation primer sequence (5'-3') |
|---|---|---|
| ***BDNF*** | rs2049046 | *CTGCATTCGAATTGCTTGTG* |
| | rs988748 | *AACCAACGCAGAGGGTCT* |
| ***GRIN2B*** | rs1806191 | *GTTTGTCGCCCGTCCCGTGCTTGAT* |
| | rs890 | *GCTTCCTCACCTAAATGAAAAGATC* |
| ***HoxB8*** | rs2303486 | *AGACTCCTGAGTGAGG* |

**Abbreviations:**[a]: identification on the NCBI SNP Database, http://www.ncbi.nlm.nih.gov/SNP/index.html**;** ***BDNF:*** brain-derived neurotrophic factor**;** ***GRIN2B***: glutamate receptor subtype 2B**;** ***HoxB8*****:** homeobox gene type B8.

#### II.5.3.1. PCR reaction clean-up

The first step in the SNaPshot reaction entails a PCR-product purification step, to remove excess dNTPs and to dephosphorylate unincorporated outer primers that may interfere with the SNaPshot reaction. Here, 5µl of the relevant PCR products were incubated with 0.33U *Exo*I (Amersham, Little Chalfont, Buckinghamshire, UK) and 0.66U shrimp alkaline phosphatase (SAP) (Roche Applied Science, Basel, Switzerland) at 37˚C for one hour, followed by an enzyme deactivation step at 75˚C for 30 minutes. The purified PCR template was subsequently stored at 4˚C until required.

#### II.5.3.2. Primer extension reaction conditions

For multiplexing, equal quantities of PCR templates were mixed in a tube. Likewise, all internal interrogation primers to be used in a single multiplex reaction were premixed to yield a final concentration of 0.2µM for each primer. The extension reaction, comprising 3µl of previously cleaned, pooled PCR products, 3µl SNaPshot Multiplex Ready Reaction mix (Applied Biosystems, Foster City, California, USA), 1µl pooled internal primers and 1µl de-ionised water, was performed by repeating the following cycle 27 times: 96˚C for 10s, 50˚C

for 5s, and 60˚C for 30s. Thereafter, a post-extension purification step was employed to avoid further primer extension. This was performed by adding 1U of SAP to the sample, which was subsequently incubated at 37˚C for one hour, and then at 72˚C for 30 minutes to deactivate the enzyme.

**II.5.3.3. Analysis on ABI Prism 3130 Genetic Analyser**

The fluorescently extended probes were separated and detected on an ABI Prism 3130 Genetic Analyser capillary electrophoresis system (Applied Biosystems, Foster City, California, USA). After an appropriate spectral matrix using materials from the matrix standard DS-02 (Applied Biosystems, Foster City, California, USA) was created, the ABI Prism 3130 Genetic Analyser was used with filter set E5 to process the data from the 5 dyes, namely dR110, dR6G, dTAMRA, dROX and LIZ.

Fluorescently labelled extension reactions were prepared for capillary electrophoresis analysis by mixing 9 µl of Hi-Di formamide (Applied Biosystems, Foster City, California, USA), 1µl of the SNaPshot product and 0.4µl of GeneScan-120 LIZ internal sizing standard (Applied Biosystems, Foster City, California, USA). The samples were then denatured by placing them at 95˚C for 2 minutes. The prepared samples were then stored on ice until loaded into the capillary electrophoresis system.

A 36cm capillary array filled with denaturing POP4 performance optimised polymer (Applied Biosystems, Foster City, California, USA) was used for DNA fragment separation. Genetic Analyser electrode running buffer with EDTA was used in 1x concentration. Typical run module parameters were: run temperature 60˚C, capillary fill volume 38000 steps, pre-run voltage 15kV, data delay 3600s and run time 14000s.

Two negative controls were electrophoresed with each multiplex: a PCR template without primers, and the internal pooled primers without template. Allele assignment was subsequently performed using ABI Prism Genotyper software (GeneMapper ID, Ver 3.7 [Applied Biosystems, Foster City, California, USA]).

**II.5.4. Allele Separation by Capillary Electrophoresis**

In order to implement quality control for the *DRD4* 48bp VNTR genotyping process, additional high precision allele analysis was conducted by capillary electrophoresis on the

ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Here, the PCR was performed using a forward primer labelled with 6-FAM. The fluorophore-labelled PCR-amplified products were subsequently electrophoresed on a 2% agarose gel to ensure that the correct fragment was amplified.

Depending on the concentration of DNA as estimated from gel electrophoresis, the products were diluted 10-fold using $ddH_2O$. 1µl of the diluted 6-FAM-labelled product was mixed with 0.5µl ROX1000 fragment size standard (Applied Biosystems, Foster City, CA, USA) and 9µl Hi-Di formamide (Applied Biosystems, Foster City, CA, USA).

For each run, the capillary column was filled with POP-7 sieving medium. The prepared products were then denatured at 95˚C for 2 minutes and snap-cooled and stored on ice until loaded onto the capillary electrophoresis system. The electrophoretic separation was performed for 20 minutes at a field strength of 415V/cm, and a capillary temperature of 60˚C for each loading.

Analysis of the resulting electropherogram was performed using ABI Prism software (GeneMapper ID, Ver 3.7 [Applied Biosystems, Foster City, California, USA]). The allele sizes (provided in Table II.3) were automatically determined by the software by use of the local Southern size calling method to establish a best-fit curve generated from the internal size standard, ROX1000 (Applied Biosystems, Foster City, California, USA).

### II.5.5. Quality Control for Genotyping

In order to ensure genotyping accuracy, replicate samples were included in the PCR-amplification reactions and subsequent genotyping procedures, and only assays that provided 100% concordance between replicates were utilised in the analyses.

## II.6. GEL ELECTROPHORESIS

### II.6.1. Agarose Gel Electrophoresis

Agarose gels were used in the present study to verify success of the PCR (**section I.4.2**), and to resolve fragments of different sizes in order to genotype selected polymorphisms (**sections I.5.1 and I.5.2**). Horizontal agarose gels, of either 15x9x1cm or 7x9x1cm dimensions, were

all impregnated with 0.5µg/ml ethidium bromide (EtBr). 1X TBE running buffer (**Appendix I**) was used to facilitate all agarose gel electrophoreses.

To verify the success of PCR-amplification, 8µl of the amplified product was mixed with 2 µl bromophenol blue loading dye (**Appendix I**), and electrophoresed in a 2% (w/v) agarose gel. For the genotyping of the VNTRs and *Alu* insertion polymorphisms (Table II.3), 10µl of the amplified product was mixed with 2µl bromophenol blue loading dye, and electrophoresed in a 2 - 3% (w/v) agarose gel.

For the genotyping of selected polymorphisms detected by ASREA, 10µl of the digested product was mixed with 2µl bromophenol blue loading dye. This mixture was subsequently electrophoresed in a 2-3% (w/v) agarose gel, depending on the size of the product after digestion with the appropriate restriction enzyme (please refer to Table II.4 for a list of those SNPs genotyped by ASREA that required agarose gel electrophoresis).

The molecular size marker, co-electrophoresed with all PCR-amplified products was one of the following: bacteriophage λ DNA, digested with the *Pst*I restriction enzyme (Promega, Madison, WI, USA) (λPst [**Appendix I**]), Marker X (Roche Applied Science, Basel, Switzerland), Marker XIV (100bp marker [Roche Applied Science, Basel, Switzerland]), Marker VI (Roche Applied Science, Basel, Switzerland) or Low Molecular Weight Marker (New England Biolabs, Beverly, MA, USA). Electrophoresis typically occurred at 10V/cm for between 30 minutes and 1 hour in 1X TBE running buffer.

All gels were visualised on a longwave ultraviolet transilluminator (3UV$^{TM}$ Transilluminator model LMS-26E), and photographed using a video printer (Sony).

### II.6.2. 12% non-denaturing Polyacrylamide Electrophoresis

Non-denaturing polyacrylamide gels were used for the resolution of fragments formed during ASREA of selected SNPs (Tables II.4[a] and [b]). Briefly, 2µl bromophenol blue dye (Appendix I) was added to 10µl of each of the samples to be electrophoresed. This mixture was then loaded into a polyacrylamide gel, of 100x80x1mm dimensions, containing 12% acrylamide and 1x TBE buffer (**Appendix I**). In all cases, either λ*Pst* marker (Appendix I), Marker X (Promega, Madison, WI, USA) or Marker XIV (100bp marker [Promega, Madison, WI, USA]) and an undigested sample were co-electrophoresed as controls.

Electrophoresis was performed in 1x TBE running buffer (**Appendix I**) at 100-120 V for 1.5 to 2 hours. After completion of electrophoresis, the polyacrylamide gel was placed immersed in a solution of 0.1% AgNO3 (**Appendix I**) and gently shaken for 10 minutes on a Labcon orbital shaker (Labcon Pty Ltd, Maraisburg, RSA). The $AgNO_3$ solution was then discarded and the gel subsequently rinsed with water. Thereafter, developing solution (**Appendix I**) then poured so as to cover the gel, which was gently shaken until stained bands became visible. The gels were then thoroughly rinsed using $dH_2O$, and viewed using a white light illuminator (Lauda Thermostat, Germany) and photographed using a video printer (Sony).

## II.7. STATISTICAL ANALYSES

R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.R) was used for all statistical analyses unless otherwise specified. Functions from base R and specific R packages were used. Specific programs were written for applications when functions could not be found. In order to test the association between the relevant phenotype and multiallelic markers (*DAT* 40bp and *DRD4* 48bp VNTRs) CLUMP (Curtis and Sham, 1995) was used.

### II.7.1. Statistical Analyses of Demographic Data

Initial demographic analyses were conducted to determine whether between-group differences existed in gender (Fisher test for equality of proportions) and age (Wilcoxon test for equality between medians).

### II.7.2. Statistical Analyses of Clinical Data

Clinical variables, such as total Y-BOCS score , age at onset of OCD, the presence or absence of specific symptom subtypes (classified under the clusters of hoarding, symmetry/ordering, sexual/religious, aggression and contamination/washing symptom dimensions) and selected co-morbid disorders (MDD, BDD, anorexia, TTM, TS, hypochondriasis, social and specific phobias, dysthymic disorder, SIB, IED, tics, GAD and panic disorder), were tabulated for OCD individuals. These main clinical variables were also compared between genders within the OCD patient subset.

Continuous variables are summarised as medians with corresponding 95% CIs, and p-values are for nonparametric tests of equality of medians (Wilcoxon when comparing two medians;

Kruskal-Wallis when comparing more than two). Categorical variables were represented as counts, with their associated frequencies and p-values for Fisher's exact test for equality of proportions.

### II.7.3. Statistical Analyses of Genetic Data

The aim of the present study was to determine whether, in each of the polymorphisms within selected candidate genes, any significant differences in allelic or genotypic distribution existed between Afrikaner OCD and control subjects, or within OCD patients in specific OCD subgroups. Increased allele or genotypic frequencies in OCD subjects, compared to controls, or in OCD subjects in a particular OCD subgroup compared to those in another subgroup, could indicate that either the allele or genotype confers increased susceptibility to OCD (or the phenotype represented by the OCD subgroup under investigation), or is in LD with such a risk variant.

However, comparing the allele frequencies between cases and controls is not statistically valid without the prior assumption that the alleles within each subject are segregating independently of one another. For this to be true, the population under investigation should obey the Hardy-Weinberg law, which states that, in a large, randomly mating population, the allelic and genotypic frequencies remain constant from one generation to the next. Thus, for a locus comprising segregating alleles *A* and *a*, with population frequencies of p and q respectively, the genotypic frequencies within a population in Hardy-Weinberg equilibrium (HWE) will be equivalent to $p^2$ (*AA*), 2pq (*Aa*) and $q^2$ (*aa*).

In the present study, exact p-values for a test of HWE were calculated using the R package *"genetics"*. Because *5-HT$_{2C}$* represents a SNP on chromosome X, resulting in hemizygous genotypes in the male population, HWE was calculated using the female population.

In order to provide a measure of informativeness of each genetic marker, heterozygosity (H) was also calculated.

### *II.7.3.1. Genetic investigations*

In all genetic analyses involving *5-HT$_{2C}$ cys23ser*, males and females were analysed separately.

#### *II.7.3.1.1. Single locus analyses*

**i. Categorical Data**

Categorical analyses involved comparing

1. allele and genotype distributions between OCD and control subjects,
2. OCD subjects experiencing certain symptom subtypes (hoarding, symmetry/ordering, sexual/religious, contamination and aggression obsessions and compulsions) and those not experiencing the relevant symptom subtype,
3. OCD subjects presenting with co-morbid MDD and those without, and
4. OCD subjects presenting with co-morbid tics and those without.

Analyses involving dichotomous categorical data were performed using the Fisher test, from which the exact p-values were calculated. The genotypic ORs, their 95% CIs and corresponding p-values were calculated for each marker under the assumption of the HWE.

For multi-allelic loci, "CLUMP" software was implemented [Sham and Curtis, 1995]. This program generates a novel chi-squared value by "clumping" columns together in a 2-by-2 table, ultimately designed so that the $\chi^2$ value is maximal. This is akin to testing a post-hoc hypothesis that a certain row has higher $\chi^2$ values than the other columns, without the need for correction for multiple testing.

Post-test powers were estimated for each single locus analysis individually. These values approximate the probability for a new study of rejecting the null hypothesis, given the sample size in our study, and assuming that the given that the specific effect size that is observed is the true effect size. Power estimates were calculated assuming the absence of any genotyping errors.

**ii. Numerical data**

The genotype and allele distributions in terms of two quantitative variables were also investigated, namely severity of OCD as measured by the total Y-BOCS score and age at onset of OCD.

Analyses involving the total Y-BOCS score were performed using the Wilcoxon test for allelic association and the Kruskal-Wallis test for genotypic association. The estimated medians and their 95% CIs constitute their effect measures.

To assess whether the genotypes of the candidate genes under investigation influenced the age at onset of OCD, Kaplan-Meier disease-free survival functions were calculated. The survival functions for each genotype were compared using the logrank test (R package "*survival*" [version 2.17]). The estimated medians of the survival functions and their 95% CIs represent the effect measures. For bi-allelic variants, where the number of individuals carrying a particular homozygous genotype was less than 5, those subjects were included in the same group as heterozygous individuals. For multi-allelic variants, rare genotypes were grouped together in the analyses.

Power analyses were not conducted for numerical data, since power calculations based on the medians of numerical values are complex and require numerous assumptions regarding the underlying distributions. These power calculations are also inaccurate for groups of below 30.

#### *II.7.3.1.2. Multi-locus analyses*

**i. Linkage disequilibrium (LD)**

Pairwise LD was assessed using the *LD* function from the R statistical genetics package.
Values of D'> 0.33 (Moffat et al., 2000; Kruglyak, 1999), and $r^2$> 0.1 (Ardlie et al., 2002; Tiret et al., 2002) were applied as a criterion for useful LD.

**ii. Haplotype analyses**

Where more than one polymorphism had been genotyped per gene, haplotype analyses were conducted using "haplo.stats" (version 1.2.0). This program assigns the probability for the occurrence of each haplotype in each individual and then directly models an individual's phenotype as a function of the inferred haplotype, weighted by their estimated probability to account for haplotype ambiguity. It then generates both global and haplotype-specific score statistics with associated p-values (Schaid et al., 2002) (a global haplotype score statistic is that which is obtained by applying a global test for association on H-1 df [where H is the number of haplotypes for which data is available], and a haplotype-specific score refers to a

test for association for that particular haplotype). The number of simulations for the empirical global and specific p-values was set at 1000.

To avoid possible errors in the haplotype estimation process, haplotype analyses were limited to those haplotypes with an expected count of more than 5.

### II.7.4. Multiple comparison considerations

P-values attained in the present study were not corrected for multiple testing, given the present uncertainty about which test is most appropriate. The statistically significant associations can only be validated when their biological meanings have been identified; moreover, p-values depend largely on the sample size; hence the emphasis is placed on the OR values (calculated for all categorical analyses) and their corresponding 95% CIs, which will provide one with a measure of the strength of association.

### II.7.5. Meta-analyses

Meta-analyses were performed on published data pertaining to the relation between OCD and the *5-$HT_{2A}$ T102C* (rs6313); *5-$HT_{2A}$ -1438A/G* (rs6311); *DRD3 ser9gly* (rs6280); *DRD2 Taq1A* (rs1800497); *5-$HT_{2C}$ ser23cys*, *COMT val158met* (rs4680) and *DAT* 40bp VNTR polymorphisms.

#### II.7.5.1. Literature search

To identify studies eligible for inclusion in the meta-analyses, a literature search using the National Library of Medicine's PubMed search engine, was conducted (www.ncbi.nih.gov/PubMed). The keywords "obsessive-compulsive disorder", "obsessive-compulsive*", "OCD", "genetics", "5-HT2A", "5HT2A', "HTR2A", "5-HT2C", "5HT2C", "HTR2C", "DRD2", "DRD3", "DAT", "DAT1", "dopamine transporter", "COMT", "serotonin", "dopamine" "receptors", "case-control" and "association", used in numerous different combinations, were used to search for the relevant articles. Retrieved articles were surveyed, and the reference sections were reviewed to identify studies that may not have been indexed by PubMed.

#### II.7.5.2. Inclusion Criteria

Only population-based case-control studies were included in the meta-analyses. Studies were only included if they investigated subjects with OCD as their primary diagnosis. Moreover,

only studies involving the relevant polymorphisms in each gene under investigation were utilised (*T102C* and *-1438A/G* in *5-HT$_{2A}$*, *ser23cys* in *5-HT$_{2C}$*, *ser9gly* in *DRD3*, *Taq1A* in *DRD2*, *val158met* in *COMT,* the 48bp VNTR in *DRD4* and the 40bp VNTR in *DAT*). The control data in each included study had to obey HWE. Studies also had to be published in a peer-reviewed journal and to represent original data.

Application of these criteria yielded four *5-HT$_{2A}$ T102C* and *5-HT$_{2A}$* -1438*A/G* studies, three *5-HT$_{2C}$ ser23cys* studies, four *DRD3 ser9gly* studies, three *DRD2 Taq1A* studies, two DRD4 48bp VNTR studies and two *DAT* 40bp VNTR studies for inclusion in the meta-analysis.

**II.7.5.3. Characteristics of the included studies**

It was also important to account for possible moderating influences that may have influenced the effect size obtained in a particular study, or which may have resulted in heterogeneity between the study samples. Each study was thus coded according to the following possible moderating influences: ethnicity of the sample, the mean age of the control and OCD groups, the mean age at onset of OCD, the gender index and the diagnostic system used. These descriptive characteristics are represented separately for each meta-analysis, in Tables II.6 (a) to (h).

***Table II.6 (a).*** *Descriptive characteristics of the studies (excluding the present study) included in the* 5-$HT_{2A}$ -1438A/G *(rs6311) meta-analysis.*

| Reference | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects (y) | Mean age at onset (y) | n (control) | Mean age control subjects (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Enoch et al. (2001) | N. American Caucasian | DSM-III-R | SADS-L, SCID-I | 101 | *Males*: 39.7±9.2 *Females*: 41.7±11.9 | 14.8±9 | 138 | *Males*: 40.25±12.4 *Females*: 39±12.9 | 0.56 |
| Tot et al. (2003) | Turkish | DSM-IV | SCID-I, Y-BOCS | 58 | 30±9 | 21±7 | 83 | 27±5 | 1.95 |
| Walitza et al. (2004) | German | DSM-IV | Y-BOCS, DIPS | 55 | 12.11±2.11 | - | 77 | 25.2 | 0.83 |

[a]Gender index= (female cases/male cases)/(female controls/male controls). **Abbreviations:** DSM: Diagnostic and Statistical Manual; SADS-L: Structured Clinical Interview for Affective Disorders and Schizophrenia-Lifetime Version (NIAAA); SCID-I: Structured Clinical Interview for DSM-IV Axis I Disorders (NIMH); Y-BOCS: Yale-Brown Obsessive-Compulsive Scale; DIPS: Diagnostisches Interview bei psychischen Storungen im Kindes-und Jugendalter.
Dashes indicate missing or insufficient data.

***Table II.6 (b).*** *Descriptive characteristics of the studies (excluding the present study) included in the* 5-$HT_{2A}$ T102C *(rs6313) meta-analysis.*

| Reference | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects (y) | Mean age at onset (y) | n (control) | Mean age control subjects (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Nicolini et al. (1996) | Mexican | DSM-III-R | DIS (Spanish version), Y-BOCS | 67 | 32.3±10.8 | - | 54 | 36.4±11.4 | 2.23 |
| Frisch et al. (2000) | Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 39 | - | - | 112 | - | 0.92 |
| Frisch et al. (2000) | non-Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 36 | - | - | 60 | - | 0.69 |
| Tot et al. (2003) | Turkish | DSM-IV | SCID-I, Y-BOCS | 58 | 30±9 | 21±7 | 83 | 27±5 | 1.95 |
| Meira-Lima et al. (2004) | Brazilian | DSM-IV | SCID, Y-BOCS | 79 | 33.6±10 | - | 202 | 33.9±9 | 0.81 |

[a]Gender index= (female cases/male cases)/(female controls/male controls). **Abbreviations:** DSM: Diagnostic and Statistical Manual; SADS-L: Structured Clinical Interview for Affective Disorders and Schizophrenia-Lifetime Version (NIAAA); SCID-I: Structured Clinical Interview for DSM-IV Axis I Disorders (NIMH); SCID-P: Structured Clinical Interview for DSM-IV Patient Version; Y-BOCS: Yale-Brown Obsessive-Compulsive Scale.
Dashes indicate missing or insufficient data.

***Table II.6 (c).*** *Descriptive characteristics of the studies (excluding the present study) included in the* 5-$HT_c$ ser23cys *(rs6318) meta-analysis.*

| Reference | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects (y) | Mean age at onset (y) | n (control) | Mean age control subjects (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Cavallini et al. (1998) | Italian | DSM-III-R | Y-BOCS | 109 | 31.41±11 | 19.86 | 107 | 33.13±11 | 0.99 |
| Frisch et al. (2000) | Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 39 | - | - | 112 | - | 0.92 |
| Frisch et al. (2000) | non-Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 36 | - | - | 60 | - | 0.70 |

[a]Gender index= (female cases/male cases)/(female controls/male controls). **Abbreviations:** DSM: Diagnostic and Statistical Manual; SADS-L: Structured Clinical Interview for Affective Disorders and Schizophrenia-Lifetime Version ; SCID-P: Structured Clinical Interview for DSM-IV Patient Version.
Dashes indicate missing or insufficient data.

***Table II.6 (d).*** *Descriptive characteristics of the studies (excluding the present study) included in the* DRD2 Taq1A *(rs1800497) meta-analysis.*

| Reference | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects (y) | Mean age at onset (y) | n (control) | Mean age control subjects (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Nicolini et al. (1996) | Mexican | DSM-III-R | DIS (Spanish version), Y-BOCS | 67 | 32.3±10.8 | - | 54 | 36.4±11.4 | 2.23 |

[a]Gender index= (female cases/male cases)/(female controls/male controls).
**Abbreviations:** DSM: Diagnostic and Statistical Manual; Y-BOCS: Yale-Brown Obsessive-Compulsive Scale; DIS: Diagnostic Interview Scale.
Dashes indicate missing or insufficient data.
Data from Billet et al.(1998) was excluded due to inconsistencies in their data.

***Table II.6 (e).*** *Descriptive characteristics of the studies (excluding the present study) included in the* DRD3 ser9gly *(rs6280) meta-analysis.*

| Reference | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects (y) | Mean age at onset | n (control) | Mean age control subjects (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Catalano et al. (1994) | N. American | DSM-III-R | Y-BOCS | 97 | 33.5±12.1 | 20.6±10.9 | 97 | 30.7±8.6 | 0.63 |
| Nicolini et al. (1996) | Mexican | DSM-III-R | DIS (Spanish version), Y-BOCS | 67 | 32.3±10.8 | - | 54 | 36.4±11.4 | 2.23 |

[a]Gender index= (female cases/male cases)/(female controls/male controls).
**Abbreviations:** DSM: Diagnostic and Statistical Manual; Y-BOCS: Yale-Brown Obsessive-Compulsive Scale; DIS: Diagnostic Interview Scale.
Dashes indicate missing or insufficient data.
Data from Billet et al.(1998) was excluded due to inconsistencies in their data.

***Table II.6 (f).*** *Descriptive characteristics of the studies (excluding the present study) included in the* DAT 40bp VNTR *meta-analysis.*

| First Author | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects (y) | Mean age at onset (y) | n (control) | Mean age control (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Frisch et al. (2000) | Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 39 | - | | 112 | - | 0.92 |
| Frisch et al. (2000) | non-Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 36 | - | | 60 | - | 0.69 |

[a]Gender index= (female cases/male cases)/(female controls/male controls). **Abbreviations:** DSM: Diagnostic and Statistical Manual; SADS-L: Structured Clinical Interview for Affective Disorders and Schizophrenia-Lifetime Version ; SCID-P: Structured Clinical Interview for DSM-IV Patient Version.
Dashes indicate missing or insufficient data.
Data from Billet et al.(1998) was excluded due to inconsistencies in their data.

***Table II.6 (g).*** *Descriptive characteristics of the studies (excluding the present study) included in the* COMT val158met *(rs4680) meta-analysis.*

| Reference | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects | Mean age at onset | n (control) | Mean age control subjects (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Karayiorgou et al. (1997) | N. American Caucasian | DSM-III-R | - | 73 | - | - | 148 | - | 0.76 |
| Ohara et al. (1998) | Japanese | DSM-IV | - | 17 | - | - | 135 | - | - |
| Meira-Lima et al. (2003) | Brazilian | DSM-IV | SCID, Y-BOCS | 79 | 33.6±10 | - | 202 | 33.9±9 | 0.81 |
| Erdal et al. (2004) | Turkish | DSM-IV | Y-BOCS, SCID-I(APA1994) | 59 | 29±9 | 21+-7 | 114 | 27±6 | 1.63 |

[a]Gender index= (female cases/male cases)/(female controls/male controls). **Abbreviations:** DSM: Diagnostic and Statistical Manual; SCID-I: Structured Clinical Interview for DSM-IV Axis I Disorders (NIMH); Y-BOCS: Yale-Brown Obsessive-Compulsive Scale
Dashes indicate missing or insufficient data.

***Table II.6 (h).*** *Descriptive characteristics of the studies (excluding the present study) included in the* DRD4 48bp VNTR *meta-analysis.*

| Reference | Ethnicity | Diagnostic System Used | Method of Assessment | n (OCD) | Mean age OCD subjects (y) | Mean age at onset (y) | n (control) | Mean age control subjects (y) | Gender Index[a] |
|---|---|---|---|---|---|---|---|---|---|
| Frisch et al. (2000) | Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 39 | - | - | 112 | - | 0.92 |
| Frisch et al. (2000) | non-Ashkenazi Jew | DSM-IV | SADS-L, SCID-P | 36 | - | - | 60 | - | 0.70 |
| Millet et al. (2003) | French | DSM-IV | DIGS, KIDDIE-SADS, Y-BOCS | 55 | 23.5+-10 | 12.9±6 | 63 | 34.8±9.7 | 0.74 |

[a]Gender index= (female cases/male cases)/(female controls/male controls). **Abbreviations:** DSM: Diagnostic and Statistical Manual; SADS-L: Structured Clinical Interview for Affective Disorders and Schizophrenia-Lifetime Version ; SCID-P: Structured Clinical Interview for DSM-IV Patient Version; DIGS: Diagnostic Interview for Genetic Studies; KIDDIE-SADS-E: Schedule for Affective Disorders and Schizophrenia for school Age Children-Epidemiological Version ;Y-BOCS: Yale-Brown Obsessive-Compulsive Scale
Dashes indicate missing or insufficient data.

### *II.7.5.4. Statistical analysis*

For each polymorphism, a two-by-two table was constructed, in which one dichotomous variable represented diagnosis (OCD/control), the other allele type. For each study, the strength of the association was summarised as the allelic OR; with the major allele assigned as the risk allele in all studies.

To test the heterogeneity of the ORs, the Cochran Q-test was performed (Cochran, 1954). The cut-off level of significance for heterogeneity was taken to be 0.1. The random effects OR was used to detect any heterogeneity across the studies involving the same polymorphisms. This model assumes that the studies under investigation represent a random sample of all possible studies, and in doing so, accounts for studies that are not available for inclusion in the meta-analyses. The random effects model thus weights each study included in the meta-analysis according to the variance of effect size for each individual study, and according to the variance of effect estimates between studies.

Pooling ORs was performed according to the methods described by DerSimonian and Laird, 1986, and the corresponding 95% CIs were calculated according to Woolf's Method (Woolf,1955).

## II.8. ANALYSIS OF AFRIKANER POPULATION STRUCTURE

Twenty-three polymorphisms were included in *Structure* analysis and, as indicated in the previous sections, some of these polymorphisms had been included in the case-control association studies, namely, *5-HT$_6$*; *DRD3*; *DAT*; *DRD1*; *5-HT$_{1D\beta}$*; *ESRα*; *DRD4*, *DRD2*; *5-HT$_{2A,,}$ INPP-1,* and *COMT* .

The genotyping procedures of those polymorphisms have been described in **section II.5.**

### II.8.1. Structure Inference

The possibility of population substructure was investigated by employing the Bayesian clustering method, *Structure* (version 2.0) (Pritchard, 2000; http://pritch.bsd.uchicago.edu/). In setting up the parameter files for structure, the admixture model was selected as an ancestry model. Pritchard et al. (2000) recommend the use of this model, given its flexibility in dealing with the complexities present in most real populations. The admixture model posits that each individual under investigation has mixed ancestry; that is, an individual would have inherited

*a fraction* of her/his genome from ancestors in population K, which is in contrast with the no admixture model in which it is assumed that the individual inherits his/her genome *solely* from ancestors in population K.

*Structure* was run for $10^6$ iterations of the Gibbs sampler, after an initial burn-in of 50 000 iterations, implementing a model of correlated allele frequencies. This model assumes that, if sub-clusters exist, their allele frequencies are likely to be similar, probably as a result of migration or shared ancestry. Prior information regarding population membership to direct clustering was not used, and the values of K were varied across runs. The value of K that maximised the posterior probability of the data was selected as representing the true number of clusters (genetically determined sub-populations) within the Afrikaner population. K was varied between one and five, and each analysis was repeated 10 times to assess convergence. Default values were used for all other parameter settings.

The individuals included in the analysis were all Afrikaans-speaking individuals, recruited by the MRC Unit on Anxiety and Stress Disorders. Although many of the individuals (both OCD and control) were included in the genetic association analyses, there remains a subset who were not included in the genetic investigations in the present study, but who were genotyped for concurrent investigations in OCD genetics. *Structure* analysis was run for the Afrikaner OCD and controls separately, and then as a combined group, using the aforementioned parameters.

# CHAPTER III

## RESULTS

**INDEX** **PAGE**

**PAGE**

**PAGE**

**PAGE**

# CHAPTER III: RESULTS

## III.1. GENOTYPING RESULTS

All polymorphisms were genotyped as described in Chapter II. The following figures (Figure III.1-III.37) depict representative gels for each polymorphism.

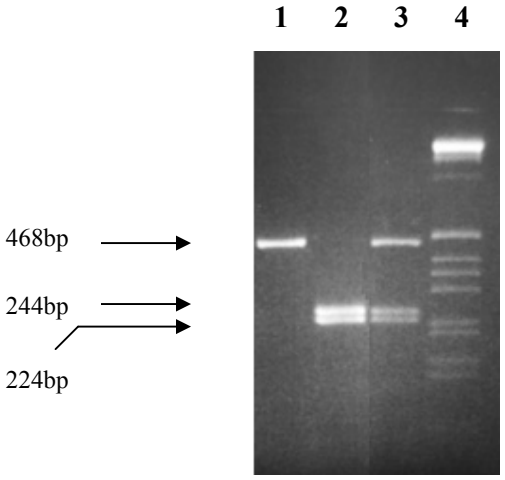



***Figure III.1. ASREA of the* 5-HT$_{2A}$ -1438A/G *(rs6311) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Msp*I. Lane 1:* -1438A/-1438A*; Lane 2:* -1438G/-1438G*; Lane 3:* -1438A/-1438G*; Lane 4: Molecular weight marker X.*

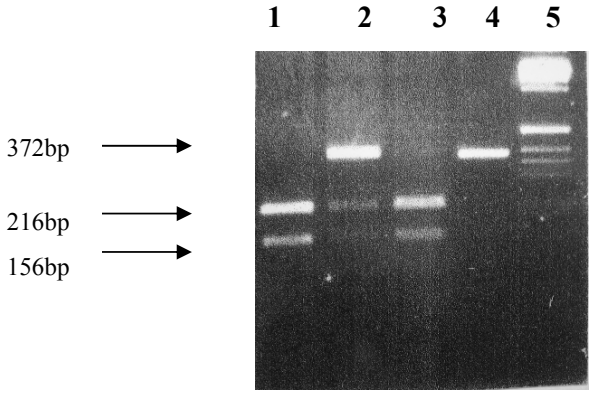



***Figure III.2. ASREA of the* 5-HT$_{2A}$ T102C *(rs6313) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Hpa*II. Lanes 1 and 3:* C102C*; Lane 2:* T102C*; Lane 4:* T102T*; Lane 5: Molecular weight marker X.*

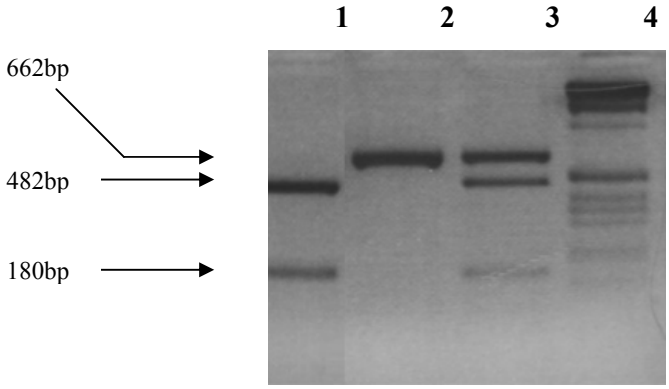



***Figure III.3. ASREA of the* 5-HT$_{1D\beta}$ G861C *(rs6296) polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Hinc*II. Lane I:* C861C*; Lane 2:* G861G*; Lane 3:*G861C*; Lane 4: Molecular weight marker X.*

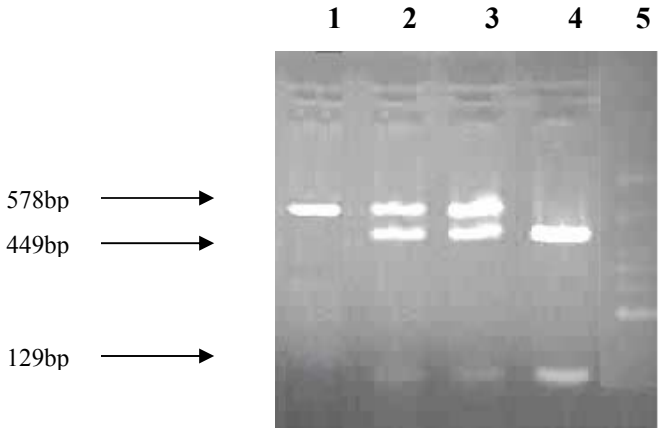



***Figure III.4. ASREA of the* 5-HT$_6$ T267C *(rs1805054) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Rsa*I. Lane I:* T267T*; Lanes 2 and 3:* T267C*; Lane 4:* C267C*; Lane 5: Low molecular weight DNA ladder.*

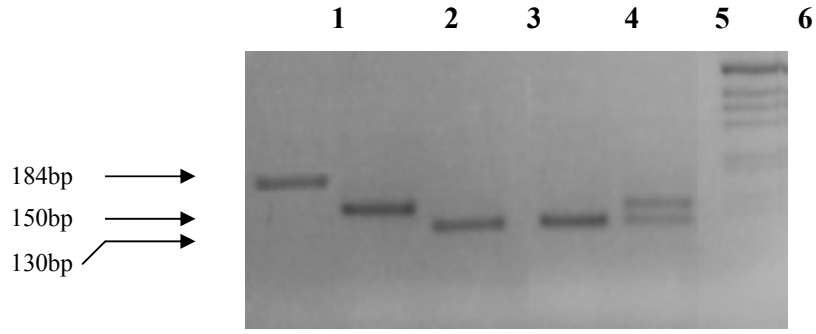



***Figure III.5. ASREA of the* 5-HT$_{2C}$ cys23ser *(rs6318) polymorphism.*** *Representative 3% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Nla*III. Lane I: undigested PCR product (control); Lane 2:* ser23ser*; Lanes 3 and 4:* cys23cys*; Lane 5:* cys23ser*; Lane 6: Molecular weight marker X.*

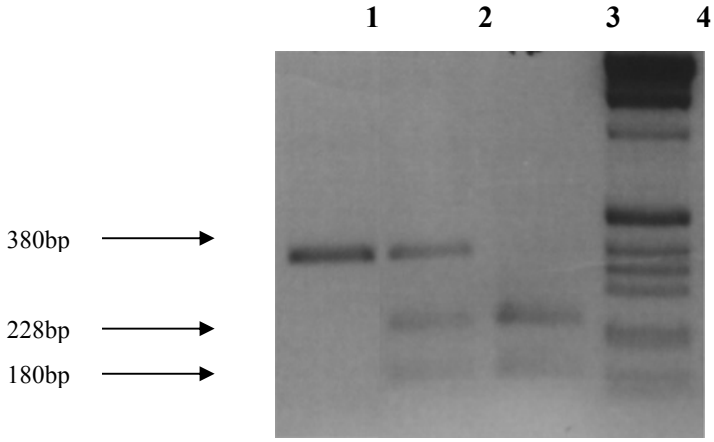



***Figure III.6. ASREA of the* DRD4 -521C/T *(rs1800955) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Fsp*I. Lane I:* -521C/C*; Lane 2:* -521C/T*; Lane 3:* -521T/T*; Lane 4: Molecular weight marker X.*

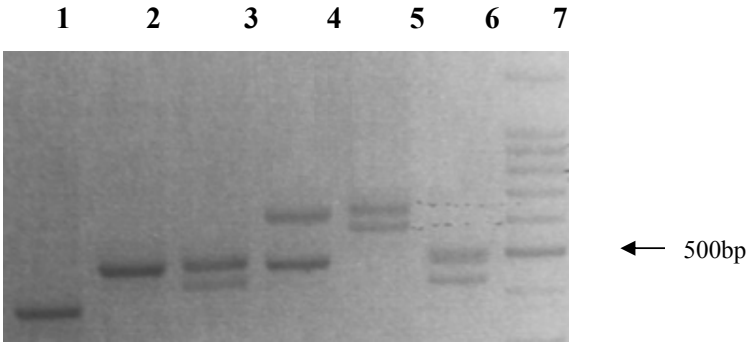



***Figure III.7. PCR amplification of the* DRD4 48bp *VNTR polymorphism.*** *Representative 3% agarose gel showing the fragment sizes generated by PCR. Lane 1:* A2/A2*; Lane 2*: A4/A4*; Lanes 3 and 6:* A4/A3*; Lane 4:* A4/A7*; Lane 5:* A6/A7*; Lane 7: Molecular weight marker XIV. (*A2*=379bp,* A3*=427bp,* A4*=475bp,* A6*=571bp,* A7*=619bp).*

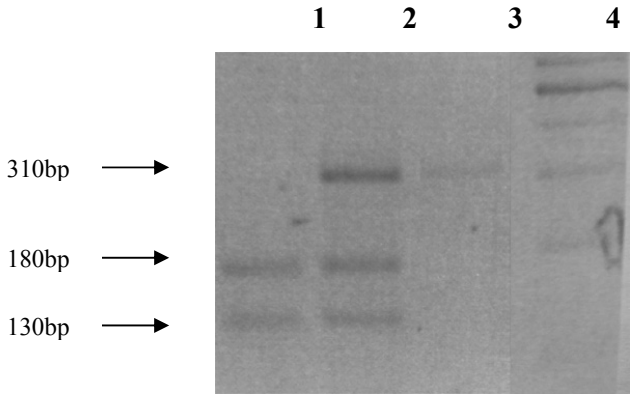



***Figure III.8. ASREA of the* DRD2 Taq*1A (rs1800497) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Taq1A *I. Lane 1:* CC*; Lane 2:* CT*; Lane 3:* TT*; Lane 4: Marker XIV*

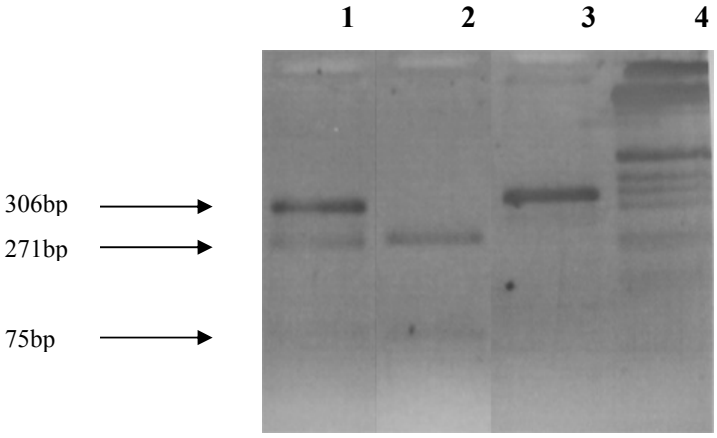



***Figure III.9. ASREA of the* COMT *promoter (rs2097603) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Hind*III. Lane 1:* AG*; Lane 2:* GG*; Lane 3:* AA*; Lane 4: Molecular weight marker X.*

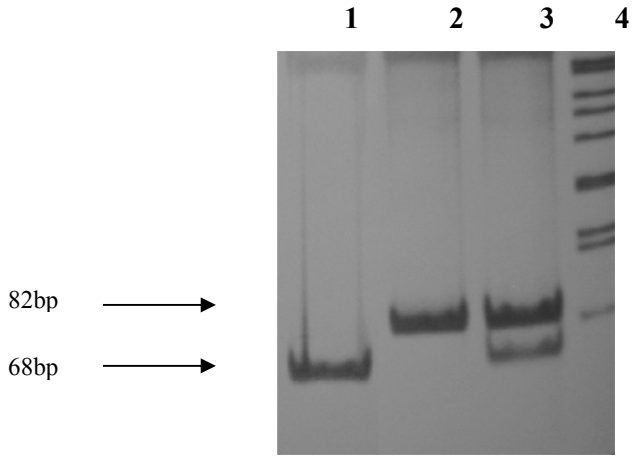



***Figure III.10. ASREA of the* COMT val158met *(rs4680) polymorphism.*** *Representative 12% polyacrylamide gel showing the fragment sizes generated by PCR and restriction digestion with* NlaIII*. Lane 1:* AA (met158met)*; Lane 2:* GG (val158val)*; Lane 3:* AG (val158met)*; Lane 4: Molecular weight marker* λPst.

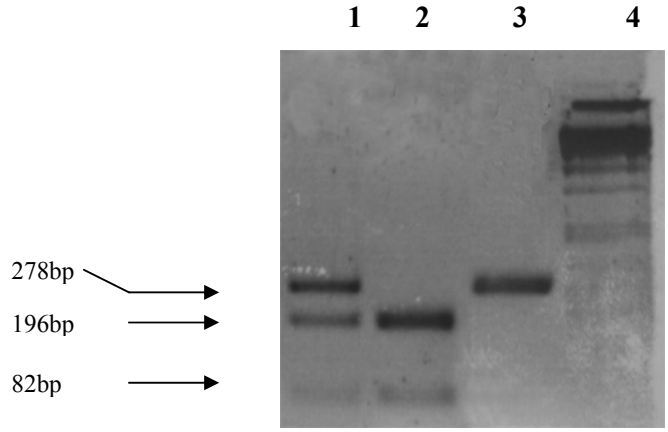



***Figure III.11. ASREA of the* COMT *exon 6 (rs362204) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Bgl*I. Lane 1:* ID *(*C+/C-*); Lane 2:* DD (C-/C-); *Lane 3:* II (C+/C+)*; Lane 4: Molecular weight marker λ*Pst*.*

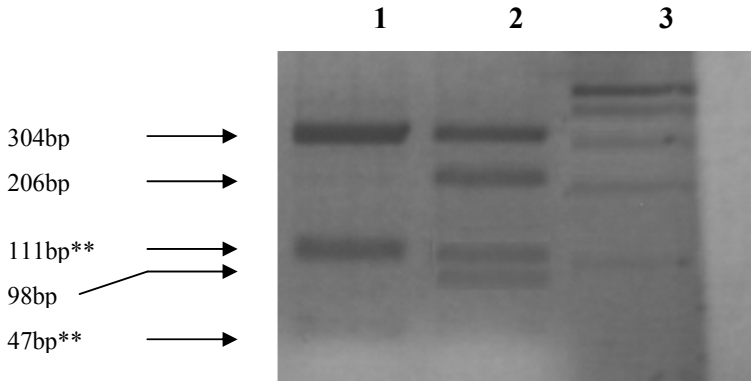



***Figure III.12. ASREA of the* DRD3 ser9gly *(rs6280) polymorphism.*** *Representative 3% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Msc*I. Lane 1:* AA *(*ser9ser*); Lane 2:* AG *(*gly9ser*) Lane 3: Molecular weight marker XIV. The visible constitutive fragments (at 47bp and 146bp) are marked with an asterisk.*

GG *(*gly9gly*) is not indicated on the gel.*

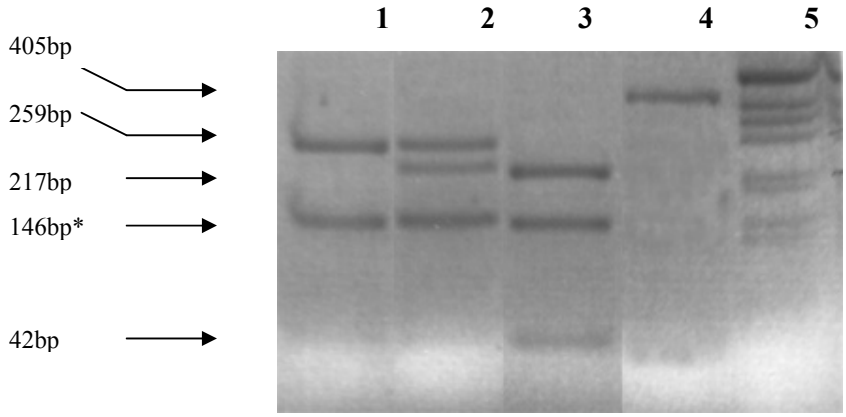



***Figure III.13. ASREA of the* DRD1 A-48G *polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Dde*I. Lane 1:* -48G/-48G*; Lane 2:* -48A/-48G*; Lane 3:* -48A/-48A*; Lane 4: undigested PCR product; Lane 5: Molecular weight marker X.*

*The constitutive fragment (at 146bp) is marked with an asterisk.*

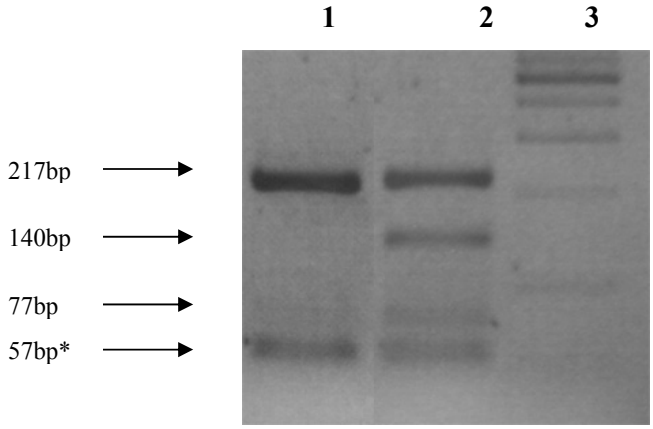



***Figure III.14. ASREA of the* BDNF val66met *(rs6265) polymorphism.*** *Representative 3% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Nla*III. Lane 1:* GG *(*val66val*); Lane 2:* GA *(*val66met*); Lane 3: Molecular weight marker XIV.*

*The constitutive fragment (at 57bp) is marked with an asterisk.*

AA (met66met) *is not indicated on this gel.*

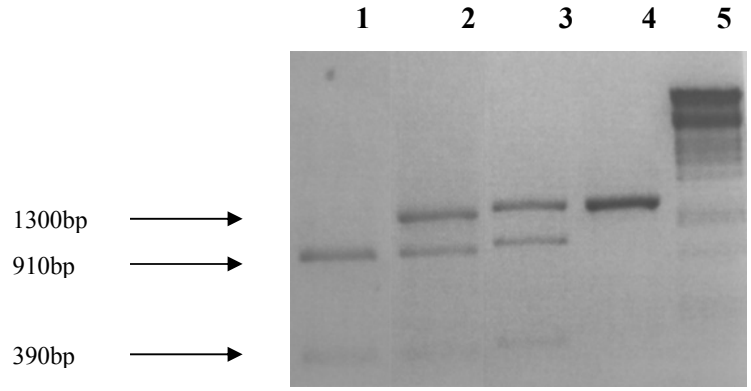



***Figure III.15. ASREA of the* ESRα *rs9430799 polymorphism.*** *Representative 1.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* XbaI*. Lane 1:* AA*; Lanes 2 and 3:* AG*; Lane 4:* GG*; Lane 5: Molecular weight marker λPst.*

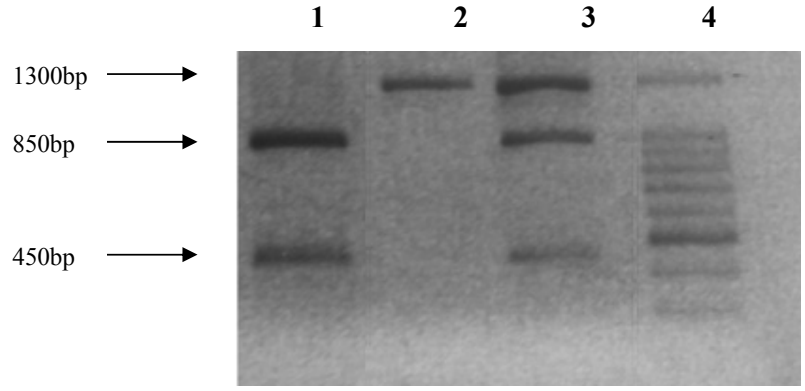



***Figure III.16. ASREA of the* ESRα *rs2234693 polymorphism.*** *Representative 1.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* PvuII*. Lane 1:* TT*; Lane 2:* CC*; Lane 3:* TC*; Lane 4: Molecular weight marker XIV.*

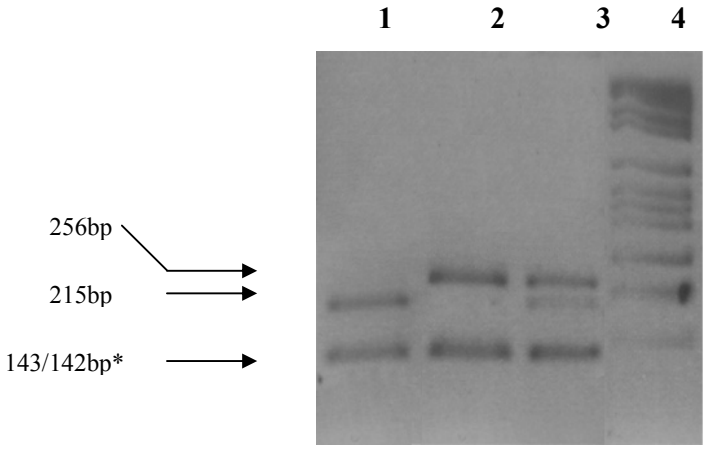



***Figure III.17. ASREA of the* INPP-1 *rs1882891 polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* DdeI*. Lane 1:* AA*; Lane 2:* CC*; Lane 3:* CA*; Lane 4: Molecular weight marker VI.*

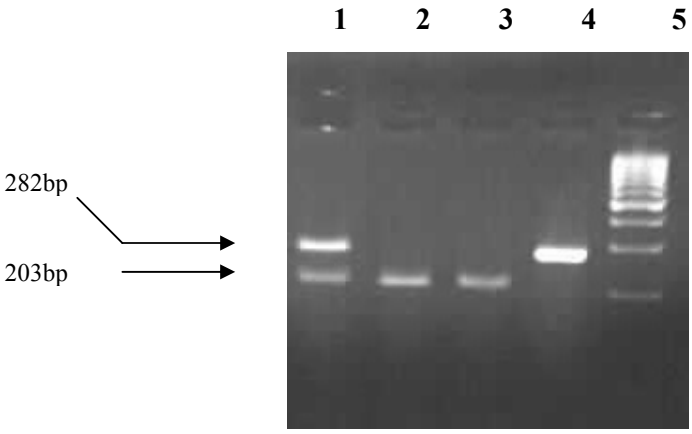



***Figure III.18. ASREA of the* PLC-γ1 *rs8192707 polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Blp*I. Lane 1:* GA *(gly279ser); Lanes 2 and 3:* AA *(ser279ser); Lane 4:* GG *(gly279gly); Lane 5: Molecular weight marker XIV.*

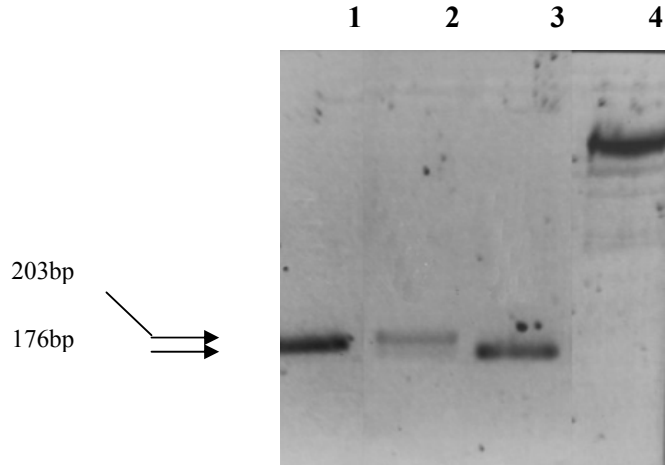



***Figure III.19. ASREA of the* DLX int1C/T *polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* ApoI*. Lane 1:* CC*; Lane 2:* CT*; Lane 3:* TT*; Lane 4: Molecular weight marker λPst.*

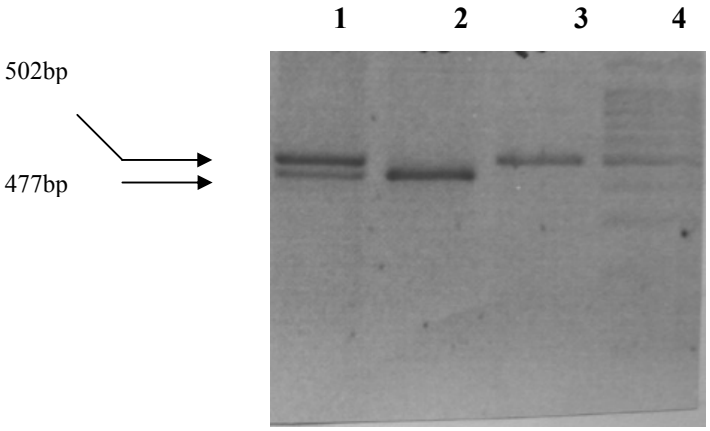



***Figure III.20. ASREA of the* ADRA1C cys492arg *polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* PstI*. Lane 1:* CT *(*cys492arg*); Lane 2:* TT *(*arg492arg*); Lane 3:* CC *(*cys492cys*); Lane 4: Molecular weight marker XIV.*

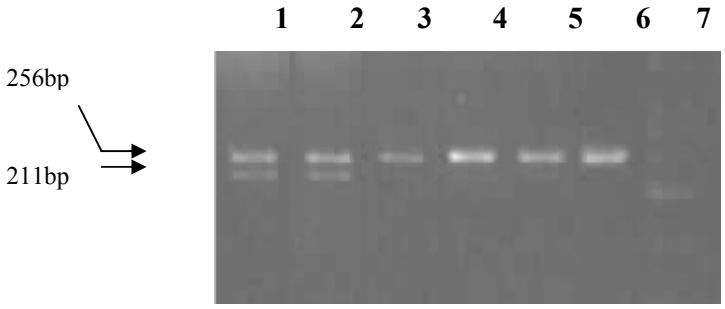



***Figure III.21. ASREA of the* SNAP25 MnlI *polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* MnlI*. Lanes 1 and 2:* TG*; Lane 3-6:* TT*. Lane 7: Low molecular weight marker.*

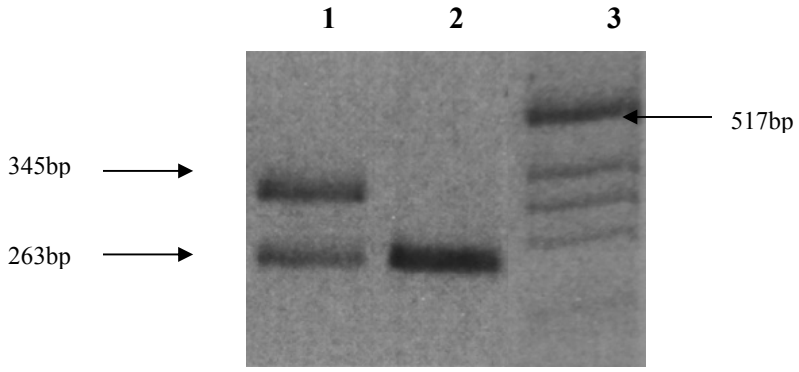



***Figure III.22. ASREA of the* GNAS FokI *(rs7121) polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Fok*I. Lane 1:* TC*; Lane 2:* CC*; Lane 3: Marker X (indicating fragment sizes from 154bp to 517bp).*

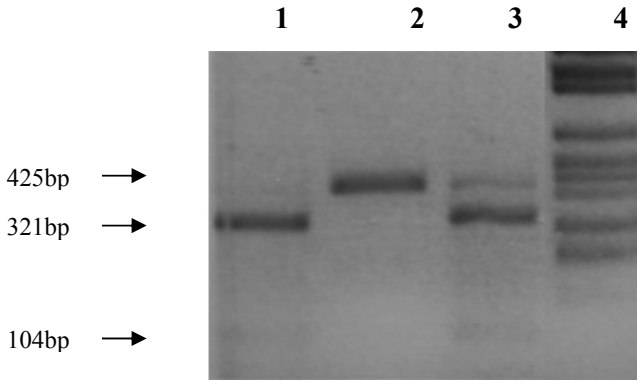



***Figure III.23. ASREA of the* ABCG1 G2457A *polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR and restriction digestion with* Hha*I. Lane 1:* GG*; Lane 2:* AA; *Lane 3:* AG*; Lane 4: Molecular weight marker VI.*

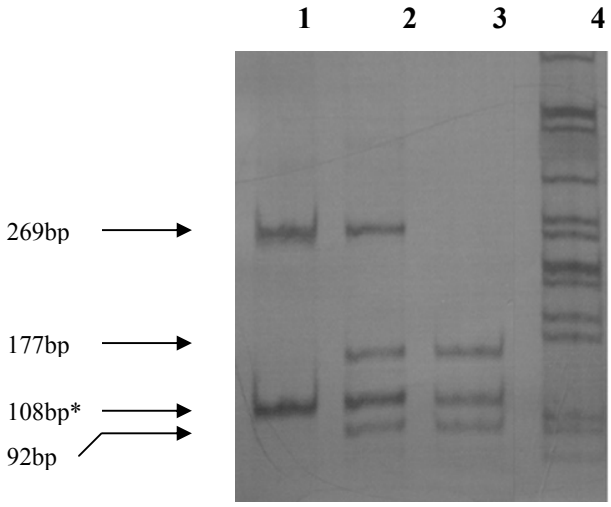



***Figure III.24. ASREA of the* SNAP29 C56T *polymorphism.*** *Representative 12% polyacrylamide gel showing the fragment sizes generated by PCR and restriction digestion with* Dde*I. Lane 1:* CC*; Lane 2:* CT*; Lane 3:* TT*; Lane 4: Molecular weight marker* λPst*. The 108bp constitutive fragment is marked with an asterisk.*

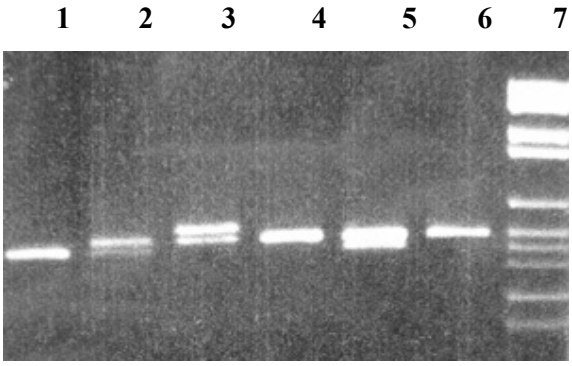



***Figure III.25. PCR amplification of the* DAT 40bp VNTR *polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR. Lane 1:* A9/A9*; Lanes 2 and 5:* A9/A10*; Lane 3:* A10/A11*; Lanes 4 and 6:* A10/A10*; Lane 7: Molecular weight marker XIV. (*A9*=440bp,* A10*=480bp,* A11*=520bp).*

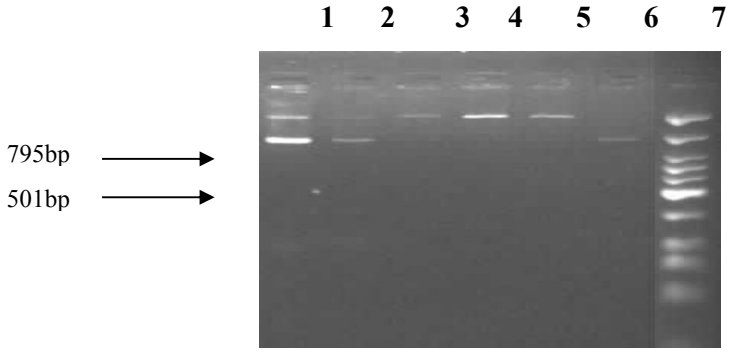



***Figure III.26. PCR amplification of the* FXIIIB Alu *ins/del polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR. Lanes 1 and 2:* I/D*; Lanes 3, 4 and 5*: I/I*; Lane 6:* D/D*; Lane 7: Low Molecular weight DNA ladder. (*I*=insertion allele;* D*=deletion allele).*

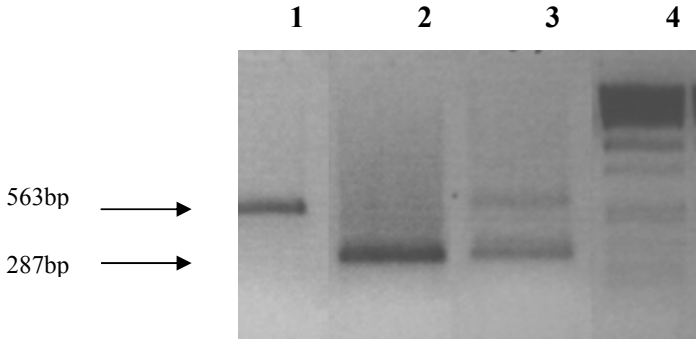



***Figure III.27. PCR amplification of the* YaNBC182 Alu *ins/del polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR. Lane 1:* I/I*; Lane 2:* D/D*; Lane 3:* I/D*; Lane 4: Low Molecular weight DNA ladder.*
*(*I*=insertion allele;* D*=deletion allele).*

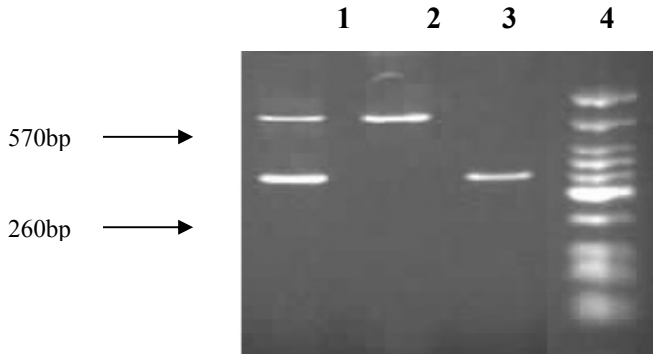



***Figure III.28. PCR amplification of the* TPA25 Alu *ins/del polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR. Lane 1:* I/D*; Lane 2:* I/I*; Lane 3:* D/D*; Lane 4: Low Molecular weight DNA ladder.*
*(*I*=insertion allele;* D*=deletion allele).*

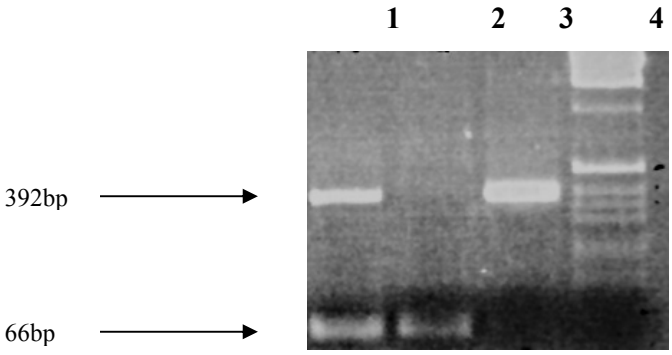



***Figure III.29. PCR amplification of the* YaNBC241 Alu *ins/del polymorphism.*** *Representative 3% agarose gel showing the fragment sizes generated by PCR. Lane 1:* I/D*; Lane 2:* D/D*; Lane 3:* I/I*; Lane 4: Molecular weight marker X.*
*(*I*=insertion allele;* D*=deletion allele).*

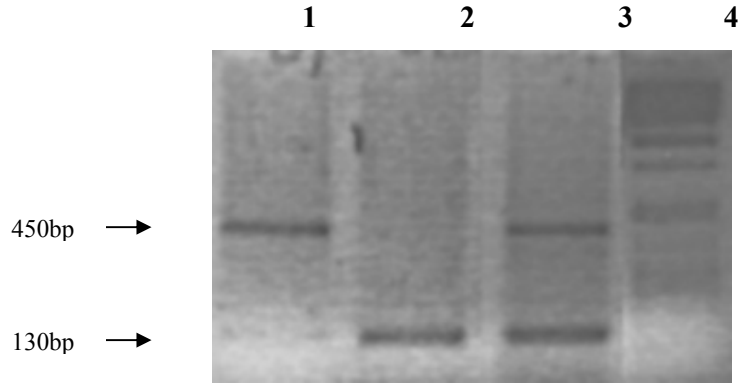



***Figure III.30. PCR amplification of the* PV92 Alu *ins/del polymorphism.*** *Representative 2.5% agarose gel showing the fragment sizes generated by PCR. Lane 1:* I/I*; Lane 2:* D/D*; Lane 3:* I/D*; Lane 4: Molecular weight marker λ*Pst*.*
*(I=insertion allele; D=deletion allele).*

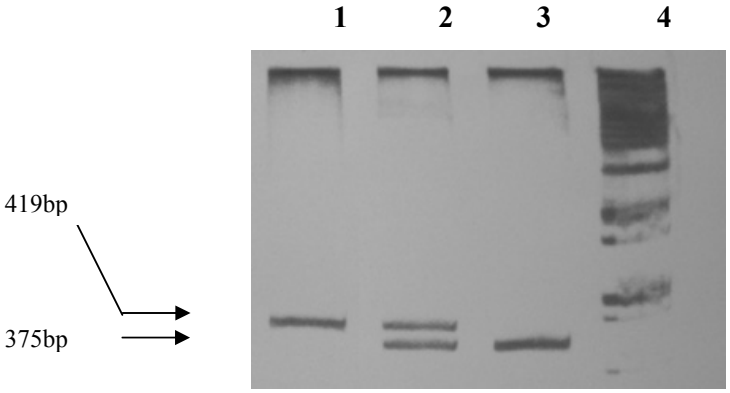



***Figure III.31. PCR amplification of the* 5-HTT 44bp *VNTR polymorphism.*** *Representative 12% polyacrylamide gel showing the fragment sizes generated by PCR. Lane 1: L/L; Lane 2: L/S; Lane 3:S/S; Lane 4: Molecular weight marker λ*Pst*.*
*(L refers to the "long" allele, S refers to the "short" allele).*

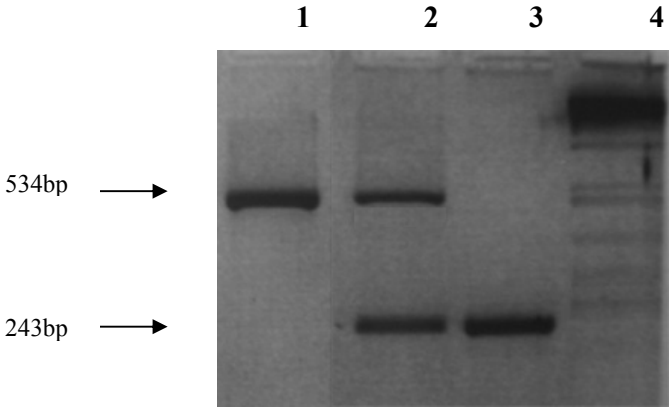
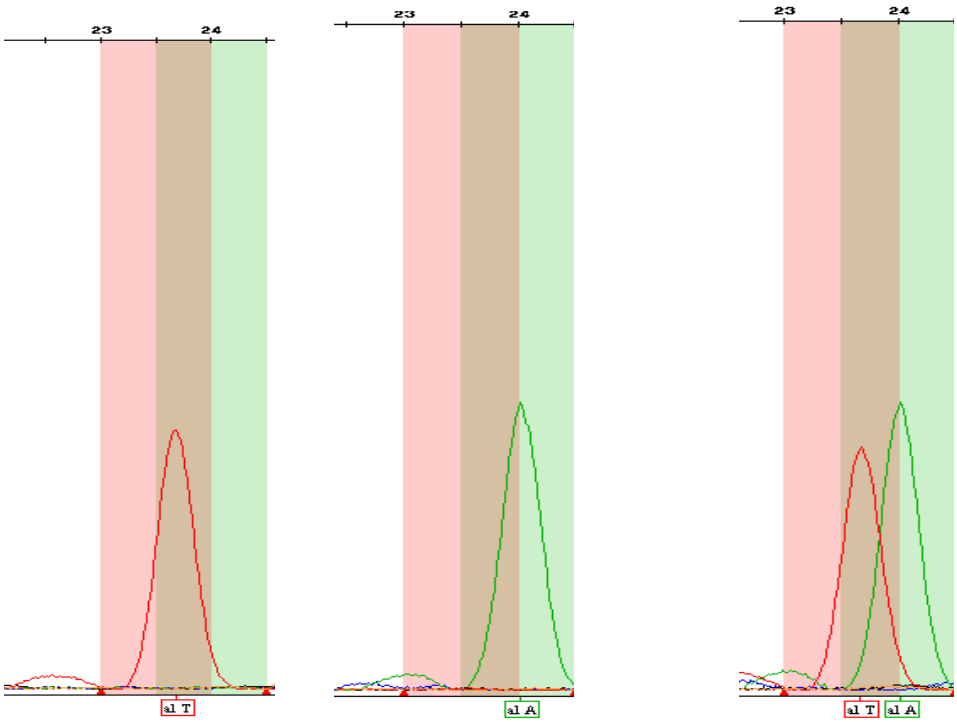



***Figure III.32. PCR amplification of the* ACE Alu *ins/del polymorphism.*** *Representative 2% agarose gel showing the fragment sizes generated by PCR. Lane 1: I/I; Lane 2: I/D; Lane 3: D/D; Lane 4: Molecular weight marker λPst.*
*(I=insertion allele; D=deletion allele).*




**A** **B** **C**

***Figure III.33. SNaPshot results for the* BDNF *rs2049046 polymorphism. (A):*** *representative snapshot result for the* TT*-genotype, (**B**): representative SNaPshot result for the* AA*-genotype and (**C**): representative snapshot result for the* TA*-genotype.*

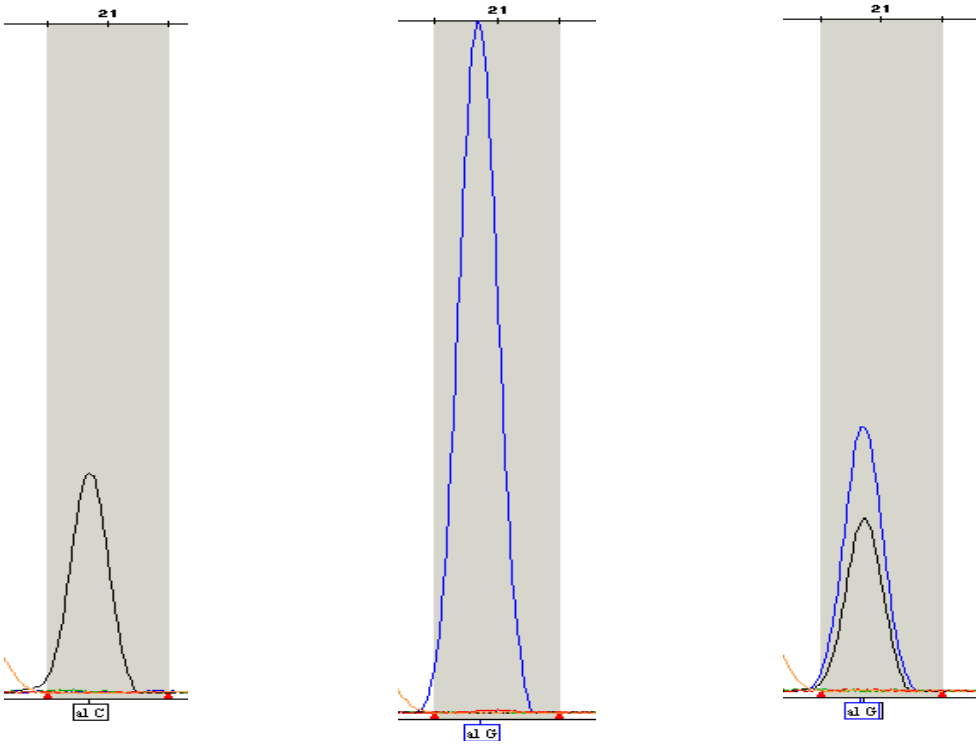



***Figure III.34. SNaPshot results for the* BDNF *rs988748 polymorphism. (A):*** *representative snapshot result for the* CC*-genotype, (**B**): representative snapshot result for the* GG*-genotype and (**C**): representative snapshot result for the* GC*-genotype.*

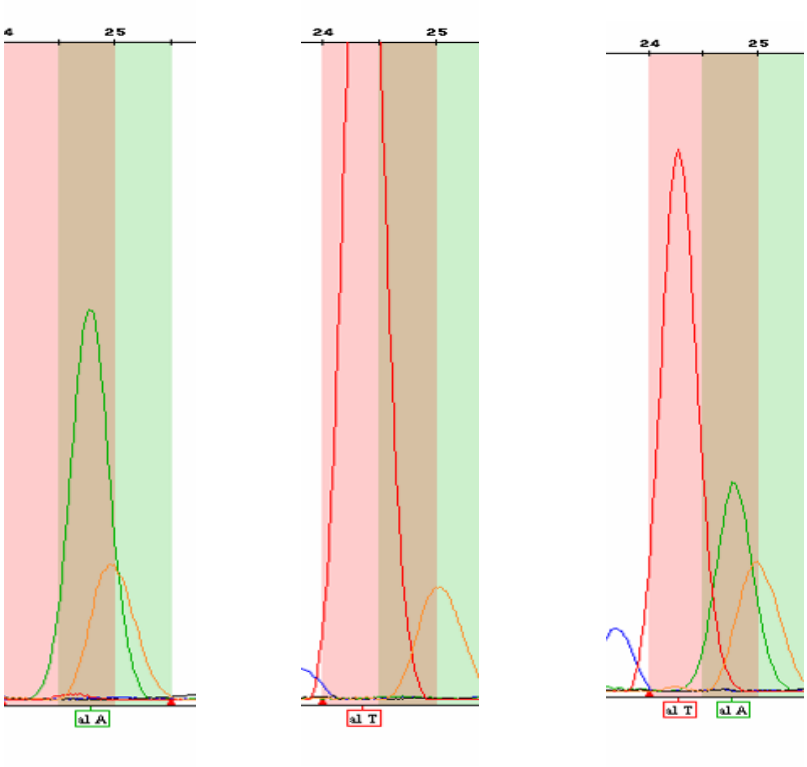



***Figure III.35. SNaPshot results for the HOXB8 rs2303486 polymorphism. (A):*** *representative snapshot result for the* AA*-genotype,* ***(B)****: representative snapshot result for the* TT*-genotype and* ***(C)****: representative snapshot result for the* AT*-genotype.*

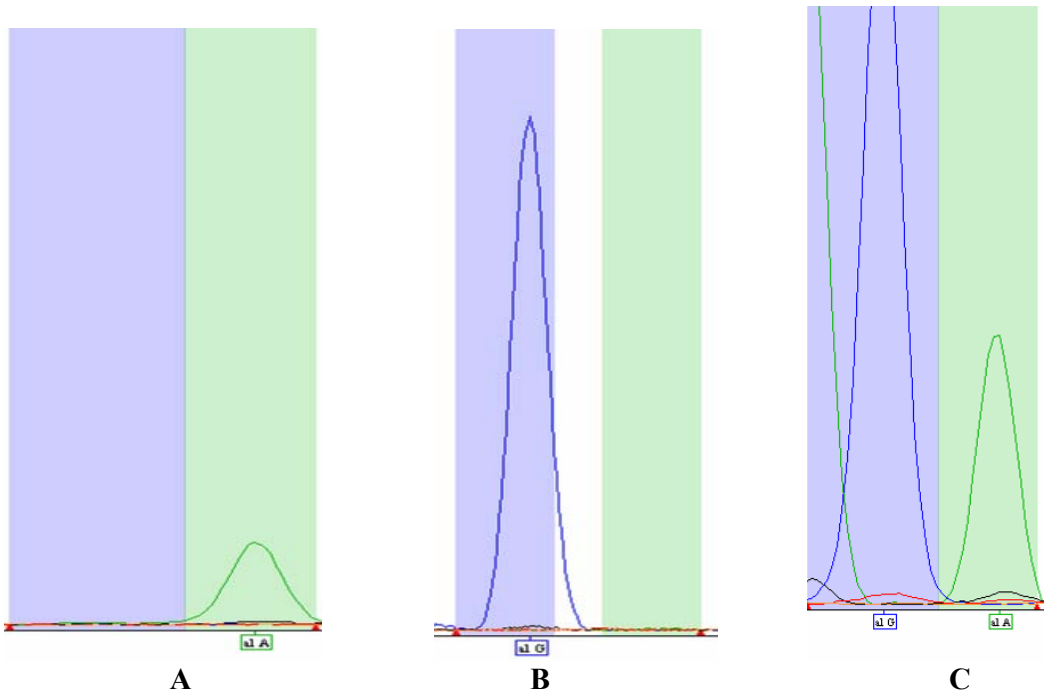
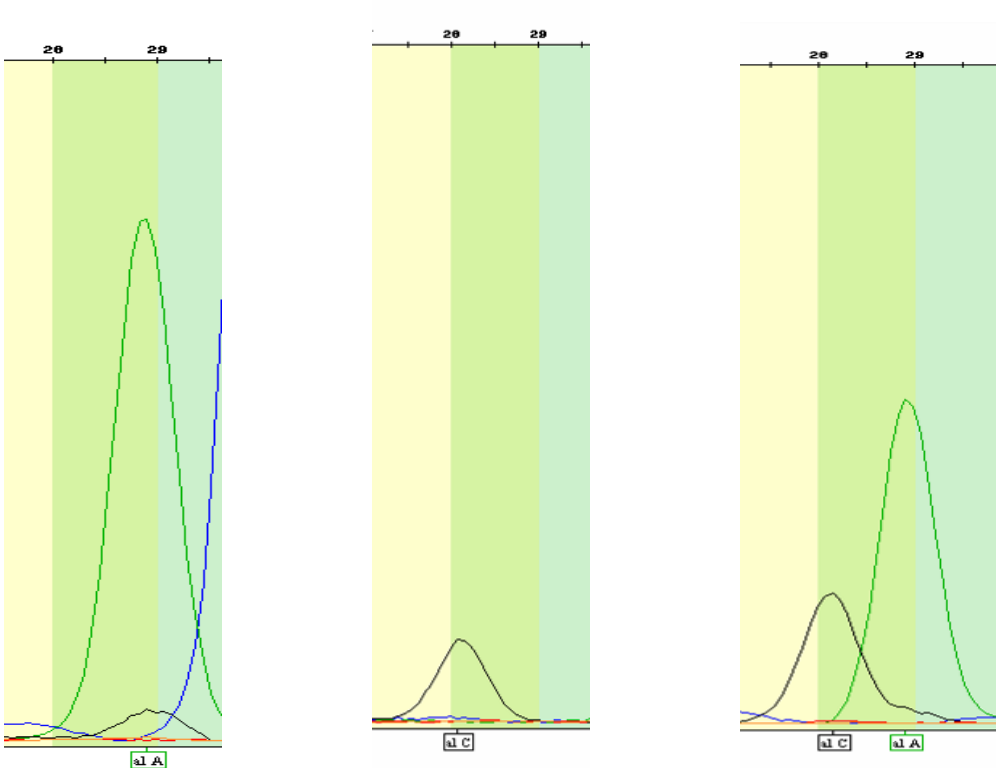



***Figure III.36. SNaPshot results for the* GRIN2B *rs1806191 polymorphism. (A):*** *representative snapshot result for the* AA*-genotype, (**B**): representative snapshot result for the* GG*-genotype and (**C**): representative snapshot result for the* GA*-genotype.*




**A** **B** **C**

***Figure III.37. SNaPshot results for the* GRIN2B *rs890 polymorphism. (A):*** *representative snapshot result for the* AA*-genotype, (**B**): representative snapshot result for the* CC*-genotype and (**C**): representative snapshot result for the* AC*-genotype.*

## III.2. INVESTIGATING SUB-STRUCTURE WITHIN THE AFRIKANER POPULATION

A total of 137 (76 controls and 61 OCD patients) Afrikaner subjects were selected for genotyping for inclusion in the determination of population substructure in the present study. These individuals were genotyped for 23 unlinked autosomal polymorphisms, selected on the basis of location. The polymorphisms, their exact HWE values and heterozygosities are portrayed in Table III.1. The mean heterozygosity for all 23 polymorphisms was 0.446. All of the polymorphisms were found to obey HWE (Table III.1).

This data was then analysed for population structure using the *Structure* program (version 2.0) (Pritchard et al., 2000; available on the Pritchard Lab website: http://pritch.bsd.uchicago.edu/). The posterior probability values of K, assuming a uniform prior on K between 1 and 5, are provided in Table III.2. These posterior probability values provide one with the probability that individuals will occupy a particular cluster (sub-population), given the observed genotypes. These values were obtained as described in "*Inference for the number of populations"* from the *Structure* user's manual (Pritchard and Wen, July 13 2004) (http://pritch.bsd.uchicago.edu). It is important to stress that these values serve only as rough guides as to the most parsimonious model, rather than accurate estimates of posterior probabilities.

The posterior probabilities favoured a K of 1 in the total population, and also when the sample was stratified according to diagnosis (i.e. control and OCD subjects) (Table III.2). This is indicative of an absence of population structure within the Afrikaner population utilised in the present study. This result is corroborated by the examining the membership coefficients (Q) for each individual for each value of K: the proportion of the population assigned to each cluster is symmetric for K=2 to K=5, as indicated by the bar plots in Figure III.38.

***Table III.1.*** *Genetic markers used in "*Structure*" analysis, indicating the chromosomal location, major allele frequency and HWE and heterozygosity values for each variant.*

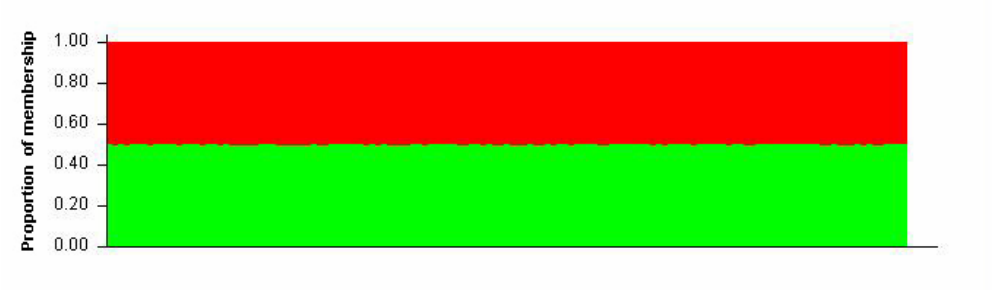
| Marker | Location | Polymorphism | HWE exact p-value | | | Heterozygosity | | |
|---|---|---|---|---|---|---|---|---|
| | | | All | Controls | OCD | All | Controls | OCD |
| ***5-HT$_6$*** | 1p35-36 | rs1805054 | 0.736 | 1.000 | 1.000 | 0.272 | 0.221 | 0.348 |
| ***FXIIIB*** | 1q31-32 | *Alu* ins/del | 0.353 | 0.804 | 0.084 | 0.453 | 0.531 | 0.358 |
| ***INPP-1*** | 2q32 | rs1882891 | 0.689 | 1.000 | 1.000 | 0.224 | 0.220 | 0.228 |
| ***DRD3*** | 3q13.3 | rs6280 (*ser9gly*) | 0.701 | 1.000 | 0.362 | 0.457 | 0.500 | 0.404 |
| ***DRD1*** | 5p35.1 | rs4532 | 0.355 | 0.445 | 0.783 | 0.496 | 0.514 | 0.475 |
| ***DAT*** | 5p15.3 | 40bp VNTR | 0.847 | 0.494 | 0.792 | 0.350 | 0.316 | 0.393 |
| ***5-HT$_{1D\beta}$*** | 6q13 | rs6296 (*G861C*) | 0.290 | 0.173 | 0.742 | 0.393 | 0.405 | 0.377 |
| ***ESRα*** | 6q25.1 | rs2234693 | 0.412 | 1.000 | 0.485 | 0.537 | 0.508 | 0.583 |
| ***DLX*** | 7q21.3 | *DLX int1C/T* | 0.658 | 0.577 | 0.162 | 0.482 | 0.549 | 0.375 |
| ***YaNBC182*** | 7 | *Alu* ins/del | 0.726 | 0.139 | 0.291 | 0.512 | 0.597 | 0.417 |
| ***TPA25*** | 8p12 | *Alu* ins/del | 0.862 | 0.450 | 0.591 | 0.492 | 0.462 | 0.527 |
| ***ADRA1C*** | 8p21 | *cys492arg* | 1.000 | 0.617 | 0.585 | 0.504 | 0.537 | 0.463 |
| ***DBH*** | 9q34 | Insertion/deletion | 0.726 | 1.000 | 0.664 | 0.464 | 0.556 | 0.421 |
| ***DRD4*** | 11p15.5 | rs1800955 | 0.211 | 0.148 | 0.792 | 0.538 | 0.589 | 0.475 |
| ***DRD2*** | 11q23.2 | rs1800497(*Taq1A*) | 0.820 | 0.560 | 1.000 | 0.382 | 0.352 | 0.417 |
| ***5-HT$_{2A}$*** | 13q14-21 | rs6311 (-1438A/G) | 0.042 | 0.082 | 0.388 | 0.391 | 0.389 | 0.393 |
| ***YaNBC241*** | 15 | *Alu* ins/del | 0.288 | 0.811 | 0.325 | 0.450 | 0.464 | 0.435 |
| ***PV92*** | 16q24 | *Alu* ins/del | 0.800 | 1.000 | 0.439 | 0.346 | 0.328 | 0.367 |
| ***5-HTT*** | 17q11 | 44bp VNTR | 0.269 | 0.330 | 0.592 | 0.441 | 0.429 | 0.455 |
| ***SNAP25*** | 20p12 | *SNAP25 Mnl*I | 0.633 | 0.697 | 0.776 | 0.467 | 0.457 | 0.474 |
| ***GNAS*** | 20q13.2 | rs7121 | 0.409 | 0.497 | 0.790 | 0.466 | 0.459 | 0.475 |
| ***SNAP29*** | 22q11.21 | *C56T* | 0.085 | 1.000 | 1.000 | 0.582 | 0.676 | 0.511 |
| ***COMT*** | 22q11.2 | rs4680 (*val158met*) | 0.431 | 0.453 | 0.453 | 0.567 | 0.565 | 0.569 |

**Abbreviations: HWE:** Hardy-Weinberg equilibrium; ***5-HT$_6$***: serotonin receptor 6; ***FXIIIB***: Factor 13B; ***INPP-1:*** inositol polyphosphate-phosphatase; ***DRD3***: dopamine receptor 3; ***DRD1*****:** dopamine receptor 1; ***DAT*****:** dopamine transporter; ***5-HT$_{1D\beta}$***: serotonin receptor 1Dβ; ***ESRα*****:** estrogen receptor α; ***DLX6***: Distal-less like homeobox 6; ***YaNBC182****:*Ya subfamily *Alu* insertion sequence ***NBC182; TPA25***: Tissue plasminogen activator *Alu* insertion; ADRA1C: Adrenergic receptor α1C; DBH: Dopa-β hydroxylase; ***DRD4:*** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***5-HT$_{2A}$*****:** serotonin receptor 2A; ***YaNBC241***: Ya subfamily of *Alu* insertion repeats; ***PV92***: predicted variant *Alu* insertion repeat; ***5-HTT***: serotonin transporter; ***SNAP25***: Synaptosomal-associated protein 25kDa; GNAS: guanine nucleotide-binding α subunit of G; ***SNAP29***: Synaptosomal-associated protein 29kDa; ***COMT***: catechol-O-methyltransferase; **VNTR**: variable number of tandem repeats.

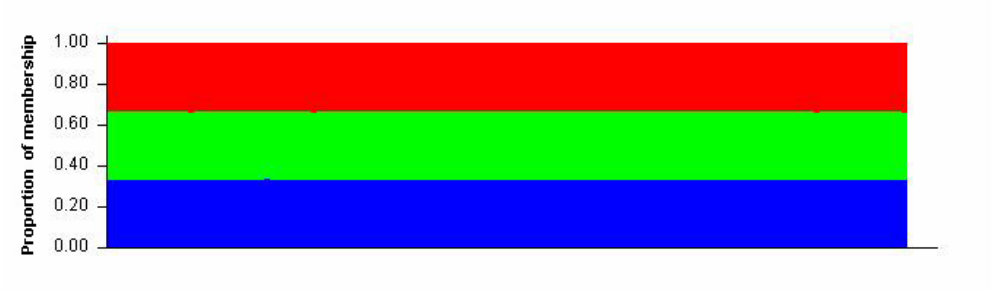
***Table III.2.*** *Estimated posterior probabilities of K for the total, control and OCD samples.*

| K | Total Sample | | Control | | OCD | |
|---|---|---|---|---|---|---|
| | ln P (X\|K)[a] | Posterior Probability (P[K\|X]) [a] | ln P (X\|K) | Posterior Probability (P[K\|X]) [a] | ln P (X\|K) | Posterior Probability (P[K\|X]) [a] |
| **1** | -3330.73 | 0.98 | -1783.86 | 0.72 | -1552.31 | 0.97 |
| **2** | -3334.64 | 0.02 | -1785.33 | 0.17 | -1555.71 | 0.03 |
| **3** | -3340.5 | 0.00 | -1786.71 | 0.04 | -1560.22 | 0.00 |
| **4** | -3340.53 | 0.00 | -1786.29 | 0.06 | -1561.54 | 0.00 |
| **5** | -3345.68 | 0.00 | -1787.98 | 0.01 | -1558.80 | 0.00 |

[a]The probability that an individual will occupy a particular cluster (sub-population), given the observed genotype data
**Abbreviation: OCD**: Obsessive-compulsive disorder.




**K=2**




**K=3**


**K=4**


**K=5**

***Figure III.38. Bar plot of estimates of membership co-efficient (Q) for K=2 to K=5 for Afrikaners.*** *The x-axis represents the Afrikaner individuals included in Structure analysis, whilst the y-axis indicates the proportion of each individual's genome that is assigned to a cluster (for the number of clusters K=2 to K=5).*

## III.3. DESCRIPTIVE OVERVIEW OF THE POLYMORPHISMS INVESTIGATED

### III.3.1. Bi-allelic Loci

Exact tests of HWE were performed on case and control individuals separately for each bi-allelic polymorphism (Table III.3[a]). All bi-allelic polymorphisms were found to obey HWE, in both the case and control populations.

Tables III.3(b), 3(c) and 3(d) provide a descriptive overview of the bi-allelic polymorphisms investigated in the present study, in the entire sample (Table III.3[b]), and stratified according to gender (males: Table III.3[c]; females: Table III.3[d]). In the control population, no bi-allelic polymorphisms had a minor allele with a frequency of below 10%, and five (21%) had a minor allele frequency between 10% and 20%.

### III.3.2. Multi-allelic Loci

#### III.3.2.1. DRD4 *48bp VNTR*

The counts and associated frequencies of the *DRD4* 48bp VNTR genotypes and alleles in the control and OCD samples are presented in Tables III.4 (a) and (b), respectively. A total of 151 controls and 89 OCD subjects were genotyped at this locus. The 4-repeat allele (*A4*) was found to be the most common allele amongst both the case and control individuals, with a frequency of 0.69 in controls and 0.64 in OCD patients (Table III.4[b]). Both the OCD and control populations are in HWE at this locus ($p = 0.999$ and $p = 0.112$, respectively). The overall heterozygosity at the locus was found to be 0.513.

***Table III.3(a).*** *Exact Hardy-Weinberg equilibrium p-values for the control and OCD populations, and heterozygosity statistics for the bi-allelic candidate loci.*

| Gene | Variant | HWE exact p-value | | Heterozygosity |
|---|---|---|---|---|
| | | Controls | OCD | |
| ***5-HT$_{2A}$*** | **rs6311** | 0.39 | 0.31 | 0.47 |
| | **rs6313** | 0.33 | 0.07 | 0.48 |
| ***5-HT$_{1Dβ}$*** | **rs6296** | 0.68 | 1.00 | 0.38 |
| ***5-HT$_{6}$*** | **rs1805054** | 1.00 | 0.73 | 0.32 |
| ***5-HT$_{2C}$**** | **rs6318** | 0.13 | 0.42 | 0.34 |
| ***DRD4*** | **rs1800955** | 1.00 | 1.00 | 0.49 |
| ***DRD2*** | **rs1800497** | 0.50 | 1.00 | 0.38 |
| ***COMT*** | **rs2097603** | 0.50 | 0.30 | 0.51 |
| | **rs4680** | 0.38 | 0.15 | 0.50 |
| | **rs362204** | 0.68 | 0.48 | 0.38 |
| ***DRD3*** | **rs6280** | 0.24 | 0.04 | 0.41 |
| ***DRD1*** | **rs4532** | 0.71 | 0.66 | 0.46 |
| ***GRIN2B*** | **rs1806191** | 0.54 | 0.78 | 0.51 |
| | **rs890**[l] | 0.76 | 0.42 | 0.49 |
| ***BDNF*** | **rs6265** | 0.37 | 0.38 | 0.32 |
| | **rs2049046** | 0.80 | 0.78 | 0.50 |
| | **rs988748** | 0.67 | 0.47 | 0.39 |
| ***HOXB8*** | **rs2303486** | 0.55 | 0.37 | 0.50 |
| ***ESRα*** | **rs9340799** | 0.20 | 0.32 | 0.45 |
| | **rs2234693** | 0.27 | 0.80 | 0.50 |
| ***INPP-1*** | **rs1882891** | 0.65 | 1.00 | 0.22 |
| ***PLCγ1*** | **rs8192707** | 0.78 | 0.77 | 0.35 |
| ***ACE*** | **Ins/del** | 0.69 | 0.07 | 0.48 |

* HWE calculated using the female population.; **Abbreviations: HWE:** Hardy-Weinberg Equilibrium**; *5-HT$_{2A}$*:** serotonin receptor 2A**; *5-HT$_{1Dβ}$*:** serotonin receptor 1Dβ**; *5-HT$_{6}$*:** serotonin receptor 6**; *5-HT$_{2C}$*:** serotonin receptor 2C**; *DRD4*:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.3(b).*** *Genotype and allele scores and frequencies of bi-allelic candidate polymorphisms in control and OCD subjects.*

| Gene | Variant | $n_1/n_2$ [a] | Control | | | | | | | | | | | OCD | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | **rs6311** | *G/A* | 57 | 39.0 | 64 | 43.8 | 25 | 17.1 | **146** | 178 | 61.0 | 114 | 39.0 | 44 | 40.7 | 46 | 42.6 | 18 | 16.7 | **108** | 134 | 62.0 | 82 | 38.0 |
| | **rs6313** | *C/T* | 66 | 40.2 | 71 | 43.3 | 27 | 16.5 | **164** | 203 | 61.9 | 125 | 38.1 | 45 | 41.7 | 42 | 38.9 | 21 | 19.4 | **108** | 132 | 61.1 | 84 | 38.9 |
| ***5-HT$_{1Dβ}$*** | **rs6296** | *G/C* | 95 | 57.6 | 59 | 35.8 | 11 | 6.7 | **165** | 249 | 75.5 | 81 | 24.5 | 55 | 52.9 | 41 | 39.4 | 8 | 7.7 | **104** | 151 | 72.6 | 57 | 27.4 |
| ***5-HT$_6$*** | **rs1805054** | *C/T* | 80 | 72.7 | 29 | 26.4 | 1 | 0.9 | **110** | 189 | 85.9 | 31 | 14.1 | 53 | 63.1 | 29 | 34.5 | 2 | 2.4 | **84** | 135 | 80.4 | 33 | 19.6 |
| ***5-HT$_{2C}$*** [b] | **rs6318** | *G/C* | 100 | 68.5 | 35 | 24.0 | 11 | 7.5 | **146** | 200 | 78.1 | 56 | 21.9 | 68 | 69.4 | 15 | 15.3 | 15 | 15.3 | **98** | 113 | 76.9 | 34 | 23.1 |
| ***DRD4*** | **rs1800955** | *T/C* | 39 | 28.5 | 69 | 50.4 | 29 | 21.2 | **137** | 147 | 53.6 | 127 | 46.4 | 32 | 35.2 | 45 | 49.5 | 14 | 15.4 | **91** | 109 | 59.9 | 73 | 40.1 |
| ***DRD2*** | **rs1800497** | *C/T* | 74 | 56.1 | 48 | 36.4 | 10 | 7.6 | **132** | 196 | 74.2 | 68 | 25.8 | 57 | 55.3 | 40 | 38.8 | 6 | 5.8 | **103** | 154 | 74.8 | 52 | 25.2 |
| ***COMT*** | **rs2097603** | *A/G* | 28 | 35.4 | 36 | 45.6 | 15 | 19.0 | **79** | 92 | 58.2 | 66 | 41.8 | 16 | 27.1 | 25 | 42.4 | 18 | 30.5 | **59** | 57 | 48.3 | 61 | 51.7 |
| | **rs4680** | *G/A* | 32 | 25.0 | 69 | 53.9 | 27 | 21.1 | **128** | 133 | 52.0 | 123 | 48.0 | 16 | 17.2 | 54 | 58.1 | 23 | 24.7 | **93** | 86 | 46.2 | 100 | 53.8 |
| | **rs362204** | *D/I* | 42 | 40.0 | 51 | 48.6 | 12 | 11.4 | **105** | 135 | 64.3 | 75 | 35.7 | 28 | 53.8 | 22 | 42.3 | 2 | 3.8 | **52** | 78 | 75.0 | 26 | 25.0 |
| ***DRD3*** | **rs6280** | *A/G* | 51 | 42.5 | 51 | 42.5 | 18 | 15.0 | **120** | 153 | 63.8 | 87 | 36.3 | 54 | 56.3 | 30 | 31.3 | 12 | 12.5 | **96** | 138 | 71.9 | 54 | 28.1 |
| ***DRD1*** | ***A-48G*** | *A/G* | 47 | 36.2 | 65 | 50.0 | 18 | 13.8 | **130** | 159 | 61.2 | 101 | 38.8 | 40 | 40.0 | 49 | 49.0 | 11 | 11.0 | **100** | 129 | 64.5 | 71 | 35.5 |
| ***GRIN2B*** | **rs1806191** | *G/A* | 10 | 25.0 | 23 | 57.5 | 7 | 17.5 | **40** | 43 | 53.8 | 37 | 46.3 | 22 | 39.3 | 25 | 44.6 | 9 | 16.1 | **56** | 69 | 61.6 | 43 | 38.4 |
| | **rs890** | *A/C* | 12 | 29.3 | 22 | 53.7 | 7 | 17.1 | **41** | 46 | 56.1 | 36 | 43.9 | 18 | 31.6 | 31 | 54.4 | 8 | 14.0 | **57** | 67 | 58.8 | 47 | 41.2 |
| ***BDNF*** | **rs6265** | *G/A* | 95 | 67.9 | 43 | 30.7 | 2 | 1.4 | **140** | 233 | 83.2 | 47 | 16.8 | 73 | 65.2 | 33 | 29.5 | 6 | 5.4 | **112** | 179 | 79.9 | 45 | 20.1 |
| | **rs2049046** | *A/T* | 19 | 30.2 | 33 | 52.4 | 11 | 17.5 | **63** | 71 | 56.3 | 55 | 43.7 | 12 | 24.0 | 24 | 48.0 | 14 | 28.0 | **50** | 48 | 48.0 | 52 | 52.0 |
| | **rs988748** | *C/G* | 39 | 63.9 | 21 | 34.4 | 1 | 1.6 | **61** | 99 | 81.1 | 23 | 18.9 | 25 | 51.0 | 22 | 44.9 | 2 | 4.1 | **49** | 72 | 73.5 | 26 | 26.5 |
| ***HOXB8*** | **rs2303486** | *T/A* | 10 | 23.8 | 24 | 57.1 | 8 | 19.0 | **42** | 44 | 52.4 | 40 | 47.6 | 10 | 20.4 | 20 | 40.8 | 19 | 38.8 | **49** | 40 | 40.8 | 58 | 59.2 |
| ***ESRα*** | **rs9340799** | *A/G* | 57 | 42.5 | 55 | 41.0 | 22 | 16.4 | **134** | 169 | 63.1 | 99 | 36.9 | 39 | 47.6 | 32 | 39.0 | 11 | 13.4 | **82** | 110 | 67.1 | 54 | 32.9 |
| | **rs2234693** | *T/C* | 32 | 27.8 | 52 | 45.2 | 31 | 27.0 | **115** | 112 | 48.7 | 118 | 51.3 | 19 | 31.1 | 29 | 47.5 | 13 | 21.3 | **61** | 67 | 54.9 | 55 | 45.1 |
| ***INPP-1*** | **rs1882891** | *C/A* | 94 | 79.0 | 23 | 19.3 | 2 | 1.7 | **119** | 211 | 88.7 | 27 | 11.3 | 71 | 74.0 | 24 | 25.0 | 1 | 1.0 | **96** | 166 | 86.5 | 26 | 13.5 |
| ***PLC-γ1*** | **rs8192707** | *A/G* | 73 | 62.4 | 40 | 34.2 | 4 | 3.4 | **117** | 186 | 79.5 | 48 | 20.5 | 56 | 60.9 | 31 | 33.7 | 5 | 5.4 | **92** | 143 | 77.7 | 41 | 22.3 |
| ***ACE*** | **Alu ins/del** | *D/I* | 63 | 47.0 | 59 | 44.0 | 12 | 9.0 | **134** | 185 | 69.0 | 83 | 31.0 | 44 | 40.4 | 43 | 39.4 | 22 | 20.2 | **109** | 131 | 60.1 | 87 | 39.9 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele. [b] Hemizygous males were grouped with females homozygous for the *ser23(C)* or *cys23(G)* alleles for descriptive purposes only

**Abbreviations: *5-HT$_{2A}$*:** serotonin receptor 2A**; *5-HT$_{1Dβ}$*:** serotonin receptor 1Dβ**; *5-HT$_6$*:** serotonin receptor 6**; *5-HT$_{2C}$*:** serotonin receptor 2C**; *DRD4*:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derived neurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme

***Table III.3(c).*** *Genotype and allele scores and frequencies of bi-allelic candidate polymorphisms in male control and OCD subjects.*

| Gene | Variant | $n_1/n_2$[a] | Control | | | | | | | | | | | OCD | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles (%) | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | **rs6311** | *G/A* | 15 | 41.7 | 15 | 41.7 | 6 | 16.7 | **36** | 45 | 62.5 | 27 | 37.5 | 27 | 49.1 | 21 | 38.2 | 7 | 12.7 | **55** | 75 | 68.2 | 35 | 31.8 |
| | **rs6313** | *C/T* | 14 | 35.0 | 20 | 50.0 | 6 | 15.0 | **40** | 48 | 60.0 | 32 | 40.0 | 26 | 47.3 | 19 | 34.5 | 10 | 18.2 | **55** | 71 | 64.5 | 39 | 35.5 |
| ***5-HT$_{1Dβ}$*** | **rs6296** | *G/C* | 26 | 63.4 | 14 | 34.1 | 1 | 2.4 | **41** | 66 | 80.5 | 16 | 19.5 | 34 | 64.2 | 15 | 28.3 | 4 | 7.5 | **53** | 83 | 78.3 | 23 | 21.7 |
| ***5-HT$_6$*** | **rs1805054** | *C/T* | 15 | 60.0 | 10 | 40.0 | 0 | 0.0 | **25** | 40 | 80.0 | 10 | 20.0 | 23 | 59.0 | 15 | 38.5 | 1 | 2.6 | **39** | 61 | 78.2 | 17 | 21.8 |
| ***5-HT$_{2C}$*** | **rs6318** | *G/C* | 35 | 97.2 | 0 | 0.0 | 1 | 2.8 | **36** | 35 | 97.2 | 1 | 2.8 | 38 | 77.6 | 0 | 0.0 | 11 | 22.4 | **49** | 38 | 77.6 | 11 | 22.4 |
| ***DRD4*** | **rs1800955** | *T/C* | 15 | 45.5 | 14 | 42.4 | 4 | 12.1 | **33** | 44 | 66.7 | 22 | 33.3 | 16 | 34.8 | 23 | 50.0 | 7 | 15.2 | **46** | 55 | 59.8 | 37 | 40.2 |
| ***DRD2*** | **rs1800497** | *C/T* | 12 | 41.4 | 13 | 44.8 | 4 | 13.8 | **29** | 37 | 63.8 | 21 | 36.2 | 28 | 58.3 | 17 | 35.4 | 3 | 6.3 | **48** | 73 | 76.0 | 23 | 24.0 |
| ***COMT*** | **rs2097603** | *A/G* | 4 | 26.7 | 8 | 53.3 | 3 | 20.0 | **15** | 16 | 53.3 | 14 | 46.7 | 9 | 33.3 | 10 | 37.0 | 8 | 29.6 | **27** | 28 | 51.9 | 26 | 48.1 |
| | **rs4680** | *G/A* | 13 | 37.1 | 18 | 51.4 | 4 | 11.4 | **35** | 44 | 62.9 | 26 | 37.1 | 9 | 19.6 | 28 | 60.9 | 9 | 19.6 | **46** | 46 | 50.0 | 46 | 50.0 |
| | **rs362204** | *D/I* | 8 | 32.0 | 15 | 60.0 | 2 | 8.0 | **25** | 31 | 62.0 | 19 | 38.0 | 15 | 65.2 | 8 | 34.8 | 0 | 0.0 | **23** | 38 | 82.6 | 8 | 17.4 |
| ***DRD3*** | **rs6280** | *A/G* | 11 | 44.0 | 12 | 48.0 | 2 | 8.0 | **25** | 34 | 68.0 | 16 | 32.0 | 29 | 61.7 | 10 | 21.3 | 8 | 17.0 | **47** | 68 | 72.3 | 26 | 27.7 |
| ***DRD1*** | ***A-48G*** | *A/G* | 11 | 34.4 | 20 | 62.5 | 1 | 3.1 | **32** | 42 | 65.6 | 22 | 34.4 | 20 | 39.2 | 27 | 52.9 | 4 | 7.8 | **51** | 67 | 65.7 | 35 | 34.3 |
| ***GRIN2B*** | **rs1806191** | *G/A* | 4 | 26.7 | 8 | 53.3 | 3 | 20.0 | **15** | 16 | 53.3 | 14 | 46.7 | 10 | 33.3 | 17 | 56.7 | 3 | 10.0 | **30** | 37 | 61.7 | 23 | 38.3 |
| | **rs890** | *A/C* | 8 | 32.0 | 13 | 52.0 | 4 | 16.0 | **25** | 29 | 58.0 | 21 | 42.0 | 7 | 23.3 | 18 | 60.0 | 5 | 16.7 | **30** | 32 | 53.3 | 28 | 46.7 |
| ***BDNF*** | **rs6265** | *G/A* | 25 | 75.8 | 8 | 24.2 | 0 | 0.0 | **33** | 58 | 87.9 | 8 | 12.1 | 33 | 57.9 | 19 | 33.3 | 5 | 8.8 | **57** | 85 | 74.6 | 29 | 25.4 |
| | **rs2049046** | *A/T* | 5 | 35.7 | 6 | 42.9 | 3 | 21.4 | **14** | 16 | 57.1 | 12 | 42.9 | 8 | 28.6 | 13 | 46.4 | 7 | 25.0 | **28** | 29 | 51.8 | 27 | 48.2 |
| | **rs988748** | *C/G* | 5 | 38.5 | 8 | 61.5 | 0 | 0.0 | **13** | 18 | 69.2 | 8 | 30.8 | 16 | 57.1 | 10 | 35.7 | 2 | 7.1 | **28** | 42 | 75.0 | 14 | 25.0 |
| ***HOXB8*** | **rs2303486** | *T/A* | 3 | 30.0 | 5 | 50.0 | 2 | 20.0 | **10** | 11 | 55.0 | 9 | 45.0 | 5 | 17.9 | 11 | 39.3 | 12 | 42.9 | **28** | 21 | 37.5 | 35 | 62.5 |
| ***ESRα*** | **rs9340799** | *A/G* | 13 | 44.8 | 12 | 41.4 | 4 | 13.8 | **29** | 38 | 65.5 | 20 | 34.5 | 19 | 46.3 | 16 | 39.0 | 6 | 14.6 | **41** | 54 | 65.9 | 28 | 34.1 |
| | **rs2234693** | *T/C* | 6 | 25 | 11 | 45.8 | 7 | 29.2 | **24** | 23 | 47.9 | 25 | 52.1 | 19 | 31.1 | 29 | 47.5 | 13 | 21.3 | **61** | 67 | 54.9 | 55 | 45.1 |
| ***INPP-1*** | **rs1882891** | *C/A* | 20 | 74.1 | 7 | 25.9 | 0 | 0.0 | **27** | 47 | 87.0 | 7 | 13.0 | 35 | 74.5 | 12 | 25.5 | 0 | 0.0 | **47** | 82 | 87.2 | 12 | 12.8 |
| ***PLC-γ1*** | **rs8192707** | *A/G* | 15 | 57.7 | 9 | 34.6 | 2 | 7.7 | **26** | 39 | 75.0 | 13 | 25.0 | 29 | 64.4 | 14 | 31.1 | 2 | 4.4 | **45** | 72 | 80.0 | 18 | 20.0 |
| ***ACE*** | **Ins/del** | *D/I* | 14 | 46.7 | 15 | 50.0 | 1 | 3.3 | **30** | 43 | 71.7 | 17 | 28.3 | 21 | 37.5 | 20 | 35.7 | 15 | 26.8 | **56** | 62 | 55.4 | 50 | 44.6 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele

**Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***5-HT$_{2C}$*****:** serotonin receptor 2C**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α;

***Table III.3(d).*** *Genotype and allele scores and frequencies of bi-allelic candidate polymorphisms in female control and OCD subjects.*

| Gene | Variant | $n_1/n_2$[a] | Control | | | | | | | | | | | OCD | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | **rs6311** | *G/A* | 42 | 38.2 | 49 | 44.5 | 19 | 17.3 | **110** | 133 | 60.5 | 87 | 39.5 | 17 | 32.1 | 25 | 47.2 | 11 | 20.8 | **53** | 59 | 55.7 | 47 | 44.3 |
| | **rs6313** | *C/T* | 52 | 41.9 | 51 | 41.1 | 21 | 16.9 | **124** | 155 | 62.5 | 93 | 37.5 | 19 | 35.8 | 23 | 43.4 | 11 | 20.8 | **53** | 61 | 57.5 | 45 | 42.5 |
| ***5-HT$_{1Dβ}$*** | **rs6296** | *G/C* | 69 | 55.6 | 45 | 36.3 | 10 | 8.1 | **124** | 183 | 73.8 | 65 | 26.2 | 21 | 41.2 | 26 | 51.0 | 4 | 7.8 | **51** | 68 | 66.7 | 34 | 33.3 |
| ***5-HT$_{6}$*** | **rs1805054** | *C/T* | 65 | 76.5 | 19 | 22.4 | 1 | 1.2 | **85** | 149 | 87.6 | 21 | 12.4 | 30 | 66.7 | 14 | 31.1 | 1 | 2.2 | **45** | 74 | 82.2 | 16 | 17.8 |
| ***5-HT$_{2C}$*** | **rs6318** | *G/C* | 65 | 59.1 | 35 | 31.8 | 10 | 9.1 | **110** | 165 | 75.0 | 55 | 25.0 | 30 | 61.2 | 15 | 30.6 | 4 | 8.2 | **49** | 75 | 76.5 | 23 | 23.5 |
| ***DRD4*** | **rs1800955** | *T/C* | 24 | 23.1 | 55 | 52.9 | 25 | 24.0 | **104** | 103 | 49.5 | 105 | 50.5 | 16 | 35.6 | 22 | 48.9 | 7 | 15.6 | **45** | 54 | 60.0 | 36 | 40.0 |
| ***DRD2*** | **rs1800497** | *C/T* | 62 | 60.2 | 35 | 34.0 | 6 | 5.8 | **103** | 159 | 77.2 | 47 | 22.8 | 29 | 52.7 | 23 | 41.8 | 3 | 5.5 | **55** | 81 | 73.6 | 29 | 26.4 |
| ***COMT*** | **rs2097603** | *A/G* | 24 | 37.5 | 28 | 43.8 | 12 | 18.8 | **64** | 76 | 59.4 | 52 | 40.6 | 7 | 21.9 | 15 | 46.9 | 10 | 31.3 | **32** | 29 | 45.3 | 35 | 54.7 |
| | **rs4680** | *G/A* | 19 | 20.4 | 51 | 54.8 | 23 | 24.7 | **93** | 89 | 47.8 | 97 | 52.2 | 7 | 14.9 | 26 | 55.3 | 14 | 29.8 | **47** | 40 | 42.6 | 54 | 57.4 |
| | **rs362204** | *D/I* | 34 | 42.5 | 36 | 45.0 | 10 | 12.5 | **80** | 104 | 65.0 | 56 | 35.0 | 13 | 44.8 | 14 | 48.3 | 2 | 6.9 | **29** | 40 | 69.0 | 18 | 31.0 |
| ***DRD3*** | **rs6280** | *A/G* | 40 | 42.1 | 39 | 41.1 | 16 | 16.8 | **95** | 119 | 62.6 | 71 | 37.4 | 25 | 51.0 | 20 | 40.8 | 4 | 8.2 | **49** | 70 | 71.4 | 28 | 28.6 |
| ***DRD1*** | **A-48G** | *A/G* | 36 | 36.7 | 45 | 45.9 | 17 | 17.3 | **98** | 117 | 59.7 | 79 | 40.3 | 20 | 40.8 | 22 | 44.9 | 7 | 14.3 | **49** | 62 | 63.3 | 36 | 36.7 |
| ***GRIN2B*** | **rs1806191** | *G/A* | 6 | 24.0 | 15 | 60.0 | 4 | 16.0 | **25** | 27 | 54.0 | 23 | 46.0 | 12 | 46.2 | 8 | 30.8 | 6 | 23.1 | **26** | 32 | 61.5 | 20 | 38.5 |
| | **rs890** | *A/C* | 4 | 25.0 | 9 | 56.3 | 3 | 18.8 | **16** | 17 | 53.1 | 15 | 46.9 | 11 | 40.7 | 13 | 48.1 | 3 | 11.1 | **27** | 35 | 64.8 | 19 | 35.2 |
| ***BDNF*** | **rs6265** | *G/A* | 70 | 65.4 | 35 | 32.7 | 2 | 1.9 | **107** | 175 | 81.8 | 39 | 18.2 | 40 | 72.7 | 14 | 25.5 | 1 | 1.8 | **55** | 94 | 85.5 | 16 | 14.5 |
| | **rs2049046** | *A/T* | 13 | 28.3 | 25 | 54.3 | 8 | 17.4 | **46** | 51 | 55.4 | 41 | 44.6 | 4 | 18.2 | 11 | 50.0 | 7 | 31.8 | **22** | 19 | 43.2 | 25 | 56.8 |
| | **rs988748** | *C/G* | 33 | 73.3 | 11 | 24.4 | 1 | 2.2 | **45** | 77 | 85.6 | 13 | 14.4 | 9 | 42.9 | 12 | 57.1 | 0 | 0.0 | **21** | 30 | 71.4 | 12 | 28.6 |
| ***HOXB8*** | **rs2303486** | *T/A* | 7 | 21.9 | 19 | 59.4 | 6 | 18.8 | **32** | 33 | 51.6 | 31 | 48.4 | 5 | 23.8 | 9 | 42.9 | 7 | 33.3 | **21** | 19 | 45.2 | 23 | 54.8 |
| ***ESRα*** | **rs9340799** | *A/G* | 44 | 41.9 | 43 | 41.0 | 18 | 17.1 | **105** | 131 | 62.4 | 79 | 37.6 | 20 | 48.8 | 16 | 39.0 | 5 | 12.2 | **41** | 56 | 68.3 | 26 | 31.7 |
| | **rs2234693** | *T/C* | 26 | 28.6 | 41 | 45.1 | 24 | 26.4 | **91** | 89 | 48.9 | 93 | 51.1 | 6 | 22.2 | 14 | 51.9 | 7 | 25.9 | **27** | 26 | 48.1 | 28 | 51.9 |
| ***INPP-1*** | **rs1882891** | *C/A* | 74 | 80.4 | 16 | 17.4 | 2 | 2.2 | **92** | 164 | 89.1 | 20 | 10.9 | 36 | 73.5 | 12 | 24.5 | 1 | 2.0 | **49** | 84 | 85.7 | 14 | 14.3 |
| ***PLCγ1*** | **rs8192707** | *A/G* | 58 | 63.7 | 31 | 34.1 | 2 | 2.2 | **91** | 147 | 80.8 | 35 | 19.2 | 27 | 57.4 | 17 | 36.2 | 3 | 6.4 | **47** | 71 | 75.5 | 23 | 24.5 |
| ***ACE*** | **Alu Ins/del** | *D/I* | 49 | 47.1 | 44 | 42.3 | 11 | 10.6 | **104** | 142 | 68.3 | 66 | 31.7 | 23 | 43.4 | 23 | 43.4 | 7 | 13.2 | **53** | 69 | 65.1 | 37 | 34.9 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele.

**Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_{6}$*****:** serotonin receptor 6**;** ***5-HT$_{2C}$*****:** serotonin receptor 2C**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derived neurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.4(a).*** *Descriptive overview of the* DRD4 *48bp VNTR polymorphism, indicating the genotype counts and frequencies in the total OCD and control populations, and stratified by gender.*

| Genotype | Control | | | | | | OCD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | Male | | Female | | Total | | Male | | Female | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| *A4/A4* | **79** | 52.3 | **18** | 47.4 | **61** | 54.0 | **34** | 38.2 | **13** | 30.2 | **21** | 45.7 |
| *A4/A7* | **24** | 15.9 | **8** | 21.1 | **16** | 14.2 | **21** | 23.6 | **13** | 30.2 | **8** | 17.4 |
| *A4/A2* | **15** | 9.9 | **2** | 5.3 | **13** | 11.5 | **15** | 16.9 | **5** | 11.6 | **10** | 21.7 |
| *A7/A7* | **12** | 7.9 | **5** | 13.2 | **7** | 6.2 | **5** | 5.6 | **1** | 2.3 | **4** | 8.7 |
| *A4/A3* | **9** | 6.0 | **2** | 5.3 | **7** | 6.2 | **9** | 10.1 | **7** | 16.3 | **2** | 4.3 |
| *A2/A2* | **5** | 3.3 | **0** | 0.0 | **5** | 4.4 | **1** | 1.1 | **1** | 2.3 | **0** | 0.0 |
| *A4/A6* | **2** | 1.3 | **2** | 5.3 | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 |
| *A7/A3* | **2** | 1.3 | **1** | 2.6 | **1** | 0.9 | **1** | 1.1 | **1** | 2.3 | **0** | 0.0 |
| *A2/A3* | **1** | 0.7 | **0** | 0.0 | **1** | 0.9 | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 |
| *A3/A3* | **1** | 0.7 | **0** | 0.0 | **1** | 0.9 | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 |
| *A7/A6* | **1** | 0.7 | **0** | 0.0 | **1** | 0.9 | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 |
| *A3/A6* | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 | **1** | 1.1 | **1** | 2.3 | **0** | 0.0 |
| *A7/A2* | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 | **2** | 2.2 | **1** | 2.3 | **1** | 2.2 |

*A2*=2-repeat allele; *A3*=3-repeat allele *A4*=4-repeat allele; *A6*=6-repeat allele; *A7*=7-repeat allele.
**Abbreviations**: **OCD**: obsessive-compulsive disorder.

***Table III.4(b).*** *Descriptive overview of the* DRD4 *48bp VNTR polymorphism, indicating the allele counts and frequencies in the total OCD and control populations, and stratified by gender.*

| Allele | Control | | | | | | OCD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | Male | | Female | | Total | | Male | | Female | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| *A4* | **208** | 68.9 | **50** | 65.8 | **158** | 69.9 | **113** | 63.5 | **51** | 59.3 | **62** | 67.4 |
| *A7* | **51** | 16.9 | **19** | 25.0 | **32** | 14.2 | **34** | 19.1 | **17** | 19.8 | **17** | 18.5 |
| *A2* | **26** | 8.6 | **2** | 2.6 | **24** | 10.6 | **19** | 10.7 | **8** | 9.3 | **11** | 12.0 |
| *A3* | **14** | 4.6 | **3** | 3.9 | **11** | 4.9 | **11** | 6.2 | **9** | 10.5 | **2** | 2.2 |
| *A6* | **3** | 1.0 | **2** | 2.6 | **1** | 0.4 | **1** | 0.6 | **1** | 1.2 | **0** | 0.0 |

*A2*=2-repeat allele; *A3*=3-repeat allele *A4*=4-repeat allele; *A6*=6-repeat allele; *A7*=7-repeat allele.
**Abbreviations**: **OCD**: obsessive-compulsive disorder.

### III.3.2.2. DAT *40bp VNTR*

The counts and associated frequencies of the DAT 40bp VNTR genotypes and alleles in the control and OCD samples are presented in Tables III.5 (a) and (b), respectively. A total of 180 controls and 110 OCD subjects were genotyped for this polymorphism. The 10-repeat allele (*A10*) was found to be the most abundant amongst both case and control individuals (with frequencies of 73.6% and 74.4%, respectively) (Table III.5[b]). Both case and control individuals were found to be in HWE for this polymorphism (p=0.07 and p=0.84, respectively). The overall heterozygosity at this locus was found to be 0.401.

***Table III.5(a).*** *Descriptive overview of the* DAT *40bp VNTR polymorphism, indicating the genotype counts and frequencies in the total OCD and control populations, and stratified by gender.*

| Genotype | Control | | | | | | OCD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | Male | | Female | | Total | | Male | | Female | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| *A10/A10* | **106** | 58.9 | **29** | 63.0 | **77** | 57.5 | **60** | 54.5 | **32** | 56.1 | **28** | 52.8 |
| *A10/A9* | **54** | 30.0 | **10** | 21.7 | **44** | 32.8 | **38** | 34.5 | **18** | 31.6 | **20** | 37.7 |
| *A9/A9* | **17** | 9.4 | **6** | 13.0 | **11** | 8.2 | **8** | 7.3 | **3** | 5.3 | **5** | 9.4 |
| *A10/A11* | **2** | 1.1 | **1** | 2.2 | **1** | 0.7 | **3** | 2.7 | **3** | 5.3 | **0** | 0.0 |
| *A10/A2* | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 | **1** | 0.9 | **1** | 1.8 | **0** | 0.0 |
| *A9/A11* | **1** | 0.6 | **0** | 0.0 | **1** | 0.7 | **0** | 0.0 | **0** | 0.0 | **0** | 0.0 |

*A2*=2-repeat allele; *A9*=9-repeat allele; *A10*=10-repeat allele; *A11*=11-repeat allele.
**Abbreviations**: **OCD**: obsessive-compulsive disorder

***Table III.5(b).*** *Descriptive overview of the* DAT *40bp VNTR polymorphism, indicating the allele counts and frequencies in the total OCD and control populations, and stratified by gender.*

| Genotype | Control | | | | | | OCD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | Male | | Female | | Total | | Male | | Female | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| *A10* | **268** | 74.4 | **69** | 75 | **199** | 74.3 | **162** | 73.6 | **86** | 75.4 | **76** | 71.7 |
| *A9* | **89** | 24.7 | **22** | 23.9 | **67** | 25 | **54** | 24.5 | **24** | 21.1 | **30** | 28.3 |
| *A11* | **3** | 0.8 | **1** | 1.1 | **2** | 0.7 | **3** | 1.4 | **3** | 2.6 | **0** | 0 |
| *A2* | **0** | 0 | **0** | 0 | **0** | 0 | **1** | 0.5 | **1** | 0.9 | **0** | 0 |

*A2*=2-repeat allele; *A9*=9-repeat allele; *A10*=10-repeat allele; *A11*=11-repeat allele.
**Abbreviations**: **OCD**: obsessive-compulsive disorder

**III.3.3. Linkage Disequilibrium**

LD analyses were performed for those candidate genes for which two or more variants were genotyped. These genes included: *5-HT$_{2A}$*, *DRD4*, *COMT*, *GRIN2B* and *BDNF* and *ESRα*. Table III.6 depicts the D' and $r^2$ values obtained when the variants were investigated for pairwise LD.

A high degree of LD was observed between the *5-HT$_{2A}$* SNPs (*-1438 A/G* [rs6313] and *T102C* [rs6311]) (D'= 0.861; $r^2$= 0.737), consistent with information obtained from previous literature (Masellis et al., 1998). On the other hand, very little pairwise LD was observed between the *DRD4 -521 C/T* (rs1800955) and exon 3 VNTR polymorphisms (D'= 0.140; $r^2$= 0.028). Pairwise LD was observed between the *COMT val158met* (rs4680) polymorphism and rs362204 (D'= 0.545; $r^2$= 0.138), but not between *val158met* and rs2097603, or rs362204 and rs2097603.

The *GRIN2B* polymorphisms under investigation in the present study (rs1806191 and rs890) were found to be in strong, although not complete, LD with one another. This is to be expected, given the relatively short distance of 1.3kb between them (D'= 0.545; $r^2$ = 0.285). For *BDNF*, a high degree of pairwise LD was observed between all the variants (BDNF rs6265, rs2049046 and rs988748) studied, consistent with results from previous studies (Hall et al., 2003; Sklar et al.,2002). A relatively high degree of pairwise LD was also noted between the two *ESRα* polymorphisms (rs9340799 and rs2234693) investigated (D'= 0.887; $r^2$ = 0.476).

***Table III.6.*** *Pairwise LD values for SNPs occurring within the same gene for the whole dataset used in the present study.*

| Gene | Variant 1 | Variant2 | \|D'\| | $r^2$ |
|---|---|---|---|---|
| ***5-HT$_{2A}$*** | **rs6311** | **rs6313** | 0.861 | 0.737 |
| ***DRD4*** | **rs1800995** | **48bp VNTR** | 0.140 | 0.028 |
| ***COMT*** | **rs2097603** | **rs4680** | 0.214 | 0.014 |
| | **rs4680** | **rs362204** | 0.545 | 0.138 |
| | **rs2097603** | **rs362204** | 0.420 | 0.072 |
| ***GRIN2B*** | **rs1806191** | **rs890** | 0.545 | 0.285 |
| ***BDNF*** | **rs6265** | **rs2049046** | 0.810 | 0.430 |
| | **rs6265** | **rs988748** | 0.920 | 0.818 |
| | **rs2049046** | **rs988748** | 0.805 | 0.204 |
| ***ESRα*** | **rs9340799** | **rs2234693** | 0.887 | 0.476 |

**Abbreviations:** ***5-HT$_{2A}$***: serotonin receptor 2A; ***DRD4***: dopamine receptor 4; ***COMT:*** Catechol-O-methyltransferase; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α.

## III.4. CLINICAL AND GENETIC ANALYSES OF DATA

The following sections pertain to the analysis of clinical and genetic data, stratified and analysed according to:

i. gender
ii. severity (as measured by total Y-BOCS score)
iii. age at onset of OCD
iv. the presence or absence of co-morbid disorders (MDD and tics)
v. symptom dimensions (hoarding, symmetry/ordering, sexual/religious, contamination/washing and aggressive symptoms).

Since numbers become particularly small when stratifying the dataset according to the selected criterion, haplotype analyses were only conducted for the case-control, severity and age at onset analyses. Moreover, as haplotype counts were reduced further when stratifying for gender, only whole group comparisons were performed for these three analyses.

### III.4.1. Stratification by gender

One hundred and thirty two Afrikaner OCD subjects (71 (54%) male and 61 (46%) female) and 218 Afrikaner control subjects (56 (26%) male and 162 (74%) female) were utilised in the present study. The proportion of each gender was found to differ significantly between the OCD and control subgroups ($p< 0.001$).

#### III.4.1.1. Age at interview

The median age at interview for the OCD subjects was 27 years (95% CI: 24.5-34.2), and for the control subjects was 36 years (95% CI: 29.5-37.9). Age at time of interview for both the OCD and control subjects, stratified according to gender, is represented by the box plot in Figure III.39. From this figure, it is evident that the distributions of the ages are skewed within the groups; this was confirmed by the Shapiro-Wilks test for normality. It was found that taking the natural logs of the ages transformed them to symmetry. The variances are, however, still very different, as confirmed by Bartlett's test. Nonparametric tests were thus used throughout the present study.

The median age for males in the control group (43 years [95% CI: 39-47]) is significantly higher than for any of the other three groups ($p< 0.001$). The median ages of the females differ by 3.5 years, and the difference is thus not statistically significant. There was also no

statistically significant difference between the median age at interview of males and females within the OCD subset (p = 0.091), although the males were on average slightly younger than the females.

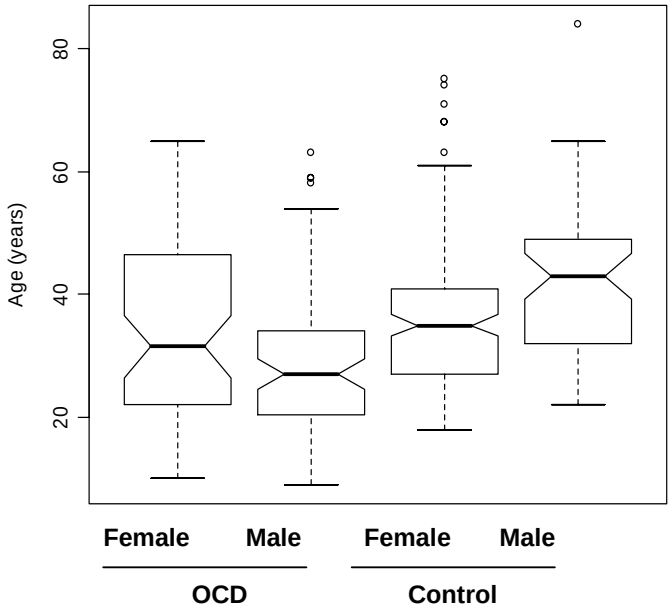



***Figure III.39.*** *Boxplot representing distribution and means of age at interview (in years) of OCD and control individuals included in the present study.*

**III.4.1.2. Clinical characteristics of males and females within the OCD sample**

The demographic and clinical characteristics, stratified by gender, of the 132 Afrikaner OCD patients used in the study are depicted in Table III.7. Quantitative data are represented as medians with 95% confidence intervals (CI), whilst categorical data are represented by counts and percentage of group.

Total Y-BOCS scores were recorded in 129 (97.7 %) of the OCD patients; the median Y-BOCS score was 21 (95% CI: 19.7-22.3). No statistically significant differences in Y-BOCS were observed between male and female subjects (p=0.548) (Table III.7). Age at onset of OCD was recorded for 119 (90.2%) of the OCD subjects, with the median age at onset of OCD found to be 14 years (95% CI: 12-15). Although the median age at onset of OCD was lower in males compared to females (14 [95% CI: 12-15] and 15 [95% CI: 12-18] years, respectively), the difference was not significant (p = 0.182) (Table III.7).

***Table III.7.*** *Demographic and clinical characteristics of male and female OCD subjects included in the present study.*

| **Clinical Characteristic** | | | **Male** | | **Female** | | |
|---|---|---|---|---|---|---|---|
| | **n** | **%** | **median** | **95% CI** | **median** | **95% CI** | **p-value** |
| Age at interview | **131** | 99.2 | **27** | 24-30 | **32** | 27-36 | 0.091 |
| Y-BOCS score | **129** | 97.7 | **20** | 18-22 | **21** | 19-23 | 0.548 |
| Age of OCD onset | **119** | 90.2 | **14** | 12-15 | **15** | 12-18 | 0.182 |
| **Family history** | **n[a]** | **%** | **n** | **%** | **n** | **%** | **p-value** |
| Family history of OCD | **20** | 25.0 | **11** | 25.6 | **9** | 24.3 | 0.897 |
| Family history of OCS | **37** | 46.3 | **23** | 53.5 | **14** | 37.8 | 0.235 |
| Family history of tics | **6** | 7.5 | **3** | 7.0 | **3** | 8.1 | 0.848 |
| **Primary symptom dimensions** | **n[b]** | **%** | **n** | **%** | **n** | **%** | **p-value** |
| Hoarding/collecting | **23** | 25.3 | **7** | 16.7 | **16** | 32.7 | 0.091 |
| Symmetry/ordering | **53** | 58.2 | **26** | 61.9 | **27** | 55.1 | 0.530 |
| Sex/religion | **38** | 41.8 | **24** | 57.1 | **14** | 28.6 | **0.010** |
| Contamination/washing | **55** | 60.4 | **26** | 61.9 | **29** | 59.2 | 0.830 |
| Aggression | **46** | 50.5 | **20** | 47.6 | **26** | 53.1 | 0.670 |
| Miscellaneous | **56** | 61.5 | **26** | 61.9 | **30** | 61.2 | 1.000 |
| **Comorbidity** | **n[c]** | **%** | **n** | **%** | **n** | **%** | **p-value** |
| MDD | **82** | 64.6 | **42** | 61.8 | **40** | 67.8 | 0.580 |
| SIB | **24** | 18.9 | **12** | 17.6 | **12** | 20.3 | 0.820 |
| Dysthymia | **20** | 15.7 | **12** | 17.6 | **8** | 13.6 | 0.630 |
| Tics | **18** | 14.2 | **13** | 19.1 | **5** | 8.5 | 0.126 |
| Specific phobia | **18** | 14.2 | **5** | 7.4 | **13** | 22.0 | **0.022** |
| GAD | **16** | 12.6 | **8** | 11.8 | **8** | 13.6 | 0.800 |
| PD | **13** | 10.2 | **4** | 5.9 | **9** | 15.3 | 0.140 |
| Social phobia | **13** | 10.2 | **6** | 8.8 | **7** | 11.9 | 0.770 |
| TTM | **12** | 9.4 | **4** | 5.9 | **8** | 13.6 | 0.220 |
| IED | **12** | 9.4 | **6** | 8.8 | **6** | 10.2 | 1.000 |
| BDD | **10** | 7.9 | **6** | 8.8 | **4** | 6.8 | 0.750 |
| TS | **7** | 5.5 | **4** | 5.9 | **3** | 5.1 | 1.000 |
| Anorexia | **5** | 3.9 | **0** | 0.0 | **5** | 8.5 | **0.020** |
| Hypochondriasis | **5** | 3.9 | **3** | 4.4 | **2** | 3.4 | 1.000 |

[a](n=80: male=43, female=37 ) [b](n=91: male=42, female=49) [c](n=127: male=68, female=59)

**Abbreviations: 95% CI:** 95% confidence intervals**; Y-BOCS:** Yale-Brown Obsessive-Compulsive Score; **OCD:** Obsessive-compulsive disorder; **OCS**: obsessive-compulsive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome.

When the family history of OCD and OCS were considered, no significant differences were noted between male and female OCD patients (p = 0.897 and p = 0.235, respectively). Likewise, no significant differences in family history of tics was observed between male and female patients (p = 0.848) (Table III.7).

Data on primary symptoms are available for 91 patients, 49 female and 42 male (Table III.7). It is interesting to note that contamination and washing represents the major primary symptom subtype, with 60.4% of the patients exhibiting this symptom with no significant difference between the genders (p = 0.830) (Figure III.40). However, significantly more males were found to suffer from sexual/ religious symptoms (57.1% of the male OCD subset compared to 28.6% of the female OCD sample; p = 0.010). No other statistically significant differences were observed when the remaining symptom subtypes were stratified according to gender (Table III.7 and Figure III.40).

Table III.7 and Figure III.41 indicate the proportions of males and females presenting with selected co-morbid disorders. The most common co-morbid disorder observed amongst the OCD subjects was major depressive disorder (MDD), diagnosed in 82 (64.6%) of the patients, with no difference in lifetime prevalence observed between males and females (61.8% and 67.8% in the male and female subsets, respectively; p = 0.580). When lifetime prevalence of other selected co-morbid disorders was compared between male and female OCD subjects, a significantly greater number of females were found to suffer from co-morbid specific phobia (7.4% versus 22.0%, for the male and female OCD subsets, respectively; p = 0.022) and anorexia nervosa (0% and 8.5%, for male and female subsets, respectively; p = 0.020).

### III.4.1.3. Single locus case-control genetic association analysis

#### *III.4.1.3.1. Single locus analysis of bi-allelic polymorphisms*

The results of the single locus case-control genetic association analyses for bi-allelic data in the whole sample are depicted in Table III.8. Tables III.9 and III.10 represent the separate statistical analyses for bi-allelic loci for males and females, respectively. When the genotype frequencies were compared between the case and control subjects, a statistically significant difference was noted in the distribution of the ACE *Alu* Ins/del genotype (p = 0.037; OR = 0.38 [95% CI: 0.16-0.91]) (Table III.8), with the *DD*- and *DI*-genotypes over-represented in

the control sample (Table III.3[b]). However, when the distribution of the allele frequencies was compared between the case and control individuals, no significant difference was observed (p = 0.057; OR = 0.69 [95% CI: 0.47-1.10]) (Table III.8), indicating that the genetic model involved in conferring protection to OCD may be dominant.

Interestingly, when the dataset was stratified according to gender, the association with *ACE* was maintained when genotype distribution of the *Alu* ins/del polymorphism was compared between male OCD subjects and controls (p = 0.020; OR = 0.10 [95% CI: 0.00-0.83]). A nominally significant association was observed when *ACE* allele distribution was compared between male OCD and control subjects (p = 0.049; OR = 0.49 [95% CI: 0.23-1.00]) (Table III.9), with the *D*-allele once again representing the protective allele. However, neither genotypic nor allelic association was observed in the female subset (p = 0.814 and p = 0.612, respectively) (Table III.10).

Comparing the allelic frequencies of the *5-HT$_{2C}$ cys23ser* (rs6318) polymorphism, statistically significant differences were observed between male OCD and control subjects (p<0.001; OR=0.10 [95% CI: 0.01-0.44]), indicating that the *G*-allele (forming part of the codon that encodes *cys23*) represents the protective allele (Table III.9). No statistically significant differences were observed when the same SNP was investigated in the female population (Table III.10).

Further significant differences between male OCD and control subjects were observed with regard to the *COMT* rs362204 and *BDNF val66met* (rs6265) allelic distributions (p=0.040 and p=0.036, respectively). For the *COMT* rs362204 polymorphism, the resultant allelic OR of 2.88 (95% CI: 1.03-8.70) indicates that the allele characterised by the deletion of a cytosine base (denoted as the *D*-allele in the present study) increases the risk of developing the disorder, compared to the *C*-insertion allele (denoted as the *I*-allele in the present study). When the genotypic distribution of the variant was considered, the OCD subjects presented with a higher frequency of *DD*-genotypes compared to controls; although this difference represented only a trend towards association (p = 0.071 [Table III.9]), it supports the notion that the *D*-allele may represent a risk factor in the development of OCD.

***Figure III.40.*** *Bar graph indicating the proportions of male and female OCD subjects experiencing certain obsessions and/or compulsions.*

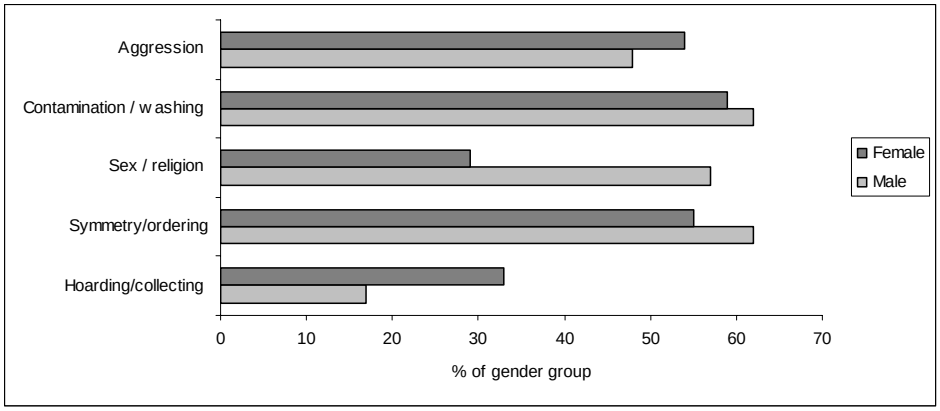
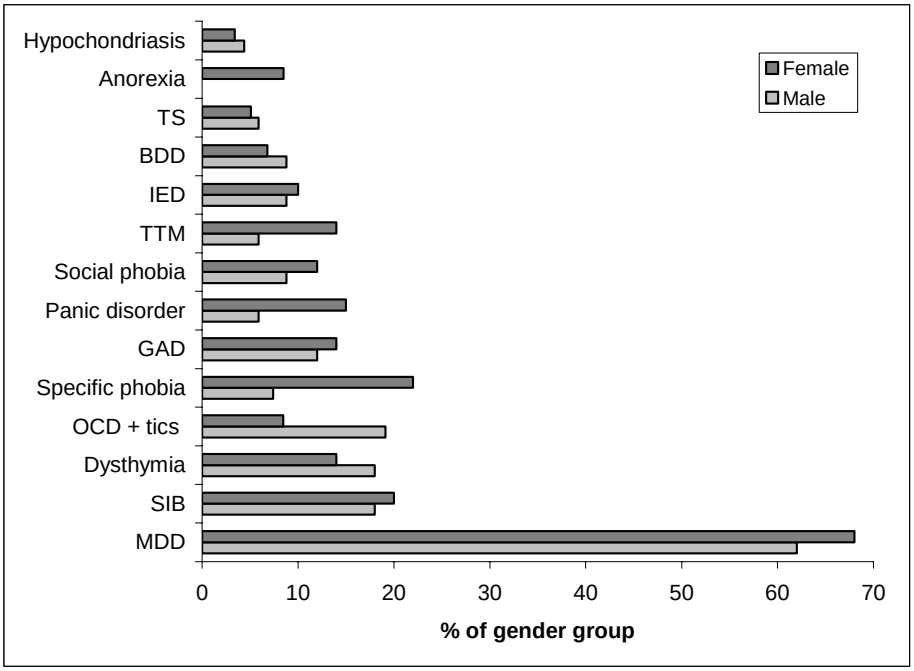



***Figure III.41.*** *Bar graph indicating the proportions of male and female OCD subjects presenting with selected co-morbid disorders.*




**Abbreviations: MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome.

***Table III.8.*** *Association analysis investigating the differences in genotype and allele distributions between OCD and control individuals in bi-allelic loci, with p-values, OR's, 95% CIs and corresponding post-test power.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.985 | 1.04 | 0.48 | 2.28 | 0.051 | 0.927 | 1.03 | 0.71 | 1.50 | 0.050 |
| | rs6313 | *C/T* | 0.686 | 0.85 | 0.41 | 1.79 | 0.060 | 0.858 | 0.96 | 0.66 | 1.38 | 0.053 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.738 | 0.79 | 0.27 | 2.41 | 0.060 | 0.480 | 0.87 | 0.57 | 1.31 | 0.098 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.289 | 0.64 | 0.05 | 9.10 | 0.050 | 0.170 | 0.68 | 0.38 | 1.19 | 0.259 |
| ***5-HT$_{2C}$***[f] | rs6318 | *G/C* | - | - | - | - | - | - | - | - | - | - |
| ***DRD4*** | rs1800955 | *T/C* | 0.479 | 1.61 | 0.69 | 3.88 | 0.170 | 0.250 | 1.26 | 0.85 | 1.87 | 0.194 |
| ***DRD2*** | rs1800497 | *C/T* | 0.769 | 1.37 | 0.42 | 4.82 | 0.060 | 0.916 | 1.05 | 0.68 | 1.55 | 0.051 |
| | rs2097603 | *A/G* | 0.275 | 0.49 | 0.16 | 1.30 | 0.290 | 0.116 | 0.67 | 0.40 | 1.10 | 0.348 |
| ***COMT*** | rs4680 | *G/A* | 0.372 | 1.72 | 0.70 | 4.19 | 0.190 | 0.249 | 1.26 | 0.85 | 1.90 | 0.193 |
| | rs362204 | *D/I* | 0.164 | 3.80 | 0.76 | 38.86 | 0.310 | 0.074 | 1.64 | 0.95 | 2.88 | 0.409 |
| ***DRD3*** | rs6280 | *A/G* | 0.173 | 1.58 | 0.65 | 3.94 | 0.150 | 0.101 | 1.43 | 0.93 | 2.20 | 0.368 |
| ***DRD1*** | *A-48G* | *A/G* | 0.792 | 1.33 | 0.50 | 3.62 | 0.070 | 0.562 | 1.13 | 0.76 | 1.69 | 0.083 |
| ***GRIN2B*** | rs1806191 | *G/A* | 0.373 | 1.76 | 0.45 | 6.89 | 0.082 | 0.252 | 1.40 | 0.76 | 2.56 | 0.137 |
| | rs890 | *A/C* | 0.548 | 1.49 | 0.37 | 6.03 | 0.059 | 0.569 | 1.19 | 0.65 | 2.16 | 0.066 |
| ***BDNF*** | rs6265 | *G/A* | 0.237 | 0.25 | 0.02 | 1.47 | 0.290 | 0.421 | 0.82 | 0.51 | 1.31 | 0.121 |
| | rs2049046 | *A/T* | 0.420 | 0.50 | 0.15 | 1.64 | 0.180 | 0.229 | 0.72 | 0.41 | 1.25 | 0.200 |
| | rs988748 | *C/G* | 0.350 | 0.33 | 0.01 | 6.60 | 0.060 | 0.194 | 0.65 | 0.32 | 1.28 | 0.224 |
| ***HOXB8*** | rs2303486 | *T/A* | 0.159 | 2.08 | 0.55 | 8.10 | 0.160 | 0.183 | 1.52 | 0.81 | 2.84 | 0.242 |
| ***ESRα*** | rs9340799 | *A/G* | 0.785 | 1.32 | 0.54 | 3.37 | 0.070 | 0.471 | 1.17 | 0.76 | 1.80 | 0.100 |
| | rs2234693 | *T/C* | 0.807 | 1.29 | 0.50 | 3.34 | 0.070 | 0.578 | 1.14 | 0.72 | 1.81 | 0.075 |
| ***INPP-1*** | rs1882891 | *C/A* | 1.000 | 1.46 | 0.08 | 87.52 | 0.059 | 0.559 | 0.82 | 0.45 | 1.52 | 0.083 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.596 | 0.61 | 0.12 | 2.97 | 0.070 | 0.722 | 0.92 | 0.56 | 1.51 | 0.057 |
| ***ACE*** | *Alu* ins/del | *D/I* | **0.037** | **0.38** | **0.16** | **0.91** | **0.600** | 0.057 | 0.69 | 0.47 | 1.02 | 0.465 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI; [f]Male and female subsets were analysed separately (dashes indicate that the variant was not analysed in the whole population).
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***5-HT$_{2C}$*****:** serotonin receptor 2C**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derived neurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

The allelic OR for the *BDNF val66met* SNP was 0.41 (95% CI: 0.15-0.99), indicating that, in the male population, the *G*-allele (encoding for part of the *val66* codon) represents the protective allele. When the genotypic distribution of *BDNF val66met* was considered, a greater number of OCD patients carrying at least one *A*-allele was observed compared to controls, although this represented only a trend towards significant association (p = 0.059) (Table III.9).

No statistically significant differences were observed when the genotype and allele frequencies of the female OCD and control subjects were compared (Table III.10).

#### *III.4.1.3.2. Single locus analysis of multi-allelic loci*

##### *i.* DRD4 *48bp VNTR*

No statistically significant differences were observed when the distribution of *DRD4* 48bp VNTR genotypes and alleles were compared between OCD subjects and controls (p = 0.059 and p = 0.679, respectively). Likewise, when the sample was stratified according to gender, no statistically significant differences in genotype or allele distributions were observed in either the male (p = 0.104 and p = 0.082, respectively) or the female (p = 0.595 and p = 0.721, respectively) (genotype and allele scores for the *DRD4* 48bp VNTR are provided in Tables III.4[a] and 4[b])

##### ii. DAT *40bp VNTR*

When the genotype and allele distributions of the *DAT* 40bp VNTR were compared between the entire OCD and control samples, no statistically significant differences were observed (p = 0.584 and p = 0.711, respectively). Moreover, when the sample was stratified according to gender, no significant differences were noted in genotype or allele distributions in the male (p = 0.512 and p = 0.649, respectively) or female (p = 0.934 and p = 0.768, respectively) subsets (genotype and allele scores for the *DAT* 40bp VNTR are provided in Tables III.5[a] and [b]).

***Table III.9.*** *Association analysis investigating the differences in genotype and allele distributions between male OCD and control individuals in bi-allelic loci, with p-values, OR's, 95% CIs and corresponding post-test power.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.802 | 1.53 | 0.35 | 6.48 | 0.06 | 0.519 | 1.27 | 0.64 | 2.49 | 0.09 |
| | rs6313 | *C/T* | 0.332 | 1.11 | 0.27 | 4.28 | 0.05 | 0.546 | 1.21 | 0.64 | 2.29 | 0.08 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.564 | 0.35 | 0.01 | 3.77 | 0.08 | 0.857 | 0.90 | 0.41 | 1.95 | 0.05 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 1.000 | 0.10 | 0.00 | 0.78 | 0.06 | 0.822 | 0.83 | 0.29 | 2.20 | 0.05 |
| ***5-HT$_{2C}$***[f] | rs6318 | *G/C* | | | - | | | **<0.001** | **0.10** | **0.01** | **0.44** | **0.92** |
| ***DRD4*** | rs1800955 | *T/C* | 0.672 | 0.62 | 0.11 | 3.04 | 0.06 | 0.408 | 0.74 | 0.36 | 1.51 | 0.11 |
| ***DRD2*** | rs1800497 | *C/T* | 0.263 | 3.03 | 0.44 | 23.98 | 0.16 | 0.140 | 1.79 | 0.83 | 3.89 | 0.30 |
| ***COMT*** | rs2097603 | *A/G* | 0.660 | 0.85 | 0.09 | 6.86 | 0.06 | 1.000 | 0.94 | 0.35 | 2.53 | 0.05 |
| | rs4680 | *G/A* | 0.183 | 0.32 | 0.05 | 1.59 | 0.25 | 0.113 | 0.59 | 0.30 | 1.17 | 0.31 |
| | rs362204 | *D/I* | 0.071 | 9.12 | 0.39 | 212.66 | 0.18 | **0.040** | **2.88** | **1.03** | **8.70** | **0.52** |
| ***DRD3*** | rs6280 | *A/G* | 0.072 | 0.66 | 0.06 | 4.13 | 0.05 | 0.701 | 1.23 | 0.54 | 2.75 | 0.06 |
| ***DRD1*** | *A-48G* | *A/G* | 0.669 | 0.46 | 0.01 | 5.50 | 0.05 | 1.000 | 1.00 | 0.49 | 2.03 | 0.05 |
| ***GRIN2B*** | rs1806191 | *G/A* | 0.538 | 3.07 | 0.16 | 61.74 | 0.06 | 0.435 | 1.60 | 0.51 | 5.04 | 0.08 |
| | rs890 | *A/C* | 0.569 | 0.26 | 0.00 | 4.40 | 0.08 | 0.439 | 0.59 | 0.18 | 1.83 | 0.10 |
| ***BDNF*** | rs6265 | *G/A* | 0.059 | 0.12 | 0.01 | 2.26 | 0.29 | **0.036** | **0.41** | **0.15** | **0.99** | **0.49** |
| | rs2049046 | *A/T* | 0.918 | 0.70 | 0.08 | 5.28 | 0.05 | 0.817 | 0.81 | 0.29 | 2.21 | 0.06 |
| | rs988748 | *C/G* | 0.435 | 0.60 | 0.03 | 14.52 | 0.07 | 0.601 | 1.33 | 0.41 | 4.14 | 0.08 |
| ***HOXB8*** | rs2303486 | *T/A* | 0.516 | 3.38 | 0.29 | 52.57 | 0.11 | 0.251 | 2.12 | 0.60 | 7.81 | 0.18 |
| ***ESRα*** | rs9340799 | *A/G* | 1.000 | 0.98 | 0.17 | 5.11 | 0.06 | 1.000 | 1.02 | 0.47 | 2.18 | 0.05 |
| | rs2234693 | *T/C* | 0.694 | 1.82 | 0.34 | 10.08 | 0.08 | 0.448 | 1.39 | 0.62 | 3.15 | 0.11 |
| ***INPP-1*** | rs1882891 | *C/A* | 1.000 | 1.73 | 0.03 | 90.61 | 0.05 | 1.000 | 1.02 | 0.32 | 3.04 | 0.06 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.777 | 1.91 | 0.13 | 28.74 | 0.05 | 0.530 | 1.33 | 0.54 | 3.23 | 0.08 |
| ***ACE*** | *Alu* ins/del | *D/I* | **0.020** | **0.10** | **0.00** | **0.83** | **0.56** | **0.049** | **0.49** | **0.23** | **1.01** | **0.49** |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI; [f]due to hemizygosity at the ***5-HT$_{2C}$*** locus, only allele frequencies were compared.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***5-HT$_{2C}$*****:** serotonin receptor 2C**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.10.*** *Association analysis investigating the differences in genotype and allele distributions between female OCD and control individuals in bi-allelic loci, with p-values, OR's, 95% CIs and corresponding post-test power.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.685 | 0.70 | 0.25 | 2.00 | 0.08 | 0.471 | 0.82 | 0.50 | 1.35 | 0.11 |
| | rs6313 | *C/T* | 0.694 | 0.70 | 0.26 | 1.92 | 0.09 | 0.406 | 0.81 | 0.50 | 1.33 | 0.12 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.191 | 0.76 | 0.19 | 3.68 | 0.05 | 0.193 | 0.71 | 0.42 | 1.21 | 0.23 |
| ***5-HT$_{6}$*** | rs1805054 | *C/T* | 0.378 | 1.15 | 0.30 | 5.45 | 0.05 | 0.264 | 0.65 | 0.30 | 1.42 | 0.17 |
| ***5-HT$_{2C}$*** | rs6318 | *G/C* | 1.000 | 0.47 | 0.01 | 37.43 | 0.06 | 0.888 | 1.09 | 0.60 | 2.00 | 0.05 |
| ***DRD4*** | rs1800955 | *T/C* | 0.228 | 2.35 | 0.75 | 8.03 | 0.28 | 0.102 | 1.53 | 0.90 | 2.61 | 0.34 |
| ***DRD2*** | rs1800497 | *C/T* | 0.598 | 0.94 | 0.18 | 6.19 | 0.06 | 0.492 | 0.83 | 0.47 | 1.47 | 0.09 |
| ***COMT*** | rs2097603 | *A/G* | 0.207 | 0.36 | 0.09 | 1.34 | 0.31 | 0.090 | 0.57 | 0.30 | 1.09 | 0.39 |
| | rs4680 | *G/A* | 0.672 | 1.64 | 0.49 | 5.85 | 0.10 | 0.447 | 1.24 | 0.73 | 2.11 | 0.11 |
| | rs362204 | *D/I* | 0.824 | 1.89 | 0.33 | 20.08 | 0.07 | 0.630 | 1.20 | 0.60 | 2.43 | 0.07 |
| ***DRD3*** | rs6280 | *A/G* | 0.326 | 2.48 | 0.69 | 11.35 | 0.24 | 0.151 | 1.49 | 0.86 | 2.64 | 0.27 |
| ***DRD1*** | *A-48G* | *A/G* | 0.869 | 1.34 | 0.44 | 4.51 | 0.06 | 0.613 | 1.16 | 0.69 | 1.99 | 0.08 |
| ***GRIN2B*** | rs1806191 | *G/A* | 1.000 | 1.32 | 0.19 | 8.53 | 0.05 | 0.541 | 1.34 | 0.56 | 3.25 | 0.07 |
| | rs890 | *A/C* | 0.656 | 2.03 | 0.26 | 18.44 | 0.06 | 0.532 | 1.40 | 0.57 | 3.43 | 0.08 |
| ***BDNF*** | rs6265 | *G/A* | 0.717 | 1.14 | 0.06 | 69.06 | 0.08 | 0.439 | 1.31 | 0.67 | 2.65 | 0.10 |
| | rs2049046 | *A/T* | 0.401 | 0.36 | 0.06 | 1.99 | 0.17 | 0.203 | 0.61 | 0.28 | 1.34 | 0.21 |
| | rs988748 | *C/G* | 0.62 | 0.85 | 0.03 | 22.63 | 0.40 | 0.054 | 0.43 | 0.17 | 1.03 | 0.52 |
| ***HOXB8*** | rs2303486 | *T/A* | 0.526 | 1.38 | 0.21 | 9.33 | 0.05 | 0.688 | 1.21 | 0.51 | 2.90 | 0.06 |
| ***ESRα*** | rs9340799 | *A/G* | 0.706 | 1.63 | 0.49 | 6.41 | 0.09 | 0.416 | 1.30 | 0.73 | 2.34 | 0.13 |
| | rs2234693 | *T/C* | 0.816 | 0.79 | 0.19 | 3.21 | 0.05 | 0.758 | 0.89 | 0.46 | 1.71 | 0.06 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.672 | 0.97 | 0.05 | 58.96 | 0.09 | 0.444 | 0.73 | 0.33 | 1.65 | 0.10 |
| ***PLCγ1*** | rs8192707 | *A/G* | 0.431 | 0.31 | 0.02 | 2.92 | 0.13 | 0.350 | 0.74 | 0.39 | 1.41 | 0.14 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.814 | 0.74 | 0.23 | 2.56 | 0.06 | 0.612 | 0.87 | 0.51 | 1.47 | 0.07 |

[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.

**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_{6}$*****:** serotonin receptor 6**;** ***5-HT$_{2C}$*****:** serotonin receptor 2C**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** ***DRD3*****:** dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

### III.4.1.4. Haplotype analyses

#### III.4.1.4.1. 5-HT$_{2A}$ *haplotype analysis*

Investigating the frequencies of the haplotypes at the *5-HT$_{2A}$* locus (Table III.11), it was found that the *G*-allele from the promoter SNP (-1438 *A/G*, or rs6311) and the *C*-allele from the SNP in exon 2 (*T102C*, or rs6313) almost always appear together, comprising more than 50% of the total haplotypes observed in the combined Afrikaner OCD and control populations. The haplotype *A-T* represents the second most common haplotype in the population, with *G-T* and *A-C* haplotypes representing roughly equivalent proportions in the total population. No statistically significant differences were observed when either the individual haplotype frequencies were compared between OCD and control subjects (represented by the corresponding individual p-values in Table III.11) or when the haplotypes were compared globally between the case and control individuals (p = 0.778).

#### III.4.1.4.2. DRD4 *haplotype analysis*

Ten haplotypes were observed in the present study, but only the haplotypes with a frequencies of >1% are presented in Table III.12. Only weakly significant differences in the overall haplotype distribution was observed between OCD subjects and controls (global p = 0.08). However, when the individual haplotypes were analysed, significantly more controls were found to carry the *C-A4* haplotype compared to cases (individual p = 0.049) (Table III.12). Moreover, a significantly increased number of *T-A2* haplotypes were observed in the OCD, compared to control population (individual p = 0.026).

#### III.4.1.4.3. COMT *haplotype analysis*

When the global test for association was considered, no statistically significant differences between OCD and control subjects were observed (p = 0.355). Likewise, no statistically significant differences in individual haplotype frequencies between OCD and control subjects (Table III.13).

#### III.4.1.4.4. GRIN2B *haplotype analysis*

When the variants were investigated as a haplotype, no statistically significant differences in haplotype frequencies were observed between case and control subjects (global p = 0.324) (Table III.14).

### III.4.1.4.5. BDNF *haplotype analysis*

The limited degree of haplotypic diversity indicates a high degree of LD between the SNPs investigated in the present study In the present study, the first three haplotypes, indicated in Table III.15, accounted for 97% of the observed haplotypes. No statistically significant differences in haplotype frequencies were observed between the OCD and control populations, with a global p-value equivalent to 0.487.

### III.4.1.4.6. ESRα *haplotype analysis*

Linkage disequilibrium was observed between rs2234693 and rs9340779 (D'= 0.911, $r^2$ = 0.492, p<0.0001), with the first three haplotypes comprising approximately 98% of those observed in the entire population. However, no statistically significant differences were noted when the haplotype frequencies were compared between OCD and control populations (global p = 0.599) (Table III.16).

***Table III.11.****Haplotype distribution of* 5-$HT_{2A}$ *SNPs -1438 A/G (rs6311) and T102C (rs6311) in OCD and control individuals.*

| Variant | | Haplotype Frequencies | | | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|---|
| rs6311 | rs6313 | Control (n= 145) | OCD (n= 104) | Total (n= 249) | | |
| *G* | *C* | 0.575 | 0.601 | 0.586 | 0.563 | 0.520 |
| *A* | *T* | 0.350 | 0.346 | 0.349 | -0.092 | 0.914 |
| *G* | *T* | 0.037 | 0.029 | 0.034 | -0.502 | 0.601 |
| *A* | *C* | 0.037 | 0.024 | 0.032 | -0.803 | 0.453 |

**Global p= 0.778**

**Abbreviations: OCD:** obsessive-compulsive disorder.

***Table III.12.*** *Haplotype distribution of* DRD4 *polymorphisms the SNP in the promoter region (-521*C/T *[rs1800955]) and the 48bp VNTR in OCD and control individuals.*

| Variant | | Haplotype Frequencies | | | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|---|
| rs1800955 | 48bp VNTR | Control (n= 124) | OCD (n=70) | Total (n= 194) | | |
| *T* | *A4* | 0.357 | 0.385 | 0.359 | 0.03 | 0.974 |
| *C* | *A4* | 0.329 | 0.207 | 0.293 | -2.03 | **0.049** |
| *T* | *A7* | 0.136 | 0.123 | 0.137 | 0.29 | 0.791 |
| *C* | *A7* | 0.045 | 0.105 | 0.062 | 1.55 | 0.134 |
| *C* | *A2* | 0.043 | 0.057 | 0.047 | 0.66 | 0.536 |
| *T* | *A2* | 0.030 | 0.072 | 0.046 | 2.16 | **0.026** |
| *C* | *A3* | 0.034 | 0.037 | 0.034 | -0.05 | 0.968 |
| *T* | *A3* | 0.014 | 0.013 | 0.015 | 0.26 | 0.845 |

**Global p=0.08**

**Abbreviations: OCD:** Obsessive-compulsive disorder; **VNTR**: variable number of tandem repeats polymorphism; ***A4***: 4-repeat allele of *DRD4* 48bp VNTR; ***A7***: 7-repeat allele of DRD4 48bp VNTR; ***A2***: 2-repeat allele of *DRD4* 48bp VNTR.

***Table III.13.*** *Haplotype distribution of the* COMT promoter *(rs2097603),* val158met *(rs4680)and rs362204 polymorphisms in OCD and control individuals.*

| Variant | | | Haplotype Frequencies | | | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|---|---|
| rs2097603 | rs4680 | rs362204 | Control (n= 48) | OCD (n= 31) | Total (n=79) | | |
| *G* | *A* | *D* | 0.230 | 0.328 | 0.259 | 1.977 | 0.054 |
| *A* | *G* | *I* | 0.233 | 0.154 | 0.200 | -1.451 | 0.131 |
| *A* | *A* | *D* | 0.124 | 0.203 | 0.163 | 0.995 | 0.346 |
| *A* | *G* | *D* | 0.196 | 0.093 | 0.151 | -1.57 | 0.116 |
| *G* | *G* | *D* | 0.096 | 0.118 | 0.111 | 0.224 | 0.841 |
| *G* | *G* | *I* | 0.048 | 0.038 | 0.044 | -0.223 | 0.843 |
| *A* | *A* | *I* | 0.051 | 0.018 | 0.037 | -0.626 | 0.557 |
| *G* | *A* | *I* | 0.023 | 0.048 | 0.036 | 0.720 | 0.481 |

**Global p=0.355**

Haplotypes with a frequency of <0.03 are not indicated.

**Abbreviations: OCD:** obsessive-compulsive disorder.

***Table III.14.*** *Haplotype distribution of* GRIN2B *SNPs rs1806191 and rs890 in OCD and control individuals.*

| Variant | | Haplotype Frequencies | | | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|---|
| rs1806191 | rs890 | Control (n= 40) | OCD (n= 55) | Total (n= 95) | | |
| *G* | *A* | 0.468 | 0.467 | 0.466 | 0.290 | 0.786 |
| *A* | *C* | 0.362 | 0.261 | 0.306 | -1.365 | 0.169 |
| *G* | *C* | 0.080 | 0.148 | 0.118 | 1.314 | 0.187 |
| *A* | *A* | 0.091 | 0.124 | 0.110 | 0.458 | 0.650 |

**Global p=0.324**

**Abbreviations: OCD:** obsessive-compulsive disorder.

***Table III.15.*** *Haplotype distribution of* BDNF *SNPs rs6265, rs2049046 and rs988748 in OCD and control individuals.*

| Variant | | | Haplotype Frequencies | | | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|---|---|
| rs6256 | rs2049046 | rs988748 | Control (n=37) | OCD (n=36) | Total (n=72) | | |
| *G* | *A* | *C* | 0.527 | 0.458 | 0.493 | -0.848 | 0.320 |
| *G* | *T* | *C* | 0.284 | 0.250 | 0.267 | -0.449 | 0.677 |
| *A* | *T* | *G* | 0.162 | 0.250 | 0.205 | 1.378 | 0.156 |

**Global p=0.487**

**Abbreviations: OCD:** obsessive-compulsive disorder.

***Table III.16.*** *Haplotype distribution of the* ESRα *SNPs, rs93407999 and* rs2234693 *in OCD and control individuals.*

| Variant | | Haplotype Frequencies | | | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|---|
| rs9340799 | rs2234693 | Control (n= 110) | OCD (n= 56) | Total (n= 166) | | |
| *A* | *T* | 0.500 | 0.518 | 0.506 | 0.299 | 0.747 |
| *G* | *C* | 0.350 | 0.339 | 0.346 | -0.186 | 0.897 |
| *A* | *C* | 0.132 | 0.134 | 0.133 | 0.055 | 1.000 |

**Global p=0.599**

**Abbreviations: OCD:** obsessive-compulsive disorder.

### III.4.2. Stratification by symptom severity (total Y-BOCS score)

#### *III.4.2.1. Analysis of clinical variables*

The relationships between selected clinical variables with symptom severity, as represented by total Y-BOCS, are presented in Table III.17. No statistically significant differences in total Y-BOCS score were noted for OCD patients with or without family history of OCD ($p = 0.084$), OCS ($p = 0.694$) or tics ($p = 0.604$). Those OCD patients experiencing aggressive obsessions and compulsions were found to have lower total Y-BOCS scores compared to those who did not experience aggressive symptoms (median Y-BOCS scores = 18 [95% CI: 15.7-20.3] and 22.5 [95% CI: 20.5-24.5], respectively; $p = 0.050$).

When the presence or absence of co-morbid disorders was investigated with regard to total Y-BOCS score (Table III.17) statistically significant differences were observed for self-injurious behaviour ($p = 0.005$), specific phobia ($p = 0.018$) and anorexia nervosa ($p = 0.012$). For all three of these disorders, the presence of the co-morbid disorder was associated with a higher total Y-BOCS score (24 [95% CI: 22.3-25.7] vs. 19.5 [95% CI: 17.8-21.2] for the presence and absence of co-morbid SIB, respectively; 23.5 [95% CI: 19.4-27.6] vs. 20.5 [95% CI: 19.0-22.0] for the presence and absence of co-morbid social phobia, respectively and 28 [95% CI: 21.0-35.1] vs. 21[95% CI: 19.6-22.5] for the presence and absence of co-morbid anorexia nervosa, respectively). Due to low numbers, the sample was not stratified, or analysed, according to gender for clinical analyses.

#### *III.4.2.2. Single locus analysis of clinical severity*

##### *III.4.2.2.1. Single locus analysis of bi-allelic polymorphisms*

The results from the Wilcoxon test for differences in medians, employed to determine whether genotype or allele distribution influenced Y-BOCS score, are presented in Tables III.18[a] to [d]. Significant differences in the distribution of Y-BOCS scores between the genotypes of the *DRD1* SNP, *A-48G*, were noted in male OCD subjects ($p = 0.045$) (Table III.18[b]). Males homozygous for the *G*-allele exhibited higher total Y-BOCS scores (median Y-BOCS score = 27.5 [95% CI: 22.4-32.6]) than those who were either heterozygous or homozygous for the *A*-allele (18.5 [95% CI: 15.4-21.6] and 18 [95% CI: 13.4-22.6], respectively) (Table III.18[b]).

Similarly, the *CC*-genotype of the *GRIN2B* rs890 was found to be associated with an increased severity of the disorder, as indicated by the higher total Y-BOCS score (median Y-BOCS score = 26 [95% CI: 20.1-31.9]) (Table III.18[c]). When the sample was stratified according to gender, both male and female individuals carrying the *CC*-genotype attained higher Y-BOCS scores compared to individuals homozygous for the *A*-allele or heterozygous individuals, although these differences did not reach statistical significance (p = 0.118 and p=0.053 for males and females, respectively).

In the female OCD subset, a nominally statistically significant difference was found in the distribution of total Y-BOCS scores amongst genotypes comprising the *BDNF val66met* (rs6265) polymorphism (p = 0.045) (Table III.18[c]). Since no conclusions could be drawn about the *AA (met66met)*-genotype and OCD severity (only one female subject was found to carry this genotype), the *AA*-carrying female was grouped with the heterozygous females. When the median Y-BOCS scores of the *AA+AG* combined group was compared to *GG(val66val)*-carrying females, those homozygous for the *G*-allele had significantly higher Y-BOCS scores (median Y-BOCS scores=23 [95% CI: 21-25] and 16 [95% CI: 11.7-21], respectively; p=0.013). No similar association was noted in the male OCD subset (p=0.677).

#### *III.4.2.2.2. Single locus analysis of multi-allelic polymorphisms*

##### *i. DRD4*

Due to the large number of *DRD4* 48bp VNTR genotypes observed in the present study, it was decided to group them according to the presence or absence of at least one *A4*-allele, as suggested by Wang et al. (2004) (**section I.6.1.2.1[i]**) (Table III.19). No statistically significant differences in median Y-BOCS scores were observed when the total OCD population was investigated (p=0.851), or when the data was stratified into male (p=0.273) and female (p=0.335) subsets.

##### *ii. DAT*

No statistically significant differences were observed in median Y-BOCS scores between the genotypes comprising the *DAT* 40bp VNTR in the total OCD population (p=0.207), or in the gender-stratified subsets (p=0.340 for males, p=0.142 for females) (Table III.20).

***Table III.17.*** *Clinical variables in the OCD patient subset according to symptom severity (as measured by total Y-BOCS score).*

| Clinical Variable | Present | | | | Absent | | | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| **Family History** | **n** | **%** | **Median** | **95% CI** | **n** | **%** | **Median** | **95% CI** | |
| Family history of OCD | **19** | 24.0 | 23.0 | 21.0-25.0 | **60** | 76.0 | 18.0 | 16.0-20.0 | 0.084 |
| Family history of OCS | **36** | 47.0 | 21.0 | 18.6-23.4 | **41** | 53.0 | 21.0 | 16.8-21.2 | 0.694 |
| Family history of tics | **6** | 7.6 | 21.0 | 15.9-21.1 | **73** | 92.0 | 19.0 | 19.2-22.8 | 0.604 |
| **Primary symptom dimensions** | | | | | | | | | |
| Hoarding/collecting | **22** | 25.0 | 22.0 | 19.7-24.3 | **67** | 75.0 | 21.0 | 19.2-22.8 | 0.394 |
| Symmetry/ordering | **53** | 59.0 | 23.0 | 21.0-25.0 | **37** | 41.0 | 19.0 | 16.9-21.1 | 0.156 |
| Sex /religion | **37** | 42.0 | 21.0 | 18.4-23.6 | **52** | 58.0 | 22.0 | 19.3-23.7 | 0.266 |
| Contamination/washing | **54** | 60.0 | 22.0 | 20.3-23.7 | **36** | 40.0 | 19.0 | 15.8-22.2 | 0.123 |
| Aggressive symptoms | **45** | 51.0 | 18.0 | 15.7-20.3 | **44** | 49.0 | 22.5 | 20.5-24.5 | **0.050** |
| **Co-morbidity** | | | | | | | | | |
| MDD | **79** | 63.0 | 22.0 | 20.2-23.8 | **46** | 37.0 | 20.0 | 17.9-22.1 | 0.515 |
| SIB | **22** | 18.0 | 24.0 | 22.3-25.7 | **103** | 82.0 | 19.5 | 17.8-21.2 | **0.005** |
| Dysthymic disorder | **20** | 16.0 | 23.5 | 20.5-26.5 | **105** | 84.0 | 21.0 | 19.5-22.5 | 0.289 |
| Tics | **17** | 14.0 | 18.0 | 14.9-21.1 | **108** | 86.0 | 22.0 | 20.4-23.6 | 0.476 |
| Specific phobia | **18** | 14.0 | 23.5 | 19.4-27.6 | **107** | 86.0 | 20.5 | 19.0-22.0 | **0.018** |
| GAD | **14** | 11.2 | 21.0 | 19.6-22.4 | **111** | 88.8 | 23.0 | 17.5-28.5 | 0.707 |
| PD | **13** | 10.0 | 22.0 | 15.4-28.6 | **112** | 90.0 | 21.0 | 19.6-22.3 | 0.313 |
| Social Phobia | **11** | 8.8 | 23.0 | 20.6-25.4 | **114** | 91.0 | 21.0 | 19.5-22.5 | 0.225 |
| TTM | **12** | 9.6 | 22.5 | 16.1-28.9 | **113** | 90.0 | 21.0 | 19.7-22.3 | 0.472 |
| IED | **11** | 8.8 | 21.0 | 13.8-28.2 | **114** | 91.0 | 21.0 | 19.7-22.3 | 0.628 |
| BDD | **10** | 8.0 | 24.5 | 21.5-27.5 | **115** | 92.0 | 21.0 | 19.7-22.3 | 0.266 |
| TS | **7** | 5.6 | 22.0 | 18.1-25.9 | **118** | 94.0 | 21.0 | 19.5-22.5 | 0.591 |
| Anorexia nervosa | **5** | 4.0 | 28.0 | 21.0-35.1 | **120** | 96.0 | 21.0 | 19.6-22.5 | **0.012** |
| Hypochondriasis | **4** | 3.2 | 13.5 | 5.6-21.4 | **121** | 97.0 | 21.0 | 19.7-22.3 | 0.340 |

Significant p-values are indicated in red, bold font.
**Abbreviations: OCD:** Obsessive-compulsive disorder; **OCS:** Obsessive-compuslive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome; **CI**: confidence interval.

***Table III.18 (a).*** *Quantitative analyses indicating the relationship between total Y-BOCS score and genotype in serotonergic candidate genes.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| ***5-HT$_{2A}$*** | **rs6311** | *A/A* | **16** | 15.2 | 20.5 | 15.8 | 25.2 | 0.390 | **6** | 11.3 | 19.0 | 7.39 | 30.6 | 0.747 | **10** | 19.2 | 21.5 | 16.5 | 26.5 | 0.399 |
| | | *G/A* | **45** | 42.9 | 21.0 | 19.1 | 22.9 | | **20** | 37.7 | 20.0 | 16.6 | 23.4 | | **25** | 48.1 | 21.0 | 18.5 | 23.5 | |
| | | *G/G* | **44** | 41.9 | 24.0 | 21.7 | 26.3 | | **27** | 50.9 | 23.0 | 19.8 | 26.2 | | **17** | 32.7 | 24.0 | 19.8 | 28.2 | |
| | **rs6313** | *C/C* | **45** | 42.5 | 22.0 | 19.4 | 24.6 | 0.210 | **26** | 49.1 | 21.5 | 18.1 | 24.9 | 0.587 | **19** | 35.8 | 22.0 | 17.8 | 26.2 | 0.37 |
| | | *C/T* | **42** | 39.6 | 20.5 | 18.6 | 22.5 | | **19** | 35.8 | 19.0 | 15.6 | 22.4 | | **23** | 43.4 | 21.0 | 18.5 | 23.5 | |
| | | *T/T* | **19** | 17.9 | 24.0 | 19.8 | 28.2 | | **8** | 15.1 | 22.5 | 14.4 | 30.6 | | **11** | 20.8 | 24.0 | 19.2 | 28.8 | |
| ***5-HT$_{1Dβ}$*** | **rs6296** | *C/C* | **8** | 7.9 | 17.5 | 13.0 | 22.0 | 0.515 | **4** | 7.8 | 15.5 | 10.0 | 21 | 0.131 | **4** | 8.0 | 23.5 | 16.4 | 30.6 | 0.784 |
| | | *G/C* | **39** | 38.6 | 21.0 | 18.0 | 24.0 | | **14** | 27.5 | 21.5 | 18.1 | 24.9 | | **25** | 50.0 | 21.0 | 15.9 | 26.1 | |
| | | *G/G* | **54** | 53.5 | 21.0 | 18.9 | 23.2 | | **33** | 64.7 | 20.0 | 16.7 | 23.3 | | **21** | 42.0 | 22.0 | 19.9 | 24.1 | |
| ***5-HT$_{6}$*** | **rs1805054** | *C/C* | **53** | 63.9 | 23.0 | 21.3 | 24.7 | 0.828 | **23** | 59.0 | 23.0 | 20.2 | 25.8 | 0.522 | **30** | 68.2 | 22.0 | 19.7 | 24.3 | 0.342 |
| | | *C/T* | **28** | 33.7 | 21.5 | 18.7 | 24.3 | | **15** | 38.5 | 19.0 | 15.3 | 22.7 | | **13** | 29.5 | 22.0 | 18.5 | 25.5 | |
| | | *T/T* | **2** | 2.4 | 24.0 | 1.7 | 46.3 | | **1** | 2.6 | 14.0 | - | - | | **1** | 2.3 | 34.0 | - | - | |
| ***5-HT$_{2C}$***[d] | **rs6318** | *G/G* | | | - | | | - | **36** | 76.6 | 20.0 | 17.0 | 23.0 | 0.435 | **30** | 61.2 | 19.0 | 15.8 | 22.2 | 0.224 |
| | | *G/C* | | | | | | | **0** | - | - | - | - | | **15** | 30.6 | 22.0 | 18.9 | 25.1 | |
| | | *C/C* | | | | | | | **11** | 23.4 | 19.0 | 15.9 | 22.1 | | **4** | 8.2 | 25.0 | 21.4 | 28.6 | |

[a] Total OCD sample for whom total Y-BOCS was recorded; [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI; [d]due to male hemizygosity at the ***5-HT$_{2C}$*** locus, genders were analysed separately. Dashes indicate that no data was generated for the whole sample for this locus.

**Abbreviations: OCD**: Obsessive-compulsive disorder; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_{6}$*****:** serotonin receptor 6**;** ***5-HT$_{2C}$*****:** serotonin receptor 2C.

***Table III.18 (b).*** *Quantitative analyses indicating the relationship between total Y-BOCS score and genotype in dopaminergic candidate genes.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| ***DRD4*** | **rs1800995** | *C/C* | **12** | 13.6 | 22.5 | 19.5 | 25.5 | 0.275 | **6** | 13.6 | 21.5 | 17 | 26 | 0.543 | **6** | 13.6 | 22.5 | 19.3 | 25.7 | 0.527 |
| | | *T/C* | **45** | 51.1 | 18.0 | 15.2 | 20.8 | | **23** | 52.3 | 18.0 | 14.2 | 21.8 | | **22** | 50.0 | 19.5 | 14.8 | 24.2 | |
| | | *T/T* | **31** | 35.2 | 22.0 | 19.5 | 24.6 | | **15** | 34.1 | 24.0 | 19.5 | 28.5 | | **16** | 36.4 | 22.0 | 17.3 | 26.7 | |
| ***DRD2*** | **rs1800497** | *C/C* | **57** | 56.4 | 22.0 | 20.1 | 23.9 | 0.923 | **28** | 59.6 | 22.0 | 19.3 | 24.7 | 0.126 | **29** | 53.7 | 22.0 | 19.1 | 24.9 | 0.460 |
| | | *C/T* | **39** | 38.6 | 22.0 | 19.5 | 24.5 | | **17** | 36.2 | 24.0 | 20.2 | 27.8 | | **22** | 40.7 | 21.5 | 18.5 | 24.5 | |
| | | *T/T* | **5** | 5.0 | 20.0 | 9.4 | 30.6 | | **2** | 4.3 | 7.5 | 4.79 | 19.8 | | **3** | 5.6 | 28.0 | 23.9 | 32.1 | |
| ***DRD3*** | **rs2097603** | *A/A* | **16** | 27.6 | 18.0 | 13.5 | 22.5 | 0.590 | **9** | 33.3 | 18.0 | 12.7 | 23.3 | 0.614 | **7** | 22.6 | 18.0 | 11.4 | 24.6 | 0.720 |
| | | *A/G* | **24** | 41.4 | 21.5 | 18.8 | 24.2 | | **10** | 37.0 | 19.5 | 15.5 | 23.5 | | **14** | 45.2 | 23.0 | 18.8 | 27.2 | |
| | | *G/G* | **18** | 31.0 | 23.0 | 20.0 | 26.0 | | **8** | 29.6 | 23.5 | 16.8 | 30.2 | | **10** | 32.3 | 23.0 | 20.0 | 26.0 | |
| ***COMT*** | **rs4680** | *A/A* | **22** | 24.2 | 21.0 | 18.3 | 23.7 | 0.330 | **8** | 18.2 | 21.0 | 15.7 | 26.3 | 0.950 | **14** | 29.8 | 21.0 | 18.5 | 23.5 | 0.198 |
| | | *A/G* | **53** | 58.2 | 22.0 | 19.2 | 24.8 | | **27** | 61.4 | 20.0 | 16.4 | 23.6 | | **26** | 55.3 | 23.0 | 18.7 | 27.3 | |
| | | *G/G* | **16** | 17.6 | 17.5 | 14.0 | 21.1 | | **9** | 20.5 | 18.0 | 12.2 | 23.8 | | **7** | 14.9 | 17.0 | 14.3 | 19.7 | |
| | **rs362204** | *D/D* | **27** | 52.9 | 21.0 | 18.1 | 23.9 | 0.437 | **14** | 63.6 | 20.0 | 14.5 | 25.5 | 0.608 | **13** | 44.8 | 22.0 | 17.2 | 26.8 | 0.657 |
| | | *D/I* | **22** | 43.1 | 23.5 | 20.1 | 26.9 | | **8** | 36.4 | 19.5 | 13.1 | 25.9 | | **14** | 48.3 | 24.0 | 16.4 | 31.6 | |
| | | *I/I* | **2** | 3.9 | 27.0 | 24.8 | 29.2 | | **0** | 0.0 | - | - | - | | **2** | 6.9 | 27.0 | 24.8 | 29.2 | |
| | **rs6280** | *A/A* | **53** | 55.8 | 22.0 | 20.1 | 24.0 | 0.931 | **29** | 61.7 | 23.0 | 20.4 | 25.6 | 0.615 | **24** | 50.0 | 22.0 | 19.6 | 24.4 | 0.534 |
| | | *A/G* | **30** | 31.6 | 22.0 | 18.5 | 25.5 | | **10** | 21.3 | 23.5 | 16.5 | 30.5 | | **20** | 41.7 | 21.0 | 17.1 | 24.9 | |
| | | *G/G* | **12** | 12.6 | 22.0 | 18.4 | 25.7 | | **8** | 17.0 | 19.0 | 13.7 | 24.3 | | **4** | 8.3 | 25.0 | 17.5 | 32.5 | |
| ***DRD1*** | ***A-48G*** | *A/A* | **40** | 40.8 | 21.5 | 18.3 | 24.8 | 0.172 | **20** | 40.0 | 18.0 | 13.4 | 22.6 | **0.045** | **20** | 41.7 | 22.0 | 17.1 | 26.9 | 0.833 |
| | | *A/G* | **47** | 48.0 | 20.0 | 17.9 | 22.1 | | **26** | 52.0 | 18.5 | 15.4 | 21.6 | | **21** | 43.8 | 23.0 | 19.9 | 26.1 | |
| | | *G/G* | **11** | 11.2 | 25.0 | 22.1 | 27.9 | | **4** | 8.0 | 27.5 | 22.4 | 32.6 | | **7** | 14.6 | 24.0 | 18.9 | 29.1 | |

Significant values are represented in red, bold font.
[*]Total OCD sample for whom total Y-BOCS was recorded**.** [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI
**Abbreviations: OCD**: Obsessive-compulsive disorder; **CI:** confidence interval; ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1.

***Table III.18 (c).*** *Quantitative analyses indicating the relationship between total Y-BOCS score and genotype in* GRIN2B, HOXB8 *and* BDNF *candidate genes.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| ***GRIN2B*** | **rs1806191** | *A/A* | **9** | 16.4 | 24.0 | 21.9 | 26.1 | | **3** | 10.0 | 26.0 | 18.7 | 33.3 | | **6** | 24.0 | 24.0 | 21.4 | 26.6 | |
| | | *A/G* | **25** | 45.5 | 21.0 | 18.5 | 23.5 | 0.230 | **17** | 56.7 | 21.0 | 17.6 | 24.5 | 0.408 | **8** | 32.0 | 20.0 | 16.7 | 23.4 | 0.373 |
| | | *G/G* | **21** | 38.2 | 19.0 | 15.6 | 22.5 | | **10** | 33.3 | 20.0 | 14.0 | 26.0 | | **11** | 44.0 | 19.0 | 16.1 | 21.9 | |
| | **rs890** | *A/A* | **18** | 32.1 | 19.0 | 14.9 | 23.1 | | **7** | 23.3 | 21.0 | 15.9 | 26.1 | | **11** | 42.3 | 18.0 | 14.7 | 21.3 | |
| | | *A/C* | **30** | 53.6 | 20.5 | 17.9 | 23.1 | **0.013** | **18** | 60.0 | 19.5 | 15.4 | 23.6 | 0.118 | **12** | 46.2 | 21.0 | 18.0 | 24.0 | 0.053 |
| | | *C/C* | **8** | 14.3 | 26.0 | 20.1 | 31.9 | | **5** | 16.7 | 26.0 | 31.7 | 31.9 | | **3** | 11.5 | 26.0 | 20.1 | 31.9 | |
| ***BDNF*** | **rs6265** | *A/A* | **6** | 5.5 | 18.5 | 13.3 | 23.7 | | **5** | 8.9 | 18.0 | 12.3 | 23.7 | | **1** | 1.9 | 19 | - | - | |
| | | *G/A* | **32** | 29.1 | 18.0 | 14.9 | 21.1 | 0.103 | **18** | 32.1 | 20.5 | 16.8 | 24.2 | 0.899 | **14** | 25.9 | 16.0 | 10.9 | 21.1 | **0.045** |
| | | *G/G* | **72** | 65.5 | 22.5 | 20.9 | 24.1 | | **33** | 58.9 | 21.0 | 17.7 | 24.3 | | **39** | 72.2 | 23.0 | 21.0 | 25.0 | |
| | | *A/A + G/A* | **38** | 34.5 | 18.0 | 15.2 | 20.8 | **0.036** | **23** | 41.1 | 18.0 | 14.9 | 21.1 | 0.677 | **15** | 27.8 | 16.0 | 11.7 | 21.0 | **0.013** |
| | **rs2049046** | *A/A* | **12** | 24.0 | 17.0 | 13.6 | 20.4 | | **8** | 28.6 | 16.5 | 11.8 | 21.2 | | **4** | 18.2 | 17.5 | 10.0 | 25.0 | |
| | | *A/T* | **24** | 48.0 | 24.0 | 20.0 | 28.0 | 0.317 | **13** | 46.4 | 24.0 | 19.2 | 28.8 | 0.251 | **11** | 50.0 | 24.0 | 19.0 | 29.0 | 0.919 |
| | | *T/T* | **14** | 28.0 | 21.0 | 17.2 | 24.8 | | **7** | 25.0 | 20.0 | 13.1 | 26.9 | | **7** | 31.8 | 22.0 | 18.7 | 25.3 | |
| | **rs988748** | *C/C* | **25** | 51.0 | 23.0 | 19.8 | 26.2 | | **16** | 57.1 | 23.0 | 19.1 | 26.9 | | **9** | 42.9 | 22.0 | 18.3 | 25.7 | |
| | | *C/G* | **22** | 44.9 | 17.0 | 13.6 | 20.4 | 0.532 | **10** | 35.7 | 17.0 | 11.0 | 23.0 | 0.452 | **12** | 57.1 | 19.5 | 15.2 | 23.8 | 0.668 |
| | | *G/G* | **2** | 4.1 | 18.0 | 0.1 | 35.9 | | **2** | 7.1 | 18.0 | 0.1 | 35.9 | | **0** | 0.0 | - | - | - | |
| ***HOXB8*** | **rs2303486** | *A/A* | **19** | 38.8 | 22.0 | 18.9 | 25.1 | | **12** | 42.9 | 20.5 | 15.9 | 25.1 | | **7** | 33.3 | 24.0 | 19.8 | 28.2 | |
| | | *A/T* | **20** | 40.8 | 19.5 | 16.3 | 22.7 | 0.797 | **11** | 39.3 | 18.0 | 13.2 | 22.8 | 0.91 | **9** | 42.9 | 21.0 | 17.3 | 24.7 | 0.384 |
| | | *T/T* | **10** | 20.4 | 20.0 | 17.0 | 23.0 | | **5** | 17.9 | 21.0 | 17.5 | 24.5 | | **5** | 23.8 | 19.0 | 14.8 | 23.2 | |

Significant values are represented in red, bold font.
[a]Total OCD sample for whom total Y-BOCS was recorded**.** [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI
**Abbreviations: OCD**: Obsessive-compulsive disorder; **CI:** confidence interval; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8.

***Table III.18 (d).*** *Quantitative analyses indicating the relationship between total Y-BOCS score and genotype in* ESRα, INPP-1, PLC-γ1 *and* ACE *candidate genes.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| *ESRα* | **rs9340799** | *A/A* | **38** | 47.5 | 21.5 | 17.7 | 25.3 | 0.647 | **18** | 46.2 | 22.0 | 17.5 | 26.5 | 0.209 | **20** | 48.8 | 21.5 | 16.4 | 26.6 | 0.094 |
| | | *A/G* | **31** | 38.8 | 20.0 | 17.6 | 22.4 | | **15** | 38.5 | 20.0 | 16.1 | 23.9 | | **16** | 39.0 | 20.5 | 17.5 | 23.5 | |
| | | *G/G* | **11** | 13.8 | 24.0 | 19.5 | 28.5 | | **6** | 15.4 | 15.0 | 9.8 | 20.2 | | **5** | 12.2 | 25.0 | 19.3 | 30.7 | |
| | **rs2234693** | *C/C* | **13** | 22.0 | 24.0 | 21.4 | 26.6 | 0.229 | **6** | 18.8 | 21.5 | 14.4 | 28.6 | 0.792 | **7** | 25.9 | 24.0 | 19.2 | 28.8 | 0.241 |
| | | *T/C* | **28** | 47.5 | 16.5 | 12.6 | 20.4 | | **14** | 43.8 | 17.0 | 11.1 | 22.9 | | **14** | 51.9 | 16.0 | 10.5 | 21.5 | |
| | | *T/T* | **18** | 30.5 | 21.0 | 16.5 | 25.5 | | **12** | 37.5 | 17.0 | 12.4 | 21.6 | | **6** | 22.2 | 25.0 | 19.8 | 30.2 | |
| *INPP-1* | **rs1882891** | *A/A* | **1** | 1.1 | 17.0 | 17.0 | 17.0 | 0.531 | **0** | 0.0 | - | - | - | 0.968 | **1** | 2.1 | 17.0 | - | - | 0.355 |
| | | *C/A* | **23** | 24.7 | 23.0 | 19.7 | 26.3 | | **11** | 24.4 | 21.0 | 15.3 | 26.7 | | **12** | 25.0 | 24.0 | 18.1 | 29.9 | |
| | | *C/C* | **69** | 74.2 | 22.0 | 20.3 | 23.7 | | **34** | 75.6 | 22.0 | 19.0 | 25.0 | | **35** | 72.9 | 22.0 | 19.7 | 24.3 | |
| *PLC-γ1* | **rs8192707** | *A/A* | **55** | 61.1 | 24.0 | 22.0 | 26.0 | 0.302 | **28** | 63.6 | 22.0 | 18.4 | 25.6 | 0.795 | **27** | 58.7 | 24.0 | 21.0 | 27.0 | 0.151 |
| | | *A/G* | **30** | 30.3 | 19.0 | 16.7 | 21.3 | | **14** | 31.8 | 19.5 | 16.1 | 22.9 | | **16** | 34.8 | 18.5 | 15.5 | 21.5 | |
| | | *G/G* | **5** | 5.1 | 22.0 | 14.9 | 29.1 | | **2** | 4.5 | 18.0 | 6.8 | 29.2 | | **3** | 6.5 | 22.0 | 11.1 | 32.9 | |
| *ACE* | ***Alu* ins/del** | *D/D* | **43** | 40.2 | 21.0 | 19.0 | 23.1 | 0.566 | **20** | 36.4 | 18.5 | 14.8 | 22.2 | 0.558 | **23** | 44.2 | 21.0 | 18.0 | 24.0 | 0.660 |
| | | *D/I* | **42** | 39.3 | 22.0 | 20.3 | 23.7 | | **20** | 36.4 | 20.0 | 16.5 | 23.5 | | **22** | 42.3 | 22.0 | 20.3 | 23.7 | |
| | | *I/I* | **22** | 20.6 | 23.0 | 16.3 | 29.7 | | **15** | 27.3 | 23.0 | 18.5 | 27.5 | | **7** | 13.5 | 19.0 | 1.4 | 36.6 | |

[a]Total OCD sample for whom total Y-BOCS was recorded**.** [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI.
**Abbreviations: OCD**: Obsessive-compulsive disorder; **CI:** confidence interval; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.19.*** *Quantitative analyses indicating the relationship between total Y-BOCS score and genotypes, categorised by DRD4 48bp VNTR A4-allele status.*

| Genotype | Total OCD population | | | | |
|---|---|---|---|---|---|
| | n | % | Median | 95% CI[a] | |
| | | | | LB[b] | UB[c] |
| *A4/A4* | **33** | 37.5 | 22.0 | 19.2 | 24.8 |
| *A4/other* | **45** | 51.1 | 21.0 | 18.6 | 23.4 |
| *other/other* | **10** | 11.4 | 21.0 | 14.0 | 28.0 |
| | **Male** | | | | |
| *A4/A4* | **12** | 28.6 | 24.5 | 21.5 | 27.5 |
| *A4/other* | **25** | 59.5 | 19.0 | 15.5 | 22.5 |
| *other/other* | **5** | 11.9 | 16.0 | 6.1 | 25.9 |
| | **Female** | | | | |
| *A4/A4* | **21** | 45.7 | 19.0 | 15.6 | 22.4 |
| *A4/other* | **20** | 43.5 | 21.0 | 18.0 | 24.0 |
| *other/other* | **5** | 10.9 | 25.0 | 15.8 | 34.2 |

**p = 0.851** for whole OCD sample**, p = 0.273** for males, **p = 0.335** for females.

*A4* = 4-repeat allele; "other" alleles comprise *A2, A3, A7*and *A6* alleles.

[a]confidence interval; [b]lower boundary of 95% CI; [c] upper boundary of 95% CI.

***Table III.20.*** *Quantitative analyses indicating the relationship between total Y-BOCS score and genotypes in the* DAT *40bp VNTR.*

| Genotype | Total OCD population | | | | |
|---|---|---|---|---|---|
| | n | % | Median | 95% CI | |
| | | | | LB | UB |
| *A10/A10* | **60** | 55.0 | 19.0 | 16.7 | 21.3 |
| *A10/A9* | **38** | 34.9 | 23.5 | 21.2 | 25.8 |
| *A9/A9* | **8** | 7.3 | 17.5 | 9.4 | 25.6 |
| *A10/A11* | **3** | 2.8 | 23.0 | 19.8 | 26.2 |
| | **Male** | | | | |
| *A10/A10* | **32** | 56.1 | 19.0 | 16.1 | 21.9 |
| *A10/A9* | **18** | 31.6 | 21.5 | 17.4 | 25.6 |
| *A9/A9* | **3** | 5.3 | 24.0 | 13.1 | 34.9 |
| *A10/A11* | **3** | 5.3 | 23.0 | 19.8 | 26.2 |
| | **Female** | | | | |
| *A10/A10* | **28** | 52.8 | 21.0 | 17.8 | 24.2 |
| *A10/A9* | **20** | 37.7 | 24.0 | 22.4 | 25.6 |
| *A9/A9* | **5** | 9.4 | 26.0 | 11.1 | 20.9 |

**p = 0.207** for whole OCD sample**, p = 0.340** for males, **p = 0.142** for females.

*A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele[c].

[a]confidence interval; [b]lower boundary of 95% CI; [c] upper boundary of 95% CI.

### *III.4.2.3. Haplotype analyses of symptom severity (total Y-BOCS score)*

#### III.4.2.3.1. 5-$HT_{2A}$ *haplotype analysis*

No statistically significant associations were observed between the haplotypes of the *5-$HT_{2A}$ -1438A/G* (rs6311) and *T102C* (rs6313) SNPs and total Y-BOCS score, with a global p-value of 0.212 (Table III.21). It should, however, be mentioned that the haplotype *G-T* did show a trend towards association with a higher total Y-BOCS score (and thus with increased severity), by the generation of a haplotype score of 1.917 and individual p-value of 0.050.

#### III.4.2.3.2. DRD4 *haplotype analysis*

When the distribution of total Y-BOCS scores was investigated in the *DRD4* haplotypes formed by the *C/T* SNP located at promoter position -521 (rs1800955) and the 48bp VNTR located in exon 3, no statistically significant differences were observed (p = 0.636) (Table III.22).

#### III.4.2.3.3. COMT *haplotype analysis*

When the distribution of Y-BOCS scores amongst *COMT* polymorphism haplotypes was investigated, no significant differences, either individually or globally (global p-value = 0.980), were noted for the haplotypes formed by the promoter polymorphism rs2097603, the functional *val158met* (rs4680) polymorphism, or the *C* insertion/deletion polymorphism in the sixth exon (rs362204) (Table III.23).

#### III.4.2.3.4. GRIN2B *haplotype analysis*

When comparing the differences in Y-BOCS score distribution between haplotypes comprised of the *GRIN2B* rs1806191 and rs890 polymorphisms, statistically significant differences were noted (global p = 0.032) (Table III.24). Closer inspection of the Y-BOCS score distribution amongst the individual haplotypes revealed that OCD patients carrying the *G-A* haplotype presented with a less severe form of the disorder, as they tended to have significantly lower Y-BOCS scores, indicated by the negative haplotype score of -2.371 (individual haplotype p-value = 0.021) (Table III.24). On the other hand, those subjects carrying the *A-C* haplotype presented with a more severe form of the disorder, indicated by higher total Y-BOCS scores (haplotype score = 2.274; individual p value = 0.022).

### III.4.2.3.5. BDNF *haplotype analysis*

The distribution of Y-BOCS scores amongst the *BDNF* rs890-rs1806191 haplotypes investigated in the present study are depicted in Table III.25. No statistically significant differences in Y-BOCS score were noted, either within individual haplotypes, or when all the haplotypes were considered globally (p = 0.408).

### III.4.2.3.6. ESRα *haplotype analysis*

No statistically significant differences in Y-BOCS score were noted for the haplotypes formed by the two intronic *ESRα* SNPs, rs1799732 and rs1800497, with a global p-value of 0.713 being attained (Table III.26).

***Table III.21.*** *Distribution of total Y-BOCS score within* 5-$HT_{2A}$ *haplotypes comprising the* -1438 A/G *(rs6311) and* T102C *(rs6313) variants.*

| Variants | | Haplotype Frequency (n=103) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| rs6311 | rs6313 | | | |
| *G* | *C* | 0.612 | 0.002 | 0.996 |
| *A* | *T* | 0.338 | -0.287 | 0.780 |
| *A* | *C* | 0.025 | -0.989 | 0.328 |
| *G* | *T* | 0.025 | 1.917 | 0.050 |

**Global p=0.212**

***Table III.22.*** *Distribution of total Y-BOCS score within haplotypes comprising the* DRD4 *promoter polymorphism (*-521C/T *[rs1800955]) and the 48bp VNTR in OCD individuals.*

| Variants | | Haplotype Frequency (n=69) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| rs1800955 | 48bp VNTR | | | |
| *T* | *A4* | 0.396 | 1.09 | 0.271 |
| *C* | *A4* | 0.191 | -1.10 | 0.269 |
| *T* | *A7* | 0.122 | 0.09 | 0.922 |
| *C* | *A7* | 0.110 | -0.37 | 0.691 |
| *T* | *A2* | 0.071 | 0.26 | 0.821 |
| *C* | *A2* | 0.059 | 0.63 | 0.528 |
| *C* | *A3* | 0.039 | -0.70 | 0.479 |

**Global p=0.636**

***A4***: 4-repeat allele of *DRD4* 48bp VNTR; ***A7***: 7-repeat allele of DRD4 48bp VNTR; ***A3***: 3-repeat allele of *DRD4* 48bp VNTR
***A2***: 2-repeat allele of *DRD4* 48bp VNTR.

***Table III.23.*** *Distribution of total Y-BOCS score within haplotypes comprising the* COMT *promoter (rs2097603),* val158met *(rs4680) and rs362204 polymorphisms in OCD individuals.*

| Variants | | | Haplotype Frequency (n=31) | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|
| rs2097603 | rs4680 | rs362204 | | | |
| *G* | *A* | *D* | 0.323 | 0.375 | 0.717 |
| *A* | *A* | *D* | 0.203 | -0.29 | 0.787 |
| *A* | *G* | *I* | 0.149 | 0.096 | 0.932 |
| *G* | *G* | *D* | 0.124 | -0.084 | 0.928 |
| *A* | *G* | *D* | 0.092 | -0.127 | 0.917 |

**Global p=0.980**

***Table III.24.*** *Distribution of total Y-BOCS score within haplotypes comprising the* GRIN2B rs890 and rs1806191 *polymorphisms in OCD individuals.*

| Variants | | Haplotype Frequency (n=54) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| rs1806191 | rs890 | | | |
| *G* | *A* | 0.464 | -2.371 | **0.021** |
| *A* | *C* | 0.270 | 2.274 | **0.022** |
| *G* | *C* | 0.138 | 1.015 | 0.306 |
| *A* | *A* | 0.128 | -1.045 | 0.294 |

Significant p-values are indicated in red, bold font.

**Global p=0.032**

***Table III.25.*** *Distribution of total Y-BOCS score within haplotypes comprising the* BDNF val66met (rs6265), rs2049046 and rs988748 *polymorphisms in OCD individuals.*

| Variants | | | Haplotype Frequency (n=36) | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|
| rs6265 | rs2049046 | rs988748 | | | |
| *G* | *A* | *C* | 0.458 | 1.136 | 0.279 |
| *A* | *T* | *G* | 0.25 | -1.699 | 0.098 |
| *G* | *T* | *C* | 0.25 | 0.076 | 0.948 |

**Global p=0.480**

***Table III.26.*** *Distribution of total Y-BOCS score within haplotypes comprising the* ESRα *rs9340799 and rs2234693 polymorphisms in OCD individuals.*

| Variants | | Haplotype Frequency (n=55) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| rs9340799 | rs2234693 | | | |
| *A* | *T* | 0.508 | -0.266 | 0.794 |
| *G* | *C* | 0.341 | 0.905 | 0.363 |
| *A* | *C* | 0.140 | -0.772 | 0.388 |

**Global p=0.713**

### III.4.3. Stratification by age at onset of OCD

#### *III.4.3.1. Analysis of clinical variables*

OCD subjects with a positive family history of OCD were found to exhibit an earlier age at onset compared to those patients without a family history of the disorder ($p < 0.001$; median ages of onset = 10 years [95% CI: 4.5-16] and 15 years [95% CI: 13-19], respectively) (Table III.27). No other statistically significant differences in age at onset of OCD were observed for presence or absence of family history of OCS or tics. Likewise, when symptom dimensions were analysed, no statistically significant differences were observed in age at onset between those subjects experiencing selected symptoms and those not experiencing the symptom (Table III.27).

When age at onset and co-morbidity were investigated, patients diagnosed with co-morbid TS were found to exhibit an earlier age at onset compared to those who were not diagnosed with TS (median age at onset=8 years [95% CI: 5.0-11.0]) and 14 years [95% CI: 12.3-15.7]), respectively; $p = 0.025$) (Table III.27). Moreover, the age at onset of OCD patients presenting with co-morbid specific phobia was lower than those who did not exhibit co-morbid specific phobia (median ages of 10 years[95% CI: 7.5-12.5] and 15 [95% CI: 13.4-16.6] years, respectively) although this difference did not reach statistical significance ($p = 0.053$).

#### *III.4.3.2. Single locus analysis of age of onset*

##### *III.4.3.2.1. Single locus analysis of bi-allelic loci*

The results of the Kaplan-Meier logrank tests for age at onset are depicted in Tables III.28 (a) to (d).

***Table III.27.*** *Clinical variables in the OCD patient subset according to age at onset of OCD.*

| Clinical Variable | Present | | | | Absent | | | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| **Family History** | **n** | **%** | **Median** | **95% CI** | **n** | **%** | **Median** | **95% CI** | |
| Family history of OCD | **17** | 22.7 | 10.0 | 4.5-16.0 | **58** | 77.3 | 15.0 | 13.0-19.0 | **<0.001** |
| Family history of OCS | **33** | 45.2 | 13.5 | 10.0-18.0 | **40** | 54.8 | 15.0 | 13.0-19.0 | 0.567 |
| Family history of tics | **5** | 6.7 | 16.0 | 15.0-α | **70** | 93.3 | 14.0 | 13.0-17.0 | 0.343 |
| **Primary symptom dimensions** | | | | | | | | | |
| Hoarding/collecting | **20** | 23.8 | 13.0 | 10.9-15.1 | **64** | 76.2 | 14.0 | 11.5-16.5 | 0.846 |
| Symmetry/ordering | **51** | 60.0 | 14.0 | 11.6-16.4 | **34** | 40.0 | 13.0 | 10.9-15.1 | 0.570 |
| Sex/religion | **34** | 40.5 | 13.0 | 10.3-15.7 | **50** | 59.5 | 15.5 | 13.3-17.7 | 0.236 |
| Contamination/washing | **51** | 60.0 | 13.0 | 10.9-15.1 | **34** | 40.0 | 15.0 | 11.7-18.3 | 0.276 |
| Aggression | **43** | 51.2 | 13.0 | 10.9-15.1 | **41** | 48.8 | 14.0 | 10.5-17.5 | 0.809 |
| **Co-morbidity** | | | | | | | | | |
| MDD | **75** | 64.7 | 14.0 | 12.2-15.8 | **41** | 35.3 | 15.0 | 12.8-17.2 | 0.337 |
| SIB | **21** | 18.1 | 13.0 | 9.9-16.1 | **95** | 81.9 | 14.0 | 12.2-15.8 | 0.802 |
| Dysthymic disorder | **20** | 17.2 | 13.5 | 9.1-17.9 | **96** | 82.8 | 14.0 | 12.4-15.6 | 0.832 |
| Tics | **16** | 13.8 | 10.0 | 5.9-14.1 | **100** | 86.2 | 14.0 | 12.3-15.7 | 0.100 |
| Specific phobia | **14** | 12.1 | 10.0 | 7.5-12.5 | **102** | 87.9 | 15.0 | 13.4-16.6 | 0.053 |
| GAD | **13** | 11.2 | 13.0 | 8.2-17.8 | **103** | 88.8 | 14.0 | 12.3-15.7 | 0.483 |
| PD | **11** | 9.5 | 14.0 | 9.0-19.0 | **105** | 90.5 | 14.0 | 12.4-15.6 | 0.713 |
| Social Phobia | **11** | 9.5 | 12.0 | 9.4-14.6 | **105** | 90.5 | 14.0 | 12.4-15.6 | 0.331 |
| TTM | **8** | 6.9 | 13.0 | 5.2-20.8 | **108** | 93.1 | 14.0 | 12.5-15.5 | 0.523 |
| IED | **11** | 9.5 | 16.0 | 12.2-19.8 | **105** | 90.5 | 14.0 | 12.5-15.5 | 0.436 |
| BDD | **10** | 8.6 | 14.5 | 11.0-18.0 | **106** | 91.4 | 14.0 | 12.5-15.5 | 0.922 |
| TS | **7** | 6.0 | 8.0 | 5.0-11.0 | **109** | 94.0 | 14.0 | 12.3-15.7 | **0.025** |
| Anorexia nervosa | **5** | 4.3 | 21.0 | 10.4-31.6 | **111** | 95.7 | 14.0 | 12.5-15.5 | 0.484 |
| Hypochondriasis | **4** | 3.4 | 16.0 | 4.9-27.1 | **112** | 96.6 | 14.0 | 12.5-15.5 | 0.617 |

Significant p-values are indicated in red, bold font.
**Abbreviations: OCD:** Obsessive-compulsive disorder; **OCS:** Obsessive-compuslive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome; **CI**: confidence interval.

***Table III.28 (a).*** *Kaplan-Meier estimates of age at onset of OCD according to genotypes of markers within serotonergic genes.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| ***5-HT$_{2A}$*** | **rs6311** | *A/A* | **14** | 14.3 | 12.5 | 10.0 | 25.0 | | **5** | 10 | 12.0 | 10.0 | α | | **9** | 18.8 | 13.0 | 10.0 | α | |
| | | *G/A* | **44** | 44.9 | 16.0 | 13.0 | 19.0 | 0.381 | **19** | 38 | 15.0 | 13.0 | 20.0 | 0.140 | **25** | 52.1 | 16.0 | 13.0 | 24.0 | 0.770 |
| | | *G/G* | **40** | 40.8 | 14.5 | 13.0 | 20.0 | | **26** | 52 | 14.0 | 13.0 | 20.0 | | **14** | 29.2 | 18.0 | 10.0 | 30.0 | |
| | **rs6313** | *C/C* | **42** | 43.3 | 15.5 | 13.0 | 20.0 | | **25** | 51 | 14.0 | 13.0 | 23.0 | | **17** | 35.4 | 17.0 | 10.0 | 24.0 | |
| | | *C/T* | **39** | 40.2 | 15.0 | 14.0 | 19.0 | 0.118 | **17** | 34.7 | 10.0 | 13.0 | 20.0 | **0.015** | **22** | 45.8 | 16.5 | 13.0 | 24.0 | 0.645 |
| | | *T/T* | **16** | 16.5 | 10.0 | 9.0 | 18.0 | | **7** | 14.3 | 10.0 | 7.0 | α | | **9** | 18.8 | 10.0 | 9.0 | α | |
| ***5-HT$_{1Dβ}$*** | **rs6296** | *C/C* | **6** | 6.4 | 13.0 | 10.0 | α | | **3** | 6.4 | 14.0 | 4.5 | α | | **3** | 6.4 | 12.0 | 10.0 | α | |
| | | *G/C* | **38** | 40.4 | 14.5 | 13.0 | 17.0 | 0.505 | **14** | 29.8 | 13.5 | 12.0 | 17.0 | 0.194 | **24** | 51.1 | 16.5 | 13.0 | 21.0 | 0.830 |
| | | *G/G* | **50** | 53.2 | 14.0 | 13.0 | 18.0 | | **30** | 63.8 | 14.5 | 13.0 | 20.0 | | **20** | 42.6 | 13.5 | 10.0 | 25.0 | |
| ***5-HT$_{6}$*** | **rs1805054** | *C/C* | **48** | 62.3 | 14.0 | 13.0 | 19.0 | | **22** | 59.5 | 14.0 | 13.0 | 20.0 | | **26** | 65 | 14.5 | 10.0 | 24.0 | |
| | | *C/T* | **28** | 36.4 | 15.0 | 11.0 | 19.0 | 0.595 | **14** | 37.8 | 12.0 | 10.0 | 17.0 | 0.121 | **14** | 35 | 18.5 | 13.0 | 30.0 | 0.966 |
| | | *T/T* | **1** | 1.3 | 20.0 | - | - | | **1** | 2.7 | 20.0 | - | - | | **0** | 0.0 | 0.0 | 0.0 | 0.0 | |
| ***5-HT$_{2C}$***[d] | **rs6318** | *G/G* | | | | | | | **34** | 77.3 | 14.5 | 13.0 | 19.0 | | **26** | 59.1 | 14.5 | 10.0 | 19.0 | |
| | | *G/C* | | | - | | | - | **0** | 0 | - | - | - | 0.688 | **15** | 34.1 | 18.0 | 10.0 | 33.0 | 0.237 |
| | | *C/C* | | | | | | | **10** | 22.7 | 15.5 | 10.0 | α | | **3** | 6.8 | 24.0 | 4.0 | α | |

Significant p-values are indicated in red, bold font.
α = infinity; [a] Total population for whom age at onset was recorded.; [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI; [d]due to male hemizygosity at the ***5-HT$_{2C}$*** locus, genders were analysed separately. Dashes indicate that no data was generated for the whole sample for this locus.
**Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_{6}$*****:** serotonin receptor 6**;** ***5-HT$_{2C}$*****:** serotonin receptor 2C

Amongst the serotonergic candidate genes investigated, significant differences in age at onset were seen only between genotypes comprising the *T102C* (rs6313) polymorphism in *5-HT$_{2A}$* (p=0.015), and only in male OCD subjects (Table III.28[a]; Figure III.42). Here, male individuals homozygous for the *5-HT$_{2A}$ T102*-allele exhibited earlier ages at onset (median age at onset=10 years [95% CI: 7-α]) compared to those who were homozygous for the *C102*-allele, or *T102C*-heterozygotes (median ages at onset=14 years for both [95% CIs: 13-23 and 13-20, respectively]).

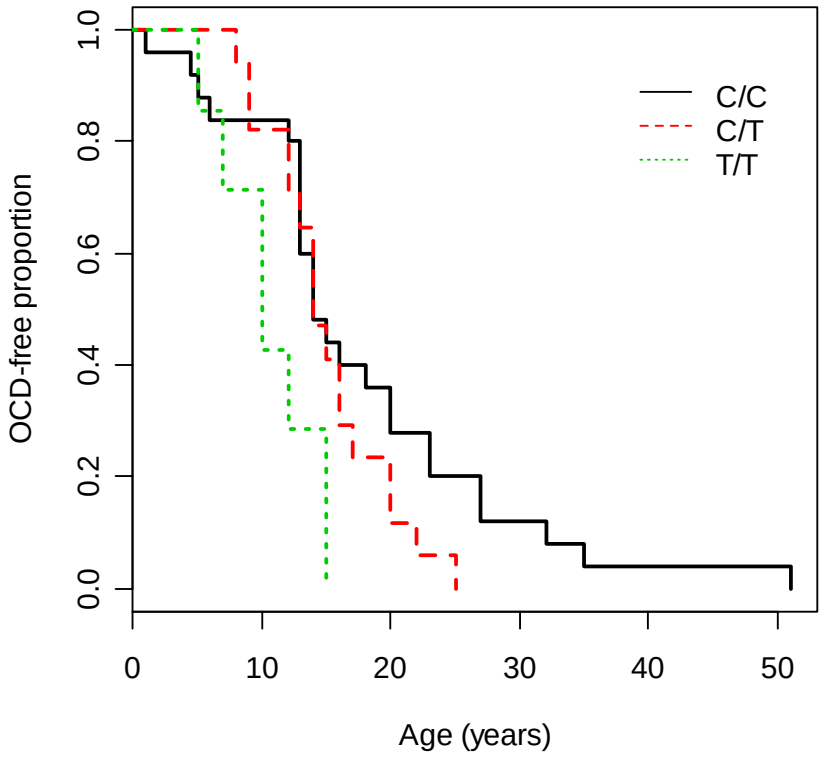



***Figure III.42. Kaplan-Meier estimation of time to age at onset of OCD, according to* 5-HT$_{2A}$ T102C *genotype in males.*** TT *homozygotes demonstrated a significantly earlier age at onset than heterozygotes or* CC *homozygotes.*

The relationship between dopaminergic candidates investigated in the present study and age at onset of OCD is depicted in Table III.28(b). An association between age at onset and the *COMT* polymorphism, rs362204, was observed in the male population. No OCD patients for whom age at onset had been recorded were found to carry the *I/I* genotype. Consequently, a pairwise comparison was made between age at onset of OCD subjects who were homozygous for the *D*-allele, and heterozygous *D/I* carriers. Males carrying the *D/D*-genotype exhibited significantly lower ages at onset (median age at onset = 14 years [95% CI: 13-20]) compared to heterozygotes (median age at onset = 20years [95% CI: 16-α]) (Table III.28[b] and Figure III.43).

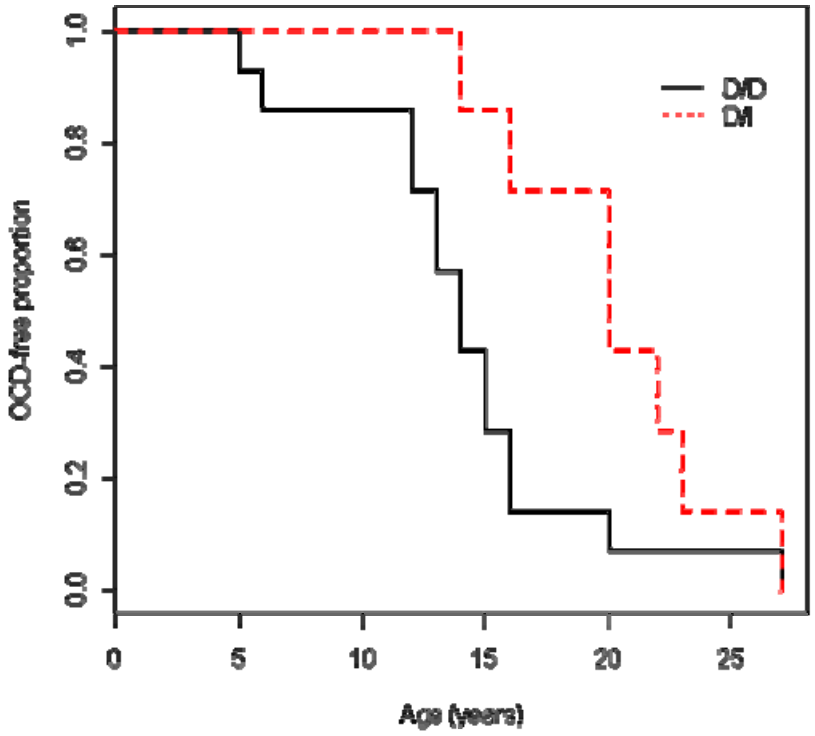



***Figure III.43. Kaplan-Meier estimation of time to age at onset of OCD, according to* COMT *rs362204 genotype in males.*** DD *homozygotes showed a significantly earlier age at onset compared to* DI *heterozygotes, while no* II *homozygotes were observed.*

For the *DRD3 ser9gly* polymorphism, heterozygous male subjects presented with earlier ages at onset (median age at onset =11 years [95% CI: 6-α]) compared to those who were homozygous for either the *A* (*ser9)*-allele or *G* (*gly9)* allele (median ages at onset = 15 years [95% CI: 13-19] and 17 years [95% CI: 13- α], respectively) (Table III.28[b] and Figure III.44). In females, however, the *GG* (*gly9gly*) homozygotes showed a weakly significant tendency for earlier age of onset (p = 0.068).

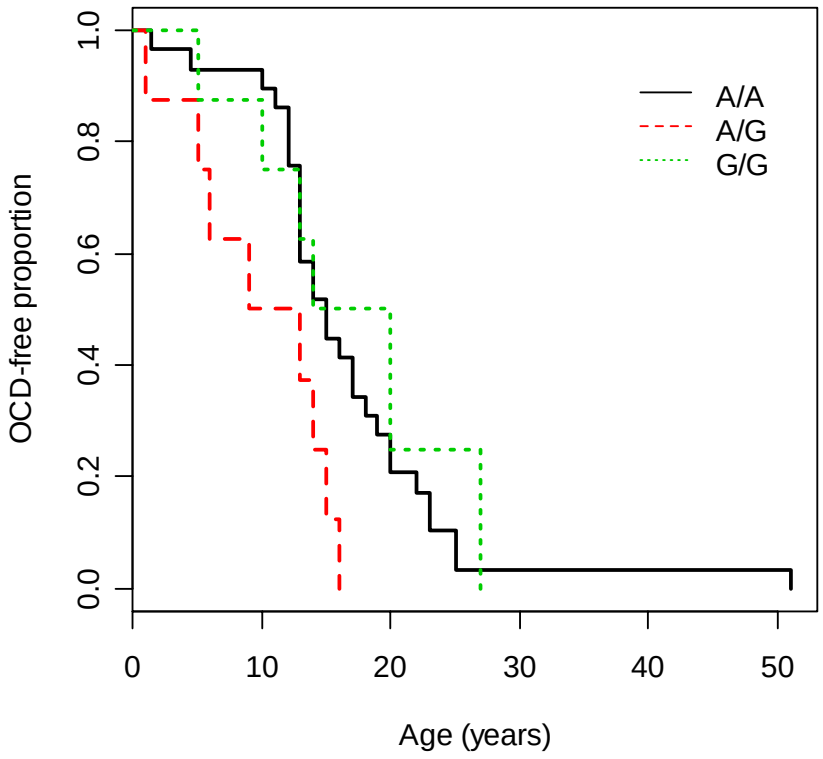



***Figure III.44. Kaplan-Meier estimation of time to age at onset of OCD, according to* DRD3 ser9gly *genotype in males***. AG (ser9gly) *heterozygotes experienced significantly lower ages at onset compared to* AA (ser9ser)- *or* GG (gly9gly)-*homozygotes.*

***Table III.28(b).*** *Kaplan-Meier estimates of age at onset of OCD according to genotypes of markers within dopaminergic candidate genes.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| ***DRD4*** | **rs1800995** | *C/C* | **14** | 16.9 | 14.0 | 13.0 | 24.0 | | **7** | 17.1 | 15.0 | 13.0 | α | | **7** | 16.7 | 13.0 | 7.0 | α | |
| | | *T/C* | **41** | 49.4 | 14.0 | 13.0 | 19.0 | 0.376 | **20** | 48.8 | 14.0 | 13.0 | 20.0 | 0.519 | **21** | 50.0 | 16.0 | 10.0 | 27.0 | 0.304 |
| | | *T/T* | **28** | 33.7 | 14.0 | 12.0 | 19.0 | | **14** | 34.1 | 13.0 | 10.0 | 20.0 | | **14** | 33.3 | 18.0 | 13.0 | α | |
| ***DRD2*** | **rs1800497** | *C/C* | **52** | 54.7 | 14.0 | 13.0 | 17.0 | | **26** | 57.8 | 13.5 | 13.0 | 17.0 | | **26** | 52.0 | 16.0 | 13.0 | 24.0 | |
| | | *C/T* | **37** | 38.9 | 15.0 | 13.0 | 19.0 | 0.614 | **16** | 35.6 | 14.5 | 9.0 | 25.0 | 0.573 | **21** | 42.0 | 17.0 | 10.0 | 21.0 | 0.632 |
| | | *T/T* | **6** | 6.3 | 17.5 | 12.0 | α | | **3** | 6.7 | 15.0 | 12.0 | α | | **3** | 6.0 | 30.0 | 9.0 | α | |
| ***COMT*** | **rs2097603** | *A/A* | **14** | 25.5 | 13.0 | 10.0 | 30.0 | | **8** | 30.8 | 13.0 | 12.0 | α | | **6** | 20.7 | 12.5 | 9.0 | α | |
| | | *A/G* | **24** | 43.6 | 16.0 | 13.0 | 24.0 | 0.597 | **10** | 38.5 | 14.0 | 9.0 | α | 0.897 | **14** | 48.3 | 22.0 | 13.0 | 30.0 | 0.625 |
| | | *G/G* | **17** | 30.9 | 13.0 | 11.0 | 20.0 | | **8** | 30.8 | 13.5 | 12.0 | α | | **9** | 31.0 | 13.0 | 10.0 | α | |
| | **rs4680** | *A/A* | **22** | 26.5 | 13.0 | 10.0 | 18.0 | | **8** | 20 | 11.0 | 6.0 | α | | **14** | 32.6 | 15.5 | 10.0 | 37.0 | |
| | | *A/G* | **47** | 56.6 | 15.0 | 14.0 | 18.0 | 0.564 | **25** | 62.5 | 15.0 | 14.0 | 18.0 | 0.223 | **22** | 51.2 | 15.0 | 13.0 | 23.0 | 0.880 |
| | | *G/G* | **14** | 16.9 | 17.0 | 13.0 | 30.0 | | **7** | 17.5 | 19.0 | 13.0 | α | | **7** | 16.3 | 12.0 | 5.0 | α | |
| | **rs362204** | *D/D* | **26** | 56.5 | 14.5 | 13.0 | 20.0 | 0.578 | **14** | 66.7 | 14.0 | 13.0 | 20.0 | **0.040** | **12** | 48.0 | 17.0 | 13.0 | α | 0.536 |
| | | *D/I* | **20** | 43.5 | 19.5 | 14.0 | 25.0 | | **7** | 33.3 | 20.0 | 16.0 | α | | **13** | 52.0 | 16.0 | 9.0 | α | |
| ***DRD3*** | **rs6280** | *A/A* | **51** | 58.0 | 15.0 | 13.0 | 19.0 | | **29** | 64.4 | 15.0 | 13.0 | 19.0 | | **22** | 51.2 | 15.0 | 10.0 | 21.0 | |
| | | *A/G* | **26** | 29.5 | 14.5 | 13.0 | 24.0 | 0.655 | **8** | 17.8 | 11.0 | 6.0 | α | **0.022** | **18** | 41.9 | 20.5 | 13.0 | 33 | 0.068 |
| | | *G/G* | **11** | 12.5 | 14.0 | 10.0 | α | | **8** | 17.8 | 17.0 | 13.0 | α | | **3** | 7.0 | 10.0 | 7.0 | α | |
| ***DRD1*** | **rs4532** | *A/A* | **37** | 40.2 | 16.0 | 14.0 | 19.0 | | **19** | 40.4 | 14.0 | 13.0 | 19.0 | | **18** | 40.0 | 17.5 | 13.0 | 30.0 | |
| | | *A/G* | **45** | 48.9 | 14.0 | 13.0 | 19.0 | 0.341 | **25** | 53.2 | 14.0 | 13.0 | 20.0 | 0.431 | **20** | 44.4 | 14.5 | 10.0 | 23.0 | 0.510 |
| | | *G/G* | **10** | 10.9 | 13.5 | 9.0 | α | | **3** | 6.4 | 13.0 | 5.0 | α | | **7** | 15.6 | 14.0 | 9.0 | α | |

Significant p-values are indicated in red, bold font; α = infinity; [a] Total population for whom age at onset was recorded.; [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI
**Abbreviations:** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; *DRD1*: dopamine receptor 1.

The relationship between age at onset of OCD and variants situated in *GRIN2B*, *BDNF* and *HOXB8* are presented in Table III.28(c). Male OCD subjects carrying the *GRIN2B* rs890 *AA*-genotype were found to experience a later age at onset (median age at onset=22 years [95% CI: 15-α]), compared to those who were heterozygous or homozygous for the *C*-allele (median ages at onset=13 years [95% CI: 9-19] and 10 years [95% CI: 7- α], respectively) (p=0.012) (Table III.28[c] and Figure III.45]). Females showed no difference in the age of onset for any of these genotypes.

In contrast, in the female subset, OCD subjects carrying the *GRIN2B* rs1806191 *GG* genotypes were found to have later ages at onset (median age at onset=19 years [95% CI: 13.0- α], compared to those who were heterozygous or homozygous for the *A*-allele (median ages at onset=11.5 years [95% CI: 7- α] and 10 years [95% CI: 10- α], respectively) (Figure III.46). However, this difference was found to be at the borderline of significance, with a p-value of 0.049. In the male subset, those individuals homozygous for the rs1806191 *AA*-genotype also experienced earlier ages at onset, compared to those who heterozygous or homozygous for the *G*-allele, although this difference did not reach statistical significance (p=0.087) (Table III.28[c]).

In addition, males homozygous for the *BDNF val66met* (rs6265) *met66*-allele (the *A*-allele) experienced earlier age at onset (median age at onset=5 years [95% CI: 4.5-α]), compared to those who are heterozygous or homozygous for the G (*val66)*-allele (median ages at onset=13 years [95% CI: 12-20] and 15 years [95% CI: 14-19], respectively) (p=0.028) (Table III.28[c] and Figure III.47).

An association between age at onset of OCD and *HoxB8* was also observed: females carrying the *HoxB8* rs2303486 *AA*-genotype exhibited much earlier ages at onset (median age at onset=9 years [95% CI: 7-α]) compared to those subjects who were either homozygous for the *T*-allele, or heterozygous (median ages at onset=25 years [95% CI: 10- α] and 16 years [95% CI: 13- α], respectively) (p=0.024) (Table III.28[c] and Figure III.48); however, no effect was observed in males.

***Table III.28(c).*** *Kaplan-Meier estimates of age at onset of OCD according to genotypes of markers within* GRIN2B, BDNF *and* HOXB8 *candidate polymorphisms.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| ***GRIN2B*** | **rs1806191** | *A/A* | **8** | 15.1 | 10.0 | 7.0 | α | | **3** | 10.3 | 4.0 | 1.4 | α | | **5** | 20.8 | 10.0 | 10.0 | α | |
| | | *G/A* | **25** | 47.2 | 13.0 | 10.0 | 19.0 | 0.090 | **17** | 58.6 | 13.0 | 12.0 | 22.0 | 0.087 | **8** | 33.3 | 11.5 | 7.0 | α | **0.049** |
| | | *G/G* | **20** | 37.7 | 14.5 | 13.0 | 30.0 | | **9** | 31.0 | 13.0 | 11.0 | α | | **11** | 45.8 | 19.0 | 13.0 | α | |
| | **rs890** | *A/A* | **18** | 33.3 | 18.5 | 15.0 | 30.0 | | **7** | 24.1 | 22.0 | 15.0 | α | | **11** | 44.0 | 18.0 | 14.0 | α | |
| | | *A/C* | **28** | 51.9 | 13.0 | 10.0 | 15.0 | 0.051 | **17** | 58.6 | 13.0 | 9.0 | 19.0 | **0.012** | **11** | 44.0 | 13.0 | 10.0 | α | 0.897 |
| | | *C/C* | **8** | 14.8 | 10.0 | 7.0 | α | | **5** | 17.2 | 10.0 | 7.0 | α | | **3** | 12.0 | 10.0 | 7.0 | α | |
| ***BDNF*** | **rs6265** | *A/A* | **6** | 5.9 | 9.5 | 4.5 | α | | **5** | 9.4 | 5.0 | 4.5 | α | | **1** | 2.0 | 45.0 | α | α | |
| | | *G/A* | **31** | 30.4 | 14.0 | 13.0 | 20.0 | 0.937 | **17** | 32.1 | 13.0 | 12.0 | 20.0 | **0.028** | **14** | 28.6 | 16.0 | 13.0 | 30.0 | 0.148 |
| | | *G/G* | **65** | 63.7 | 15.0 | 14.0 | 18.0 | | **31** | 58.5 | 15.0 | 14.0 | 19.0 | | **34** | 69.4 | 15.5 | 12.0 | 21.0 | |
| | **rs2049046** | *A/A* | **11** | 24.4 | 13.0 | 13.0 | α | | **7** | 28.0 | 13.0 | 11.0 | α | | **4** | 20.0 | 17.0 | 10.0 | α | |
| | | *A/T* | **20** | 44.4 | 13.5 | 10.0 | 22.0 | 0.964 | **11** | 44.0 | 13.0 | 9.0 | α | 0.908 | **9** | 45.0 | 14.0 | 10.0 | α | 0.993 |
| | | *T/T* | **14** | 31.1 | 11.0 | 7.0 | 30.0 | | **7** | 28.0 | 13.0 | 5.0 | α | | **7** | 35.0 | 8.0 | 7.0 | α | |
| | **rs988748** | *C/C* | **23** | 52.3 | 13.0 | 11.0 | 23.0 | 0.966 | **15** | 60.0 | 13.0 | 13.0 | 25.0 | 0.572 | **8** | 42.1 | 12.0 | 10.0 | α | 0.939 |
| | | *G/G+C/G* | **21** | 47.7 | 13.0 | 9.0 | 23.0 | | **10** | 40.0 | 10.5 | 5.0 | α | | **11** | 57.9 | 14.0 | 10.0 | α | |
| ***HOXB8*** | **rs2303486** | *A/A* | **18** | 40.0 | 14.0 | 9.0 | 21.0 | | **12** | 46.2 | 15.0 | 13.0 | α | | **6** | 31.6 | 9.0 | 7.0 | α | |
| | | *A/T* | **17** | 37.8 | 13.0 | 13.0 | 23.0 | 0.923 | **9** | 34.6 | 13.0 | 10.0 | α | 0.253 | **8** | 42.1 | 16.0 | 13.0 | α | **0.024** |
| | | *T/T* | **10** | 22.2 | 12.0 | 7.0 | α | | **5** | 19.2 | 11.0 | 5.0 | α | | **5** | 26.3 | 25.0 | 10.0 | α | |

Significant p-values are indicated in red, bold font.
α = infinity; [a] Total population for whom age at onset was recorded.; [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI.
**Abbreviations:** **OCD**: Obsessive-compulsive disorder; **CI:** confidence interval; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8

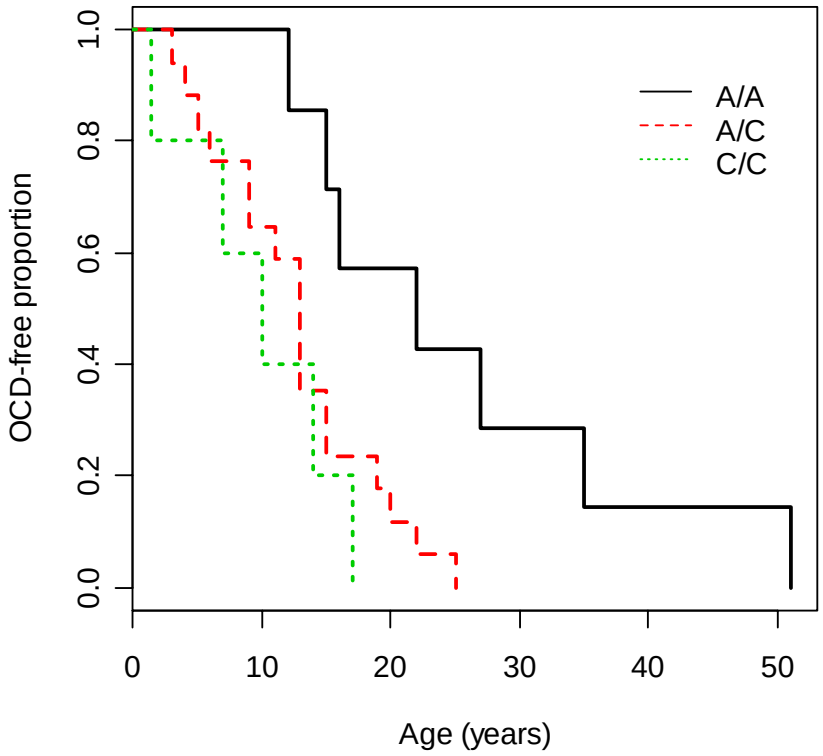



***Figure III.45. Kaplan-Meier estimation of time to age at onset of OCD, according to* GRIN2B rs890 *genotype in males****. Those carrying at least one C-allele experienced significantly earlier ages at onset compared to those homozygous for the A-allele.***

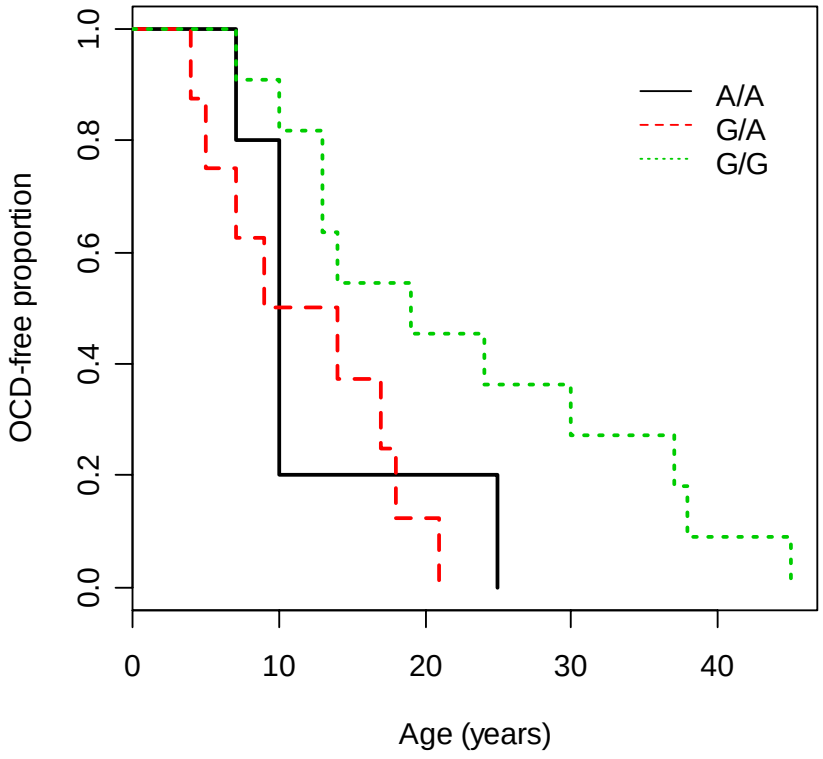



***Figure III.46 Kaplan-Meier estimation of time to age at onset of OCD, according to* GRIN2B rs1806191 *genotype in females.*** *Those carrying at least one* A*-allele experienced significantly earlier ages at onset compared to* GG*-homozygotes.*

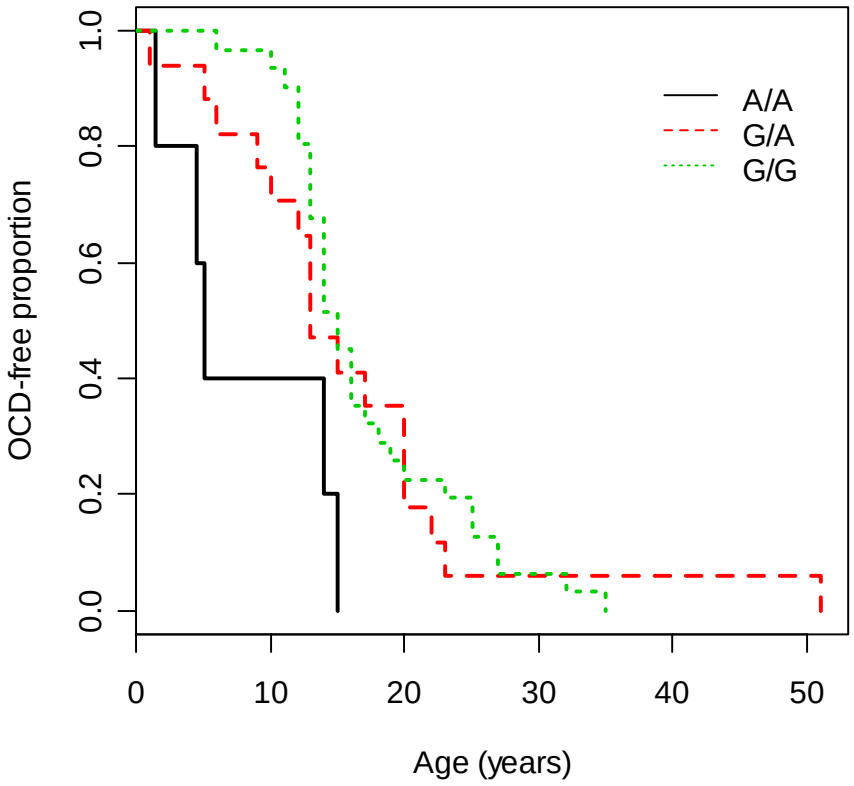



***Figure III.47. Kaplan-Meier estimation of time to age at onset of OCD, according to* BDNF val66met *genotype in males***. AA(met66met)-*homozygotes experienced significantly earlier ages at onset compared to* AG(val66met) - *or* GG(val66val)- *carriers.*

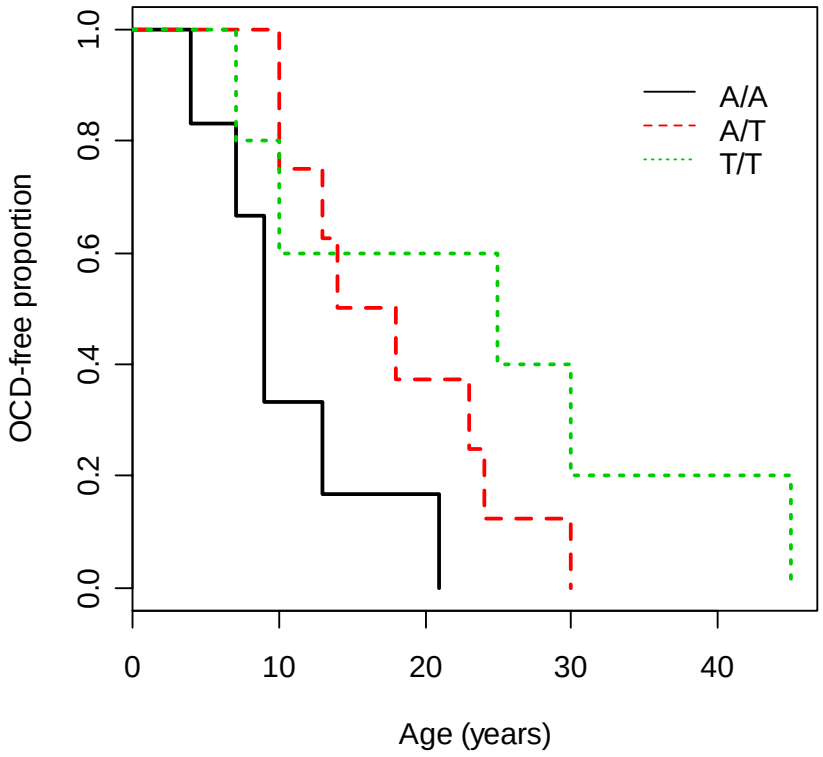



***Figure III.48. Kaplan-Meier estimation of time to age at onset of OCD, according to* HOXB8 rs2303486 *genotype in females.*** AA-*carriers experienced significantly earlier ages at onset compared to* AT- *or* TT-*carriers.*

When the unstratified OCD group was investigated, a significant association was noted between the *PLC-γ1 ser279gly* variant (rs8192707) and age at onset (p=0.008) (Table III.28[d]; Figure 49[a]). Here, subjects homozygous for the *A (ser279)*-allele were found to exhibit a later age at onset (median age at onset=16 years [95% CI: 14-20]), compared to those who were heterozygous, or homozygous for the *G (gly279)*-allele (median ages at onset=13 years [95% CI: 10-17] and 10 years [95% CI: 10- α], respectively).

Because the numbers within the genotype groups were reduced to below 5 when the sample was stratified according to gender, individuals carrying the *GG (gly279gly)*- or *GA* (*gly279ser)*-genotypes were grouped together in their respective gender subsets. Once again, when the total OCD sample was considered with this genotype-grouping, individuals carrying at least one *G*(*ser279*)-allele were found to experience significantly lower ages at onset (median=13 years [95% CI: 10-15] compared to those who were homozygous for the *A*(*gly279*)-allele (p=0.005) (Table III.28[d]; Figure 49[b]). When the grouped genotype dataset was stratified according to gender, both male and female individuals homozygous for the *A(ser279)*-allele were found to present with later ages at onset (median ages at onset=15 [95%CI: 14-20] and 18.5 years [95% CI: 13-24], respectively), compared to those who carried at least one copy of the *G*-allele (median ages at onset=13 years for both male and female, with male 95% CI: 9-16 and female 95% CI: 10-23) (Table III.28[d]; Figures III.49 [c] and [d]).

***Table III.28(d).*** *Kaplan-Meier estimates of age at onset of OCD according to genotypes of markers within bi-allelic* ESRα, INPP-1 and PLC-γ1 *candidate polymorphisms.*

| Gene | Variant | Genotype | Total OCD sample[a] | | | | | | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value | n | % | median | 95% CI | | p-value |
| | | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | | | | | LB[b] | UB[c] | |
| ***ESRα*** | **rs9340799** | *A/A* | **35** | 47.3 | 17.0 | 14.0 | 19.0 | | **17** | 47.2 | 15.0 | 14.0 | 23.0 | | **18** | 47.4 | 17.0 | 13.0 | 22.0 | |
| | | *A/G* | **30** | 40.5 | 13.0 | 10.0 | 17.0 | 0.646 | **15** | 41.7 | 14.0 | 13.0 | 18.0 | 0.284 | **15** | 39.5 | 10.0 | 9.0 | 27.0 | 0.928 |
| | | *G/G* | **9** | 12.2 | 16.0 | 12.0 | α | | **4** | 11.1 | 17.0 | 12 | α | | **5** | 13.2 | 16.0 | 10.0 | α | |
| | **rs2234693** | *C/C* | **13** | 23.2 | 15.0 | 13.0 | α | | **6** | 20.0 | 14.5 | 14.0 | α | | **7** | 26.9 | 16.0 | 8.0 | α | |
| | | *T/C* | **26** | 46.4 | 13.5 | 10.0 | 16.0 | 0.389 | **13** | 43.3 | 14.0 | 13.0 | α | 0.740 | **13** | 50.0 | 13.0 | 7.0 | α | 0.547 |
| | | *T/T* | **17** | 30.4 | 19.0 | 16.0 | 24.0 | | **11** | 36.7 | 17.0 | 16.0 | α | | **6** | 23.1 | 21.5 | 13.0 | α | |
| ***INPP-1*** | **rs1882891** | *C/A* | **24** | 27.0 | 14.5 | 13.0 | 23.0 | 0.708 | **12** | 26.7 | 13.5 | 12.0 | α | 0.783 | **12** | 27.3 | 20.0 | 13.0 | α | 0.848 |
| | | *C/C* | **65** | 73.0 | 15.0 | 13.0 | 17.0 | | **33** | 73.3 | 15.0 | 14.0 | 20.0 | | **32** | 72.7 | 14.0 | 10.0 | 21.0 | |
| ***PLC-γ1*** | **rs8192707** | *A/A* | **51** | 60.0 | 16.0 | 14.0 | 20.0 | | **27** | 64.3 | 15.0 | 14.0 | 20.0 | | **24** | 55.8 | 18.5 | 13.0 | 24.0 | |
| | | *A/G* | **29** | 34.1 | 13.0 | 10.0 | 17.0 | **0.008** | **13** | 31.0 | 13.0 | 9.0 | α | 0.091 | **16** | 37.2 | 14.0 | 7.0 | 25.0 | 0.075 |
| | | *G/G* | **5** | 5.9 | 10.0 | 10.0 | α | | **2** | 4.8 | 8.5 | 1.0 | α | | **3** | 7.0 | 10.0 | 10.0 | α | |
| | | *GG+AG*[d] | **34** | 40.0 | 13.0 | 10.0 | 15.0 | **0.005** | **15** | 35.7 | 13.0 | 9.0 | 16.0 | **0.030** | **19** | 44.2 | 13.0 | 10.0 | 23.0 | **0.039** |
| ***ACE*** | **Alu ins/del** | *D/D* | **40** | 40.4 | 19.0 | 15.0 | 22.0 | | **19** | 36.5 | 17.0 | 15.0 | 25.0 | | **21** | 44.7 | 19.0 | 13.0 | 25.0 | |
| | | *D/I* | **38** | 38.4 | 13.0 | 10.0 | 18.0 | 0.296 | **19** | 36.5 | 14.0 | 13.0 | 20.0 | 0.225 | **19** | 40.4 | 10.0 | 10.0 | 24.0 | 0.371 |
| | | *I/I* | **21** | 21.2 | 14.0 | 13.0 | 16.0 | | **14** | 26.9 | 14.0 | 13.0 | 16.0 | | **7** | 14.9 | 14.0 | 7.0 | α | |

Significant p-values are indicated in red, bold font; α = infinity

[a] Total population for whom age at onset was recorded.; [b]**LB:** lower boundary of the 95% CI; [c]**UB:**upper boundary of the 95% CI; [d]*GG* and *AG* were grouped together for this analysis.

**Abbreviations: OCD**: Obsessive-compulsive disorder; **CI:** confidence interval; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

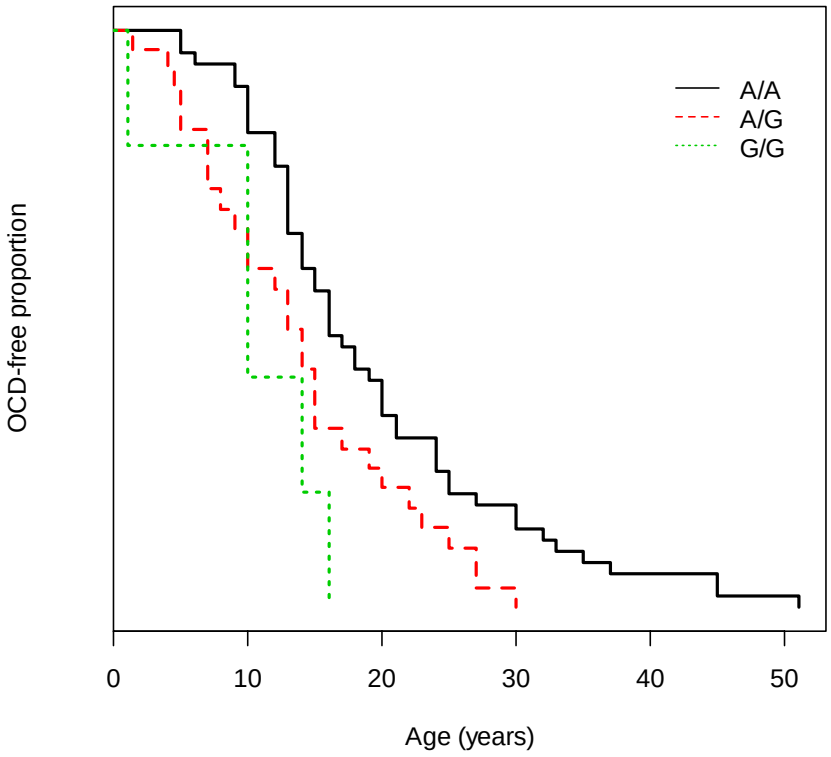



***Figure III.49(a). Kaplan-Meier estimation of time to age at onset of OCD, according to* PLC-γ1 *genotype, in the whole OCD sample.*** GG (gly279gly) *and* AG (ser279gly*)- carriers experienced significantly earlier ages at onset compared to* AA (ser279ser*)-carriers.*

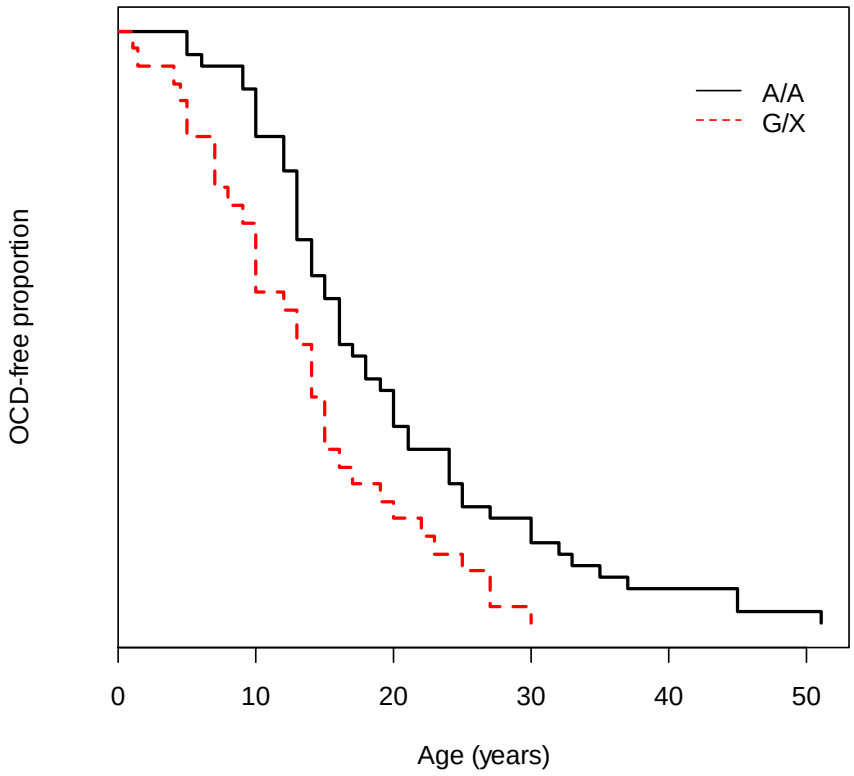



***Figure III.49(b): Kaplan-Meier estimation of time to age at onset, according to the presence or absence of the* G(gly279) PLC-γ1 *allele in the whole OCD sample.*** *Subjects carrying at least one* G (gly279)*-allele were found to experience lower ages at onset compared to those carrying the* AA (ser279ser)*-genotype (*X *refers to either the* G *or* A *alleles*).

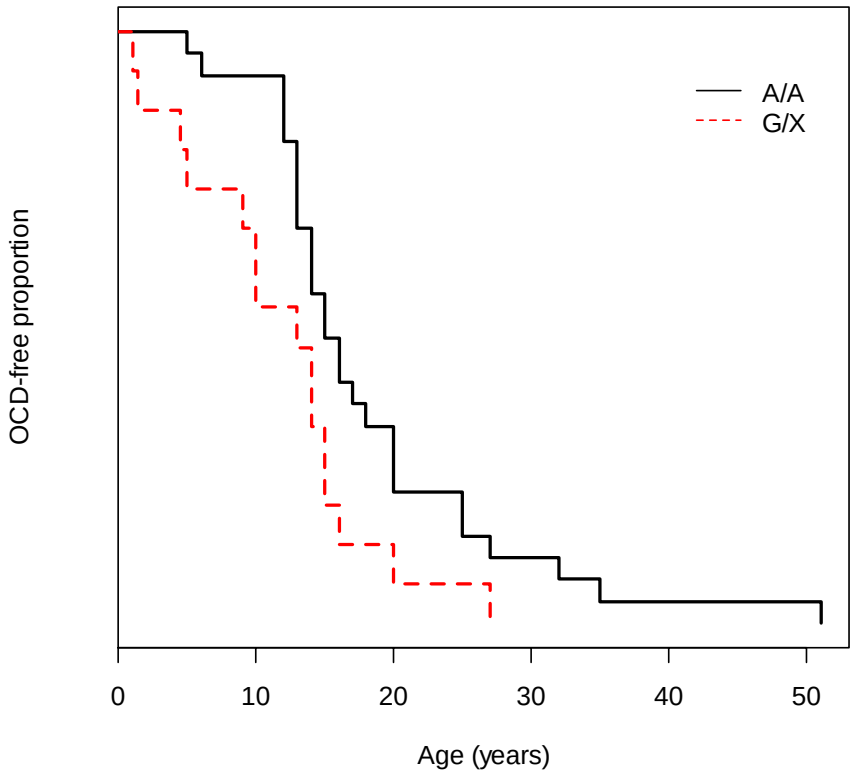



***Figure III.49(c). Kaplan-Meier estimation of time to age at onset of OCD in males, grouped according to the presence or absence of the* G *(gly279) allele.*** *Males carrying at least one* G (ser279)*-allele experienced significantly earlier ages at onset compared to those homozygous for the* A*-allele (*X *refers to either the* G *or* A *alleles*).

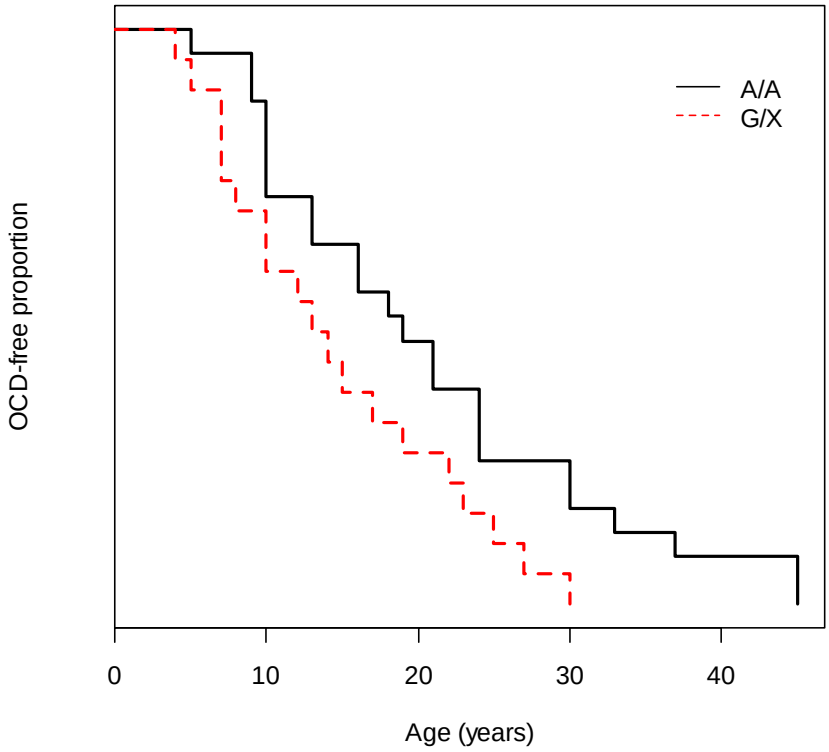



***Figure III.49 (d). Kaplan-Meier estimation of time to age at onset of OCD in females, grouped according to the presence or absence of the* G *(gly279) allele.*** *Females carrying at least one* G(gly279)*-allele experienced significantly earlier ages at onset compared to those homozygous for the* A*-allele (X refers to either* G *or* A*).*

***III.4.3.2.2. Single locus analysis of multi-allelic loci***

***i. DRD4***

A large number of genotypes (9 in total) were observed for the *DRD4* 48bp VNTR polymorphism amongst OCD subjects for whom age at onset was recorded, at least two thirds of which could be considered rare (<10%) in the present study. The 95% CIs corresponding to the median ages at onset in individuals carrying the rare genotypes are thus very wide, with the result that very little reliable information can be derived from them. Consequently, as for the Y-BOCS analyses (**section III.4.2.2.2[i]**), it was decided to group the genotypes according to the presence of at least one *A4*-allele (Table III.29). However, the analyses was not found to yield a statistically significant result when the whole OCD population was considered ($p = 0.917$), or when stratified by gender ($p = 0.963$ and $p = 0.838$ for males and females, respectively).

***ii. DAT***

Five genotypes were observed in the total OCD sample for whom age at onset was recorded. As one of these, the *A10/A2* genotype, was observed in only one person, it was decided that this genotype should be excluded from the analysis; the age at onset of this individual had been 12 years.

Results generated for the remaining genotypes are presented, with the caution that they should be viewed as preliminary. OCD subjects carrying the *A10/A11* genotypes experienced significantly earlier ages at onset ($p < 0.001$) compared to those homozygous for either the *A10* or *A9*-alleles, and *A9/A10* heterozygotes (Table III.30; Figure III.50[a]). Upon stratification, however, this association was only observed in the male population ($p < 0.001$) (Figure III.50 [b]) and not in the female population ($p=0.860$).

***Table III.29.*** *Kaplan-Meier estimates of the ages at onset of OCD according to DRD4 48bp VNTR genotypes, grouped according to the presence or absence of at least one A4-allele.*

| Genotype | Total OCD sample[a] | | | | |
|---|---|---|---|---|---|
| | n | % | median | 95% CI[a] | |
| | | | | LB[b] | UB[c] |
| *A4/A4* | **30** | 36.6 | 14.0 | 13 | 22 |
| *A4/other* | **43** | 52.4 | 15.0 | 13 | 18 |
| *other/other* | **9** | 11.0 | 23.0 | 12 | α |
| | **Male** | | | | |
| *A4/A4* | **12** | 28.6 | 15.0 | 13 | α |
| *A4/other* | **23** | 59.5 | 15.0 | 13 | 20 |
| *other/other* | **5** | 11.9 | 13.0 | 4 | α |
| | **Female** | | | | |
| *A4/A4* | **21** | 45.7 | 13.5 | 10 | 25 |
| *A4/other* | **20** | 43.5 | 16.5 | 13 | 24 |
| *other/other* | **5** | 10.9 | 23.5 | 12 | α |

**Total p = 0.917; males p = 0.963 female p = 0.838**

α=infinity

*A4* = 4-repeat allele; "other" alleles comprise *A2, A3, A7*and *A6* alleles

[a]confidence interval; [b]lower boundary of 95% CI; [c] upper boundary of 95% CI.

***Table III.30*** *Kaplan-Meier estimates of the ages at onset of OCD according to DAT 40bp VNTR genotypes.*

| Genotype | Total OCD sample[a] | | | | |
|---|---|---|---|---|---|
| | n | % | median | 95% CI[a] | |
| | | | | LB[b] | UB[c] |
| *A10/A10* | **50** | 50.5 | 16.0 | 14.0 | 18.0 |
| *A10/A9* | **38** | 38.4 | 14.0 | 12.0 | 19.0 |
| *A9/A9* | **8** | 8.1 | 11.0 | 7.0 | α |
| *A10/A11* | **4** | 3.0 | 7.3 | 1.0 | α |
| | **Male** | | | | |
| *A10/A10* | **26.0** | 52.0 | 16.5 | 14.0 | 20.0 |
| *A10/A9* | **18.0** | 36.0 | 13.5 | 13.0 | 16.0 |
| *A9/A9* | **3.0** | 6.0 | 13.0 | 9.0 | α |
| *A10/A11* | **3.0** | 6.0 | 7.3 | 1.0 | α |
| | **Female** | | | | |
| *A10/A10* | **24.0** | 49.0 | 15.5 | 13.0 | 18.0 |
| *A10/A9* | **20.0** | 40.8 | 19.0 | 10.0 | 25.0 |
| *A9/A9* | **5.0** | 10.2 | 7.0 | 5.0 | α |

**Total $p < 0.001$; male $p < 0.001$; female $p = 0.860$**

*A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele

[a]confidence interval; [b]lower boundary of 95% CI; [c] upper boundary of 95% CI.

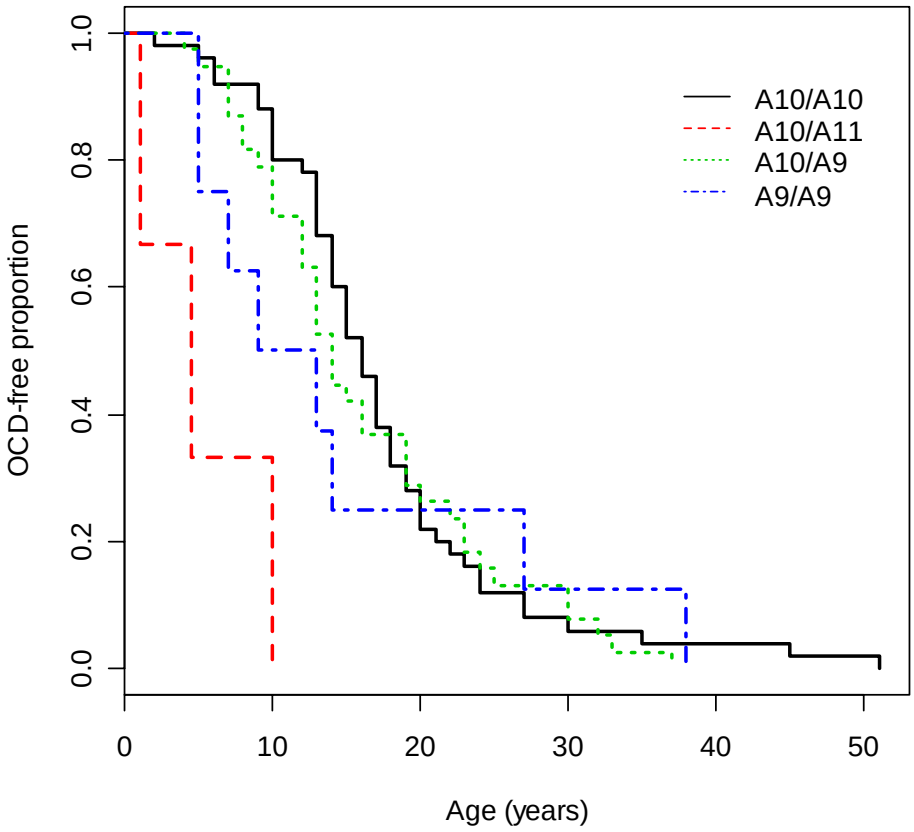



***Figure III.50(a). Kaplan-Meier estimation of time to age at onset of OCD, according to* DAT *40bp VNTR genotypes observed in the present sample (excluding* A10/A2*).*** *Individuals carrying the* A10/A11 *genotype experienced significantly earlier ages at onset.*

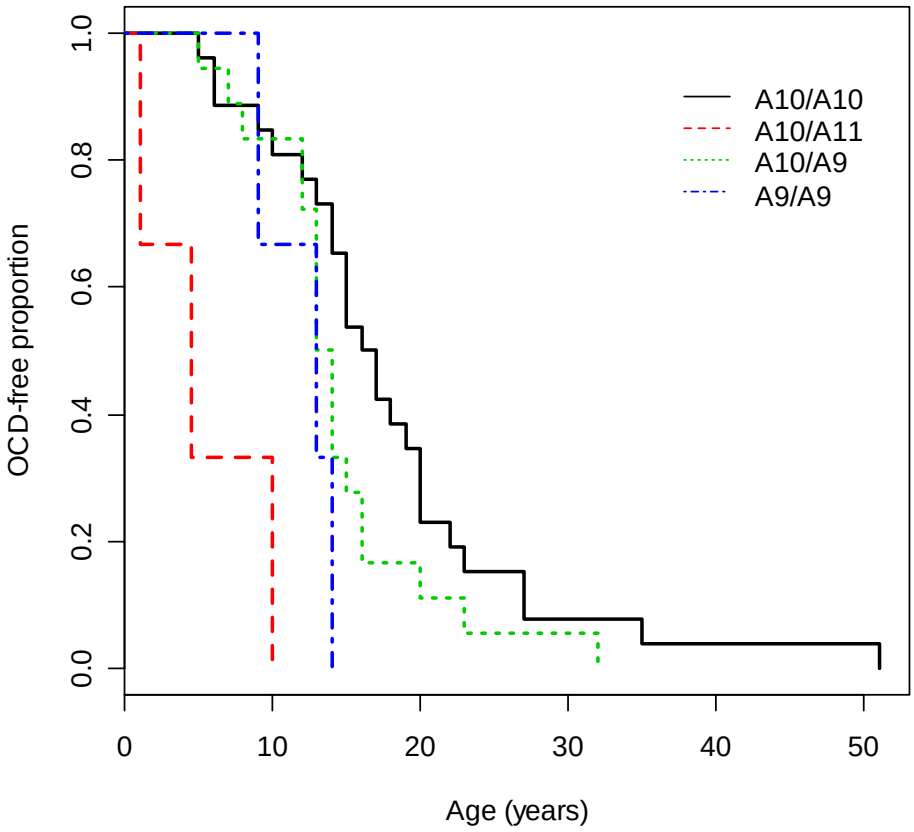



***Figure III.50(b). Kaplan-Meier estimation of time to age at onset of OCD in males, according to* DAT *40bp VNTR genotypes observed in the present sample (excluding* A10/A2*).*** *Individuals carrying the* A10/A11 *genotype experienced significantly earlier ages at onset.*

### III.4.3.3. Haplotype analyses: age at onset

#### *III.4.3.3.1.* 5-HT$_{2A}$ *haplotype analysis*

When the haplotypes comprising the SNPs genotyped in *5-HT$_{2A}$* were analysed for any differences in median ages at onset, the global p-value of 0.443 indicated that no significant differences were present (Table III.31).

#### *III.4.3.3.2.* DRD4 *haplotype analysis*

Haplotype frequencies for the two DRD4 polymorphisms investigated are presented in Table III.32. No statistically significant differences were observed, with a global p-value of 0.319.

#### *III.4.3.3.3.* COMT *haplotype analysis*

The results from the age at onset haplotype analysis involving the three *COMT* variants genotyped are represented in Table III.33. No statistically significant differences in age at onset between haplotypes were noted, with global p-value of 0.850.

#### *III.4.3.3.4.* GRIN2B *haplotype analysis*

Although the global p-value indicated only a trend towards significant differences in age at onset (p = 0.060) between the four possible haplotypes comprising the *GRIN2B* rs890 and rs1806191 variants, it was found that those OCD patients carrying the *G-A* haplotype experienced significantly later onset compared to those carrying the remaining haplotypes (haplotype score=2.541; individual p-value = 0.011). Conversely, those OCD patients carrying the *A-C* haplotype exhibited significantly earlier ages at onset (haplotype score = -2.382; individual p-value = 0.018) (Table III.34).

#### *III.4.3.3.5.* BDNF *haplotype analysis*

Although the *BDNF val66met* (rs6265) variant was found to be associated with age at onset of OCD in males when single loci were analysed (Table III.28[c]), no statistically significant differences were observed when haplotype analysis of the three *BDNF* markers, *val66met* (rs6265), rs2049046 and rs988748 was conducted (global p-value = 0.584) (Table III.35).

### *III.4.3.3.6.* ESRα *haplotype analysis*

No statistically significant differences in age at onset between haplotypes formed by the *ESRα* intronic SNPs, rs9340799 and rs2234693, was noted, with global p-value of 0.829 (Table III.36).

***Table III.31.*** *Haplotype analysis of candidate markers within* 5-HT2A *and age at onset distribution.*

| Variants | | Haplotype Frequency (n=95) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| rs6311 | rs6313 | | | |
| *G* | *C* | 0.622 | 0.992 | 0.320 |
| *A* | *T* | 0.329 | -0.862 | 0.409 |
| *A* | *C* | 0.027 | 0.669 | 0.503 |

**Global p=0.443**

***Table III.32.*** *Haplotype analysis of candidate markers within* DRD4 *and age at onset distribution.*

| Variants | | Haplotype Frequency (n=65) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| rs1800955 | 48bp VNTR | | | |
| *T* | *A4* | 0.396 | 1.09 | 0.271 |
| *C* | *A4* | 0.191 | -1.10 | 0.269 |
| *T* | *A7* | 0.122 | 0.09 | 0.922 |
| *C* | *A7* | 0.110 | -0.37 | 0.691 |
| *T* | *A2* | 0.071 | 0.26 | 0.821 |
| *C* | *A2* | 0.059 | 0.63 | 0.528 |
| *C* | *A3* | 0.039 | -0.70 | 0.479 |

**Global p=0.319**

*A4*=4-repeat allele; *A2*=2-repeat allele; *A3*=3-repeat allele; *A7*=7-repeat allele

***Table III.33.*** *Haplotype analysis of candidate markers within* COMT *and age at onset distribution.*

| Variants | | | Haplotype Frequency (n=29) | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|
| **rs2097603** | **rs4680** | **rs362204** | | | |
| *G* | *A* | *D* | 0.363 | 0.516 | 0.630 |
| *A* | *A* | *D* | 0.194 | 0.209 | 0.833 |
| *A* | *G* | *I* | 0.132 | -0.503 | 0.631 |
| *G* | *G* | *D* | 0.132 | 0.433 | 0.682 |

**Global p=0.850**

***Table III.34.*** *Haplotype analysis of candidate markers within* GRIN2B *and age at onset distribution.*

| Variants | | Haplotype Frequency (n=52) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| **rs1806191** | **rs890** | | | |
| *G* | *A* | 0.475 | 2.541 | **0.011** |
| *A* | *C* | 0.273 | -2.382 | **0.018** |
| *G* | *C* | 0.131 | -0.137 | 0.902 |
| *A* | *A* | 0.122 | 0.110 | 0.916 |

**Global p=0.060**

Significant p-values are indicated in red, bold font.

***Table III.35.*** *Haplotype analysis of candidate markers within* BDNF *and age at onset distribution.*

| Variants | | | Haplotype Frequency (n=32) | Haplotype Score | Individual p-value |
|---|---|---|---|---|---|
| **rs6265** | **rs2049046** | **rs988748** | | | |
| *G* | *A* | *C* | 0.453 | 0.486 | 0.631 |
| *A* | *T* | *G* | 0.266 | -1.112 | 0.266 |
| *G* | *T* | *C* | 0.250 | 0.664 | 0.509 |

**Global p=0.584**

***Table III.36.*** *Haplotype analysis of candidate markers within* ESRα *and age at onset distribution.*

| Variants | | Haplotype Frequency (n=55) | Haplotype Score | Individual p-value |
|---|---|---|---|---|
| rs9340799 | rs2234693 | | | |
| *A* | *T* | 0.510 | 0.614 | 0.556 |
| *G* | *C* | 0.343 | -0.270 | 0.761 |
| *A* | *C* | 0.147 | -0.519 | 0.643 |

**Global p=0.829**

### III.4.4. Stratification by co-morbidity

The OCD sample was also stratified according to the presence or absence of selected co-morbid disorder. Since the sample sizes become small after stratification of the OCD sample, genetic analyses were conducted using the most common co-morbid disorders observed in the present study; namely, co-morbid major depressive disorder (MDD), and the presence or absence of tics. Following this logic, haplotype analyses were not conducted, due to the reduced numbers after stratification for co-morbidity.

#### III.4.4.1. Major depressive disorder (MDD)

##### *III.4.4.1.1. Analysis of clinical variables*

Major depressive disorder (MDD) has been found to be the most prevalent disorder occurring co-morbidly with OCD: in the present study, 64.6% of the OCD patients presented with co-morbid MDD (Table III.7). For this reason, it was of interest to determine whether OCD patients exhibiting co-morbid MDD (OCD+MDD) differed genetically or clinically from those patients who did not exhibit MDD (OCD-MDD).

Initial comparisons were made between the two groups of patients and selected demographic and clinical factors, and certain aspects of a family history of psychiatric disorders (Table III.37). Male OCD patients presenting with co-morbid MDD were, on average, younger than female OCD patients with co-morbid MDD, although this difference did not reach statistical significance (median ages at onset=13 years [95% CI: 12-15] and 16 years [95% CI: 13-21] for males and females, respectively; p=0.198). No statistically significant differences were observed when gender (p = 0.580 [Table III.7]), total Y-BOCS score (p = 0.515 [Table

III.17]) or age at onset of OCD (p = 0.337 [Table III.27]) were compared between the two patient groups. Moreover, no significant differences in presence or absence of family history of OCD (p = 0.182), OCS (p = 0.062) or tics (p = 0.605) were noted between the groups (Table III.37).

However, when the frequencies of symptom subtypes were compared between the groups, significantly more patients with MDD experienced sexual/religious symptoms compared to those without MDD (50.0% versus 25.8%, respectively; p = 0.042) (Table III.37).

***III.4.4.1.2. Single locus analysis according to the presence or absence of co-morbid MDD***

***III.4.4.1.2.1. Single locus analysis of bi-allelic polymorphisms***

The genotype and allele scores and their corresponding frequencies for each bi-allelic polymorphism investigated are represented in Table III.38. The results of the genetic association analyses comparing genotype and allele frequencies between OCD patients with MDD and those without, are depicted in Table III.39(a), and between OCD patients exhibiting co-morbid MDD and controls are presented in Table III.39(b).

Two significant associations were observed: firstly, the *C*-allele of the *5-HT$_6$* SNP, rs1805054, was found to be significantly overrepresented in the OCD-MDD group (p=0.004; OR=0.22 [95% CI: 0.05-0.68]) (Tables III.38 and III.39[a]), and in the control group (p=0.008; OR=0.47 [95% CI: 0.27-0.83]) (Tables III.3[b], III.38 and III.39[b]), indicating that this allele may confer protection against the development of co-morbid MDD.

Secondly, when the genotypic distribution of the *COMT* promoter SNP, rs2097603, was compared between the two OCD subsets, significantly more OCD-MDD patients possessed the *AA*-genotype compared to OCD+MDD patients (p = 0.038; OR = 0.21 [95% CI: 0.04-0.97]) indicating that this genotype may confer protection against the development of co-morbid MDD. The allelic distribution was also found to differ significantly between OCD+MDD and the OCD-MDD subsets (p = 0.035; OR = 0.43 [95% CI: 0.18-0.98]) (Table III.39[a]). The OR value of the aforementioned association indicates that the *A*-allele may represent a protective allele.

***Table III.37****. Clinical characteristics in the OCD patient subset according to the presence (OCD+MDD) or absence of MDD (OCD-MDD) as a co-morbid disorder.*

| Clinical Variables<br>Family History[a] | OCD+MDD | | OCD-MDD | | p-value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **Family history of OCD** | **10** | 20.0 | **10** | 33.3 | 0.182 |
| **Family history of OCS** | **19** | 38.0 | **18** | 60.0 | 0.062 |
| **Family history of tics** | **4** | 8.0 | **2** | 6.7 | 0.605 |
| **Primary Symptom Dimensions** [b] | | | | | |
| **Hoarding** | **14** | 24.1 | **8** | 25.8 | 0.862 |
| **Symmetry/ ordering** | **36** | 62.1 | **17** | 54.8 | 0.571 |
| **Sexual/religious** | **29** | 50.0 | **8** | 25.8 | **0.042** |
| **Contamination** | **37** | 63.8 | **17** | 54.8 | 0.469 |
| **Aggression** | **31** | 53.4 | **14** | 45.2 | 0.456 |

[a]n(OCD+MDD)=50; n(OCD-MDD)=30; [b]n(OCD+MDD)=58; n(OCD-MDD)=31

**Abbreviations: MDD**: Major depressive disorder **OCD:** obsessive-compulsive disorder**; OCS:** Obsessive-compulsive symptoms.

***Table III.38.*** *Genotype and allele scores and frequencies in bi-allelic candidate polymorphisms in OCD patients presenting with co-morbid MDD (OCD+MDD) and those without co-morbid MDD (OCD-MDD).*

| Gene | Variant | $n_1/n_2$[a] | OCD+MDD | | | | | | | | | | | OCD-MDD | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | **rs6311** | *G/A* | 26 | 37.7 | 32 | 46.4 | 11 | 15.9 | **69** | 84 | 60.9 | 54 | 39.1 | 17 | 45.9 | 13 | 35.1 | 7 | 18.9 | **37** | 47 | 63.5 | 27 | 36.5 |
| | **rs6313** | *C/T* | 26 | 39.4 | 27 | 40.9 | 13 | 19.7 | **66** | 79 | 59.8 | 53 | 40.2 | 18 | 48.6 | 12 | 32.4 | 7 | 18.9 | **37** | 48 | 64.9 | 26 | 35.1 |
| ***5-HT$_{1D\beta}$*** | **rs6296** | *G/C* | 29 | 46.0 | 30 | 47.6 | 4 | 6.3 | **63** | 88 | 69.8 | 38 | 30.2 | 23 | 63.9 | 10 | 27.8 | 3 | 8.3 | **36** | 56 | 77.8 | 16 | 22.2 |
| ***5-HT$_6$*** | **rs1805054** | *C/T* | 29 | 51.8 | 25 | 44.6 | 2 | 3.6 | **56** | 83 | 74.1 | 29 | 25.9 | 24 | 85.7 | 4 | 14.3 | 0 | 0.0 | **28** | 52 | 92.9 | 4 | 7.1 |
| ***DRD4*** | **rs1800955** | *T/C* | 20 | 35.1 | 28 | 49.1 | 9 | 15.8 | **57** | 68 | 59.6 | 46 | 40.4 | 10 | 31.3 | 17 | 53.1 | 5 | 15.6 | **32** | 37 | 57.8 | 27 | 42.2 |
| ***DRD2*** | **rs1800497** | *C/T* | 39 | 59.1 | 25 | 37.9 | 2 | 3.0 | **66** | 103 | 78.0 | 29 | 22.0 | 18 | 50.0 | 15 | 41.7 | 3 | 8.3 | **36** | 51 | 70.8 | 21 | 29.2 |
| ***COMT*** | **rs2097603** | *A/G* | 8 | 21.1 | 15 | 39.5 | 15 | 39.5 | **38** | 31 | 40.8 | 45 | 59.2 | 8 | 38.1 | 10 | 47.6 | 3 | 14.3 | **21** | 26 | 61.9 | 16 | 38.1 |
| | **rs4680** | *A/G* | 14 | 25.9 | 29 | 53.7 | 11 | 20.4 | **54** | 57 | 52.8 | 51 | 47.2 | 9 | 26.5 | 21 | 61.8 | 4 | 11.8 | **34** | 39 | 57.4 | 29 | 42.6 |
| | **rs362204** | *D/I* | 16 | 44.4 | 18 | 50.0 | 2 | 5.6 | **36** | 50 | 69.4 | 22 | 30.6 | 12 | 75.0 | 4 | 25.0 | 0 | 0.0 | **16** | 28 | 87.5 | 4 | 12.5 |
| ***DRD3*** | **rs6280** | *A/G* | 36 | 56.3 | 22 | 34.4 | 6 | 9.4 | **64** | 94 | 73.4 | 34 | 26.6 | 17 | 54.8 | 8 | 25.8 | 6 | 19.4 | **31** | 42 | 67.7 | 20 | 32.3 |
| ***DRD1*** | ***A-48G*** | *A/G* | 22 | 33.8 | 37 | 56.9 | 6 | 9.2 | **65** | 81 | 62.3 | 49 | 37.7 | 17 | 50.0 | 12 | 35.3 | 5 | 14.7 | **34** | 46 | 67.6 | 22 | 32.4 |
| ***GRIN2B*** | **rs1806191** | *G/A* | 14 | 41.2 | 15 | 44.1 | 5 | 14.7 | **34** | 43 | 63.2 | 25 | 36.8 | 8 | 40.0 | 8 | 40.0 | 4 | 20.0 | **20** | 24 | 60.0 | 16 | 40.0 |
| | **rs890** | *A/C* | 12 | 35.3 | 18 | 52.9 | 4 | 11.8 | **34** | 42 | 61.8 | 26 | 38.2 | 6 | 28.6 | 12 | 57.1 | 3 | 14.3 | **21** | 24 | 57.1 | 18 | 42.9 |
| ***BDNF*** | **rs6265** | *G/A* | 44 | 60.3 | 26 | 35.6 | 3 | 4.1 | **73** | 114 | 78.1 | 32 | 21.9 | 27 | 73.0 | 7 | 18.9 | 3 | 8.1 | **37** | 61 | 82.4 | 13 | 17.6 |
| | **rs2049046** | *T/A* | 8 | 26.7 | 16 | 53.3 | 6 | 20.0 | **30** | 32 | 53.3 | 28 | 46.7 | 6 | 30.0 | 8 | 40.0 | 6 | 30.0 | **20** | 20 | 50.0 | 20 | 50.0 |
| | **rs988748** | *C/G* | 14 | 45.2 | 17 | 54.8 | 0 | 0.0 | **31** | 45 | 72.6 | 17 | 27.4 | 11 | 61.1 | 5 | 27.8 | 2 | 11.1 | **18** | 27 | 75.0 | 9 | 25.0 |
| ***HOXB8*** | **rs2303486** | *A/T* | 11 | 37.9 | 12 | 41.4 | 6 | 20.7 | **29** | 34 | 58.6 | 24 | 41.4 | 8 | 40.0 | 8 | 40.0 | 4 | 20.0 | **20** | 24 | 60.0 | 16 | 40.0 |
| ***ESRα*** | **rs9340799** | *A/G* | 24 | 48.0 | 19 | 38.0 | 7 | 14.0 | **50** | 67 | 67.0 | 33 | 33.0 | 15 | 48.4 | 13 | 41.9 | 3 | 9.7 | **31** | 43 | 69.4 | 19 | 30.6 |
| | **rs2234693** | *T/C* | 12 | 33.3 | 18 | 50.0 | 6 | 16.7 | **36** | 42 | 58.3 | 30 | 41.7 | 7 | 28.0 | 11 | 44.0 | 7 | 28.0 | **25** | 25 | 50.0 | 25 | 50.0 |
| ***INPP-1*** | **rs1882891** | *C/A* | 45 | 76.3 | 13 | 22.0 | 1 | 1.7 | **59** | 103 | 87.3 | 15 | 12.7 | 25 | 69.4 | 11 | 30.6 | 0 | 0.0 | **36** | 61 | 84.7 | 11 | 15.3 |
| ***PLC-γ1*** | **rs8192707** | *A/G* | 34 | 59.6 | 18 | 31.6 | 5 | 8.8 | **57** | 86 | 75.4 | 28 | 24.6 | 21 | 63.6 | 12 | 36.4 | 0 | 0.0 | **33** | 54 | 81.8 | 12 | 18.2 |
| ***ACE*** | ***Alu* Ins/del** | *D/I* | 29 | 40.8 | 25 | 35.2 | 17 | 23.9 | **71** | 83 | 58.5 | 59 | 41.5 | 13 | 36.1 | 18 | 50.0 | 5 | 13.9 | **36** | 44 | 61.1 | 28 | 38.9 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele.

**Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1D\beta}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT***: Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.39(a).*** *Association analyses investigating the differences in genotype and allele distributions between OCD subjects with co-morbid MDD and those without co-morbid MDD in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 1.000 | 0.97 | 0.26 | 3.42 | 0.054 | 0.768 | 0.89 | 0.48 | 1.66 | 0.054 |
| | rs6313 | *C/T* | 0.784 | 0.78 | 0.22 | 2.62 | 0.052 | 0.551 | 0.81 | 0.43 | 1.51 | 0.075 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 1.000 | 0.95 | 0.13 | 6.22 | 0.059 | 0.250 | 0.66 | 0.31 | 1.35 | 0.140 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.499 | 0.00 | 0.00 | 6.87 | 0.073 | **0.004** | **0.22** | **0.05** | **0.68** | **0.667** |
| ***DRD4*** | rs1800955 | *T/C* | 1.000 | 1.11 | 0.23 | 4.98 | 0.053 | 0.874 | 1.08 | 0.55 | 2.10 | 0.051 |
| ***DRD2*** | rs1800497 | *C/T* | 0.325 | 3.18 | 0.33 | 41.20 | 0.101 | 0.307 | 1.46 | 0.72 | 2.95 | 0.128 |
| ***COMT*** | rs2097603 | *A/G* | **0.038** | **0.21** | **0.04** | **0.97** | **0.313** | **0.035** | **0.43** | **0.18** | **0.98** | **0.417** |
| | rs4680 | *A/G* | 0.501 | 0.57 | 0.10 | 2.79 | 0.065 | 0.641 | 0.83 | 0.43 | 1.60 | 0.065 |
| | rs362204 | *D/I* | 0.503 | 0.00 | 0.00 | 8.01 | 0.065 | 0.054 | 0.33 | 0.08 | 1.10 | 0.317 |
| ***DRD3*** | rs6280 | *A/G* | 0.319 | 2.09 | 0.48 | 9.15 | 0.107 | 0.493 | 1.31 | 0.64 | 2.67 | 0.083 |
| ***DRD1*** | *A-48G* | *A/G* | 1.000 | 1.08 | 0.22 | 5.07 | 0.054 | 0.533 | 0.79 | 0.40 | 1.53 | 0.077 |
| ***GRIN2B*** | rs1806191 | *G/A* | 0.704 | 1.39 | 0.21 | 8.75 | 0.050 | 0.838 | 1.15 | 0.47 | 2.75 | 0.051 |
| | rs890 | *A/C* | 0.673 | 1.48 | 0.16 | 12.30 | 0.050 | 0.691 | 1.21 | 0.51 | 2.84 | 0.056 |
| ***BDNF*** | rs6265 | *G/A* | 0.673 | 1.62 | 0.20 | 13.00 | 0.052 | 0.485 | 0.76 | 0.34 | 1.62 | 0.076 |
| | rs2049046 | *T/A* | 1.000 | 1.32 | 0.22 | 8.15 | 0.050 | 0.839 | 1.14 | 0.48 | 2.75 | 0.051 |
| | rs988748 | *C/G* | 0.222 | α | 0.21 | α | 0.100 | 1.000 | 0.88 | 0.30 | 2.46 | 0.050 |
| ***HOXB8*** | rs2303486 | *A/T* | 1.000 | 0.92 | 0.14 | 5.54 | 0.056 | 1.000 | 0.95 | 0.38 | 2.31 | 0.050 |
| ***ESRα*** | rs9340799 | *A/G* | 0.726 | 0.69 | 0.10 | 3.64 | 0.051 | 0.863 | 0.90 | 0.43 | 1.86 | 0.051 |
| | rs2234693 | *T/C* | 0.473 | 1.96 | 0.38 | 10.60 | 0.077 | 0.460 | 1.40 | 0.64 | 3.08 | 0.092 |
| ***INPP-1*** | rs1882891 | *C/A* | 1.000 | 0.00 | 0.00 | 71.70 | 0.057 | 0.666 | 1.24 | 0.48 | 3.10 | 0.056 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.152 | 0.00 | 0.00 | 1.97 | 0.178 | 0.357 | 0.68 | 0.29 | 1.53 | 0.102 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.568 | 0.66 | 0.16 | 2.43 | 0.062 | 0.769 | 0.90 | 0.48 | 1.66 | 0.054 |

Significant p-values are indicated in red, bold font.

[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI

**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8: homeobox gene B8; ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme

***Table III.39(b).*** *Association analyses investigating the differences in genotype and allele distributions between OCD subjects with co-morbid MDD and controls in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.623 | 0.82 | 0.37 | 1.83 | 0.050 | 0.684 | 0.92 | 0.61 | 1.39 | 0.050 |
| | rs6313 | *C/T* | 0.933 | 1.04 | 0.44 | 2.42 | 0.062 | 0.985 | 1.00 | 0.66 | 1.51 | 0.068 |
| ***5-HT$_{1D\beta}$*** | rs6296 | *G/C* | 0.777 | 0.84 | 0.25 | 2.84 | 0.189 | 0.222 | 0.75 | 0.48 | 1.19 | 0.246 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.126 | 0.18 | 0.02 | 2.08 | 0.660 | **0.008** | **0.47** | **0.27** | **0.83** | **0.770** |
| ***DRD4*** | rs1800955 | *T/C* | 0.283 | 1.65 | 0.66 | 4.15 | 0.148 | 0.278 | 1.28 | 0.82 | 1.99 | 0.192 |
| ***DRD2*** | rs1800497 | *C/T* | 0.210 | 2.64 | 0.55 | 12.63 | 0.111 | 0.408 | 1.23 | 0.75 | 2.02 | 0.140 |
| | rs2097603 | *A/G* | **0.012** | **0.49** | **0.28** | **0.86** | **0.572** | **0.018** | **0.29** | **0.10** | **0.83** | **0.684** |
| ***COMT*** | rs4680 | *G/A* | 0.390 | 0.66 | 0.26 | 1.70 | 0.111 | 0.409 | 0.83 | 0.53 | 1.30 | 0.141 |
| | rs362204 | *D/I* | 0.302 | 2.29 | 0.46 | 11.37 | 0.097 | 0.426 | 1.26 | 0.71 | 2.25 | 0.120 |
| ***DRD3*** | rs6280 | *A/G* | 0.143 | 2.12 | 0.77 | 5.86 | 0.340 | 0.060 | 1.57 | 0.98 | 2.52 | 0.452 |
| ***DRD1*** | *A-48G* | *A/G* | 0.526 | 1.40 | 0.49 | 4.03 | 0.154 | 0.825 | 1.05 | 0.68 | 1.62 | 0.058 |
| ***GRIN2B*** | rs1806191 | *G/A* | 0.345 | 1.96 | 0.48 | 7.99 | 0.617 | 0.243 | 1.48 | 0.77 | 2.87 | 0.736 |
| | rs890 | *A/C* | 0.452 | 1.75 | 0.40 | 7.58 | 0.092 | 0.483 | 1.26 | 0.66 | 2.44 | 0.105 |
| | rs6265 | *G/A* | 0.184 | 0.31 | 0.05 | 1.91 | 0.256 | 0.195 | 0.72 | 0.44 | 1.19 | 0.267 |
| ***BDNF*** | rs2049046 | *A/T* | 0.201 | 0.43 | 0.12 | 1.58 | 0.183 | 0.216 | 0.68 | 0.37 | 1.26 | 0.228 |
| | rs988748 | *C/G* | 0.550 | 1.10 | 0.04 | 28.53 | 0.404 | 0.183 | 0.62 | 0.30 | 1.26 | 0.265 |
| ***HOXB8*** | rs2303486 | *T/A* | 0.229 | 0.44 | 0.11 | 1.70 | 0.345 | 0.197 | 0.64 | 0.33 | 1.26 | 0.252 |
| ***ESRα*** | rs9340799 | *A/G* | 0.573 | 1.32 | 0.50 | 3.51 | 0.080 | 0.483 | 1.19 | 0.73 | 1.93 | 0.096 |
| | rs2234693 | *T/C* | 0.233 | 1.94 | 0.65 | 5.81 | 0.180 | 0.242 | 1.38 | 0.81 | 2.35 | 0.199 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.972 | 0.96 | 0.09 | 10.84 | 0.067 | 0.706 | 0.88 | 0.45 | 1.72 | 0.070 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.146 | 0.37 | 0.09 | 1.48 | 0.207 | 0.391 | 0.79 | 0.47 | 1.35 | 0.160 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.245 | 0.50 | 0.15 | 1.65 | 0.701 | 0.203 | 0.71 | 0.41 | 1.21 | 0.566 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI;

**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1D\beta}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF: brain-derivedneurotrophic factor; HOXB8: homeobox gene B8; ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme

Indeed, when the allele and genotype frequencies of the same SNP were compared between the OCD+MDD and control samples (Table III.3[b], Table III.38 and Table III.39[b]), significant differences in both the genotypic (p = 0.012; OR = 0.49 [95% CI: 0.28-0.86]) and allelic (p = 0.018; OR = 0.29 [95% CI: 0.10-0.83]) (Table III.39[b]) distributions were observed, re-inforcing the assumption that the rs2097603 *AA*-genotype confers protection against the development of co-morbid MDD.

***III.4.4.1.2.2. Single locus analysis of multi-allelic polymorphisms***

***i. DRD4***

The genotype and allele counts for the *DRD4* 48bp VNTR in OCD+MDD and OCD-MDD subgroups are provided in Tables III.40 (a) and (b). No statistically significant differences in genotype (p = 0.884) or allele (p = 0.976) were observed between the two OCD subsets.

Likewise, when the genotype and allele frequencies were compared between OCD+MDD and control samples, no statistically significant differences were observed (p = 0.384 and p = 0.851 for genotype and allele analyses, respectively (Tables III.4[a] and [b] and III.40[a] and [b]).

***ii. DAT***

Genotype and allele counts and frequencies for the *DAT* 40bp VNTR in the OCD patients presenting with co-morbid MDD, and those not, are provided in Tables III.41[a] and [b]), no statistically significant differences could be observed between OCD patients with co-morbid MDD and those without (p = 0.388 and p = 0.253, respectively).

Likewise, when the *DAT* 40bp VNTR genotype and allele frequencies were compared between the OCD+MDD and control samples, no statistically significant differences were observed (p = 0.367 and p = 0.378 for genotype and allele analyses, respectively) (Tables III.5[a] and [b] and III.41[a] and [b]).

***Table III.40(a).*** *The genotype counts and associated frequencies in the* DRD4 *48bp VNTR OCD subjects presenting with co-morbid MDD (OCD+MDD) and those without co-morbid MDD (OCD-MDD).*

| Genotype | OCD+MDD | | OCD-MDD | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A4* | **21** | 40.4 | **11** | 32.4 |
| *A4/A7* | **11** | 21.2 | **10** | 29.4 |
| *A4/A2* | **8** | 15.4 | **7** | 20.6 |
| *A4/A3* | **6** | 11.5 | **3** | 8.8 |
| *A7/A7* | **3** | 5.8 | **1** | 2.9 |
| *A2/A2* | **1** | 1.9 | **0** | 0.0 |
| *A3/A6* | **1** | 1.9 | **0** | 0.0 |
| *A7/A2* | **1** | 1.9 | **1** | 2.9 |
| *A7/A3* | **0** | 0.0 | **1** | 2.9 |

**p=0.884**

*A4*=4-repeat allele; "other" alleles comprise *A2, A3, A7*and *A6* alleles

**Abbreviations: OCD:** obsessive-compulsive disorder**; MDD:** major depressive disorder

***Table III.40(b).*** *The allele counts and associated frequencies in the* DRD4 *48bp VNTR in OCD subjects presenting with co-morbid MDD (OCD+MDD) and those without co-morbid MDD (OCD-MDD).*

| Allele | OCD+MDD | | OCD-MDD | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **67** | 64.4 | **42** | 61.8 |
| *A7* | **18** | 17.3 | **14** | 20.6 |
| *A2* | **11** | 10.6 | **8** | 11.8 |
| *A3* | **7** | 6.7 | **4** | 5.9 |
| *A6* | **1** | 1.0 | **0** | 0.0 |

**p = 0.976**

*A4* = 4-repeat allele; "other" alleles comprise *A2, A3, A7*and *A6* alleles

**Abbreviations: OCD:** obsessive-compulsive disorder**; MDD:** major depressive disorder

***Table III.41(a).*** *The genotype counts and associated frequencies in the* DAT *40bp VNTR OCD subjects presenting with co-morbid MDD (OCD+MDD) and those without co-morbid MDD (OCD-MDD).*

| Genotype | OCD+MDD | | OCD-MDD | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10/A10* | **34** | 48.6 | **23** | 97.1 |
| *A9/A10* | **27** | 38.6 | **9** | 77.1 |
| *A9/A9* | **6** | 8.6 | **2** | 17.1 |
| *A10/A11* | **2** | 2.9 | **1** | 5.7 |
| *A2/A10* | **1** | 1.4 | **0** | 2.9 |

**p = 0.388**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele

**Abbreviations: OCD:** obsessive-compulsive disorder**; MDD:** major depressive disorder

***Table III.41(b).*** *The allele counts and associated frequencies in the* DAT *40bp VNTR in OCD subjects presenting with co-morbid MDD (OCD+MDD) and those without co-morbid MDD (OCD-MDD).*

| Allele | OCD+MDD | | OCD-MDD | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10* | **98** | 70.0 | **56** | 80.0 |
| *A9* | **39** | 27.9 | **13** | 18.6 |
| *A11* | **2** | 1.4 | **1** | 1.4 |
| *A2* | **1** | 0.7 | **0** | 0.0 |

**p = 0.253**

*A2*= 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele

**Abbreviations: OCD:** obsessive-compulsive disorder**; MDD:** major depressive disorder

### III.4.4.2. Co-morbid tics

#### *III.4.4.2.1. Clinical variables*

Although more males did present with co-morbid tics compared to female OCD subjects (19.1% versus 8.5%), this difference did not reach statistical significance (p = 0.126) (Table III.7 and Figure III.41). In addition, no significant differences in total Y-BOCS score were observed (p = 0.476 [Table III.17]). Although the OCD subjects exhibiting co-morbid tics did experience, on average, earlier ages at onset than those subjects who were not diagnosed with co-morbid tic disorders, this difference was not statistically significant (median ages at onset=10 years [95% CI: 5.9-14.1] and 14 years [95% CI: 12.3-15.7], respectively; p=0.100 [Table III.27]).

The family history and symptom dimensions of OCD subjects with co-morbid tics (OCD+tics) and those without (OCD-tics) are presented in Table III.42. When the family history of OCD, OCS and tics was considered, no significant differences were observed with regard to the frequency of co-morbid tic disorders. When the frequency of co-morbid tics amongst patients with certain symptom dimensions was investigated, significantly more patients with contamination symptoms presented with tics, compared to those subjects without contamination symptoms (90.9% versus 57.5%, respectively; p=0.045).

#### *III.4.4.2.2. Single locus analysis according to the presence or absence of co-morbid tics*

Since the maximum number of OCD patients presenting with co-morbid tics amounted to 18 (i.e. 14% of the population), only those variants for which more than 60 subjects had been genotyped were utilised in the genetic analyses.

##### *III.4.4.2.2.1. Single locus analysis of bi-allelic polymorphisms*

The genotype and allele distributions of the selected variants in the OCD+tics and OCD-tics subsets are represented in Table III.43. The results of the association analyses between the two OCD subsets are represented in Table III.44(a), whilst those for the genetic analyses between the OCD+tics and control samples are presented in Table III.44(b) (the genotype and allele distributions of the control subjects are represented in Table II.3[b]).

***Table III.42.*** *Clinical characteristics in the OCD patient subset according to the presence (OCD+tics) or absence (OCD-tics) of tics.*

| Clinical Variable | OCD+tics | | OCD-tics | | p-value |
|---|---|---|---|---|---|
| **Family History**[a] | **n** | **%** | **n** | **%** | |
| **Family history of OCD** | **5** | 41.7 | **15** | 22.1 | 0.163 |
| **Family history of OCS** | **5** | 41.7 | 32 | 47.1 | 1.000 |
| **Family history of tics** | **2** | 16.7 | **4** | 5.9 | 0.219 |
| **Primary Symptom Dimensions**[b] | | | | | |
| **Hoarding** | **3** | 27.3 | **20** | 25.0 | 1.000 |
| **Symmetry/ ordering** | **7** | 63.6 | **47** | 58.8 | 1.000 |
| **Sexual/religious** | **5** | 45.5 | **33** | 41.3 | 1.000 |
| **Contamination** | **10** | 90.9 | **46** | 57.5 | **0.045** |
| **Aggression** | **6** | 54.5 | **41** | 51.3 | 1.000 |

[a]n(OCD+tics) =12, n(OCD-tics) =68; [b]n (OCD+tics)=11, n(OCD-tics) =80.

**Abbreviations: OCD:** Obsessive-compulsive disorder**; OCS:** Obsessive-compulsive symptoms

A statistically significant difference in genotype and allele distribution between the two OCD subgroups was observed for the *COMT val158met* (rs4680) polymorphism (p=0.026; OR=0.10 [95% CI: 0.00-0.86] and p = 0.004; OR = 0.30 [95% CI: 0.11-0.74]) (Table III.44[a]), with the *G (val158*)-allele assumed to confer protection against the development of co-morbid tics. When the genotype and allele frequencies between the OCD+tics and control samples were compared, differences in frequencies were also observed (p=0.006; OR=0.08 [95% CI: 0.01-0.70]) and p = 0.002; OR = 0.29 [95% CI: 0.12-0.65], respectively) (Table III.44[b]). Once again, the *G* (*val158*)-allele was found to be underrepresented amongst the OCD+tics group, and thus might be assumed to confer protection against the development of this subtype.

The *BDNF val66met* (rs6265) polymorphism was also found to exhibit significantly different genotype frequencies in the two OCD subsets under investigation (p = 0.035; OR = 0.13 [95% CI: 0.02-1.16]) (Table III.44[a]), with an increase in *AA* homozygotes in the OCD+tics group indicating that the possession of the *G*(*val66*)-allele confers protection against developing co-morbid tics. When the *BDNF val66met* allele frequencies were analysed, the frequency of the *A*(*met66*)-allele was greater amongst the OCD+tics subset, although this difference did not reach statistical significance (p=0.059; OR=0.45 [95% CI: 0.18-1.18]). When the allele and

genotype frequencies in the OCD+tics and control samples were compared, significant differences in both the allele and genotype distributions were observed (p=0.026; OR=0.40 [95% CI: 0.18-0.92] and p<0.001; OR=0.06 [95% CI: 0.01-0.39] for allele and genotype association analyses, respectively) (Table III.44[b]). Once again, the *G*(*val66*)-allele was found at a lower frequency in the OCD+tics group, and implying that it confers protection against the development of co-morbid tics.

No further significant differences in genotype or allele distributions were observed between the two OCD subsets, or between the OCD+tics subset and controls.

***III.4.4.2.2.2. Single locus analysis of multi-allelic polymorphisms***

***i. DRD4***

Tables III.45 (a) and (b) depicts the genotype and allele frequencies, respectively, in OCD patients presenting with co-morbid tic disorder and those without, for the *DRD4* 48bp VNTR polymorphism. When the two OCD subsets were analysed, no statistically significant differences in genotype (p = 0.427) or allele (p = 0.513) frequencies were detected

When the genotype and allele frequencies of the VNTR were compared between the OCD+tics and control samples, no statistically significant differences in distribution for either were observed (p = 0.224 and p = 0.292 for genotype and allele analyses, respectively ) (Tables III.4[a] and [b] and III.45[a] and [b]).

***ii. DAT***

The genotype and allele counts and frequencies for the DAT 40bp VNTR are presented in Tables 46(a) and (b), respectively. No statistically significant differences were observed for genotype or allele frequencies when the two OCD subsets were investigated (p = 0.747 and p = 0.900, respectively. Likewise, when the OCD+tics and control samples were compared, no significant distributions in *DAT* 40bp VNTR genotype or allele frequencies were observed (p = 0.550 and p = 0.527, respectively) (Tables III.5[a] and [b] and III.46[a] and [b]) .

***Table III.43*** *Genotype and allele scores and frequencies in bi-allelic candidate polymorphisms in OCD patients presenting with co-morbid tics (OCD+tics) and those without co-morbid tics (OCD-tics).*

| Gene | Variant | $n_1/n_2$[a] | OCD+tics | | | | | | | | | | | OCD-tics | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | **rs6311** | *G/A* | 9 | 50.0 | 6 | 33.3 | 3 | 16.7 | **18** | 24 | 66.7 | 12 | 33.3 | 34 | 38.6 | 39 | 44.3 | 15 | 17.0 | **88** | 107 | 60.8 | 69 | 39.2 |
| | **rs6313** | *C/T* | 8 | 44.4 | 7 | 38.9 | 3 | 16.7 | **18** | 23 | 63.9 | 13 | 36.1 | 36 | 42.4 | 32 | 37.6 | 17 | 20.0 | **85** | 104 | 61.2 | 66 | 38.8 |
| ***5-HT$_{1D\beta}$*** | **rs6296** | *G/C* | 13 | 72.2 | 4 | 22.2 | 1 | 5.6 | **18** | 30 | 83.3 | 6 | 16.7 | 39 | 48.1 | 36 | 44.4 | 6 | 7.4 | **81** | 114 | 70.4 | 48 | 29.6 |
| ***5-HT$_6$*** | **rs1805054** | *C/T* | 8 | 72.7 | 3 | 27.3 | 0 | 0.0 | **11** | 19 | 86.4 | 3 | 13.6 | 45 | 61.6 | 26 | 35.6 | 2 | 2.7 | **73** | 116 | 79.5 | 30 | 20.5 |
| ***DRD4*** | **rs1800955** | *T/C* | 5 | 33.3 | 7 | 46.7 | 3 | 20.0 | **15** | 17 | 56.7 | 13 | 43.3 | 25 | 33.8 | 38 | 51.4 | 11 | 14.9 | **74** | 88 | 59.5 | 60 | 40.5 |
| ***DRD2*** | **rs1800497** | *C/T* | 6 | 46.2 | 7 | 53.8 | 0 | 0.0 | **13** | 19 | 73.1 | 7 | 26.9 | 51 | 57.3 | 33 | 37.1 | 5 | 5.6 | **89** | 135 | 75.8 | 43 | 24.2 |
| ***COMT*** | **rs4680** | *G/A* | 1 | 5.9 | 6 | 35.3 | 10 | 58.8 | **17** | 8 | 23.5 | 26 | 76.5 | 14 | 19.7 | 44 | 62.0 | 13 | 18.3 | **71** | 72 | 50.7 | 70 | 49.3 |
| ***DRD3*** | **rs6280** | *A/G* | 5 | 41.7 | 3 | 25.0 | 4 | 33.3 | **12** | 13 | 54.2 | 11 | 45.8 | 48 | 57.8 | 27 | 32.5 | 8 | 9.6 | **83** | 123 | 74.1 | 43 | 25.9 |
| ***DRD1*** | ***A-48G*** | *A/G* | 5 | 33.3 | 9 | 60.0 | 1 | 6.7 | **15** | 19 | 63.3 | 11 | 36.7 | 34 | 40.5 | 40 | 47.6 | 10 | 11.9 | **84** | 108 | 64.3 | 60 | 35.7 |
| ***BDNF*** | **rs6265** | *G/A* | 8 | 53.3 | 4 | 26.7 | 3 | 20.0 | **15** | 20 | 66.7 | 10 | 33.3 | 63 | 66.3 | 29 | 30.5 | 3 | 3.2 | **95** | 155 | 81.6 | 35 | 18.4 |
| ***ESRα*** | **rs9340799** | *A/G* | 7 | 53.8 | 4 | 30.8 | 2 | 15.4 | **13** | 18 | 69.2 | 8 | 30.8 | 32 | 47.1 | 28 | 41.2 | 8 | 11.8 | **68** | 92 | 67.6 | 44 | 32.4 |
| | **rs2234693** | *T/C* | 5 | 41.7 | 5 | 41.7 | 2 | 16.7 | **12** | 15 | 62.5 | 9 | 37.5 | 14 | 28.6 | 24 | 49.0 | 11 | 22.4 | **49** | 52 | 53.1 | 46 | 46.9 |
| ***INPP-1*** | **rs1882891** | *C/A* | 12 | 80.0 | 3 | 20.0 | 0 | 0.0 | **15** | 27 | 90.0 | 3 | 10.0 | 58 | 72.5 | 21 | 26.3 | 1 | 1.3 | **80** | 137 | 85.6 | 23 | 14.4 |
| ***PLC-γ1*** | **rs8192707** | *A/G* | 10 | 71.4 | 4 | 28.6 | 0 | 0.0 | **14** | 24 | 85.7 | 4 | 14.3 | 45 | 59.2 | 26 | 34.2 | 5 | 6.6 | **76** | 116 | 76.3 | 36 | 23.7 |
| ***ACE*** | **Alu Ins/del** | *D/I* | 6 | 37.5 | 7 | 43.8 | 3 | 18.8 | **16** | 19 | 59.4 | 13 | 40.6 | 36 | 39.6 | 36 | 39.6 | 19 | 20.9 | **91** | 108 | 59.3 | 74 | 40.7 |

[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele. **Abbreviations: *5-HT$_{2A}$*:** serotonin receptor 2A**; *5-HT$_{1D\beta}$*:** serotonin receptor 1Dβ**; *5-HT$_6$*:** serotonin receptor 6**; *5-HT$_{2C}$*:** serotonin receptor 2C**;** ***DRD4*:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; COMT: Catechol-O-methyltransferase; DRD3: dopamine receptor 3; DRD1: dopamine receptor 1; GRIN2B: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.44(a).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects with co-morbid tics (OCD+tics) and those without co-morbid tics (OCD-tics) in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 1.000 | 1.32 | 0.27 | 8.63 | 0.050 | 0.575 | 1.29 | 0.58 | 3.02 | 0.067 |
| | rs6313 | *C/T* | 1.000 | 1.25 | 0.26 | 8.26 | 0.050 | 0.851 | 1.12 | 0.50 | 2.59 | 0.051 |
| ***5-HT$_{1D\beta}$*** | rs6296 | *G/C* | 1.000 | 1.98 | 0.21 | 99.00 | 0.052 | 0.148 | 2.10 | 0.79 | 6.57 | 0.215 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.552 | 0.72 | 0.04 | 14.58 | 0.064 | 0.573 | 1.63 | 0.44 | 9.18 | 0.067 |
| ***DRD4*** | rs1800955 | *T/C* | 0.695 | 0.74 | 0.12 | 5.60 | 0.050 | 0.840 | 0.89 | 0.38 | 2.16 | 0.051 |
| ***DRD2*** | rs1800497 | *C/T* | 0.445 | 1.39 | 0.07 | 28.11 | 0.050 | 0.808 | 0.87 | 0.32 | 2.61 | 0.050 |
| ***COMT*** | rs4680 | *G/A* | **0.026** | **0.10** | **0.00** | **0.86** | 0.445 | **0.004** | **0.30** | **0.11** | **0.74** | 0.663 |
| ***DRD3*** | rs6280 | *A/G* | 0.052 | 0.22 | 0.04 | 1.32 | 0.312 | 0.054 | 0.42 | 0.16 | 1.11 | 0.338 |
| ***DRD1*** | *A-48G* | *A/G* | 1.000 | 1.46 | 0.14 | 76.40 | 0.053 | 1.000 | 0.96 | 0.40 | 2.39 | 0.051 |
| ***BDNF*** | rs6265 | *G/A* | **0.035** | **0.13** | **0.02** | **1.16** | 0.414 | **0.059** | **0.45** | **0.18** | **1.18** | 0.291 |
| ***ESRα*** | rs9340799 | *A/G* | 1.000 | 0.88 | 0.13 | 10.20 | 0.057 | 1.000 | 1.08 | 0.41 | 3.09 | 0.050 |
| | rs2234693 | *T/C* | 0.670 | 1.92 | 0.25 | 23.90 | 0.057 | 0.495 | 1.47 | 0.54 | 4.20 | 0.079 |
| ***INPP-1*** | rs1882891 | *C/A* | 1.000 | 1.06 | 0.23 | 4.70 | 0.114 | 0.772 | 1.51 | 0.41 | 8.40 | 0.059 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.296 | 2.54 | 0.13 | 45.57 | 0.063 | 0.331 | 1.86 | 0.58 | 7.85 | 0.109 |
| ***ACE*** | *Alu* ins/del | *D/I* | 1.000 | 1.05 | 0.20 | 7.24 | 0.057 | 1.000 | 1.00 | 0.44 | 2.35 | 0.053 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.

**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1D\beta}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.44(b).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects with co-morbid tics (OCD+tics) and controls, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.904 | 1.09 | 0.27 | 4.43 | 0.173 | 0.815 | 1.09 | 0.53 | 2.23 | 0.098 |
| | rs6313 | *C/T* | 0.697 | 1.32 | 0.33 | 5.28 | 0.085 | 0.506 | 1.28 | 0.62 | 2.66 | 0.056 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.705 | 1.51 | 0.18 | 12.64 | 0.197 | 0.292 | 1.63 | 0.65 | 4.05 | 0.174 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.751 | 0.32 | 0.01 | 8.40 | 0.070 | 1.000 | 1.04 | 0.29 | 3.72 | 0.050 |
| ***DRD4*** | rs1800955 | *T/C* | 0.780 | 1.24 | 0.27 | 5.61 | 0.060 | 0.753 | 1.13 | 0.53 | 2.42 | 0.062 |
| ***DRD2*** | rs1800497 | *C/T* | 0.370 | 1.83 | 0.10 | 34.94 | 0.202 | 0.896 | 0.94 | 0.38 | 2.34 | 0.052 |
| ***COMT*** | rs4680 | *G/A* | **0.006** | **0.08** | **0.01** | **0.70** | 0.832 | **0.002** | **0.29** | **0.12** | **0.65** | 0.877 |
| ***DRD3*** | rs6280 | *A/G* | 0.249 | 0.44 | 0.11 | 1.83 | 0.423 | 0.354 | 0.67 | 0.29 | 1.56 | 0.153 |
| ***DRD1*** | *A-48G* | *A/G* | 0.559 | 1.92 | 0.21 | 17.54 | 0.141 | 0.816 | 1.10 | 0.50 | 2.40 | 0.055 |
| ***BDNF*** | rs6265 | *G/A* | **<0.001** | **0.06** | **0.01** | **0.39** | 0.791 | **0.026** | **0.40** | **0.18** | **0.92** | 0.604 |
| ***ESRα*** | rs9340799 | *A/G* | 0.719 | 1.35 | 0.26 | 7.01 | 0.154 | 0.532 | 1.32 | 0.55 | 3.14 | 0.094 |
| | rs2234693 | *T/C* | 0.299 | 2.42 | 0.44 | 13.43 | 0.219 | 0.260 | 1.64 | 0.69 | 3.89 | 0.251 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.645 | 0.56 | 0.03 | 12.36 | 0.064 | 0.555 | 0.77 | 0.25 | 2.38 | 0.053 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.461 | 1.29 | 0.06 | 25.63 | 0.113 | 0.435 | 1.55 | 0.51 | 4.68 | 0.122 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.217 | 0.38 | 0.08 | 1.74 | 0.175 | 0.268 | 0.66 | 0.31 | 1.39 | 0.193 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.45 (a).*** *Genotype frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with co-morbid tic disorder (OCD+tics) and those without co-morbid tic disorder (OCD-tics).*

| Genotype | OCD+tics | | OCD-tics | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A7* | **4** | 40.0 | **17** | 22.4 |
| *A4/A2* | **3** | 30.0 | **12** | 15.8 |
| *A4/A4* | **2** | 20.0 | **30** | 39.5 |
| *A7/A7* | **1** | 10.0 | **3** | 3.9 |
| *A2/A2* | **0** | 0.0 | **1** | 1.3 |
| *A3/A6* | **0** | 0.0 | **1** | 1.3 |
| *A4/A3* | **0** | 0.0 | **9** | 11.8 |
| *A7/A2* | **0** | 0.0 | **2** | 2.6 |
| *A7/A3* | **0** | 0.0 | **1** | 1.3 |

**p=0.427**

*A2*=2-repeat allele; *A9*=9-repeat allele; *A10*=10-repeat allele; *A11*=11-repeat allele

**Abbreviations**: OCD: Obsessive-compulsive disorder.

***Table III.45 (b).*** *Genotype frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with co-morbid tic disorder (OCD+tics) and those without co-morbid tic disorder (OCD-tics).*

| Allele | OCD+tics | | OCD-tics | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **11** | 55.0 | **98** | 64.5 |
| *A7* | **6** | 30.0 | **26** | 17.1 |
| *A2* | **3** | 15.0 | **16** | 10.5 |
| *A3* | **0** | 0.0 | **11** | 7.2 |
| *A6* | **0** | 0.0 | **1** | 0.7 |

**p = 0.513**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele

**Abbreviations**: OCD: Obsessive-compulsive disorder

***Table III.46 (a).*** *Genotype frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with co-morbid tic disorder (OCD+tics) and those without co-morbid tic disorder (OCD-tics).*

| Genotype | OCD+tics | | OCD-tics | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10/A10* | **11** | 61.1 | **46** | 0.5 |
| *A9/A10* | **5** | 27.8 | **31** | 0.4 |
| *A9/A9* | **1** | 5.6 | **7** | 0.1 |
| *A10/A11* | **1** | 5.6 | **2** | 0.0 |
| *A10/A2* | **0** | 0.0 | **1** | 0.0 |

**p = 0.747**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele

**Abbreviations**: OCD: Obsessive-compulsive disorder

***Table III.46(b).*** *Allele counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with co-morbid tic disorder (OCD+tics) and those without co-morbid tic disorder (OCD-tics).*

| Allele | OCD+tics | | OCD-tics | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10* | **28** | 77.8 | **126** | 72.4 |
| *A9* | **7** | 19.4 | **45** | 25.9 |
| *A11* | **1** | 2.8 | **2** | 1.1 |
| *A2* | **0** | 0.0 | **1** | 0.6 |

**p = 0.900**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele

**Abbreviations**: OCD: Obsessive-compulsive disorder

### III.4.5. Stratification by primary symptom dimension

The OCD population was also stratified according to primary symptom subtype. Clinical variables and genotype and allele frequencies were investigated in, and compared between, those OCD patients experiencing a particular symptom subtype, and those who did not experience the particular symptom subtype. The symptom subtypes that were investigated were:

i. Hoarding and collecting obsessions and/or compulsions
ii. Symmetry and ordering obsessions and/or compulsions
iii. Sexual/ religious obsessions and/or compulsions
iv. Contamination and washing obsessions and/or compulsions
v. Aggression obsessions and/or compulsions

#### *III.4.5.1. Hoarding symptoms*

##### *III.4.5.1.1. Analysis of clinical variables*

No statistically significant differences were observed in the frequency of hoarders between male and female subjects (p=0.091 [Table III.7]), although more females were found to present with the hoarding subtype (32.7% females versus 16.7% males). Moreover, no statistically significant differences were observed with regard to severity (total Y-BOCS score) (p=0.394 [Table III.17]) or age at onset (p=0.846 [Table III.27]).

When family history was investigated, those OCD patients experiencing hoarding obsessions and/or compulsions had more family members with OCD compared to those patients who did not experience hoarding symptoms (58.3% and 19.0%, respectively, p=0.008) (Table III.47). No statistically significant differences with regard to family history of OCS and family history of tics was observed (p=0.752 and p=0.266, respectively) (Table III.47). When the lifetime prevalence of co-morbid disorders was investigated, it was found that significantly more subjects with co-morbid SIB and BDD experienced hoarding symptomatology (p=0.034 and p=0.011, respectively) (Table III.47).

***Table III.47.*** *Clinical characteristics in the OCD patient subset according to the presence or absence of hoarding symptoms.*

| Family history[a] | HOARDING | | | | p-value |
|---|---|---|---|---|---|
| | Present | | Absent | | |
| | n | % | n | % | |
| Family history of OCD | **7** | 58.3 | **11** | 19.0 | **0.008** |
| Family history of OCS | **7** | 58.3 | **28** | 48.3 | 0.752 |
| Family history of tics | **2** | 16.7 | **4** | 6.9 | 0.266 |
| **Co-morbidity[b]** | | | | | |
| MDD | **15** | 65.2 | **44** | 64.7 | 0.965 |
| SIB | **6** | 26.1 | **6** | 8.8 | **0.034** |
| Dysthymia | **5** | 21.7 | **11** | 16.2 | 0.545 |
| OCD + tics | **3** | 13.0 | **8** | 11.8 | 0.871 |
| Specific phobia | **6** | 26.1 | **8** | 11.8 | 0.177 |
| GAD | **4** | 17.4 | **5** | 7.4 | 0.163 |
| Panic disorder | **3** | 13.0 | **6** | 8.8 | 0.558 |
| Social phobia | **2** | 8.7 | **6** | 8.8 | 0.985 |
| TTM | **2** | 8.7 | **4** | 5.9 | 0.638 |
| IED | **2** | 8.7 | **5** | 7.4 | 0.835 |
| BDD | **5** | 21.7 | **3** | 4.4 | **0.011** |
| TS | **2** | 8.7 | **2** | 2.9 | 0.245 |
| Anorexia | **1** | 4.3 | **4** | 5.9 | 0.78 |
| Hypochondriasis | **1** | 4.3 | **1** | 1.5 | 0.416 |

Statistically significant differences are indicated in red, bold font. [a] n(hoarding present) = 12, n(hoarding absent) = 58; [b] n(hoarding present) = 23, n(hoarding absent) = 68**.**

**Abbreviations:** **OCD:** Obsessive-compulsive disorder; **OCS**: obsessive-compulsive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome.

***III..4.5.1.2. Single locus analysis of hoarding symptomatology***

As for the genetic analyses in the previous section, only OCD subjects for whom more than 60 samples were genotyped per variant were included in the analyses.

***III.4.5.1.2.1. Single locus genetic investigation of bi-allelic polymorphisms***

For each candidate marker investigated, the genotype and allele counts and frequencies of OCD subjects experiencing hoarding symptoms, and those not, are presented in Table III.48. Control genotype and allele frequencies for each variant are presented in Table III.3b. The results from the association analyses, whereby the genotype and allele frequencies of individual markers were compared between hoarders and non-hoarders are presented in Table III.49(a), and between hoarders and control individuals in Table III.49(b).

The alleles of the *DRD4* -521*C/T* promoter polymorphism (rs1800955) were found to differ significantly in frequency between hoarders and non-hoarders (p=0.043), with an OR of 2.86 (95% CI: 0.99-9.53) indicating that the -*521T*-allele may represent a risk allele for developing hoarding symptoms (Table III.49[a]). A similar association was noted when the genotype and allele frequencies of the variant were compared between hoarders and controls (p = 0.047; OR = 6.69 [95% CI: 0.80-55.82] for the genotype analysis, and p = 0.011; OR = 3.17 [95% CI: 1.25-8.06] for the allele analysis) (Tables III.3[b], III.48 and III.49[b]). One should, however, not dismiss the relatively wide 95% CI that was obtained for the genotype analysis, indicating that chance could have played a factor in the observed result.

In addition, statistically significant differences in both genotypic and allelic frequencies between hoarders and non-hoarders were observed for the *COMT val158met* (rs4680) variant (p = 0.008; OR = 0.04 [95% CI: 0.00-0.86] and p = 0.006; OR = 0.29 [95% CI: 0.10-0.77], respectively). The OR of below zero suggests that the *G*(*val158*)-allele possesses a protective effect (Table III.49[a]). Indeed, when the genotype and allele frequencies were compared between the hoarding and control samples, the *G* (*val158*)-allele was once again found to confer protection against the development of hoarding obsessions and compulsions (p=0.004; OR=0.05 [95% CI: 0.00-0.90] for the genotype analysis, and p=0.004; OR=0.31 [95% CI: 0.13-0.71] for the allele analysis) (Tables III.3[b], III.48 and III.49[b]).

Interestingly, both of the *ESRα* variants investigated (rs9340799 and rs2234693) produced significant differences in genotypic frequency between OCD patients experiencing hoarding

symptoms and those not (p = 0.004 and p = 0.041, respectively) (Table III.49[a]). The distribution of alleles in rs9340799 was also found to differ significantly between hoarders and non-hoarders (p = 0.002) (Table III.49[a]). However, no significant association was noted when the genotype and allele frequencies of the two *ESRα* variants were compared between the hoarding and control samples (Table III.49[b]), indicating that the variants may play a role in the development of a characteristic specific to the non-hoarding OCD group. Indeed, when both of the variants were compared between the OCD *non*-hoarding and control samples, statistically significant differences in genotype and allele frequencies for both the *ESRα* rs9340799 and rs2234693 polymorphisms were generated (Table III.49[c]).

#### *III.4.5.1.2.2. Single locus analysis of multi-allelic loci*

##### *i. DRD4*

The genotype and allele frequencies and scores, respectively, for the hoarding and non-hoarding subsets are presented in Table III.50(a) and (b). No statistically significant differences were noted between hoarders and non-hoarders (p = 0.341 and p = 0.282 for genotype and allele analyses, respectively), or between hoarders and controls (p = 0.339 and p = 0.673 for genotype and allele analyses, respectively) (Tables III.4[a] and [b] and III.50[a] and [b]).

##### *ii. DAT*

No statistically significant differences in the genotypic or allelic distribution of the *DAT* 40bp VNTR were observed when OCD patients with hoarding symptoms were compared to those without hoarding symptoms (p = 0.833 and p = 0.796, respectively). (Tables III.51[a] and [b]). Likewise, no significant differences in the genotypic or allelic frequencies were observed when the distribution of the VNTR was compared between hoarders and controls (p = 0.634 and p = 0.604 for genotype and allele analyses, respectively) (Tables III.5[a] and [b] and III.51[a] and [b]).

***Table III.48.*** *Genotype and allele scores and frequencies in bi-allelic candidate polymorphisms in OCD patients presenting hoarding symptoms and those without.*

| Gene | Variant | $n_1/n_2$* | Hoarding | | | | | | | | | | | No hoarding | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 10 | 47.6 | 7 | 33.3 | 4 | 19.0 | **21** | 27 | 64.3 | 15 | 35.7 | 20 | 39.2 | 24 | 47.1 | 7 | 13.7 | **51** | 64 | 62.7 | 38 | 37.3 |
| | rs6313 | *C/T* | 11 | 52.4 | 7 | 33.3 | 3 | 14.3 | **21** | 29 | 69.0 | 13 | 31.0 | 21 | 42.9 | 20 | 40.8 | 8 | 16.3 | **49** | 62 | 63.3 | 36 | 36.7 |
| ***5-HT$_{1D\beta}$*** | rs6296 | *G/C* | 12 | 63.2 | 7 | 36.8 | 0 | 0.0 | **19** | 31 | 81.6 | 7 | 18.4 | 21 | 44.7 | 21 | 44.7 | 5 | 10.6 | **47** | 63 | 67.0 | 31 | 33.0 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 11 | 68.8 | 5 | 31.3 | 0 | 0.0 | **16** | 27 | 84.4 | 5 | 15.6 | 28 | 65.1 | 15 | 34.9 | 0 | 0.0 | **43** | 71 | 82.6 | 15 | 17.4 |
| ***DRD4*** | rs1800955 | *T/C* | 9 | 64.3 | 4 | 28.6 | 1 | 7.1 | **14** | 22 | 78.6 | 6 | 21.4 | 12 | 28.6 | 23 | 54.8 | 7 | 16.7 | **42** | 47 | 56.0 | 37 | 44.0 |
| ***DRD2*** | rs1800497 | *C/T* | 10 | 52.6 | 7 | 36.8 | 2 | 10.5 | **19** | 27 | 71.1 | 11 | 28.9 | 31 | 59.6 | 19 | 36.5 | 2 | 3.8 | **52** | 81 | 77.9 | 23 | 22.1 |
| ***COMT*** | rs4680 | *G/A* | 0 | 0.0 | 8 | 50.0 | 8 | 50.0 | **16** | 8 | 25.0 | 24 | 75.0 | 11 | 28.2 | 20 | 51.3 | 8 | 20.5 | **39** | 42 | 53.8 | 36 | 46.2 |
| ***DRD3*** | rs6280 | *A/G* | 10 | 52.6 | 6 | 31.6 | 3 | 15.8 | **19** | 26 | 68.4 | 12 | 31.6 | 28 | 57.1 | 16 | 32.7 | 5 | 10.2 | **49** | 72 | 73.5 | 26 | 26.5 |
| ***DRD1*** | *A-48G* | *A/G* | 7 | 35.0 | 9 | 45.0 | 4 | 20.0 | **20** | 23 | 57.5 | 17 | 42.5 | 19 | 41.3 | 25 | 54.3 | 2 | 4.3 | **46** | 63 | 68.5 | 29 | 31.5 |
| ***BDNF*** | rs6265 | *G/A* | 16 | 72.7 | 6 | 27.3 | 0 | 0.0 | **22** | 38 | 86.4 | 6 | 13.6 | 33 | 62.3 | 16 | 30.2 | 4 | 7.5 | **53** | 82 | 77.4 | 24 | 22.6 |
| ***ESRα*** | rs9340799 | *A/G* | 2 | 13.3 | 10 | 66.7 | 3 | 20.0 | **15** | 14 | 46.7 | 16 | 53.3 | 20 | 60.6 | 13 | 39.4 | 0 | 0.0 | **33** | 53 | 80.3 | 13 | 19.7 |
| | rs2234693 | *T/C* | 1 | 10.0 | 6 | 60.0 | 3 | 30.0 | **10** | 8 | 40.0 | 12 | 60.0 | 9 | 40.9 | 12 | 54.5 | 1 | 4.5 | **22** | 30 | 68.2 | 14 | 31.8 |
| ***INPP-1*** | rs1882891 | *C/A* | 16 | 84.2 | 3 | 15.8 | 0 | 0.0 | **19** | 35 | 92.1 | 3 | 7.9 | 32 | 69.6 | 13 | 28.3 | 1 | 2.2 | **46** | 77 | 83.7 | 15 | 16.3 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 13 | 72.2 | 3 | 16.7 | 2 | 11.1 | **18** | 29 | 80.6 | 7 | 19.4 | 25 | 56.8 | 17 | 38.6 | 2 | 4.5 | **44** | 67 | 76.1 | 21 | 23.9 |
| ***ACE*** | Ins/del | *D/I* | 10 | 50.0 | 7 | 35.0 | 3 | 15.0 | **20** | 27 | 67.5 | 13 | 32.5 | 22 | 40.7 | 21 | 38.9 | 11 | 20.4 | **54** | 65 | 60.2 | 43 | 39.8 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele. **Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1D\beta}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; COMT: Catechol-O-methyltransferase; DRD3: dopamine receptor 3; DRD1: dopamine receptor 1; GRIN2B: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.49(a).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects with hoarding symptoms and those without, indicating corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 1.000 | 0.88 | 0.17 | 5.09 | 0.053 | 1.000 | 1.07 | 0.48 | 2.45 | 0.050 |
| | rs6313 | *C/T* | 1.000 | 1.39 | 0.26 | 9.75 | 0.050 | 0.566 | 1.29 | 0.57 | 3.07 | 0.067 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.103 | 6.39 | 0.32 | 125.64 | 0.154 | 0.094 | 2.18 | 0.86 | 5.50 | 0.238 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 1.000 | 0.40 | 0.01 | 21.60 | 0.054 | 1.000 | 1.14 | 0.38 | 4.40 | 0.056 |
| ***DRD4*** | rs1800955 | *T/C* | 0.124 | 5.00 | 0.49 | 50.64 | 0.152 | **0.043** | **2.86** | **0.99** | **9.53** | **0.382** |
| ***DRD2*** | rs1800497 | *C/T* | 0.286 | 0.33 | 0.02 | 5.13 | 0.070 | 0.505 | 0.70 | 0.28 | 1.80 | 0.080 |
| ***COMT*** | rs4680 | *G/A* | **0.008** | **0.04** | **0.00** | **0.86** | **0.553** | **0.006** | **0.29** | **0.10** | **0.77** | **0.621** |
| ***DRD3*** | rs6280 | *A/G* | 0.669 | 0.60 | 0.10 | 4.59 | 0.053 | 0.670 | 0.78 | 0.32 | 1.96 | 0.061 |
| ***DRD1*** | *A-48G* | *A/G* | 0.148 | 0.20 | 0.01 | 1.71 | 0.214 | 0.239 | 0.63 | 0.27 | 1.45 | 0.136 |
| ***BDNF*** | rs6265 | *G/A* | 0.303 | 4.43 | 0.29 | 87.33 | 0.102 | 0.265 | 1.85 | 0.66 | 5.99 | 0.138 |
| ***ESRα*** | rs9340799 | *A/G* | **0.004** | **0.00** | **0.00** | **0.45** | **0.748** | **0.002** | **0.22** | **0.08** | **0.61** | **0.795** |
| | rs2234693 | *T/C* | **0.041** | **0.06** | **0.00** | **1.29** | **0.337** | 0.054 | 0.32 | 0.09 | 1.06 | 0.363 |
| ***INPP-1*** | rs1882891 | *C/A* | 1.000 | 1.52 | 0.01 | 39.50 | 0.061 | 0.271 | 2.26 | 0.59 | 13.00 | 0.130 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.608 | 0.53 | 0.03 | 8.07 | 0.051 | 0.645 | 1.30 | 0.46 | 4.02 | 0.057 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.724 | 1.65 | 0.33 | 11.20 | 0.058 | 0.451 | 1.37 | 0.60 | 3.23 | 0.081 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.

**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.49(b).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects with hoarding symptoms and controls, indicating corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.555 | 1.50 | 0.39 | 5.80 | 0.06 | 0.366 | 1.37 | 0.69 | 2.74 | 0.07 |
| | rs6313 | *C/T* | 0.885 | 1.10 | 0.31 | 3.82 | 0.07 | 0.678 | 1.15 | 0.59 | 2.26 | 0.11 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.242 | 3.01 | 0.17 | 54.29 | 0.12 | 0.402 | 3.01 | 0.61 | 3.40 | 0.19 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.711 | 0.43 | 0.02 | 11.16 | 0.06 | 0.789 | 0.89 | 0.32 | 2.47 | 0.11 |
| ***DRD4*** | rs1800955 | *T/C* | **0.047** | **6.69** | **0.80** | **55.82** | **0.72** | **0.011** | **3.17** | **1.25** | **8.06** | **0.67** |
| ***COMT*** | rs4680 | *G/A* | **0.004** | **0.05** | **0.00** | **0.90** | **0.76** | **0.004** | **0.31** | **0.13** | **0.71** | **0.82** |
| ***DRD3*** | rs6280 | *A/G* | 0.819 | 1.18 | 0.29 | 4.76 | 0.08 | 0.576 | 1.23 | 0.59 | 2.56 | 0.08 |
| ***DRD1*** | *A-48G* | *A/G* | 0.557 | 0.67 | 0.18 | 2.57 | 0.05 | 0.659 | 0.86 | 0.44 | 1.69 | 0.08 |
| ***BDNF*** | rs6265 | *G/A* | 0.562 | 0.86 | 0.04 | 18.82 | 0.07 | 0.599 | 1.28 | 0.51 | 3.19 | 0.08 |
| ***ESRα*** | rs9340799 | *A/G* | 0.127 | 0.26 | 0.04 | 1.65 | 0.63 | 0.081 | 1.51 | 0.24 | 1.10 | 0.42 |
| | rs2234693 | *T/C* | 0.317 | 0.32 | 0.03 | 3.27 | 0.15 | 0.371 | 0.66 | 0.26 | 1.66 | 0.11 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.561 | 0.87 | 0.04 | 19.02 | 0.05 | 0.781 | 1.49 | 0.43 | 5.19 | 0.08 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.243 | 0.36 | 0.06 | 2.15 | 0.05 | 0.882 | 1.07 | 0.44 | 2.59 | 0.05 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.531 | 0.64 | 0.15 | 2.65 | 0.05 | 0.845 | 0.93 | 0.46 | 1.90 | 0.05 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI. **Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*:** serotonin receptor 1Dβ**;** ***5-HT$_6$*:** serotonin receptor 6**;** ***DRD4*:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** ***DRD3***: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.49(c).*** *Association analysis investigating the differences in genotype and allele distributions in the* ESRα *rs9340799 and rs2234693 polymorphisms between OCD subjects without hoarding symptoms and controls, indicating corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | Allele | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | p-value | OR[c] | 95% CI | |
| | | | | | LB[d] | UB[e] | | | LB[d] | UB[e] |
| ***ESRα*** | rs9340799 | *A/G* | **<0.001** | **0.02** | **0.00** | **0.45** | **<0.001** | **0.22** | **0.08** | **0.55** |
| | rs2234693 | *T/C* | **0.016** | **0.04** | **0.002** | **0.80** | **0.033** | **0.31** | **0.10** | **0.93** |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI. **Abbreviations:** ***ESRα***: estrogen receptor α.

***Table III.50(a).*** *Genotype counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with hoarding symptoms and those without.*

| Genotype | Hoarding | | No Hoarding | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A4* | **7** | 43.8 | **17** | 40.5 |
| *A4/A7* | **5** | 31.3 | **8** | 19.0 |
| *A4/A2* | **4** | 25.0 | **5** | 11.9 |
| *A2/A2* | **0** | 0.0 | **1** | 2.4 |
| *A3/A6* | **0** | 0.0 | **1** | 2.4 |
| *A4/A3* | **0** | 0.0 | **6** | 14.3 |
| *A7/A2* | **0** | 0.0 | **1** | 2.4 |
| *A7/A3* | **0** | 0.0 | **1** | 2.4 |
| *A7/A7* | **0** | 0.0 | **2** | 4.8 |

**p = 0.341**

*A2* = 2-repeat allele; *A3* = 3-repeat allele; *A4* = 4-repeat allele; *A6* = 6-repeat allele; *A7* = 7-repeat allele.

***Table III.50(b).*** *Allele counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with hoarding symptoms and those without.*

| Allele | Hoarding | | No Hoarding | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **23** | 71.9 | **53** | 63.1 |
| *A7* | **5** | 15.6 | **14** | 16.7 |
| *A2* | **4** | 12.5 | **8** | 9.5 |
| *A3* | **0** | 0.0 | **8** | 9.5 |
| *A6* | **0** | 0.0 | **1** | 1.2 |

**p = 0.282**

*A2* = 2-repeat allele; *A3* = 3-repeat allele; *A4* = 4-repeat allele; *A6* = 6-repeatallele; *A7* = 7-repeat allele.

***Table III.51(a).*** *Genotype counts and frequencies in the DAT 40bp VNTR polymorphism for OCD patients presenting with hoarding symptoms and those without.*

| Genotype | Hoarding | | No Hoarding | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10/A10* | **10** | 47.6 | **23** | 46.9 |
| *A9/A10* | **9** | 42.9 | **18** | 36.7 |
| *A9/A9* | **2** | 9.5 | **5** | 10.2 |
| *A10/A11* | **0** | 0.0 | **2** | 4.1 |
| *A2/A10* | **0** | 0.0 | **1** | 2.0 |

**p=0.833**
*A2*=2-repeat allele; *A9*=9-repeat allele; *A10*=10-repeat allele; *A11*=11-repeat allele.

***Table III.51(b).*** *Allele counts and frequencies in the DAT 40bp VNTR polymorphism for OCD patients presenting with hoarding symptoms and those without.*

| Allele | Hoarding | | No Hoarding | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10* | **29** | 69.0 | **67** | 68.4 |
| *A9* | **13** | 31.0 | **28** | 28.6 |
| *A2* | **0** | 0.0 | **1** | 1.0 |
| *A11* | **0** | 0.0 | **2** | 2.0 |

**p=0.796**

*A2*=2-repeat allele; *A9*=9-repeat allele; *A10*=10-repeat allele; *A11*=11-repeat allele.

### *III.4.5.2. Symmetry and ordering symptoms*

#### *III.4.5.2.1. Analysis of clinical variables*

No statistically significant differences between male and female OCD subjects were noted in the frequency of symmetry/ordering obsessions and compulsions (p = 0.530 [Table III.7]; Figure III.40). In addition, no differences in Y-BOCS (p = 0.156 [Table III.17]) or age at onset (p = 0.570 [Table III.27]) were observed between those subjects presenting with symmetry/ordering symptoms and those that did not.

A comparison of clinical variables (family history of OCD, OCS and tics, and the prevalence of selected co-morbid disorders) is presented in Table III.52. Those OCD patients with a positive family history of tics were found to experience significantly more symmetry/ordering symptoms than those without a family history of tics (2.3% versus 17.9%, p = 0.032). Investigating the prevalence of certain co-morbid disorders, fewer of the OCD subjects who also experienced symmetry/ordering symptom dimensions were diagnosed with co-morbid social phobia (3.7%), compared to those patients who did not present with symmetry/ordering symptoms (18.4%) (p = 0.019) (Table III.52). There were no other statistically significant differences in the prevalence of the remaining co-morbid disorders.

#### *III.4.5.2.2. Single locus analysis of hoarding behaviour symmetry/ordering symptomatology*

##### *III.4.5.2.2.1. Single locus genetic investigation of bi-allelic polymorphisms*

The genotypic and allele counts and frequencies of those OCD patients experiencing symmetry/ordering symptoms, and those who did not, are presented in Table III.53. No statistically significant differences in genotypic or allelic frequencies were observed between the two OCD subsets (Table III.54[a]). A marginally significant difference in the *ACE Alu* ins/del genotype distribution was observed when the symmetry/ordering OCD subset was compared to controls (p = 0.048; OR = 0.38 [95% CI: 0.14-1.01]) (Table III.3[b], Table III.53 and Table III.54[b]). Here, an increased number of individuals carrying at least one *D*-allele were observed in the control sample compared to OCD subset (91.0% and 78.8%), suggesting that this genotype may represent a protective factor against the development of the symmetry/ordering symptom subtype, probably in a dominant manner.

### *III.4.5.2.2.2. Single locus analysis of multi-allelic polymorphisms*

#### *i. DRD4*

The genotype and allele scores and frequencies in OCD patients experiencing symmetry/ordering and those not are presented in Table III.55(a) and (b) respectively. No statistically significant differences in either genotype or allele frequencies were observed ($p = 0.066$ and $p = 0.726$ for genotype and allele analyses, respectively). Likewise, no statistically significant differences in genotype or allele frequencies were noted between the symmetry/ordering OCD group and control subjects ($p = 0.067$ and $p = 0.885$ for genotype and allele analyses, respectively.

#### *ii. DAT*

The genotype and allele scores and frequencies, respectively, in OCD patients experiencing symmetry/ordering and those not are presented in Tables III.56(a) and (b). No statistically significant differences in the genotype or allele distributions between OCD patients with symmetry/ordering obsessions and compulsions and those without were observed ($p = 0.496$ and $p = 0.811$, respectively). Also, no statistically significant differences were observed when the *DAT* VNTR genotype and allele frequencies were compared between the symmetry/ordering OCD subset and control subjects ($p = 0.576$ and $p = 0.302$ for genotype and allele analyses, respectively.

***Table III.52.*** *Clinical characteristics in the OCD patient subset according to the presence or absence of symmetry/ ordering symptoms.*

| Clinical Variable | SYMMETRY / ORDERING | | | | p-value |
|---|---|---|---|---|---|
| | Present | | Absent | | |
| **Family history**[a] | **n** | **%** | **n** | **%** | |
| Family history of OCD | **11** | 25.6 | **7** | 25.0 | 0.592 |
| Family history of OCS | **22** | 51.2 | **13** | 46.4 | 1.000 |
| Family history of tics | **1** | 2.3 | **5** | 17.9 | **0.032** |
| **Co-morbidity**[b] | | | | | |
| MDD | **37** | 68.5 | **23** | 60.5 | 0.428 |
| SIB | **6** | 11.1 | **7** | 18.4 | 0.322 |
| Dysthymia | **12** | 22.2 | **4** | 10.5 | 0.145 |
| OCD + tics | **7** | 13.0 | **4** | 10.5 | 0.723 |
| Specific phobia | **6** | 11.1 | **8** | 21.1 | 0.191 |
| GAD | **5** | 9.3 | **4** | 10.5 | 0.840 |
| Panic disorder | **7** | 13.0 | **2** | 5.3 | 0.221 |
| Social phobia | **2** | 3.7 | **7** | 18.4 | **0.019** |
| TTM | **4** | 7.4 | **3** | 7.9 | 0.931 |
| IED | **6** | 11.1 | **2** | 5.3 | 0.327 |
| BDD | **5** | 9.3 | **3** | 7.9 | 0.819 |
| TS | **2** | 3.7 | **2** | 5.3 | 0.718 |
| Anorexia | **5** | 9.3 | **0** | 0.0 | 0.054 |
| Hypochondriasis | **0** | 0.0 | **2** | 5.3 | 0.088 |

Statistically significant differences are indicated in red, bold font.
[a] n(symmetry/ordering present) = 43, n(symmetry/ordering absent) = 28; [b] n(symmetry/ordering present) = 54, n(hoarding absent) = 38**.**
**Abbreviations: OCD:** Obsessive-compulsive disorder; **OCS**: obsessive-compulsive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome.

***Table III.53.*** *Genotype and allele scores and frequencies in bi-allelic candidate polymorphisms in OCD patients presenting with symmetry/ordering symptoms and those without.*

| Gene | Variant | $n_1/n_2$[a] | Symmetry/ordering symptoms | | | | | | | | | | | No symmetry/ordering symptoms | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 20 | 45.5 | 18 | 40.9 | 6 | 13.6 | **44** | 58 | 65.9 | 30 | 34.1 | 10 | 34.5 | 14 | 48.3 | 5 | 17.2 | **29** | 34 | 58.6 | 24 | 41.4 |
| | rs6313 | *C/T* | 21 | 48.8 | 16 | 37.2 | 6 | 14.0 | **43** | 58 | 67.4 | 28 | 32.6 | 11 | 39.3 | 12 | 42.9 | 5 | 17.9 | **28** | 34 | 60.7 | 22 | 39.3 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 22 | 55.0 | 16 | 40.0 | 2 | 5.0 | **40** | 60 | 75.0 | 20 | 25.0 | 11 | 40.7 | 13 | 48.1 | 3 | 11.1 | **27** | 35 | 64.8 | 19 | 35.2 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 25 | 64.1 | 14 | 35.9 | 0 | 0.0 | **39** | 64 | 82.1 | 14 | 17.9 | 14 | 66.7 | 7 | 33.3 | 0 | 0.0 | **21** | 35 | 83.3 | 7 | 16.7 |
| ***DRD4*** | rs1800955 | *T/C* | 14 | 41.2 | 15 | 44.1 | 5 | 14.7 | **34** | 43 | 63.2 | 25 | 36.8 | 7 | 30.4 | 13 | 56.5 | 3 | 13.0 | **23** | 27 | 58.7 | 19 | 41.3 |
| ***DRD2*** | rs1800497 | *C/T* | 27 | 58.7 | 17 | 37.0 | 2 | 4.3 | **46** | 71 | 77.2 | 21 | 22.8 | 15 | 57.7 | 9 | 34.6 | 2 | 7.7 | **26** | 39 | 75.0 | 13 | 25.0 |
| ***COMT*** | rs4680 | *A/G* | 7 | 21.9 | 19 | 59.4 | 6 | 18.8 | **32** | 33 | 51.6 | 31 | 48.4 | 9 | 37.5 | 10 | 41.7 | 5 | 20.8 | **24** | 28 | 58.3 | 20 | 41.7 |
| ***DRD3*** | rs6280 | *A/G* | 28 | 60.9 | 13 | 28.3 | 5 | 10.9 | **46** | 69 | 75.0 | 23 | 25.0 | 11 | 47.8 | 9 | 39.1 | 3 | 13.0 | **23** | 31 | 67.4 | 15 | 32.6 |
| ***DRD1*** | A-48G | *A/G* | 14 | 35.0 | 20 | 50.0 | 6 | 15.0 | **40** | 48 | 60.0 | 32 | 40.0 | 12 | 44.4 | 15 | 55.6 | 0 | 0.0 | **27** | 39 | 72.2 | 15 | 27.8 |
| ***BDNF*** | rs6265 | *G/A* | 31 | 66.0 | 14 | 29.8 | 2 | 4.3 | **47** | 76 | 80.9 | 18 | 19.1 | 19 | 65.5 | 8 | 27.6 | 2 | 6.9 | **29** | 46 | 79.3 | 12 | 20.7 |
| ***ESRα*** | rs9340799 | *A/G* | 14 | 46.7 | 14 | 46.7 | 2 | 6.7 | **30** | 42 | 70.0 | 18 | 30.0 | 8 | 42.1 | 9 | 47.4 | 2 | 10.5 | **19** | 25 | 65.8 | 13 | 34.2 |
| | rs2234693 | *T/C* | 5 | 23.8 | 12 | 57.1 | 4 | 19.0 | **21** | 22 | 52.4 | 20 | 47.6 | 5 | 41.7 | 6 | 50.0 | 1 | 8.3 | **12** | 16 | 66.7 | 8 | 33.3 |
| ***INPP-1*** | rs1882891 | *C/A* | 31 | 73.8 | 10 | 23.8 | 1 | 2.4 | **42** | 72 | 85.7 | 12 | 14.3 | 18 | 75.0 | 6 | 25.0 | 0 | 0.0 | **24** | 42 | 87.5 | 6 | 12.5 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 22 | 55.0 | 16 | 40.0 | 2 | 5.0 | **40** | 60 | 75.0 | 20 | 25.0 | 17 | 73.9 | 4 | 17.4 | 2 | 8.7 | **23** | 38 | 82.6 | 8 | 17.4 |
| ***ACE*** | *Alu* Ins/del | *D/I* | 20 | 42.6 | 17 | 36.2 | 10 | 21.3 | **47** | 57 | 60.6 | 37 | 39.4 | 12 | 42.9 | 11 | 39.3 | 5 | 17.9 | **28** | 35 | 62.5 | 21 | 37.5 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele.

**Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; COMT: Catechol-O-methyltransferase; DRD3: dopamine receptor 3; DRD1: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.54(a).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects with symmetry/ordering symptoms and those without, indicating corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.491 | 1.65 | 0.31 | 8.42 | 0.059 | 0.387 | 1.36 | 0.65 | 2.85 | 0.091 |
| | rs6313 | *C/T* | 0.719 | 1.57 | 0.31 | 7.89 | 0.057 | 0.473 | 1.34 | 0.62 | 2.86 | 0.082 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.337 | 2.91 | 0.29 | 39.70 | 0.084 | 0.246 | 1.62 | 0.71 | 3.70 | 0.148 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 1.000 | 1.76 | 0.03 | 93.42 | 0.052 | 1.000 | 0.92 | 0.29 | 2.70 | 0.054 |
| ***DRD4*** | rs1800955 | *T/C* | 1.000 | 1.19 | 0.14 | 8.49 | 0.054 | 0.696 | 1.21 | 0.52 | 2.79 | 0.057 |
| ***DRD2*** | rs1800497 | *C/T* | 0.619 | 1.78 | 0.12 | 26.80 | 0.050 | 0.839 | 1.13 | 0.46 | 2.66 | 0.051 |
| ***COMT*** | rs4680 | *A/G* | 0.704 | 0.66 | 0.11 | 3.90 | 0.052 | 0.566 | 0.76 | 0.33 | 1.73 | 0.071 |
| ***DRD3*** | rs6280 | *A/G* | 0.680 | 1.51 | 0.20 | 9.44 | 0.051 | 0.419 | 1.45 | 0.61 | 3.36 | 0.094 |
| ***DRD1*** | *A-48G* | *A/G* | 0.061 | 0.00 | 0.00 | 1.25 | 0.291 | 0.196 | 0.58 | 0.25 | 1.29 | 0.189 |
| ***BDNF*** | rs6265 | *G/A* | 0.638 | 1.62 | 0.11 | 24.00 | 0.050 | 0.836 | 1.10 | 0.44 | 2.67 | 0.050 |
| ***ESRα*** | rs9340799 | *A/G* | 0.625 | 1.71 | 0.11 | 28.00 | 0.050 | 0.663 | 1.21 | 0.46 | 3.14 | 0.053 |
| | rs2234693 | *T/C* | 0.580 | 0.27 | 0.00 | 4.25 | 0.074 | 0.308 | 0.56 | 0.17 | 1.74 | 0.111 |
| ***INPP-1*** | rs1882891 | *C/A* | 1.000 | 0.00 | 0.00 | 69.30 | 0.057 | 1.000 | 0.86 | 0.25 | 2.69 | 0.050 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 1.000 | 1.29 | 0.09 | 19.40 | 0.056 | 0.379 | 0.63 | 0.22 | 1.69 | 0.097 |
| ***ACE*** | *Alu* ins/del | *D/I* | 1.000 | 0.84 | 0.18 | 3.54 | 0.050 | 0.864 | 0.93 | 0.44 | 1.92 | 0.050 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.54(b).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects with symmetry/ordering symptoms and controls, indicating corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.467 | 1.46 | 0.524 | 4.08 | 0.108 | 0.401 | 1.24 | 0.751 | 2.04 | 0.135 |
| | rs6313 | *C/T* | 0.485 | 1.43 | 0.521 | 3.94 | 0.109 | 0.342 | 1.28 | 0.77 | 2.11 | 0.137 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.763 | 1.27 | 0.26 | 6.16 | 0.051 | 0.932 | 0.98 | 0.56 | 1.72 | 0.051 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.576 | 0.95 | 0.04 | 24.06 | 0.105 | 0.413 | 0.75 | 0.38 | 1.50 | 0.131 |
| ***DRD4*** | rs1800955 | *T/C* | 0.197 | 2.08 | 0.67 | 6.44 | 0.217 | 0.155 | 1.49 | 0.86 | 2.57 | 0.282 |
| ***DRD2*** | rs1800497 | *C/T* | 0.450 | 1.82 | 0.38 | 8.86 | 0.080 | 0.576 | 1.17 | 0.67 | 2.05 | 0.093 |
| ***COMT*** | rs4680 | *G/A* | 0.597 | 0.72 | 0.22 | 2.41 | 0.075 | 0.615 | 0.87 | 0.34 | 1.01 | 0.088 |
| ***DRD3*** | rs6280 | *A/G* | 0.216 | 1.98 | 0.66 | 5.90 | 0.381 | 0.051 | 1.71 | 0.99 | 2.93 | 0.479 |
| ***DRD1*** | *A-48G* | *A/G* | 0.841 | 0.89 | 0.30 | 2.68 | 0.052 | 0.853 | 0.95 | 0.57 | 1.59 | 0.052 |
| ***BDNF*** | rs6265 | *G/A* | 0.250 | 0.33 | 0.04 | 2.42 | 0.650 | 0.601 | 0.85 | 0.47 | 1.56 | 0.073 |
| ***ESRα*** | rs9340799 | *A/G* | 0.198 | 2.70 | 0.57 | 12.87 | 0.134 | 0.310 | 1.37 | 0.75 | 2.50 | 0.172 |
| | rs2234693 | *T/C* | 0.789 | 1.21 | 0.30 | 4.93 | 0.065 | 0.817 | 1.08 | 0.56 | 2.09 | 0.072 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.735 | 0.66 | 0.06 | 7.53 | 0.092 | 0.478 | 0.77 | 0.37 | 1.59 | 0.113 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.570 | 0.60 | 0.10 | 3.51 | 0.095 | 0.400 | 0.77 | 0.43 | 1.41 | 0.116 |
| ***ACE*** | *Alu* ins/del | *D/I* | **0.048** | **0.38** | **0.14** | **1.01** | **0.226** | 0.137 | 0.70 | 0.42 | 1.13 | 0.294 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.55(a).*** *Genotype counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with symmetry/ordering symptoms and those without.*

| Genotype | Symmetry/ordering | | No Symmetry/ordering | |
|---|---|---|---|---|
| | n | % | n | % |
| *A2/A2* | **0** | 0.0 | **1** | 3.7 |
| *A3/A6* | **0** | 0.0 | **1** | 3.7 |
| *A4/A2* | **4** | 12.5 | **5** | 18.5 |
| *A4/A3* | **4** | 12.5 | **2** | 7.4 |
| *A4/A4* | **11** | 34.4 | **13** | 48.1 |
| *A4/A7* | **12** | 37.5 | **2** | 7.4 |
| *A7/A2* | **1** | 3.1 | **0** | 0.0 |
| *A7/A3* | **0** | 0.0 | **1** | 3.7 |
| *A7/A7* | **0** | 0.0 | **2** | 7.4 |

**p = 0.066**

*A2* = 2-repeat allele; *A3* = 3-repeat allele; *A4* = 4-repeat allele; *A6* = 6-repeat allele; *A7* = 7-repeat allele.

***Table III.55(b).*** *Allele counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with symmetry/ordering symptoms and those without.*

| Allele | Symmetry/ordering | | No Symmetry/ordering | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **42** | 65.6 | **35** | 64.8 |
| *A7* | **13** | 20.3 | **7** | 13.0 |
| *A2* | **5** | 7.8 | **7** | 13.0 |
| *A3* | **4** | 6.3 | **4** | 7.4 |
| *A6* | **0** | 0.0 | **1** | 1.9 |

**p = 0.726**

*A2* = 2-repeat allele; *A3* = 3-repeat allele; *A4* = 4-repeat allele; *A6* = 6-repeat allele; *A7* = 7-repeat allele.

***Table III.56(a).*** *Genotype counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with symmetry/ordering symptoms and those without.*

| Genotype | Symmetry/ordering | | No symmetry ordering | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10/A10* | **20** | 47.6 | **14** | 48.3 |
| *A9/A10* | **15** | 35.7 | **12** | 41.4 |
| *A9/A9* | **6** | 14.3 | **1** | 3.4 |
| *A10/A11* | **1** | 2.4 | **1** | 3.4 |
| *A10/A2* | **0** | 0.0 | **1** | 3.4 |

**p = 0.496**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele

***Table III.56(b).*** *Allele counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with symmetry/ordering symptoms and those without.*

| Allele | Symmetry/ordering | | No symmetry ordering | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10* | **56** | 66.7 | **42** | 72.4 |
| *A9* | **27** | 32.1 | **14** | 24.1 |
| *A11* | **1** | 1.2 | **1** | 1.7 |
| *A2* | **0** | 0.0 | **1** | 1.7 |

**p = 0.811.**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele.

### *III.4.5.3. Sexual and religious symptoms*

#### *III.4.5.3.1. Analysis of clinical variables*

When the frequency of sexual/religious symptoms was compared between male and female OCD subjects, significantly more males were found to experience this group of symptoms (57.1% versus 28.6%; p = 0.010 [Table III.7). No significant differences in median Y-BOCS score (Table III.17) or age at onset of OCD (Table III.27) were observed between those patients experiencing the symptoms and those not (p = 0.266 and 0.236, respectively).

Table III.57 shows the comparison of clinical variables between OCD subjects experiencing sexual/religious symptoms and those not. No significant differences in proportion of family members suffering from OCD, OCS or tics was noted. However, when the proportion of patients diagnosed with selected co-morbid disorders was investigated, statistically significant differences were observed for MDD (p = 0.017), dysthymic disorder (p = 0.016) and BDD (p = 0.019). Significantly more OCD patients with sexual and religious symptoms were diagnosed with co-morbid MDD and dysthymic disorder compared to those OCD subjects who did not exhibit sexual/religious symptomatology (78.9% versus 54.7% and 28.9% versus 9.4%, respectively). On the other hand, a greater proportion of OCD subjects *without* sexual/religious symptoms were diagnosed with BDD compared to those with sexual/religious symptoms (15.1% versus 0%, respectively).

***Table III.57.*** *Clinical characteristics in the OCD patient subset according to the presence or absence of sexual/religious symptoms.*

| Clinical Variable | Sexual/religious symptoms | | | | p-value |
|---|---|---|---|---|---|
| | Present | | Absent | | |
| **Family history**[a] | **n** | **%** | **n** | **%** | |
| Family history of OCD | **5** | 17.2 | **13** | 31.0 | 0.269 |
| Family history of OCS | **12** | 41.4 | **23** | 54.8 | 0.332 |
| Family history of tics | **4** | 13.8 | **2** | 4.8 | 0.218 |
| **Co-morbidity**[b] | | | | | |
| MDD | **30** | 78.9 | **29** | 54.7 | **0.017** |
| SIB | **4** | 10.5 | **8** | 15.1 | 0.755 |
| Dysthymia | **11** | 28.9 | **5** | 9.4 | **0.016** |
| OCD + tics | **5** | 13.2 | **6** | 11.3 | 1.000 |
| Specific phobia | **4** | 10.5 | **10** | 18.9 | 0.381 |
| GAD | **2** | 5.3 | **7** | 13.2 | 0.295 |
| Panic disorder | **5** | 13.2 | **4** | 7.5 | 0.483 |
| Social phobia | **5** | 13.2 | **3** | 5.7 | 0.271 |
| TTM | **2** | 5.3 | **4** | 7.5 | 1.000 |
| IED | **4** | 10.5 | **3** | 5.7 | 0.446 |
| BDD | **0** | 0.0 | **8** | 15.1 | **0.019** |
| TS | **1** | 2.6 | **3** | 5.7 | 0.638 |
| Anorexia | **1** | 2.6 | **4** | 7.5 | 0.396 |
| Hypochondriasis | **0** | 0.0 | **2** | 3.8 | 0.508 |

Statistically significant differences are indicated in red, bold font. [a] n(sexual/religious symptoms present) = 29, n(sexual/religious symptoms absent) =42; [b] n(sexual/religious symptoms present) = 38, n(sexual/religious symptoms absent) = 53**.** **Abbreviations:** **OCD:** Obsessive-compulsive disorder; **OCS**: obsessive-compulsive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome.

### *III.4.5.3.2. Single locus analysis of sexual/religious symptomatology*

#### *III.4.5.3.2.1. Single locus genetic investigation of bi-allelic polymorphisms*

Table III.58 represents the genotypic and allelic counts in selected markers, in those OCD subjects experiencing sexual/religious symptoms and those who did not. Table III.59(a) depicts the resultant p-values and the corresponding ORs after comparing the distribution of these genotypic and allelic frequencies between the two groups, whilst Table III.59(b) presents the p-values and ORs obtained when the genotype and allele frequencies were compared between OCD patients experiencing sexual/religious symptoms and controls.

No statistically significant differences in genotype or allele distribution were observed when the two OCD subsets were analysed (Table III.59[a]). However, when the OCD subset with sexual/religious symptoms was compared to the control sample, significant differences in the *BDNF val66met* (rs6265) genotype distribution (Table III.59[b]). Here, OCD patients experiencing sexual/religious symptoms exhibited a significantly higher frequency of the *AA*(*met66met*)–genotype compared to controls (10% versus 1.4%, respectively; p=0.015 [Table III.3(b), Table III.58 and Table III.59(b)]). Although not statistically significant, the same pattern was noted when the two OCD subsets were analysed (Table III.58), with the frequency of *AA* (*met66met6*) carriers in the OCD subset characterised by no sexual/religious symptoms amounting to only 2.2%.

Furthermore, significantly more controls were found to carry the *ACE Alu* ins/del *DD*-genotype compared to OCD patients who experienced sexual/religious symptoms (p = 0.021; OR = 0.27 [95% CI: 0.09-0.85]) (Tables III.3[b], III.58 and III.59[b]). Significant differences in allelic distribution were also observed between the two samples (p = 0.042; OR = 0.55 [95% CI: 0.31-0.99]) (Tables III.3[b], III.58 and III.59[b]), indicating that the *D*-allele may be functioning in a dominant manner in conferring protection against the development of sexual/religious symptoms .

***Table III.58.*** *Genotype and allele counts and frequencies in bi-allelic candidate polymorphisms in OCD patients presenting with sexual/religious symptoms and those without.*

| Gene | Variant | $n_1/n_2$[a] | Sexual/religious symptoms | | | | | | | | | | | No sexual/religious symptoms | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | **rs6311** | *G/A* | 14 | 48.3 | 13 | 44.8 | 2 | 6.9 | **29** | 41 | 70.7 | 17 | 29.3 | 16 | 37.2 | 18 | 41.9 | 9 | 20.9 | **43** | 50 | 58.1 | 36 | 41.9 |
| | **rs6313** | *C/T* | 14 | 51.9 | 10 | 37.0 | 3 | 11.1 | **27** | 38 | 70.4 | 16 | 29.6 | 18 | 41.9 | 17 | 39.5 | 8 | 18.6 | **43** | 53 | 61.6 | 33 | 38.4 |
| ***5-HT$_{1Dβ}$*** | **rs6296** | *G/C* | 14 | 56.0 | 9 | 36.0 | 2 | 8.0 | **25** | 37 | 74.0 | 13 | 26.0 | 19 | 46.3 | 19 | 46.3 | 3 | 7.3 | **41** | 57 | 69.5 | 25 | 30.5 |
| ***5-HT$_6$*** | **rs1805054** | *C/T* | 15 | 57.7 | 11 | 42.3 | 0 | 0.0 | **26** | 41 | 78.8 | 11 | 21.2 | 24 | 72.7 | 9 | 27.3 | 0 | 0.0 | **33** | 57 | 86.4 | 9 | 13.6 |
| ***DRD4*** | **rs1800955** | *T/C* | 8 | 36.4 | 11 | 50.0 | 3 | 13.6 | **22** | 27 | 61.4 | 17 | 38.6 | 13 | 38.2 | 16 | 47.1 | 5 | 14.7 | **34** | 42 | 61.8 | 26 | 38.2 |
| ***DRD2*** | **rs1800497** | *C/T* | 15 | 55.6 | 10 | 37.0 | 2 | 7.4 | **27** | 40 | 74.1 | 14 | 25.9 | 26 | 59.1 | 16 | 36.4 | 2 | 4.5 | **44** | 68 | 77.3 | 20 | 22.7 |
| ***COMT*** | **rs4680** | *A/G* | 7 | 35.0 | 8 | 40.0 | 5 | 25.0 | **20** | 22 | 55.0 | 18 | 45.0 | 9 | 25.7 | 20 | 57.1 | 6 | 17.1 | **35** | 38 | 54.3 | 32 | 45.7 |
| ***DRD3*** | **rs6280** | *A/G* | 17 | 58.6 | 8 | 27.6 | 4 | 13.8 | **29** | 42 | 72.4 | 16 | 27.6 | 21 | 53.8 | 14 | 35.9 | 4 | 10.3 | **39** | 56 | 71.8 | 22 | 28.2 |
| ***DRD1*** | ***A-48G*** | *A/G* | 11 | 40.7 | 16 | 59.3 | 0 | 0.0 | **27** | 38 | 70.4 | 16 | 29.6 | 15 | 38.5 | 18 | 46.2 | 6 | 15.4 | **39** | 48 | 61.5 | 30 | 38.5 |
| ***BDNF*** | **rs6265** | *G/A* | 19 | 63.3 | 8 | 26.7 | 3 | 10.0 | **30** | 46 | 76.7 | 14 | 23.3 | 30 | 66.7 | 14 | 31.1 | 1 | 2.2 | **45** | 74 | 82.2 | 16 | 17.8 |
| ***ESRα*** | **rs9340799** | *A/G* | 6 | 33.3 | 10 | 55.6 | 2 | 11.1 | **18** | 22 | 61.1 | 14 | 38.9 | 16 | 53.3 | 13 | 43.3 | 1 | 3.3 | **30** | 45 | 75.0 | 15 | 25.0 |
| | **rs2234693** | *T/C* | 2 | 18.2 | 6 | 54.5 | 3 | 27.3 | **11** | 10 | 45.5 | 12 | 54.5 | 8 | 38.1 | 12 | 57.1 | 1 | 4.8 | **21** | 28 | 66.7 | 14 | 33.3 |
| ***INPP-1*** | **rs1882891** | *C/A* | 20 | 74.1 | 7 | 25.9 | 0 | 0.0 | **27** | 47 | 87.0 | 7 | 13.0 | 28 | 73.7 | 9 | 23.7 | 1 | 2.6 | **38** | 65 | 85.5 | 11 | 14.5 |
| ***PLC-γ1*** | **rs8192707** | *A/G* | 11 | 52.4 | 9 | 42.9 | 1 | 4.8 | **21** | 31 | 73.8 | 11 | 26.2 | 27 | 65.9 | 11 | 26.8 | 3 | 7.3 | **41** | 65 | 79.3 | 17 | 20.7 |
| ***ACE*** | **Ins/del** | *D/I* | 10 | 34.5 | 12 | 41.4 | 7 | 24.1 | **29** | 32 | 55.2 | 26 | 44.8 | 22 | 48.9 | 16 | 35.6 | 7 | 15.6 | **45** | 60 | 66.7 | 30 | 33.3 |

[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele. **Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; COMT: Catechol-O-methyltransferase; ***DRD3***: dopamine receptor 3; DRD1: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.59(a).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects experiencing sexual/religious symptoms, and those not, in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.152 | 3.82 | 0.63 | 42.10 | 0.195 | 0.159 | 1.73 | 0.81 | 3.79 | 0.210 |
| | rs6313 | *C/T* | 0.480 | 2.04 | 0.39 | 14.20 | 0.079 | 0.363 | 1.47 | 0.68 | 3.30 | 0.112 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 1.000 | 1.10 | 0.11 | 14.90 | 0.061 | 0.693 | 1.25 | 0.53 | 3.01 | 0.060 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 1.000 | 0.63 | 0.01 | 33.56 | 0.149 | 0.328 | 0.59 | 0.20 | 1.73 | 0.193 |
| ***DRD4*** | rs1800955 | *T/C* | 1.000 | 1.02 | 0.15 | 8.45 | 0.062 | 1.000 | 0.98 | 0.42 | 2.32 | 0.052 |
| ***DRD2*** | rs1800497 | *C/T* | 0.626 | 0.58 | 0.04 | 8.84 | 0.050 | 0.689 | 0.84 | 0.36 | 2.01 | 0.054 |
| ***COMT*** | rs4680 | *A/G* | 1.000 | 0.94 | 0.16 | 5.74 | 0.057 | 1.000 | 1.03 | 0.44 | 2.42 | 0.051 |
| ***DRD3*** | rs6280 | *A/G* | 1.000 | 0.81 | 0.13 | 5.07 | 0.051 | 1.000 | 1.03 | 0.45 | 2.38 | 0.051 |
| ***DRD1*** | *A-48G* | *A/G* | 0.071 | 9.65 | 0.68 | 189.11 | 0.247 | 0.354 | 1.48 | 0.67 | 3.36 | 0.110 |
| ***BDNF*** | rs6265 | *G/A* | 0.295 | 0.22 | 0.00 | 2.93 | 0.114 | 0.413 | 0.71 | 0.29 | 1.74 | 0.081 |
| ***ESRα*** | rs9340799 | *A/G* | 0.231 | 0.20 | 0.00 | 4.55 | 0.090 | 0.173 | 0.53 | 0.20 | 1.41 | 0.174 |
| | rs2234693 | *T/C* | 0.095 | 0.11 | 0.00 | 2.05 | 0.202 | 0.116 | 0.42 | 0.13 | 1.36 | 0.215 |
| ***INPP-1*** | rs1882891 | *C/A* | 1.000 | 2.16 | 0.08 | 55.68 | 0.053 | 1.000 | 1.14 | 0.37 | 3.73 | 0.050 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 1.000 | 1.22 | 0.09 | 69.90 | 0.063 | 0.504 | 0.74 | 0.29 | 1.97 | 0.066 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.321 | 0.46 | 0.10 | 2.01 | 0.113 | 0.169 | 0.62 | 0.30 | 1.28 | 0.180 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.59(b).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects experiencing sexual/religious symptoms and controls in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.332 | 1.91 | 0.51 | 7.18 | 0.30 | 0.23 | 1.46 | 0.78 | 2.73 | 0.23 |
| | rs6313 | *C/T* | 0.141 | 3.07 | 0.65 | 14.53 | 0.20 | 0.162 | 1.55 | 0.84 | 2.85 | 0.16 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.798 | 0.81 | 0.16 | 4.05 | 0.06 | 0.824 | 0.93 | 0.47 | 1.83 | 0.09 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.665 | 0.58 | 0.02 | 14.85 | 0.24 | 0.205 | 0.61 | 0.28 | 1.32 | 0.19 |
| ***DRD4*** | rs1800955 | *T/C* | 0.335 | 1.98 | 0.48 | 8.13 | 0.15 | 0.339 | 1.37 | 0.72 | 2.63 | 0.12 |
| ***DRD2*** | rs1800497 | *C/T* | 0.987 | 1.01 | 0.20 | 5.10 | 0.05 | 0.979 | 0.99 | 0.51 | 1.93 | 0.05 |
| ***COMT*** | rs4680 | *G/A* | 0.426 | 0.60 | 0.17 | 2.12 | 0.13 | 0.413 | 0.76 | 0.39 | 1.48 | 0.10 |
| ***DRD3*** | rs6280 | *A/G* | 0.511 | 1.50 | 0.45 | 5.05 | 0.23 | 0.213 | 1.49 | 0.79 | 2.81 | 0.17 |
| ***DRD1*** | *A-48G* | *A/G* | 0.045 | 8.96 | 0.50 | 159.86 | 0.24 | 0.202 | 1.51 | 0.80 | 2.85 | 0.18 |
| ***BDNF*** | rs6265 | *G/A* | **0.015** | **0.13** | **0.02** | **0.85** | 0.20 | 0.230 | 0.66 | 0.34 | 1.30 | 0.15 |
| ***ESRα*** | rs9340799 | *A/G* | 0.863 | 1.16 | 0.22 | 6.18 | 0.06 | 0.820 | 0.92 | 0.45 | 1.88 | 0.05 |
| | rs2234693 | *T/C* | 0.642 | 0.65 | 0.10 | 4.13 | 0.06 | 0.655 | 0.82 | 0.34 | 1.97 | 0.06 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.514 | 1.10 | 0.05 | 23.45 | 0.06 | 0.738 | 0.86 | 0.35 | 2.09 | 0.07 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.660 | 0.60 | 0.06 | 5.90 | 0.14 | 0.408 | 0.73 | 0.34 | 1.55 | 0.11 |
| ***ACE*** | *Alu* ins/del | *D/I* | **0.021** | **0.27** | **0.09** | **0.86** | **0.54** | **0.042** | **0.55** | **0.31** | **0.99** | **0.43** |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

#### *III.4.5.3.2.2. Single locus analysis of multi-allelic polymorphisms*

##### *i. DRD4*

The genotypic and allelic distributions of the *DRD4* 48bp are presented in Tables III.60(a) and (b). No statistically significant differences in genotype or allele distribution were noted for either of the investigations ($p = 0.891$ and $p = 0.524$ for genotype and allele analyses, respectively). Likewise, when the genotype and allele frequencies were compared between OCD patients with sexual/religious symptoms and the control group, no significant differences were observed ($p = 0.647$ and $p = 0.563$ for genotype and allele analyses, respectively) (Tables III.4[a] and [b] and III.60[a] and [b]).

##### *ii. DAT*

The genotype and allele counts and frequencies for the *DAT* 40bp VNTR in OCD subjects experiencing sexual/religious symptoms and those who did not, are represented in Tables III.61(a) and (b). No significant differences in either genotype or allele distribution between the two subsets was observed ($p = 0.648$ and $p = 0.786$, respectively). Likewise, when the genotype and allele distributions were compared between OCD patients with sexual/religious symptoms and the control group, no significant differences were observed ($p = 0.253$ and $p = 0.151$ for genotype and allele analyses, respectively) (Tables III.5[a] and [b] and III.61[a] and [b]).

***Table III.60(a).*** *Genotype counts and frequencies in the* DRD4 *40bp VNTR polymorphism for OCD patients presenting with sexual/ religious symptoms and those without.*

| Genotype | Sexual/religious symptoms | | No sexual/religious symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A4* | **9** | 42.9 | **15** | 40.5 |
| *A4/A7* | **4** | 19.0 | **9** | 24.3 |
| *A4/A2* | **3** | 14.3 | **6** | 16.2 |
| *A4/A3* | **3** | 14.3 | **3** | 8.1 |
| *A2/A2* | **1** | 4.8 | **0** | 0.0 |
| *A3/A6* | **1** | 4.8 | **0** | 0.0 |
| *A7/A2* | **0** | 0.0 | **1** | 2.7 |
| *A7/A3* | **0** | 0.0 | **1** | 2.7 |
| *A7/A7* | **0** | 0.0 | **2** | 5.4 |

**p=0.891**

*A2*=2-repeat allele; *A3*=3-repeat allele; *A4*=4-repeat allele; *A6*=6-repeat allele; *A7*=7-repeat allele.

***Table III.60(b).*** *Allele counts and frequencies in the*DRD4 *40bp VNTR polymorphism for OCD patients presenting with sexual/ religious symptoms and those without.*

| Allele | Sexual/religious symptoms | | No sexual/religious symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **28** | 66.7 | **48** | 64.9 |
| *A7* | **4** | 9.5 | **15** | 20.3 |
| *A2* | **5** | 11.9 | **7** | 9.5 |
| *A3* | **4** | 9.5 | **4** | 5.4 |
| *A6* | **1** | 2.4 | **0** | 0.0 |

**p=0.524**

*A2*=2-repeat allele; *A3*=3-repeat allele; *A4*=4-repeat allele; *A6*=6-repeat allele; *A7*=7-repeat allele.

***Table III.61(a).*** *Genotype counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with sexual/ religious symptoms and those without.*

| Allele | Sexual/religious symptoms | | No sexual/religious symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10* | **38** | 67.9 | **58** | 69.0 |
| *A9* | **16** | 28.6 | **25** | 29.8 |
| *A2* | **1** | 1.8 | **0** | 0.0 |
| *A11* | **1** | 1.8 | **1** | 1.2 |

**p=0.648**

*A9*=9-repeat allele; *A10*=10-repeat allele; *A11*=11-repeat allele.

***Table III.61(b).*** *Allele counts and frequencies in the DAT 40bp VNTR polymorphism for OCD patients presenting with sexual/religious symptoms and those without.*

| Genotype | Sexual/religious symptoms | | No sexual/religious symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10/A10* | **14** | 50.0 | **19** | 45.2 |
| *A9/A10* | **8** | 28.6 | **19** | 45.2 |
| *A9/A9* | **4** | 14.3 | **3** | 7.1 |
| *A10/A11* | **1** | 3.6 | **1** | 2.4 |
| *A10/A2* | **1** | 3.6 | **0** | 0.0 |

**p=0.786**

*A9*=9-repeat allele; *A10*=10-repeat allele; *A11*=11-repeat allele.

### *III.4.5.4. Contamination Symptoms*

#### *III.4.5.4.1. Analysis of clinical variables*

Contamination obsessions and washing compulsions (comprising the contamination/washing symptom dimension) were found to be the most common symptom subtype experienced in the present study, with 60.4% of the patients reporting these obsessions and compulsions (Table III.7). No significant differences were observed with regard to gender ($p = 0.830$ [Table III.7]). Moreover, no significant differences in median total Y-BOCS scores or median ages at onset were observed between subjects experiencing contamination/washing symptoms and those not ($p = 0.123$ [Table III.17] and $p = 0.276$ [Table III.27], respectively).

The comparison of selected clinical variables is presented in Table III.62. No significant differences were observed when family history of OCD, OCS or tics was considered. When selected co-morbid disorders were investigated, significantly more patients experiencing contamination/washing symptoms were found to be diagnosed with tics, compared to those OCD subjects who did not experience contamination fears (17.9% versus 2.8%, respectively, $p = 0.045$).

#### *III.4.5.4.2. Single locus analysis of contamination symptomatology*

##### *III.4.5.4.2.1. Single locus analysis of bi-allelic polymorphisms*

Table III.63 presents the genotypic and allelic counts of the candidate polymorphisms in OCD subjects with contamination fears and those without. No statistically significant differences in either genotypic or allelic counts were noted when the two OCD subsets were compared (Table III.64[a]). When the OCD subset characterised by contamination symptoms and the control sample were compared, a statistically significant difference in the genotypic frequency of the *ACE Alu* ins/del variant was observed ($p = 0.038$; OR = 0.36 [95% CI: 0.14-0.97]), with an overrepresentation of the *DD*- and *DI*-genotypes amongst control individuals compared to contamination/washing OCD subset (91% versus 78.7%, respectively) (Tables III.3[b], III.63 and III.64[b]). No significant differences in allele distribution were noted, indicating that the *D* allele may be functioning in a dominant manner to confer protection against the development of contamination symptoms

***Table III.62.*** *Clinical characteristics in the OCD patient subset according to the presence or absence of contamination symptoms.*

| **Family history**[a] | **Contamination symptoms** | | | | **p-value** |
|---|---|---|---|---|---|
| | **Present** | | **Absent** | | |
| | **n** | **%** | **n** | **%** | |
| Family history of OCD | **13** | 29.5 | **5** | 18.5 | 0.403 |
| Family history of OCS | **21** | 47.7 | **14** | 51.9 | 1.000 |
| Family history of tics | **4** | 9.1 | **2** | 7.4 | 1.000 |
| **Co-morbidity**[b] | | | | | |
| MDD | **38** | 67.9 | **22** | 61.1 | 0.512 |
| SIB | **9** | 16.1 | **4** | 11.1 | 0.558 |
| Dysthymia | **13** | 23.2 | **3** | 8.3 | 0.092 |
| Tics | **10** | 17.9 | **1** | 2.8 | **0.045** |
| Specific phobia | **8** | 14.3 | **6** | 16.7 | 0.773 |
| GAD | **5** | 8.9 | **4** | 11.1 | 0.733 |
| Panic disorder | **3** | 5.4 | **6** | 16.7 | 0.147 |
| Social phobia | **5** | 8.9 | **4** | 11.1 | 0.733 |
| TTM | **6** | 10.7 | **1** | 2.8 | 0.245 |
| IED | **6** | 10.7 | **2** | 5.6 | 0.475 |
| BDD | **4** | 7.1 | **4** | 11.1 | 0.707 |
| TS | **4** | 7.1 | **0** | 0.0 | 0.153 |
| Anorexia | **4** | 7.1 | **1** | 2.8 | 0.645 |
| Hypochondriasis | **0** | 0.0 | **2** | 5.6 | 0.151 |

Statistically significant differences are indicated in red, bold font.
[a]n(contamination symptoms present)=44, n(contamination symptoms absent)=27; [b]n(contamination symptoms present)=56, n(contamination symptoms absent)=36**.**
**Abbreviations:** **OCD:** Obsessive-compulsive disorder; **OCS**: obsessive-compulsive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome.

***Table III.63.*** *Genotype and allele counts and frequencies in bi-allelic candidate markers in OCD patients presenting with contamination symptoms and those without.*

| Gene | Variant | $n_1/n_2$[a] | Contamination symptoms | | | | | | | | | | | No contamination symptoms | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 19 | 41.3 | 20 | 43.5 | 7 | 15.2 | **46** | 58 | 63.0 | 34 | 37.0 | 11 | 40.7 | 12 | 44.4 | 4 | 14.8 | **27** | 34 | 63.0 | 20 | 37.0 |
| | rs6313 | *C/T* | 19 | 42.2 | 20 | 44.4 | 6 | 13.3 | **45** | 58 | 64.4 | 32 | 35.6 | 13 | 50.0 | 8 | 30.8 | 5 | 19.2 | **26** | 34 | 65.4 | 18 | 34.6 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 21 | 50.0 | 17 | 40.5 | 4 | 9.5 | **42** | 59 | 70.2 | 25 | 29.8 | 12 | 48.0 | 12 | 48.0 | 1 | 4.0 | **25** | 36 | 72.0 | 14 | 28.0 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 22 | 61.1 | 14 | 38.9 | 0 | 0.0 | **36** | 58 | 80.6 | 14 | 19.4 | 17 | 70.8 | 7 | 29.2 | 0 | 0.0 | **24** | 41 | 85.4 | 7 | 14.6 |
| ***DRD4*** | rs1800955 | *T/C* | 13 | 36.1 | 16 | 44.4 | 7 | 19.4 | **36** | 42 | 58.3 | 30 | 41.7 | 8 | 38.1 | 12 | 57.1 | 1 | 4.8 | **21** | 28 | 66.7 | 14 | 33.3 |
| ***DRD2*** | rs1800497 | *C/T* | 27 | 62.8 | 14 | 32.6 | 2 | 4.7 | **43** | 68 | 79.1 | 18 | 20.9 | 15 | 51.7 | 12 | 41.4 | 2 | 6.9 | **29** | 42 | 72.4 | 16 | 27.6 |
| ***COMT*** | rs4680 | *A/G* | 10 | 27.8 | 17 | 47.2 | 9 | 25.0 | **36** | 37 | 51.4 | 35 | 48.6 | 6 | 30.0 | 12 | 60.0 | 2 | 10.0 | **20** | 24 | 60.0 | 16 | 40.0 |
| ***DRD3*** | rs6280 | *A/G* | 24 | 57.1 | 11 | 26.2 | 7 | 16.7 | **42** | 59 | 70.2 | 25 | 29.8 | 15 | 55.6 | 11 | 40.7 | 1 | 3.7 | **27** | 41 | 75.9 | 13 | 24.1 |
| ***DRD1*** | *A-48G* | *A/G* | 16 | 38.1 | 22 | 52.4 | 4 | 9.5 | **42** | 54 | 64.3 | 30 | 35.7 | 10 | 40.0 | 13 | 52.0 | 2 | 8.0 | **25** | 33 | 66.0 | 17 | 34.0 |
| ***BDNF*** | rs6265 | *G/A* | 33 | 70.2 | 13 | 27.7 | 1 | 2.1 | **47** | 79 | 84.0 | 15 | 16.0 | 17 | 58.6 | 9 | 31.0 | 3 | 10.3 | **29** | 43 | 74.1 | 15 | 25.9 |
| ***ESRα*** | rs9340799 | *A/G* | 12 | 38.7 | 15 | 48.4 | 4 | 12.9 | **31** | 39 | 62.9 | 23 | 37.1 | 10 | 55.6 | 8 | 44.4 | 0 | 0.0 | **18** | 28 | 77.8 | 8 | 22.2 |
| | rs2234693 | *T/C* | 6 | 27.3 | 11 | 50.0 | 5 | 22.7 | **22** | 23 | 52.3 | 21 | 47.7 | 4 | 36.4 | 7 | 63.6 | 0 | 0.0 | **11** | 15 | 68.2 | 7 | 31.8 |
| ***INPP-1*** | rs1882891 | *C/A* | 33 | 80.5 | 8 | 19.5 | 0 | 0.0 | **41** | 74 | 90.2 | 8 | 9.8 | 16 | 64.0 | 8 | 32.0 | 1 | 4.0 | **25** | 40 | 80.0 | 10 | 20.0 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 24 | 61.5 | 12 | 30.8 | 3 | 7.7 | **39** | 60 | 76.9 | 18 | 23.1 | 15 | 62.5 | 8 | 33.3 | 1 | 4.2 | **24** | 38 | 79.2 | 10 | 20.8 |
| ***ACE*** | Ins/del | *D/I* | 19 | 40.4 | 18 | 38.3 | 10 | 21.3 | **47** | 56 | 59.6 | 38 | 40.4 | 13 | 46.4 | 10 | 35.7 | 5 | 17.9 | **28** | 36 | 64.3 | 20 | 35.7 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele.

**Abbreviations:** ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; COMT: Catechol-O-methyltransferase; DRD3: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***BDNF***: brain-derivedneurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.64(a).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects experiencing contamination symptoms, and those not, in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.985 | 1.10 | 0.24 | 4.26 | 0.050 | 0.992 | 1.00 | 0.50 | 2.00 | 0.050 |
| | rs6313 | *C/T* | 0.779 | 1.22 | 0.31 | 4.84 | 0.051 | 0.910 | 0.96 | 0.47 | 1.96 | 0.052 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.472 | 2.29 | 0.23 | 22.87 | 0.055 | 0.828 | 1.09 | 0.50 | 2.63 | 0.057 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 1.000 | 1.29 | 0.02 | 68.08 | 0.086 | 0.492 | 0.71 | 0.26 | 1.91 | 0.104 |
| ***DRD4*** | rs1800955 | *T/C* | 0.182 | 4.31 | 0.44 | 41.82 | 0.158 | 0.377 | 1.43 | 0.65 | 3.20 | 0.124 |
| ***DRD2*** | rs1800497 | *C/T* | 0.572 | 1.80 | 0.23 | 14.11 | 0.118 | 0.356 | 1.44 | 0.66 | 3.13 | 0.150 |
| ***COMT*** | rs4680 | *A/G* | 0.281 | 0.37 | 0.06 | 2.32 | 0.112 | 0.380 | 0.71 | 0.32 | 1.54 | 0.141 |
| ***DRD3*** | rs6280 | *A/G* | 0.158 | 0.23 | 0.03 | 2.05 | 0.082 | 0.465 | 0.75 | 0.34 | 1.63 | 0.098 |
| ***DRD1*** | *A-48G* | *A/G* | 0.815 | 0.80 | 0.12 | 5.20 | 0.054 | 0.841 | 0.93 | 0.44 | 1.94 | 0.055 |
| ***BDNF*** | rs6265 | *G/A* | 0.102 | 5.82 | 0.56 | 60.3 | 0.243 | 0.136 | 1.83 | 0.82 | 4.11 | 0.319 |
| ***ESRα*** | rs9340799 | *A/G* | 0.090 | 0.13 | 0.01 | 2.75 | 0.258 | 0.127 | 0.48 | 0.19 | 1.24 | 0.338 |
| | rs2234693 | *T/C* | 0.100 | 7.62 | 0.33 | 175.10 | 0.180 | 0.217 | 1.96 | 0.68 | 5.70 | 0.234 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.159 | 6.10 | 0.24 | 157.80 | 0.279 | 0.096 | 2.31 | 0.85 | 6.32 | 0.366 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.595 | 1.87 | 0.18 | 19.72 | 0.055 | 0.770 | 1.14 | 0.50 | 2.73 | 0.058 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.631 | 1.37 | 0.38 | 5.00 | 0.068 | 0.570 | 1.22 | 0.62 | 2.42 | 0.078 |

Significant p-values are indicated in red, bold font.

[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.

**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme

***Table III.64(b).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects experiencing contamination symptoms and controls in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.620 | 1.30 | 0.47 | 3.60 | 0.06 | 0.657 | 1.12 | 0.69 | 1.81 | 0.06 |
| | rs6313 | *C/T* | 0.728 | 1.19 | 0.44 | 3.19 | 0.06 | 0.721 | 1.09 | 0.67 | 1.77 | 0.07 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.383 | 0.60 | 0.17 | 2.00 | 0.16 | 0.271 | 0.74 | 0.44 | 1.26 | 0.20 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 0.601 | 0.84 | 0.03 | 21.29 | 0.14 | 0.275 | 0.68 | 0.34 | 1.36 | 0.18 |
| ***DRD4*** | rs1800955 | *T/C* | 0.542 | 1.38 | 0.49 | 3.89 | 0.08 | 0.477 | 1.21 | 0.72 | 2.05 | 0.09 |
| ***DRD2*** | rs1800497 | *C/T* | 0.450 | 1.82 | 0.38 | 8.86 | 0.12 | 0.367 | 1.31 | 0.73 | 2.36 | 0.15 |
| ***COMT*** | rs4680 | *G/A* | 0.902 | 0.94 | 0.33 | 2.64 | 0.07 | 0.932 | 0.98 | 0.58 | 1.65 | 0.07 |
| ***DRD3*** | rs6280 | *A/G* | 0.708 | 1.21 | 0.45 | 3.28 | 0.13 | 0.282 | 1.34 | 0.80 | 2.30 | 0.17 |
| ***DRD1*** | *A-48G* | *A/G* | 0.492 | 1.53 | 0.45 | 5.20 | 0.07 | 0.607 | 1.14 | 0.67 | 1.91 | 0.08 |
| ***BDNF*** | rs6265 | *G/A* | 0.768 | 0.70 | 0.06 | 7.91 | 0.06 | 0.852 | 1.06 | 0.56 | 2.00 | 0.05 |
| ***ESRα*** | rs9340799 | *A/G* | 0.815 | 1.20 | 0.34 | 3.98 | 0.05 | 0.982 | 0.99 | 0.56 | 1.76 | 0.05 |
| | rs2234693 | *T/C* | 0.818 | 1.16 | 0.32 | 4.20 | 0.06 | 0.823 | 1.08 | 0.56 | 2.05 | 0.07 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.405 | 1.77 | 0.08 | 37.87 | 0.05 | 0.691 | 1.18 | 0.52 | 2.72 | 0.06 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.291 | 0.44 | 0.09 | 2.10 | 0.08 | 0.631 | 0.86 | 0.46 | 1.59 | 0.09 |
| ***ACE*** | *Alu* ins/del | *D/I* | **0.038** | **0.36** | **0.14** | **0.97** | 0.33 | 0.100 | 0.66 | 0.41 | 1.08 | 0.42 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***III.4.5.4.2.2. Single locus analysis of multi-allelic polymorphisms***

**i. *DRD4***

The genotype and allele scores and frequencies for the *DRD4* 48bp VNTR are provided in Tables III.65[a] and [b], respectively. Statistically significant differences in distribution were noted for the genotype analysis ($p = 0.015$), but not for the allele analysis ($p = 0.076$). When the genotype combinations were combined according to the presence of at least one *A4* allele, it was found that significantly more OCD patients carrying the *A4/A4* genotype did not experience contamination symptoms, compared to those who did (58.3% versus 28.6%, respectively; $p = 0.035$) (Table III.65[c]).

This association was, however, only observed when the genotype frequencies were compared between the two OCD subsets. When the frequencies of the *A4*-allele were compared between the subsets, no statistically significant differences were observed ($p = 0.172$) (Table III.65[d]).

Likewise, when the genotype and allele frequencies of the ungrouped *DRD4* 48bp VNTR were compared between OCD patients presenting with contamination symptoms and controls, statistically significant differences were noted with regard to genotype distribution ($p = 0.042$), but not for allele distribution ($p = 0.392$) (Tables III.4[a] and [b] and III.65[a] and [b]). Moreover, when the genotypes were combined according to the presence or absence of at least one *A4*-allele, the control subjects presented with a significantly higher frequency of *A4/A4* genotypes (52.3% versus 28.6%; $p = 0.005$) (Table III.65[e]). Again, when the frequency of *A4*-alleles were compared against non-*A4*-alleles, however, no statistically significant differences were observed ($p = 0.158$) (Table III.65[f]).

**ii. *DAT***

Tables III.66(a) and (b) provide the genotype and allele counts and frequencies for the *DAT* 40bp VNTR polymorphism in OCD subjects with contamination symptoms and those without. No significant differences in either the genotype or allele distributions were noted between the two OCD patient subsets ($p = 0.348$ and $p = 0.743$, respectively).

Similarly, no statistically significant differences in either genotype ($p = 0.121$) or allele ($p = 0.207$) frequencies were observed when the OCD patients experiencing contamination symptoms were compared to controls.

***Table III.65(a).*** *Genotype scores and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with contamination symptoms and those without.*

| Genotype | Contamination symptoms | | No contamination symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A7* | **12** | 34.3 | **2** | 8.3 |
| *A4/A4* | **10** | 28.6 | **14** | 58.3 |
| *A4/A2* | **7** | 20.0 | **2** | 8.3 |
| *A4/A3* | **3** | 8.6 | **3** | 12.5 |
| *A7/A7* | **2** | 5.7 | **0** | 0.0 |
| *A2/A2* | **1** | 2.9 | **0** | 0.0 |
| *A3/A6* | **0** | 0.0 | **1** | 4.2 |
| *A7/A2* | **0** | 0.0 | **1** | 4.2 |
| *A7/A3* | **0** | 0.0 | **1** | 4.2 |

**p=0.015**

*A2*=2-repeat allele; *A3*=3-repeat allele; *A4*=4-repeat allele; *A6*=6-repeat allele; *A7*=7-repeat allele.

***Table III.65(b).*** *Allele counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with contamination symptoms and those without.*

| Genotype | Contamination symptoms | | No contamination symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A4* | **10** | 28.6 | **14** | 58.3 |
| *A4/other* | **22** | 62.9 | **7** | 29.2 |
| *other/other* | **3** | 8.6 | **3** | 12.5 |

**p= 0.076**

*A2* = 2-repeat allele; *A3* = 3-repeat allele; *A4* = 4-repeat allele; *A6* = 6-repeat allele; *A7* = 7-repeat allele

***Table III.65(c).*** *Genotype counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with contamination symptoms and those without, according to the presence or absence of at least one* A4*-allele.*

| Allele | Contamination symptoms | | No contamination symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **42** | 60.0 | **35** | 72.9 |
| *A7* | **16** | 22.9 | **4** | 8.3 |
| *A2* | **9** | 12.9 | **3** | 6.3 |
| *A3* | **3** | 4.3 | **5** | 10.4 |
| *A6* | **0** | 0.0 | **1** | 2.1 |

**p= 0.035**

*A4* = 4-repeat allele; "other" genotypes comprise *A2, A3, A7*and *A6* alleles.

***Table III.65(d).*** *Allele counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with contamination symptoms and those without, according to the presence or absence of the* A4*-allele.*

| Allele | Contamination symptoms | | No contamination symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **42** | 60.0 | **35** | 72.9 |
| *other* | **28** | 40.0 | **13** | 27.1 |

**p= 0.172**

*A4* = 4-repeat allele; "other" alleles comprise *A2, A3, A7*and *A6* allele.

***Table III.65(e).*** *Genotype counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with contamination symptoms and controls, according to the presence or absence of at least one* A4*-allele.*

| Genotype | Contamination symptoms | | Control | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A4* | 10 | 28.6 | 79 | 52.3 |
| *A4/other* | 22 | 62.9 | 50 | 33.1 |
| *other/other* | 3 | 8.6 | 22 | 14.6 |

**p= 0.005**

*A4* = 4-repeat allele; "other" genotypes comprise *A2, A3, A7*and *A6* alleles

***Table III.65(f).*** *Allele counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with contamination symptoms and controls, according to the presence or absence of the A4-allele.*

| Allele | Contamination symptoms | | Control | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | 42 | 60.0 | 208 | 68.9 |
| *other* | 28 | 40.0 | 94 | 31.1 |

**p = 0.158**

*A4* = 4 -repeat allele; "other" genotypes comprise *A2, A3, A7*and *A6* alleles

***Table III.66(a).*** *Genotype counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with contamination symptoms and those without.*

| Genotype | Contamination symptoms | | No contaminationsymptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10/A10* | **19** | 42.2 | **15** | 57.7 |
| *A9/A10* | **20** | 44.4 | **7** | 26.9 |
| *A9/A9* | **4** | 8.9 | **3** | 11.5 |
| *A10/A11* | **2** | 4.4 | **0** | 0.0 |
| *A2/A10* | **0** | 0.0 | **1** | 3.8 |

**p = 0.348**

*A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele.

***Table III.66(b).*** *Allele counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with contamination symptoms and those without.*

| Allele | Contamination symptoms | | No contamination symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10* | **60** | 66.7 | **38** | 45.2 |
| *A9* | **28** | 31.1 | **13** | 15.5 |
| *A11* | **2** | 2.2 | **0** | 0.0 |
| *A2* | **0** | 0.0 | **1** | 1.2 |

**p = 0.743**

*A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele.

### *III.4.5.5. Aggressive Symptoms*

#### *III.4.5.5.1. Analysis of clinical variables*

No statistically significant differences in the frequency of aggressive symptoms were observed amongst males and females (p = 0.670 [Table III.7). Likewise, no significant differences in age at onset were observed between those OCD subjects experiencing aggressive symptoms and those not (p = 0.809, [Table III.27]). Interestingly, the subtype was found to be nominally associated with Y-BOCS score (Table III.17): those patients experiencing aggressive symptoms were found to have a lower Y-BOCS score (decreased severity) (median Y-BOCS = 18 [95% CI: 15.7-20.3]) compared to those without these symptoms (median Y-BOCS score = 22.5 [95% CI: 20.5-24.5]) (p = 0.050).

The comparison of selected clinical variables between OCD subjects experiencing aggressive symptoms and those not, is depicted in Table III.67. No significant differences were observed with regard to family history of OCD, OCS or tics, or selected co-morbid disorders.

#### *III.4.5.5.2. Single locus analysis of aggressive symptomatology*

##### *III.4.5.5.2.1. Single locus genetic investigation of bi-allelic polymorphisms*

The genotypic and allelic counts of the bi-allelic polymorphisms are represented in Table III.68. Table III.69(a) indicates the p-values and corresponding ORs obtained from comparing the distributions of genotypes and alleles between the two OCD subsets, and Table III.69(b) presents the p-values and corresponding ORs obtained from comparing the genotype and allele frequencies between OCD subjects presenting with aggressive symptoms, and controls. No statistically significant differences were detected for either of the groups of analyses.

***Table III.67.*** *Clinical characteristics in the OCD patient subset according to the presence or absence of aggressive symptoms.*

| Family history[a] | Aggressive symptoms | | | | p-value |
|---|---|---|---|---|---|
| | Present | | Absent | | |
| | n | % | n | % | |
| Family history of OCD | **12** | 32.4 | **6** | 17.6 | 0.181 |
| Family history of OCS | **20** | 54.1 | **15** | 44.1 | 0.473 |
| Family history of tics | **3** | 8.1 | **3** | 8.8 | 1.000 |
| **Co-morbidity[b]** | | | | | |
| MDD | **32** | 68.1 | **27** | 61.4 | 0.519 |
| SIB | **7** | 14.9 | **5** | 11.4 | 0.760 |
| Dysthymia | **8** | 17.0 | **8** | 18.2 | 1.000 |
| OCD + tics | **6** | 12.8 | **5** | 11.4 | 1.000 |
| Specific phobia | **9** | 19.1 | **5** | 11.4 | 0.388 |
| GAD | **5** | 10.6 | **4** | 9.1 | 1.000 |
| Panic disorder | **6** | 12.8 | **3** | 6.8 | 0.487 |
| Social phobia | **4** | 8.5 | **4** | 9.1 | 1.000 |
| TTM | **3** | 6.4 | **3** | 6.8 | 1.000 |
| IED | **4** | 8.5 | **3** | 6.8 | 1.000 |
| BDD | **5** | 10.6 | **3** | 6.8 | 0.715 |
| TS | **1** | 2.1 | **3** | 6.8 | 0.350 |
| Anorexia | **4** | 8.5 | **1** | 2.3 | 0.362 |
| Hypochondriasis | **1** | 2.1 | **1** | 2.3 | 1.000 |

Statistically significant differences are indicated in red, bold font.
[a] n(aggression symptoms present)=37, n(aggression symptoms absent)=34; [b] n(aggression symptoms present)=47, n(aggression symptoms absent)=44**.**
**Abbreviations:** **OCD:** Obsessive-compulsive disorder; **OCS**: obsessive-compulsive symptoms; **MDD**: major depressive disorder; **SIB**: self-injurious behaviour; **GAD**: generalised anxiety disorder; **PD**: panic disorder; **TTM**: Trichotillomania; **IED**: intermittent explosive disorder; **BDD**: body dysmorphic disorder; **TS**: Tourette Syndrome.

***Table III.68.*** *Genotype and allele scores and frequencies in bi-allelic candidate polymorphisms in OCD patients presenting with aggressive symptoms and those without.*

| Gene | Variant | $n_1/n_2$[a] | Aggressive symptoms | | | | | | | | | | | No aggressive symptoms | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Genotype | | | | | | Total | Alleles | | | | Genotype | | | | | | Total | Alleles | | | |
| | | | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % | $n_{11}$ | % | $n_{12}$ | % | $n_{22}$ | % | | $n_1$ | % | $n_2$ | % |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 14 | 37.8 | 17 | 45.9 | 6 | 16.2 | **37** | 45 | 60.8 | 29 | 39.2 | 16 | 45.7 | 14 | 40.0 | 5 | 14.3 | **35** | 46 | 65.7 | 24 | 34.3 |
| | rs6313 | *C/T* | 15 | 40.5 | 15 | 40.5 | 7 | 18.9 | **37** | 45 | 60.8 | 29 | 39.2 | 17 | 51.5 | 12 | 36.4 | 4 | 12.1 | **33** | 46 | 69.7 | 20 | 30.3 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 13 | 38.2 | 18 | 52.9 | 3 | 8.8 | **34** | 44 | 64.7 | 24 | 35.3 | 20 | 62.5 | 10 | 31.3 | 2 | 6.3 | **32** | 50 | 78.1 | 14 | 21.9 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 16 | 53.3 | 14 | 46.7 | 0 | 0.0 | **30** | 46 | 76.7 | 14 | 23.3 | 23 | 79.3 | 6 | 20.7 | 0 | 0.0 | **29** | 52 | 89.7 | 6 | 10.3 |
| ***DRD4*** | rs1800955 | *T/C* | 9 | 31.0 | 16 | 55.2 | 4 | 13.8 | **29** | 34 | 58.6 | 24 | 41.4 | 12 | 44.4 | 11 | 40.7 | 4 | 14.8 | **27** | 35 | 64.8 | 19 | 35.2 |
| ***DRD2*** | rs1800497 | *C/T* | 16 | 44.4 | 18 | 50.0 | 2 | 5.6 | **36** | 50 | 69.4 | 22 | 30.6 | 25 | 71.4 | 8 | 22.9 | 2 | 5.7 | **35** | 58 | 82.9 | 12 | 17.1 |
| ***COMT*** | rs4680 | *A/G* | 7 | 25.0 | 15 | 53.6 | 6 | 21.4 | **28** | 29 | 51.8 | 27 | 48.2 | 9 | 33.3 | 13 | 48.1 | 5 | 18.5 | **27** | 31 | 57.4 | 23 | 42.6 |
| ***DRD3*** | rs6280 | *A/G* | 21 | 61.8 | 10 | 29.4 | 3 | 8.8 | **34** | 52 | 76.5 | 16 | 23.5 | 17 | 50.0 | 12 | 35.3 | 5 | 14.7 | **34** | 46 | 67.6 | 22 | 32.4 |
| ***DRD1*** | A-48G | *A/G* | 14 | 40.0 | 18 | 51.4 | 3 | 8.6 | **35** | 46 | 65.7 | 24 | 34.3 | 12 | 38.7 | 16 | 51.6 | 3 | 9.7 | **31** | 40 | 64.5 | 22 | 35.5 |
| ***BDNF*** | rs6265 | *G/A* | 26 | 68.4 | 11 | 28.9 | 1 | 2.6 | **38** | 63 | 82.9 | 13 | 17.1 | 23 | 62.2 | 11 | 29.7 | 3 | 8.1 | **37** | 57 | 77.0 | 17 | 23.0 |
| ***ESRα*** | rs9340799 | *A/G* | 10 | 43.5 | 12 | 52.2 | 1 | 4.3 | **23** | 32 | 69.6 | 14 | 30.4 | 12 | 48.0 | 11 | 44.0 | 2 | 8.0 | **25** | 35 | 70.0 | 15 | 30.0 |
| | rs2234693 | *T/C* | 7 | 36.8 | 11 | 57.9 | 1 | 5.3 | **19** | 25 | 65.8 | 13 | 34.2 | 3 | 23.1 | 7 | 53.8 | 3 | 23.1 | **13** | 13 | 50.0 | 13 | 50.0 |
| ***INPP-1*** | rs1882891 | *C/A* | 28 | 80.0 | 7 | 20.0 | 0 | 0.0 | **35** | 63 | 90.0 | 7 | 10.0 | 20 | 66.7 | 9 | 30.0 | 1 | 3.3 | **30** | 49 | 81.7 | 11 | 18.3 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 21 | 65.6 | 9 | 28.1 | 2 | 6.3 | **32** | 51 | 79.7 | 13 | 20.3 | 17 | 56.7 | 11 | 36.7 | 2 | 6.7 | **30** | 45 | 75.0 | 15 | 25.0 |
| ***ACE*** | Alu Ins/del | *D/I* | 18 | 46.2 | 14 | 35.9 | 7 | 17.9 | **39** | 50 | 64.1 | 28 | 35.9 | 14 | 40.0 | 14 | 40.0 | 7 | 20.0 | **35** | 42 | 60.0 | 28 | 40.0 |

[a] $n_1$ refers to the major allele, $n_2$ refers to the minor allele.

**Abbreviations: *5-HT$_{2A}$*:** serotonin receptor 2A**; *5-HT$_{1Dβ}$*:** serotonin receptor 1Dβ**; *5-HT$_6$*:** serotonin receptor 6**; *DRD4*:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; COMT: Catechol-O-methyltransferase; DRD3: dopamine receptor 3; ***DRD1***: dopamine receptor 1; ***GRIN2B***: glutamate receptor subunit 2B; ***BDNF***: brain-derivedneurotrophic factor; ***HOXB8***: homeobox gene B8; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLCγ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.69(a).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects experiencing aggressive symptoms, and those not, in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.734 | 0.74 | 0.14 | 3.62 | 0.051 | 0.606 | 0.81 | 0.39 | 1.69 | 0.065 |
| | rs6313 | *C/T* | 0.488 | 0.51 | 0.09 | 2.50 | 0.079 | 0.292 | 0.68 | 0.31 | 1.44 | 0.120 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.632 | 0.44 | 0.03 | 4.43 | 0.061 | 0.123 | 0.52 | 0.22 | 1.19 | 0.252 |
| ***5-HT$_6$*** | rs1805054 | *C/T* | 1.000 | 0.71 | 0.01 | 37.21 | 0.370 | 0.085 | 0.38 | 0.13 | 1.17 | 0.474 |
| ***DRD4*** | rs1800955 | *T/C* | 1.000 | 0.76 | 0.11 | 5.30 | 0.050 | 0.562 | 0.77 | 0.33 | 1.77 | 0.068 |
| ***DRD2*** | rs1800497 | *C/T* | 1.000 | 0.65 | 0.04 | 9.76 | 0.051 | 0.077 | 0.47 | 0.19 | 1.12 | 0.303 |
| ***COMT*** | rs4680 | *A/G* | 0.704 | 0.66 | 0.11 | 3.90 | 0.052 | 0.572 | 0.80 | 0.35 | 1.81 | 0.062 |
| ***DRD3*** | rs6280 | *A/G* | 0.451 | 2.03 | 0.34 | 15.00 | 0.071 | 0.339 | 1.55 | 0.68 | 3.57 | 0.125 |
| ***DRD1*** | *A-48G* | *A/G* | 1.000 | 1.16 | 0.13 | 10.40 | 0.056 | 1.000 | 1.05 | 0.48 | 2.30 | 0.050 |
| ***BDNF*** | rs6265 | *G/A* | 0.351 | 3.32 | 0.25 | 185.00 | 0.074 | 0.418 | 1.44 | 0.60 | 3.54 | 0.088 |
| ***ESRα*** | rs9340799 | *A/G* | 1.000 | 1.63 | 0.07 | 108.00 | 0.054 | 1.000 | 0.98 | 0.37 | 2.57 | 0.052 |
| | rs2234693 | *T/C* | 0.245 | 5.99 | 0.33 | 417.00 | 0.122 | 0.300 | 1.90 | 0.62 | 6.02 | 0.133 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.243 | 4.17 | 0.16 | 107.62 | 0.052 | 0.207 | 2.01 | 0.66 | 6.60 | 0.155 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 1.000 | 1.23 | 0.08 | 18.60 | 0.058 | 0.668 | 1.30 | 0.52 | 3.34 | 0.063 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.755 | 1.28 | 0.30 | 5.44 | 0.050 | 0.615 | 1.19 | 0.58 | 2.44 | 0.059 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_6$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

***Table III.69(b).*** *Association analysis investigating the differences in genotype and allele distributions between OCD subjects experiencing aggressive symptom and controls in bi-allelic loci, with corresponding p-values, ORs and 95% CIs.*

| Gene | Variant | $n_1/n_2$[a] | Genotype | | | | | Allele | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p-value | OR[b] | 95% CI | | Power | p-value | OR[c] | 95% CI | | Power |
| | | | | | LB[d] | UB[e] | | | | LB[d] | UB[e] | |
| ***5-HT$_{2A}$*** | rs6311 | *G/A* | 0.966 | 1.02 | 0.35 | 2.97 | 0.05 | 0.981 | 0.99 | 0.59 | 1.68 | 0.05 |
| | rs6313 | *C/T* | 0.796 | 0.88 | 0.32 | 2.40 | 0.05 | 0.863 | 0.95 | 0.57 | 1.60 | 0.05 |
| ***5-HT$_{1Dβ}$*** | rs6296 | *G/C* | 0.327 | 0.50 | 0.12 | 2.04 | 0.37 | 0.070 | 0.60 | 0.34 | 1.04 | 0.47 |
| ***5-HT$_{6}$*** | rs1805054 | *C/T* | 0.655 | 0.62 | 0.02 | 38.50 | 0.31 | 0.084 | 0.54 | 0.27 | 1.10 | 0.39 |
| ***DRD4*** | rs1800955 | *T/C* | 0.425 | 1.67 | 0.47 | 5.97 | 0.09 | 0.489 | 1.22 | 0.69 | 2.17 | 0.11 |
| ***DRD2*** | rs1800497 | *C/T* | 0.924 | 1.08 | 0.22 | 5.42 | 0.11 | 0.415 | 0.78 | 0.45 | 1.40 | 0.14 |
| ***COMT*** | rs4680 | *A/G* | 0.597 | 0.72 | 0.22 | 2.41 | 0.07 | 0.612 | 0.86 | 0.48 | 1.54 | 0.08 |
| ***DRD3*** | rs6280 | *A/G* | 0.170 | 2.47 | 0.66 | 9.28 | 0.42 | 0.050 | 1.85 | 1.00 | 3.42 | 0.52 |
| ***DRD1*** | *A-48G* | *A/G* | 0.398 | 1.79 | 0.46 | 6.96 | 0.10 | 0.485 | 1.22 | 0.70 | 2.12 | 0.12 |
| ***BDNF*** | rs6265 | *G/A* | 0.623 | 0.55 | 0.05 | 6.23 | 0.05 | 0.947 | 0.98 | 0.50 | 1.92 | 0.05 |
| ***ESRα*** | rs9340799 | *A/G* | 0.182 | 3.86 | 0.47 | 31.95 | 0.12 | 0.395 | 1.34 | 0.68 | 2.63 | 0.15 |
| | rs2234693 | *T/C* | 0.050 | 6.78 | 0.79 | 58.38 | 0.40 | 0.080 | 1.89 | 0.92 | 3.87 | 0.50 |
| ***INPP-1*** | rs1882891 | *C/A* | 0.441 | 1.51 | 0.07 | 32.33 | 0.05 | 0.752 | 1.15 | 0.48 | 1.96 | 0.05 |
| ***PLC-γ1*** | rs8192707 | *A/G* | 0.535 | 0.58 | 0.10 | 3.36 | 0.05 | 0.971 | 1.01 | 0.51 | 2.01 | 0.05 |
| ***ACE*** | *Alu* ins/del | *D/I* | 0.185 | 0.49 | 0.17 | 1.43 | 0.13 | 0.412 | 0.80 | 0.47 | 1.36 | 0.11 |

Significant p-values are indicated in red, bold font.
[a]$n_1$ refers to the major allele, $n_2$ refers to the minor allele [b]genotypic OR is calculated with the major genotype representing the risk genotype; [c]allelic OR is calculated assuming the major allele to be the risk allele;[d]lower boundary of the 95% CI; [e]Upper boundary of the 95% CI.
**Abbreviations**: **OR:** Odds ratio; **CI:** confidence interval; ***5-HT$_{2A}$*****:** serotonin receptor 2A**;** ***5-HT$_{1Dβ}$*****:** serotonin receptor 1Dβ**;** ***5-HT$_{6}$*****:** serotonin receptor 6**;** ***DRD4*****:** dopamine receptor 4; ***DRD2***: dopamine receptor 2; ***COMT:*** Catechol-O-methyltransferase**;** *DRD3*: dopamine receptor 3; *DRD1*: dopamine receptor 1; ***BDNF***: brain-derived neurotrophic factor; ***ESRα***: estrogen receptor α; ***INPP-1:*** inositol polyphosphate-phosphatase 1; ***PLC-γ1***: phospholipase-gamma; ***ACE***: Angiotensin-converting enzyme.

### *III.4.5.5.2.2. Single locus analysis of multi-allelic polymorphisms*

#### *i .DRD4*

The *DRD4* 48bp VNTR genotypic and allelic scores in those OCD patients experiencing aggressive symptoms, and those without are presented in Tables III.70(a) and (b). No statistically significant differences in either the genotypic ($p = 0.267$) or allelic ($p = 0.616$) distributions were noted. Similarly, when the OCD subset experiencing aggressive symptoms was compared to the control sample, no significant differences in genotypic ($p = 0.265$) or allelic ($p = 0.350$) distributions were observed (Tables III.4[a] and [b] and III.70[a] and [b]).

#### *ii. DAT*

The *DAT* 40bp VNTR genotypic and allelic scores in those OCD patients experiencing aggressive symptoms, and those without are presented in Tables III.71(a) and (b). No statistically significant differences in either the genotypic ($p = 0.638$) or allelic ($p = 0.612$) distributions were noted. Similarly, when the OCD subset experiencing aggressive symptoms was compared to the control sample, no significant differences in genotypic ($p = 0.214$) or allelic ($p = 0.146$) distributions were observed (Tables III.5[a] and[b] and III.71[a] and [b]).

***Table III.70(a).*** *Genotype counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with aggressive symptoms and those without.*

| Genotype | Aggressive symptoms | | No aggressive symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4/A4* | **11** | 37.9 | **13** | 44.8 |
| *A4/A2* | **5** | 17.2 | **4** | 13.8 |
| *A4/A3* | **5** | 17.2 | **1** | 3.4 |
| *A4/A7* | **5** | 17.2 | **8** | 27.6 |
| *A7/A7* | **2** | 6.9 | **0** | 0.0 |
| *A7/A3* | **1** | 3.4 | **0** | 0.0 |
| *A2/A2* | **0** | 0.0 | **1** | 3.4 |
| *A3/A6* | **0** | 0.0 | **1** | 3.4 |
| *A7/A2* | **0** | 0.0 | **1** | 3.4 |

**p = 0.267**

*A2* = 2-repeat allele; *A3* = 3-repeat allele; *A4* = 4-repeat allele; *A6* = 6-repeat allele; *A7* = 7-repeat allele

***Table III.70(b).*** *Allele counts and frequencies in the* DRD4 *48bp VNTR polymorphism for OCD patients presenting with aggressive symptoms and those without.*

| Allele | Aggressive symptoms | | No aggressive symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A4* | **37** | 63.8 | **39** | 67.2 |
| *A7* | **10** | 17.2 | **9** | 15.5 |
| *A2* | **5** | 8.6 | **7** | 12.1 |
| *A3* | **6** | 10.3 | **2** | 3.4 |
| *A6* | **0** | 0.0 | **1** | 1.7 |

**p = 0.616**

*A2* = 2-repeat allele; *A3* = 3-repeat allele; *A4* = 4-repeat allele; *A6* = 6-repeat allele; *A7* = 7-repeat allele.

***Table III.71(a).*** *Genotype counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with aggressive symptoms and those without.*

| Genotype | Aggressive symptoms | | No aggressive symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10/A10* | **15** | 40.5 | **18** | 54.5 |
| *A9/A10* | **16** | 43.2 | **11** | 33.3 |
| *A9/A9* | **4** | 10.8 | **3** | 9.1 |
| *A10/A11* | **2** | 5.4 | **0** | 0.0 |
| *A2/A10* | **0** | 0.0 | **1** | 3.0 |

**p = 0.638**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele.

***Table III.71(b).*** *Allele counts and frequencies in the* DAT *40bp VNTR polymorphism for OCD patients presenting with aggressive symptoms and those without.*

| Allele | Aggressive symptoms | | No aggressive symptoms | |
|---|---|---|---|---|
| | n | % | n | % |
| *A10* | **48** | 64.9 | **48** | 72.7 |
| *A9* | **24** | 32.4 | **17** | 25.8 |
| *A2* | **0** | 0.0 | **1** | 1.5 |
| *A11* | **2** | 2.7 | **0** | 0.0 |

**p = 0.612**

*A2* = 2-repeat allele; *A9* = 9-repeat allele; *A10* = 10-repeat allele; *A11* = 11-repeat allele.

## III.5. META-ANALYSES

### III.5.1. *5-HT$_{2A}$ -1438 A/G* (rs6311)

Four studies, including the present one, were included in the meta-analysis investigating the role that the *5-HT$_{2A}$* promoter polymorphism, *-1438 A/G* (rs6311), may play in mediating the development of OCD. The total number of OCD patients and controls amounted to 322 and 444, respectively. The frequencies of the -1438*G*-allele in the control and OCD subjects in each included study are represented in Table III.72. All of the studies were in HWE, and no heterogeneity of ORs across the studies could be detected (Q = 6.37, 3df, p = 0.100).

Figure III.51 presents a Forest Plot of the individual and summary ORs. Although a significant association was detected by Enoch et al. (2001), implicating the *G*-allele as a protective factor (therefore, the *A*-allele is assumed to represent the risk factor in their study), the summary OR generated by the present analysis indicates that no association exists between the variant and OCD (summary OR= 0.83 [95% CI: 0.62-1.11]).

### III.5.2. *5-HT$_{2A}$ T102C* (rs6313)

The five studies included in the meta-analysis of the *5-HT$_{2A}$* polymorphism *T102C* represented six independent samples and effect sizes, with an aggregate of 387 OCD patients and 657 controls. All studies were found to be in HWE. The frequency of the most prevalent allele, *C102*, in each study included in the meta-analysis is indicated in Table III.73.

The individual and summary ORs and their corresponding 95% CIs are presented in Figure III.52, with neither the individual studies nor the meta-analysis achieving statistical significance (summary OR=0.97 [95% CI: 0.79-1.20]). There was no evidence for heterogeneity of ORs across the studies (Q = 6.36, 5df, p = 0.273).

***Table III.72.*** *Frequency of the* 5-$HT_{2A}$ -1438G-*allele in case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of *-1438G* | |
|---|---|---|
| | OCD | Control |
| Enoch et al. (2001) | 0.50 | 0.62 |
| Tot et al. (2003) | 0.54 | 0.51 |
| Walitza et al. (2004) | 0.51 | 0.60 |
| Present Study | 0.62 | 0.61 |

**Abbreviations:** OCD: obsessive-compulsive disorder.

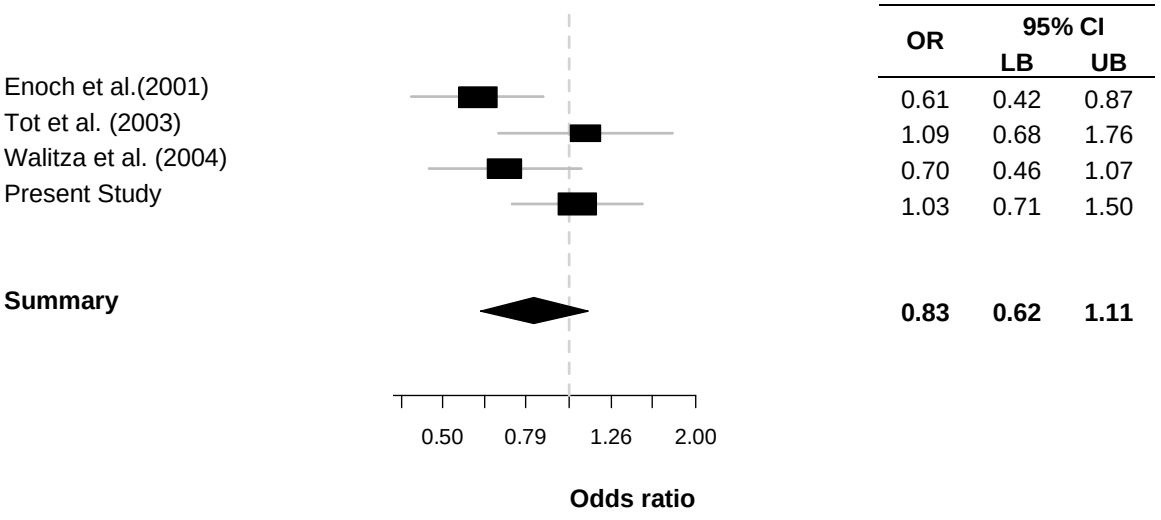
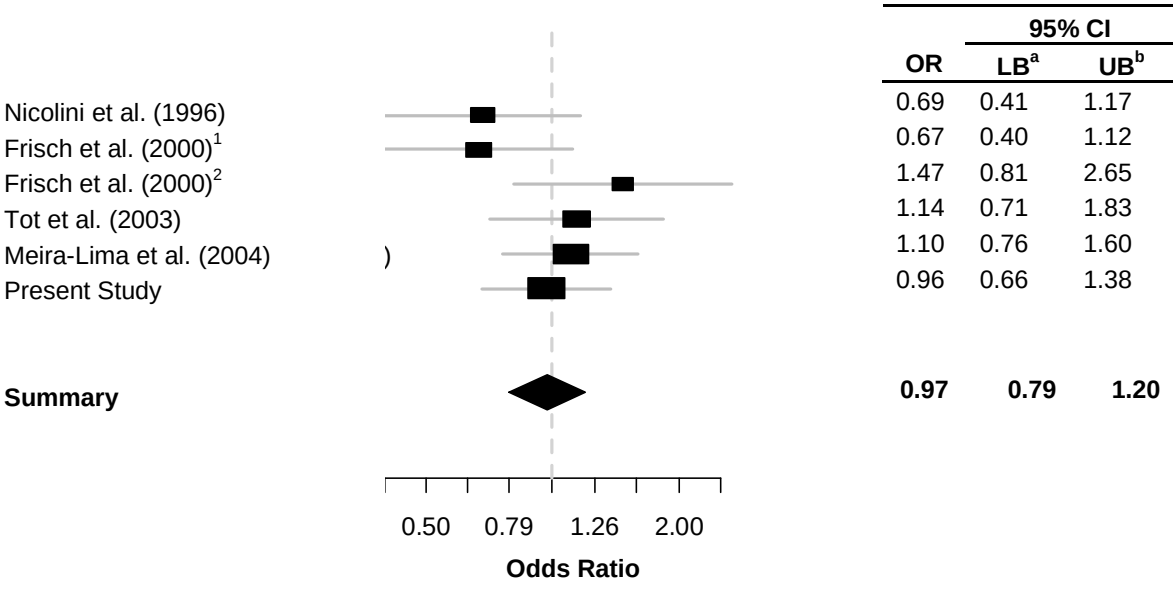



***Figure III.51.*** *Forest plot of the association between the* G-*allele of the* 5-$HT_{2A}$ -1438A/G *(rs6311) variant and OCD. Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

***Table III.73.*** *Frequency of the* 5-$HT_{2A}$ C102*-allele in case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of *5-$HT_{2A}$ C102* | |
|---|---|---|
| | OCD | Control |
| Nicolini et al. (1996) | 0.61 | 0.69 |
| Frisch et al. (2000) [1] | 0.45 | 0.55 |
| Frisch et al. (2000) [2] | 0.53 | 0.43 |
| Tot et al. (2003) | 0.51 | 0.48 |
| Meira-Lima et al. (2004) | 0.55 | 0.52 |
| Present Study | 0.61 | 0.62 |

**Abbreviations:** OCD: obsessive-compulsive disorder.




[1]*Ashkenazi Jews,* [2]*non-Ashkenazi Jews.*

***Figure III.52. Forest plot of the association between the* C*-allele of the* 5-$HT_{2A}$ T102C ***(rs6313) variant and OCD.*** *Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

### III.5.3. *5-HT$_{2C}$ cys23ser* (rs6318)

Three studies were included in the *5-HT$_{2C}$ cys23ser* meta-analysis, yielding a total of 281 OCD patients (135 males, 146 females) and 425 (162 males, 263 females) controls. All studies were found to be in HWE, and no heterogeneity was observed across the ORs (Q = 5.05, 3df; p = 0.17 for males and Q = 0.25, 3df; p = 0.97 for females). Given the location of *5-HT$_{2C}$* on the X-chromosome, male and female meta-analyses were conducted separately.

The frequencies of the *cys23*-allele (the *G*-allele) in the male and female case and control samples for each study included in the meta-analysis are presented in Table III.74. The individual and summary ORs and their corresponding 95% CIs pertaining to the male subsets in each analysis are presented in Figure III.53(a). Although a significant association between the variant and OCD was observed in the present study (**section III.4.1.3.1.**), no overall significance was detected when the studies were combined (OR = 0.85 [95% CI: 0.36-0.82]. As for the male subset, no statistically significant differences were noted for the female subset (OR = 1.09 [95% CI: 0.76-1.57]) (Figure III.53[b]).

### III.5.4 *DAT* 40bp VNTR

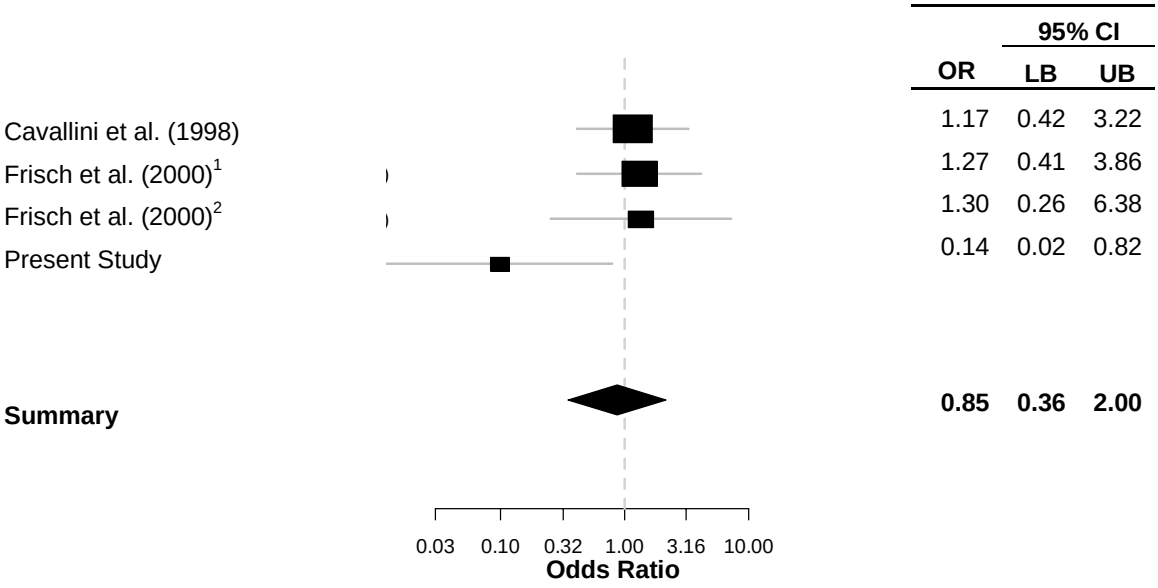
Two studies (Frisch et al., 2000 and the present study) were included in the meta-analysis, and only the two most common alleles (*A10* and *A9*) were used to construct the summary OR. One study was excluded due to inconsistencies in the data (Billet et al., 1998). The meta-analysis comprised a total of 181 OCD patients and 349 controls. The *A10*-allele was found to be most prevalent in both studies included in the meta-analysis. The frequencies of this allele in the case and control populations of the included studies are provided in Table III.75.

The individual and summary ORs and their 95% CIs are provided in Figure III.54. The summary OR and its 95% CI indicate the lack of association between the *DAT* 40bp VNTR and OCD. No heterogeneity across the ORs was noted(Q = 0.38, 2df, p = 0.826).

***Table III.74.*** *Frequency of the* 5-$HT_{2C}$ cys23-*allele in male and female case and control subjects in each of the studies included in the meta-analysis.*

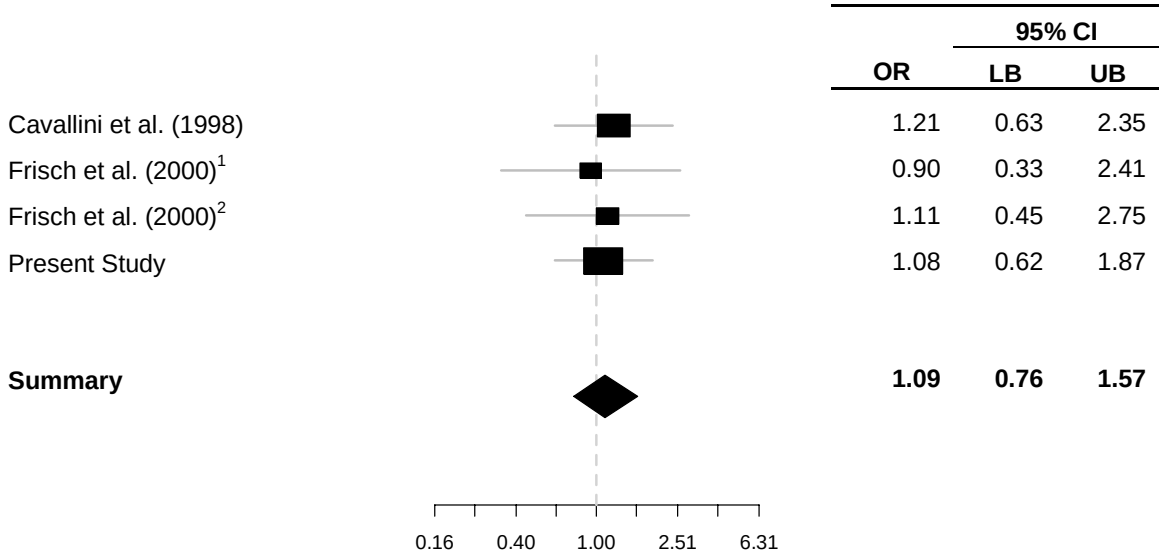
| Reference | Male | | Female | |
|---|---|---|---|---|
| | OCD | Control | OCD | Control |
| Cavallini et al. | 0.85 | 0.83 | 0.82 | 0.79 |
| Frisch et al. (2000) [1] | 0.76 | 0.71 | 0.83 | 0.84 |
| Frisch et al. (2000) [2] | 0.75 | 0.69 | 0.83 | 0.81 |
| Present Study | 0.78 | 0.97 | 0.77 | 0.75 |

[1]Ashkenazi Jews, [2]non-Ashkenazi Jews.




*[1]Ashkenazi Jews, [2]non-Ashkenazi Jews.*

***Figure III.53(a). Forest plot of the association between the*** **cys23-*allele of the* 5-$HT_{2c}$ cys23ser *(rs6318) variant and male OCD subjects***. *Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*



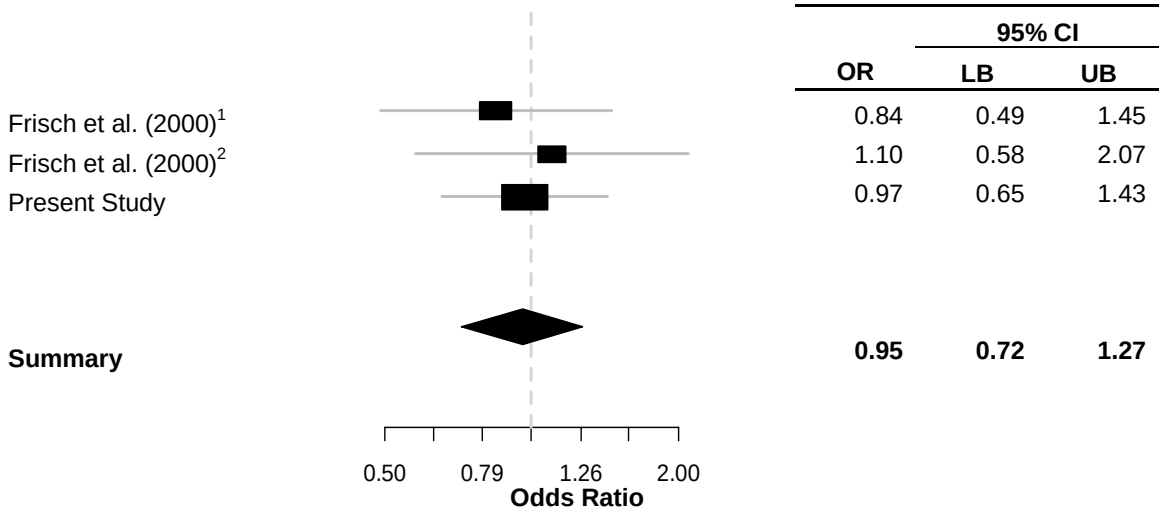

*[1]Ashkenazi Jews, [2]non-Ashkenazi Jews.*

***Figure III.53(b). Forest plot of the association between the* cys23-*allele of the* 5-HT$_{2c}$ cys23ser *(rs6318) variant and female OCD subjects**. Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

***Table III.75.*** *Frequency of the* DAT A10*-allele in male and female case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of *A10* | |
|---|---|---|
| | OCD | Control |
| Frisch et al. (2000) [1] | 0.61 | 0.65 |
| Frisch et al. (2000) [2] | 0.67 | 0.65 |
| Present Study | 0.75 | 0.75 |

[1]Ashkenazi Jews, [2]non-Ashkenazi Jews.




*[1]Ashkenazi Jews, [2]non-Ashkenazi Jews.*

***Figure III.54. Forest plot of the association between the* A10*-allele of the* DAT 40bp VNTR *variant and OCD.*** *Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

### III.5.5. DRD2 Taq1A (rs1800497)

Two studies (Nicolini et al., 1996 and the present study) were included in the meta-analysis investigating the role that the *DRD2 Taq1*A polymorphism (rs1800497) may play in the development of OCD. Data presented by Billet et al. (1998) was excluded, due to inconsistencies. The total number of OCD patients amounted to 173, whilst the controls reached 231. The frequencies of the *C*-allele in the case and control populations of each study included in the meta-analysis are presented in Table III.76.

The individual and summary ORs and 95% CIs are presented in Figure III.55. The 95% CIs indicate that no statistically significant associations were observed, either in the individual studies, or the meta-analysis. No heterogeneity across ORs was noted (Q = 0.84, 1df, p = 0.361), and both studies were in HWE.

No statistically significant differences were noted in *DRD2 Taq1A C*-allele frequency between the pooled case and control subjects (summary OR = 1.18 [95% CI: 0.86-1.63]).

### III.5.6. DRD3 *ser9gly*

Data from three studies were included in the meta-analysis (Catalano et al., 1994; Nicolini et al., 1996, and the present study). Data from one study (Billet et al., 1998) was excluded due to inconsistencies in their data. The total number of OCD and control subjects amounted to 260 and 275, respectively. The frequencies of the *A* (*ser9*)- allele in the OCD and control samples of each study included in the meta-analysis are represented in Table III.77. All studies were in HWE, and no heterogeneity across the individual ORs (presented in Figure III.56) was detected (Q = 1.92, 2 df, p = 0.382).

Although none of the included studies reported a significant association between the variant and OCD, the summary OR (OR = 1.30 [95% CI: 1.01-1.68]; Figure III.56) indicates a significant association between the *DRD3 ser9*gly variant, with the *A (ser9)*-allele representing a risk factor in the development of OCD.

***Table III.76.*** *Frequency of the* DRD2 C(A1)*-allele in male and female case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of *C* (*A1*) | |
|---|---|---|
| | OCD | Control |
| Nicolini et al. (1996) | 0.56 | 0.47 |
| Present | 0.75 | 0.74 |

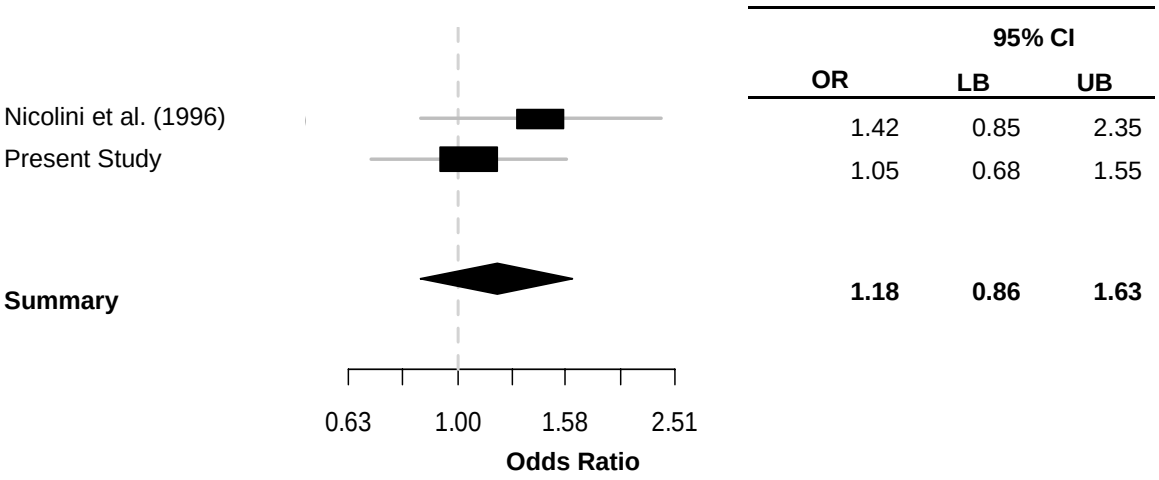



***Figure III.55. Forest plot of the association between the* C*-allele of the* DRD2 Taq1A (rs180094) *variant and OCD.*** *Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

***Table III.77.*** *Frequency of the* DRD3 A (ser9)*-allele in case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of *ser9* | |
|---|---|---|
| | OCD | Control |
| Catalano et al. (1994) | 0.66 | 0.57 |
| Nicolini et al. (1996) | 0.59 | 0.60 |
| Present Study | 0.72 | 0.64 |

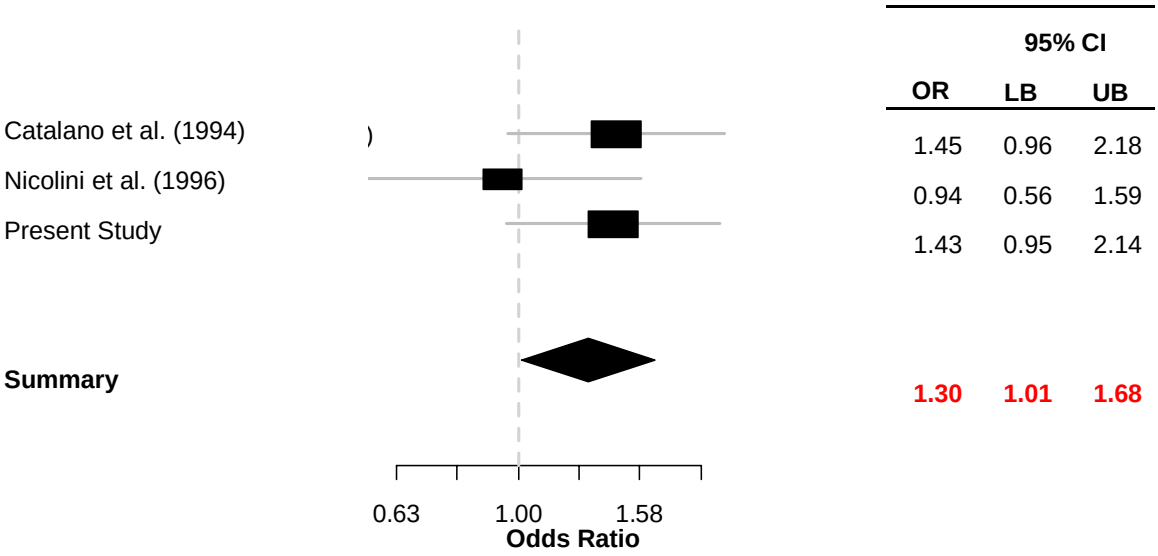



***Figure III.56. Forest plot of the association between the* ser9*-allele of the* DRD3 ser9gly (rs6280) ***variant and OCD****. Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

### III.5.7 *COMT val158met* (rs4680) meta-analysis

Five studies, including the present, were pooled in the *COMT val158met* (rs4680) meta-analysis, amounting to 321 OCD patients and 727 controls. No heterogeneity was detected across the ORs of the individual studies (Q = 5.46; 4df; p = 0.24). All studies were in HWE. Table III.78 presents the *G*(*val158*)-allele frequencies in the OCD and control populations in each of the studies. No evidence of association between the *val158met* variant and OCD was observed in the meta-analysis (summary OR = 0.81 [95% CI: 0.65-1.02]) (Figure III.57[a]).

Since previously reported associations between the *COMT val158met* variant and OCD have been found to be gender dimorphic (Karayiourgou et al., 1997; 1999; Alsobrook et al., 2002), it was of interest to stratify the sample according to gender. Unfortunately, due to the inability to extract gender data from some of the investigations, only the present study and that by Karayiorgou et al. (1997) could be included in the gender-specific meta-analyses. Table III.78(b) presents the frequency of the *G*(*val158*)-allele in male OCD and control subjects in these two investigations, which included a total of 88 male OCD and 110 male control individuals were included in the meta-analysis. No significant heterogeneity across ORs was noted (Q=1.43; 1df; p=0.23). The meta-analysis revealed a significant difference in allelic distribution of the variant, with the *G*(*val158*)-allele conferring protection against developing the disorder (Figure III.57[b]).

The genotype data from a total of 78 female OCD and 166 female control subjects was available for inclusion in the meta-analysis of the female subset. No significant differences in ORs between the two studies included in the meta-analysis were detected (Q=0.45; 1df; p=0.50). The *G*(*val158*)-allele frequency in the female OCD and control subsets in both studies is provided in Table III.78(c). From the Forest plot (Figure III.57[c], it is clear that, in contrast to the male subset, no significant differences in allele frequency were observed in the pooled female subset (summary OR = 0.90 [95% CI:0.62-1.35]).

***Table III.78(a).*** *Frequency of the* COMT G(val158)-*allele in case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of met*158 (G)* | |
|---|---|---|
| | OCD | Control |
| Karayiorgou et al. (1997) | 0.44 | 0.58 |
| Ohara et al. (1998) | 0.62 | 0.65 |
| Meira-Lima et al. (2003) | 0.59 | 0.62 |
| Erdal et al. (2004) | 0.60 | 0.57 |
| Present Study | 0.46 | 0.52 |

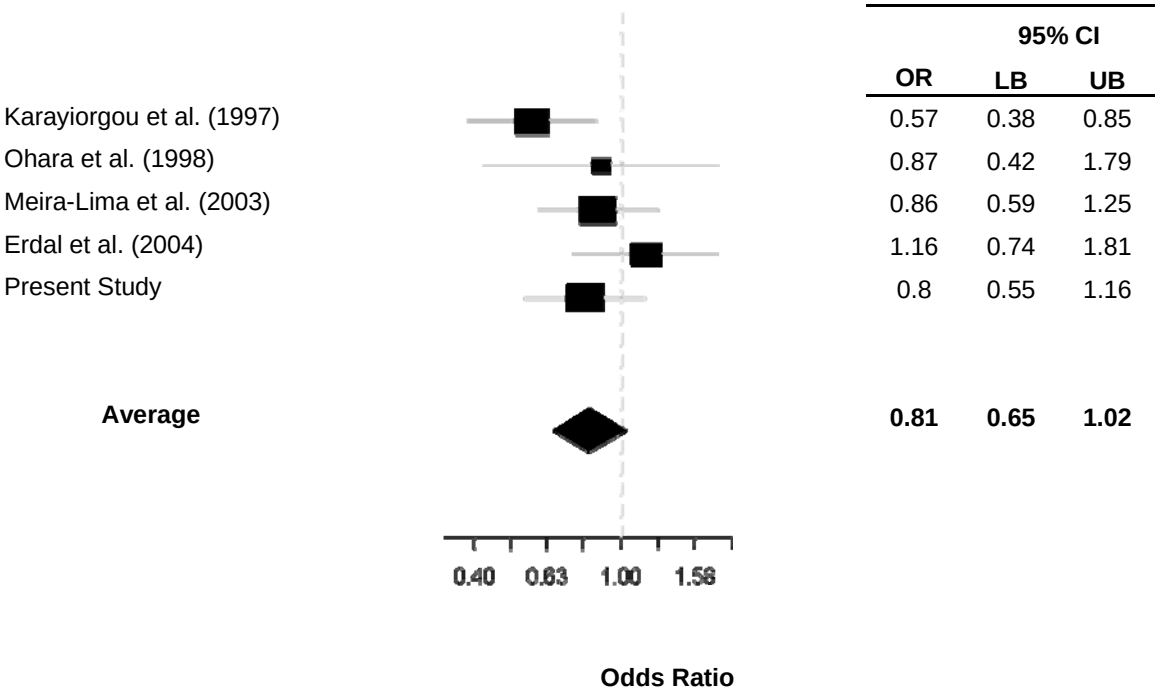



***Figure III.57(a). Forest plot of the association between the*** **val158-*allele of the* COMT val158met (rs4680)** ***variant and OCD.*** *Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

***Table III.78(b).*** *Frequency of the* COMT G(val158)*-allele in male case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of met*158 (G)* | |
|---|---|---|
| | OCD | Control |
| Karayiorgou et al. (1997) | 0.32 | 0.57 |
| Present Study | 0.50 | 0.63 |

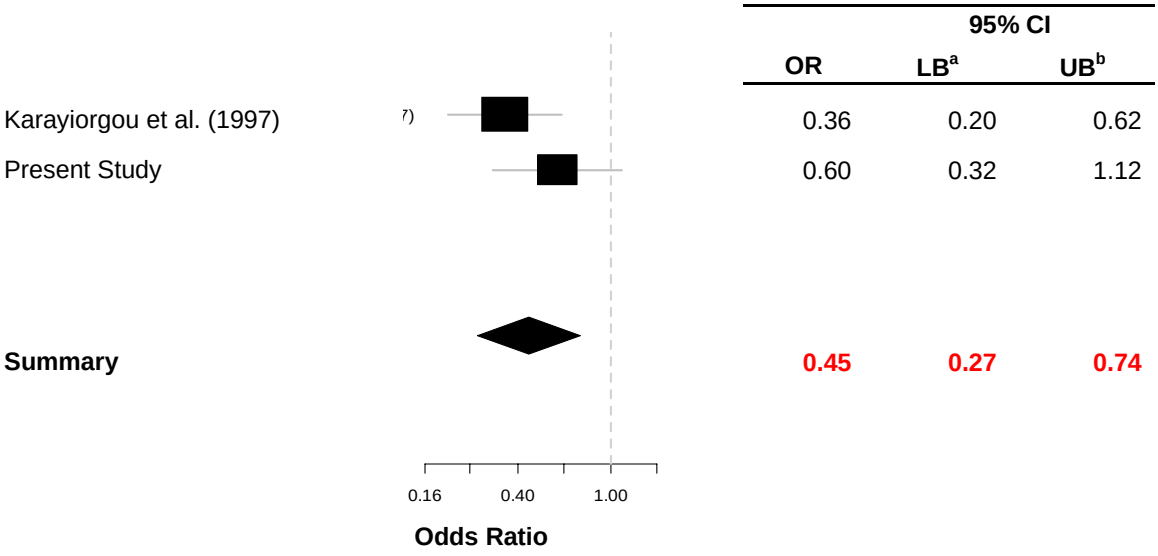



***Figure III.57(b). Forest plot of the association between the* val158*-allele of the* COMT **val158met (rs4680)** ***variant and OCD in males****. Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

***Table III.78(c).*** *Frequency of the* COMT G(val158)*-allele in female case and control subjects in each of the studies included in the meta-analysis.*

| Reference | Frequency of met*158 (G)* | |
|---|---|---|
| | OCD | Control |
| Karayiorgou et al. (1997) | 0.60 | 0.58 |
| Present Study | 0.43 | 0.48 |

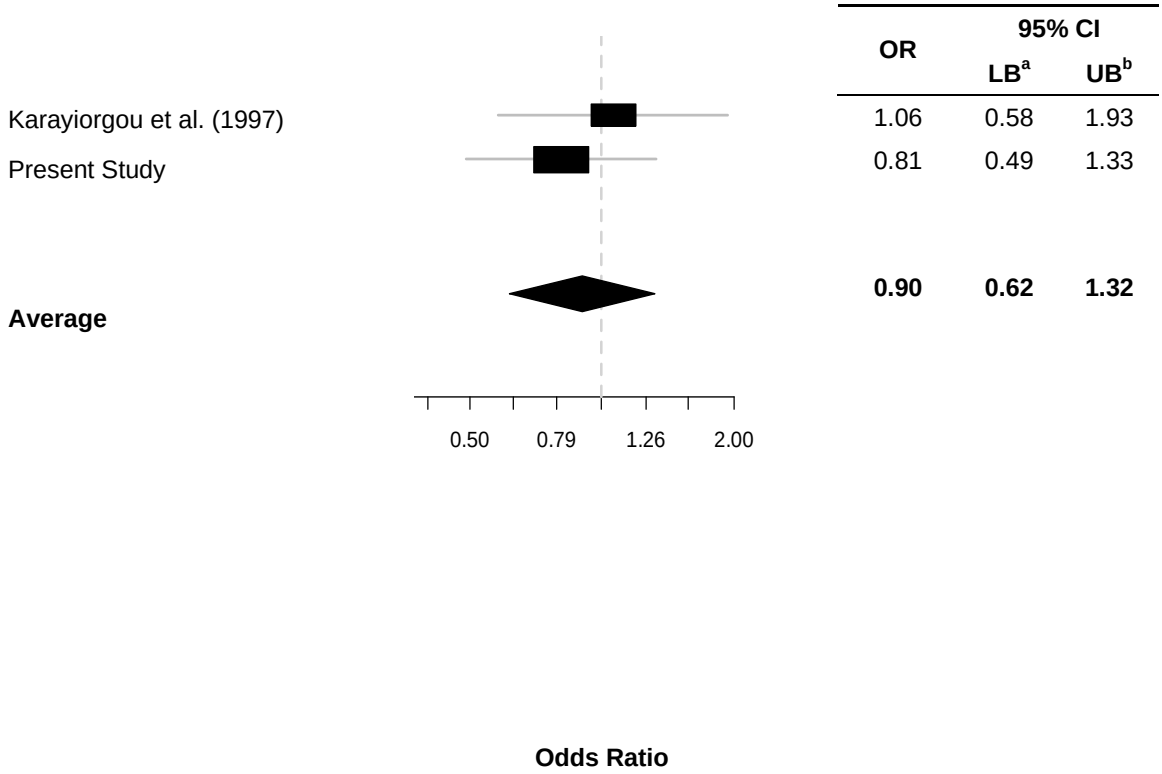



***Figure III.57(c). Forest plot of the association between the*** **val158-*allele of the* COMT val158met (rs4680)** ***variant and OCD in females.*** *Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

### III.5.8. *DRD4* 48bp VNTR

Three studies were included in the present meta-analysis of the *DRD4* VNTR polymorphism, amounting to 209 OCD and 386 control subjects. The effects of the three most prevalent alleles, *A2*, *A4* and *A7*, were investigated in separate analyses. The *A4*-allele was found to be the most prevalent in all of the included studies. The frequencies of the *A2*, *A4* and *A7*-alleles in the OCD and control samples of all studies included in the meta-analyses are presented in Table III.79.

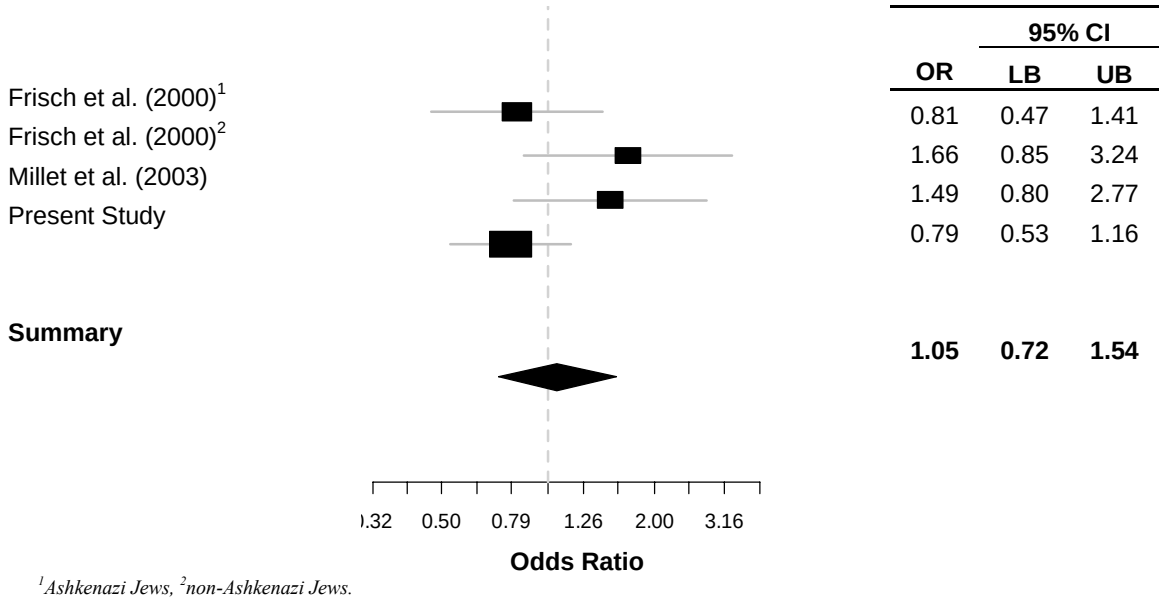
All studies were in HWE, and no heterogeneity across the individual ORs were detected when the meta-analyses were conducted according to the presence or absence of the *A4*-allele (Q = 5.76, 3df, p = 0.12). However, a significant amount of heterogeneity was noted between the studies when data was grouped according to the presence or absence of the *A2*- and *A7*-alleles (Q = 6.63, 3df, p = 0.08 for the *A2*-analysis; Q = 11.88; 3df, p=0.01 for the *A7*-analysis). Meta-analyses were thus not performed according to the presence or absence of either *DRD4 A2* or *A7*-alleles.

Figure III.58 represents the ORs and corresponding 95% CIs for the individual studies included in the meta-analysis to determine whether the *A4*-allele conferred an increased risk to the development of OCD. The pooled OR and corresponding 95% CIs indicate that the *A4*-allele did not play a role in the mediating the development of OCD (OR = 1.05 [95% CI: 0.72-1.54].

***Table III.79.*** *Frequency of the* DRD4 48bp VNTR A2, A4 and A7 *alleles in case and control subjects in each of the studies included in the meta-analysis.*

| Reference | *A2*-allele | | *A4*-allele | | *A7*-allele | |
|---|---|---|---|---|---|---|
| | OCD | Control | OCD | Control | OCD | Control |
| Frisch et al. (2000) [1] | 0.08 | 0.04 | 0.67 | 0.71 | 0.20 | 0.21 |
| Frisch et al. (2000) [2] | 0.11 | 0.09 | 0.76 | 0.65 | 0.09 | 0.23 |
| Millet et al. (2003) | 0.02 | 0.10 | 0.80 | 0.72 | 0.10 | 0.01 |
| Present Study | 0.11 | 0.09 | 0.63 | 0.69 | 0.19 | 0.17 |

[1]Ashkenazi Jews, [2]non-Ashkenazi Jews.




*[1]Ashkenazi Jews, [2]non-Ashkenazi Jews.*

***Figure III.58. Forest plot of the association between the* A4-*allele of the* DRD4 48bp VNTR *and OCD.*** *Each study is indicated by the reference, OR point estimate and 95% CI. The summary refers to the meta-analysis OR by random effects model. Studies are ordered by year of publication.*

# CHAPTER IV
# DISCUSSION

**INDEX** **PAGE**

**PAGE**

# CHAPTER IV: DISCUSSION

*"The difficulty lies, not in the new ideas, but in escaping the old ones"*
*John Maynard Keynes, English economist*

OCD represents a psychiatric disorder with a complex multifactorial inheritance. Presently, it is believed that individual susceptibility to the disorder is governed by the conjoint effects of variation at an, as of yet, undetermined number of genes, and the collective effect of various environmental exposures encountered during an individual's life. Case-control association studies represent an important tool in dissecting the genetic aetiology of complex disorders. Over the last decade, a large number of association studies have been dedicated to disentangling the genetic components that may be involved in the aetiology of OCD. However, numerous potential caveats limit the success of case-control association studies; not surprisingly, very few of the initially exciting positive findings in the OCD field have been replicated, with inconsistent results between studies. The paucity of robust findings is probably the result of a mixture of a lack of sufficient phenotypic resolution to identify genetic risk factors, and the lack of application of the appropriate statistical methodology.

The aim of the present study was thus two-fold: firstly, to assess the factors that may confound case-control genetic association studies of complex disorders, and secondly, to investigate the genetic aetiology of OCD, a complex psychiatric disorder, and clinically-defined OCD-related subtypes, avoiding the identified pitfalls.

## IV.1. POPULATION STRATIFICATION

Generally, in this type of study, it is customary for subjects to be classified as being of a particular ancestral grouping based on self-reported ancestry: in this study, following convention, at least three of their four grandparents had to be of Afrikaner descent (**section II.1**). This self-reported ancestry method is thought by some investigators to be adequate to prevent stratification (Morton and Collins, 1998), but others have disagreed (Freedman et al., 2004).

The Afrikaner population has often been used in case-control association studies, as the Afrikaner is thought to be a homogeneous group. However, until now, no empirical data existed to support the absence of cryptic subpopulations within the Afrikaner population that may confound an association study. Recently, proof of substructure in populations originally

thought to be genetically homogeneous has been reported, for example, the Icelandic population (Helgason et al., 2005). Hence, to *a priori* assess the absence of population stratification and thus the validity of using the Afrikaner population in a case-control association study, *Structure*, a model-based clustering algorithm that identifies clusters of related individuals from multilocus genotypes, was implemented in the present study. The number of clusters, which was chosen by the author to vary from K=1 to K=5, was based on the reported ancestry of the Afrikaners: the population has been proposed to originate from five Northern European populations: Dutch, German, French, Belgian and British (Botha and Beighton, 1983).

No evidence was observed for cryptic population substructure in either the Afrikaner OCD or control population used in the current genetic analyses: classification of the Afrikaner individuals into clusters demonstrated symmetry, with roughly the same proportion of each individual's genome assigned to each cluster (Table III.2). Varying K to larger values (up to 12) had no effect on the result (results not shown). The evidence thus suggested that cases and controls were ethnically and genetically matched, and not subject to population stratification with respect to each other, supporting the use of at least these particular samples in case-control association studies.

Of course, *Structure* does not represent the only means by which one can detect and control for population structure in genetic association studies. Other methods, each with their respective merits, do exist, for example the GC method (**section I.4.1.1.3[ii]**). However, *Structure* was chosen over the GC method in the present study, largely because it provides one with a more meaningful characterisation of individuals into specific subpopulations (if they exist), which could not only be used in subsequent genetic investigations, but may also aid consequent genealogical investigations. The GC method, on the other hand, provides one with only an average correction factor that can be implemented in subsequent genetic association studies.

A limiting factor to detecting population substructure in the present investigation is that, if substructure within the Afrikaner population does exist, it is likely to be very subtle, given their past geographical and cultural isolation, and derivation from geographically closely related Northern European groups. Detection of such subtle substructure may require the use of many more markers, increasing the amount and cost of genotyping to a very large degree.

It should also be noted that the most informative marker to use when investigating whether population stratification exists would be those that possess large frequency differences between the proposed subpopulations (Campbell et al., 2005). It is thought that the so-called ancestry-informative markers that are currently available will not be sufficient to detect structure in closely related populations: identification of such markers in the Afrikaner population will entail assessing the frequencies of a large number of variants across the proposed contributing sub-populations. However, even if, as yet undetected subtle population substructure amongst Afrikaners does exist, it is unlikely that the frequency of OCD between these subpopulations will be significantly different, decreasing the potential for confounding by population stratification.

## IV.2. GENETIC ANALYSES

OCD is a clinically heterogeneous disorder: many clinical subtypes of the disorder, which may be mediated by different aetiological mechanisms, have been reported (Ball et al., 1996; Mataix-Cols et al., 2000; Ravindran et al., 1999). These subtypes may thus represent intermediate phenotypes that are more proximal to a particular genetic substrate than the higher order construct of the disorder, and may therefore provide a stronger genetic signal, with correspondingly greater effect size.

However, although the phenotypic heterogeneity of OCD is fast becoming recognised, and indeed utilised, in genetic association studies (as discussed in the forthcoming sections), the formal, dichotomous diagnosis of OCD may still provide insight into which genes play a role in contributing to the overall aetiology, and may provide important clues with regard to the pathophysiology of the disorder. The following sections highlight important findings from the present population-based genetic association study, beginning with the overall case-control association analyses, and moving onto the various subtype analyses performed.

### IV.2.1. Unstratified Case-control Association Analyses

No statistically significant associations were noted when any of the candidate markers were analysed in a single locus context in the unstratified sample. However, when the *DRD4* VNTR and *-521C/T* (rs1800955) variants were analysed as a haplotype, significantly more controls were found to carry the *-521C/T*-VNTR *C-A4* haplotype ($p = 0.049$). Moreover, significantly more cases were found to carry the *T-A2* haplotype compared to controls ($p = 0.026$) (Table III.12).

It is interesting to note that both polymorphisms possess proposed functionality, with the alleles implicated in susceptibility to OCD depressing the functionality of the DRD4 receptor: the *T*-allele of the *-521C/T* SNP has been found to result in a decrease in expression of *DRD4* by as much as 40% in comparison to the *C*-allele (Okuyama et al., 1999), while, compared to the *A4*-allele, the VNTR *A2*-allele has been found to possess a blunted cAMP response (Asghari et al., 1995) (**section I.6.1.2.1[i]**). As neither of the alleles was found to contribute to OCD when analysed separately, the haplotypic association may be due to *cis*-interaction between the alleles in the promoter variant and the exon 3 VNTR. This points to a threshold effect, where OCD susceptibility is increased when available DRD4s are reduced both in number and in function.

One should, however, bear in mind that, whilst haplotype-based analyses may represent a somewhat statistically more robust model compared to single locus analysis, the unambiguous construction of haplotypes from genotypic population-based data is not possible, especially for heterozygotes. Consequently, the eventual reverse classification into haplotype-based diplotypes is approximate, based on haplotype estimates and posterior probabilities. It is thus likely that the eventual statistical significance may be overstated, signifying the requirement for independent replication of the present observation.

### IV.2.2. Subtype Analyses

One of the main reasons for lack of replication amongst association studies investigating complex disorders such as OCD may be the phenotypic heterogeneity of the disorder. Subtyping according to clinically-defined variables reduces background "noise" and consequently increases the power to detect small effects. In the present investigation, OCD was subtyped according to gender, severity of the symptoms (assessed using the total Y-BOCS score), age at onset of the disorder, co-morbidity (MDD and tics) and symptom dimensions (hoarding, symmetry/ordering, sexual/religious, contamination and aggressive symptoms).

#### IV.2.2.1. Stratification by gender

Although epidemiological studies have indicated that the male:female ratio in OCD is approximately 1:1 (**section I.2.1**), it has been hypothesised that OCD may exhibit features of gender dimorphism, particularly with regard to symptomatology and co-morbidity (Karayiorgou et al., 1999; Bogetto et al., 1999; Castle et al., 1995; Enoch et al., 2001; Baer et

al., 1993). Clinically, male OCD patients in previous studies have often been found to have a higher frequency of co-morbid social phobia, alcohol abuse and tic disorders, whilst females have been found to experience a higher frequency of co-morbid eating disorders and MDD (Bogetto et al., 1999). As far as symptomatology is concerned, prominent sexual, exactness and symmetry obsessions have previously been found to be more prominent amongst male OCD patients (Lensi et al., 1996), whilst female OCD subjects have been found to exhibit more frequent contamination and washing rituals compared to male OCD patients (Castle et al., 1995; Minichiello et al., 1990; Rasmussen and Eisen, 1990). Indeed, in the present study, significantly more males were found to exhibit sexual and religious obsessions, corroborating results from previous studies (Lensi et al., 1996), whilst females were found to experience significantly higher frequencies of specific phobia and anorexia as co-morbid disorders (**section III.4.1.2;** Table III.7).

It has been proposed that such gender differences may be due to epigenetic hormonal influences that affect the manifestation of the disorder (Karayiorgou et al., 1999) and are apparent at a genetic level as well. Indeed, sexually dimorphic effects have been noted in genetic association studies of the role of *COMT* (Karayiorgou et al., 1997; 1999), *MAO-A* (Camarena et al., 1998; 2001; Karayiorgou et al., 1999) and *5-$HT_{2A}$* (Enoch et al., 2001). In the present study, when the total sample was stratified by gender, differences in genotype and allele frequencies of the *5-$HT_{2C}$ cys23ser* (rs6318), *COMT* rs362204, *BDNF val66met* (rs6265) and *ACE Alu* ins/del variants were noted between male OCD and control individuals; these findings are discussed below.

#### *IV.2.2.1.1. 5-$HT_{2C}$ cys23ser*

In the present study, the X-linked *5-$HT_{2C}$ cys23ser* (rs6318) *G* (*cys23*)-allele was found to confer protection against the development of OCD in males (Table III.9). This gene has been proposed to play a role in grooming behaviour (Heisler and Tecott, 1999; Graf et al., 2003), and in the manifestation of compulsive-like syndromes (Chou-Green et al., 2003).

Contradictory results have been obtained with regard to the functionality of the *cys23ser* variant: it has been found to increase CSF MHPG (the major metabolite of norepinephrine) in Finnish males carrying the *C* (*ser23*)-allele (Lappalainen et al., 1999), but this result was not replicated in a more recent study conducted on a Swedish sample (Jonsson et al., 2004) (**section I.6.1.1.1[iib]**). On the other hand, a recent functional analysis suggest that the 5-

$HT_{2C}$ receptor containing the *C* (*ser23*)-allele may be constitutively more active than one containing the *G* (*cys23*)-allele (Okada et al., 2004).

In the light of the uncertainty regarding the functionality of the variant, the reasons for the gender-biased effects require speculation, although it is tempting to consider that the *5-$HT_{2C}$* gene being X-linked may play a role. Indeed, previous studies have indicated a sexually dimorphic effect between another X-linked gene, *MAO-A*, and OCD in males (Camarena et al., 1998; Karayiorgou et al., 1999). Although in general, X-linked genes undergo random X-inactivation in females, a mechanism which is proposed to increase similarity amongst males and females, recent evidence suggests that at least one out of every five X-linked genes escapes X-inactivation (Carrel et al., 1999), which could result in differences between the sexes due to the imbalanced expression of the gene product. Therefore, one could hypothesise that, if *5-$HT_{2C}$* does indeed harbour the disease susceptibility variant (assumed to decrease expression of the gene), and it represents one of the genes that escapes X-inactivation, females carrying the disease susceptibility variant may express sufficient amounts of the product due to the presence of two copies of the gene. On the other hand, males carrying the disease susceptibility variant may express lower levels of 5-$HT_{2C}$, which, in conjunction with other environmental and genetic factors, may deem them more susceptible to OCD than their female counterparts.

The present finding does contradict results from two previous case-control association studies, where no evidence to support a role for the variant in mediating the development of OCD was found (Cavallini et al., 1998; Frisch et al., 2000). The inconsistencies could be due to a number of factors, including ethnic differences: Cavallini et al. (1998) utilised an Italian population, whilst Frisch et al. (2000) utilised non-Ashkenazi and Ashkenazi Jewish populations). Given the present uncertainty regarding the functionality of the variant, a possible explanation for the discrepancies in the genetic findings is that the *cys23ser* variant is not the causal variant, but may be in LD with the susceptibility variant in the *5-$HT_{2C}$* gene, or one nearby, in the Afrikaner population, but not in the Italian or Jewish populations.

One cannot, of course, dismiss the possibility that the present observation represents a Type I error, due to the relatively small number of male control subjects. On the other hand, the possibility does also exist that the two negative associations (Cavallini et al., 1998; Frisch et al., 2000) were not sufficiently powered to accept the null hypothesis with certainty. In an

attempt to resolve the discrepancies regarding the results of the association studies, at the same time increasing the power of the study, a meta-analysis was conducted, the results of which indicated no role for the variant in mediating the development of OCD, in either males or females. However, it would be unwise to completely dismiss the association, as again, the meta-analysis comprises a relatively small sample size, and thus probably does not possess the power to reject the alternative hypothesis with absolute certainty. The results can thus best be described as inconclusive: a suggestion would be to perform independent studies using larger samples of males and females, and to eventually pool the data appropriately in a meta-analysis that does possess sufficient power to refute or accept the null hypothesis.

#### *IV.2.2.1.2. COMT val158met and rs362204*

The allelic distribution of the *COMT* rs362204 insertion/deletion polymorphism was found to be significantly different in male cases and controls. Specifically, the *D*-allele (characterised by the deletion of a cytosine base) was found to confer an increased risk to developing OCD in males (OR = 2.88) (Table III.9). The rs362204 variant has thus far not been found to be functional (DeMille et al., 2002), although it is situated immediately 3' to the stop codon in exon 6, and as such, may affect gene transcription. Moreover, in the present study, the *D*-allele was found to exhibit a measure of pairwise LD with the *val158met A*-allele, which has been found to be associated with an increased susceptibility OCD in males in previous studies (Karayiorgou et al., 1997; 1999), and in a meta-analysis performed in the present study.

Disappointingly, however, haplotype analyses involving the *COMT val158met*, rs362204 and promoter rs2097603 variants yielded no statistically significant differences (p = 0.355) (Table III.13), although the frequency of the *val158met*-rs362204 *A*(*met158*)-*D*-haplotype was found to be increased in the OCD population compared to controls (32.8% versus 23%, respectively) (data not shown). It may thus be that the sample size included in the haplotype analysis is too small to attain sufficient power to reject the null hypothesis completely. On the other hand, the lack of complete LD between the *val158met* and rs362204 variants with the promoter variant (rs2097603), which was also included in the haplotype analysis, could result in the "dilution" of any association the *COMT val158met*-rs362204 haplotype has with OCD. However, haplotype analysis involving only the latter two variants also yielded no statistically significant results, although, once again, the frequency of the *val158met*-rs362204 *A-D* variant was higher in the OCD, compared to the control, population (data not shown).

Therefore further investigation, in a dataset large enough to retain optimal power after stratifying according to gender and haplotype, is warranted.

The *COMT val158met* (rs4680) variant has, to date, been one of the most extensively genotyped *COMT* polymorphism in psychiatric association studies, including studies of OCD. The present study represents an extension of an original investigation involving the *val158met* polymorphism and OCD in an Afrikaner population (Niehaus et al., 2001). In the latter, an association was observed between the heterozygous *val158met* genotype and the disorder. In the present study, however, this association disappears; no statistically significant differences in allele or genotype frequency were observed between the OCD subjects and controls of either gender. A possible reason for the discrepant results may be the preliminary nature of the Niehaus et al. (2001) investigation: only 54 OCD and 54 control subjects were included in the study (compared to 93 OCD and 128 controls in the present study (Table III.3[b]). This indicates, as the authors themselves stated, the possibility that the results may represent a spurious association between the *COMT val158met* variant and OCD.

Indeed, two recently conducted meta-analyses (one based on available case-control information, one based on available family-based information) yielded little evidence for the involvement of the *COMT val158met* polymorphism in the development of OCD (Azzam et al., 2003). However, it should be mentioned that those authors did note an association between the *A*(*met158*)-allele and OCD in the case-control meta-analysis, but only when using the less conservative fixed-effects model (Azzam et al., 2003). Due to the extra data obtained from the present study, and two studies investigating the relationship between the *COMT val158met* variant and OCD, being published subsequent to the publication of the meta-analysis by Azzam et al. (2003) (Erdal et al., 2003; Meira-Lima et al., 2004), a case-control meta-analysis was conducted in the present study.

As was found by Azzam et al. (2003), when the data was analysed in its entirety, no statistically significant differences were observed, although the present meta-analysis did result in narrower 95% CIs than were obtained by Azzam et al. (2003). In view of the fact that gender bias that has often been noted in associations between *COMT val158met* and OCD, the meta-analytic data was stratified according to gender, where possible. Interestingly, in contrast to the results attained by Azzam et al. (2003), who observed no significant differences when meta-analyses were conducted according to gender, results from the present

study indicate that a significantly higher proportion of male controls carry the *G*(*val158*)-allele, compared to male OCD subjects, thus again implicating the *A*(*met158*)-allele allele as a susceptibility allele (**Figure III.57[b]**).

These results are intriguing for a number of reasons. The *COMT val158met* polymorphism has been found to be functional: the *A*-allele (coding for the *met158* codon) is associated with a three-to fourfold reduction in COMT enzymatic activity (**section I.6.1.2.1[vi]**). The main function of this enzyme is to catabolise certain catecholamines, including dopamine. Consequently, a reduction in activity may result in the accumulation of extraneuronal dopamine, eventually resulting in a hyperdopaminergic state, which, in mice, has been found to elicit behaviour reminiscent of OCD or TS (Berridge et al., 2005) (**section I.6.1.2**).

The gender-biased results are also interesting, given that the influence of gonadal steroids on brain development has been found to be associated with sex differences in brain organisation, neuropsychological performance and learning and memory function (McEwen et al., 1997). Indeed, the expression of MB-COMT is driven by an ERE, indicating that estrogen regulates the expression of the gene. Moreover, unpublished results, presented at the Endocrine Society's annual meeting in San Diego (2005) indicated that male mice who were deficient in aromatase (which converts testosterone to estrogen) exhibited OCD-like behaviour: they ran excessively on their running wheels and spent twice as long grooming themselves compared to control littermates. Interestingly, they also possessed lower levels of *COMT*. Female mice deficient in estrogen did not exhibit the same patterns of behaviour, suggesting that estrogen interacts with certain components in the male and female brain differently. It may well be these differences in the interactions between estrogen and COMT that bring about the gender-biased association observed in the present study. Further investigation is, however, required to elucidate the exact mechanism by which changes in the estrogen-COMT interaction may bring about the clinical phenotype in males more often than in females.

#### *IV.2.2.1.3. BDNF val66met*

*BDNF* was selected as a candidate gene in the present study on the basis of its pivotal role in neurodevelopment in the brain. Three SNPs (rs988748, rs2049046 and *val66met* [rs6265]), each exhibiting a high degree of pairwise LD (Table III.6), were included in the present study. Significantly different *val66met* allele frequencies were observed between male case and control individuals, with the *G*(*val66*)-allele found to represent the protective allele, with a

greater frequency amongst the male control individuals (Table III.9). The *A* (*met66*)-allele has previously been found to affect the intracellular processing of the pro-BDNF polypeptide, thereby inhibiting the release of BDNF from activated neurons (Egan et al., 2003). This allele has also been implicated as the risk allele in a restrictive subtype of anorexia nervosa (Ribases et al., 2003; 2004; 2005), which has features in common with OCD. In a more recent study, Jiang et al. (2005) concluded that the *met66*-allele may act as a risk factor in the development of anxiety disorders.

The sex-selective effect of the *BDNF val66met* on OCD is interesting, since it has recently been proposed that estradiol may bring about its structural and functional effects in the brain via the action of intermediate signalling molecules such as growth factors (Miranda et al., 1994; Scharfman and MacLusky, 2005). Indeed, *BDNF* has been found to possess a sequence similar to the EREs noted in other genes that are estrogen-regulated, such as *COMT* (Sohrabji et al., 1995). In the same study, the authors observed an estrogen-mediated increase in *BDNF* mRNA expression in the cerebral cortex and olfactory bulb in rats. Further impetus for the notion that BDNF is regulated by estrogen via ERE is the observation that ESRα have been found to co-localise with BDNF in male and female rat brains (Solum and Handa, 2001).

Moreover, BDNF and estradiol have been found to possess similar effects, including the modulation of NMDA receptors (Lu, 2003; Foy et al., 1999), most notably GRIN2B (Yamada and Nabeshima, 2003; Adams et al., 2004) which has been investigated as a candidate gene in the present study, and has subsequently been found to be associated with severity and age at onset of OCD (**section IV.2.3.1 and IV.2.4.1**). Furthermore, like BDNF, estrogen has been found to be associated with neuronal differentiation and survival, and to play an important role in brain development by influencing the maturation of neural systems and by affecting sexual differentiation of brain structure and function (Solum and Handa, 2001). Moreover, in males with epilepsy, a disorder thought to be mediated via a dysfunction in estrogen-BDNF interaction, serum estrogen concentrations have been found to rise, and it is thought that this might contribute to the subsequent seizures (Herzog, 1999; Murialdo et al., 1994). Thus, given the close relationship between BDNF and estrogen, it is plausible that the gender-specific effects in OCD are mediated via a dysfunction in the estrogen-BDNF interaction.

***IV.2.2.1.4. ACE Alu ins/del***

One of the genes characterised as a "novel" candidate in the present study, *ACE*, was found to be associated with the formal, dichotomous diagnosis of OCD. Here, the *DD*-genotype was found be protective against the development of OCD (Table III.8). This observation is interesting, given that *ACE D*-allele carriers have been shown to have higher plasma ACE concentrations (Rigat et al., 1990), although this effect has been proposed to be a result of polymorphisms in LD with the *ACE Alu* ins/del (Tiret et al., 1992; Cox et al., 2002), and the effect of these polymorphisms on brain ACE levels have yet to be determined. However, the possibility does exist that *ACE D*-allele carriers will possess a higher capability for the hydrolysis of angiotensin I into AngII, and for the degradation of SP and other neuropeptides (**section I.6.1.5.4**).

AngII has been found to co-localise and indeed stimulate, certain types of neurotransmission, including dopamine, in various regions of the CNS, including the striatum and substantia nigra (Jenkins et al., 1995; Hasenörhl et al., 2000; Mendelsohn et al., 1993; Reardon et al., 2000). To this end, it has also been found that ACE inhibitors increase dopaminergic neurotransmission in the brain (Jenkins et al., 1997; Reardon et al., 2000). From these results it could be suggested that a decrease in activity in ACE is likely to result in an increase in dopaminergic neurotransmission. It may thus be that *ACE Alu* ins/del *II*-carriers, due to their lower ACE activity, may experience an increase in dopaminergic neurotransmission, which is thought to underlie some aspects of OCD pathology. However, this remains speculative, because, at present, the exact mechanism by which ACE interacts with the dopaminergic system is unclear, and the role of other substrates upon which ACE acts cannot be excluded.

Indeed, the role that ACE plays in the degradation of SP may also be relevant to the present association: *D*-allele carriers will possess higher SP degradation capabilities, which may confer protection against the development of OCD. Indeed, antagonism of the SP-NK1R pathway has been found to result in antidepressant and anxiolytic effects (Kramer et al., 1998); a decreased SP concentration in *D*-allele carriers may thus have resulted in the observed protective effect. Moreover, in a recent genetic association study, Baghai et al. (2004) observed that depressed females carrying at least one *ACE D*-allele exhibited a better therapeutic outcome with different antidepressants, compared to *II*-carrying females, and males.

A gender-biased effect in the direction of males was also observed in the present study, with the *D*-allele and *DI*- and *DD*-genotypes implicated as the protective factors (Table III.9). The sex-specific effect may be due to the influence of gonadal steroids on the expression of *ACE.* For example, estrogen has been found to reduce *ACE* mRNA concentrations, thereby regulating tissue ACE protein activity (Gallagher et al., 1999), whilst testosterone has been found to enhance the activities of *ACE* in various animal studies (Jaiswal et al., 1985; Freshour et al., 2002). However, these investigations concentrated on the expression of *ACE* in peripheral tissues, and not in the CNS. The role that the gonadal hormones may play in the regulation of ACE activity in the brain thus remains speculative.

The present study represents the first to show an association between OCD and the *ACE Alu* ins/del polymorphism, and requires independent replication in a larger sample. Moreover, given the recent proposition that the *ACE Alu* ins/del does not function independently in determining ACE levels, it would be of interest to perform haplotype-based investigations, utilising polymorphisms across the length of the gene (Cox et al., 2002).

### IV.2.3. Severity of OCD (as assessed by total Y-BOCS score)

The quantitative phenotype of OCD, as defined by the total Y-BOCS score, was used in addition to the formal diagnosis of OCD in genetic analyses, as the use of quantitative instead of categorical variables can increase the power of a study. This is because categorising a continuous variable results in a reduction of the amount of information available, and a consequent lack of sensitivity. In the present study, associations were noted between three genes and total Y-BOCS score; these are discussed below.

#### IV.2.3.1. *GRIN2B*

First, when the entire OCD population was considered, the *GRIN2B* rs890 gene (analogous to the *5072T/G* polymorphism investigated in a family-based study by Arnold et al. [2004]) was found to be associated with the severity of the disorder. OCD subjects carrying the *CC*-genotype were found to exhibit more severe forms of the disorder, as evidenced by the higher total Y-BOCS score (Table III.18[c]). Interestingly, Arnold et al. (2004) found that an increased Y-BOCS score in their family-based sample was associated with a trend for increased transmission of the *5072G*-allele (corresponding to the rs890 *C*-allele in the present study) under the recessive model, and with a decreased transmission of the *5072T*-allele under the dominant model.

The *GRIN2B* rs1806191 variant, situated in exon 13, was not found to be associated with severity of the disorder in the single locus analysis; however, haplotype analysis revealed that the rs1806191-rs890 *A-C* haplotype was associated with a significantly higher Y-BOCS score (and subsequently a more severe form of OCD) compared to the other haplotypes ($p = 0.021$) (Table III.24). On the other hand, the more common *G-A* haplotype was found to be significantly associated with a lower Y-BOCS score compared to the other haplotypes ($p = 0.022$) (Table III.24).

This finding is interesting, since a two-locus haplotype (*G-T*), combining the rs890 (*5072G/T*) variant and the *5988T/C* variant situated in the 3'UTR, was found by Arnold et al. (2004) to be associated with the dichotomous diagnosis of OCD, and nominally associated with lifetime severity of the disorder. Although the *5988T/C* variant was not included in the present study, the findings indicate that *GRIN2B* may be involved in the development of at least some aspects of OCD. However, the present results are difficult to interpret, since no function has, as yet, been elucidated for either of the two variants under investigation in the present study. The rs1806191 SNP represents a synonymous mutation in exon 13, which is unlikely to affect function, and rs890 represents a variant in the 3'UTR. However, it can be speculated that rs890, by virtue of its position in the 3'UTR, may well affect mRNA processing, thereby altering the quantity of protein. In order to delineate the role that the gene (and, in particular, rs890) may play in mediating the development of severe OCD, additional variants, throughout the gene, need to be genotyped to determine whether rs890 exhibits LD with a functional variant that increases susceptibility to OCD characterised by more severe symptomatology. In the absence of such a functional variant being detected, functional analyses to determine whether the rs890 variant does, in fact, alter the levels of expression of *GRIN2B*, would be warranted.

The lack of association of the rs890 variant with the formal, dichotomous, diagnosis in the present study may be due to a number of factors, the most probable being the lack of sufficient power in the case-control analysis to reject the alternative hypothesis with certainty. Indeed, the power for the case-control genotypic analyses of rs890 and rs1806191 amounted to 5.9% and 8.2% respectively (Table III.8), indicating that there is a possibility that chance may have played a role in obtaining those results. The association may be observed in the severity subtype analysis due to the increase in power afforded by the quantitative analysis. Moreover, no significantly gender-biased effects were observed, with the same trend noted in

both genders (i.e. the association of the *CC*-genotype with increased severity) (Table III.18[c]). This observation could thus lead one to conclude that the association is not sex-specific, although further studies using larger male and female OCD subsets would be required to determine whether the association is, in fact, gender-dependent.

### IV.2.3.2. *BDNF*

Associations were also observed between the *BDNF val66met* variant and total Y-BOCS score, although this association was only observed amongst the female subset. Here, individuals carrying the *GG*(*val66val*)-genotype were found to be possess higher Y-BOCS score compared to female individuals carrying at least one *A*(*met66*)-allele ($p = 0.013$) (Table III.18[c]). This finding contradicts observations from the present case-control analysis, where an association between the same variant and a dichotomous diagnosis of OCD in males was noted, with the *A(met66)*-allele representing the risk allele, and may be indicative of the gender differences that have been found to exist between male and female OCD patients. Indeed, both the *A(met66*) and *G*(*val66*)- alleles have been implicated as risk factors in various neuropsychiatric disorders (**section I.6.1.4.1[i]**), and BDNF has been found to interact in a complex manner with estrogen to modulate certain neurodevelopmental aspects (**section IV.2.2.1.3**). It may be that the *G*(*val66*)-allele, by means of female-specific epigenetic interaction, increases the risk for a more severe form of OCD in females, but not in males. Alternatively, it must be considered that the association may not have been detected in the case-control association studies due to a lack of power of afforded by the categorical analysis.

### IV.2.3.3. *DRD1*

Finally, a marginal association was noted between *DRD1 A-48G* and severity of OCD, with male OCD patients homozygous for the *G*-allele exhibiting significantly higher Y-BOCS scores compared to those carrying the *AG*- or *AA*-genotypes (Table III.18[c]). Interestingly, evidence from a transgenic model has indicated that the chronic potentiation of DRD1-containing neurons in the cortex and amygdala, known to induce efferent glutamatergic neurotransmission to the striatum, induces compulsive behaviours in mice (Campbell et al., 1999; McGrath et al., 2000). From these results, it could be hypothesised that an increase in dopaminergic stimulation of DRD1 may result in an efflux of glutamate in the striatum, which could result in increased severity of the disorder. Indeed, it has been suggested that DRD1-antagonists, which could attenuate the glutamatergic output to the striatum, may be more

efficacious in treating OCD. This is an exciting result, given that the *GRIN2B* rs890 was found to be associated with the severity of OCD in the present study (**section IV.2.3.1**).

The gender-specific effect of the *DRD1 A-48G* variant is intriguing: it has been found that male mice exhibit a marked overproduction and elimination of striatal dopaminergic receptors during childhood and adolescence, but that extensive pruning of the dopamine receptors in the striatum occurs after puberty (Andersen et al., 2000). It may be that a dysfunction in the *DRD1* gene prevents the pre-programmed pruning in males, resulting in the increased severity of the disorder, although this requires further investigation. Nonetheless, the observation by Andersen et al. (2000) indicates a sex-specific effect in brain structure, which may, at least partially, explain the present results.

The present study represents the first to investigate the role that the *A-48G* variant may play in mediating the development of OCD and OCD-related subtypes. However, the functional role of the *DRD1 A-48G* promoter variant has not yet been elucidated; it is thus difficult to determine whether the variant itself, or one in LD with it, mediates the development of more severe forms of OCD. Therefore, the present result requires replication in a larger sample. In addition, future studies to investigate the possibility of an epistatic interaction between the *DRD1* and *GRIN2B* genes should be conducted to elucidate the role that they may play in the development of OCD.

### IV.2.4. Age at Onset of OCD

Family history of OCD was more prevalent for patients with EO OCD (median = 10 years), compared to those with LO OCD (median = 15 years) ($p<0.001$) (Table III.27), in line with previous investigations where significantly higher rates of OCD amongst first degree relatives of EO probands were observed, compared to LO probands (Pauls et al., 1995; Nestadt et al., 2000[a]; Grados et al., 2001; Rosario-Campos et al., 2005). However, in contrast to previous studies reporting a gender bias in age at onset (with EO OCD more prevalent in males [Geller et al., 1998; Millet et al., 2004; Fontenelle et al., 2003]), ages at onset were not found to differ between the sexes in the present study, in fact, they were remarkably similar (median age at onset was 14 years for males and 15 years for females).

Although the median age at onset for patients presenting with co-morbid tics was found to be lower than those without co-morbid tic disorders, this did not reach statistical significance in

the present sample (Table III.27), in contrast to previous results where co-morbid tics have been found to be associated with a significantly earlier age at onset (Swedo et al., 1992; Rosario-Campos et al., 2001). However, it should be mentioned that patients presenting with co-morbid TS, a disorder which presents with tics, experienced significantly earlier ages at onset compared to those who did not present with co-morbid TS. The cumulative data thus point towards the possibility that EO OCD may represent a clinically (and, perhaps a genetically) homogeneous OCD subtype. The present study thus investigated the role that selected genetic variants may play in mediating the development of EO OCD.

Since there is no agreement as to the precise age at onset threshold that constitutes early- and late-onset of OCD, Kaplan-Meier survival function analyses were implemented in the present study. This provides a quantitative measure of the differences in age at onset for the genotypes of each variant, and as such, represents a more powerful analysis than if one were to categorically divide patients into early- and late onset, based on some arbitrary threshold. The Kaplan Meier logrank test provides additional power compared to numerical univariate analyses in that it investigates not only the difference between the medians of onset for each genotype, but also compares the number of observed OCD patients at each age group with the number of patients that would be expected based on the number of OCD patients in the combined groups.

#### IV.2.4.1. *GRIN2B rs890* and *rs1806191* variants

When the OCD sample was stratified according to gender, males carrying the *GRIN2B* rs890 *CC*-genotype exhibited a significantly earlier age at onset compared to *AA*- or *AC*-carrying males (Table III.28[c]). This result is particularly interesting in light of the fact that individuals homozygous for the *C*-allele were also found to experience more severe forms of the disorder in the present study (**section IV.2.2.1**), and that EO OCD has often been found to present with a more severe phenotype, and, at least in other studies, to occur in males more often than females (Geller et al., 1998; Millet et al., 2004; Fontenelle et al., 2003; **section I.4.2.2.4**). This finding underscores the gender-differentiated development and manifestation of OCD, and, in the light of the common age at onset between male and female OCD in the present study, may indicate that other or additional factors may be participating in the development of severe and/or early onset OCD in females. However, it should be noted that, for the rs890 variant, although no association was observed between age at onset and genotype for the female subset, only three females were found to carry the *CC*-genotype,

which may have skewed the results slightly. Indeed, females with the *CC*-genotype, like their male counterparts, were found to experience earlier ages at onset compared to those carrying the *AA*- or *AC*-genotypes (Table III.28[c]).

A marginally significant association was also observed between *GRIN2B* rs1806191 and age at onset in the *female* subgroup (p = 0.049) (Table III.28[c]), with *AA*-carrying females presenting with earlier ages at onset compared to either those homozygous for the *G*-allele, or to heterozygous females. A trend towards association of the *AA*-genotype with earlier onset was also noted for the male subset (p = 0.087) and the total OCD population (p = 0.090). Like the association between rs890 *CC*-genotype and age at onset, female OCD subjects carrying the *AA*-genotype also presented with higher Y-BOCS scores than *AG*-or *GG*- carrying females, although this difference was not found to be statistically significant (p = 0.373) (Table III.18[c]).

Haplotype analysis, performed on the entire OCD dataset, revealed that the rs1806191-rs890 *G-A* haplotype was significantly associated with a later age at onset (p = 0.010), whilst the *A-C* haplotype was associated with a significantly earlier age at onset (p = 0.018). Unfortunately, given the small dataset available for haplotype analysis, the data could not be stratified according to gender. It is thus difficult to determine whether the single variant effects are indeed gender-specific or not; further work using larger samples would be required to answer this question.

#### IV.2.4.2. *PLC-γ1 ser279gly*

Another intriguing finding was the association of the *PLC-γ1* variant with age at onset of OCD. When the total OCD population was considered, the individuals carrying the *PLC-γ1 GG(gly279gly)*-genotype were found to experience significantly earlier ages at onset compared to those carrying either the *GA*(*ser279gly*)- or *AA*(*ser279ser*)-genotypes. Although male and female subjects homozygous for the *G*(*gly279*)-allele were found to experience earlier ages at onset when analysed separately, these differences were not significant (Table III.28[d]). However, due to the low numbers of the *GG*-genotype in both the male and female subsets, the gender-stratified subjects were grouped according to the presence or absence of at least one *G*(*gly279*)-allele. Here, subjects of either gender who carried at least one *G*(*gly279*)-allele were found to experience significantly earlier ages at onset of OCD compared to those who were homozygous for the *A*(*ser279*)-allele.

Although no functional role has, as yet, been ascribed to the *PLC-γ1* variant under investigation, the association with EO OCD is intriguing, considering the role that PLC-γ1 may play in the intracellular signalling cascades activated by growth factors, including BDNF (**section I.6.1.4.1[i]**), which was shown to be associated with EO OCD in the male OCD subset in the present study (discussed in **section IV.2.4.3**). The possible functional relationship between *PLC-γ1* and *BDNF* is thus relevant to the present investigation. It may be that a dysfunction in BDNF-mediated responses involving PLC-γ1 could result in an abnormality in the activation of downstream nuclear transcription factors, which may be responsible for the activation of genes necessary for neurodevelopment, ultimately resulting in the expression of OCD at an early age.

### IV.2.4.3. *BDNF val66met*

The *BDNF val66met* variant was found to be associated with EO OCD in males, with *AA*(*met66met*)-carriers found to exhibit significantly lower ages at onset (median = 5 years) compared to those who were homozygous for the *G(val66)*-allele, or heterozygotes (Table III.28[c]). The findings are intriguing not only because of the gender-dependent effect that was noted, but also because, in a recent investigation by Hall et al. (2003), it was found that the *BDNF val66met G*(*val66*)-allele, rather than the *A*(*met66*)-allele, was overtransmitted to EO OCD probands (although their sample was not stratified according to gender).

As already discussed in **section IV.2.2.1.3**, one possible explanation for the gender-specific effects of BDNF is a dysfunction in the estrogen-mediated regulation of BDNF expression. Moreover, with relevance to its association with EO OCD, it has been hypothesised that the manner in which estrogen and BDNF interact is highly dependent on developmental stage, because concentrations of nuclear estrogen receptors, which are thought to mediate the estrogen-BDNF interactions, have been found to vary drastically during postnatal life (Solum and Handa, 2001).

No evidence of association with the age at onset of OCD was found for *BDNF* rs2049046 or rs988748 variants in the present study (Table III.28[d]), although Hall et al. (2003) demonstrated a significant overtransmission of the rs988748 *C*- and rs2049046 *T*-alleles to EO OCD probands. Moreover, haplotype analysis in the present study, involving all three of the above *BDNF* polymorphisms, revealed no significant associations with any of the phenotypes (Table III.35). However, in the present study, the 3-locus haplotype analysis

involved only 32 OCD subjects; therefore it is possible that the analyses are underpowered to detect any small effects that the variants may have on the development of EO OCD.

The cumulative findings from the present study and that by Hall et al. (2003) suggest that *BDNF* may play a role in mediating the development of EO OCD. The question remains, however, as to why the discrepancy in *val66met* risk alleles for the variant exists between the two separate studies. One reason for the contradictory results may be population-based differences in allele frequencies: although the alleles associated with age at onset differ between the studies, it may be that the *val66met* variant is not itself the causal variant, but that either of these alleles are in LD with the disease-susceptibility variant depending on the population involved. Indeed, although in both the present study and that by Hall et al. (2003), a high degree of LD was observed across *BDNF*, the LD was not complete. Therefore, the possibility remains that, whilst the *G*(*val66*)-allele is in LD with the risk allele in a North American Caucasian population, the *A*(*met66*)-allele may be in LD with the risk allele in the Afrikaner population.

Appropriate to this discussion, another potential OCD candidate gene has been found to be located approximately 140kb upstream from *BDNF*. This gene, named *MALS3*, encodes a protein that plays an important role in recruiting enzymes and receptors to specific synaptic sites (Jo et al., 1999). Interestingly, one of the functions of the gene is to ensure the proper localisation of GRIN2B to neuronal postsynaptic density. This relationship is potentially important to the aetiology of EO OCD, given that GRIN2B was also found, in the present study, to be associated with EO OCD (**section IV.2.4.1**).

It is therefore possible that variant(s) within *MALS3*, in LD with the *val66met* variant in *BDNF*, may represent the actual risk factor in the development of EO OCD. Alternatively, functional *MALS3* variants may be in LD with those in *BDNF*, creating a "super-allele" the possession of which represents a risk factor in developing EO OCD. Given the aforementioned role that BDNF and MALS3 play in regulating GRIN2B functioning, it may well be that variants occurring in all three of genes (and possibly more) interact epistatically, and together may be required for the expression of the EO OCD phenotype. The cumulative results are exciting, and necessitate the further investigation of more densely-spaced SNPs in BDNF, in conjunction with those in the nearby *MALS3* gene, in order to elucidate the role that the gene(s) may play in mediating the development of EO OCD in males.

### IV.2.4.4. *DAT 40bp VNTR*

Three dopaminergic candidates (*DAT, DRD3* and *COMT*) were also found to play a role in mediating the development of EO OCD in males. First, the *DAT* 40bp VNTR was found to be associated with age at onset, but only in the male population. The results are difficult to interpret due to the small number of males carrying the *A10/A11* and *A9/A9*-genotypes (Table III.31). Nonetheless, it was decided not to group the genotypes, since the function of the polymorphism has not yet been clearly elucidated: Heinz et al. (2000) found that the *A10/A10*-genotype resulted in a higher density of DAT, whilst Jacobsen et al. (2000) found that the *A10/A10* genotype yielded a lower DAT density than the *A9/A10* repeat. On the other hand, a study by Martinez et al. (2001) suggested that the polymorphism did not play a role in affecting the density of DAT. Consequently, pooling the genotypes may have resulted in a loss of information. Ideally, in order to maximise the likelihood of relating the functional variant to the phenotype under investigation, the grouping of genotypes should be performed on the basis of function, or allele grouping should be based on the evolutionary relationship.

It is clear from the Kaplan-Meier curve in Figure III.50(b) that the males with the *A10/A11* genotypes experience significantly earlier ages at onset compared to those carrying any of the other genotypes. This finding is difficult to reconcile, since firstly, the *A11*-allele is a rare allele, and as already mentioned, has no function ascribed to it. Secondly, it is evident that the *A10*-allele is associated with a later age at onset, albeit non-significantly, in the male population. Therefore, if the *A10/A11* genotype really is associated with early age at onset, it implies that it is the *A11* allele that brings about the effect in a dominant manner. However, while it may be that the observation is due to chance, one cannot exclude the possibility that the genotype does indeed play a role in mediating the development of EO OCD; or is in LD with a variant that is associated with the phenotype. Indeed, although not directly associated with age at onset in OCD, recent evidence from a DAT-knockdown experiment in mice suggests that a decrease in DAT (resulting in an increase in dopamine) results in excessive sequential stereotypic behaviours that are characteristic of disorders such as OCD and TS (Berridge et al., 2005). Thus the role that the gene plays in mediating OCD, or aspects of the phenotype, cannot be negated; further studies conducted using a larger population are required to reach a definitive conclusion.

**IV.2.4.5. *DRD3 ser9gly***

The *DRD3 ser9gly* polymorphism was also found to be associated with age at onset in the male population, with the *ser9gly* heterozygotes exhibiting a significantly lower age at onset than either of the homozygotes. The present observation of the association of the heterozygote with EO OCD may be representative of molecular heterosis (Comings and MacMurray, 2000). Molecular heterosis refers to the phenomenon whereby heterozygous individuals show a significantly greater (positive heterosis) or lesser (negative heterosis) effect than homozygous individuals. Since the mean age at onset for male *ser9gly*-carriers is significantly lower than *DRD3 ser9gly* homozygotes, the present association could perhaps be considered a form of negative heterosis. Molecular heterosis has been previously reported for the *DRD3 ser9gly* polymorphism: a decrease in heterozygote frequency has been observed amongst schizophrenic subjects in three separate studies (Crocq et al., 1992; Mant et al., 1994; Asherson et al., 1996). Interestingly, all three of these associations were noted only in male subsets, indicating that molecular heterosis at the *DRD3 ser9gly* locus may be gender-specific.

However, although the phenomenon of heterosis represents an appealing explanation for the association between *DRD3 ser9gly* heterozygote genotype and OCD, functional studies are required to support the hypothesis. Presently, the *in vivo* functional significance of this polymorphism is unknown; however, *in vitro* analysis suggests that *gly9*-homozygotes may have a higher binding affinity for dopamine (Lundstrom and Turpin, 1996). Thus, although the variant may indeed be functional, in order to support the hypothesis of negative heterosis, one requires proof that the heterozygote variant functions differently to either of the homozygote variants.

The present observation represents the first to suggest a sexually dimorphic relationship between the *DRD3 ser9gly* variant and EO OCD in males. The finding is interesting, given that a relationship has been suggested to exist between BDNF and DRD3: BDNF has been found to be responsible for the appearance and maintenance of DRD3 during development and adulthood (Guillin et al., 2003). In the present study, *BDNF val66met* variant was also found to be associated with age at onset of the disorder (**section IV.2.4.3**), indicating the possibility of an epistatic interaction existing between the two genes. The effect that BDNF has in regulating DRD3 may also explain, at least in part, the gender-specifc effects that were observed: estrogen has been found to regulate the transcription of *BDNF* mRNA, and as such,

may also play a role in mediating the transcription of *DRD3* mRNA. Indeed, in the female population in the present study, although non-significant, the effects that genotype have on age at onset of OCD are opposite to those observed for males, with the heterozygotes exhibiting the latest ages at onset. These observations may reflect the effect that estrogen, via BDNF, has on DRD3 in males and females.

The present findings are particularly interesting, given that, as will be discussed (**section IV.3.1.2**), the *DRD3* meta-analysis also indicated that an association existed between the *DRD3 ser9gly* polymorphism and the diagnosis of OCD. Taken together, the results are suggestive of a role for the *DRD3 ser9gly* variant, or one in LD with it, in mediating the development of OCD, and perhaps EO OCD in particular. Further analysis is, however, required to elucidate the precise role the variant(s) play.

#### IV.2.4.6. *COMT rs362204*

The final dopaminergic candidate found to be associated with EO OCD in males was the *COMT* rs362204 variant, where males homozygous for the *D*-allele experienced significantly earlier ages at onset compared to heterozygous males (Table III.28[b]). As already discussed, this variant was also found to be associated with the dichotomous diagnosis of OCD in the male sample. The variant has not been found to be functional (DeMille et al., 2002), and, although it is in LD with the *val158met* variant in the present study, the latter was not found to be associated with EO OCD in the present study. The possibility thus exists that either the COMT rs362204 variant is functional (this possibility is mentioned in **section IV.2.2.1.2**) and is thus responsible for the observation, or that the variant is in LD with another functional variant that contributes to the development of EO OCD in males. The observed gender dimorphism may be due to the effect that estrogen has on the transcription, and thus activity of *COMT* (discussed previously in **section IV.2.2.1.2**).

#### IV.2.4.7. *5-HT$_{2A}$*

When the serotonergic candidate genes were investigated for the role they may play in mediating EO OCD, the only association observed was with the *5-HT$_{2A}$ T102C* (rs6313) variant in the male subset (Table III.28[a]). Here, the *T102T* genotype was found to be associated with a significantly earlier age at onset. This finding contradicts that of Walitza et al. (2004), who observed an association between the *A*-allele of the *5-HT$_{2A}$ -1438A/G* (rs6311) variant and OCD in a juvenile OCD sample. Interestingly, the *5-HT$_{2A}$ -1438A/G* and *T102C*

variants were found to exhibit a high degree of pairwise LD in the present study, with the *T102* allele mostly co-occurring with the *-1438A* allele. However, no statistically significant association between the *-1438A/G* variant and age at onset was noted in the present study, although individuals carrying the *AA*-genotype were found to experience earlier ages at onset compared to heterozygotes, or *GG*-homozygotes. Both the *5-$HT_{2A}$ T102T* genotype and *-1438A* allele have been found to be associated with increased 5-$HT_{2A}$ receptor density (Parsons et al., 2004; Khait et al., 2005; Polesskaya and Sokolov, 2002) (**section I.6.1.1.1[iia]**). On the contrary, however, Bray et al. (2004) and Spurlock et al. (1998) observed no functional differences between the alleles comprising the *5-$HT_{2A}$ T102C* and *-1438A/G* variants, respectively. The present findings are difficult to interpret, given the abovementioned inconsistent findings with respect to functional analyses conducted on the *5-$HT_{2A}$ T102C* polymorphism.

Moreover, when haplotype analysis involving the two variants was conducted in the present study, no association with age at onset was noted. However, although the variants were found to exhibit a high degree of pairwise LD, this was not complete; consequently, it may be that the *5-$HT_{2A}$ T102C* (rs6313) variant, in the Afrikaner population, is in LD with the causal variant, whilst the *-1438 A/G* (rs6311) variant is not. Obviously, the converse may be true for the German population studied by Walitza et al. (2004).

The sexually dimorphic effects may be due to the effect that estrogen has been found to exert on the serotonergic system; indeed, the administration of estrogen has been found to increase the density (Sumner et al., 1999; Cyr et al. 1998; 2000) and ligand binding (Kugaya et al., 2003) of prefrontal 5-$HT_{2A}$ receptors. Clearly, further investigations involving variants within the *5-$HT_{2A}$* gene are required in order to elucidate the role that this gene may play in mediating the development of EO OCD.

#### IV.2.4.8. *HoxB8 rs2303486*

An exploratory investigation was undertaken in order to determine the role that the *HOXB8* variant, rs2303486, may play in the categorical diagnosis of OCD, severity of OCD or the age at onset of OCD. Indeed, in the female OCD population, those carrying the *AA*-genotype experienced significantly earlier ages at onset compared to heterozygous or *TT*-homozygous females ($p = 0.024$) (Table III.28[c**]**). However, these results are best viewed in a cautionary light, since no function has yet been ascribed to the investigated polymorphism, and the wide

95% CIs (the upper limits of the medians for all genotypes could not be calculated, as they approximated infinity) indicate that chance may play a role in the observed results. Thus, although an interesting finding in light of the developmental aspects of OCD (**section 1.6.1.4**), replication in a larger sample, and perhaps in a haplotypic context, is required.

### IV.2.5. Subtyping According to Co-morbidity

#### IV.2.5.1. Co-morbid MDD

MDD has been found to be the most common disorder occurring co-morbidly with OCD in a number of studies (**section 1.4.2.2.1**), indicating that the two disorders may share some functional commonality, which may extend to the genetic level. Indeed, in line with previous studies, MDD was found to be the most frequent co-morbid disorder amongst the OCD patient sample, with 64.6% of the patients presenting with this disorder. However, in contrast to results from other studies, no significant differences in family history of OCD, OCS or tic disorders were observed between OCD patients with co-morbid MDD and those without; this may be an effect of sample size.

The frequency of symptom dimensions was also found to differ between the two OCD subsets: significantly more OCD subjects with co-morbid MDD experienced sexual/religious obsessions and compulsions, compared to those without co-morbid MDD ($p = 0.042$), consistent with recent observations by Hasler et al. (2005). This association appears to be the only clinical distinction between OCD patients exhibiting co-morbid MDD and those not, although the limited sample size may prevent the detection of further clinical associations. Therefore, based on previous evidence indicating the possible genetic contribution to OCD and selected co-morbid disorders, including MDD (Nestadt et al., 2003), the genetic aetiology of the OCD subtype characterised by co-morbid MDD was warranted.

##### *IV.2.5.1.1.* 5-HT$_6$ T267C

The significant differences in allele distribution of the *5-HT$_6$ T267C* between OCD patients with co-morbid MDD (Table III.39[a]), and those without co-morbid MDD is interesting, given that the current study represents the first time that *5-HT$_6$* has been investigated as a candidate gene in OCD-related analyses. Indeed, it represents a plausible candidate, due to its proposed involvement in the regulation of dopaminergic release in the CNS, and in its role in mediating the development of 5-HT neurons (**section 1.6.1.1.1[iii]**).

Although the gene has not yet been investigated for the role that it may play in major depressive disorder *per se*, the receptor has been shown to exhibit a high affinity for tricyclic antidepressants (Monsma et al., 1993; Roth et al., 1994; Boess et al.,1997), implicating its possible involvement in the aetiology of the MDD or related disorders. In the present study, the *C267*-allele was observed at a significantly greater frequency amongst the group of OCD-MDD patients, indicating that it may confer protection against developing co-morbid MDD.

The *T267C* variant does not alter the predicted amino acid sequence of the receptor and is thus unlikely to be functional. It is, however, possible that a causal variant is in LD with the *T267C* polymorphism, resulting in the observed association.

#### *IV.2.5.1.2.* COMT rs2097603

When the allelic frequencies of *COMT* rs2097603 SNP in individuals with MDD were compared to those without MDD, a statistically significant difference was noted, with the *A*-allele representing a possible protective effect against the development of co-morbid MDD (Table III.39[a]). Since the function, if any, of the polymorphism has yet to be elucidated, the interpretation of the role that *COMT* itself may play in the pathophysiology of the development of co-morbid MDD is complex. The variant does, however, lie within the estrogen-sensitive P2 promoter region of the gene, and as such, may play a role in regulating its expression. However, further functional studies are required to elucidate the role (if any) that this variant may play in regulating the expression of *COMT*. The rs2097603 variant was not found to be in LD with either the rs4680 or rs362204 *COMT* variants in the present study; it may thus be that functional variants situated at the 5' end of the gene may be responsible for susceptibility to co-morbid MDD.

The collective results do, however, provide putative evidence for the distinct clinical and genetic aetiology of OCD+MDD, which warrant further investigation using a suitably sized dataset.

### IV.2.5.2. Co-morbid tics

Research has indicated a substantial overlap between tic disorders and OCD, implicating the possibility of shared neurobiological and genetic underpinnings (Pauls et al., 1995; Leckman et al., 1995; Pauls, 1992), prompting the present genetic investigation. The present data indicates that a larger number of males exhibit co-morbid tics compared to females (Table

III.7), and that the age at onset of those patients exhibiting co-morbid tic disorders is earlier than those without co-morbid tic disorders (Table III.27). These data, although not statistically significant in the present study, are in line with results from previous investigations (Leonard et al., 1992; Grados et al., 2001). In addition, significantly more OCD patients presenting with co-morbid tics also experienced contamination obsessions and compulsions (90.9%) compared to those who did not exhibit co-morbid tic disorder (56.8%) (Table III.42).

The *COMT val158met* and *BDNF val66met* variants were found to be associated with the presence or absence of co-morbid tics in the present study. For the *COMT val158met* variant, the functionality of which has been discussed in **section IV.2.2.1.2**., the frequency of genotypes containing at least one *G*(*val158*)-allele was found to be significantly higher in the OCD-tics group (Table III.39[a]), suggesting that the *G(val158)*-allele may confer protection against the development of co-morbid tics. This finding is interesting, given the results from the *COMT val158met* meta-analysis conducted in the present study, where this allele was found to confer protection against the development of the global OCD phenotype in males (**section IV.2.2.1.2**).

The association between the presence of tics and the *BDNF val66met* variant is also interesting, in view of the fact that, in the present study, the *A*(*met66*) variant (the functionality of which has been discussed in **section IV.2.2.1.3**) was found to be associated with both the formal diagnosis of OCD and with EO OCD in males.

Unfortunately, because of the small sample sizes after stratifying the OCD sample according to the presence or absence of tics, it was not feasible to further stratify the sample by gender; consequently, it is not known whether the association of the *COMT val158met* and *BDNF val66met* variants with co-morbid tics is gender-specific. However, a non-significant trend towards a higher frequency of co-morbid tics in males, compared to females, was noted (19.1% versus 8.5%; p=0.126) (Table III.7). The cumulative, preliminary, results thus suggest that the COMT *val158met A*(*met158*) allele may be involved in increasing susceptibility to tic-related OCD in males, and that the *BDNF A*(*met66*)-allele (or variants in LD with it) may increase the risk for developing EO (**section IV.2.4.3**), tic-related OCD in males.

### IV.2.6. Subtyping According to Symptom Dimensions

#### IV.2.6.1. Hoarding

In previous studies, the hoarding symptom dimension has been found to correlate with increased co-morbidity, familial aggregation and poor treatment response (Mataix-Cols et al., 2005; 2002; 1999; Black et al., 1998; Abramowitz et al., 2003; Baer, 1994; Saxena et al., 2002; Winsberg et al., 1999; Samuels et al., 2002). It was thus relevant to investigate this symptom dimension as a putative OCD subtype that may be linked to a specific genetic aetiology.

Although no significant demographic correlates of hoarding were observed in the present study, more females were found to present with hoarding symptoms (32.7%) compared to male subjects (16.7%) (Table III.7), consistent with previous data (Hogstel, 1993). The present findings also support previous data, indicating familial aggregation of hoarding symptoms (Samuels et al., 2002): more than half of the OCD subjects presenting with hoarding symptoms also had a family history of OCD, whereas only 16% of the non-hoarders presented with a family history of OCD (p=0.008) (Table III.47). Interestingly, significant correlations were observed between hoarding and BDD and SIB, although not with co-morbid major depressive or tic disorder, as has previously been found (Zhang et al., 2002; Frost et al., 2000).

Single locus analysis of the *DRD4 -521C/T* (rs1800955) variant revealed an allelic association when the OCD patient group was stratified according to the hoarding symptom dimension. Here, the *-521T*-allele was found to be more prevalent amongst hoarders compared to non-hoarders ($p = 0.043$), and to controls ($p = 0.011$) (Tables III.49[a] and [b]), and thus contributes to the risk of developing hoarding symptoms. In addition, the *met158* allele of the *COMT val158met* variant was found to increase the risk of developing hoarding obsessions and compulsions. Given the putative functional roles of each of the associated polymorphisms (the *T*-allele of the *DRD4 -521C/T* polymorphism has been associated with lower *DRD4* transcriptional activity, and the *met158* allele of the *COMT val158met* polymorphism has been associated with lower COMT enzymatic activity [and thus possibly an increase in dopamine levels]), the present results, taken together, suggest that the dopaminergic system may play a role in the pathophysiology of hoarding symptoms in OCD. This may provide an

explanation as to why OCD patients who present with hoarding symptoms exhibit a worse response to traditional SSRI treatment.

It should also be noted that, when the OCD hoarders and non-hoarders were compared, significant differences in genotypic and allelic frequencies were noted for the *ESRα* rs9340799 variant, while a significant difference in genotypic frequency was noted for the *ESRα* 2234693 variant. However, when the genotypic and allelic frequencies of these two polymorphisms were compared between the hoarders and healthy controls, no differences in genotypic or allelic frequencies were observed for either of the variants, suggesting that some characteristic within the non-hoarding OCD group may be associated with variation in *ESRα*. Nevertheless, given the already small sample size of this group, it was decided not to stratify the group to investigate the putative association further. This does, however, remain an aspect of future work, and also highlights the importance of using a control population against which to test the association observed within case-only investigations.

### IV.2.6.2. Symmetry/ordering symptom subtype

Symmetry/ordering obsessive-compulsive symptoms, along with those characterised by aggressive, sexual and religious obsessions and checking compulsions, have previously been found to possess a strong familial component (Alsobrook et al., 1999; Leckman et al., 2003). This implies, indirectly, that these symptom dimensions may comprise a genetic component. Although Leckman et al. (2003) provided evidence consistent with a dominant major gene effect for both of the aforementioned symptoms, the present study represents the first to investigate which genes may contribute to their genetic aetiologies.

From a clinical perspective, very few significant differences in family history or co-morbidity were observed between patients with the symmetry/ordering phenotype and those without (Table III.52). Interestingly, with regard to family history of tic disorders, a significantly lower number of patients with symmetry/ordering symptoms reported a positive family history of tic disorders ($p = 0.032$). In addition, less OCD patients suffering from symmetry/ordering obsessions and compulsions were found to present with co-morbid social phobia ($p = 0.019$).

When the genetic aetiology of the symmetry/ordering dimension was investigated, no statistically significant differences were detected between the OCD subjects experiencing

symmetry/ordering symptoms and those not. However, when the OCD subset characterised by symmetry/ordering was compared to controls, a marginal genotypic association was noted for the *ACE Alu* ins/del variant (Table III.54[b]). As with the case-control analysis (**section IV.2.2.1.4**), a significantly increased frequency of individuals carrying the *DD*- and *DI*-genotypes was noted amongst the control population, indicative of the possible dominant role that the *D*-allele may play in conferring protection against the development of OCD, particularly with regard to symmetry/ordering symptoms. The larger control size, compared to the size of the OCD subset lacking symmetry/ordering symptoms (n=134 and n=28, respectively), may have facilitated the detection of the effect that *ACE* has on symmetry/ordering in the OCD+symmetry/ordering vs control analysis.

### IV.2.6.3. Sexual/religious symptom subtype

Demographically, significantly more males were found to experience sexual/religious symptoms compared to females in the present study. Clinically, OCD subjects experiencing sexual/religious obsessions and compulsions were found to exhibit significantly higher frequencies of co-morbid MDD ($p = 0.017$) and dysthymia ($p = 0.016$), and a significantly lower frequency of BDD ($p = 0.019$) compared to subjects who did not experience the symptoms (no subjects with sexual and religious symptoms were diagnosed with co-morbid BDD) (Table III.57).

Investigating the genetic correlates yielded no significant results when the genotype and allele frequencies of selected candidate markers in individuals experiencing sexual/religious symptoms and those who did not, were compared. However, when the sexual/religious OCD subset was compared to the control sample, significant differences in genotypic frequencies were noted for the *BDNF val66met* and *ACE Alu ins/del* variants (Table III.59[a]).

The significantly increased frequency of *BDNF G*(*val66*)-allele carriers amongst the control sample compared to the OCD sexual/religious subset is suggestive of the dominant mode in which this allele may protect against the development of sexual/religious symptoms in OCD. As mentioned in previous sections, this allele has also been found to be associated with protection against the formal diagnosis of OCD, EO OCD and the development of tics in males in the present study. It is interesting to note that, in the present study, the males experiencing sexual/religious symptoms far outweigh females experiencing the same symptoms. Although no formal gender stratification was performed for the current analysis

due to already small sample sizes, it may be that the association with the *BDNF val66met* variant is only present in the male subset. This would also provide an explanation as to why the difference is only observable between the OCD sexual/religious subset and controls, and not between the two OCD subsets: due to the larger control sample, detection of the small effect may be facilitated, given the increased power of the analysis. Indeed, if the association is, in fact, sexually dimorphic, it is possible that the development of the symptom subtype is mediated via the previously discussed estrogen-BDNF interaction.

An association was also noted between the *ACE Alu ins/del*, against the larger control sample, and consequently increased power of analysis, making it possible to detect the small effect that the variant may have on the phenotype. Once again, the increased frequency of *DD* and *DI*-carriers amongst controls may point towards the dominant effect that this variant has in protecting against the development of sexual/religious symptoms.

**IV.2.6.4. Contamination symptom subtype**

Although the contamination/washing obsessions and compulsions represented the most frequently experienced symptom dimension in the present dataset, very few significant clinical differences were noted between those OCD subjects presenting with the subtype, and those not (Table III.62). Indeed, only one nominally significant difference in the frequency of co-morbid tic disorders was noted between the two groups ($p = 0.045$), with OCD subjects experiencing contamination/washing obsessions and compulsions also experiencing a greater frequency of tic disorders.

In the genetic analyses, a significantly decreased frequency of the *DRD4* 48bp VNTR *A4/A4*-genotypes was noted in the OCD subset characterised by the presence of contamination symptoms compared to the subset characterised by the absence of the symptoms, and to the control sample (Tables III.65[a], [b], [c] and [d].). This suggests that the *A4*-allele may be functioning in a recessive manner to protect against the development of contamination/washing symptoms. On the other hand, the *A7*-allele (in homozygous and heterozygous forms with *A2* and *A4*) was present at an increased frequency in the OCD subset experiencing contamination symptoms (Table III.65[a]). This could be indicative of the *A7*-allele functioning as a risk allele in a dominant mode.

The *DRD4* 48bp VNTR polymorphism has been found to be functional, although the most appropriate means of grouping alleles for genetic analyses is still under debate. The *A4*-allele has been found to possess a normal cAMP response, compared to the *A7*-allele, which has been found to encode a protein that has a blunted cAMP response, requiring at least three times more dopamine to induce a normal response compared to *A4*-containing DRD4 receptors (Asghari et al., 1995). In addition, recent evidence has also indicated that the *A2*-allele codes for a DRD4 receptor that has a blunted cAMP response that lies midway between that of *A4*- and *A7*-containing receptors, indicating a dissociation between length of the VNTR region and functionality (Wang et al., 2004). Therefore, on the basis of the cumulative biochemical and genetic data, Wang et al. (2004) have proposed that the most biologically efficient manner to group genotypes and/or alleles for genetic association studies may by grouping the *A4*-alleles and comparing them to other genotypes and/or alleles. However, although Wang et al's proposed method of pooling alleles was employed in the present study, one must be mindful that grouping alleles and/or genotypes in polymorphisms for which functionality has not been completely established remains essentially arbitrary. Consequently, the involvement of the *A7*-or *A2*-allele in the observed association cannot be negated.

Results obtained from the present study, following Wang et al's proposal for allele-grouping, indicate that the development of contamination symptoms may be a result of a blunted cAMP response of DRD4 receptors, due to allelic composition. Since DRD4 acts principally to downregulate postsynaptic cAMP (**section I.6.1.2.1[i]**), the possession of a non-*A4*-containing DRD4 receptor (i.e. one containing the *A7*- or *A2*-repeat allele) may result in an increase in cAMP levels. Rat models have suggested that an activation of cAMP/protein kinase A second messenger systems in the PFC may impair certain prefrontal functions (Taylor et al., 1999). Thus, it is possible that decreased cAMP inhibition in patients with contamination/washing symptoms may result in neural cAMP-induced changes in cellular regulation and downstream events, which could manifest as contamination/washing symptomatology.

A second association was observed when the OCD contamination/washing subset was compared to the control sample. Here, the *ACE Alu* ins/del polymorphism *D*-allele appeared to be acting in a dominant manner to protect against the development of OCD with contamination/washing symptoms. Given the involvement of ACE (albeit indirectly) in controlling dopaminergic concentrations in the brain (**section I.6.1.5.4**), the cumulative data

point towards the possibility that a dopaminergic mechanism may be involved in the aetiopathology of OCD with contamination/washing symptoms.

### IV.2.6.5. Aggressive symptom subtype

As mentioned in the previous section, evidence exists to suggest that aggressive obsessions and compulsions are genetically transmitted in a dominant manner. This symptom dimension has also been found to be associated with an increase in social phobia co-morbidity (Hasler et al., 2005). In the present study, however, no association between aggressive symptoms and co-morbidity with the any disorders considered in this study were observed. These apparently discrepant results may be due to the fact that in the Hasler et al. (2005) model, four main symptom dimensions were described: aggressive symptoms were grouped with sexual and religious symptoms, whereas in the present study the aggressive symptoms were grouped separately.

Despite the lack of clinical distinctions between OCD subjects experiencing aggressive symptoms and those not, the possibility exists that the sample numbers investigated in the present study are too small to detect an effect of small to moderate size. Thus, the compelling evidence that aggressive symptoms possess a familial (and thus perhaps, genetic) component (Leckman et al., 2003; Alsobrook et al.,1999) prompted the investigation into the possibility of a genetic aetiology of this symptom subtype.

Disappointingly, but perhaps not surprisingly considering the sample numbers, no candidate markers investigated in the present study were found to be associated with the aggressive symptom subtype. Further studies comprising greater statistical power are required to determine whether the candidates can be excluded as playing a role in the development of the subtype.

## IV.2.7. Summary of Subtype Genetic Association Studies

There is no doubt that some interesting results, worthy of further discussion, have emerged from the present genetic study. These results are summarised below to provide a greater overview of the implications of the findings of the present study.

Firstly, the association of *BDNF val66met* polymorphism with the formal, dichotomous diagnosis and age at onset of OCD in males, and the concurrent finding that it is also

associated with the tic-related phenotype, are potentially indicative of a distinct EO, tic-related OCD phenotype in males that is a distinct clinical and genetic entity. Moreover, these results represent a partial replication of those obtained by Hall et al. (2003), who investigated the role that *BDNF* polymorphisms may play in mediating the development of EO OCD. Although different *val66met* alleles were implicated as risk factors in both studies, this is likely to be the result of population-based differences, as already discussed in **section IV.2.4.3**.

Secondly, the association of *COMT* rs362204 variant with the formal diagnosis of OCD and EO OCD, both in males, also represents a potentially interesting finding. It was noted that the *COMT val158met* variant (found to be in pairwise LD with rs362204 in the present study) was found to be associated with the tic-related phenotype (the *COMT* rs362204 variant was not included in the OCD+tics subtype analysis, due to small sample size). It is thus tempting to speculate that, as stated in the previous paragraph, the EO, tic-related phenotype in males may represent a genetically distinct subtype, and, although it may not be the rs362204 and *val158met* variants *per se* that are involved, they may be in LD with the causative polymorphism.

The finding that both genes are regulated, at least in part, by estrogen, via EREs makes for an even more intriguing result, in that one could thus hypothesise that sex-specific estrogen-mediated effects, perhaps at certain stages during neurodevelopment, may underlie the aetiology of some aspects (particularly the age at onset and development of co-morbid tics) of the disorder. It would also be interesting, and perhaps fruitful, to conduct an investigation into possible epistatic interactions between the *COMT* and *BDNF* genes, to determine whether they function synergistically to increase susceptibility to the EO, tic-related OCD phenotype.

The third result worthy of further mention is the observation that the *GRIN2B* rs890, in particular, and possibly rs1806191, were found to play a role in severity and age at onset of the disorder. The results are interesting, since not only were they corroborated by the results obtained in the haplotype investigation in the present study, but also in a family-based study by Arnold et al. (2004), who observed an association between the *GRIN2B* rs890 and the severity of OCD. The glutamatergic system has only recently gained popularity as one of the systems that may be affected in OCD, and, given the present results, it seems ever more likely

that investigation will continue into the role that the system may play in the aetiology of OCD.

Overall, the gender-specificity of the observed associations is striking, especially for the age at onset analyses, where approximately two-thirds of the observed associations were observed only in males. This may imply that a gender-specific dysfunction, perhaps at different stages of neural development, may be playing a role in mediating certain aspects of the disorder. Certainly, the role that estrogen may play in regulating many of the products encoded by the candidate genes investigated has been touched upon, but it is suspected that the interactions may be highly complex. The notion that *ESRα* may play a role in the development of OCD and related subtypes was also investigated in the present study. Although no statistically significant associations between either of the selected *ESRα* intronic variants and OCD, or subtypes of OCD, were observed in the present study, including variants in genes encoding gonadal hormones and related products in future studies is probably a worthwhile endeavour.

### IV.2.8. Limitations to Categorisation by Subtype

It is important to note that the classification of OCD subjects into the respective symptom dimensions is not mutually exclusive: a single patient can present with one or more of these symptom dimensions. The focus of the study was thus on the genetic aetiology of each group of symptom dimensions, not on groups of patients.

It is also notable that one of the major disadvantages when subtyping a patient dataset is a reduction in sample size, and consequent reduction in the power of the analysis. One way of overcoming this would be to focus beyond the diagnosis of OCD, and on individuals in the broader population who may suffer from sub-syndromal OCD. In addition, the possibility exists that one could include patients suffering from other psychiatric disorders that are known to present with similar obsessions and compulsions; for example, TS and TTM. Unfortunately, such data was not available for investigation in the present study, but the acquisition thereof remains a future goal.

It is also important to bear in mind that the aforementioned subtypes are by no means the only ones that characterise the disorder. Recently, a large amount of interest has been invested in identifying “endophenotypes” of complex disorders. Endophenotypes are described as “measurable components unseen by the unaided eye along the pathway between disease and

distal phenotype" (Gottesman and Gould, 2003). Simply put, they are intermediate phenotypes that are more closely linked to genetic substrates than the global OCD phenotype. Presently, very few reliable endophenotypes for OCD have been identified, although proposals include the use of neuroimaging data to identify structural and functional indices of brain function in OCD patients, and measurements from neurocognitive tasks that highlight cognitive and behavioural inhibitory processes at play in OCD (Chamberlain et al., 2005; Miguel et al., 2005).

## IV.3. FAILURE TO REPLICATE POSITIVE CASE-CONTROL ASSOCIATIONS

Failure to confirm previously identified susceptibility loci seems to be fairly commonplace in association studies. Indeed, in the present study, a number of non-replications of previously reported positive results were observed. In analysing these discrepancies, it is necessary to consider both procedural and statistical issues (Owen et al., 1997). Statistical issues include the power of the individual studies and correction for multiple testing, whilst procedural issues involve investigating the possibility of population stratification, differences in diagnostic procedures and sample ascertainment.

### IV.3.1. Meta-analyses of Case-control Association Studies

Meta-analyses were conducted using the present data obtained in case-control analyses for the *5-$HT_{2A}$ -1438A/G*, *5-$HT_{2A}$ T102C*, *5-$HT_{2C}$ cys23ser*, *DRD3 ser9gly*, *DAT* 40 bp VNTR, *COMT val158met* and *DRD4* 48bp VNTR polymorphisms, and combining this with previously published data from population-based case-control studies investigating the same variants. Results obtained from the *5-$HT_{2C}$ cys23ser* and *COMT val158met* meta-analyses have already been discussed in the appropriate sections (sections **IV.2.2.1.1** and **IV.2.2.1.2,** respectively). Data generated for the remaining meta-analyses will be discussed in the following subsections.

#### IV.3.1.1. *5-$HT_{2A}$ -1438A/G* (rs6311)

The results obtained from the present case-control analysis involving the *5-$HT_{2A}$ -1438A/G* (rs6311) variant indicated that no significant association between the variant and OCD existed, in line with those obtained using a Turkish population (Tot et al., 2003). These results are in contrast to two other studies, in which evidence for the *A*-allele as a possible risk factor in increasing susceptibility to OCD, was provided (Enoch et al., 2001; Walitza et al., 2004). However, Enoch et al. (2001) observed this association only in the female population,

comprising 48 cases and 77 controls, whilst Walitza et al. (2002) observed only a marginally significant association for genotype distribution (p=0.046). Interestingly, when calculating the OR values of each study prior to the present meta-analysis, our analyses of Walitza et al's data indicated a non-significant allelic association, with an OR=0.70 (95% CI: 0.46-1.07). As mentioned, Walitza et al. (2002) had originally reported only a nominally significant finding (p=0.046); it is likely that the difference in methods in calculating significance values between the present meta-analysis and the Walitza et al. (2002) investigation is responsible for this slight discrepancy.

Nonetheless, it is of note that Walitza et al. (2002) utilised an OCD population comprising adolescents, with an average age at onset of 12.11 years, lower than that obtained for any of the other studies. Such differences in datasets are important to account for, given that they may result in inconsistent findings, and perhaps even Type I or Type II errors in a meta-analysis (the limiting factors in a meta-analysis are discussed in **section IV.3.1.6**). However, given that no significant heterogeneity was observed between the studies, the results of their study were included in the current meta-analysis.

Combining the results of the association studies in a meta-analysis revealed no evidence for association between the variant and OCD (Figure III.51). However, it is interesting that an association between EO OCD in males and *5-$HT_{2A}$ T102C*, found to be in LD with the *-1438 A/G* variant, was observed in the present study. The reasons for the contradictory results have already been discussed (**section IV.2.4.7**): it may thus be that, by stratifying OCD according to age at onset, residual background "noise" is eliminated, enabling the identification of the variant involved in this OCD subtype. However, the information available from the published studies was, unfortunately, insufficient to allow an analysis into the relationship between *5-$HT_{2A}$* and EO OCD. Further haplotype-based studies should nonetheless be conducted in order to determine whether the gene plays any role in mediating development of EO OCD.

### IV.3.1.2. *5-$HT_{2A}$ T102C* (rs6313)

When the genotype and allele frequencies of the *5-$HT_{2A}$ T102C* (rs6313) variant were compared between the non-stratified OCD and control subjects in the present study, no statistically significant differences were noted. These findings are in line with results obtained from previous investigations in Jewish (Frisch et al., 2000), Turkish (Tot et al., 2003) and Mexican (Meira-Lima et al., 2004) populations. However, all of these case-control association

investigations (including the present) were of modest size: indeed, in the present study, the power to accept the null hypothesis with certainty was only 6% for the genotypic association, and 5.3% for the allelic association. It is thus likely that none of the aforementioned studies possessed sufficient power to exclude *5-$HT_{2A}$ T102C* as a candidate marker.

Results from the meta-analysis, which investigated the relationship between the dichotomous diagnosis of OCD and the *5-$HT_{2A}$ T102C* variant, did not reveal any significant association between the variant and OCD (Figure III.52). However, given the observation, in the present study, that the variant may be linked to EO OCD in males, provides sufficient support for the variant (or, indeed, the gene) to continue to be investigated as a potential candidate.

#### IV.3.1.3. *DRD2 Taq*1A polymorphism (rs1800497)

Only two studies were included in the meta-analysis investigating the role that the *DRD2 Taq*1A variant may play in OCD. Another study, conducted by Billet et al. (1998) had also been conducted, but the author found that their data proved to be unreliable (the genotype frequencies reported totalled more than one for the OCD population; hence reliable genotypic counts could not be derived from the published data). This highlights the importance of ensuring that all data reported, such as frequencies of genotypes and alleles, are correct. No statistically significant associations between the variant and OCD were noted for either of the studies included in the meta-analysis (Table III.55), but, due to the relatively small sample sizes attained even in the meta-analysis (173 cases and 231 controls), the possibility that the variant did not play a role could not be excluded. However, an increase in power and accuracy of the observation was noted by the corresponding decrease in width of the 95% CIs.

#### IV.3.1.4. *DRD3 ser9gly* (rs6280)

Although no studies (including the present) have yielded evidence for the association of the *DRD3 ser9gly* polymorphism with OCD (Nicolini et al., 1996; Catalano et al., 1994) including these data in a meta-analysis indicated otherwise (Figure III.56). With a combined sample of 260 cases and 275 controls available for use in the meta-analysis, it was found that the *ser9*-allele was associated with an increased susceptibility to OCD. It is interesting to note that the present study and that by Catalano et al. (1994) present with very similar ORs and corresponding 95% CIs, and, although both individually produced a result with no association; it thus seems that, by increasing the sample size, sufficient power was attained, enabling the narrowing of the CI so that the significant effect could be detected.

This is an intriguing finding, and represents the first to find an association between the *DRD3* gene and OCD. Moreover, given the proposed functionality of the polymorphism, with the *gly9*-homozygote and heterozygote exhibiting a higher binding affinity for dopamine than the *ser9*-homozygote (Lundstrom and Turpin, 1996), it is possible that this may translate into a hyperdopaminergic state, which may play a role in the expression of OCD. In addition, it has recently been found that DRD2 and DRD3 form functional heterodimers in the brain that are able to inhibit cellular inhibition of AC more effectively than DRD2 alone (Scarselli et al., 2001). It could therefore be hypothesised that the *ser9*-containing DRD3 variant exhibits a reduced capacity to form functional heterodimers, possibly leading to less efficient signal transduction processes. It may thus be worthwhile, in future studies, to investigate the possible epistatic interaction that may occur between *DRD2* and *DRD3* to result in increased susceptibility to OCD.

#### IV.3.1.5. *DAT* 40bp VNTR

Two previous studies, conducted in a Jewish (Frisch et al., 2000) and a North American Caucasian (Billet et al. 1998) population had previously indicated that no association existed between the *DAT* 40bp VNTR and OCD. However, given the relatively small sample sizes of these studies (103 cases and controls in Billet et al. [1998] and 75 cases and 172 controls in Frisch et al. [2000]), it may well have been that the sample sizes were too small to detect an effect of small to moderate size that the *DAT* variant played in the development of OCD. A meta-analysis, combining the study performed by Frisch et al. (2000) and the present study was thus performed in an effort to improve power of the individual analyses. The Billet et al. [1998] data was not included, due to inconsistencies in their data: the investigators only reported genotype frequencies, which when added, exceeded 1 for both the OCD and control groups). Only the three most common alleles (the *A9/A9, A9/A10* and *A10/A10*) were included in the meta-analysis, due to lack of insufficient data concerning the other genotypes in the study by Frisch et al. (2000). However, the meta-analysis revealed no association between the variant and the development of OCD (Table III.54).

#### IV.3.1.6. *DRD4* 48bp VNTR

The *A2*-allele of the *DRD4* 48bp VNTR has been found to confer protection against the development of OCD in a French population- and family-based association study (Millet et al., 2002), whilst in a previous population-based study in a Jewish population, the *A7*-allele

was found to be less frequent in OCD Jews of non-Ashkenazi origin (Frisch et al., 2000). Data by Billet et al. (1998) was excluded (please refer to **section IV.3.1.3** for explanation). In contrast, in the present study, no significant differences in genotype or allele frequencies were noted between OCD and controls.

Due to lack of information regarding the nature and amounts of the observed genotypes by Frisch et al. (2000), it was decided that three separate meta-analyses should be conducted: one investigating the role that the *A2*-allele (given the findings by Millet et al. [2002]), one investigating the role that the *A7*-allele may play (given the preliminary findings by Frisch et al. [2000]), and one investigating the role that the *A4*-allele may play, a grouping suggested by Wang et al. [2004] and Grady et al. [2003] (**section I.6.1.21[i]**).

Although no heterogeneity was observed amongst ORs for the *DRD4 A4*-allele meta-analysis, a significant amount of heterogeneity was observed between studies for the *A2*-*A7*-allele meta-analyses, hence these were not conducted. Heterogeneity in a meta-analysis refers to the diversity of characteristics of the investigations and can arise as a result of numerous factors, including differences in sample selection (e.g. age, gender, method of diagnosis), differences in methods (e.g. genotyping methods), or it may be due to real differences between the populations, such as race, or interaction with other risk factors (this would include differences in allele frequencies and/or LD between populations). In meta-analyses including a large number of studies, it is possible to determine the source of heterogeneity by stratifying the data according to sources of potential heterogeneity. This was not plausible in the present study, given the small number of investigations included.

When the meta-analysis was conducted according to the presence or absence of the *DRD4 A4*-allele, no statistically significant differences were noted. However, the fact that the finding pertaining to the protective effect of the *DRD4 A2*-allele by Millet et al (2003) was replicated in a subsequent family-based analysis by the same investigators, and that the polymorphism was found to be associated with OCD in a haplotypic context in the present study (**section IV. 2.1**), provides sufficient evidence for further studies, perhaps using more densely-spaced markers across the whole *DRD4* gene, to be undertaken.

### IV.3.1.7. Limitations of meta-analyses

As already mentioned, meta-analyses are systematic reviews that are aimed at increasing the sample size, and consequently the power, of an association study. However, although they represent a replicable and defensible method of synthesising findings across studies, the method is not without limitations. First, the quality and usefulness of any meta-analysis is dependent on the quality of the component studies. The data in each study included in the meta-analysis should be carefully assessed for methodological errors; for example, HWE should preferably exist amongst the control individuals. A departure from HWE amongst controls can indicate, amongst other things, genotyping error; any studies with controls in HW disequilibrium should thus be excluded from the meta-analysis. In the present meta-analyses, all studies were re-assessed for HWE, and were only included if HWE was met.

Secondly, meta-analyses require some degree of commonality between studies, since the pooling of data is done under the assumption that variations between studies are minimal, and are due to chance. As already discussed (**section IV.3.1.6**), departures from homogeneity amongst studies may be a result of different phenotypic measurements, genotypic measurements, differing disease definition, variations in gene-environment interactions, and variations in LD with functional alleles in different populations (Davey, 2003). Where no statistical heterogeneity was observed in any of the present meta-analyses, it is reasonable to assume that differences between studies are due to chance fluctuations. However, in larger meta-analyses, a more detailed examination of heterogeneity should be conducted, stratifying data according to all potential sources of heterogeneity and performing the meta-analyses accordingly. Obviously, the small number of studies included in each meta-analysis in the present investigation did not allow for such stratification.

A third limiting factor may occur in the form of publication bias, which refers to publishing of significant associations more readily than non-significant ones. In the present meta-analyses, no formal test for publication bias was performed (**section I.4.5.1**) given the small numbers of studies in each meta-analysis, and the fact that, on average, more negative studies were published compared to positive associations.

The method employed in the present study, i.e. the pooling of individual studies by allele frequencies, also introduces a bias. Generally, the odds ratios in such a case represent the odds that an individual has the allele, given that he is a case or control subjects, which does not

easily translate into a risk of disease given the genotype. On the other hand, as previously discussed (section **IV.2.6.4**), to pool genotypes, one requires a validated biological model in order to know which genotypes to pool. Moreover, one is required to test different modes of inheritance, which introduces the problem of multiple testing.

In addition, it should be considered that when performing meta-analyses using data from different populations, in which discrepant results have been found, that one may in fact be diluting the signal of the genetic susceptibility factor. In association studies, one is often not looking at the susceptibility gene itself, but at an allele or locus that may be in LD with the disease gene. Subsequently, divergent findings may well be real, and reflect differences in population histories and genetic make-up. Combining such populations in meta-analyses would then affect the power to detect association of a particular allele with the phenotype. Moreover, if population admixture or stratification is present in some samples, which itself could lead to conflicting results being generated between research groups utilising different populations, this effect would be perpetuated in any meta-analyses utilising such data.

Positive meta-analyses findings point the way to future studies; however, the present meta-analyses still did not generate sample sizes that were sufficiently large to conclusively exclude any of the genes as potential candidates. This may simply be a reflection of the infancy of genetic studies in OCD; hopefully as more statistically sound genetic association studies are conducted, they will enable the implementation of more powerful meta-analyses.

## IV.4. STATISTICAL POWER

The power of a genetic association study depends on numerous interrelated variables, including the underlying genetic model that is assumed, effect size, the level of agreement between the susceptibility and marker allele frequencies, the degree of linkage disequilibrium between the susceptibility and marker alleles, case and control sample sizes and the end-point (or phenotype) under investigation (**Section I.4.4.2**). By virtue of the design of the association study, many of these variables will always be unknown, and therefore need to be approximated, rendering the calculation of the actual power of the study very difficult.

Moreover, in complex, multifactorial disorders, it is speculated that the genetic component of risk may be spread across several loci, necessitating analyses of sufficient power to detect a modest (and most likely, small) contribution by the individual genes. Where negative

associations are observed, a review of the power of the study to detect effects of small to moderate size is imperative, since one can only truly reject the null hypothesis (and thus conclusively state that no association exists between the two parameters) if certain limitations are set. Post-test power calculations were therefore performed in the present study, and it is clear from the values obtained that the power to detect small effects (especially for less common variants) is low. It should be mentioned, however, that post-test power is simply an indication of the power of the study, assuming that the observed effect size in that study is the true effect size. Consequently, one cannot compare such post-test powers between studies, unless the effect sizes that are observed are exactly the same; they thus simply serve the purpose of indicating the degree to which a Type II error might occur in that particular analysis.

For example, assuming the control:case ratio is 2:1, and the risk allele has a frequency of 0.55 in the control population, a case sample of 316 and control sample of 632 is required to attain a power of 80% (DuPont and Plummer, 1990) (Figure IV.1) (these power calculations are based on the CD/CV hypothesis. If the "geneticists nightmare" is true, with many rare alleles contributing to the aetiology of the disorder in a population-specific manner, the sample size required to achieve 80% power to detect an OR of 1.2 is in the region of 20 000!). One also needs to remember that multiple testing will result in a decrease in the significance level, which ultimately decreases the power of the study, hence the sample sizes reported here are optimistically based on a significance level of 0.05, and assume that the marker and disease alleles possess the same frequency. Indeed, in a recent study, it was estimated that the minimal number of cases and controls required to achieve a power of 80% power at $\alpha$=0.05 is usually far greater than 200 when the disease-susceptibility allele is not tested directly, even under the most favourable of circumstances (Pfeiffer and Gail, 2003).

It can thus be stated that the analyses in the present study in which candidate markers that were found to have no statistically significant association with the phenotype can, at best, be described as inconclusive. Further studies using larger sample sizes are required to determine whether one can, with certainty, exclude the marker as a potential candidate.

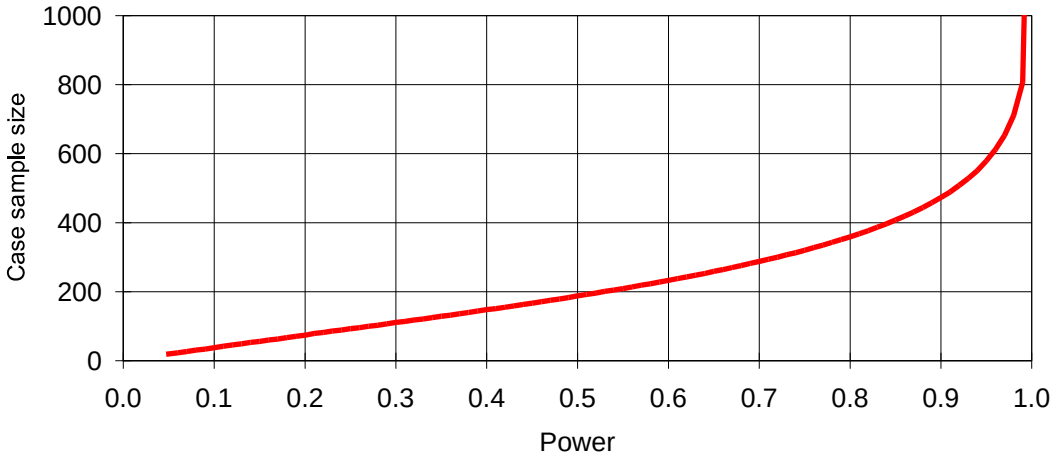



***Figure IV.1.*** *Power calculations for a risk allele frequency of 0.55 to detect an OR of at least 1.5 at a significance level of 0.05. The control sample size required is twice that of the size of the required case sample.*

The small sample sizes obtained in this study are a result of the difficulty experienced in recruiting OCD cases from the general community. Most patients are too ashamed of their symptoms to seek help, viewing their disorder as a purely psychological "taint". This places severe restrictions on the number of patients who are willing to participate in the investigative research. This desire for secrecy also limits the type of association analysis that can be performed, since it confines the number of relatives participating in the study, placing restrictions on conducting family-based association analyses. Moreover, more female than male controls seemed to be recruited for the study (n=56 versus n=162). There may be many reasons to explain this phenomenon, one being that female subjects (both case and control), when approached, were more willing to participate and more ready to offer personal information more readily compared ro male subjects. The excess of female control subjects compared to male control subjects could represent a potential limitation in the current investigation, but the fact that the OCD case subjects were matched for gender should all but eliminate this restraining factor.

In addition, when planning to perform haplotype analyses, where the same individuals need to be investigated at a host of different variants and different genes, a renewable source of DNA for these individuals, such as cell lines, is crucial, particularly if the time-span of the project is of the duration of several years. Insufficient DNA of good quality can eventually result in

small sample numbers for particular analyses, even if the number of patients enrolled at first were greater.

One way to overcome small sample sizes typical of OCD case-control association studies, including the present study, is to conduct meta-analyses where possible. Due to the combination of samples from various studies, meta-analyses present one with a means of increasing the power of a study, at the same time collating the data to extract useful information.

**IV.5. MULTIPLE COMPARISONS**

It has been found that the most likely reason for failure to replicate the results of association studies is Type I errors: many investigators fail to account, in some way, for number of tests performed (Berry et al., 1998; Colhoun et al., 2003). The appropriate means of correcting for multiple testing remains a point of contention amongst researchers. The present belief is that applying the Bonferroni correction produces a p-value that is too conservative (Perneger et al., 1998; Macciardi et al., 2003), and may thus lead to false negative (Type II error) results, especially where data is not independent.

The current study utilises both single locus and haplotype analyses in an effort to determine the underlying genetic aetiology of OCD, and thus not all of the genetic data presented is strictly independent; consequently, employing the Bonferroni correction would constitute overadjustment. Moreover, in the present study, an extensive amount of subtyping has been conducted in an effort to determine whether certain genetic substrates are more closely related to selected clinical subtypes than to the higher order construct of OCD. Although the degree to which these phenotypic subtypes may correlate is presently not known, they are all likely to be related in some way, given that they are all clinical manifestations of OCD. It is thus difficult to determine how many independent factors need to be corrected for. Indeed, correcting for multiple testing using Bonferroni's correction would have resulted in many of the associations observed in the present study being represented as non-significant, with the exception of the "very" significant association between the *DAT* 40bp VNTR and age at onset.

Given the present uncertainty regarding the most appropriate means of adjusting for multiple comparisons, the p-values were presented as is in the present study, with the obvious caution

that most of the observed associations remain to be validated by means of replication, using larger samples. In the present study, p-values for haplotype tests are also presented uncorrected, since the correction for multiple testing for haplotype investigations has not yet been well-established (Chapman et al., 2003).

## IV.6. CHOICE OF CANDIDATE GENES

The role that genes may play in mediating the development of OCD was investigated by means of candidate gene association studies, in which an *a priori* hypothesis between exposure to a given factor (the genotype in this case) and the disorder is assessed. However, one of the most important confounding factors in any psychiatric association analysis is that the research relies, to a large extent, on the knowledge and understanding of the genetic aetiology of the disorder, which, at least for OCD, is currently incomplete.

The result is that most of the candidate genes that are investigated possess low prior probability of being involved in the disorder; unless the prior probability of a candidate gene is reasonably high (in the order of 1/1000), Bayes theorem indicates that most of the positive associations observed are likely to be false positives. Unfortunately there are, as of yet, no criteria that are agreed upon for setting a value for the prior probability of a candidate gene.
The candidates in the present study included the “usual suspects” from the serotonergic and dopaminergic pathways, due to the role that they play in encoding products that are targeted by the pharmacological agents used to treat the disorder. However, while the acute effect of anti-OCD drugs is located at the neurotransmitter synapse, this may not be the region of their therapeutic effect. Indeed, they may interact with other neurotransmitters and/or initiate a cascade of events, including the activation of secondary messenger signalling pathways and subsequent gene transcription that alleviate the symptomatology.

Obviously, the use of functional polymorphisms in association studies provides the investigator with the advantage that, if a significant association between the candidate gene and OCD is detected, it could be ascribed to altered function of the encoded protein. However, many of the previous endeavours to identify OCD susceptibility genes have been hindered by attempts of researchers to transfer the Mendelian model of single gene inheritance to the model of polygenic inheritance proposed for complex disorders, by focussing on mutations occurring in the exons of candidate genes (Comings, 1998).

It is hypothesised that the susceptibility loci for many of the psychiatric disorders may, in fact, be positioned in the non-exonic gene regulatory regions (Comings, 1998; Comings et al., 1991). These regulatory regions are presently viewed as "black boxes" by most molecular biologists, even in the present era of technological advance. It is hoped that the non-functional polymorphisms used in genetic association studies might be in LD with, and thus point the way to, the identification of functional polymorphisms in the regulatory regions of genes which may be involved in the aetiology of OCD.

Indeed, in the present study, the functionality of many of the variants shown to be associated with OCD, or related subtypes, has only previously been speculated upon, especially those occurring within regulatory and 3' UTRs. Thus promoter assays to determine whether variants situated in (or near) the 5' region affect gene expression levels, and mRNA studies to assess the effect of synonymous intragenic or 3'UTR variants on mRNA stability are required. Moreover, it will also be relevant to screen and/or sequence whole gene transcripts, in order to identify potentially functional variants which may be in LD with the OCD-associated variants.

## IV.7. EPISTATIC INTERACTIONS

It is speculated that susceptibility genes may operate in unison with one another such that a particular combination of alleles may have a much stronger effect on vulnerability to OCD than each separate allele (Nothen et al, 1993; Souery et al., 2001). In other words, the susceptibility loci could be acting in unison to bring about the clinical phenotype in such a way that a particular combination of alleles may have a much stronger effect on susceptibility to OCD than a single susceptibility locus on its own. Consequently, epistatic effects could understate the impact that a single locus may have in contributing to the development of OCD, and looking at just a single candidate marker may thus lack power and reproducibility. It has been stated that epistasis may, in fact, be one of the factors that contributes to failure to replicate genetic association studies (Williams et al., 2004): therefore, genetic association studies should not only focus on investigating potential candidate genes, but also explore the possible pleiotropic, epistatic and environment dependent effects of the candidate gene.

Such analyses would, however, require sufficient knowledge regarding the genes involved in the aetiology of the disorder, and the genetic environment and functional pathways in which those genes occur (Comings, 1998), something which is currently lacking in the realm of

psychiatric disorders (including OCD). Moreover, the identification and characterisation of genes that increase susceptibility to complex disorders is a statistically and computationally intensely challenging. Data becomes sparse when multiple genetic risk factors are considered simultaneously, increasing the possibility of a false positive error using methods such as logistic regression (Peduzzi et al., 1996). On the other hand, by implementing methods such as step-wise model-fitting procedures, the risk of a Type II error increases, given that interaction effects are only tested for those variables found to have an independent main effect; polymorphisms with an interaction, but not main, effect will consequently be overlooked. However, alternative analytical strategies, including those based on neural network algorithms (Ritchie et al., 2003) have been, and are presently being, developed.

An investigation into the epistatic interactions between candidate genes in the current study would perhaps have facilitated an understanding of the genetic aetiology of the disorder. For example, the possible epistatic interactions between *GRIN2B* and *BDNF* (**section IV.2.3.1**), and between *BDNF* and *DRD3* (**section IV.2.4.5**) would be important to investigate. In addition, epistatic interactions between genes encoding gonadal hormones and *COMT* and *BDNF* represent important ones to follow up. Moreover, given the possible interaction, on a physiological level, between ACE and dopaminergic neurotransmission, it is also relevant to investigate whether these interactions translate to their genetic substrates. However, due to the limited sample size and consequently, power, of the present dataset, such an investigation could not, at this stage, be conducted. Nonetheless, as this is an ongoing study, the analysis of epistatic interactions remains a definite possibility in the near future.

## IV.8. FUTURE RESEARCH DIRECTIONS

The present study forms part of a larger ongoing collaborative research investigation in which polymorphisms in candidate genes in the relatively genetically homogeneous Afrikaner population are screened, to elucidate the potential role they might play in the aetiology of OCD.

Evidence is presently being gathered to support the notion of susceptibility alleles that are common to related psychiatric disorders. A single common genetic foundation seems improbable, but it may be that certain “building blocks” could be shared. In this regard it might be useful to look beyond traditional diagnostic boundaries for shared genetic aetiologies to certain symptom subtypes. As the numbers of Afrikaner OCD subjects recruited

in the coming years increases, the genetic analyses within such subgroups should yield sufficient statistical power to rule out the possibility of Type I or Type II errors.

It may also be of use to employ other quantitative approaches in the design of the investigation. For example, many of the obsessions experienced by OCD patients are endorsed as "worries" by psychologically normal individuals - the possibility should therefore be considered that OCD symptoms can be broken down into multiple dimensions that are continuous with the normal population (Leckman et al., 2001; Leboyer et al., 1998). This would represent an important route to disentangling the complex inheritance of OCD. The results obtained from genetic investigations should be incorporated with clinical and epidemiological parameters to correctly elucidate the cause of OCD.

Moreover, future studies should be extended to incorporate the screening of more polymorphisms within the context of a larger Afrikaner population, and high resolution mapping within specific chromosomes will improve knowledge regarding the impact of genetic diversity within the genes or linked chromosomal regions in OCD. The advantages of a "gene-based" instead of a "SNP-based" approach are becoming ever more apparent (Neale and Sham, 2004): therefore, a more complete assessment of candidate genes, possibly utilising haplotype blocks that span larger regions, is proposed. A systematic genome-wide approach is also required in order to identify currently unknown susceptibility genes for OCD. To this end, an exciting ongoing collaboration with the New York State Psychiatric Institute has presented our teams with the opportunity to conduct genome-wide association studies, using the Afrikaner population.

Increasing the amount of information on human genome sequences and polymorphisms will make it possible to characterise the amount of sequence variation expressed in the brain, and to delineate the potential effects that these variations may have on the development of OCD. Knowledge of new functional variants will emerge as researchers gain an appreciation of the potential for genetic variants in the coding and regulatory regions to impact on gene expression.

It seems that, presently, technological advances in the molecular biology arena are superseding those in the statistical genetics arena. Novel statistical methods capable of identifying genes with small to moderate effects amongst a variety of homogeneous factors is

needed. Available statistical methodology needs to be re-examined and improved to make it more applicable to genetics and molecular biology. They should be empirically tested in order to compare and contrast the relative strengths and weaknesses of methods specific to particular genetic association questions.

Finally, a serious effort at collaboration should be made between neurogeneticists, neurologists, psychiatrists, psychologists, bioinformaticians and statisticians involved in unravelling the complex aetiology of OCD. This would, however, require a more critical, standardised use of clinical information in order to increases the comparability of global data, and facilitate the assimilation of the data. The competitive nature of many research teams, and lack of incentive to re-analyse old data may mar the initiation of such collaborations, but they may be the only hope we have of identifying disease-susceptibility genes amidst a labyrinth of genetic, clinical and environmental heterogeneity.

## IV.9. CONCLUSION

The preliminary and frequently inconsistent nature of the data represented in the majority of psychiatric genetic association studies attempting to find genetically-based aetiological factors for OCD might seem discouraging. However, these studies represent the initial forays into what promises to be an exciting frontier. The maze of genetic susceptibility and environmental factors contributing to the development of OCD has posed an enormous challenge to psychiatric geneticists, but many researchers have responded to this challenge by developing increasingly powerful molecular and statistical tools.

Given the small effects that each identified, validated, susceptibility genotype will have in contributing to OCD, the predictive value of any single susceptibility variant is going to be very limited. However identifying susceptibility genes may afford researchers a better understanding of the role that a dysfunction in a particular protein may play in disease causation. These may include the interaction of the gene product with other protein or genetic substrates and the possible effect of environmental modification on protein levels and functions. So far, many of the environmental risk factors that play a role in OCD have proven elusive: hopefully, they will be identified as a result of the elimination of genetic “noise” as identification and comprehension of predisposing genetic factors improves. Such genetic characterisation of OCD individuals may offer insight into molecular and biochemical sub-categories of the disorder, previously indiscernible by clinicians. This, in turn, could lead to

the improvement in diagnostic and treatment approaches, since treatments may be better tailored to target the major contributing sub-phenotype comprising the disorder, instead of implementing a more global treatment regime, which may prove to be ineffective.

The present results yield some exciting and interesting preliminary observations, which are to be followed up, in a larger sample and with additional gene variants in the associated genes, and will employ additional LD tests and functional assays to verify the causal implications of verified associations. Indeed, the small sample size (especially after stratification) remains the most important limitation, and efforts are underway to improve and increase the Afrikaner OCD sample size. However, although certain characteristics of the Afrikaner population, such as genetic, cultural and diagnostic homogeneity may have a major impact on simplifying the identification of susceptibility loci involved in the aetiology of OCD, the probability exists that several genetic and environmental factors with relatively small individual effect contribute to the risk of developing OCD, even in the Afrikaner population. In the forthcoming years, researchers will hopefully gain insight into these aspects of OCD, which should prove to be invaluable to establishing the aetiology, with the subsequent introduction of new treatment strategies for this incapacitating psychiatric disorder.

# APPENDIX I

## BUFFERS, MARKERS AND SOLUTIONS

### 1. DNA EXTRACTION SOLUTIONS

**Cell Lysis Buffer**

| | |
|---|---|
| Sucrose | 0.32M |
| Triton-X-100 | 1% |
| $MgCl_2$ | 5mM |
| Tris-HCl | 10mM |
| $H_2O$ | 1litre |

**3M Sodium acetate**

| | |
|---|---|
| Sodium Acetate | 40.81g |
| $H_2O$ | 50ml |

Adjust pH to 5.2 with glacial acetic acid (Merck) and adjust volume to 100ml with $ddH_2O$

**Na-EDTA solution**

| | |
|---|---|
| NaCl (Merck) | 18.75ml of 4M stock solution |
| EDTA (B&M Scientific) | 250ml of a 100mM stock solution |

Mix well

**Phenol/Chloroform**

| | |
|---|---|
| Phenol (saturated with 1XTE) (Merck) | 50ml |
| Chloroform (Merck) | 48ml |
| 8-hydroxyquinone (Merck) | 2ml |

Mix well, store at 4˚C

**Chloroform/octanol (24:1)**

| | |
|---|---|
| Chloroform (Merck) | 96ml |
| Octanol (Merck) | 4ml |
| Mix well, store at 4˚C | |

**TE-Buffer (10x stock)**

| | |
|---|---|
| Tris OH | 0.1M(pH 8.00) |
| EDTA(pH8) | 0.01M (pH 8.00) |
| $H_2O$ | 150ml |

**2. ELECTROPHORESIS STOCK SOLUTIONS**

**TBE-buffer (10x stock)**

| | |
|---|---|
| Tris-HCl (Biorad) | 108g |
| Boric Acid (Merck) | 58g |
| $Na_2EDTA$ (Merck) | 9.3g |
| ddH2O to a final volume of 1 litre | |

**Bromophenol Blue**

| | |
|---|---|
| Bromophenol blue | 0.2%(w/v) |
| Glycerol | 50% |
| Tris (pH8) | 10mM |

**Ethidium Bromide**

| | |
|---|---|
| Ethidium Bromide | 500mg |
| ddH2O | 50ml |

**3. MOLECULAR SIZE MARKERS**

| | |
|---|---|
| Bacteriophage Lambda DNA (250µg) | 100µl |
| Buffer M (Boerhinger Mannhein) | 15µl |
| *Pst*I (Boerhinger Mannheim) | 11µl |
| $H_2O$ | 32µl |

Incubate at 37°C for 2 hours followed by heat inactivation at 65°C for 5 minutes.

Load 2µl onto polyacrylamide gels.

**4. SOLUTIONS FOR SILVER STAINING**

**0.1% $AgNO_3$ (Solution B)**

| | |
|---|---|
| $AgNO_3$ | 1g |
| $H_2O$ | 1litre |

**Developing Solution (Solution C)**

| | |
|---|---|
| NaOH | 15g |
| $NaBH_4$ | 0.1g |
| Formaldehyde | 4ml |

$H_2O$ up to a final volume of 1 litre

**5. SOLUTIONS FOR POLYACRYLAMIDE GELS**

**10% Ammonium persulphate (APS)**

| | |
|---|---|
| APS | 2g |
| $H_2O$ | 20ml |

## 6. GELS

### 12% POLYACRYLAMIDE GEL

| | |
|---|---|
| 30 acrylamide/0.8% bis-acrylamide stock | 4ml |
| 10xTBE | 1ml |
| Distilled $H_2O$ | 5ml |
| APS | 80µl |
| TEMED | 30µl |

# APPENDIX II

## LIST OF SUPPLIERS

| | |
|---|---|
| 6-FAM | Applied Biosystmes |
| Acrylamide | Merck |
| $AgNO_3$ | Merck |
| Ammonium persulphate | Merck |
| *Apo*I | New England Biolabs |
| *Bgl*I | Promega |
| *Blp*I | New England Biolabs |
| Bis-acrylamide | Merck |
| Boric acid | Merck |
| Bromophenol blue | Merck |
| *Dde*I | Roche Applied Science |
| dGTP | Boerhinger Mannheim |
| dCTP | Boerhinger Mannheim |
| dTTP | Boerhinger Mannheim |
| dATP | Boerhinger Mannheim |
| *EcoR*V | Promega |
| EDTA | Boerhinger Mannheim |
| Ethanol | Boerhinger Mannheim |
| *Exo*I | Amersham |
| *Fok*I | New England Biolabs |
| Formamide | Merck |
| Formaldeyde | Merck |
| *Fsp*I | New England Biolabs |
| GeneScan-120 LIZ internal sizing standard | Applied Biosystmes |
| Glycerol | FMC |
| *Hha*I | Promega |
| *Hind*III | Promega |
| Hi-Di formamide | Applied Biosystmes |

| | |
|---|---|
| *Hinc*II | Promega |
| *Hpa*II | Promega |
| K-acetate | Sigma |
| KCl | Merck |
| Lambda DNA | Promega |
| *Mnl*I | New England Biolabs |
| *Msc*I | Roche Applied Science |
| *Msp*I | Promega |
| NaAc | Merck |
| NaCl | BDH Chemicals |
| NaOH | Sigma |
| *Nla*III | New England Biolabs |
| Oligonucleotide primers | Department of Biochemistry, University of Cape Town School |
| PBS | Sigma |
| POP4 (performance optimizimg polymer) | Applied Biosystmes |
| Phenol/Chloroform | Merck |
| Phenol/Octanol | Merck |
| Proteinase K | Sigma |
| *Pst*I | Promega |
| *Pvu*II | Promega |
| Qiagen Kit | Stratagene |
| *Rsa*I | Promega |
| ROX100 fragment size standard | Applied Biosystmes |
| SAP | Roche Applied Science |
| SDS | Sigma |
| SNaPshot Multiplex Ready Reactiohn mix | Applied Biosystems |
| *Taq*I | Promega |
| Taq polymerase | Bioline |
| TEMED | Sigma |

| | |
|---|---|
| Tris | Merck |
| Tris-OH | Merck |
| Tris-HCl | Merck |
| *Xba*I | Promega |

# APPENDIX III

## PATIENT INFORMATION AND INFORMED CONSENT
## Genetics of Anxiety Disorders

**PURPOSE**:

This study is part of a research project we are conducting to learn more about the genetic causes and symptoms of anxiety disorders (including obsessive-compulsive and spectrum disorders, panic disorder or social phobia). We would like to discuss your life experiences and those of your other family members with you. Doctors and scientists at the MRC Unit on Anxiety and Stress Disorders and the University of Stellenbosch, in collaboration with qualified researchers from other research institutions worldwide, hope to identify the genes that may increase susceptibility to these disorders.
This is not a treatment study. Information is being collected for research purposes only.

**STUDY PROCEDURE:**

If you decide to participate, we shall ask you to attend an interview (which may be videotaped) with a researcher. This interview will include neuropsychological tasks and a number of questions related to your current illness, your prior history of treatment for psychiatric conditions, and particular symptoms you may have experienced as part of your illness. In addition, we may ask to take photographs of your face and hands. This whole procedure will last about 4-5 hours (two 2-hour sessions with a break in-between).

You will also be asked to have your blood drawn. Approximately 48 ml (3 tablespoons) of blood will be drawn from your arm. We may need to contact you again to get another blood sample should we fail to get a DNA sample from your blood. The blood sample you give may be used to create a cell line. This is done by changing some of your blood cells so that they can grow forever. The cell line is living tissue and it can be used to make more of your DNA at any time in the future. This process will take place at the MRC Centre for Molecular and Cellular Biology and the Division of Medical Biochemistry, Faculty of Health Sciences, at the University of Stellenbosch. The DNA will then be taken from the cell line and saved for scientific analyses which will be performed now, and possibly in the future.

We may contact you later for further information, or request you to complete another interview at a later date, in order to obtain follow-up information that may be of use in our genetic analyses. This may involve an assessment similar to the current assessment, including a series of interviews and/or another blood sample. Your current participation is in no way binding to your future participation.

We would like your permission to contact your relatives in order to get more information about any family history of mental illness. You can still participate in the study even if your relatives do not.

Personal information that could be used to identify you (such as your name,

contact information, etc) will not be given out. Your data and DNA is likely to be made available to qualified scientists around the world to study your particular anxiety disorder. Your cell line and DNA will be maintained permanently, unless you request to have it removed. If at any time in the future you wish to have your DNA, cell lines or clinical data removed from the storage site, you may do so by contacting the researchers conducting this study (Christine Lochner at 021 - 938 9179).

The researchers who will have access to your DNA include those who work with private and/or for profit companies. These researchers may be interested in eventually developing commercial medical products using the DNA from you and other participants. They may sell or patent discoveries based on this research and thus benefit financially. Please note that you or your heirs will not receive any compensation if this occurs.

We do not expect to discover any information of direct benefit to your condition, or your treatment, during the next few years. If later on, diagnostic tests or new ways to treat your condition are discovered, this information will have to be obtained from properly licensed clinical labs, clinics, or your physician, and will not be available from the research team.

If you are hospitalized at a psychiatry facility or have received any treatment from a mental health professional, we would like your permission to review your treatment records, which will be obtained from your doctor.

**RISKS:**

There are no more than minimal medical or psychological risks associated with this study. If you feel fatigued, tired, uncomfortable, or in any way upset during any part of the session(s), you may ask to stop for a rest break or have the interview discontinued. The research interview does not take the place of a full psychiatric evaluation. You may experience some emotional discomfort when answering some questions. If any particular question makes you feel uncomfortable, you may discuss its importance with the specially trained interviewer. You may choose not to answer any question which you are still uncomfortable with.

You may feel some pain associated with having blood withdrawn from a vein. You may experience discomfort, bruising and/or other bleeding at the site where the needle is inserted. Occasionally, some people experience fleeting dizziness or feel faint when their blood is drawn.

Some insurance companies may mistakenly assume that your participation in this study is an indication that you are at higher risk of a genetic disease, and this could hurt your access to health or other insurance. We will not share any information about you, or your family, with an insurance company. However, if you discuss your participation in this study with your doctor, and he or she records it in your medical record, it is possible that an insurance company may access the information as part of a medical record review. It is the opinion of the investigators that participation in this study does not constitute genetic testing. Although one long-term goal of this research is the development of a genetic test for the anxiety disorders, at the current time, no

information from your DNA sample that would be useful in the treatment of your disorder will be obtained. Therefore, participation in this study should not be reported as genetic testing.

Your unidentified DNA and cell line will be available to qualified researchers permanently.

## BENEFITS:

There are no direct benefits to you. However, individuals who might develop one or more of these anxiety disorders in the future, their family members, and future generations may benefit if we can locate the genes that lead to such disorders. That knowledge could then lead to the development of methods for prevention and new treatments for curing these diseases.

### CONFIDENTIALITY:

If you consent to participate in this study, your identity will be kept confidential. Your answers will not be shared with other family members or anyone else except for staff members involved in this study. All data will be kept in locked file cabinets accessible only to the research staff. All research information obtained will not be associated with your name; research staff will use only a coded number and/or your initials. Blood samples will be safely stored and identified by code number and access will be limited to authorized scientific investigators. Copies of treatment records from hospitals or mental health professionals are kept in locked files and are reviewed by members of the research team only. Any publications resulting from this study will not identify you by name.

## VOLUNTARY PARTICIPATION:

Your participation in this study is voluntary and you may refuse to participate or withdraw from the study at any time without any loss of benefits to which you are otherwise entitled. Some members of the team of investigators conducting this study may be responsible for your clinical care. Refusal to participate in this study will not change your clinical care.

## RESEARCH QUESTIONS AND CONTACTS:

If you are interested in genetic counseling, you will be given information about where you can receive such counseling and a new blood sample may be required at that time. DNA information about a relative will be released only if the genetic counsellor confirms that the relative in question is deceased or cannot be found and that the information is essential for clinical counseling.

The researchers will answer any questions you might have about the procedures described above, or about the results of the study. If you have any questions, you may call Christine Lochner at (021) 938 9179.

The University of Stellenbosch Research Subcommittee C has approved recruitment and participation of individuals for this study.

You have been given a copy of this consent form to keep.

**INFORMED CONSENT:**

I have read the above patient information, my questions have been answered, and I consent voluntarily to participate in this study.

Print name: _____ Signature: _____

Date: _____

I have discussed the proposed research with this subject and, in my opinion, this patient understands the benefits, risks, and alternatives (including non-participation) and is capable of consenting to voluntary participation.

Print name: _____ Signature: _____
Study Investigator or Designee

Date: _____

Print name: _____ Signature: _____
Witness (if applicable)

Date: _____

**PASIëNTINLIGTING EN INGELIGTE TOESTEMMING**
**Genetika van Angssteurings**

## DOELWIT:

Hierdie projek is deel van 'n navorsingsprojek wat tans onderneem word om meer uit te vind oor die genetiese oorsake en simptome van angssteurings (insluitend obsessief-kompulsiewe- en spektrumversteurings, paniek-, of sosiale angssteuring). Ons wil graag oor u lewenservarings en dié van u gesinslede met u gesels. Dokters en wetenskaplikes by die MNR Eenheid vir Angs- en Stressteurings en die Universiteit van Stellenbosch, in samewerking met gekwalifiseerde navorsers van ander navorsingsinstellings wêreldwyd, hoop om die gene wat vatbaarheid vir hierdie angssteurings laat toeneem, te identifiseer. Dit is nie 'n behandelingstudie nie. Inligting word alleenlik vir navorsingsdoeleindes versamel.

## PROJEKPROSEDURE:

Indien u besluit om deel te neem, sal ons u vra om 'n onderhoud (wat moontlik op videoband vasgelê kan word,) met 'n navorser te voer. Hierdie onderhoud sluit neurosielkundige take en 'n aantal vrae in wat met die volgende aspekte verband kan hou: u huidige siekte, u geskiedenis van behandeling vir psigiatriese steurings, en spesifieke simptome wat u dalk kon ervaar as deel van u siekte. Daarmee saam, kan ons u vra om foto's van u hande en gesig te neem. Hierdie hele prosedure sal ongeveer 4-5 ure duur (twee 2-uur sessies met 'n pouse tussen-in).

U sal ook gevra word om toe te laat dat u bloed getrek word. Ons kan dalk weer met u in verbinding moet tree om nog 'n bloedmonster te trek in geval ons nie daarin kon slaag om 'n DNA monster van u bloed te verkry nie. Die bloedmonster wat u gee, kan gebruik word om 'n sellyn te skep. Dit word gedoen deur sommige van u bloedselle te verander sodat dit vir altyd kan groei. Die sellyn is lewende weefsel en dit kan gebruik word om meer van u DNA in die toekoms te maak. Hierdie proses sal plaasvind by die MNR Sentrum vir Molekulêre en Sellulêre Biologie en die Afdeling Geneeskundige Biochemie, Fakulteit Gesondheidswetenskappe, Universiteit van Stellenbosch. Die DNA sal dan van die sellyn geneem en gehou word vir wetenskaplike analise wat nou, en moontlik in die toekoms gedoen sal word.

Ons kan met u in aanraking kom vir verdere inligting, of u vra om nog 'n onderhoud te voltooi op 'n latere stadium, ten einde opvolg-inligting te bekom wat gebruik kan word in ons genetika-analise. Dit kan 'n soortgelyke assessering as die huidige wees, insluitend 'n reeks van onderhoude en/of ander bloedmonsters behels. U huidige deelname verbind u onder geen omstandighede tot toekomstige deelname nie.

Ons wil graag u toestemming hê om met u familielede in aanraking te kom ten einde meer inligting oor enige familiegeskiedenis van geestessiekte te bekom. U kan steeds deelneem aan die projek selfs al is u familielede nie betrokke nie.

Persoonlike inligting wat gebruik kan word om u te identifiseer (soos u naam, kontakbesonderhede, ens.), sal nie uitgegee word nie. U data en DNA sal moontlik aan gekwalifiseerde wetenskaplikes regoor die wêreld beskikbaar gestel word om u betrokke angssteuring te bestudeer. U sellyn en DNA sal permanent gehou word, behalwe wanneer u vereis dat dit verwyder word. Indien u op enige stadium in die toekoms besluit om u DNA, sellyne of kliniese inligting uit die bergingsplek te laat verwyder, kan u dit doen deur die navorsers wat hierdie projek behartig, te vra om dit te doen (Christine Lochner by 021 - 938 9179).

Die navorsers wat tot u DNA toegang het, sluit diegene in wat werk met private en/of winsgeoriënteerde maatskappye. Hierdie navorsers kan ook daarin geïnteresseerd wees om uiteindelik kommersiële mediese produkte te ontwikkel deur van u en die ander deelnemers se DNA gebruik te maak. Hulle kan hierdie uitvindings, wat op hierdie navorsing gebaseer is, verkoop of patenteer en sodoende finansieel daaruit voordeel trek. Let asseblief daarop dat u of u erfgename nie enige kompensasie hiervoor sal ontvang indien dit wel gebeur nie.

Ons verwag nie om enige inligting te bekom wat van direkte nut vir u toestand of u behandeling gedurende die volgende paar jare sal wees nie. Indien daar in die toekoms diagnostiese toetse of nuwe wyses om u toestand te behandel, ontdek word, sal hierdie inligting van behoorlik gelisensieërde kliniese laboratoria, klinieke, of u mediese dokter verkry moet word, en dus nie van die navorsingspan nie.

Indien u by 'n psigiatrie fasiliteit gehospitaliseer word, of behandeling van 'n geestesgesondheidswerker ontvang, wil ons graag u toestemming hê om u behandelingsrekords, wat van u dokter verkry sal word, na te gaan.

**RISIKO'S:**

Daar is nie meer as die minimum mediese en sielkudige risiko's geassosieer met hierdie projek nie. Indien u uitgeput, ongemaklik, of ontsteld raak tydens enige gedeelte van die sessie(-s), kan u vra om te onderbreek vir 'n ruskansie of om die onderhoud te beëindig. Die onderhoud wat met u gevoer word, neem nie die plek van 'n deeglike psigiatriese evaluasie nie. U kan dalk 'n mate van emosionele ongemak verduur wanneer u sommige van die vrae beantwoord. Indien enige vraag u ongemaklik laat voel, kan u die belang daarvan met die spesiaal opgeleide onderhoudvoerder bespreek. U kan verkies om enige vraag waarmee u steeds ongemaklik voel, nie te beantwoord nie.

U kan moontlik 'n mate van pyn ervaar wanneer die bloed getrek word. U kan ongemak, kneusing en/of bloeding by die plek waar die naald ingesteek word, ervaar. Soms ervaar sommige persone verbygaande duiseligheid of 'n flou gevoel wanneer hulle bloed getrek word.

Sommige versekeringsmaatskappye kan verkeerdelik aanneem dat u deelname aan hierdie projek 'n aanduiding is dat u 'n verhoogde risiko het vir 'n genetiese siekte, en dit kan u toegang tot gesondheid- of ander versekering skaad. Ons sal nie enige inligting oor u, of u familie aan 'n versekeringsmaatskappy bekendmaak nie. Indien u egter u deelname met u dokter bepreek, en hy/sy maak 'n nota daarvan in u mediese rekord, is dit moontlik dat 'n versekeringsmaatskappy hierdie inligting as deel van 'n hersiening van mediese rekords kan bekom. Dit is die mening van die navorsers dat deelname aan hierdie studie nie genetiese toetsing is nie. Alhoewel een langtermyn-doelwit van hierdie navorsing die ontwikkeling van 'n genetiese toets vir die angssteurings is, sal geen inligting van u DNA-monster wat nuttig kan wees in die behandeling van u toestand, tans verkry word nie. Daarom behoort deelname aan hierdie studie nie as genetiese toetsing beskryf te word nie.

U ongeïdentifiseerde DNA en sellyn sal <u>permanent</u> aan gekwalifiseerde navorsers beskikbaar wees.

**<u>VOORDELE:</u>**

Daar is geen direkte voordele vir u nie. Individue wat egter in die toekoms een of meer van hierdie angssteurings ontwikkel, hulle familielede, en toekomstige generasies, kan voordeel daaruit put as ons die gene wat tot sulke versteurings aanleiding kan gee, kan identifiseer. Hierdie kennis kan dan lei tot die ontwikkeling van metodes vir voorkoming en nuwe behandelingswyses vir genesing van die siektes.

**<u>VERTROULIKHEID:</u>**

Indien u toestem tot deelname aan die projek, sal u identiteit vertroulik gehou word. U antwoorde sal nie met u familielede of enige iemand anders behalwe die personeellede wat gemoeid is met hierdie projek, gedeel word nie. Alle inligting sal in geslote liasseringskabinette wat slegs vir navorsingspersoneel toeganklik is, gehou word. Alle navorsingsinligting wat verkry word, sal nie met u naam verbind kan word nie; navorsingspersoneel sal bloot 'n kodenommer en/of u voorletters gebruik. Bloedmonsters sal veilig gestoor en geïdentifiseer word deur die kodenommer, en toegang sal tot die gemagtigde wetenskaplike navorsers beperk wees. Kopieë van behandelingsrekords van hospitale of geestesgesondheidswerkers word in geslote lêers gehou en word slegs deur lede van die navorsingspan deurgegaan. Enige publikasie wat uit hierdie projek voorspruit, sal u nie by name identifiseer nie.

**VRYWILLIGE DEELNAME:**

U deelname aan hierdie projek is vrywillig en u kan deelname weier of u op enige stadium van die projek onttrek sonder verlies van enige voordele waartoe u andersins geregtig is. Sommige lede van die span navorsers wat hierdie projek uitvoer, kan moontlik verantwoordelik wees vir u kliniese versorging. Weiering om deel te neem aan hierdie studie sal nie u kliniese versorging verander nie.

**VRAE OOR DIE NAVORSING EN KONTAKBESONDERHEDE:**

Indien u wel in genetiese berading geïnteresseerd is, sal u inligting oor waar sodanige berading beskikbaar is, ontvang en 'n nuwe bloedmonster kan op daardie stadium vereis word. DNA-inligting van 'n familielid sal slegs beskikbaar gestel word indien die genetiese berader bevestig dat die familielid oorlede is of nie opgespoor kan word nie en dat die inligting noodsaaklik is vir kliniese berading.

Die navorsers sal enige vrae wat u mag hê oor bogenoemde prosedures of oor die resultate van die projek, beantwoord. Indien u enige navrae het, kan u Christine Lochner by 021 - 938 9179 skakel.

Die Navorsingsubkomitee C van die Universiteit van Stellenbosch het die werwing en deelname van individue aan hierdie projek goedgekeur.

U het 'n afskrif van hierdie toestemmingsvorm ontvang om te bewaar.

**INGELIGTE TOESTEMMING:**

Ek het die bostaande pasiëntinligting gelees, my vrae is beantwoord, en ek stem vrywillig in om aan hierdie projek deel te neem.

Naam:_____ Handtekening:
_____

Datum: _____

Ek het die voorgestelde projek met die deelnemer bespreek en, na my mening, verstaan die deelnemer die voordele, risiko's, en alternatiewe (inlsuitend nie-deelname) en is in staat om toestemming te gee vir vrywillige deelname.

Naam:_____ Handtekening:
_____
Navorser of Gemagtigde

Datum: _____

Naam:_____ Handtekening: _____

Getuie (indien van toepassing)

Datum: _____

# APPENDIX IV

## PUBLICATIONS

**Lochner C, Hemmings SM, Kinnear CJ, Niehaus DJ, Nel DG, Corfield VA, Moolman-Smook JC, Seedat S, Stein DJ**. **2005.** Cluster analysis of obsessive-compulsive spectrum disorders in patients with obsessive-compulsive disorder: clinical and genetic correlates. *Compr.Psychiatry* **46:** 14-19.

**Hemmings SM, Kinnear CJ, Lochner C, Niehaus DJ, Knowles JA, Moolman-Smook JC, Corfield VA, Stein DJ**. **2004.** Early- versus late-onset obsessive-compulsive disorder: investigating genetic and clinical correlates. *Psychiatry Res.* **128:** 175-182.

**Lochner C, Hemmings SM, Kinnear CJ, Moolman-Smook JC, Corfield VA, Knowles JA, Niehaus DJ, Stein DJ**. **2004.** Corrigendum to "gender in obsessive-compulsive disorder: clinical and genetic findings" [Eur. Neuropsychopharmacol. 14 (2004) 105-113]. *Eur.Neuropsychopharmacol.* **14:** 437-445.

**Lochner C, Hemmings SM, Kinnear CJ, Moolman-Smook JC, Corfield VA, Knowles JA, Niehaus DJ, Stein DJ**. **2004.** Gender in obsessive-compulsive disorder: clinical and genetic findings. *Eur.Neuropsychopharmacol.* **14:** 105-113.

**Hemmings SM, Kinnear CJ, Niehaus DJ, Moolman-Smook JC, Lochner C, Knowles JA, Corfield VA, Stein DJ**. **2003.** Investigating the role of dopaminergic and serotonergic candidate genes in obsessive-compulsive disorder. *Eur.Neuropsychopharmacol.* **13:** 93-98.

# REFERENCES

**Abdolmaleky HM, Smith CL, Faraone SV, Shafa R, Stone W, Glatt SJ, Tsuang MT**. **2004.** Methylomics in psychiatry: Modulation of gene-environment interactions may be through DNA methylation. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **127:** 51-59.

**Abecasis GR, Noguchi E, Heinzmann A, Traherne JA, Bhattacharyya S, Leaves NI, Anderson GG, Zhang Y, Lench NJ, Carey A, Cardon LR, Moffatt MF, Cookson WO**. **2001.** Extent and distribution of linkage disequilibrium in three genomic regions. *Am.J.Hum.Genet.* **68:** 191-197.

**Abramowitz JS, Franklin ME, Schwartz SA, Furr JM**. **2003.** Symptom presentation and outcome of cognitive-behavioral therapy for obsessive-compulsive disorder. *J.Consult Clin.Psychol.* **71:** 1049-1057.

**Accili D, Fishburn CS, Drago J, Steiner H, Lachowicz JE, Park BH, Gauda EB, Lee EJ, Cool MH, Sibley DR, Gerfen CR, Westphal H, Fuchs S**. **1996.** A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. *Proc.Natl.Acad.Sci.U.S.A* **93:** 1945-1949.

**Ackerman DL, Greenland S, Bystritsky A, Morgenstern H, Katz RJ**. **1994.** Predictors of treatment response in obsessive-compulsive disorder: multivariate analyses from a multicenter trial of clomipramine. *J.Clin.Psychopharmacol.* **14:** 247-254.

**Adams MM, Fink SE, Janssen WG, Shah RA, Morrison JH**. **2004.** Estrogen modulates synaptic N-methyl-D-aspartate receptor subunit distribution in the aged hippocampus. *J.Comp Neurol.* **474:** 419-426.

**Adamson MD, Kennedy J, Petronis A, Dean M, Virkkunen M, Linnoila M, Goldman D**. **1995.** DRD4 dopamine receptor genotype and CSF monoamine metabolites in Finnish alcoholics and controls. *Am.J.Med.Genet.* **60:** 199-205.

**Adkins RM**. **2004.** Comparison of the accuracy of methods of computational haplotype inference using a large empirical dataset. *BMC.Genet.* **5:** 22.

**Aguiar MS, Brandao ML**. **1994.** Conditioned place aversion produced by microinjections of substance P into the periaqueductal gray of rats. *Behav.Pharmacol.* **5:** 369-373.

**Akey J, Jin L, Xiong M**. **2001.** Haplotypes vs single marker linkage disequilibrium tests: what do we gain? *Eur.J.Hum.Genet.* **9:** 291-300.

**Albert U, Maina G, Ravizza L, Bogetto F**. **2002.** An exploratory study on obsessive-compulsive disorder with and without a familial component: are there any phenomenological differences? *Psychopathology* **35:** 8-16.

**Alexander GE, DeLong MR, Strick PL**. **1986.** Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu.Rev.Neurosci.* **9:** 357-381.

**Alsobrook II JP, Leckman JF, Goodman WK, Rasmussen SA, Pauls DL**. **1999.** Segregation analysis of obsessive-compulsive disorder using symptom-based factor scores. *Am.J.Med.Genet.* **88:** 669-675.

**Alsobrook JP, Pauls DL**. **1998.** Molecular approaches to child psychopathology. *Hum.Biol.* **70:** 413-432.

**Alsobrook JP, Zohar AH, Leboyer M, Chabane N, Ebstein RP, Pauls DL**. **2002.** Association between the COMT locus and obsessive-compulsive disorder in females but not males. *Am.J.Med.Genet.* **114:** 116-120.

**Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, Lindsay RM, Wiegand SJ**. **1997.** Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature* **389:** 856-860.

**Altar CA, DiStefano PS**. **1998.** Neurotrophin trafficking by anterograde transport. *Trends Neurosci.* **21:** 433-437.

**American Psychiatric Association. 1994.** Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Press, Washington DC.

**Ananth J, Pecknold JC, van den SN, Engelsmann F**. **1981.** Double-blind comparative study of clomipramine and amitriptyline in obsessive neurosis. *Prog.Neuropsychopharmacol.* **5:** 257-262.

**Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH**. **2000.** Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse* **37:** 167-169.

**Andrade C**. **1998.** Risperidone may worsen fluoxetine-treated OCD. *J.Clin.Psychiatry* **59:** 255-256.

**Angius A, Melis PM, Morelli L, Petretto E, Casu G, Maestrale GB, Fraumene C, Bebbere D, Forabosco P, Pirastu M**. **2001.** Archival, demographic and genetic studies define a Sardinian sub-isolate as a suitable model for mapping complex traits. *Hum.Genet.* **109:** 198-209.

**Angst J, Gamma A, Endrass J, Goodwin R, Ajdacic V, Eich D, Rossler W**. **2004.** Obsessive-compulsive severity spectrum in the community: prevalence, comorbidity, and course. *Eur.Arch.Psychiatry Clin.Neurosci.* **254:** 156-164.

**Antony MM, Downie F, Swinson RP. 1998**. Diagnostic issues and epidemiology in obsessive-compulsive disorder. **In**: Swinson RP, Antony MM, Rachman S, Richter MA (eds). Obsessive-compulsive disorder: Theory, Research and Treatment. New York: Guilford. pp3-27

**Apter A, Pauls DL, Bleich A, Zohar AH, Kron S, Ratzoni G, Dycian A, Kotler M, Weizman A, Gadot N, . 1993.** An epidemiologic study of Gilles de la Tourette's syndrome in Israel. *Arch.Gen.Psychiatry* **50:** 734-738.

**Arcot SS, Shaikh TH, Kim J, Bennett L, Alegria-Hartman M, Nelson DO, Deininger PL, Batzer MA**. **1995.** Sequence diversity and chromosomal distribution of "young" Alu repeats. *Gene* **163:** 273-278.

**Ardlie KG, Kruglyak L, Seielstad M**. **2002.** Patterns of linkage disequilibrium in the human genome. *Nat.Rev.Genet.* **3:** 299-309.

**Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR**. **1997.** Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res.* **752:** 26-34.

**Arinami T, Li L, Mitsushio H, Itokawa M, Hamaguchi H, Toru M**. **1996.** An insertion/deletion polymorphism in the angiotensin converting enzyme gene is associated with both brain substance P contents and affective disorders. *Biol.Psychiatry* **40:** 1122-1127.

**Arinami T, Gao M, Hamaguchi H, Toru M**. **1997.** A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. *Hum.Mol.Genet.* **6:** 577-582.

**Arnold PD, Rosenberg DR, Mundo E, Tharmalingam S, Kennedy JL, Richter MA**. **2004.** Association of a glutamate (NMDA) subunit receptor gene (GRIN2B) with obsessive-compulsive disorder: a preliminary study. *Psychopharmacology (Berl)* **174:** 530-538.

**Arranz MJ, Munro J, Owen MJ, Spurlock G, Sham PC, Zhao J, Kirov G, Collier DA, Kerwin RW**. **1998.** Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine. *Mol.Psychiatry* **3:** 61-66.

**Asghari V, Schoots O, van Kats S, Ohara K, Jovanovic V, Guan HC, Bunzow JR, Petronis A, Van Tol HH**. **1994.** Dopamine D4 receptor repeat: analysis of different native and mutant forms of the human and rat genes. *Mol.Pharmacol.* **46:** 364-373.

**Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH**. **1995.** Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J.Neurochem.* **65:** 1157-1165.

**Asherson P, Walsh C, Williams J, Sargeant M, Taylor C, Clements A, Gill M, Owen M, McGuffin P**. **1994.** Imprinting and anticipation. Are they relevant to genetic studies of schizophrenia? *Br.J.Psychiatry* **164:** 619-624.

**Asherson P, Mant R, Holmans P, Williams J, Cardno A, Murphy K, Jones L, Collier D, McGuffin P, Owen MJ**. **1996.** Linkage, association and mutational analysis of the dopamine D3 receptor gene in schizophrenia. *Mol.Psychiatry* **1:** 125-132.

**Axelrod J, Tomchick R**. **1958.** Enzymatic O-methylation of epinephrine and other catechols. *J.Biol.Chem.* **233:** 702-705.

**Azzam A, Mathews CA**. **2003.** Meta-analysis of the association between the catecholamine-O-methyl-transferase gene and obsessive-compulsive disorder. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **123:** 64-69.

**Bacanu SA, Devlin B, Roeder K**. **2000.** The power of genomic control. *Am.J.Hum.Genet.* **66:** 1933-1944.

**Baer L**. **1993.** Behavior therapy for obsessive compulsive disorder in the office-based practice. *J.Clin.Psychiatry* **54 Suppl:** 10-15.

**Baer L**. **1994.** Factor analysis of symptom subtypes of obsessive compulsive disorder and their relation to personality and tic disorders. *J.Clin.Psychiatry* **55 Suppl:** 18-23.

**Baghai TC, Schule C, Zwanzger P, Minov C, Schwarz MJ, de Jonge S, Rupprecht R, Bondy B**. **2001.** Possible influence of the insertion/deletion polymorphism in the angiotensin I-converting enzyme gene on therapeutic outcome in affective disorders. *Mol.Psychiatry* **6:** 258-259.

**Baghai TC, Schule C, Zill P, Deiml T, Eser D, Zwanzger P, Ella R, Rupprecht R, Bondy B**. **2004.** The angiotensin I converting enzyme insertion/deletion polymorphism influences therapeutic outcome in major depressed women, but not in men. *Neurosci.Lett.* **363:** 38-42.

**Baker RW, Chengappa KN, Baird JW, Steingard S, Christ MA, Schooler NR**. **1992.** Emergence of obsessive compulsive symptoms during treatment with clozapine. *J.Clin.Psychiatry* **53:** 439-442.

**Ball SG, Baer L, Otto MW**. **1996.** Symptom subtypes of obsessive-compulsive disorder in behavioral treatment studies: a quantitative review. *Behav.Res.Ther.* **34:** 47-51.

**Bamshad MJ, Wooding S, Watkins WS, Ostler CT, Batzer MA, Jorde LB**. **2003.** Human population genetic structure and inference of group membership. *Am.J.Hum.Genet.* **72:** 578-589.

**Barbujani G, Magagni A, Minch E, Cavalli-Sforza LL**. **1997.** An apportionment of human DNA diversity. *Proc.Natl.Acad.Sci.U.S.A* **94:** 4516-4519.

**Bardelay C, Mach E, Worcel M, Hunt P**. **1989.** Angiotensin-converting enzyme in rat brain and extraneural tissues visualized by quantitative autoradiography using 3H-trandolaprilate. *J.Cardiovasc.Pharmacol.* **14:** 511-518.

**Barondes SH**. **1998.** Will genetics revolutionize psychiatry? *Harv.Ment.Health Lett.* **15:** 4-6.

**Barr CL, Feng Y, Wigg K, Bloom S, Roberts W, Malone M, Schachar R, Tannock R, Kennedy JL**. **2000.** Identification of DNA variants in the SNAP-25 gene and linkage study of these polymorphisms and attention-deficit hyperactivity disorder. *Mol.Psychiatry* **5:** 405-409.

**Barr CL, Wigg K, Zai G, Roberts W, Malone M, Schachar R, Tannock R, Kennedy JL**. **2001.** Attention-deficit hyperactivity disorder and the adrenergic receptors alpha 1C and alpha 2C. *Mol.Psychiatry* **6:** 334-337.

**Barr LC, Goodman WK, Price LH, McDougle CJ, Charney DS**. **1992.** The serotonin hypothesis of obsessive compulsive disorder: implications of pharmacologic challenge studies. *J.Clin.Psychiatry* **53 Suppl:** 17-28.

**Barr LC, Goodman WK, Price LH**. **1993.** The serotonin hypothesis of obsessive compulsive disorder. *Int.Clin.Psychopharmacol.* **8 Suppl 2:** 79-82.

**Basile VS, Masellis M, Potkin SG, Kennedy JL**. **2002.** Pharmacogenomics in schizophrenia: the quest for individualized therapy. *Hum.Mol.Genet.* **11:** 2517-2530.

**Batzer MA, Kilroy GE, Richard PE, Shaikh TH, Desselle TD, Hoppens CL, Deininger PL**. **1990.** Structure and variability of recently inserted Alu family members. *Nucleic Acids Res.* **18:** 6793-6798.

**Batzer MA, Stoneking M, Alegria-Hartman M, Bazan H, Kass DH, Shaikh TH, Novick GE, Ioannou PA, Scheer WD, Herrera RJ, . 1994.** African origin of human-specific polymorphic Alu insertions. *Proc.Natl.Acad.Sci.U.S.A* **91:** 12288-12292.

**Batzer MA, Arcot SS, Phinney JW, Alegria-Hartman M, Kass DH, Milligan SM, Kimpton C, Gill P, Hochmeister M, Ioannou PA, Herrera RJ, Boudreau DA, Scheer WD, Keats BJ, Deininger PL, Stoneking M**. **1996.** Genetic variation of recent Alu insertions in human populations. *J.Mol.Evol.* **42:** 22-29.

**Baxter LR, Jr., Phelps ME, Mazziotta JC, Guze BH, Schwartz JM, Selin CE**. **1987.** Local cerebral glucose metabolic rates in obsessive-compulsive disorder. A comparison with rates in unipolar depression and in normal controls. *Arch.Gen.Psychiatry* **44:** 211-218.

**Baxter LR, Jr., Schwartz JM, Mazziotta JC, Phelps ME, Pahl JJ, Guze BH, Fairbanks L**. **1988.** Cerebral glucose metabolic rates in nondepressed patients with obsessive-compulsive disorder. *Am.J.Psychiatry* **145:** 1560-1563.

**Baxter LR, Jr., Schwartz JM, Bergman KS, Szuba MP, Guze BH, Mazziotta JC, Alazraki A, Selin CE, Ferng HK, Munford P, . 1992.** Caudate glucose metabolic rate changes with both drug and behavior therapy for obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **49:** 681-689.

**Baxter LR, Jr. 1994.** Positron emission tomography studies of cerebral glucose metabolism in obsessive compulsive disorder. *J.Clin.Psychiatry* **55 Suppl:** 54-59.

**Baxter LR, Jr., Saxena S, Brody AL, Ackermann RF, Colgan M, Schwartz JM, Allen-Martinez Z, Fuster JM, Phelps ME**. **1996.** Brain Mediation of Obsessive-Compulsive Disorder Symptoms: Evidence From Functional Brain Imaging Studies in the Human and Nonhuman Primate. *Semin.Clin.Neuropsychiatry* **1:** 32-47.

**Bebbington PE**. **1998.** Epidemiology of obsessive-compulsive disorder. *Br.J.Psychiatry Suppl* 2-6.

**Begg CB, Mazumdar M**. **1994.** Operating characteristics of a rank correlation test for publication bias. *Biometrics* **50:** 1088-1101.

**Bellodi L, Sciuto G, Diaferia G, Ronchi P, Smeraldi E**. **1992.** Psychiatric disorders in the families of patients with obsessive-compulsive disorder. *Psychiatry Res.* **42:** 111-120.

**Bellodi L, Cavallini MC, Bertelli S, Chiapparino D, Riboldi C, Smeraldi E**. **2001.** Morbidity risk for obsessive-compulsive spectrum disorders in first-degree relatives of patients with eating disorders. *Am.J.Psychiatry* **158:** 563-569.

**Bender R, Lange S**. **1999.** Multiple test procedures other than Bonferroni's deserve wider use. *BMJ* **318:** 600-601.

**Benjamin J, Levine J, Fux M, Aviv A, Levy D, Belmaker RH** . **1995.** Double-blind, placebo-controlled, crossover trial of inositol treatment for panic disorder. *Am.J.Psychiatry* **152:** 1084-1086.

**Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH**. **1996.** Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nat.Genet.* **12:** 81-84.

**Benkelfat C, Mefford IN, Masters CF, Nordahl TE, King AC, Cohen RM, Murphy DL**. **1991.** Plasma catecholamines and their metabolites in obsessive-compulsive disorder. *Psychiatry Res.* **37:** 321-331.

**Berridge KC, Aldridge JW, Houchard KR, Zhuang X**. **2005.** Sequential super-stereotypy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive compulsive disorder and Tourette's. *BMC.Biol.* **3:** 4.

**Berridge MJ, Irvine RF**. **1984.** Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312:** 315-321.

**Berridge MJ, Irvine RF**. **1989.** Inositol phosphates and cell signalling. *Nature* **341:** 197-205.

**Berridge MJ**. **1993.** Inositol trisphosphate and calcium signalling. *Nature* **361:** 315-325.

**Berry EM, Coustere-Yakir C, Grover NB**. **1998.** The significance of non-significance. *QJM.* **91:** 647-653.

**Bertocci B, Miggiano V, Da Prada M, Dembic Z, Lahm HW, Malherbe P**. **1991.** Human catechol-O-methyltransferase: cloning and expression of the membrane-associated form. *Proc.Natl.Acad.Sci.U.S.A* **88:** 1416-1420.

**Bestor TH, Chandler VL, Feinberg AP**. **1994.** Epigenetic effects in eukaryotic gene expression. *Dev.Genet.* **15:** 458-462.

**Bilder RM, Volavka J, Czobor P, Malhotra AK, Kennedy JL, Ni X, Goldman RS, Hoptman MJ, Sheitman B, Lindenmayer JP, Citrome L, McEvoy JP, Kunz M, Chakos M, Cooper TB, Lieberman JA**. **2002.** Neurocognitive correlates of the COMT Val(158)Met polymorphism in chronic schizophrenia. *Biol.Psychiatry* **52:** 701-707.

**Billet EA, Richter MA, Sam F, Swinson RP, Dai X-Y, King N, Badri F, Sasaki T, Buchanan JA, Kennedy JL. 1998**. Investigation of dopamine system genes in obsessive-compulsive disorder. *Psychiatric Genetics.* **8:** 163-169

**Bird A**. **2002.** DNA methylation patterns and epigenetic memory. *Genes Dev.* **16:** 6-21.

**Birken DL, Oldendorf WH**. **1989.** N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. *Neurosci.Biobehav.Rev.* **13:** 23-31.

**Bjornsson HT, Fallin MD, Feinberg AP**. **2004.** An integrated epigenetic and genetic approach to common human disease. *Trends Genet.* **20:** 350-358.

**Black DW, Noyes R, Jr., Goldstein RB, Blum N**. **1992.** A family study of obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **49:** 362-368.

**Black DW, Monahan P, Gable J, Blum N, Clancy G, Baker P**. **1998.** Hoarding and treatment response in 38 nondepressed subjects with obsessive-compulsive disorder. *J.Clin.Psychiatry* **59:** 420-425.

**Black DW, Gaffney GR, Schlosser S, Gabel J**. **2003.** Children of parents with obsessive-compulsive disorder -- a 2-year follow-up study. *Acta Psychiatr.Scand.* **107:** 305-313.

**Michell RH 1997** The multiplying roles of inositol lipids and phosphates in cell control processes. *Ess Biochem* 32; 31-40

**Blier P, De Montigny C**. **1994.** Current advances and trends in the treatment of depression. *Trends Pharmacol.Sci.* **15:** 220-226.

**Blier P, Bouchard C**. **1994.** Modulation of 5-HT release in the guinea-pig brain following long-term administration of antidepressant drugs. *Br.J.Pharmacol.* **113:** 485-495.

**Bliss TV, Collingridge GL**. **1993.** A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361:** 31-39.

**Blundell JE, Herberg LJ**. **1973.** Effectiveness of lateral hypothalamic stimulation, arousal, and food deprivation in the initiation of hoarding behaviour in naive rats. *Physiol Behav.* **10:** 763-767.

**Boess FG, Monsma FJ, Jr., Carolo C, Meyer V, Rudler A, Zwingelstein C, Sleight AJ**. **1997.** Functional and radioligand binding characterization of rat 5-HT6 receptors stably expressed in HEK293 cells. *Neuropharmacology* **36:** 713-720.

**Bogetto F, Venturello S, Albert U, Maina G, Ravizza L**. **1999.** Gender-related clinical differences in obsessive-compulsive disorder. *Eur.Psychiatry* **14:** 434-441.

**Boghosian-Sell L, Comings DE, Overhauser J**. **1996.** Tourette syndrome in a pedigree with a 7;18 translocation: identification of a YAC spanning the translocation breakpoint at 18q22.3. *Am.J.Hum.Genet.* **59:** 999-1005.

**Bolton J, Moore GJ, MacMillan S, Stewart CM, Rosenberg DR**. **2001.** Case study: caudate glutamatergic changes with paroxetine persist after medication discontinuation in pediatric OCD. *J.Am.Acad.Child Adolesc.Psychiatry* **40:** 903-906.

**Bolton PF, Pickles A, Murphy M, Rutter M**. **1998.** Autism, affective and other psychiatric disorders: patterns of familial aggregation. *Psychol.Med.* **28:** 385-395.

**Bondy B, Baghai TC, Zill P, Schule C, Eser D, Deiml T, Zwanzger P, Ella R, Rupprecht R**. **2005.** Genetic variants in the angiotensin I-converting-enzyme (ACE) and angiotensin II receptor (AT1) gene and clinical outcome in depression. *Prog.Neuropsychopharmacol.Biol.Psychiatry* **29:** 1094-1099.

**Bonnier B, Gorwood P, Hamon M, Sarfati Y, Boni C, Hardy-Bayle MC**. **2002.** Association of 5-HT(2A) receptor gene polymorphism with major affective disorders: the case of a subgroup of bipolar disorder with low suicide risk. *Biol.Psychiatry* **51:** 762-765.

**Botha MC, Beighton P**. **1983.** Inherited disorders in the Afrikaner population of southern Africa. Part I. Historical and demographic background, cardiovascular, neurological, metabolic and intestinal conditions. *S.Afr.Med.J.* **64:** 609-612.

**Brambilla F, Bellodi L, Perna G, Arancio C, Bertani A**. **1997.** Dopamine function in obsessive-compulsive disorder: growth hormone response to apomorphine stimulation. *Biol.Psychiatry* **42:** 889-897.

**Brambilla F, Perna G, Bussi R, Bellodi L**. **2000.** Dopamine function in obsessive compulsive disorder: cortisol response to acute apomorphine stimulation. *Psychoneuroendocrinology* **25:** 301-310.

**Branchek TA, Blackburn TP**. **2000.** 5-HT6 receptors as emerging targets for drug discovery. *Annu.Rev.Pharmacol.Toxicol.* **40:** 319-334.

**Bray NJ, Buckland PR, Hall H, Owen MJ, O'Donovan MC**. **2004.** The serotonin-2A receptor gene locus does not contain common polymorphism affecting mRNA levels in adult brain. *Mol.Psychiatry* **9:** 109-114.

**Brink PA, Ferreira A, Moolman JC, Weymar HW, van der Merwe PL, Corfield VA**. **1995.** Gene for progressive familial heart block type I maps to chromosome 19q13. *Circulation* **91:** 1633-1640.

**Bristol A, Hall SM, Kriz RW, Stahl ML, Fan YS, Byers MG, Eddy RL, Shows TB, Knopf JL**. **1988.** Phospholipase C-148: chromosomal location and deletion mapping of functional domains. *Cold Spring Harb.Symp.Quant.Biol.* **53 Pt 2:** 915-920.

**Broocks A, Pigott TA, Hill JL, Canter S, Grady TA, L'Heureux F, Murphy DL**. **1998.** Acute intravenous administration of ondansetron and m-CPP, alone and in combination, in patients with obsessive-compulsive disorder (OCD): behavioral and biological results. *Psychiatry Res.* **79:** 11-20.

**Burgner D, Usen S, Rockett K, Jallow M, Ackerman H, Cervino A, Pinder M, Kwiatkowski DP**. **2003.** Nucleotide and haplotypic diversity of the NOS2A promoter region and its relationship to cerebral malaria. *Hum.Genet.* **112:** 379-386.

**Busatto GF, Buchpiguel CA, Zamignani DR, Garrido GE, Glabus MF, Rosario-Campos MC, Castro CC, Maia A, Rocha ET, McGuire PK, Miguel EC**. **2001.** Regional cerebral blood flow abnormalities in early-onset obsessive-compulsive disorder: an exploratory SPECT study. *J.Am.Acad.Child Adolesc.Psychiatry* **40:** 347-354.

**Calamari JE, Wiegartz PS, Janeck AS**. **1999.** Obsessive-compulsive disorder subgroups: a symptom-based clustering approach. *Behav.Res.Ther.* **37:** 113-125.

**Calamari JE, Wiegartz PS, Riemann BC, Cohen RJ, Greer A, Jacobi DM, Jahn SC, Carmin C**. **2004.** Obsessive-compulsive disorder subtypes: an attempted replication and extension of a symptom-based taxonomy. *Behav.Res.Ther.* **42:** 647-670.

**Camarena B, Cruz C, de lF, Jr., Nicolini H**. **1998.** A higher frequency of a low activity-related allele of the MAO-A gene in females with obsessive-compulsive disorder. *Psychiatr.Genet.* **8:** 255-257.

**Camarena B, Rinetti G, Cruz C, Gomez A, de lF, Jr., Nicolini H**. **2001.** Additional evidence that genetic variation of MAO-A gene supports a gender subtype in obsessive-compulsive disorder. *Am.J.Med.Genet.* **105:** 279-282.

**Camarena B, Aguilar A, Loyzaga C, Nicolini H**. **2004.** A family-based association study of the 5-HT-1Dbeta receptor gene in obsessive-compulsive disorder. *Int.J.Neuropsychopharmacol.* **7:** 49-53.

**Cambien F, Alhenc-Gelas F, Herbeth B, Andre JL, Rakotovao R, Gonzales MF, Allegrini J, Bloch C**. **1988.** Familial resemblance of plasma angiotensin-converting enzyme level: the Nancy Study. *Am.J.Hum.Genet.* **43:** 774-780.

**Campbell CD, Ogburn EL, Lunetta KL, Lyon HN, Freedman ML, Groop LC, Altshuler D, Ardlie KG, Hirschhorn JN**. **2005.** Demonstrating stratification in a European American population. *Nat.Genet.* **37:** 868-872.

**Campbell H, Rudan I**. **2002.** Interpretation of genetic association studies in complex disease. *Pharmacogenomics.J.* **2:** 349-360.

**Campbell KM, de Lecea L, Severynse DM, Caron MG, McGrath MJ, Sparber SB, Sun LY, Burton FH**. **1999(a).** OCD-Like behaviors caused by a neuropotentiating transgene targeted to cortical and limbic D1+ neurons. *J.Neurosci.* **19:** 5044-5053.

**Campbell KM, McGrath MJ, Burton FH**. **1999(b).** Behavioral effects of cocaine on a transgenic mouse model of cortical-limbic compulsion. *Brain Res.* **833:** 216-224.

**Canossa M, Gartner A, Campana G, Inagaki N, Thoenen H**. **2001.** Regulated secretion of neurotrophins by metabotropic glutamate group I (mGluRI) and Trk receptor activation is mediated via phospholipase C signalling pathways. *EMBO J.* **20:** 1640-1650.

**Capecchi MR**. **1997.** Hox genes and mammalian development. *Cold Spring Harb.Symp.Quant.Biol.* **62:** 273-281.

**Cardon LR, Bell JI**. **2001.** Association study designs for complex diseases. *Nat.Rev.Genet.* **2:** 91-99.

**Cardon LR, Palmer LJ**. **2003.** Population stratification and spurious allelic association. *Lancet* **361:** 598-604.

**Carey and Gottesman II. 1981.** Twin and Family Studies of anxiety, phobic and obsessive disorders. **In:** Klein DF, Rabkin J (eds). Anxiety: new Research and Changing Concepts. Raven Press, NY . pp 117-36.

**Carrel L, Cottle AA, Goglin KC, Willard HF**. **1999.** A first-generation X-inactivation profile of the human X chromosome. *Proc.Natl.Acad.Sci.U.S.A* **96:** 14440-14444.

**Carroll ML, Roy-Engel AM, Nguyen SV, Salem AH, Vogel E, Vincent B, Myers J, Ahmad Z, Nguyen L, Sammarco M, Watkins WS, Henke J, Makalowski W, Jorde LB, Deininger PL, Batzer MA**. **2001.** Large-scale analysis of the Alu Ya5 and Yb8 subfamilies and their contribution to human genomic diversity. *J.Mol.Biol.* **311:** 17-40.

**Carey and Gottesman II.** Twin and Family Studies of anxiety, phobic and obsessive disorders. **In:** Klein DF, Rabkin J (eds). Anxiety: new Research and Changing Concepts. Raven Press, NY 1981. pp 117-36.

**Castle DJ, Deale A, Marks IM**. **1995.** Gender differences in obsessive compulsive disorder. *Aust.N.Z.J.Psychiatry* **29:** 114-117.

**Catalano M, Sciuto G, Di Bella D, Novelli E, Nobile M, Bellodi L**. **1994.** Lack of association between obsessive-compulsive disorder and the dopamine D3 receptor gene: some preliminary considerations. *Am.J.Med.Genet.* **54:** 253-255.

**Cath DC, Spinhoven P, Hoogduin CA, Landman AD, van Woerkom TC, van de Wetering BJ, Roos RA, Rooijmans HG**. **2001.** Repetitive behaviors in Tourette's syndrome and OCD with and without tics: what are the differences? *Psychiatry Res.* **101:** 171-185.

**Cavalli-Sforza LL, Piazza A**. **1975.** Analysis of evolution: evolutionary rates, independence and treeness. *Theor.Popul.Biol.* **8:** 127-165.

**Cavallini MC, Di Bella D, Pasquale L, Henin M, Bellodi L** . **1998.** 5HT2C CYS23/SER23 polymorphism is not associated with obsessive-compulsive disorder. *Psychiatry Res.* **77:** 97-104.

**Cavallini MC, Pasquale L, Bellodi L, Smeraldi E**. **1999.** Complex segregation analysis for obsessive compulsive disorder and related disorders. *Am.J.Med.Genet.* **88:** 38-43.

**Cavallini MC, Di Bella D, Siliprandi F, Malchiodi F, Bellodi L**. **2002.** Exploratory factor analysis of obsessive-compulsive patients and association with 5-HTTLPR polymorphism. *Am.J.Med.Genet.* **114:** 347-353.

**Chacon P, Rosario-Campos MC, Hounie AG, Lopes AC, Curi M, Miguel EC**. **2004.** Comment on "the identification of OCD-related subgroups based on comorbidity". *Biol.Psychiatry* **55:** 960.

**Chamberlain SR, Blackwell AD, Fineberg NA, Robbins TW, Sahakian BJ. 2005**. The neuropsychology of obsessive compulsive disorder: the importance of failures in cognitive and behavioural inhibition as candidate endophenotypic markers. *Neurosci. Biobehav. Rev.* **29**:399-41.

**Chang FM, Kidd JR, Livak KJ, Pakstis AJ, Kidd KK**. **1996.** The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus. *Hum.Genet.* **98:** 91-101.

**Chapman JM, Cooper JD, Todd JA, Clayton DG**. **2003.** Detecting disease associations due to linkage disequilibrium using haplotype tags: a class of tests and the determinants of statistical power. *Hum.Hered.* **56:** 18-31.

**Chaput Y, De Montigny C, Blier P**. **1991.** Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. *Neuropsychopharmacology* **5:** 219-229.

**Charney DS, Goodman WK, Price LH, Woods SW, Rasmussen SA, Heninger GR**. **1988.** Serotonin function in obsessive-compulsive disorder. A comparison of the effects of tryptophan and m-chlorophenylpiperazine in patients and healthy subjects. *Arch.Gen.Psychiatry* **45:** 177-185.

**Chee IS, Lee SW, Kim JL, Wang SK, Shin YO, Shin SC, Lee YH, Hwang HM, Lim MR**. **2001.** 5-HT2A receptor gene promoter polymorphism -1438A/G and bipolar disorder. *Psychiatr.Genet.* **11:** 111-114.

**Chen CH, Lee YR, Liu MY, Wei FC, Koong FJ, Hwu HG, Hsiao KJ**. **1996.** Identification of a BglI polymorphism of catechol-O-methyltransferase (COMT) gene, and association study with schizophrenia. *Am.J.Med.Genet.* **67:** 556-559.

**Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR**. **2004.** Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am.J.Hum.Genet.* **75:** 807-821.

**Chen K, Yang W, Grimsby J, Shih JC**. **1992.** The human 5-HT2 receptor is encoded by a multiple intron-exon gene. *Brain Res.Mol.Brain Res.* **14:** 20-26.

**Cho YM, Ritchie MD, Moore JH, Park JY, Lee KU, Shin HD, Lee HK, Park KS**. **2004.** Multifactor-dimensionality reduction shows a two-locus interaction associated with Type 2 diabetes mellitus. *Diabetologia* **47:** 549-554.

**Choi DS, Birraux G, Launay JM, Maroteaux L**. **1994.** The human serotonin 5-HT2B receptor: pharmacological link between 5-HT2 and 5-HT1D receptors. *FEBS Lett.* **352:** 393-399.

**Chopin P, Briley M**. **1987**. Animal models of anxiety: the effects of compounds that modify 5serotonergic neurotransmission. *Trends Pharmacol Sci*. **8**: 383-388

**Chou-Green JM, Holscher TD, Dallman MF, Akana SF**. **2003.** Compulsive behavior in the 5-HT2C receptor knockout mouse. *Physiol Behav.* **78:** 641-649.

**Christenson GA, Mackenzie TB, Mitchell JE, Callies AL**. **1991.** A placebo-controlled, double-blind crossover study of fluoxetine in trichotillomania. *Am.J.Psychiatry* **148:** 1566-1571.

**Cichon S, Nothen MM, Rietschel M, Korner J, Propping P**. **1994.** Single-strand conformation analysis (SSCA) of the dopamine D1 receptor gene (DRD1) reveals no significant mutation in patients with schizophrenia and manic depression. *Biol.Psychiatry* **36:** 850-853.

**Cichon S, Nothen MM, Stober G, Schroers R, Albus M, Maier W, Rietschel M, Korner J, Weigelt B, Franzek E, Wildenauer D, Fimmers R, Propping P**. **1996.** Systematic screening for mutations in the 5'-regulatory region of the human dopamine D1 receptor (DRD1) gene in patients with schizophrenia and bipolar affective disorder. *Am.J.Med.Genet.* **67:** 424-428.

**Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB, Levey AI**. **1995.** The dopamine transporter: immunochemical characterization and localization in brain. *J.Neurosci.* **15:** 1714-1723.

**Clark AG**. **1990.** Inference of haplotypes from PCR-amplified samples of diploid populations. *Mol.Biol.Evol.* **7:** 111-122.

**Clark AG, Weiss KM, Nickerson DA, Taylor SL, Buchanan A, Stengard J, Salomaa V, Vartiainen E, Perola M, Boerwinkle E, Sing CF**. **1998.** Haplotype structure and population genetic inferences from nucleotide-sequence variation in human lipoprotein lipase. *Am.J.Hum.Genet.* **63:** 595-612.

**Clark D, White FJ**. **1987.** D1 dopamine receptor--the search for a function: a critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. *Synapse* **1:** 347-388.

**Clifford CA, Murray RM, Fulker DW**. **1984.** Genetic and environmental influences on obsessional traits and symptoms. *Psychol.Med.* **14:** 791-800.

**Cochran WG. 1954**. The combination of estimates from different experiments. *Biometrics*. **3**: 101-129.

**Cohen AI, Todd RD, Harmon S, O'Malley KL**. **1992.** Photoreceptors of mouse retinas possess D4 receptors coupled to adenylate cyclase. *Proc.Natl.Acad.Sci.U.S.A* **89:** 12093-12097.

**Colhoun HM, McKeigue PM, Davey SG**. **2003.** Problems of reporting genetic associations with complex outcomes. *Lancet* **361:** 865-872.

**Coling JG, Herberg LJ**. **1982.** Effect of ovarian and exogenous hormones on defended body weight, actual body weight, and the paradoxical hoarding of food by female rats. *Physiol Behav.* **29:** 687-691.

**Collier DA, Arranz MJ, Li T, Mupita D, Brown N, Treasure J**. **1997.** Association between 5-HT2A gene promoter polymorphism and anorexia nervosa. *Lancet* **350:** 412.

**Collins A, Lonjou C, Morton NE**. **1999.** Genetic epidemiology of single-nucleotide polymorphisms. *Proc.Natl.Acad.Sci.U.S.A* **96:** 15173-15177.

**Collins FS, Guyer MS, Charkravarti A**. **1997.** Variations on a theme: cataloging human DNA sequence variation. *Science* **278:** 1580-1581.

**Comas D, Plaza S, Calafell F, Sajantila A, Bertranpetit J**. **2001.** Recent insertion of an Alu element within a polymorphic human-specific Alu insertion. *Mol.Biol.Evol.* **18:** 85-88.

**Comings DE, Comings BG**. **1987.** A controlled study of Tourette syndrome. IV. Obsessions, compulsions, and schizoid behaviors. *Am.J.Hum.Genet.* **41:** 782-803.

**Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E, Kocsis P, Baumgarten R, Kovacs BW. 1991.** The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *JAMA* **266:** 1793-1800.

**Comings DE, Gade R, Wu S, Chiu C, Dietz G, Muhleman D, Saucier G, Ferry L, Rosenthal RJ, Lesieur HR, Rugle LJ, MacMurray P**. **1997.** Studies of the potential role of the dopamine D1 receptor gene in addictive behaviors. *Mol.Psychiatry* **2:** 44-56.

**Comings DE**. **1998.** Polygenic inheritance and micro/minisatellites. *Mol.Psychiatry* **3:** 21-31.

**Comings DE, Muhleman D, Johnson P, MacMurray JP**. **1999.** Potential role of the estrogen receptor gene (ESR1) in anxiety. *Mol.Psychiatry* **4:** 374-377.

**Comings DE, MacMurray JP**. **2000.** Molecular heterosis: a review. *Mol.Genet.Metab* **71:** 19-31.

**Cordell HJ, Todd JA**. **1995.** Multifactorial inheritance in type 1 diabetes. *Trends Genet.* **11:** 499-504.

**Cordell HJ, Todd JA, Hill NJ, Lord CJ, Lyons PA, Peterson LB, Wicker LS, Clayton DG**. **2001.** Statistical modeling of interlocus interactions in a complex disease: rejection of the multiplicative model of epistasis in type 1 diabetes. *Genetics* **158:** 357-367.

**Cordell HJ**. **2002.** Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. *Hum.Mol.Genet.* **11:** 2463-2468.

**Cox NJ, Bell GI**. **1989.** Disease associations. Chance, artifact, or susceptibility genes? *Diabetes* **38:** 947-950.

**Cox NJ, Frigge M, Nicolae DL, Concannon P, Hanis CL, Bell GI, Kong A**. **1999.** Loci on chromosomes 2 (NIDDM1) and 15 interact to increase susceptibility to diabetes in Mexican Americans. *Nat.Genet.* **21:** 213-215.

**Cox R, Bouzekri N, Martin S, Southam L, Hugill A, Golamaully M, Cooper R, Adeyemo A, Soubrier F, Ward R, Lathrop GM, Matsuda F, Farrall M**. **2002.** Angiotensin-1-converting enzyme (ACE) plasma concentration is influenced by multiple ACE-linked quantitative trait nucleotides. *Hum.Mol.Genet.* **11:** 2969-2977.

**Crocq MA, Mant R, Asherson P, Williams J, Hode Y, Mayerova A, Collier D, Lannfelt L, Sokoloff P, Schwartz JC, . 1992.** Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. *J.Med.Genet.* **29:** 858-860.

**Creese I, Iversen SD**. **1974.** The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. *Psychopharmacologia*. **39(4):** 345-357

**Crowe RR**. **1993.** Candidate genes in psychiatry: an epidemiological perspective. *Am.J.Med.Genet.* **48:** 74-77.

**Cruz C, Camarena B, King N, Paez F, Sidenberg D, de lF, Jr., Nicolini H**. **1997.** Increased prevalence of the seven-repeat variant of the dopamine D4 receptor gene in patients with obsessive-compulsive disorder with tics. *Neurosci.Lett.* **231:** 1-4.

**Cuker A, State MW, King RA, Davis N, Ward DC**. **2004.** Candidate locus for Gilles de la Tourette syndrome/obsessive compulsive disorder/chronic tic disorder at 18q22. *Am.J.Med.Genet.A* **130:** 37-39.

**Cummings JL, Frankel M**. **1985.** Gilles de la Tourette syndrome and the neurological basis of obsessions and compulsions. *Biol.Psychiatry* **20:** 117-126.

**Cyr M, Bosse R, Di Paolo T**. **1998.** Gonadal hormones modulate 5-hydroxytryptamine2A receptors: emphasis on the rat frontal cortex. *Neuroscience* **83:** 829-836.

**Cyr M, Landry M, Di Paolo T**. **2000.** Modulation by estrogen-receptor directed drugs of 5-hydroxytryptamine-2A receptors in rat brain. *Neuropsychopharmacology* **23:** 69-78.

**Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES**. **2001.** High-resolution haplotype structure in the human genome. *Nat.Genet.* **29:** 229-232.

**Davey SG**. **2003.** Uncertainty and significance. *Int.J.Epidemiol.* **32:** 683-684.

**Dawson B., Trapp R.G**. **2004**. Basic and Clinical Biostatistics. 4th edition. Lange Medical Books, Mcgraw-Hill, New York.

**de Brabander JM, de Bruin JP, van Eden CG**. **1991.** Comparison of the effects of neonatal and adult medial prefrontal cortex lesions on food hoarding and spatial delayed alternation. *Behav.Brain Res.* **42:** 67-75.

**de Jager T, Corbett CH, Badenhorst JC, Brink PA, Corfield VA**. **1996.** Evidence of a long QT founder gene with varying phenotypic expression in South African families. *J.Med.Genet.* **33:** 567-573.

**Defesche JC, Van Diermen DE, Hayden MR, Kastelein JP**. **1996.** Origin and migration of an Afrikaner founder mutation FHAfrikaner-2 (V408M) causing familial hypercholesterolemia. *Gene Geogr*. **10:** 1-10.

**Deininger PL, Batzer MA**. **1999.** Alu repeats and human disease. *Mol.Genet.Metab* **67:** 183-193.

**Delmas P, Wanaverbecq N, Abogadie FC, Mistry M, Brown DA** . **2002.** Signaling microdomains define the specificity of receptor-mediated InsP(3) pathways in neurons. *Neuron* **34:** 209-220.

**Delorme R, Krebs MO, Chabane N, Roy I, Millet B, Mouren-Simeoni MC, Maier W, Bourgeron T, Leboyer M**. **2004.** Frequency and transmission of glutamate receptors GRIK2 and GRIK3 polymorphisms in patients with obsessive compulsive disorder. *Neuroreport* **15:** 699-702.

**Delorme R, Chabane N, Callebert J, Falissard B, Mouren-Simeoni MC, Rouillon F, Launay JM, Leboyer M**. **2004.** Platelet serotonergic predictors of clinical improvement in obsessive compulsive disorder. *J.Clin.Psychopharmacol.* **24:** 18-23.

**Demchyshyn L, Sunahara RK, Miller K, Teitler M, Hoffman BJ, Kennedy JL, Seeman P, Van Tol HH, Niznik HB**. **1992.** A human serotonin 1D receptor variant (5HT1D beta) encoded by an intronless gene on chromosome 6. *Proc.Natl.Acad.Sci.U.S.A* **89:** 5522-5526.

**DeMille MM, Kidd JR, Ruggeri V, Palmatier MA, Goldman D, Odunsi A, Okonofua F, Grigorenko E, Schulz LO, Bonne-Tamir B, Lu RB, Parnas J, Pakstis AJ, Kidd KK**. **2002.** Population variation in linkage disequilibrium across the COMT gene considering promoter region and coding region variation. *Hum.Genet.* **111:** 521-537.

**Dempster A.P., Laird N.M., Rubin D.B**. **1977**. Maximum likelihood from Incomplete data via the EM algorithm. *J.Roy.Statist.Soc* B39: 1-38.

**Dempster E, Toulopoulou T, McDonald C, Bramon E, Walshe M, Filbey F, Wickham H, Sham PC, Murray RM, Collier DA**. **2005.** Association between BDNF val66 met genotype and episodic memory. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **134:** 73-75.

**Deng HW**. **2001.** Population admixture may appear to mask, change or reverse genetic effects of genes underlying complex traits. *Genetics* **159:** 1319-1323.

**Denys D, De Geus F, van Megen HJ, Westenberg HG**. **2004(a).** Symptom dimensions in obsessive-compulsive disorder: factor analysis on a clinician-rated scale and a self-report measure. *Psychopathology* **37:** 181-189.

**Denys D, Tenney N, van Megen HJ, De Geus F, Westenberg HG**. **2004(b).** Axis I and II comorbidity in a large sample of patients with obsessive-compulsive disorder. *J.Affect.Disord.* **80:** 155-162.

**Denys D, van der Wee N, Janssen J, De Geus F, Westenberg HG**. **2004(c).** Low level of dopaminergic D2 receptor binding in obsessive-compulsive disorder. *Biol.Psychiatry* **55:** 1041-1045.

**Devinsky O**. **1983.** Neuroanatomy of Gilles de la Tourette's syndrome. Possible midbrain involvement. *Arch.Neurol.* **40:** 508-514.

**Devlin B, Roeder K**. **1999.** Genomic control for association studies. *Biometrics* **55:** 997-1004.

**Devlin B, Roeder K, Wasserman L**. **2001.** Genomic control, a new approach to genetic-based association studies. *Theor.Popul.Biol.* **60:** 155-166.

**Di Bella D, Cavallini MC, Bellodi L**. **2002.** No association between obsessive-compulsive disorder and the 5-HT(1Dbeta) receptor gene. *Am.J.Psychiatry* **159:** 1783-1785.

**Di M, V, De Blasi A, Di Giulio C, Esposito E**. **2001.** Role of 5-HT(2C) receptors in the control of central dopamine function. *Trends Pharmacol.Sci.* **22:** 229-232.

**Ding YC, Chi HC, Grady DL, Morishima A, Kidd JR, Kidd KK, Flodman P, Spence MA, Schuck S, Swanson JM, Zhang YP, Moyzis RK**. **2002.** Evidence of positive selection acting at the human dopamine receptor D4 gene locus. *Proc.Natl.Acad.Sci.U.S.A* **99:** 309-314.

**do Rosario-Campos MC, Leckman JF, Curi M, Quatrano S, Katsovitch L, Miguel EC, Pauls DL**. **2005.** A family study of early-onset obsessive-compulsive disorder. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **136:** 92-97.

**Dodt JE, Byerly MJ, Cuadros C, Christensen RC**. **1997.** Treatment of risperidone-induced obsessive-compulsive symptoms with sertraline. *Am.J.Psychiatry* **154:** 582.

**Dolberg OT, Iancu I, Sasson Y, Zohar J**. **1996.** The pathogenesis and treatment of obsessive-compulsive disorder. *Clin.Neuropharmacol.* **19:** 129-147.

**Doucette-Stamm LA, Blakely DJ, Tian J, Mockus S, Mao JI**. **1995.** Population genetic study of the human dopamine transporter gene (DAT1). *Genet.Epidemiol.* **12:** 303-308.

**Doyle AE, Goodman JE, Silber PM, Yager JD**. **2004.** Catechol-O-methyltransferase low activity genotype (COMTLL) is associated with low levels of COMT protein in human hepatocytes. *Cancer Lett.* **214:** 189-195.

**Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV**. **2003.** Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum.Mol.Genet.* **12:** 205-216.

**Duarte FS, Testolin R, De Lima TC**. **2004.** Further evidence on the anxiogenic-like effect of substance P evaluated in the elevated plus-maze in rats. *Behav.Brain Res.* **154:** 501-510.

**Dunning AM, Durocher F, Healey CS, Teare MD, McBride SE, Carlomagno F, Xu CF, Dawson E, Rhodes S, Ueda S, Lai E, Luben RN, Van Rensburg EJ, Mannermaa A, Kataja V, Rennart G, Dunham I, Purvis I, Easton D, Ponder BA**. **2000.** The extent of linkage disequilibrium in four populations with distinct demographic histories. *Am.J.Hum.Genet.* **67:** 1544-1554.

**DuPont RL, Rice DP, Shiraki S, Rowland CR**. **1995.** Economic costs of obsessive-compulsive disorder. *Med.Interface* **8:** 102-109.

**Du Pont WD, Plummer WD. 1990.** Power and sample size calculations: a review and computer program . *Controlled Clinical Trials* **11**: 116-128.

**du Toit, P. L., J. van Kradenburg, D. Niehaus, and D. J. Stein**. 2001. Comparison of obsessive-compulsive disorder patients with and without comorbid putative obsessive-compulsive spectrum disorders using a structured clinical interview. *Compr.Psychiatry* **42**:291-300.

**Eapen V, Pauls DL, Robertson MM**. **1993.** Evidence for autosomal dominant transmission in Tourette's syndrome. United Kingdom cohort study. *Br.J.Psychiatry* **162:** 593-596.

**Eapen V, Robertson MM, Alsobrook JP, Pauls DL**. **1997.** Obsessive compulsive symptoms in Gilles de la Tourette syndrome and obsessive compulsive disorder: differences by diagnosis and family history. *Am.J.Med.Genet.* **74:** 432-438.

**Eapen V, O'Neill J, Gurling HM, Robertson MM**. **1997.** Sex of parent transmission effect in Tourette's syndrome: evidence for earlier age at onset in maternally transmitted cases suggests a genomic imprinting effect. *Neurology* **48:** 934-937.

**Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR**. **1991.** Publication bias in clinical research. *Lancet* **337:** 867-872.

**Ebstein RP, Nemanov L, Klotz I, Gritsenko I, Belmaker RH** . **1997.** Additional evidence for an association between the dopamine D4 receptor (D4DR) exon III repeat polymorphism and the human personality trait of Novelty Seeking. *Mol.Psychiatry* **2:** 472-477.

**Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR**. **2001.** Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc.Natl.Acad.Sci.U.S.A* **98:** 6917-6922.

**Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR**. **2003.** The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112:** 257-269.

**Egger M, Ebrahim S, Smith GD**. **2002.** Where now for meta-analysis? *Int.J.Epidemiol.* **31:** 1-5.

**Egger M, Smith GD, Schneider M, Minder C.1997**. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 315: 629-34

**Eichstedt JA, Arnold SL**. **2001.** Childhood-onset obsessive-compulsive disorder: a tic-related subtype of OCD? *Clin.Psychol.Rev.* **21:** 137-157.

**Eilam D, Golani I, Szechtman H**. **1989.** D2-agonist quinpirole induces perseveration of routes and hyperactivity but no perseveration of movements. *Brain Res.* **490:** 255-267.

**Einat H, Szechtman H**. **1995.** Perseveration without hyperlocomotion in a spontaneous alternation task in rats sensitized to the dopamine agonist quinpirole. *Physiol Behav.* **57:** 55-59.

**Einat H, Karbovski H, Korik J, Tsalah D, Belmaker RH**. **1999.** Inositol reduces depressive-like behaviors in two different animal models of depression. *Psychopharmacology (Berl)* **144:** 158-162.

**Eisenberg J, Zohar A, Mei-Tal G, Steinberg A, Tartakovsky E, Gritsenko I, Nemanov L, Ebstein RP**. **2000.** A haplotype relative risk study of the dopamine D4 receptor (DRD4) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). *Am.J.Med.Genet.* **96:** 258-261.

**el Mansari M, Bouchard C, Blier P**. **1995.** Alteration of serotonin release in the guinea pig orbito-frontal cortex by selective serotonin reuptake inhibitors. Relevance to treatment of obsessive-compulsive disorder. *Neuropsychopharmacology* **13:** 117-127.

**Enoch MA, Kaye WH, Rotondo A, Greenberg BD, Murphy DL, Goldman D**. **1998.** 5-HT2A promoter polymorphism -1438G/A, anorexia nervosa, and obsessive-compulsive disorder. *Lancet* **351:** 1785-1786.

**Enoch MA, Goldman D, Barnett R, Sher L, Mazzanti CM, Rosenthal NE**. **1999.** Association between seasonal affective disorder and the 5-HT2A promoter polymorphism, -1438G/A. *Mol.Psychiatry* **4:** 89-92.

**Enoch MA, Greenberg BD, Murphy DL, Goldman D**. **2001.** Sexually dimorphic relationship of a 5-HT2A promoter polymorphism with obsessive-compulsive disorder. *Biol.Psychiatry* **49:** 385-388.

**Erdal ME, Tot S, Yazici K, Yazici A, Herken H, Erdem P, Derici E, Camdeviren H**. **2003.** Lack of association of catechol-O-methyltransferase gene polymorphism in obsessive-compulsive disorder. *Depress.Anxiety.* **18:** 41-45.

**Ernfors P, Wetmore C, Olson L, Persson H**. **1990.** Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron* **5:** 511-526.

**Espejo EF**. **1997.** Selective dopamine depletion within the medial prefrontal cortex induces anxiogenic-like effects in rats placed on the elevated plus maze. *Brain Res.* **762:** 281-284.

**Eubanks JH, Djabali M, Selleri L, Grandy DK, Civelli O, McElligott DL, Evans GA**. **1992.** Structure and linkage of the D2 dopamine receptor and neural cell adhesion molecule genes on human chromosome 11q23. *Genomics* **14:** 1010-1018.

**Excoffier L, Slatkin M**. **1995.** Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol.Biol.Evol.* **12:** 921-927.

**Fahy TA, Osacar A, Marks I**. **1993.** History of eating disorders in female patients with obsessive-compulsive disorder. *Int.J.Eat.Disord.* **14:** 439-443.

**Falk CT, Rubinstein P**. **1987.** Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. *Ann.Hum.Genet.* **51 ( Pt 3):** 227-233.

**Fallin D, Schork NJ**. **2000.** Accuracy of haplotype frequency estimation for biallelic loci, via the expectation-maximization algorithm for unphased diploid genotype data. *Am.J.Hum.Genet.* **67:** 947-959.

**Fallin D, Cohen A, Essioux L, Chumakov I, Blumenfeld M, Cohen D, Schork NJ**. **2001.** Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res.* **11:** 143-151.

**Falzone TL, Gelman DM, Young JI, Grandy DK, Low MJ, Rubinstein M**. **2002.** Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. *Eur.J.Neurosci.* **15:** 158-164.

**Fantino M, Boucher H, Faion F, Mathiot P**. **1988.** Dexfenfluramine and body weight regulation: experimental study with hoarding behavior. *Clin.Neuropharmacol.* **11 Suppl 1:** S97-104.

**Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Frazier J, Cleves M, Sakai J**. **1999.** Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. *Am.J.Psychiatry* **156:** 768-770.

**Farde L, Hall H, Pauli S, Halldin C**. **1995.** Variability in D2-dopamine receptor density and affinity: a PET study with [11C]raclopride in man. *Synapse* **20:** 200-208.

**Favazza AR. 1992.** Repetitive self-mutilation. *Psychiatric Annals*. **22**: 60-63.

**Feng JF, Rhee SG, Im MJ**. **1996.** Evidence that phospholipase delta1 is the effector in the Gh (transglutaminase II)-mediated signaling. *J.Biol.Chem.* **271:** 16451-16454.

**File SE**. **1997.** Anxiolytic action of a neurokinin1 receptor antagonist in the social interaction test. *Pharmacol.Biochem.Behav.* **58:** 747-752.

**Fineberg N, Roberts A. 2002.** Obsessive-Compulsive Disorder: a twenty-first century perspective **In**: Fineberg N., Marazziti D, Stein DJ (eds).Obsessive-compulsive disorder: A Practical Guide. Martin Dunitz London UK; pp1-14

**Fireman B, Koran LM, Leventhal JL, Jacobson A**. **2001.** The prevalence of clinically recognized obsessive-compulsive disorder in a large health maintenance organization. *Am.J.Psychiatry* **158:** 1904-1910.

**First MB, Spitzer RL, Gobbon M, Williams JBW. 1998.** Structured Clinical Interview For DSM-IV Axis I Disorders—Patient Edition (SCID-I/P, Version 2.0, 8/98 Revision). New York State Psychiatric Institute, Biometrics Research Department, New York (1998).

**Fisher SK, Heacock AM, Agranoff BW**. **1992.** Inositol lipids and signal transduction in the nervous system: an update. *J.Neurochem.* **58:** 18-38.

**Fitzgerald KD, Moore GJ, Paulson LA, Stewart CM, Rosenberg DR**. **2000.** Proton spectroscopic imaging of the thalamus in treatment-naive pediatric obsessive-compulsive disorder. *Biol.Psychiatry* **47:** 174-182.

**Flament MF, Rapoport JL, Murphy DL, Berg CJ, Lake CR**. **1987.** Biochemical changes during clomipramine treatment of childhood obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **44:** 219-225.

**Flament MF, Whitaker A, Rapoport JL, Davies M, Berg CZ, Kalikow K, Sceery W, Shaffer D**. **1988.** Obsessive compulsive disorder in adolescence: an epidemiological study. *J.Am.Acad.Child Adolesc.Psychiatry* **27:** 764-771.

**Florijn WJ, Tarazi FI, Creese I**. **1997.** Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. *J.Pharmacol.Exp.Ther.* **280:** 561-569.

**Fog R. 1972.** On stereotypy and catalepsy: studies on the effect of amphetamines and neuroleptics in rats. Acta Neurol Scandanavica. **Suppl 50**. 3-66.

**Fontenelle LF, Mendlowicz MV, Marques C, Versiani M**. **2003.** Early- and late-onset obsessive-compulsive disorder in adult patients: an exploratory clinical and therapeutic study. *J.Psychiatr.Res.* **37:** 127-133.

**Foster MW, Sharp RR**. **2002.** Race, ethnicity, and genomics: social classifications as proxies of biological heterogeneity. *Genome Res.* **12:** 844-850.

**Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Berger TW**. **1999.** 17beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J.Neurophysiol.* **81:** 925-929.

**Frankel M, Cummings JL, Robertson MM, Trimble MR, Hill MA, Benson DF**. **1986.** Obsessions and compulsions in Gilles de la Tourette's syndrome. *Neurology* **36:** 378-382.

**Frankel WN, Schork NJ**. **1996.** Who's afraid of epistasis? *Nat.Genet.* **14:** 371-373.

**Frankenburg FR**. **1984.** Hoarding in anorexia nervosa. *Br.J.Med.Psychol.* **57 ( Pt 1):** 57-60.

**Frazer A, Gerhardt GA, Daws LC**. **1999.** New views of biogenic amine transporter function: implications for neuropsychopharmacology. *Int.J.Neuropsychopharmcol.* **2:** 305-320.

**Freedman ML, Reich D, Penney KL, McDonald GJ, Mignault AA, Patterson N, Gabriel SB, Topol EJ, Smoller JW, Pato CN, Pato MT, Petryshen TL, Kolonel LN, Lander ES, Sklar P, Henderson B, Hirschhorn JN, Altshuler D**. **2004.** Assessing the impact of population stratification on genetic association studies. *Nat.Genet.* **36:** 388-393.

**Freeman MP, Freeman SA, McElroy SL**. **2002.** The comorbidity of bipolar and anxiety disorders: prevalence, psychobiology, and treatment issues. *J.Affect.Disord.* **68:** 1-23.

**Freimer N, Sabatti C**. **2004.** The use of pedigree, sib-pair and association studies of common diseases for genetic mapping and epidemiology. *Nat.Genet.* **36:** 1045-1051.

**Freshour JR, Chase SE, Vikstrom KL**. **2002.** Gender differences in cardiac ACE expression are normalized in androgen-deprived male mice. *Am.J.Physiol Heart Circ.Physiol* **283:** H1997-H2003.

**Frisch A, Michaelovsky E, Rockah R, Amir I, Hermesh H, Laor N, Fuchs C, Zohar J, Lerer B, Buniak SF, Landa S, Poyurovsky M, Shapira B, Weizman R**. **2000.** Association between obsessive-compulsive disorder and polymorphisms of genes encoding components of the serotonergic and dopaminergic pathways. *Eur.Neuropsychopharmacol.* **10:** 205-209.

**Frisse L, Hudson RR, Bartoszewicz A, Wall JD, Donfack J, Di Rienzo A**. **2001.** Gene conversion and different population histories may explain the contrast between polymorphism and linkage disequilibrium levels. *Am.J.Hum.Genet.* **69:** 831-843.

**Froger N, Gardier AM, Moratalla R, Alberti I, Lena I, Boni C, De Felipe C, Rupniak NM, Hunt SP, Jacquot C, Hamon M, Lanfumey L**. **2001.** 5-hydroxytryptamine (5-HT)1A autoreceptor adaptive changes in substance P (neurokinin 1) receptor knock-out mice mimic antidepressant-induced desensitization. *J.Neurosci.* **21:** 8188-8197.

**Frost RO, Gross RC**. **1993.** The hoarding of possessions. *Behav.Res.Ther.* **31:** 367-381.

**Frost RO, Krause MS, Steketee G**. **1996.** Hoarding and obsessive-compulsive symptoms. *Behav.Modif.* **20:** 116-132.

**Frost RO, Steketee G, Williams LF, Warren R**. **2000.** Mood, personality disorder symptoms and disability in obsessive compulsive hoarders: a comparison with clinical and nonclinical controls. *Behav.Res.Ther.* **38:** 1071-1081.

**Ftouhi-Paquin N, Alda M, Grof P, Chretien N, Rouleau G, Turecki G**. **2001.** Identification of three polymorphisms in the translated region of PLC-gamma1 and their investigation in lithium responsive bipolar disorder. *Am.J.Med.Genet.* **105:** 301-305.

**Fux M, Levine J, Aviv A, Belmaker RH**. **1996.** Inositol treatment of obsessive-compulsive disorder. *Am.J.Psychiatry* **153:** 1219-1221.

**Fux M, Benjamin J, Belmaker RH**. **1999.** Inositol versus placebo augmentation of serotonin reuptake inhibitors in the treatment of obsessive-compulsive disorder: a double-blind cross-over study. *Int.J.Neuropsychopharmcol.* **2:** 193-195.

**Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D**. **2002.** The structure of haplotype blocks in the human genome. *Science* **296:** 2225-2229.

**Gallagher PE, Li P, Lenhart JR, Chappell MC, Brosnihan KB**. **1999.** Estrogen regulation of angiotensin-converting enzyme mRNA. *Hypertension* **33:** 323-328.

**Gallinat J, Bajbouj M, Sander T, Schlattmann P, Xu K, Ferro EF, Goldman D, Winterer G**. **2003.** Association of the G1947A COMT (Val(108/158)Met) gene polymorphism with prefrontal P300 during information processing. *Biol.Psychiatry* **54:** 40-48.

**Gambaro G, Anglani F, D'Angelo A**. **2000.** Association studies of genetic polymorphisms and complex disease. *Lancet* **355:** 308-311.

**Gawin FH, Ellinwood EH, Jr. 1988.** Cocaine and other stimulants. Actions, abuse, and treatment. *N.Engl.J.Med.* **318:** 1173-1182.

**Gelernter J, Kennedy JL, Van Tol HH, Civelli O, Kidd KK**. **1992.** The D4 dopamine receptor (DRD4) maps to distal 11p close to HRAS. *Genomics* **13:** 208-210.

**Gelernter J, Kranzler H, Cubells JF**. **1997.** Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African- and European-American and Japanese populations and in alcohol-dependent subjects. *Hum.Genet.* **101:** 243-246.

**Gelernter J, Page GP, Bonvicini K, Woods SW, Pauls DL, Kruger S**. **2003.** A chromosome 14 risk locus for simple phobia: results from a genomewide linkage scan. *Mol.Psychiatry* **8:** 71-82.

**Gelernter J, Page GP, Stein MB, Woods SW**. **2004.** Genome-wide linkage scan for loci predisposing to social phobia: evidence for a chromosome 16 risk locus. *Am.J.Psychiatry* **161:** 59-66.

**Geller DA, Biederman J, Griffin S, Jones J, Lefkowitz TR** . **1996.** Comorbidity of juvenile obsessive-compulsive disorder with disruptive behavior disorders. *J.Am.Acad.Child Adolesc.Psychiatry* **35:** 1637-1646.

**Geller DA, Biederman J, Jones J, Shapiro S, Schwartz S, Park KS**. **1998.** Obsessive-compulsive disorder in children and adolescents: a review. *Harv.Rev.Psychiatry* **5:** 260-273.

**Geller DA, Biederman J, Faraone S, Agranat A, Cradock K, Hagermoser L, Kim G, Frazier J, Coffey BJ**. **2001.** Developmental aspects of obsessive compulsive disorder: findings in children, adolescents, and adults. *J.Nerv.Ment.Dis.* **189:** 471-477.

**George MS, Trimble MR, Ring HA, Sallee FR, Robertson MM**. **1993.** Obsessions in obsessive-compulsive disorder with and without Gilles de la Tourette's syndrome. *Am.J.Psychiatry* **150:** 93-97.

**Gerard C, el Mestikawy S, Lebrand C, Adrien J, Ruat M, Traiffort E, Hamon M, Martres MP**. **1996.** Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse* **23:** 164-173.

**Gerard C, Martres MP, Lefevre K, Miquel MC, Verge D, Lanfumey L, Doucet E, Hamon M, el Mestikawy S**. **1997.** Immuno-localization of serotonin 5-HT6 receptor-like material in the rat central nervous system. *Brain Res.* **746:** 207-219.

**Ghosh S, Schork NJ**. **1996.** Genetic analysis of NIDDM. The study of quantitative traits. *Diabetes* **45:** 1-14.

**Gillot A, Furniss F, Walter A. 2001.** Anxiety in high functioning children with autism. *Autism.* 5(3). 277-286.

**Giros B, el Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, Caron MG**. **1992.** Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol.Pharmacol.* **42:** 383-390.

**Giros B, Jaber M, Jones SR, Wightman RM, Caron MG**. **1996.** Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **379:** 606-612.

**Glass GV. 1976.** Primary secondary and meta-analysis *EducRes* 5: 3-8.

**Glatt CE, DeYoung JA, Delgado S, Service SK, Giacomini KM, Edwards RH, Risch N, Freimer NB**. **2001.** Screening a large reference sample to identify very low frequency sequence variants: comparisons between two genes. *Nat.Genet.* **27:** 435-438.

**Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, Goldman D, Weinberger DR**. **2003.** Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. *Arch.Gen.Psychiatry* **60:** 889-896.

**Golden RN, Morris JE, Sack DA**. **1988.** Combined lithium-tricyclic treatment of obsessive-compulsive disorder. *Biol.Psychiatry* **23:** 181-185.

**Golden RN, Gilmore JH, Corrigan MH, Ekstrom RD, Knight BT, Garbutt JC**. **1991.** Serotonin, suicide, and aggression: clinical studies. *J.Clin.Psychiatry* **52 Suppl:** 61-69.

**Goldman A, Krause A, Ramsay M, Jenkins T**. **1996.** Founder effect and prevalence of myotonic dystrophy in South Africans: molecular studies. *Am.J.Hum.Genet.* **59:** 445-452.

**Goodman AH**. **2000.** Why genes don't count (for racial differences in health). *Am.J.Public Health* **90:** 1699-1702.

**Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, Heneniger GR, Charney DS. 1989**. The Yale-Brown Obsessive Compulsive Scale I. Development, use and reliability. *Archives of General Psychiatry*. **46**: 1006-1011.

**Goodman WK, McDougle CJ, Price LH, Riddle MA, Pauls DL, Leckman JF**. **1990.** Beyond the serotonin hypothesis: a role for dopamine in some forms of obsessive compulsive disorder? *J.Clin.Psychiatry* **51 Suppl:** 36-43.

**Goodman WK, McDougle CJ, Price LH, Barr LC, Hills OF, Caplik JF, Charney DS, Heninger GR**. **1995.** m-Chlorophenylpiperazine in patients with obsessive-compulsive disorder: absence of symptom exacerbation. *Biol.Psychiatry* **38:** 138-149.

**Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, Heneniger GR, Charney DS. 1989**. The Yale-Brown Obsessive Compulsive Scale I. Development, use and reliability. *Archives of General Psychiatry*. **46**: 1006-1011.

**Goodwin DW, Guze SB, Robins E**. **1969.** Follow-up studies in obsessional neurosis. *Arch.Gen.Psychiatry* **20:** 182-187.

**Gordon D, Simonic I, Ott J**. **2000.** Significant evidence for linkage disequilibrium over a 5-cM region among Afrikaners. *Genomics* **66:** 87-92.

**Gothert M, Schlicker E**. **1987.** Classification of serotonin receptors. *J.Cardiovasc.Pharmacol.* **10 Suppl 3:** S3-S7.

**Gothert M**. **1990.** Presynaptic serotonin receptors in the central nervous system. *Ann.N.Y.Acad.Sci.* **604:** 102-112.

**Gottesman II, Gould TD**. **2003.** The endophenotype concept in psychiatry: etymology and strategic intentions. *Am.J.Psychiatry* **160:** 636-645.

**Grad LR, Pelcovitz D, Olson M, Matthews M, Grad GJ**. **1987.** Obsessive-compulsive symptomatology in children with Tourette's syndrome. *J.Am.Acad.Child Adolesc.Psychiatry* **26:** 69-73.

**Grados MA, Riddle MA, Samuels JF, Liang KY, Hoehn-Saric R, Bienvenu OJ, Walkup JT, Song D, Nestadt G**. **2001.** The familial phenotype of obsessive-compulsive disorder in relation to tic disorders: the Hopkins OCD family study. *Biol.Psychiatry* **50:** 559-565.

**Grady DL, Chi HC, Ding YC, Smith M, Wang E, Schuck S, Flodman P, Spence MA, Swanson JM, Moyzis RK**. **2003.** High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. *Mol.Psychiatry* **8:** 536-545.

**Graf M, Kantor S, Anheuer ZE, Modos EA, Bagdy G**. **2003.** m-CPP-induced self-grooming is mediated by 5-HT2C receptors. *Behav.Brain Res.* **142:** 175-179.

**Graham J, Thompson EA**. **1998.** Disequilibrium likelihoods for fine-scale mapping of a rare allele. *Am.J.Hum.Genet.* **63:** 1517-1530.

**Grandy DK, Zhou QY, Allen L, Litt R, Magenis RE, Civelli O, Litt M**. **1990.** A human D1 dopamine receptor gene is located on chromosome 5 at q35.1 and identifies an EcoRI RFLP. *Am.J.Hum.Genet.* **47:** 828-834.

**Graybiel AM, Rauch SL**. **2000.** Toward a neurobiology of obsessive-compulsive disorder. *Neuron* **28:** 343-347.

**Green E, Craddock N**. **2003.** Brain-derived neurotrophic factor as a potential risk locus for bipolar disorder: evidence, limitations, and implications. *Curr.Psychiatry Rep.* **5:** 469-476.

**Greenberg BD, Ziemann U, Cora-Locatelli G, Harmon A, Murphy DL, Keel JC, Wassermann EM**. **2000.** Altered cortical excitability in obsessive-compulsive disorder. *Neurology* **54:** 142-147.

**Greenberg D, Witztum E, Levy A**. **1990.** Hoarding as a psychiatric symptom. *J.Clin.Psychiatry* **51:** 417-421.

**Greenberg DA**. **1993.** Linkage analysis of "necessary" disease loci versus "susceptibility" loci. *Am.J.Hum.Genet.* **52:** 135-143.

**Greenberg DA**. **1993.** Linkage analysis of "necessary" disease loci versus "susceptibility" loci. *Am.J.Hum.Genet.* **52:** 135-143.

**Greer JM, Capecchi MR**. **2002.** Hoxb8 is required for normal grooming behavior in mice. *Neuron* **33:** 23-34.

**Greist JH, Jefferson JW, Kobak KA, Katzelnick DJ, Serlin RC**. **1995.** Efficacy and tolerability of serotonin transport inhibitors in obsessive-compulsive disorder. A meta-analysis. *Arch.Gen.Psychiatry* **52:** 53-60.

**Greist JH, Jefferson JW**. **1998.** Pharmacotherapy for obsessive-compulsive disorder. *Br.J.Psychiatry Suppl* 64-70.

**Griebel G. 1999**. Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders. *Pharmacol Ther.* **82**(1):1-61

**Grimaldi B, Bonnin A, Fillion MP, Ruat M, Traiffort E, Fillion G**. **1998.** Characterization of 5-ht6 receptor and expression of 5-ht6 mRNA in the rat brain during ontogenetic development. *Naunyn Schmiedebergs Arch.Pharmacol.* **357:** 393-400.

**Groenewald JZ, Liebenberg J, Groenewald IM, Warnich L**. **1998.** Linkage disequilibrium analysis in a recently founded population: evaluation of the variegate porphyria founder in South African Afrikaners. *Am.J.Hum.Genet.* **62:** 1254-1258.

**Grossman MH, Szumlanski C, Littrell JB, Weinstein R, Weinshilboum RM**. **1992.** Electrophoretic analysis of low and high activity forms of catechol-O-methyltransferase in human erythrocytes. *Life Sci.* **50:** 473-480.

**Gross-Isseroff, R., R. Cohen, Y. Sasson, H. Voet, and J. Zohar**. **2004**. Serotonergic dissection of obsessive compulsive symptoms: a challenge study with m-chlorophenylpiperazine and sumatriptan. *Neuropsychobiology* **50**:200-205.

**Guillin O, Griffon N, Bezard E, Leriche L, Diaz J, Gross C, Sokoloff P**. **2003.** Brain-derived neurotrophic factor controls dopamine D3 receptor expression: therapeutic implications in Parkinson's disease. *Eur.J.Pharmacol.* **480:** 89-95.

**Guo N, Klitenick MA, Tham CS, Fibiger HC**. **1995.** Receptor mechanisms mediating clozapine-induced c-fos expression in the forebrain. *Neuroscience* **65:** 747-756.

**Gusfield D**. **2001.** Inference of haplotypes from samples of diploid populations: complexity and algorithms. *J.Comput.Biol.* **8:** 305-323.

**Hall D, Wijsman EM, Roos JL, Gogos JA, Karayiorgou M**. **2002.** Extended intermarker linkage disequilibrium in the Afrikaners. *Genome Res.* **12:** 956-961.

**Hall D, Dhilla A, Charalambous A, Gogos JA, Karayiorgou M**. **2003.** Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am.J.Hum.Genet.* **73:** 370-376.

**Hallbook F, Ibanez CF, Persson H**. **1991.** Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. *Neuron* **6:** 845-858.

**Hamblin MW, Metcalf MA, McGuffin RW, Karpells S**. **1992.** Molecular cloning and functional characterization of a human 5-HT1B serotonin receptor: a homologue of the rat 5-HT1B receptor with 5-HT1D-like pharmacological specificity. *Biochem.Biophys.Res.Commun.* **184:** 752-759.

**Hamilton SP, Fyer AJ, Durner M, Heiman GA, Baisre dL, Hodge SE, Knowles JA, Weissman MM**. **2003.** Further genetic evidence for a panic disorder syndrome mapping to chromosome 13q. *Proc.Natl.Acad.Sci.U.S.A* **100:** 2550-2555.

**Hanes KR**. **1996.** Serotonin, psilocybin, and body dysmorphic disorder: a case report. *J.Clin.Psychopharmacol.* **16:** 188-189.

**Hanna GL, Yuwiler A, Cantwell DP**. **1991.** Whole blood serotonin in juvenile obsessive-compulsive disorder. *Biol.Psychiatry* **29:** 738-744.

**Hanna GL**. **1995.** Demographic and clinical features of obsessive-compulsive disorder in children and adolescents. *J.Am.Acad.Child Adolesc.Psychiatry* **34:** 19-27.

**Hanna GL, Veenstra-VanderWeele J, Cox NJ, Boehnke M, Himle JA, Curtis GC, Leventhal BL, Cook EH, Jr. 2002.** Genome-wide linkage analysis of families with obsessive-compulsive disorder ascertained through pediatric probands. *Am.J.Med.Genet.* **114:** 541-552.

**Hanna GL, Fischer DJ, Chadha KR, Himle JA, Van Etten M**. **2005.** Familial and sporadic subtypes of early-onset Obsessive-Compulsive disorder. *Biol.Psychiatry* **57:** 895-900.

**Hartl DL and Clark AG. 1997**. Principles of population genetics. 3rd edition. Sinauer Associates Inc. Publishers, Sunderland, Massachussets.

**Harvey BH, Scheepers A, Brand L, Stein DJ**. **2001.** Chronic inositol increases striatal D(2) receptors but does not modify dexamphetamine-induced motor behavior. Relevance to obsessive-compulsive disorder. *Pharmacol.Biochem.Behav.* **68:** 245-253.

**Harvey BH, Brink CB, Seedat S, Stein DJ**. **2002.** Defining the neuromolecular action of myo-inositol: application to obsessive-compulsive disorder. *Prog .Neuropsychopharmacol. Biol. Psychiatry* **26:** 21-32.

**Hasenohrl RU, Souza-Silva MA, Nikolaus S, Tomaz C, Brandao ML, Schwarting RK, Huston JP**. **2000.** Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides* **34:** 272-280.

**Hashimoto K, Shimizu E, Iyo M**. **2004.** Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Res.Brain Res.Rev.* **45:** 104-114.

**Hasler G, LaSalle-Ricci VH, Ronquillo JG, Crawley SA, Cochran LW, Kazuba D, Greenberg BD, Murphy DL**. **2005.** Obsessive-compulsive disorder symptom dimensions show specific relationships to psychiatric comorbidity. *Psychiatry Res.* **135:** 121-132.

**Hawley ME, Kidd KK**. **1995.** HAPLO: a program using the EM algorithm to estimate the frequencies of multi-site haplotypes. *J.Hered.* **86:** 409-411.

**Hedrick PW**. **1987.** Gametic disequilibrium measures: proceed with caution. *Genetics* **117:** 331-341.

**Heinz A, Sander T, Harms H, Finckh U, Kuhn S, Dufeu P, Dettling M, Graf K, Rolfs A, Rommelspacher H, Schmidt LG**. **1996.** Lack of allelic association of dopamine D1 and D2 (TaqIA) receptor gene polymorphisms with reduced dopaminergic sensitivity to alcoholism. *Alcohol Clin.Exp.Res.* **20:** 1109-1113.

**Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, Lee KS, Linnoila M, Weinberger DR**. **2000.** Genotype influences in vivo dopamine transporter availability in human striatum. *Neuropsychopharmacology* **22:** 133-139.

**Heisler LK, Tecott LH**. **1999.** Knockout Corner: Neurobehavioural consequences of a serotonin 5-HT(2C) receptor gene mutation. *Int.J.Neuropsychopharmcol.* **2:** 67-69.

**Heisler LK, Tecott LH**. **2000.** A paradoxical locomotor response in serotonin 5-HT(2C) receptor mutant mice. *J.Neurosci.* **20:** RC71.

**Helgason A, Yngvadottir B, Hrafnkelsson B, Gulcher J, Stefansson K**. **2005.** An Icelandic example of the impact of population structure on association studies. *Nat.Genet.* **37:** 90-95.

**Hemmings SM, Kinnear CJ, Niehaus DJ, Moolman-Smook JC, Lochner C, Knowles JA, Corfield VA, Stein DJ**. **2003.** Investigating the role of dopaminergic and serotonergic candidate genes in obsessive-compulsive disorder. *Eur.Neuropsychopharmacol.* **13:** 93-98.

**Hemmings SM, Kinnear CJ, Lochner C, Niehaus DJ, Knowles JA, Moolman-Smook JC, Corfield VA, Stein DJ**. **2004.** Early- versus late-onset obsessive-compulsive disorder: investigating genetic and clinical correlates. *Psychiatry Res.* **128:** 175-182.

**Henrietta LL, Rapoport JL. 1987.** Relief of obsessive-compulsive symptoms by LSD and psilocybin. Am J Psychiatry. **144:** 1239-1240

**Herzog AG**. **1999.** Psychoneuroendocrine aspects of temporolimbic epilepsy. Part I. Brain, reproductive steroids, and emotions. *Psychosomatics* **40:** 95-101.

**Hettema JM, Neale MC, Kendler KS**. **2001.** A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am.J.Psychiatry* **158:** 1568-1578.

**Heutink P, Oostra BA**. **2002.** Gene finding in genetically isolated populations. *Hum.Mol.Genet.* **11:** 2507-2515.

**Hill AB. 1965.** The environmental disease: association or causation? Proc.R.Soc.Med. **58**: 295-300

**Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K**. **2002.** A comprehensive review of genetic association studies. *Genet.Med.* **4:** 45-61.

**Hirschhorn JN, Daly MJ**. **2005.** Genome-wide association studies for common diseases and complex traits. *Nat.Rev.Genet.* **6:** 95-108.

**Hirvonen M, Laakso A, Nagren K, Rinne JO, Pohjalainen T, Hietala J**. **2004.** C957T polymorphism of the dopamine D2 receptor (DRD2) gene affects striatal DRD2 availability in vivo. *Mol.Psychiatry* **9:** 1060-1061.

**Hmadcha A, Bedoya FJ, Sobrino F, Pintado E**. **1999.** Methylation-dependent gene silencing induced by interleukin 1beta via nitric oxide production. *J.Exp.Med.* **190:** 1595-1604.

**Hodge SE**. **1993.** Linkage analysis versus association analysis: distinguishing between two models that explain disease-marker associations. *Am.J.Hum.Genet.* **53:** 367-384.

**Hoglinger GU, Sautter J, Meyer M, Spenger C, Seiler RW, Oertel WH, Widmer HR**. **1998.** Rat fetal ventral mesencephalon grown as solid tissue cultures: influence of culture time and BDNF treatment on dopamine neuron survival and function. *Brain Res.* **813:** 313-322.

**Hogstel MO**. **1993.** Understanding hoarding behaviors in the elderly. *Am.J.Nurs.* **93:** 42-45.

**Hoh J, Wille A, Ott J**. **2001.** Trimming, weighting, and grouping SNPs in human case-control association studies. *Genome Res.* **11:** 2115-2119.

**Hollander E, Benzaquen SD. 1997.** The obsessive-compulsive spectrum disorders. *Int Rev Psychiatry*. **9**: 99-109

**Hollander E, Liebowitz MR, Winchel R, Klumker A, Klein DF**. **1989.** Treatment of body-dysmorphic disorder with serotonin reuptake blockers. *Am.J.Psychiatry* **146:** 768-770.

**Hollander E, DeCaria C, Gully R, Nitescu A, Suckow RF, Gorman JM, Klein DF, Liebowitz MR**. **1991.** Effects of chronic fluoxetine treatment on behavioral and neuroendocrine responses to meta-chlorophenylpiperazine in obsessive-compulsive disorder. *Psychiatry Res.* **36:** 1-17.

**Hollander E, Stein DJ. 1997.** Obsessive-compulsive disorders: diagnosis, etiology, treatment. NY: Dekker.

**Hollander E, DeCaria CM, Nitescu A, Gully R, Suckow RF, Cooper TB, Gorman JM, Klein DF, Liebowitz MR**. **1992(a).** Serotonergic function in obsessive-compulsive disorder. Behavioral and neuroendocrine responses to oral m-chlorophenylpiperazine and fenfluramine in patients and healthy volunteers. *Arch.Gen.Psychiatry* **49:** 21-28.

**Hollander E, Stein DJ, Saoud JB, DeCaria CM, Cooper TB, Trungold S, Stanley M, Liebowitz MR**. **1992(b).** Effects of fenfluramine on plasma HVA in OCD. *Psychiatry Res.* **42:** 185-188.

**Hollander E, Wong CM**. **1995.** Body dysmorphic disorder, pathological gambling, and sexual compulsions. *J.Clin.Psychiatry* **56 Suppl 4:** 7-12.

**Hollander E, Kwon JH, Stein DJ, Broatch J, Rowland CT, Himelein CA**. **1996.** Obsessive-compulsive and spectrum disorders: overview and quality of life issues. *J.Clin.Psychiatry* **57 Suppl 8:** 3-6.

**Hollander, E. and Cohen 1996**. **In**: Oldham J Hollander E, Skodol AE (eds) Psychobiology and psychopharmacology of compulsive spectrum disorders in impulsivity and compulsivity Washington DC APA pp167-195.

**Hollander E**. **1998.** Treatment of obsessive-compulsive spectrum disorders with SSRIs. *Br.J.Psychiatry Suppl* 7-12.

**Hollander E, Baldini RN, Sood E, Pallanti S**. **2003(a).** Risperidone augmentation in treatment-resistant obsessive-compulsive disorder: a double-blind, placebo-controlled study. *Int.J.Neuropsychopharmacol.* **6:** 397-401.

**Hollander E, King A, Delaney K, Smith CJ, Silverman JM**. **2003(b).** Obsessive-compulsive behaviors in parents of multiplex autism families. *Psychiatry Res.* **117:** 11-16.

**Holmes C, Smith H, Ganderton R, Arranz M, Collier D, Powell J, Lovestone S**. **2001.** Psychosis and aggression in Alzheimer's disease: the effect of dopamine receptor gene variation. *J.Neurol.Neurosurg.Psychiatry* **71:** 777-779.

**Holzer JC, Goodman WK, McDougle CJ, Baer L, Boyarsky BK, Leckman JF, Price LH**. **1994.** Obsessive-compulsive disorder with and without a chronic tic disorder. A comparison of symptoms in 70 patients. *Br.J.Psychiatry* **164:** 469-473.

**Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, et al.**. **2000.** Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat.Genet.* **26:** 163-175.

**Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP**. **1994.** International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol.Rev.* **46:** 157-203.

**Hoyer D, Martin GR**. **1996.** Classification and nomenclature of 5-HT receptors: a comment on current issues. *Behav.Brain Res.* **73:** 263-268.

**Hu FB, Doria A, Li T, Meigs JB, Liu S, Memisoglu A, Hunter D, Manson JE**. **2004.** Genetic variation at the adiponectin locus and risk of type 2 diabetes in women. *Diabetes* **53:** 209-213.

**Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S**. **1999.** BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* **98:** 739-755.

**Hudziak JJ, Van Beijsterveldt CE, Althoff RR, Stanger C, Rettew DC, Nelson EC, Todd RD, Bartels M, Boomsma DI**. **2004.** Genetic and environmental contributions to the Child Behavior Checklist Obsessive-Compulsive Scale: a cross-cultural twin study. *Arch.Gen.Psychiatry* **61:** 608-616.

**Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G**. **2001.** Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411:** 599-603.

**Huttenlocher PR**. **1979.** Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Res.* **163:** 195-205.

**Hwang SJ, Beaty TH, Liang KY, Coresh J, Khoury MJ**. **1994.** Minimum sample size estimation to detect gene-environment interaction in case-control designs. *Am.J.Epidemiol.* **140:** 1029-1037.

**Inada T, Sugita T, Dobashi I, Inagaki A, Kitao Y, Matsuda G, Kato S, Takano T, Yagi G, Asai M**. **1996.** Dopamine transporter gene polymorphism and psychiatric symptoms seen in schizophrenic patients at their first episode. *Am.J.Med.Genet.* **67:** 406-408.

**Inhorn RC, Majerus PW**. **1988.** Properties of inositol polyphosphate 1-phosphatase. *J.Biol.Chem.* **263:** 14559-14565.

**Inouye E**. **1965**. Similar and dissimilar manifestations of obsessive-compulsive neurosis in monozygotic twins. *Am J Psychiatry*. **121**: 1171

**Insel TR, Kalin NH, Guttmacher LB, Cohen RM, Murphy DL**. **1982.** The dexamethasone suppression test in patients with primary obsessive-compulsive disorder. *Psychiatry Res.* **6:** 153-160.

**Insel TR, Mueller EA, Alterman I, Linnoila M, Murphy DL**. **1985.** Obsessive-compulsive disorder and serotonin: is there a connection? *Biol.Psychiatry* **20:** 1174-1188.

**Insel TR**. **1992.** Toward a neuroanatomy of obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **49:** 739-744.

**Insel TR, Winslow JT**. **1992.** Neurobiology of obsessive compulsive disorder. *Psychiatr.Clin.North Am.* **15:** 813-824.

**Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG**. **2003.** Genetic associations in large versus small studies: an empirical assessment. *Lancet* **361:** 567-571.

**Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M, . 1993.** Molecular characterization of the family of the N-methyl-D-aspartate receptor subunits. *J.Biol.Chem.* **268:** 2836-2843.

**Iyer RN, Bradberry CW**. **1996.** Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. *J.Pharmacol.Exp.Ther.* **277:** 40-47.

**Jaber M, Jones S, Giros B, Caron MG**. **1997.** The dopamine transporter: a crucial component regulating dopamine transmission. *Mov Disord.* **12:** 629-633.

**Jacobs BL**. **1991.** Serotonin and behavior: emphasis on motor control. *J.Clin.Psychiatry* **52 Suppl:** 17-23.

**Jacobsen FM**. **1995.** Risperidone in the treatment of affective illness and obsessive-compulsive disorder. *J.Clin.Psychiatry* **56:** 423-429.

**Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB, Gelernter J**. **2000.** Prediction of dopamine transporter binding availability by genotype: a preliminary report. *Am.J.Psychiatry* **157:** 1700-1703.

**Jaiswal AK, Panda JN, Kumar MV, Joshi P**. **1985.** Androgen dependence of testicular and epididymal angiotensin converting enzyme. *Andrologia* **17:** 92-97.

**Jarvie KR, Caron MG**. **1993.** Heterogeneity of dopamine receptors. *Adv.Neurol.* **60:** 325-333.

**Jarvik GP**. **1998.** Complex segregation analyses: uses and limitations. *Am.J.Hum.Genet.* **63:** 942-946.

**Jenike MA, Buttolph L, Baer L, Ricciardi J, Holland A**. **1989.** Open trial of fluoxetine in obsessive-compulsive disorder. *Am.J.Psychiatry* **146:** 909-911.

**Jenike MA**. **2001.** An update on obsessive-compulsive disorder. *Bull.Menninger Clin.* **65:** 4-25.

**Jenkins T**. **1990.** Medical genetics in South Africa. *J.Med.Genet.* **27:** 760-779.

**Jenkins TA, Allen AM, Chai SY, Mendelsohn FA**. **1995.** Interactions of angiotensin II with central catecholamines. *Clin.Exp.Hypertens.* **17:** 267-280.

**Jenkins TA, Mendelsohn FA, Chai SY**. **1997.** Angiotensin-converting enzyme modulates dopamine turnover in the striatum. *J.Neurochem.* **68:** 1304-1311.

**Jenuwein T, Allis CD**. **2001.** Translating the histone code. *Science* **293:** 1074-1080.

**Jernigan TL, Tallal P**. **1990.** Late childhood changes in brain morphology observable with MRI. *Dev.Med.Child Neurol.* **32:** 379-385.

**Jiang H, Xie T, Ramsden DB, Ho SL**. **2003.** Human catechol-O-methyltransferase down-regulation by estradiol. *Neuropharmacology* **45:** 1011-1018.

**Jiang X, Xu K, Hoberman J, Tian F, Marko AJ, Waheed JF, Harris CR, Marini AM, Enoch MA, Lipsky RH**. **2005.** BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology* **30:** 1353-1361.

**Jo K, Derin R, Li M, Bredt DS**. **1999.** Characterization of MALS/Velis-1, -2, and -3: a family of mammalian LIN-7 homologs enriched at brain synapses in association with the postsynaptic density-95/NMDA receptor postsynaptic complex. *J.Neurosci.* **19:** 4189-4199.

**Joel D, Avisar A**. **2001.** Excessive lever pressing following post-training signal attenuation in rats: a possible animal model of obsessive compulsive disorder? *Behav.Brain Res.* **123:** 77-87.

**Joel D, Doljansky J**. **2003.** Selective alleviation of compulsive lever-pressing in rats by D1, but not D2, blockade: possible implications for the involvement of D1 receptors in obsessive-compulsive disorder. *Neuropsychopharmacology* **28:** 77-85.

**Johnson GC, Esposito L, Barratt BJ, Smith AN, Heward J, Di Genova G, Ueda H, Cordell HJ, Eaves IA, Dudbridge F, Twells RC, Payne F, Hughes W, Nutland S, Stevens H, Carr P, Tuomilehto-Wolf E, Tuomilehto J, Gough SC, Clayton DG, Todd JA**. **2001.** Haplotype tagging for the identification of common disease genes. *Nat.Genet.* **29:** 233-237.

**Johnston CI**. **1990.** Biochemistry and pharmacology of the renin-angiotensin system. *Drugs* **39 Suppl 1:** 21-31.

**Jonnal AH, Gardner CO, Prescott CA, Kendler KS**. **2000.** Obsessive and compulsive symptoms in a general population sample of female twins. *Am.J.Med.Genet.* **96:** 791-796.

**Jonsson EG, Bah J, Melke J, Abou JR, Schumacher J, Westberg L, Ivo R, Cichon S, Propping P, Nothen MM, Eriksson E, Sedvall GC**. **2004.** Monoamine related functional gene variants and relationships to monoamine metabolite concentrations in CSF of healthy volunteers. *BMC.Psychiatry* **4:** 4.

**Jorde LB**. **1995.** Linkage disequilibrium as a gene-mapping tool. *Am.J.Hum.Genet.* **56:** 11-14.

**Jorde LB**. **2000.** Linkage disequilibrium and the search for complex disease genes. *Genome Res.* **10:** 1435-1444.

**Joseph J. 2000.** Potential confounds in psychiatric genetic research: the case of pellagra. *New Ideas in Psychology*. **18**: 83-91.

**Kringlen E. 1965.** Obsessional neurotics: a long-term follow-up. *Br J Psychiatry*. **111**: 709-722.

**Joyce PR, Rogers GR, Miller AL, Mulder RT, Luty SE, Kennedy MA**. **2003.** Polymorphisms of DRD4 and DRD3 and risk of avoidant and obsessive personality traits and disorders. *Psychiatry Res.* **119:** 1-10.

**Kaasinen V, Kemppainen N, Nagren K, Helenius H, Kurki T, Rinne JO**. **2002.** Age-related loss of extrastriatal dopamine D(2) -like receptors in women. *J.Neurochem.* **81:** 1005-1010.

**Kahn RS, Wetzler S**. **1991.** m-Chlorophenylpiperazine as a probe of serotonin function. *Biol.Psychiatry* **30:** 1139-1166.

**Kalivas PW, Duffy P, Barrow J**. **1989.** Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. *J.Pharmacol.Exp.Ther.* **251:** 378-387.

**Kalsbeek A, de Bruin JP, Feenstra MG, Matthijssen MA, Uylings HB**. **1988.** Neonatal thermal lesions of the mesolimbocortical dopaminergic projection decrease food-hoarding behavior. *Brain Res.* **475:** 80-90.

**Kamboh MI, Sanghera DK, Ferrell RE, DeKosky ST**. **1995.** APOE*4-associated Alzheimer's disease risk is modified by alpha 1-antichymotrypsin polymorphism. *Nat.Genet.* **10:** 486-488.

**Kang AM, Palmatier MA, Kidd KK**. **1999.** Global variation of a 40-bp VNTR in the 3'-untranslated region of the dopamine transporter gene (SLC6A3). *Biol.Psychiatry* **46:** 151-160.

**Kaplan Z, Amir M, Swartz M, Levine J**. **1996.** Inositol treatment of post-traumatic stress disorder. *Anxiety.* **2:** 51-52.

**Kaptchuk TJ**. **2003.** Effect of interpretive bias on research evidence. *BMJ* **326:** 1453-1455.

**Kapur S, Remington G**. **1996.** Serotonin-dopamine interaction and its relevance to schizophrenia. *Am.J.Psychiatry* **153:** 466-476.

**Karayiorgou M, Morris MA, Morrow B, Shprintzen RJ, Goldberg R, Borrow J, Gos A, Nestadt G, Wolyniec PS, Lasseter VK, . 1995.** Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. *Proc.Natl.Acad.Sci.U.S.A* **92:** 7612-7616.

**Karayiorgou M, Altemus M, Galke BL, Goldman D, Murphy DL, Ott J, Gogos JA**. **1997.** Genotype determining low catechol-O-methyltransferase activity as a risk factor for obsessive-compulsive disorder. *Proc.Natl.Acad.Sci.U.S.A* **94:** 4572-4575.

**Karayiorgou M, Gogos JA, Galke BL, Wolyniec PS, Nestadt G, Antonarakis SE, Kazazian HH, Housman DE, Pulver AE**. **1998.** Identification of sequence variants and analysis of the role of the catechol-O-methyl-transferase gene in schizophrenia susceptibility. *Biol.Psychiatry* **43:** 425-431.

**Karayiorgou M, Sobin C, Blundell ML, Galke BL, Malinova L, Goldberg P, Ott J, Gogos JA**. **1999.** Family-based association studies support a sexually dimorphic effect of COMT and MAOA on genetic susceptibility to obsessive-compulsive disorder. *Biol.Psychiatry* **45:** 1178-1189.

**Karayiorgou M, Torrington M, Abecasis GR, Pretorius H, Robertson B, Kaliski S, Lay S, Sobin C, Moller N, Lundy SL, Blundell ML, Gogos JA, Roos JL**. **2004.** Phenotypic characterization and genealogical tracing in an Afrikaner schizophrenia database. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **124:** 20-28.

**Karno M, Golding JM, Sorenson SB, Burnam MA**. **1988.** The epidemiology of obsessive-compulsive disorder in five US communities. *Arch.Gen.Psychiatry* **45:** 1094-1099.

**Kavaliers M, Hirst M**. **1985.** Differential opiate influences on food hoarding and intake in the deer mouse, Peromyscus maniculatus. *Life Sci.* **37:** 2213-2220.

**Keavney B, McKenzie CA, Connell JM, Julier C, Ratcliffe PJ, Sobel E, Lathrop M, Farrall M**. **1998.** Measured haplotype analysis of the angiotensin-I converting enzyme gene. *Hum.Mol.Genet.* **7:** 1745-1751.

**Kelley AE, Stinus L**. **1985.** Disappearance of hoarding behavior after 6-hydroxydopamine lesions of the mesolimbic dopamine neurons and its reinstatement with L-dopa. *Behav.Neurosci.* **99:** 531-545.

**Kelsoe JR, Sadovnick AD, Kristbjarnarson H, Bergesch P, Mroczkowski-Parker Z, Drennan M, Rapaport MH, Flodman P, Spence MA, Remick RA**. **1996.** Possible locus for bipolar disorder near the dopamine transporter on chromosome 5. *Am.J.Med.Genet.* **67:** 533-540.

**Kennedy JL, Sidenberg DG, Van Tol HH, Kidd KK**. **1991.** A HincII RFLP in the human D4 dopamine receptor locus (DRD4). *Nucleic Acids Res.* **19:** 5801.

**Keuthen NJ, Savage CR, O'Sullivan RL, Brown HD, Shera DM, Cyr P, Jenike MA, Baer L**. **1996.** Neuropsychological functioning in trichotillomania. *Biol.Psychiatry* **39:** 747-749.

**Khait VD, Huang YY, Zalsman G, Oquendo MA, Brent DA, Harkavy-Friedman JM, Mann JJ**. **2005.** Association of serotonin 5-HT2A receptor binding and the T102C polymorphism in depressed and healthy Caucasian subjects. *Neuropsychopharmacology* **30:** 166-172.

**Khanna S, John JP, Reddy LP**. **2001.** Neuroendocrine and behavioral responses to mCPP in Obsessive-Compulsive Disorder. *Psychoneuroendocrinology* **26:** 209-223.

**Kidd JR, Pakstis AJ, Zhao H, Lu RB, Okonofua FE, Odunsi A, Grigorenko E, Tamir BB, Friedlaender J, Schulz LO, Parnas J, Kidd KK**. **2000.** Haplotypes and linkage disequilibrium at the phenylalanine hydroxylase locus, PAH, in a global representation of populations. *Am.J.Hum.Genet.* **66:** 1882-1899.

**Kidd KK, Morar B, Castiglione CM, Zhao H, Pakstis AJ, Speed WC, Bonne-Tamir B, Lu RB, Goldman D, Lee C, Nam YS, Grandy DK, Jenkins T, Kidd JR**. **1998.** A global survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus. *Hum.Genet.* **103:** 211-227.

**Kilty JE, Lorang D, Amara SG**. **1991.** Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science* **254:** 578-579.

**Kim CH, Koo MS, Cheon KA, Ryu YH, Lee JD, Lee HS**. **2003.** Dopamine transporter density of basal ganglia assessed with [123I]IPT SPET in obsessive-compulsive disorder. *Eur.J.Nucl.Med.Mol.Imaging* **30:** 1637-1643.

**Kim SW, Dysken MW, Kline MD**. **1990.** Monozygotic twins with obsessive-compulsive disorder. *Br.J.Psychiatry* **156:** 435-438.

**Knowles JA, Fyer AJ, Vieland VJ, Weissman MM, Hodge SE, Heiman GA, Haghighi F, de Jesus GM, Rassnick H, Preud'homme-Rivelli X, Austin T, Cunjak J, Mick S, Fine LD, Woodley KA, Das K, Maier W, Adams PB, Freimer NB, Klein DF, Gilliam TC**. **1998.** Results of a genome-wide genetic screen for panic disorder. *Am.J.Med.Genet.* **81:** 139-147.

**Kofman O, Belmaker RH**. **1993.** Ziskind-Somerfeld Research Award 1993. Biochemical, behavioral, and clinical studies of the role of inositol in lithium treatment and depression. *Biol.Psychiatry* **34:** 839-852.

**Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, Meltzer HY, Sibley DR, Roth BL, Hamblin MW**. **1996.** Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. *J.Neurochem.* **66:** 47-56.

**Kojima H, Ohmori O, Shinkai T, Terao T, Suzuki T, Abe K**. **1999.** Dopamine D1 receptor gene polymorphism and schizophrenia in Japan. *Am.J.Med.Genet.* **88:** 116-119.

**Kolb B**. **1974.** Prefrontal lesions alter eating and hoarding behavior in rats. *Physiol Behav.* **12:** 507-511.

**Koran, L. M., S. Pallanti, and L. Quercioli**. **2001**. Sumatriptan, 5-HT(1D) receptors and obsessive-compulsive disorder. *Eur.Neuropsychopharmaco*l. **11**:169-172.

**Kotler M, Cohen H, Segman R, Gritsenko I, Nemanov L, Lerer B, Kramer I, Zer-Zion M, Kletz I, Ebstein RP**. **1997.** Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. *Mol.Psychiatry* **2:** 251-254.

**Kouzmenko AP, Pereira AM, Singh BS**. **1997.** Intronic sequences are involved in neural targeting of human dopamine transporter gene expression. *Biochem.Biophys.Res.Commun.* **240:** 807-811.

**Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hale JJ, Mills SG, MacCoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR, Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NM**. **1998.** Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* **281:** 1640-1645.

**Kringlen E. 1965.** Obsessional neurotics: a long-term follow-up. *Br J Psychiatry*. **111**: 709-722

**Kruglyak L**. **1999(a).** Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nat.Genet.* **22:** 139-144.

**Kruglyak L**. **1999(b).** Genetic isolates: separate but equal? *Proc.Natl.Acad.Sci.U.S.A* **96:** 1170-1172.

**Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, Abi-Saab W, Madonick S**. **1999.** NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. *Harv.Rev.Psychiatry* **7:** 125-143.

**Kugaya A, Epperson CN, Zoghbi S, van Dyck CH, Hou Y, Fujita M, Staley JK, Garg PK, Seibyl JP, Innis RB**. **2003.** Increase in prefrontal cortex serotonin 2A receptors following estrogen treatment in postmenopausal women. *Am.J.Psychiatry* **160:** 1522-1524.

**Kuno S, Taniguchi A, Saito A, Tsuchida-Otsuka S, Kamatani N**. **2004.** Comparison between various strategies for the disease-gene mapping using linkage disequilibrium analyses: studies on adenine phosphoribosyltransferase deficiency used as an example. *J.Hum.Genet.* **49:** 463-473.

**Kuppers E, Beyer C**. **2001.** Dopamine regulates brain-derived neurotrophic factor (BDNF) expression in cultured embryonic mouse striatal cells. *Neuroreport* **12:** 1175-1179.

**Laan M, Paabo S**. **1997.** Demographic history and linkage disequilibrium in human populations. *Nat.Genet.* **17:** 435-438.

**Lacerda AL, Dalgalarrondo P, Caetano D, Camargo EE, Etchebehere EC, Soares JC**. **2003.** Elevated thalamic and prefrontal regional cerebral blood flow in obsessive-compulsive disorder: a SPECT study. *Psychiatry Res.* **123:** 125-134.

**Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM**. **1996.** Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* **6:** 243-250.

**LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, Kennedy JL**. **1996.** Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol.Psychiatry* **1:** 121-124.

**Laine TP, Ahonen A, Rasanen P, Pohjalainen T, Tiihonen J, Hietala J**. **2001.** The A1 allele of the D2 dopamine receptor gene is associated with high dopamine transporter density in detoxified alcoholics. *Alcohol Alcohol* **36:** 262-265.

**Lalouel JM, Rohrwasser A**. **2001.** Development of genetic hypotheses in essential hypertension. *J.Hum.Genet.* **46:** 299-306.

**Lammers CH, Diaz J, Schwartz JC, Sokoloff P**. **2000.** Selective increase of dopamine D3 receptor gene expression as a common effect of chronic antidepressant treatments. *Mol.Psychiatry* **5:** 378-388.

**Lanau F, Zenner MT, Civelli O, Hartman DS**. **1997.** Epinephrine and norepinephrine act as potent agonists at the recombinant human dopamine D4 receptor. *J.Neurochem.* **68:** 804-812.

**Lander ES**. **1996.** The new genomics: global views of biology. *Science* **274:** 536-539.

**Landwehrmeyer B, Mengod G, Palacios JM**. **1993.** Dopamine D3 receptor mRNA and binding sites in human brain. *Brain Res.Mol.Brain Res.* **18:** 187-192.

**Lane HY, Lin CC, Huang CH, Chang YC, Hsu SK, Chang WH**. **2004.** Risperidone response and 5-HT6 receptor gene variance: genetic association analysis with adjustment for nongenetic confounders. *Schizophr.Res.* **67:** 63-70.

**Langley CH, Lazzaro BP, Phillips W, Heikkinen E, Braverman JM**. **2000.** Linkage disequilibria and the site frequency spectra in the su(s) and su(w(a)) regions of the Drosophila melanogaster X chromosome. *Genetics* **156:** 1837-1852.

**Laplane D, Levasseur M, Pillon B, Dubois B, Baulac M, Mazoyer B, Tran DS, Sette G, Danze F, Baron JC**. **1989.** Obsessive-compulsive and other behavioural changes with bilateral basal ganglia lesions. A neuropsychological, magnetic resonance imaging and positron tomography study. *Brain* **112 ( Pt 3):** 699-725.

**Lappalainen J, Dean M, Charbonneau L, Virkkunen M, Linnoila M, Goldman D**. **1995(a).** Mapping of the serotonin 5-HT1D beta autoreceptor gene on chromosome 6 and direct analysis for sequence variants. *Am.J.Med.Genet.* **60:** 157-161.

**Lappalainen J, Zhang L, Dean M, Oz M, Ozaki N, Yu DH, Virkkunen M, Weight F, Linnoila M, Goldman D**. **1995(b).** Identification, expression, and pharmacology of a Cys23-Ser23 substitution in the human 5-HT2C receptor gene (HTR2C). *Genomics* **27:** 274-279.

**Lappalainen J, Long JC, Virkkunen M, Ozaki N, Goldman D, Linnoila M**. **1999.** HTR2C Cys23Ser polymorphism in relation to CSF monoamine metabolite concentrations and DSM-III-R psychiatric diagnoses. *Biol.Psychiatry* **46:** 821-826.

**Last JM. 2000.** A Dictionary of Epidemiology (4th Edition). New York: Oxford University Press.

**Le Coniat M, Sokoloff P, Hillion J, Martres MP, Giros B, Pilon C, Schwartz JC, Berger R**. **1991.** Chromosomal localization of the human D3 dopamine receptor gene. *Hum.Genet.* **87:** 618-620.

**Leboyer M, Bellivier F, Nosten-Bertrand M, Jouvent R, Pauls D, Mallet J**. **1998.** Psychiatric genetics: search for phenotypes. *Trends Neurosci.* **21:** 102-105.

**Leckman JF, Riddle M, Hardin M, Ort S, Swartz K, Stevenson J *et al.* 1989**. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J. Am. Acad. Child Adolesc. Psychiatry* **28** pp. 566–573.

**Leckman JF, McDougle CJ, Pauls DL, Peterson BS, Grice DE, King RA, Scahill L Price LH Rasmussen SA. 2000.** Tic-related vs. non-tic-related obsessive-compulsive disorder **In**: Goodman WK, Rudorfer MY, Maser JD (eds). Obsessive-compulsive disorder: contemporary issues in treatment; p43-68.

**Leckman JF, Goodman WK, Anderson GM, Riddle MA, Chappell PB, McSwiggan-Hardin MT, McDougle CJ, Scahill LD, Ort SI, Pauls DL. 1995.** Cerebrospinal fluid biogenic amines in obsessive compulsive disorder, Tourette's syndrome, and healthy controls. *Neuropsychopharmacology* **12:** 73-86.

**Leckman JF, Grice DE, Boardman J, Zhang H, Vitale A, Bondi C, Alsobrook J, Peterson BS, Cohen DJ, Rasmussen SA, Goodman WK, McDougle CJ, Pauls DL**. **1997.** Symptoms of obsessive-compulsive disorder. *Am.J.Psychiatry* **154:** 911-917.

**Leckman JF, Zhang H, Alsobrook JP, Pauls DL**. **2001.** Symptom dimensions in obsessive-compulsive disorder: toward quantitative phenotypes. *Am.J.Med.Genet.* **105:** 28-30.

**Leckman JF, Pauls DL, Zhang H, Rosario-Campos MC, Katsovich L, Kidd KK, Pakstis AJ, Alsobrook JP, Robertson MM, McMahon WM, Walkup JT, van de Wetering BJ, King RA, Cohen DJ**. **2003.** Obsessive-compulsive symptom dimensions in affected sibling pairs diagnosed with Gilles de la Tourette syndrome. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **116:** 60-68.

**Lee R, Kermani P, Teng KK, Hempstead BL**. **2001.** Regulation of cell survival by secreted proneurotrophins. *Science* **294:** 1945-1948.

**Lees AJ, Robertson M, Trimble MR, Murray NM**. **1984.** A clinical study of Gilles de la Tourette syndrome in the United Kingdom. *J.Neurol.Neurosurg.Psychiatry* **47:** 1-8.

**Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde YA**. **1989.** Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* **341:** 149-152.

**LeMoal M**. **1995**. Mesocorticolimbic dopaminergic neurons: Functional and regulatory roles. **In**: Bloom FE. Kupfer DJ (eds). Psychopharmacology: the Fourth Generation of Progress. Raven, New York. pp 283-294

**Lenane MC, Swedo SE, Leonard H, Pauls DL, Sceery W, Rapoport JL**. **1990.** Psychiatric disorders in first degree relatives of children and adolescents with obsessive compulsive disorder. *J.Am.Acad.Child Adolesc.Psychiatry* **29:** 407-412.

**Lensi P, Cassano GB, Correddu G, Ravagli S, Kunovac JL, Akiskal HS**. **1996.** Obsessive-compulsive disorder. Familial-developmental history, symptomatology, comorbidity and course with special reference to gender-related differences. *Br.J.Psychiatry* **169:** 101-107.

**Leonard, H. L. and J. L. Rapoport**. **1987**. Relief of obsessive-compulsive symptoms by LSD and psilocin. *Am.J.Psychiatry* **144**:1239-1240.

**Leonard HL, Rapoport JL**. **1989.** Pharmacotherapy of childhood obsessive-compulsive disorder. *Psychiatr.Clin.North Am.* **12:** 963-970.

**Leonard HL, Lenane MC, Swedo SE, Rettew DC, Gershon ES, Rapoport JL**. **1992.** Tics and Tourette's disorder: a 2- to 7-year follow-up of 54 obsessive-compulsive children. *Am.J.Psychiatry* **149:** 1244-1251.

**Leonard HL, Swedo SE**. **2001.** Paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS). *Int.J.Neuropsychopharmacol.* **4:** 191-198.

**Levine J, Rapaport A, Lev L, Bersudsky Y, Kofman O, Belmaker RH, Shapiro J, Agam G**. **1993.** Inositol treatment raises CSF inositol levels. *Brain Res.* **627:** 168-170.

**Levine J, Barak Y, Gonzalves M, Szor H, Elizur A, Kofman O, Belmaker RH**. **1995.** Double-blind, controlled trial of inositol treatment of depression. *Am.J.Psychiatry* **152:** 792-794.

**Levine J**. **1997.** Controlled trials of inositol in psychiatry. *Eur.Neuropsychopharmacol.* **7:** 147-155.

**Levine J, Mishori A, Susnosky M, Martin M, Belmaker RH**. **1999.** Combination of inositol and serotonin reuptake inhibitors in the treatment of depression. *Biol.Psychiatry* **45:** 270-273.

**Lewin GR, Barde YA**. **1996.** Physiology of the neurotrophins. *Annu.Rev.Neurosci.* **19:** 289-317.

**Lewontin RC**. **1964.** The interaction of selection and linkage. ii. optimum models. *Genetics* **50:** 757-782.

**Li T, Xu K, Deng H, Cai G, Liu J, Liu X, Wang R, Xiang X, Zhao J, Murray RM, Sham PC, Collier DA**. **1997.** Association analysis of the dopamine D4 gene exon III VNTR and heroin abuse in Chinese subjects. *Mol.Psychiatry* **2:** 413-416.

**Lichter JB, Barr CL, Kennedy JL, Van Tol HH, Kidd KK, Livak KJ**. **1993.** A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. *Hum.Mol.Genet.* **2:** 767-773.

**Lidow MS, Goldman-Rakic PS**. **1994.** A common action of clozapine, haloperidol, and remoxipride on D1- and D2-dopaminergic receptors in the primate cerebral cortex. *Proc.Natl.Acad.Sci.U.S.A* **91:** 4353-4356.

**Lidow MS, Wang F, Cao Y, Goldman-Rakic PS**. **1998.** Layer V neurons bear the majority of mRNAs encoding the five distinct dopamine receptor subtypes in the primate prefrontal cortex. *Synapse* **28:** 10-20.

**Lilford RJ, Braunholtz D**. **1996.** The statistical basis of public policy: a paradigm shift is overdue. *BMJ* **313:** 603-607.

**Lindholm D, Carroll P, Tzimagiogis G, Thoenen H**. **1996.** Autocrine-paracrine regulation of hippocampal neuron survival by IGF-1 and the neurotrophins BDNF, NT-3 and NT-4. *Eur.J.Neurosci.* **8:** 1452-1460.

**Little J, Bradley L, Bray MS, Clyne M, Dorman J, Ellsworth DL, Hanson J, Khoury M, Lau J, O'Brien TR, Rothman N, Stroup D, Taioli E, Thomas D, Vainio H, Wacholder S, Weinberg C**. **2002.** Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations. *Am.J.Epidemiol.* **156:** 300-310.

**Liu Q, Sobell JL, Heston LL, Sommer SS**. **1995.** Screening the dopamine D1 receptor gene in 131 schizophrenics and eight alcoholics: identification of polymorphisms but lack of functionally significant sequence changes. *Am.J.Med.Genet.* **60:** 165-171.

**Liu QR, Walther D, Drgon T, Polesskaya O, Lesnick TG, Strain KJ, de Andrade M, Bower JH, Maraganore DM, Uhl GR**. **2005.** Human brain derived neurotrophic factor (BDNF) genes, splicing patterns, and assessments of associations with substance abuse and Parkinson's Disease. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **134:** 93-103.

**Lochner C, Hemmings SM, Kinnear CJ, Moolman-Smook JC, Corfield VA, Knowles JA, Niehaus DJ, Stein DJ**. **2004.** Gender in obsessive-compulsive disorder: clinical and genetic findings. *Eur.Neuropsychopharmacol.* **14:** 105-113.

**Loftis JM, Janowsky A**. **2003.** The N-methyl-D-aspartate receptor subunit NR2B: localization, functional properties, regulation, and clinical implications. *Pharmacol.Ther.* **97:** 55-85.

**Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN**. **2003.** Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat.Genet.* **33:** 177-182.

**Lohof AM, Ip NY, Poo MM**. **1993.** Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* **363:** 350-353.

**Long AD, Langley CH**. **1999.** The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits. *Genome Res.* **9:** 720-731.

**Long JC, Williams RC, Urbanek M**. **1995.** An E-M algorithm and testing strategy for multiple-locus haplotypes. *Am.J.Hum.Genet.* **56:** 799-810.

**Lopez-Ibor JJ, Jr. 1988.** The involvement of serotonin in psychiatric disorders and behaviour. *Br.J.Psychiatry Suppl* 26-39.

**Lopez I, Mak EC, Ding J, Hamm HE, Lomasney JW**. **2001.** A novel bifunctional phospholipase c that is regulated by Galpha 12 and stimulates the Ras/mitogen-activated protein kinase pathway. *J.Biol.Chem.* **276:** 2758-2765.

**Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, Taskinen J**. **1995.** Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* **34:** 4202-4210.

**Lovlie R, Gulbrandsen AK, Molven A, Steen VM**. **1999.** Genomic structure and sequence analysis of a human inositol polyphosphate 1-phosphatase gene (INPP1). *Pharmacogenetics* **9:** 517-528.

**Lu B**. **2003.** BDNF and activity-dependent synaptic modulation. *Learn.Mem.* **10:** 86-98.

**Luchins DJ, Goldman MB, Lieb M, Hanrahan P**. **1992.** Repetitive behaviors in chronically institutionalized schizophrenic patients. *Schizophr.Res.* **8:** 119-123.

**Lucki I, Kucharik RF. 1988.** Selectrive enhancement of grooming behaviour by the D1-agoinst SKF 38393 in rats following the destruction of serotonin neurons **In:** Neural mechanisms and biological significance of grooming behaviour New York: New York Academy of Sciences; pp420-422].

**Lundstrom K, Tenhunen J, Tilgmann C, Karhunen T, Panula P, Ulmanen I**. **1995.** Cloning, expression and structure of catechol-O-methyltransferase. *Biochim.Biophys.Acta* **1251:** 1-10.

**Lundstrom K, Turpin MP**. **1996.** Proposed schizophrenia-related gene polymorphism: expression of the Ser9Gly mutant human dopamine D3 receptor with the Semliki Forest virus system. *Biochem.Biophys.Res.Commun.* **225:** 1068-1072.

**Luxenberg JS, Swedo SE, Flament MF, Friedland RP, Rapoport J, Rapoport SI**. **1988.** Neuroanatomical abnormalities in obsessive-compulsive disorder detected with quantitative X-ray computed tomography. *Am.J.Psychiatry* **145:** 1089-1093.

**Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW, Bora SH, Wihler C, Koliatsos VE, Tessarollo L**. **1999.** Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc.Natl.Acad.Sci.U.S.A* **96:** 15239-15244.

**Macciardi F. 2003.** Multiple testing in genetic association studies: to correct or not to correct? *Am.J.Med.Genet.* 122B. 31-32.

**Madras BK, Miller GM, Fischman AJ**. **2005.** The dopamine transporter and attention-deficit/hyperactivity disorder. *Biol.Psychiatry* **57:** 1397-1409.

**Maina G, Albert U, Bogetto F, Ravizza L**. **1999.** Obsessive-compulsive syndromes in older adolescents. *Acta Psychiatr.Scand.* **100:** 447-450.

**Majerus PW, Connolly TM, Bansal VS, Inhorn RC, Ross TS, Lips DL**. **1988.** Inositol phosphates: synthesis and degradation. *J.Biol.Chem.* **263:** 3051-3054.

**Makalowski W, Mitchell GA, Labuda D**. **1994.** Alu sequences in the coding regions of mRNA: a source of protein variability. *Trends Genet.* **10:** 188-193.

**Malhotra AK, Kestler LJ, Mazzanti C, Bates JA, Goldberg T, Goldman D**. **2002.** A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. *Am.J.Psychiatry* **159:** 652-654.

**Mamounas LA, Blue ME, Siuciak JA, Altar CA**. **1995.** Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. *J.Neurosci.* **15:** 7929-7939.

**Mancini F, D'Olimpio F, Del Genio M, Didonna F, Prunetti E**. **2002.** Obsessions and compulsions and intolerance for uncertainty in a non-clinical sample. *J.Anxiety.Disord.* **16:** 401-411.

**Mandich P, Schito AM, Bellone E, Antonacci R, Finelli P, Rocchi M, Ajmar F**. **1994.** Mapping of the human NMDAR2B receptor subunit gene (GRIN2B) to chromosome 12p12. *Genomics* **22:** 216-218.

**Mant R, Williams J, Asherson P, Parfitt E, McGuffin P, Owen MJ**. **1994.** Relationship between homozygosity at the dopamine D3 receptor gene and schizophrenia. *Am.J.Med.Genet.* **54:** 21-26.

**Marazziti D, Hollander E, Lensi P, Ravagli S, Cassano GB** . **1992.** Peripheral markers of serotonin and dopamine function in obsessive-compulsive disorder. *Psychiatry Res.* **42:** 41-51.

**Marazziti D, Masala I, Rossi A, Hollander E, Presta S, Giannaccini G, Mazzoni MR, Dell'Osso L, Lucacchini A, Cassano GB**. **2000.** Increased inhibitory activity of protein kinase C on the serotonin transporter in OCD. *Neuropsychobiology* **41:** 171-177.

**March JS, Leonard HL**. **1996.** Obsessive-compulsive disorder in children and adolescents: a review of the past 10 years. *J.Am.Acad.Child Adolesc.Psychiatry* **35:** 1265-1273.

**Marchini J, Cardon LR, Phillips MS, Donnelly P**. **2004.** The effects of human population structure on large genetic association studies. *Nat.Genet.* **36:** 512-517.

**Marek GJ, Carpenter LL, McDougle CJ, Price LH**. **2003.** Synergistic action of 5-HT2A antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. *Neuropsychopharmacology* **28:** 402-412.

**Marini AM, Jiang X, Wu X, Tian F, Zhu D, Okagaki P, Lipsky RH**. **2004.** Role of brain-derived neurotrophic factor and NF-kappaB in neuronal plasticity and survival: From genes to phenotype. *Restor.Neurol.Neurosci.* **22:** 121-130.

**Marth G, Yeh R, Minton M, Donaldson R, Li Q, Duan S, Davenport R, Miller RD, Kwok PY**. **2001.** Single-nucleotide polymorphisms in the public domain: how useful are they? *Nat.Genet.* **27:** 371-372.

**Martinez D, Gelernter J, Abi-Dargham A, van Dyck CH, Kegeles L, Innis RB, Laruelle M**. **2001.** The variable number of tandem repeats polymorphism of the dopamine transporter gene is not associated with significant change in dopamine transporter phenotype in humans. *Neuropsychopharmacology* **24:** 553-560.

**Masellis, M., V. Basile, H. Y. Meltzer, J. A. Lieberman, S. Sevy, F. M. Macciardi, P. Cola, A. Howard, F. Badri, M. M. Nothen, W. Kalow, and J. L. Kennedy**. **1998**. Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenia patients. *Neuropsychopharmacology* **19**:123-132.

**Masellis, M., V. S. Basile, H. Y. Meltzer, J. A. Lieberman, S. Sevy, D. A. Goldman, M. W. Hamblin, F. M. Macciardi, and J. L. Kennedy**. **2001**. Lack of association between the T-->C 267 serotonin 5-HT6 receptor gene (HTR6) polymorphism and prediction of response to clozapine in schizophrenia. *Schizophr.Res.* **47**:49-58.

**Mataix-Cols D, Rauch SL, Manzo PA, Jenike MA, Baer L**. **1999.** Use of factor-analyzed symptom dimensions to predict outcome with serotonin reuptake inhibitors and placebo in the treatment of obsessive-compulsive disorder. *Am.J.Psychiatry* **156:** 1409-1416.

**Mataix-Cols D, Baer L, Rauch SL, Jenike MA**. **2000.** Relation of factor-analyzed symptom dimensions of obsessive-compulsive disorder to personality disorders. *Acta Psychiatr.Scand.* **102:** 199-202.

**Mataix-Cols D, Rauch SL, Baer L, Eisen JL, Shera DM, Goodman WK, Rasmussen SA, Jenike MA**. **2002.** Symptom stability in adult obsessive-compulsive disorder: data from a naturalistic two-year follow-up study. *Am.J.Psychiatry* **159:** 263-268.

**Mataix-Cols D, Wooderson S, Lawrence N, Brammer MJ, Speckens A, Phillips ML**. **2004.** Distinct neural correlates of washing, checking, and hoarding symptom dimensions in obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **61:** 564-576.

**Matsumoto M, Togashi H, Mori K, Ueno K, Miyamoto A, Yoshioka M**. **1999.** Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. *Eur.J.Pharmacol.* **383:** 39-48.

**Matsunaga H, Kiriike N, Matsui T, Miyata A, Iwasaki Y, Fujimoto K, Kasai S, Kojima M**. **2000.** Gender differences in social and interpersonal features and personality disorders among Japanese patients with obsessive-compulsive disorder. *Compr.Psychiatry* **41:** 266-272.

**Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF, Kolachana B, Callicott JH, Weinberger DR**. **2003.** Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc.Natl.Acad.Sci.U.S.A* **100:** 6186-6191.

**Mayer ML, Westbrook GL**. **1987.** The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog.Neurobiol.* **28:** 197-276.

**McDougle CJ, Goodman WK, Leckman JF, Lee NC, Heninger GR, Price LH**. **1994.** Haloperidol addition in fluvoxamine-refractory obsessive-compulsive disorder. A double-blind, placebo-controlled study in patients with and without tics. *Arch.Gen.Psychiatry* **51:** 302-308.

**McDougle CJ, Fleischmann RL, Epperson CN, Wasylink S, Leckman JF, Price LH**. **1995.** Risperidone addition in fluvoxamine-refractory obsessive-compulsive disorder: three cases. *J.Clin.Psychiatry* **56:** 526-528.

**McDougle CJ**. **1997.** Update on pharmacologic management of OCD: agents and augmentation. *J.Clin.Psychiatry* **58 Suppl 12:** 11-17.

**McDougle CJ, Epperson CN, Pelton GH, Wasylink S, Price LH**. **2000.** A double-blind, placebo-controlled study of risperidone addition in serotonin reuptake inhibitor-refractory obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **57:** 794-801.

**McElroy SL, Phillips KA, Keck PE, Jr. 1994.** Obsessive compulsive spectrum disorder. *J.Clin.Psychiatry* **55 Suppl:** 33-51.

**McElroy SL, Phillips KA, Keck PE, Jr. 1994.** Obsessive compulsive spectrum disorder. *J.Clin.Psychiatry* **55 Suppl:** 33-51.

**McEwen BS, Alves SE, Bulloch K, Weiland NG**. **1997.** Ovarian steroids and the brain: implications for cognition and aging. *Neurology* **48:** S8-15.

**McGrath MJ, Campbell KM, Parks CR, Burton FH**. **2000.** Glutamatergic drugs exacerbate symptomatic behavior in a transgenic model of comorbid Tourette's syndrome and obsessive-compulsive disorder. *Brain Res.* **877:** 23-30.

**McGuffin P, Mawson D**. **1980.** Obsessive-compulsive neurosis: two identical twin pairs. *Br.J.Psychiatry* **137:** 285-287.

**McKay D, Abramowitz JS, Calamari JE, Kyrios M, Radomsky A, Sookman D, Taylor S, Wilhelm S**. **2004.** A critical evaluation of obsessive-compulsive disorder subtypes: symptoms versus mechanisms. *Clin.Psychol.Rev.* **24:** 283-313.

**McKenzie CA, Julier C, Forrester T, McFarlane-Anderson N, Keavney B, Lathrop GM, Ratcliffe PJ, Farrall M**. **1995.** Segregation and linkage analysis of serum angiotensin I-converting enzyme levels: evidence for two quantitative-trait loci. *Am.J.Hum.Genet.* **57:** 1426-1435.

**McKeon J, McGuffin P, Robinson P**. **1984.** Obsessive-compulsive neurosis following head injury. A report of four cases. *Br.J.Psychiatry* **144:** 190-192.

**McKeon P, Murray R**. **1987.** Familial aspects of obsessive-compulsive neurosis. *Br.J.Psychiatry* **151:** 528-534.

**Meador-Woodruff JH, Grandy DK, Van Tol HH, Damask SP, Little KY, Civelli O, Watson SJ, Jr. 1994.** Dopamine receptor gene expression in the human medial temporal lobe. *Neuropsychopharmacology* **10:** 239-248.

**Meador-Woodruff JH, Damask SP, Wang J, Haroutunian V, Davis KL, Watson SJ**. **1996.** Dopamine receptor mRNA expression in human striatum and neocortex. *Neuropsychopharmacology* **15:** 17-29.

**Meira-Lima I, Shavitt RG, Miguita K, Ikenaga E, Miguel EC, Vallada H**. **2004.** Association analysis of the catechol-o-methyltransferase (COMT), serotonin transporter (5-HTT) and serotonin 2A receptor (5HT2A) gene polymorphisms with obsessive-compulsive disorder. *Genes Brain Behav.* **3:** 75-79.

**Mendelsohn FA, Jenkins TA, Berkovic SF**. **1993.** Effects of angiotensin II on dopamine and serotonin turnover in the striatum of conscious rats. *Brain Res.* **613:** 221-229.

**Messina D, Annesi G, Serra P, Nicoletti G, Pasqua A, Annesi F, Tomaino C, Ciro-Candiano IC, Carrideo S, Caracciolo M, Spadafora P, Zappia M, Savettieri G, Quattrone A**. **2002.** Association of the 5-HT6 receptor gene polymorphism C267T with Parkinson's disease. *Neurology* **58:** 828-829.

**Michalatos-Beloin S, Tishkoff SA, Bentley KL, Kidd KK, Ruano G**. **1996.** Molecular haplotyping of genetic markers 10 kb apart by allele-specific long-range PCR. *Nucleic Acids Res.* **24:** 4841-4843.

**Michell RH**. **1997.** The multiplying roles of inositol lipids and phosphates in cell control processes. *Essays Biochem.* **32:** 31-47.

**Middleton FA, Pato MT, Gentile KL, Morley CP, Zhao X, Eisener AF, Brown A, Petryshen TL, Kirby AN, Medeiros H, Carvalho C, Macedo A, Dourado A, Coelho I, Valente J, Soares MJ, Ferreira CP, Lei M, Azevedo MH, Kennedy JL, Daly MJ, Sklar P, Pato CN**. **2004.** Genomewide linkage analysis of bipolar disorder by use of a high-density single-nucleotide-polymorphism (SNP) genotyping assay: a comparison with microsatellite marker assays and finding of significant linkage to chromosome 6q22. *Am.J.Hum.Genet.* **74:** 886-897.

**Miguel EC, Baer L, Coffey BJ, Rauch SL, Savage CR, O'Sullivan RL, Phillips K, Moretti C, Leckman JF, Jenike MA**. **1997.** Phenomenological differences appearing with repetitive behaviours in obsessive-compulsive disorder and Gilles de la Tourette's syndrome. *Br.J.Psychiatry* **170:** 140-145.

**Miguel EC, Leckman JF, Rauch S, do Rosario-Campos MC, Hounie AG, Mercadante MT, Chacon P, Pauls DL. 2005.** Obsessive-compulsive disorder phenotypes: implications for genetic studies. *Mol. Psychiatry* **10**:258-75

**Milatovich, A., C. L. Hsieh, G. Bonaminio, L. Tecott, D. Julius, and U. Francke**. **1992**. Serotonin receptor 1c gene assigned to X chromosome in human (band q24) and mouse (bands D-F4). *Hum.Mol.Genet.* **1**:681-684.

**Millet B, Chabane N, Delorme R, Leboyer M, Leroy S, Poirier MF, Bourdel MC, Mouren-Simeoni MC, Rouillon F, Loo H, Krebs MO**. **2003.** Association between the dopamine receptor D4 (DRD4) gene and obsessive-compulsive disorder. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **116:** 55-59.

**Millet B, Kochman F, Gallarda T, Krebs MO, Demonfaucon F, Barrot I, Bourdel MC, Olie JP, Loo H, Hantouche EG**. **2004.** Phenomenological and comorbid features associated in obsessive-compulsive disorder: influence of age of onset. *J.Affect.Disord.* **79:** 241-246.

**Minichiello WE, Baer L, Jenike MA, Holland A. 1990.** Age of onset of major subtypes of obsessive-compulsive disorder .*J Anxiety Disorders*. **4**: 147-150.

**Minichiello L, Calella AM, Medina DL, Bonhoeffer T, Klein R, Korte M**. **2002.** Mechanism of TrkB-mediated hippocampal long-term potentiation. *Neuron* **36:** 121-137.

**Miranda RC, Sohrabji F, Toran-Allerand D**. **1994.** Interactions of estrogen with the neurotrophins and their receptors during neural development. *Horm.Behav.* **28:** 367-375.

**Missale C, Nash SR, Robinson SW, Jaber M, Caron MG**. **1998.** Dopamine receptors: from structure to function. *Physiol Rev.* **78:** 189-225.

**Modell JG, Mountz JM, Curtis GC, Greden JF**. **1989.** Neurophysiologic dysfunction in basal ganglia/limbic striatal and thalamocortical circuits as a pathogenetic mechanism of obsessive-compulsive disorder. *J.Neuropsychiatry Clin.Neurosci.* **1:** 27-36.

**Moffatt MF, Traherne JA, Abecasis GR, Cookson WO**. **2000.** Single nucleotide polymorphism and linkage disequilibrium within the TCR alpha/delta locus. *Hum.Mol.Genet.* **9:** 1011-1019.

**Mogenson GJ, Wu M**. **1988.** Disruption of food hoarding by injections of procaine into mediodorsal thalamus, GABA into subpallidal region and haloperidol into accumbens. *Brain Res.Bull.* **20:** 247-251.

**Moll GH, Eysenbach K, Woerner W, Banaschewski T, Schmidt MH, Rothenberger A**. **2000.** Quantitative and qualitative aspects of obsessive-compulsive behaviour in children with attention-deficit hyperactivity disorder compared with tic disorder. *Acta Psychiatr.Scand.* **101:** 389-394.

**Molloy AG, Waddington JL**. **1987.** Assessment of grooming and other behavioural responses to the D-1 dopamine receptor agonist SK & F 38393 and its R- and S-enantiomers in the intact adult rat. *Psychopharmacology (Berl)* **92:** 164-168.

**Montgomery, S. A. and N. Fineberg**. **1989**. Is there a relationship between serotonin receptor subtypes and selectivity of response in specific psychiatric illnesses? *Br.J.Psychiatry Suppl* 63-69.

**Monsma FJ, Jr., Shen Y, Ward RP, Hamblin MW, Sibley DR**. **1993.** Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol.Pharmacol.* **43:** 320-327.

**Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH**. **1992.** Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* **256:** 1217-1221.

**Moolman-Smook JC, De Lange WJ, Bruwer EC, Brink PA, Corfield VA**. **1999.** The origins of hypertrophic cardiomyopathy-causing mutations in two South African subpopulations: a unique profile of both independent and founder events. *Am.J.Hum.Genet.* **65:** 1308-1320.

**Moon IS, Apperson ML, Kennedy MB**. **1994.** The major tyrosine-phosphorylated protein in the postsynaptic density fraction is N-methyl-D-aspartate receptor subunit 2B. *Proc.Natl.Acad.Sci.U.S.A* **91:** 3954-3958.

**Moore GJ, MacMaster FP, Stewart C, Rosenberg DR**. **1998.** Case study: caudate glutamatergic changes with paroxetine therapy for pediatric obsessive-compulsive disorder. *J.Am.Acad.Child Adolesc.Psychiatry* **37:** 663-667.

**Moreno FA, Delgado PL**. **1997.** Hallucinogen-induced relief of obsessions and compulsions. *Am.J.Psychiatry* **154:** 1037-1038.

**Moret C, Briley M**. **2000.** The possible role of 5-HT(1B/D) receptors in psychiatric disorders and their potential as a target for therapy. *Eur.J.Pharmacol.* **404:** 1-12.

**Morilak DA, Somogyi P, Lujan-Miras R, Ciaranello RD. 1994**. Neurons expressing 5-HT2 receptors in the rat brain: neurochemical identification of cell types by immunocytochemistry. *Neuropsychopharmacology*. **11(3):** 157-166

**Morton NE, Collins A**. **1998.** Tests and estimates of allelic association in complex inheritance. *Proc.Natl.Acad.Sci.U.S.A* **95:** 11389-11393.

**Mossner R, Daniel S, Albert D, Heils A, Okladnova O, Schmitt A, Lesch KP**. **2000.** Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF). *Neurochem.Int.* **36:** 197-202.

**Mowla SJ, Farhadi HF, Pareek S, Atwal JK, Morris SJ, Seidah NG, Murphy RA**. **2001.** Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J.Biol.Chem.* **276:** 12660-12666.

**Munafo MR, Flint J**. **2004.** Meta-analysis of genetic association studies. *Trends Genet.* **20:** 439-444.

**Mundo E, Richter MA, Sam F, Macciardi F, Kennedy JL**. **2000.** Is the 5-HT(1Dbeta) receptor gene implicated in the pathogenesis of obsessive-compulsive disorder? *Am.J.Psychiatry* **157:** 1160-1161.

**Mundo E, Richter MA, Zai G, Sam F, McBride J, Macciardi F, Kennedy JL**. **2002.** 5HT1Dbeta Receptor gene implicated in the pathogenesis of Obsessive-Compulsive Disorder: further evidence from a family-based association study. *Mol.Psychiatry* **7:** 805-809.

**Murer MG, Yan Q, Raisman-Vozari R**. **2001.** Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog.Neurobiol.* **63:** 71-124.

**Murialdo G, Galimberti CA, Fonzi S, Manni R, Costelli P, Parodi C, Torre F, Solinas GP, Polleri A, Tartara A**. **1994.** Sex hormones, gonadotropins and prolactin in male epileptic subjects in remission: role of the epileptic syndrome and of antiepileptic drugs. *Neuropsychobiology* **30:** 29-36.

**Murray AM, Hyde TM, Knable MB, Herman MM, Bigelow LB, Carter JM, Weinberger DR, Kleinman JE**. **1995.** Distribution of putative D4 dopamine receptors in postmortem striatum from patients with schizophrenia. *J.Neurosci.* **15:** 2186-2191.

**Murray CJ, Lopez AD**. **1997.** Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* **349:** 1498-1504.

**Murray GD**. **1991.** Statistical aspects of research methodology. *Br.J.Surg.* **78:** 777-781.

**Nabi R, Zhong H, Serajee FJ, Huq AH**. **2003.** No association between single nucleotide polymorphisms in DLX6 and Piccolo genes at 7q21-q22 and autism. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **119:** 98-101.

**Nakanishi S, Nakajima Y, Masu M, Ueda Y, Nakahara K, Watanabe D, Yamaguchi S, Kawabata S, Okada M**. **1998.** Glutamate receptors: brain function and signal transduction. *Brain Res.Brain Res.Rev.* **26:** 230-235.

**Nakatome M, Honda K, Islam MN, Terada M, Yamazaki M, Kuroki H, Ogura Y, Bai H, Wakasugi C**. **1995.** Amplification of DAT1 (human dopamine transporter gene) 3' variable region in the Japanese population. *Hum.Hered.* **45:** 262-265.

**Nakatome M, Honda K, Tun Z, Kato Y, Harihara S, Omoto K, Misawa S, Gerelsaikhan T, Nyamkhishig S, Dashnyam B, Batsuuri J, Wakasugi C**. **1996.** Genetic polymorphism of the 3' VNTR region of the human dopaminergic function gene DAT1 (human dopamine transporter gene) in the Mongolian population. *Hum.Biol.* **68:** 509-515.

**Neale BM, Sham PC**. **2004.** The future of association studies: gene-based analysis and replication. *Am.J.Hum.Genet.* **75:** 353-362.

**Nee LE, Caine ED, Polinsky RJ, Eldridge R, Ebert MH**. **1980.** Gilles de la Tourette syndrome: clinical and family study of 50 cases. *Ann.Neurol.* **7:** 41-49.

**Nelson E, Rice J**. **1997.** Stability of diagnosis of obsessive-compulsive disorder in the Epidemiologic Catchment Area study. *Am.J.Psychiatry* **154:** 826-831.

**Nestadt G, Samuels J, Riddle M, Bienvenu OJ, III, Liang KY, LaBuda M, Walkup J, Grados M, Hoehn-Saric R**. **2000(a).** A family study of obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **57:** 358-363.

**Nestadt G, Lan T, Samuels J, Riddle M, Bienvenu OJ, III, Liang KY, Hoehn-Saric R, Cullen B, Grados M, Beaty TH, Shugart YY**. **2000(b).** Complex segregation analysis provides compelling evidence for a major gene underlying obsessive-compulsive disorder and for heterogeneity by sex. *Am.J.Hum.Genet.* **67:** 1611-1616.

**Nestadt G, Addington A, Samuels J, Liang KY, Bienvenu OJ, Riddle M, Grados M, Hoehn-Saric R, Cullen B**. **2003.** The identification of OCD-related subgroups based on comorbidity. *Biol.Psychiatry* **53:** 914-920.

**Neville MJ, Johnstone EC, Walton RT**. **2004.** Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum.Mutat.* **23:** 540-545.

**Neyman J., Pearson E.1933**. On the problem of the most efficient tests of statistical hypotheses. *Philos Trans Roy Soc*. **A231**: 289-337.

**Nibuya M, Morinobu S, Duman RS**. **1995.** Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J.Neurosci.* **15:** 7539-7547.

**Nicolini H, Cruz C, Camarena B, Orozco B, Kennedy JL, King N, Weissbecker K, de lF, Jr., Sidenberg D**. **1996.** DRD2, DRD3 and 5HT2A receptor genes polymorphisms in obsessive-compulsive disorder. *Mol.Psychiatry* **1:** 461-465.

**Nicolini H, Cruz C, Paez F, Camarena B**. **1998.** [Dopamine D2 and D4 receptor genes distinguish the clinical presence of tics in obsessive-compulsive disorder]. *Gac.Med.Mex.* **134:** 521-527.

**Nicolini H, Hanna G, Baxter L, Schwartz J, Weissbecker K, Spence M, 1991.** Segregation analyses of obsessive-compulsive and associatied disorders: preliminary results *Ursus Medicus* **1:** 25-28**.**

**Niehaus DJ, Kinnear CJ, Corfield VA, du Toit PL, van Kradenburg J, Moolman-Smook JC, Weyers JB, Potgieter A, Seedat S, Emsley RA, Knowles JA, Brink PA, Stein DJ**. **2001.** Association between a catechol-o-methyltransferase polymorphism and obsessive-compulsive disorder in the Afrikaner population. *J.Affect.Disord.* **65:** 61-65.

**Niu T, Qin ZS, Xu X, Liu JS**. **2002.** Bayesian haplotype inference for multiple linked single-nucleotide polymorphisms. *Am.J.Hum.Genet.* **70:** 157-169.

**Nordborg M, Tavare S**. **2002.** Linkage disequilibrium: what history has to tell us. *Trends Genet.* **18:** 83-90.

**Nordstrom EJ, Burton FH**. **2002.** A transgenic model of comorbid Tourette's syndrome and obsessive-compulsive disorder circuitry. *Mol.Psychiatry* **7:** 617-25, 524.

**Noshirvani HF, Kasvikis Y, Marks IM, Tsakiris F, Monteiro WO**. **1991.** Gender-divergent aetiological factors in obsessive-compulsive disorder. *Br.J.Psychiatry* **158:** 260-263.

**Nothen MM, Propping P, Fimmers R**. **1993.** Association versus linkage studies in psychosis genetics. *J.Med.Genet.* **30:** 634-637.

**Novelli E, Nobile M, Diaferia G, Sciuto G, Catalano M**. **1994.** A molecular investigation suggests no relationship between obsessive-compulsive disorder and the dopamine D2 receptor. *Neuropsychobiology* **29:** 61-63.

**Novick GE, Novick CC, Yunis J, Yunis E, Antunez de Mayolo P, Scheer WD, Deininger PL, Stoneking M, York DS, Batzer MA, Herrera RJ. 1998.** Polymorphic Alu insertions and the Asian origin of Native American populations. *Hum Biol*. **70**: 23-39.

**Nutt DJ, Forshall S, Bell C, Rich A, Sandford J, Nash J, Argyropoulos S**. **1999.** Mechanisms of action of selective serotonin reuptake inhibitors in the treatment of psychiatric disorders. *Eur.Neuropsychopharmacol.* **9 Suppl 3:** S81-S86.

**Nyholt DR**. **2001.** Genetic case-control association studies--correcting for multiple testing. *Hum.Genet.* **109:** 564-567.

**O'Malley KL, Harmon S, Tang L, Todd RD**. **1992.** The rat dopamine D4 receptor: sequence, gene structure, and demonstration of expression in the cardiovascular system. *New Biol.* **4:** 137-146.

**O'Sullivan RL, Rauch SL, Breiter HC, Grachev ID, Baer L, Kennedy DN, Keuthen NJ, Savage CR, Manzo PA, Caviness VS, Jenike MA**. **1997.** Reduced basal ganglia volumes in trichotillomania measured via morphometric magnetic resonance imaging. *Biol.Psychiatry* **42:** 39-45.

**Oak JN, Oldenhof J, Van Tol HH**. **2000.** The dopamine D(4) receptor: one decade of research. *Eur.J.Pharmacol.* **405:** 303-327.

**Ohara K, Xu HD, Mori N, Suzuki Y, Xu DS, Ohara K, Wang ZC**. **1997.** Anticipation and imprinting in schizophrenia. *Biol.Psychiatry* **42:** 760-766.

**Ohara K, Nagai M, Suzuki Y, Ochiai M, Ohara K**. **1998.** No association between anxiety disorders and catechol-O-methyltransferase polymorphism. *Psychiatry Res.* **80:** 145-148.

**Ohtsuki T, Sakurai K, Dou H, Toru M, Yamakawa-Kobayashi K, Arinami T**. **2001.** Mutation analysis of the NMDAR2B (GRIN2B) gene in schizophrenia. *Mol.Psychiatry* **6:** 211-216.

**Okada M, Northup JK, Ozaki N, Russell JT, Linnoila M, Goldman D**. **2004.** Modification of human 5-HT(2C) receptor function by Cys23Ser, an abundant, naturally occurring amino-acid substitution. *Mol.Psychiatry* **9:** 55-64.

**Okuyama Y, Ishiguro H, Toru M, Arinami T**. **1999.** A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia. *Biochem.Biophys.Res.Commun.* **258:** 292-295.

**Omkumar RV, Kiely MJ, Rosenstein AJ, Min KT, Kennedy MB**. **1996.** Identification of a phosphorylation site for calcium/calmodulindependent protein kinase II in the NR2B subunit of the N-methyl-D-aspartate receptor. *J.Biol.Chem.* **271:** 31670-31678.

**Otano A, Frechilla D, Cobreros A, Cruz-Orive LM, Insausti A, Insausti R, Hamon M, Del Rio J**. **1999.** Anxiogenic-like effects and reduced stereological counting of immunolabelled 5-hydroxytryptamine6 receptors in rat nucleus accumbens by antisense oligonucleotides. *Neuroscience* **92:** 1001-1009.

**Jurg Ott: 1999.** Analysis of Human Linkage**.** 3rd edition. Johns Hopkins University Press, Baltimore MD

**Ott 1999**. Analysis of human genetic linkage, 3rd ed. Johns Hopkins University Press.

**Otto M**. **1990**. Neuropsychology of Obsessive-Compulsive Disorders: **In:** Jenike MA, Baer L, Minichiello WE, (eds). Obsessive-compulsive disorder. Theory and Management (2nd ed) Yearbook Medical Publishing. Chicago. pp 132-148

**Owen MJ, Holmans P, McGuffin P**. **1997.** Association studies in psychiatric genetics. *Mol.Psychiatry* **2:** 270-273.

**Owen MJ, Cardno AG**. **1999.** Psychiatric genetics: progress, problems, and potential. *Lancet* **354 Suppl 1:** SI11-SI14.

**Owen MJ, Cardno AG, O'Donovan MC**. **2000.** Psychiatric genetics: back to the future. *Mol.Psychiatry* **5:** 22-31.

**Pani L, Porcella A, Gessa GL**. **2000.** The role of stress in the pathophysiology of the dopaminergic system. *Mol.Psychiatry* **5:** 14-21.

**Papolos DF, Faedda GL, Veit S, Goldberg R, Morrow B, Kucherlapati R, Shprintzen RJ**. **1996.** Bipolar spectrum disorders in patients diagnosed with velo-cardio-facial syndrome: does a hemizygous deletion of chromosome 22q11 result in bipolar affective disorder? *Am.J.Psychiatry* **153:** 1541-1547.

**Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, Malek TB, Johnson GS, DeFrance HB, Ostrander EA, Kruglyak L**. **2004.** Genetic structure of the purebred domestic dog. *Science* **304:** 1160-1164.

**Parsons MJ, D'Souza UM, Arranz MJ, Kerwin RW, Makoff AJ**. **2004.** The -1438A/G polymorphism in the 5-hydroxytryptamine type 2A receptor gene affects promoter activity. *Biol.Psychiatry* **56:** 406-410.

**Paschou P, Feng Y, Pakstis AJ, Speed WC, DeMille MM, Kidd JR, Jaghori B, Kurlan R, Pauls DL, Sandor P, Barr CL, Kidd KK**. **2004.** Indications of linkage and association of Gilles de la Tourette syndrome in two independent family samples: 17q25 is a putative susceptibility region. *Am.J.Hum.Genet.* **75:** 545-560.

**Patapoutian A, Reichardt LF**. **2001.** Trk receptors: mediators of neurotrophin action. *Curr.Opin.Neurobiol.* **11:** 272-280.

**Pathak S, Cottingham EM, McConville BJ**. **2003.** The use of sumatriptan in the treatment of obsessive-compulsive disorder in an adolescent. *J.Child Adolesc.Psychopharmacol.* **13 Suppl 1:** S93-S94.

**Patil N, Berno AJ, Hinds DA, Barrett WA, Doshi JM, Hacker CR, Kautzer CR, Lee DH, Marjoribanks C, McDonough DP, Nguyen BT, Norris MC, Sheehan JB, Shen N, Stern D, Stokowski RP, Thomas DJ, Trulson MO, Vyas KR, Frazer KA, Fodor SP, Cox DR**. **2001.** Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21. *Science* **294:** 1719-1723.

**Patil VJ**. **1992.** Development of transient obsessive-compulsive symptoms during treatment with clozapine. *Am.J.Psychiatry* **149:** 272.

**Pauls DL, Towbin KE, Leckman JF, Zahner GE, Cohen DJ**. **1986.** Gilles de la Tourette's syndrome and obsessive-compulsive disorder. Evidence supporting a genetic relationship. *Arch.Gen.Psychiatry* **43:** 1180-1182.

**Pauls DL, Raymond CL, Stevenson JM, Leckman JF**. **1991.** A family study of Gilles de la Tourette syndrome. *Am.J.Hum.Genet.* **48:** 154-163.

**Pauls DL**. **1992.** The genetics of obsessive compulsive disorder and Gilles de la Tourette's syndrome. *Psychiatr.Clin.North Am.* **15:** 759-766.

**Pauls DL, Alsobrook JP, Goodman W, Rasmussen S, Leckman JF**. **1995.** A family study of obsessive-compulsive disorder. *Am.J.Psychiatry* **152:** 76-84.

**Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR** . **1996.** A simulation study of the number of events per variable in logistic regression analysis. *J.Clin.Epidemiol.* **49:** 1373-1379.

**Peltonen L**. **2000.** Positional cloning of disease genes: advantages of genetic isolates. *Hum.Hered.* **50:** 66-75.

**Perneger TV**. **1998.** What's wrong with Bonferroni adjustments. *BMJ* **316:** 1236-1238.

**Persico AM, Wang ZW, Black DW, Andreasen NC, Uhl GR, Crowe RR**. **1995.** Exclusion of close linkage of the dopamine transporter gene with schizophrenia spectrum disorders. *Am.J.Psychiatry* **152:** 134-136.

**Perugi G, Akiskal HS, Pfanner C, Presta S, Gemignani A, Milanfranchi A, Lensi P, Ravagli S, Cassano GB**. **1997.** The clinical impact of bipolar and unipolar affective comorbidity on obsessive-compulsive disorder. *J.Affect.Disord.* **46:** 15-23.

**Petek E, Windpassinger C, Vincent JB, Cheung J, Boright AP, Scherer SW, Kroisel PM, Wagner K**. **2001.** Disruption of a novel gene (IMMP2L) by a breakpoint in 7q31 associated with Tourette syndrome. *Am.J.Hum.Genet.* **68:** 848-858.

**Petralia RS, Wenthold RJ**. **1992.** Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. *J.Comp Neurol.* **318:** 329-354.

**Petronis A, Van Tol HH, Lichter JB, Livak KJ, Kennedy JL** . **1993.** The D4 dopamine receptor gene maps on 11p proximal to HRAS. *Genomics* **18:** 161-163.

**Petronis A**. **2001.** Human morbid genetics revisited: relevance of epigenetics. *Trends Genet.* **17:** 142-146.

**Pfeiffer RM, Gail MH**. **2003.** Sample size calculations for population- and family-based case-control association studies on marker genotypes. *Genet.Epidemiol.* **25:** 136-148.

**Phillips ML, Marks IM, Senior C, Lythgoe D, O'Dwyer AM, Meehan O, Williams SC, Brammer MJ, Bullmore ET, McGuire PK**. **2000.** A differential neural response in obsessive-compulsive disorder patients with washing compared with checking symptoms to disgust. *Psychol.Med.* **30:** 1037-1050.

**Pian, K. L., H. G. Westenberg, H. J. van Megen, and J. A. den Boer**. **1998**. Sumatriptan (5-HT1D receptor agonist) does not exacerbate symptoms in obsessive compulsive disorder. *Psychopharmacology (Berl)* **140**:365-370.

**Piccinelli M, Pini S, Bellantuono C, Wilkinson G**. **1995.** Efficacy of drug treatment in obsessive-compulsive disorder. A meta-analytic review. *Br.J.Psychiatry* **166:** 424-443.

**Pitman RK, Green RC, Jenike MA, Mesulam MM**. **1987.** Clinical comparison of Tourette's disorder and obsessive-compulsive disorder. *Am.J.Psychiatry* **144:** 1166-1171.

**Pogarell O, Hamann C, Popperl G, Juckel G, Chouker M, Zaudig M, Riedel M, Moller HJ, Hegerl U, Tatsch K**. **2003.** Elevated brain serotonin transporter availability in patients with obsessive-compulsive disorder. *Biol.Psychiatry* **54:** 1406-1413.

**Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J**. **1998.** The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Mol.Psychiatry* **3:** 256-260.

**Polesskaya OO, Sokolov BP**. **2002.** Differential expression of the "C" and "T" alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. *J.Neurosci.Res.* **67:** 812-822.

**Popoli M, Gennarelli M, Racagni G**. **2002.** Modulation of synaptic plasticity by stress and antidepressants. *Bipolar.Disord.* **4:** 166-182.

**Potkin SG, Basile VS, Jin Y, Masellis M, Badri F, Keator D, Wu JC, Alva G, Carreon DT, Bunney WE, Jr., Fallon JH, Kennedy JL**. **2003.** D1 receptor alleles predict PET metabolic correlates of clinical response to clozapine. *Mol.Psychiatry* **8:** 109-113.

**Pouzet B, Didriksen M, Arnt J**. **2002.** Effects of the 5-HT(6) receptor antagonist, SB-271046, in animal models for schizophrenia. *Pharmacol.Biochem.Behav.* **71:** 635-643.

**Poyurovsky M, Hermesh H, Weizman A**. **1996.** Fluvoxamine treatment in clozapine-induced obsessive-compulsive symptoms in schizophrenic patients. *Clin.Neuropharmacol.* **19:** 305-313.

**Pozzo-Miller LD, Gottschalk W, Zhang L, McDermott K, Du J, Gopalakrishnan R, Oho C, Sheng ZH, Lu B**. **1999.** Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. *J.Neurosci.* **19:** 4972-4983.

**Prichard Z, Jorm AF, Prior M, Sanson A, Smart D, Zhang Y, Huttley G, Easteal S**. **2002.** Association of polymorphisms of the estrogen receptor gene with anxiety-related traits in children and adolescents: a longitudinal study. *Am.J.Med.Genet.* **114:** 169-176.

**Prisco S, Esposito E**. **1995.** Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopaminergic neurones in the ventral tegmental area. *Br.J.Pharmacol.* **116:** 1923-1931.

**Prichard, Z., A. F. Jorm, M. Prior, A. Sanson, D. Smart, Y. Zhang, G. Huttley, and S. Easteal**. **2002**. Association of polymorphisms of the estrogen receptor gene with anxiety-related traits in children and adolescents: a longitudinal study. *Am.J.Med.Genet*. **114**:169-176.

**Pritchard JK, Rosenberg NA**. **1999.** Use of unlinked genetic markers to detect population stratification in association studies. *Am.J.Hum.Genet.* **65:** 220-228.

**Pritchard JK, Stephens M, Donnelly P**. **2000.** Inference of population structure using multilocus genotype data. *Genetics* **155:** 945-959.

**Pritchard JK**. **2001.** Are rare variants responsible for susceptibility to complex diseases? *Am.J.Hum.Genet.* **69:** 124-137.

**Pritchard JK, Przeworski M**. **2001.** Linkage disequilibrium in humans: models and data. *Am.J.Hum.Genet.* **69:** 1-14.

**Pritchard JK, Cox NJ**. **2002.** The allelic architecture of human disease genes: common disease-common variant...or not? *Hum.Mol.Genet.* **11:** 2417-2423.

**Pronk JC, Gibson RA, Savoia A, Wijker M, Morgan NV, Melchionda S, Ford D, Temtamy S, Ortega JJ, Jansen S, . 1995.** Localisation of the Fanconi anaemia complementation group A gene to chromosome 16q24.3. *Nat.Genet.* **11:** 338-340.

**Propping P, Nothen MM, Fimmers R et al. 1993.** Linkage versus association studies in complex diseases. *Psychiatr Genet.* **3**: 136

**Puffenberger EG, Kauffman ER, Bolk S, Matise TC, Washington SS, Angrist M, Weissenbach J, Garver KL, Mascari M, Ladda R, . 1994.** Identity-by-descent and association mapping of a recessive gene for Hirschsprung disease on human chromosome 13q22. *Hum.Mol.Genet.* **3:** 1217-1225.

**Pujol J, Soriano-Mas C, Alonso P, Cardoner N, Menchon JM, Deus J, Vallejo J**. **2004.** Mapping structural brain alterations in obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **61:** 720-730.

**Pulver AE, Nestadt G, Goldberg R, Shprintzen RJ, Lamacz M, Wolyniec PS, Morrow B, Karayiorgou M, Antonarakis SE, Housman D, . 1994.** Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. *J.Nerv.Ment.Dis.* **182:** 476-478.

**Purcell S, Sham P**. **2004.** Properties of structured association approaches to detecting population stratification. *Hum.Hered.* **58:** 93-107.

**Qin ZS, Niu T, Liu JS**. **2002.** Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms. *Am.J.Hum.Genet.* **71:** 1242-1247.

**Quartara L, Maggi CA**. **1998.** The tachykinin NK1 receptor. Part II: Distribution and pathophysiological roles. *Neuropeptides* **32:** 1-49.

**Rannala B**. **2001.** Finding genes influencing susceptibility to complex diseases in the post-genome era. *Am.J.Pharmacogenomics.* **1:** 203-221.

**Rapoport JL, Wise SP**. **1988.** Obsessive-compulsive disorder: evidence for basal ganglia dysfunction. *Psychopharmacol.Bull.* **24:** 380-384.

**Rapoport JL**. **1991.** Recent advances in obsessive-compulsive disorder. *Neuropsychopharmacology* **5:** 1-10.

**Rapoport JL, Inoff-Germain G**. **2000.** Treatment of obsessive-compulsive disorder in children and adolescents. *J.Child Psychol.Psychiatry* **41:** 419-431.

**Rasmussen SA, Tsuang MT**. **1986.** Clinical characteristics and family history in DSM-III obsessive-compulsive disorder. *Am.J.Psychiatry* **143:** 317-322.

**Rasmussen SA, Eisen JL**. **1988.** Clinical and epidemiologic findings of significance to neuropharmacologic trials in OCD. *Psychopharmacol.Bull.* **24:** 466-470.

**Rasmussen SA, Eisen JL**. **1990.** Epidemiology of obsessive compulsive disorder. *J.Clin.Psychiatry* **51 Suppl:** 10-13.

**Rasmussen SA, Eisen JL**. **1992.** The epidemiology and clinical features of obsessive compulsive disorder. *Psychiatr.Clin.North Am.* **15:** 743-758.

**Rasmussen SA, Eisen JL**. **1994.** The epidemiology and differential diagnosis of obsessive compulsive disorder. *J.Clin.Psychiatry* **55 Suppl:** 5-10.

**Rasmussen S, Eisen JL, 1998**. The epidemiology and clinical features of obsessive-compulse disorders. **In**: Jenike M, Baer I, Minichiello W.(eds).Obsessive-compulsive disorder: practical management. St Louis Mosby; pp12-43.

**Rauch S, Baxter L**. 1998. Neuroimaging of OCD and related disorders. **In**: M Jenike, l Baer, W Minichiello (eds). Obsessive-Compulsive Disorders: Practical Management. M Jenike, l Baer, W Minichiello (eds). Boston: Mosby pp289-317.

**Rauch SL, Dougherty DD, Shin, LM, Alpert NM, Manzo, P, Leahy, L, Fischman, AJ, Jenike, MA, Baer, L 1998**. Neural correlates of factor-analyzed OCD symptom dimensions: a PET study. *CNS.Spectr*.3: 37-43.

**Rauch SL, Jenike MA, Alpert NM, Baer L, Breiter HC, Savage CR, Fischman AJ**. **1994.** Regional cerebral blood flow measured during symptom provocation in obsessive-compulsive disorder using oxygen 15-labeled carbon dioxide and positron emission tomography. *Arch.Gen.Psychiatry* **51:** 62-70.

**Rauch SL, Savage CR, Alpert NM, Dougherty D, Kendrick A, Curran T, Brown HD, Manzo P, Fischman AJ, Jenike MA**. **1997.** Probing striatal function in obsessive-compulsive disorder: a PET study of implicit sequence learning. *J.Neuropsychiatry Clin.Neurosci.* **9:** 568-573.

**Rauch SL**. **2003.** Neuroimaging and neurocircuitry models pertaining to the neurosurgical treatment of psychiatric disorders. *Neurosurg.Clin.N.Am.* **14:** 213-viii.

**Ravindran AV**. **1999.** Obsessive-compulsive spectrum disorders. *J.Psychiatry Neurosci.* **24:** 10-12.

**Ravizza L, Barzega G, Bellino S, Bogetto F, Maina G**. **1995.** Predictors of drug treatment response in obsessive-compulsive disorder. *J.Clin.Psychiatry* **56:** 368-373.

**Reardon KA, Mendelsohn FA, Chai SY, Horne MK**. **2000.** The angiotensin converting enzyme (ACE) inhibitor, perindopril, modifies the clinical features of Parkinson's disease. *Aust.N.Z.J.Med.* **30:** 48-53.

**Redden DT, Allison DB**. **2003.** Nonreplication in genetic association studies of obesity and diabetes research. *J.Nutr.* **133:** 3323-3326.

**Reich DE, Lander ES**. **2001.** On the allelic spectrum of human disease. *Trends Genet.* **17:** 502-510.

**Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES**. **2001.** Linkage disequilibrium in the human genome. *Nature* **411:** 199-204.

**Rhee SG, Suh PG, Ryu SH, Lee SY**. **1989.** Studies of inositol phospholipid-specific phospholipase C. *Science* **244:** 546-550.

**Rhee SG**. **2001.** Regulation of phosphoinositide-specific phospholipase C. *Annu.Rev.Biochem.* **70:** 281-312.

**Ribases M, Gratacos M, Armengol L, de Cid R, Badia A, Jimenez L, Solano R, Vallejo J, Fernandez F, Estivill X**. **2003.** Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol.Psychiatry* **8:** 745-751.

**Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, Cavallini MC, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Wagner G, Treasure J, Collier DA, Estivill X**. **2004.** Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations. *Hum.Mol.Genet.* **13:** 1205-1212.

**Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, Cristina CM, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Tomori M, Wagner G, Treasure J, Collier DA, Estivill X**. **2005.** Association of BDNF with restricting anorexia nervosa and minimum body mass index: a family-based association study of eight European populations. *Eur.J.Hum.Genet.* **13:** 428-434.

**Riddle MA, Scahill L, King R, Hardin MT, Towbin KE, Ort SI, Leckman JF, Cohen DJ**. **1990.** Obsessive compulsive disorder in children and adolescents: phenomenology and family history. *J.Am.Acad.Child Adolesc.Psychiatry* **29:** 766-772.

**Rieder MJ, Taylor SL, Clark AG, Nickerson DA**. **1999.** Sequence variation in the human angiotensin converting enzyme. *Nat.Genet.* **22:** 59-62.

**Rietschel M, Naber D, Fimmers R, Moller HJ, Propping P, Nothen MM**. **1997.** Efficacy and side-effects of clozapine not associated with variation in the 5-HT2C receptor. *Neuroreport* **8:** 1999-2003.

**Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F**. **1990.** An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J.Clin.Invest* **86:** 1343-1346.

**Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ**. **2001.** Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat.Genet.* **29:** 223-228.

**Risch N, Merikangas K**. **1996.** The future of genetic studies of complex human diseases. *Science* **273:** 1516-1517.

**Risch N, Botstein D**. **1996.** A manic depressive history. *Nat.Genet.* **12:** 351-353.

**Risch N**. **2000.** Searching for genes in complex diseases: lessons from systemic lupus erythematosus. *J.Clin.Invest* **105:** 1503-1506.

**Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, Moore JH**. **2001.** Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am.J.Hum.Genet.* **69:** 138-147.

**Ritchie MD, White BC, Parker JS, Hahn LW, Moore JH**. **2003.** Optimization of neural network architecture using genetic programming improves detection and modeling of gene-gene interactions in studies of human diseases. *BMC.Bioinformatics.* **4:** 28.

**Rivett AJ, Francis A, Roth JA**. **1983.** Distinct cellular localization of membrane-bound and soluble forms of catechol-O-methyltransferase in brain. *J.Neurochem.* **40:** 215-219.

**Roberts JC, Reavill C, East SZ, Harrison PJ, Patel S, Routledge C, Leslie RA**. **2002.** The distribution of 5-HT(6) receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT(6) receptor antagonist [(125)I]SB-258585. *Brain Res.* **934:** 49-57.

**Robertson MM, Trimble MR, Lees AJ**. **1988.** The psychopathology of the Gilles de la Tourette syndrome. A phenomenological analysis. *Br.J.Psychiatry* **152:** 383-390.

**Robins LN, Helzer JE, Weissman MM, Orvaschel H, Gruenberg E, Burke JD, Jr., Regier DA**. **1984.** Lifetime prevalence of specific psychiatric disorders in three sites. *Arch.Gen.Psychiatry* **41:** 949-958.

**Robinson D, Wu H, Munne RA, Ashtari M, Alvir JM, Lerner G, Koreen A, Cole K, Bogerts B**. **1995.** Reduced caudate nucleus volume in obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **52:** 393-398.

**Rosario-Campos MC, Leckman JF, Mercadante MT, Shavitt RG, Prado HS, Sada P, Zamignani D, Miguel EC**. **2001.** Adults with early-onset obsessive-compulsive disorder. *Am.J.Psychiatry* **158:** 1899-1903.

**Rosenberg DR, Keshavan MS, O'Hearn KM, Dick EL, Bagwell WW, Seymour AB, Montrose DM, Pierri JN, Birmaher B**. **1997.** Frontostriatal measurement in treatment-naive children with obsessive-compulsive disorder. *Arch.Gen.Psychiatry* **54:** 824-830.

**Rosenberg DR, Keshavan MS**. **1998.** A.E. Bennett Research Award. Toward a neurodevelopmental model of of obsessive--compulsive disorder. *Biol.Psychiatry* **43:** 623-640.

**Rosenberg DR, Hanna GL**. **2000.** Genetic and imaging strategies in obsessive-compulsive disorder: potential implications for treatment development. *Biol.Psychiatry* **48:** 1210-1222.

**Rosenberg DR, MacMaster FP, Keshavan MS, Fitzgerald KD, Stewart CM, Moore GJ**. **2000.** Decrease in caudate glutamatergic concentrations in pediatric obsessive-compulsive disorder patients taking paroxetine. *J.Am.Acad.Child Adolesc.Psychiatry* **39:** 1096-1103.

**Rosenberg DR, Mirza Y, Russell A, Tang J, Smith JM, Banerjee SP, Bhandari R, Rose M, Ivey J, Boyd C, Moore GJ**. **2004.** Reduced anterior cingulate glutamatergic concentrations in childhood OCD and major depression versus healthy controls. *J.Am.Acad.Child Adolesc.Psychiatry* **43:** 1146-1153.

**Rosenberg NA, Burke T, Elo K, Feldman MW, Freidlin PJ, Groenen MA, Hillel J, Maki-Tanila A, Tixier-Boichard M, Vignal A, Wimmers K, Weigend S**. **2001.** Empirical evaluation of genetic clustering methods using multilocus genotypes from 20 chicken breeds. *Genetics* **159:** 699-713.

**Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, Feldman MW**. **2002.** Genetic structure of human populations. *Science* **298:** 2381-2385.

**Rosendorff J, Bernstein R, Macdougall L, Jenkins T**. **1987.** Fanconi anemia: another disease of unusually high prevalence in the Afrikaans population of South Africa. *Am.J.Med.Genet.* **27:** 793-797.

**Rosenthal A, Goeddel DV, Nguyen T, Lewis M, Shih A, Laramee GR, Nikolics K, Winslow JW**. **1990.** Primary structure and biological activity of a novel human neurotrophic factor. *Neuron* **4:** 767-773.

**Roth BL, Craigo SC, Choudhary MS, Uluer A, Monsma FJ, Jr., Shen Y, Meltzer HY, Sibley DR**. **1994.** Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J.Pharmacol.Exp.Ther.* **268:** 1403-1410.

**Rothman KJ**. **1986.** Significance questing. *Ann.Intern.Med.* **105:** 445-447.

**Rothman KJ**. **1990.** No adjustments are needed for multiple comparisons. *Epidemiology* **1:** 43-46.

**Rowe DC, Stever C, Giedinghagen LN, Gard JM, Cleveland HH, Terris ST, Mohr JH, Sherman S, Abramowitz A, Waldman ID**. **1998.** Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Mol.Psychiatry* **3:** 419-426.

**Roy AM, Carroll ML, Nguyen SV, Salem AH, Oldridge M, Wilkie AO, Batzer MA, Deininger PL**. **2000.** Potential gene conversion and source genes for recently integrated Alu elements. *Genome Res.* **10:** 1485-1495.

**Ruano G, Kidd KK**. **1989.** Direct haplotyping of chromosomal segments from multiple heterozygotes via allele-specific PCR amplification. *Nucleic Acids Res.* **17:** 8392.

**Ruano G, Kidd KK, Stephens JC**. **1990.** Haplotype of multiple polymorphisms resolved by enzymatic amplification of single DNA molecules. *Proc.Natl.Acad.Sci.U.S.A* **87:** 6296-6300.

**Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R, Schwartz JC**. **1993.** A novel rat serotonin (5-HT6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem.Biophys.Res.Commun.* **193:** 268-276.

**Rubenstein CS, Pigott TA, L'Heureux F, Hill JL, Murphy DL**. **1992.** A preliminary investigation of the lifetime prevalence of anorexia and bulimia nervosa in patients with obsessive compulsive disorder. *J.Clin.Psychiatry* **53:** 309-314.

**Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ, Grandy DK**. **1997.** Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. *Cell* **90:** 991-1001.

**Rubinstein M, Cepeda C, Hurst RS, Flores-Hernandez J, Ariano MA, Falzone TL, Kozell LB, Meshul CK, Bunzow JR, Low MJ, Levine MS, Grandy DK**. **2001.** Dopamine D4 receptor-deficient mice display cortical hyperexcitability. *J.Neurosci.* **21:** 3756-3763.

**Rudolf GD, Cronin CA, Landwehrmeyer GB, Standaert DG, Penney JB, Jr., Young AB**. **1996.** Expression of N-methyl-D-aspartate glutamate receptor subunits in the prefrontal cortex of the rat. *Neuroscience* **73:** 417-427.

**Rufer M, Grothusen A, Mass R, Peter H, Hand I**. **2005.** Temporal stability of symptom dimensions in adult patients with obsessive-compulsive disorder. *J.Affect.Disord.* **88:** 99-102.

**Rybakowski JK, Borkowska A, Czerski PM, Hauser J**. **2001.** Dopamine D3 receptor (DRD3) gene polymorphism is associated with the intensity of eye movement disturbances in schizophrenic patients and healthy subjects. *Mol.Psychiatry* **6:** 718-724.

**Saito T, Guan F, Papolos DF, Rajouria N, Fann CS, Lachman HM**. **2001.** Polymorphism in SNAP29 gene promoter region associated with schizophrenia. *Mol.Psychiatry* **6:** 193-201.

**Samuels J, Bienvenu OJ, III, Riddle MA, Cullen BA, Grados MA, Liang KY, Hoehn-Saric R, Nestadt G**. **2002.** Hoarding in obsessive compulsive disorder: results from a case-control study. *Behav.Res.Ther.* **40:** 517-528.

**Samuels J, Nestadt G**. **1997**. Epidemiology and genetics of obsessive-compulsive disorder. *Int Rev Psychiatry*. **9:** 61-71

**Sano A, Kondoh K, Kakimoto Y, Kondo I**. **1993.** A 40-nucleotide repeat polymorphism in the human dopamine transporter gene. *Hum.Genet.* **91:** 405-406.

**Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P, Hen R, Heath MJ**. **2001.** Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc.Natl.Acad.Sci.U.S.A* **98:** 1912-1917.

**Santarelli L, Gobbi G, Blier P, Hen R**. **2002.** Behavioral and physiologic effects of genetic or pharmacologic inactivation of the substance P receptor (NK1). *J.Clin.Psychiatry* **63 Suppl 11:** 11-17.

**Sasson Y, Zohar J, Chopra M, Lustig M, Iancu I, Hendler T**. **1997.** Epidemiology of obsessive-compulsive disorder: a world view. *J.Clin.Psychiatry* **58 Suppl 12:** 7-10.

**Satel SL, McDougle CJ**. **1991.** Obsessions and compulsions associated with cocaine abuse. *Am.J.Psychiatry* **148:** 947.

**Sato M, Soma M, Nakayama T, Kanmatsuse K**. **2000.** Dopamine D1 receptor gene polymorphism is associated with essential hypertension. *Hypertension* **36:** 183-186.

**Satten GA, Flanders WD, Yang Q**. **2001.** Accounting for unmeasured population substructure in case-control studies of genetic association using a novel latent-class model. *Am.J.Hum.Genet.* **68:** 466-477.

**Sautel F, Griffon N, Sokoloff P, Schwartz JC, Launay C, Simon P, Costentin J, Schoenfelder A, Garrido F, Mann A, . 1995.** Nafadotride, a potent preferential dopamine D3 receptor antagonist, activates locomotion in rodents. *J.Pharmacol.Exp.Ther.* **275:** 1239-1246.

**Saxena S, Brody AL, Schwartz JM, Baxter LR**. **1998.** Neuroimaging and frontal-subcortical circuitry in obsessive-compulsive disorder. *Br.J.Psychiatry Suppl* 26-37.

**Saxena S, Maidment KM, Vapnik T, Golden G, Rishwain T, Rosen RM, Tarlow G, Bystritsky A**. **2002.** Obsessive-compulsive hoarding: symptom severity and response to multimodal treatment. *J.Clin.Psychiatry* **63:** 21-27.

**Saxena S, Brody AL, Maidment KM, Smith EC, Zohrabi N, Katz E, Baker SK, Baxter LR, Jr. 2004.** Cerebral glucose metabolism in obsessive-compulsive hoarding. *Am.J.Psychiatry* **161:** 1038-1048.

**Scalzitti JM, Cervera LS, Smith C, Hensler JG**. **1999.** Serotonin2A receptor modulation of D1 dopamine receptor-mediated grooming behavior. *Pharmacol.Biochem.Behav.* **63:** 279-284.

**Scarselli M, Novi F, Schallmach E, Lin R, Baragli A, Colzi A, Griffon N, Corsini GU, Sokoloff P, Levenson R, Vogel Z, Maggio R**. **2001.** D2/D3 dopamine receptor heterodimers exhibit unique functional properties. *J.Biol.Chem.* **276:** 30308-30314.

**Schaid DJ**. **1998.** Transmission disequilibrium, family controls, and great expectations. *Am.J.Hum.Genet.* **63:** 935-941.

**Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. 2002.** Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet*. 70(2):425-434

**Schall U, Schon A, Zerbin D, Eggers C, Oades RD**. **1996.** Event-related potentials during an auditory discrimination with prepulse inhibition in patients with schizophrenia, obsessive-compulsive disorder and healthy subjects. *Int.J.Neurosci.* **84:** 15-33.

**Scharfman HE, Maclusky NJ**. **2005.** Similarities between actions of estrogen and BDNF in the hippocampus: coincidence or clue? *Trends Neurosci.* **28:** 79-85.

**Schilder P**. **1938**. The organic background of obsessions and compulsions. *Am J Psychiatry*. **94**: 1397-1414.

**Schindler KM, Richter MA, Kennedy JL, Pato MT, Pato CN**. **2000.** Association between homozygosity at the COMT gene locus and obsessive compulsive disorder. *Am.J.Med.Genet.* **96:** 721-724.

**Schito AM, Pizzuti A, Di Maria E, Schenone A, Ratti A, Defferrari R, Bellone E, Mancardi GL, Ajmar F, Mandich P**. **1997.** mRNA distribution in adult human brain of GRIN2B, a N-methyl-D-aspartate (NMDA) receptor subunit. *Neurosci.Lett.* **239:** 49-53.

**Schmidt CJ, Sorensen SM, Kehne JH, Carr AA, Palfreyman MG. 1995**. The role of 5-HT2A receptors in antipsychotic activity. Life Sci. **56(25):** 2209-2222

**Schmidt CJ, Fadayel GM**. **1995.** The selective 5-HT2A receptor antagonist, MDL 100,907, increases dopamine efflux in the prefrontal cortex of the rat. *Eur.J.Pharmacol.* **273:** 273-279.

**Schoenbach VJ, Rosamond WD. 2000**. Understanding the fundamentals of epidemiology, an evolving text. Chapel Hill, North Carolina, USA.

**Schork NJ**. **2002.** Power calculations for genetic association studies using estimated probability distributions. *Am.J.Hum.Genet.* **70:** 1480-1489.

**Schulze TG, McMahon FJ**. **2002.** Genetic association mapping at the crossroads: which test and why? Overview and practical guidelines. *Am.J.Med.Genet.* **114:** 1-11.

**Seedat S, Stein DJ, Harvey BH**. **2001.** Inositol in the treatment of trichotillomania and compulsive skin picking. *J.Clin.Psychiatry* **62:** 60-61.

**Segawa M**. **2003.** Neurophysiology of Tourette's syndrome: pathophysiological considerations. *Brain Dev.* **25 Suppl 1:** S62-S69.

**Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M**. **2003.** A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology* **28:** 397-401.

**Sham PC, Curtis D. 1995**. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet.* **59**: 97-105.

**Shapira NA, Ward HE, Mandoki M, Murphy TK, Yang MC, Blier P, Goodman WK**. **2004.** A double-blind, placebo-controlled trial of olanzapine addition in fluoxetine-refractory obsessive-compulsive disorder. *Biol.Psychiatry* **55:** 553-555.

**Sherman SL**. **1997.** Evolving methods in genetic epidemiology. IV. Approaches to non-Mendelian inheritance. *Epidemiol.Rev.* **19:** 44-51.

**Shifman S, Darvasi A**. **2001.** The value of isolated populations. *Nat.Genet.* **28:** 309-310.

**Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A, Reznik I, Spivak B, Grisaru N, Karp L, Schiffer R, Kotler M, Strous RD, Swartz-Vanetik M, Knobler HY, Shinar E, Beckmann JS, Yakir B, Risch N, Zak NB, Darvasi A**. **2002.** A highly significant association between a COMT haplotype and schizophrenia. *Am.J.Hum.Genet.* **71:** 1296-1302.

**Shimada S, Kitayama S, Lin CL, Patel A, Nanthakumar E, Gregor P, Kuhar M, Uhl G**. **1991.** Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science* **254:** 576-578.

**Shriver MD, Jin L, Ferrell RE, Deka R**. **1997.** Microsatellite data support an early population expansion in Africa. *Genome Res.* **7:** 586-591.

**Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL**. **1984.** A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. *Peptides* **5:** 1097-1128.

**Sidenberg DG, Bassett AS, Demchyshyn L, Niznik HB, Macciardi F, Kamble AB, Honer WG, Kennedy JL**. **1993.** New polymorphism for the human serotonin 1D receptor variant (5-HT1D beta) not linked to schizophrenia in five Canadian pedigrees. *Hum.Hered.* **43:** 315-318.

**Silverman EK, Palmer LJ**. **2000.** Case-control association studies for the genetics of complex respiratory diseases. *Am.J.Respir.Cell Mol.Biol.* **22:** 645-648.

**Simon H, Scatton B, Moal ML**. **1980.** Dopaminergic A10 neurones are involved in cognitive functions. *Nature* **286:** 150-151.

**Simonic I, Gericke GS, Ott J, Weber JL**. **1998.** Identification of genetic markers associated with Gilles de la Tourette syndrome in an Afrikaner population. *Am.J.Hum.Genet.* **63:** 839-846.

**Simonic I, Nyholt DR, Gericke GS, Gordon D, Matsumoto N, Ledbetter DH, Ott J, Weber JL**. **2001.** Further evidence for linkage of Gilles de la Tourette syndrome (GTS) susceptibility loci on chromosomes 2p11, 8q22 and 11q23-24 in South African Afrikaners. *Am.J.Med.Genet.* **105:** 163-167.

**Simpson HB, Lombardo I, Slifstein M, Huang HY, Hwang DR, Abi-Dargham A, Liebowitz MR, Laruelle M**. **2003.** Serotonin transporters in obsessive-compulsive disorder: a positron emission tomography study with [(11)C]McN 5652. *Biol.Psychiatry* **54:** 1414-1421.

**Siuciak JA, Altar CA, Wiegand SJ, Lindsay RM**. **1994.** Antinociceptive effect of brain-derived neurotrophic factor and neurotrophin-3. *Brain Res.* **633:** 326-330.

**Siuciak JA, Boylan C, Fritsche M, Altar CA, Lindsay RM**. **1996.** BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. *Brain Res.* **710:** 11-20.

**Skidgel RA, Engelbrecht S, Johnson AR, Erdos EG**. **1984.** Hydrolysis of substance p and neurotensin by converting enzyme and neutral endopeptidase. *Peptides* **5:** 769-776.

**Skidgel RA, Erdos EG**. **1987.** The broad substrate specificity of human angiotensin I converting enzyme. *Clin.Exp.Hypertens.A* **9:** 243-259.

**Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, Schaffner S, Kirov G, Jones I, Owen M, Craddock N, DePaulo JR, Lander ES**. **2002.** Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. *Mol.Psychiatry* **7:** 579-593.

**Skre I, Onstad S, Torgersen S, Lygren S, Kringlen E**. **1993.** A twin study of DSM-III-R anxiety disorders. *Acta Psychiatr.Scand.* **88:** 85-92.

**Smalley SL, Bailey JN, Palmer CG, Cantwell DP, McGough JJ, Del'Homme MA, Asarnow JR, Woodward JA, Ramsey C, Nelson SF**. **1998.** Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. *Mol.Psychiatry* **3:** 427-430.

**Smith DJ, Lusis AJ**. **2002.** The allelic structure of common disease. *Hum.Mol.Genet.* **11:** 2455-2461.

**Snyder LH. 1951**. Old and new pathways in human genetics. **In**: LC Dunn (ed). Genetics and the twentieth century: Essays on the progress of genetics during its first 50 years New Macmillan, New York; pp369-92.

**Sobin C, Blundell M, Weiller F, Gavigan C, Haiman C, Karayiorgou M**. **1999.** Phenotypic characteristics of Obsessive-Compulsive Disorder ascertained in adulthood. *J.Psychiatr.Res.* **33:** 265-273.

**Sobin C, Blundell ML, Karayiorgou M**. **2000.** Phenotypic differences in early- and late-onset obsessive-compulsive disorder. *Compr.Psychiatry* **41:** 373-379.

**Sohrabji F, Miranda RC, Toran-Allerand CD**. **1995.** Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. *Proc.Natl.Acad.Sci.U.S.A* **92:** 11110-11114.

**Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC**. **1990.** Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* **347:** 146-151.

**Sokoloff P, Giros B, Martres MP, Andrieux M, Besancon R, Pilon C, Bouthenet ML, Souil E, Schwartz JC**. **1992.** Localization and function of the D3 dopamine receptor. *Arzneimittelforschung.* **42:** 224-230.

**Solum DT, Handa RJ**. **2001.** Localization of estrogen receptor alpha (ER alpha) in pyramidal neurons of the developing rat hippocampus. *Brain Res.Dev.Brain Res.* **128:** 165-175.

**Song C, Hu CD, Masago M, Kariyai K, Yamawaki-Kataoka Y, Shibatohge M, Wu D, Satoh T, Kataoka T**. **2001.** Regulation of a novel human phospholipase C, PLCepsilon, through membrane targeting by Ras. *J.Biol.Chem.* **276:** 2752-2757.

**Sorbi S, Nacmias B, Tedde A, Ricca V, Mezzani B, Rotella CM**. **1998.** 5-HT2A promoter polymorphism in anorexia nervosa. *Lancet* **351:** 1785.

**Souery D, Lipp O, Mahieu B, Mendelbaum K, De M, V, Van Broeckhoven C, Mendlewicz J**. **1996.** Association study of bipolar disorder with candidate genes involved in catecholamine neurotransmission: DRD2, DRD3, DAT1, and TH genes. *Am.J.Med.Genet.* **67:** 551-555.

**Souery D, Rivelli SK, Mendlewicz J**. **2001.** Molecular genetic and family studies in affective disorders: state of the art. *J.Affect.Disord.* **62:** 45-55.

**Sparkes RS, Lan N, Klisak I, Mohandas T, Diep A, Kojis T, Heinzmann C, Shih JC**. **1991.** Assignment of a serotonin 5HT-2 receptor gene (HTR2) to human chromosome 13q14-q21 and mouse chromosome 14. *Genomics* **9:** 461-465.

**Spielman RS, McGinnis RE, Ewens WJ**. **1993.** Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am.J.Hum.Genet.* **52:** 506-516.

**Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, Rozov A, Hvalby O, Jensen V, Paulsen O, Andersen P, Kim JJ, Thompson RF, Sun W, Webster LC, Grant SG, Eilers J,**

**Konnerth A, Li J, McNamara JO, Seeburg PH**. **1998.** Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. *Cell* **92:** 279-289.

**Spurlock G, Heils A, Holmans P, Williams J, D'Souza UM, Cardno A, Murphy KC, Jones L, Buckland PR, McGuffin P, Lesch KP, Owen MJ**. **1998.** A family based association study of T102C polymorphism in 5HT2A and schizophrenia plus identification of new polymorphisms in the promoter. *Mol.Psychiatry* **3:** 42-49.

**Stahl SM (Ed). 1996.** Essential Psychopharmacology. Cambridge University Press. pp 216-248.

**Stahl SM (Ed). 2000.** Essential Psychopharmacology. Cambridge University Press. pp 375.

**Standaert DG, Testa CM, Young AB, Penney JB, Jr. 1994.** Organization of N-methyl-D-aspartate glutamate receptor gene expression in the basal ganglia of the rat. *J.Comp Neurol.* **343:** 1-16.

**Stanley MA, Hannay HJ, Breckenridge JK**. **1997.** The neuropsychology of trichotillomania. *J.Anxiety.Disord.* **11:** 473-488.

**Starfield M, Hennies HC, Jung M, Jenkins T, Wienker T, Hull P, Spurdle A, Kuster W, Ramsay M, Reis A**. **1997.** Localization of the gene causing keratolytic winter erythema to chromosome 8p22-p23, and evidence for a founder effect in South African Afrikaans-speakers. *Am.J.Hum.Genet.* **61:** 370-378.

**State MW, Greally JM, Cuker A, Bowers PN, Henegariu O, Morgan TM, Gunel M, DiLuna M, King RA, Nelson C, Donovan A, Anderson GM, Leckman JF, Hawkins T, Pauls DL, Lifton RP, Ward DC**. **2003.** Epigenetic abnormalities associated with a chromosome 18(q21-q22) inversion and a Gilles de la Tourette syndrome phenotype. *Proc.Natl.Acad.Sci.U.S.A* **100:** 4684-4689.

**Steen VM, Lovlie R, Osher Y, Belmaker RH, Berle JO, Gulbrandsen AK**. **1998.** The polymorphic inositol polyphosphate 1-phosphatase gene as a candidate for pharmacogenetic prediction of lithium-responsive manic-depressive illness. *Pharmacogenetics* **8:** 259-268.

**Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, et al. 2002.** Neuregulin 1 and susceptibility to schizophrenia. *Am.J.Hum.Genet.* **71:** 877-892.

**Stein, D.J., Hollander E**. **1993**. The spectrum of obsessive-compuslve disorder-related disorders. **In:** Obsessive-compulsive related disorders. Hollander E (ed) APA, Washington DC pp241-72 .

**Stein DJ, Hollander E**. **1995.** Obsessive-compulsive spectrum disorders. *J.Clin.Psychiatry* **56:** 265-266.

**Stein DJ, Spadaccini E, Hollander E**. **1995.** Meta-analysis of pharmacotherapy trials for obsessive-compulsive disorder. *Int.Clin.Psychopharmacol.* **10:** 11-18.

**Stein DJ, Bouwer C, Hawkridge S, Emsley RA**. **1997.** Risperidone augmentation of serotonin reuptake inhibitors in obsessive-compulsive and related disorders. *J.Clin.Psychiatry* **58:** 119-122.

**Stein DJ, Van Heerden B, Wessels CJ, van Kradenburg J, Warwick J, Wasserman HJ**. **1999.** Single photon emission computed tomography of the brain with Tc-99m HMPAO during sumatriptan challenge in obsessive-compulsive disorder: investigating the functional role of the serotonin auto-receptor. *Prog.Neuropsychopharmacol.Biol.Psychiatry* **23:** 1079-1099.

**Stein DJ**. **2000.** Advances in the neurobiology of obsessive-compulsive disorder. Implications for conceptualizing putative obsessive-compulsive and spectrum disorders. *Psychiatr.Clin.North Am.* **23:** 545-562.

**Steingard R, Dillon-Stout D**. **1992.** Tourette's syndrome and obsessive compulsive disorder. Clinical aspects. *Psychiatr.Clin.North Am.* **15:** 849-860.

**Stengler-Wenzke K, Muller U, Angermeyer MC, Sabri O, Hesse S**. **2004.** Reduced serotonin transporter-availability in obsessive-compulsive disorder (OCD). *Eur.Arch.Psychiatry Clin.Neurosci.* **254:** 252-255.

**Stephens JC, Schneider JA, Tanguay DA, Choi J, Acharya T, Stanley SE, Jiang R, Messer CJ, Chew A, Han JH, Duan J, Carr JL, Lee MS, Koshy B, Kumar AM, Zhang G, Newell WR, Windemuth A, Xu C, Kalbfleisch TS, Shaner SL, Arnold K, Schulz V, Drysdale CM, Nandabalan K, Judson RS, Ruano G, Vovis GF**. **2001.** Haplotype variation and linkage disequilibrium in 313 human genes. *Science* **293:** 489-493.

**Stephens M, Smith NJ, Donnelly P**. **2001.** A new statistical method for haplotype reconstruction from population data. *Am.J.Hum.Genet.* **68:** 978-989.

**Stern L, Zohar J, Cohen R, Sasson Y**. **1998.** Treatment of severe, drug resistant obsessive compulsive disorder with the 5HT1D agonist sumatriptan. *Eur.Neuropsychopharmacol.* **8:** 325-328.

**Stern TA, Jenike MA**. **1983.** Treatment of obsessive-compulsive disorder with lithium carbonate. *Psychosomatics* **24:** 671-673.

**Sterne JA, Davey SG**. **2001.** Sifting the evidence-what's wrong with significance tests? *BMJ* **322:** 226-231.

**Stoll M, Corneliussen B, Costello CM, Waetzig GH, Mellgard B, Koch WA, Rosenstiel P, Albrecht M, Croucher PJ, Seegert D, Nikolaus S, Hampe J, Lengauer T, Pierrou S, Foelsch UR, Mathew CG, Lagerstrom-Fermer M, Schreiber S**. **2004.** Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat.Genet.* **36:** 476-480.

**Stoltenberg SF, Burmeister M**. **2000.** Recent progress in psychiatric genetics-some hope but no hype. *Hum.Mol.Genet.* **9:** 927-935.

**Stoneking M, Fontius JJ, Clifford SL, Soodyall H, Arcot SS, Saha N, Jenkins T, Tahir MA, Deininger PL, Batzer MA**. **1997.** Alu insertion polymorphisms and human evolution: evidence for a larger population size in Africa. *Genome Res.* **7:** 1061-1071.

**Sullivan PF, Neale BM, van den OE, Miles MF, Neale MC, Bulik CM, Joyce PR, Straub RE, Kendler KS**. **2004.** Candidate genes for nicotine dependence via linkage, epistasis, and bioinformatics. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* **126:** 23-36.

**Summerfeldt LJ, Richter MA, Antony MM, Swinson RP**. **1999.** Symptom structure in obsessive-compulsive disorder: a confirmatory factor-analytic study. *Behav.Res.Ther.* **37:** 297-311.

**Sumner BE, Grant KE, Rosie R, Hegele-Hartung C, Fritzemeier KH, Fink G**. **1999.** Effects of tamoxifen on serotonin transporter and 5-hydroxytryptamine(2A) receptor binding sites and mRNA levels in the brain of ovariectomized rats with or without acute estradiol replacement. *Brain Res.Mol.Brain Res.* **73:** 119-128.

**Sunahara RK, Niznik HB, Weiner DM, Stormann TM, Brann MR, Kennedy JL, Gelernter JE, Rozmahel R, Yang YL, Israel Y, . 1990.** Human dopamine D1 receptor encoded by an intronless gene on chromosome 5. *Nature* **347:** 80-83.

**Susser M**. **1990.** Disease, illness, sickness; impairment, disability and handicap. *Psychol.Med.* **20:** 471-473.

**Suzuki M, Hurd YL, Sokoloff P, Schwartz JC, Sedvall G**. **1998.** D3 dopamine receptor mRNA is widely expressed in the human brain. *Brain Res.* **779:** 58-74.

**Svensson K, Carlsson A, Waters N**. **1994.** Locomotor inhibition by the D3 ligand R-(+)-7-OH-DPAT is independent of changes in dopamine release. *J.Neural Transm.Gen.Sect.* **95:** 71-74.

**Swanson JM, Sunohara GA, Kennedy JL, Regino R, Fineberg E, Wigal T, Lerner M, Williams L, LaHoste GJ, Wigal S**. **1998.** Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. *Mol.Psychiatry* **3:** 38-41.

**Swedo SE, Rapoport JL, Leonard H, Lenane M, Cheslow D**. **1989(a).** Obsessive-compulsive disorder in children and adolescents. Clinical phenomenology of 70 consecutive cases. *Arch.Gen.Psychiatry* **46:** 335-341.

**Swedo SE, Rapoport JL, Cheslow DL, Leonard HL, Ayoub EM, Hosier DM, Wald ER**. **1989(b).** High prevalence of obsessive-compulsive symptoms in patients with Sydenham's chorea. *Am.J.Psychiatry* **146:** 246-249.

**Swedo SE, Rapoport JL, Cheslow DL, Leonard HL, Ayoub EM, Hosier DM, Wald ER**. **1989(c).** High prevalence of obsessive-compulsive symptoms in patients with Sydenham's chorea. *Am.J.Psychiatry* **146:** 246-249.

**Swedo SE, Leonard HL**. **1992.** Trichotillomania. An obsessive compulsive spectrum disorder? *Psychiatr.Clin.North Am.* **15:** 777-790.

**Swedo SE, Leonard HL, Garvey M, Mittleman B, Allen AJ, Perlmutter S, Lougee L, Dow S, Zamkoff J, Dubbert BK**. **1998.** Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. *Am.J.Psychiatry* **155:** 264-271.

**Sweet RA, Nimgaonkar VL, Kamboh MI, Lopez OL, Zhang F, DeKosky ST**. **1998.** Dopamine receptor genetic variation, psychosis, and aggression in Alzheimer disease. *Arch.Neurol.* **55:** 1335-1340.

**Swerdlow NR, Geyer MA**. **1993.** Prepulse inhibition of acoustic startle in rats after lesions of the pedunculopontine tegmental nucleus. *Behav.Neurosci.* **107:** 104-117.

**Szechtman H, Sulis W, Eilam D**. **1998.** Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). *Behav.Neurosci.* **112:** 1475-1485.

**Szechtman H, Eckert MJ, Tse WS, Boersma JT, Bonura CA, McClelland JZ, Culver KE, Eilam D**. **2001.** Compulsive checking behavior of quinpirole-sensitized rats as an animal model of Obsessive-Compulsive Disorder(OCD): form and control. *BMC.Neurosci.* **2:** 4.

**Szeszko PR, MacMillan S, McMeniman M, Chen S, Baribault K, Lim KO, Ivey J, Rose M, Banerjee SP, Bhandari R, Moore GJ, Rosenberg DR**. **2004.** Brain structural abnormalities in psychotropic drug-naive pediatric patients with obsessive-compulsive disorder. *Am.J.Psychiatry* **161:** 1049-1056.

**Taber MT, Fibiger HC**. **1993.** Electrical stimulation of the medial prefrontal cortex increases dopamine release in the striatum. *Neuropsychopharmacology* **9:** 271-275.

**Tabor HK, Risch NJ, Myers RM**. **2002.** Opinion: Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat.Rev.Genet.* **3:** 391-397.

**Takahashi M, Shirakawa O, Toyooka K, Kitamura N, Hashimoto T, Maeda K, Koizumi S, Wakabayashi K, Takahashi H, Someya T, Nawa H**. **2000.** Abnormal expression of brain-derived neurotrophic factor and its receptor in the corticolimbic system of schizophrenic patients. *Mol.Psychiatry* **5:** 293-300.

**Tao X, West AE, Chen WG, Corfas G, Greenberg ME**. **2002.** A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. *Neuron* **33:** 383-395.

**Tarazi FI, Kula NS, Baldessarini RJ**. **1997(a).** Regional distribution of dopamine D4 receptors in rat forebrain. *Neuroreport* **8:** 3423-3426.

**Tarazi FI, Yeghiayan SK, Baldessarini RJ, Kula NS, Neumeyer JL**. **1997(b).** Long-term effects of S(+)N-n-propylnorapomorphine compared with typical and atypical antipsychotics: differential increases of cerebrocortical D2-like and striatolimbic D4-like dopamine receptors. *Neuropsychopharmacology* **17:** 186-196.

**Tarazi FI, Campbell A, Yeghiayan SK, Baldessarini RJ**. **1998.** Localization of ionotropic glutamate receptors in caudate-putamen and nucleus accumbens septi of rat brain: comparison of NMDA, AMPA, and kainate receptors. *Synapse* **30:** 227-235.

**Tarazi FI, Baldessarini RJ**. **1999.** Dopamine D4 receptors: significance for molecular psychiatry at the millennium. *Mol.Psychiatry* **4:** 529-538.

**Taylor JR, Birnbaum S, Ubriani R, Arnsten AF**. **1999.** Activation of cAMP-dependent protein kinase A in prefrontal cortex impairs working memory performance. *J.Neurosci.* **19:** RC23.

**Taylor LD, Krizman DB, Jankovic J, Hayani A, Steuber PC, Greenberg F, Fenwick RG, Caskey CT**. **1991.** 9p monosomy in a patient with Gilles de la Tourette's syndrome. *Neurology* **41:** 1513-1515.

**Templeton AR, Clark AG, Weiss KM, Nickerson DA, Boerwinkle E, Sing CF**. **2000.** Recombinational and mutational hotspots within the human lipoprotein lipase gene. *Am.J.Hum.Genet.* **66:** 69-83.

**Teng J, Risch N**. **1999.** The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases. II. Individual genotyping. *Genome Res.* **9:** 234-241.

**Tenhunen J, Salminen M, Lundstrom K, Kiviluoto T, Savolainen R, Ulmanen I**. **1994.** Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters. *Eur.J.Biochem.* **223:** 1049-1059.

**Terwilliger JD, Ott J**. **1992.** A haplotype-based 'haplotype relative risk' approach to detecting allelic associations. *Hum.Hered.* **42:** 337-346.

**Terwilliger JD, Zollner S, Laan M, Paabo S**. **1998.** Mapping genes through the use of linkage disequilibrium generated by genetic drift: 'drift mapping' in small populations with no demographic expansion. *Hum.Hered.* **48:** 138-154.

**Theal GM. 1964.** History of South Africa. Vol 4. Struik Publishers, Cape Town. pp346-364

**Thomas DC, Witte JS**. **2002.** Point: population stratification: a problem for case-control studies of candidate-gene associations? *Cancer Epidemiol.Biomarkers Prev.* **11:** 505-512.

**Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA**. **1997.** D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics* **7:** 479-484.

**Thompson M, Comings DE, Feder L, George SR, O'Dowd BF**. **1998.** Mutation screening of the dopamine D1 receptor gene in Tourette's syndrome and alcohol dependent patients. *Am.J.Med.Genet.* **81:** 241-244.

**Thomsen PH**. **1993.** Obsessive-compulsive disorder in children and adolescents. Self-reported obsessive-compulsive behaviour in pupils in Denmark. *Acta Psychiatr.Scand.* **88:** 212-217.

**Thoren P, Asberg M, Bertilsson L, Mellstrom B, Sjoqvist F, Traskman L**. **1980.** Clomipramine treatment of obsessive-compulsive disorder. II. Biochemical aspects. *Arch.Gen.Psychiatry* **37:** 1289-1294.

**Thyer BA, Parrish RT, Curtis GC, Nesse RM, Cameron OG**. **1985.** Ages of onset of DSM-III anxiety disorders. *Compr.Psychiatry* **26:** 113-122.

**Tingley WG, Roche KW, Thompson AK, Huganir RL**. **1993.** Regulation of NMDA receptor phosphorylation by alternative splicing of the C-terminal domain. *Nature* **364:** 70-73.

**Tipping AJ, Pearson T, Morgan NV, Gibson RA, Kuyt LP, Havenga C, Gluckman E, Joenje H, de Ravel T, Jansen S, Mathew CG**. **2001.** Molecular and genealogical evidence for a founder effect in Fanconi anemia families of the Afrikaner population of South Africa. *Proc.Natl.Acad.Sci.U.S.A* **98:** 5734-5739.

**Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, Soubrier F**. **1992.** Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am.J.Hum.Genet.* **51:** 197-205.

**Tishkoff SA, Dietzsch E, Speed W, Pakstis AJ, Kidd JR, Cheung K, Bonne-Tamir B, Santachiara-Benerecetti AS, Moral P, Krings M**. **1996.** Global patterns of linkage disequilibrium at the CD4 locus and modern human origins. *Science* **271:** 1380-1387.

**Toledo-Aral JJ, Brehm P, Halegoua S, Mandel G**. **1995.** A single pulse of nerve growth factor triggers long-term neuronal excitability through sodium channel gene induction. *Neuron* **14:** 607-611.

**Tot S, Erdal ME, Yazici K, Yazici AE, Metin O**. **2003.** T102C and -1438 G/A polymorphisms of the 5-HT2A receptor gene in Turkish patients with obsessive-compulsive disorder. *Eur.Psychiatry* **18:** 249-254.

**Trifiletti RR, Packard AM**. **1999.** Immune mechanisms in pediatric neuropsychiatric disorders. Tourette's syndrome, OCD, and PANDAS. *Child Adolesc.Psychiatr.Clin.N.Am.* **8:** 767-775.

**Tsai G, Coyle JT**. **1995.** N-acetylaspartate in neuropsychiatric disorders. *Prog.Neurobiol.* **46:** 531-540.

**Tsai SJ, Chiu HJ, Wang YC, Hong CJ**. **1999.** Association study of serotonin-6 receptor variant (C267T) with schizophrenia and aggressive behavior. *Neurosci.Lett.* **271:** 135-137.

**Tsaltas E, Kontis D, Chrysikakou S, Giannou H, Biba A, Pallidi S, Christodoulou A, Maillis A, Rabavilas A**. **2005.** Reinforced spatial alternation as an animal model of obsessive-compulsive disorder (OCD): investigation of 5-HT2C and 5-HT1D receptor involvement in OCD pathophysiology. *Biol.Psychiatry* **57:** 1176-1185.

**Tukel R, Polat A, Ozdemir O, Aksut D, Turksoy N**. **2002.** Comorbid conditions in obsessive-compulsive disorder. *Compr.Psychiatry* **43:** 204-209.

**Tukel R, Polat A, Genc A, Bozkurt O, Atli H**. **2004.** Gender-related differences among Turkish patients with obsessive-compulsive disorder. *Compr.Psychiatry* **45:** 362-366.

**Turner CA, Presti MF, Newman HA, Bugenhagen P, Crnic L, Lewis MH**. **2001.** Spontaneous stereotypy in an animal model of Down syndrome: Ts65Dn mice. *Behav.Genet.* **31:** 393-400.

**Turner D, Choudhury F, Reynard M, Railton D, Navarrete C**. **2002.** Typing of multiple single nucleotide polymorphisms in cytokine and receptor genes using SNaPshot. *Hum.Immunol.* **63:** 508-513.

**Turner CA, Lewis MH**. **2003.** Environmental enrichment: effects on stereotyped behavior and neurotrophin levels. *Physiol Behav.* **80:** 259-266.

**Tycko B, Ashkenas J**. **2000.** Epigenetics and its role in disease. *J.Clin.Invest* **105:** 245-246.

**Uhl GR, Grow RW**. **2004.** The burden of complex genetics in brain disorders. *Arch.Gen.Psychiatry* **61:** 223-229.

**Ulloa RE, Nicolini H, Fernandez-Guasti A**. **2004.** Age differences in an animal model of obsessive-compulsive disorder: participation of dopamine: dopamine in an animal model of OCD. *Pharmacol.Biochem.Behav.* **78:** 661-666.

**Ullu E, Tschudi C**. **1984.** Alu sequences are processed 7SL RNA genes. *Nature* **312:** 171-172.

**Uphouse L**. **1997.** Multiple serotonin receptors: too many, not enough, or just the right number? *Neurosci.Biobehav.Rev.* **21:** 679-698.

**Valleni-Basile LA, Garrison CZ, Jackson KL, Waller JL, McKeown RE, Addy CL, Cuffe SP**. **1994.** Frequency of obsessive-compulsive disorder in a community sample of young adolescents. *J.Am.Acad.Child Adolesc.Psychiatry* **33:** 782-791.

**Vallone PM, Butler JM**. **2004**. AutoDimer: a screening tool for primer-dimer and hairpin structures. Biotechniques**. 37(2):**226-31.

**Van der Wee, N. Stevens, H. Hardeman, H. Denys, D. van Megen, H.J., Kahn, R.S., Westenberg, H.G**. 2001. Enhanced densities of dopamine but not of serotonin transporters in psychotropic-naïve patients with obsessive-compulsive disorder. *J.Nucl.Med*. **42**: 238P

**Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O**. **1991.** Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* **350:** 610-614.

**Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, Uhl GR**. **1992.** Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. *Genomics* **14:** 1104-1106.

**Vandenbergh DJ, Thompson MD, Cook EH, Bendahhou E, Nguyen T, Krasowski MD, Zarrabian D, Comings D, Sellers EM, Tyndale RF, George SR, O'Dowd BF, Uhl GR**. **2000.** Human dopamine transporter gene: coding region conservation among normal, Tourette's disorder, alcohol dependence and attention-deficit hyperactivity disorder populations. *Mol.Psychiatry* **5:** 283-292.

**Vecchio TJ. 1966**. Predictive value of a single diagnostic test in unselected populations. *N. Engl. J. Med*. **274:**1171-1173.

**Veenstra-VanderWeele J, Kim SJ, Gonen D, Hanna GL, Leventhal BL, Cook EH, Jr. 2001.** Genomic organization of the SLC1A1/EAAC1 gene and mutation screening in early-onset obsessive-compulsive disorder. *Mol.Psychiatry* **6:** 160-167.

**Verkerk AJ, Mathews CA, Joosse M, Eussen BH, Heutink P, Oostra BA**. **2003.** CNTNAP2 is disrupted in a family with Gilles de la Tourette syndrome and obsessive compulsive disorder. *Genomics* **82:** 1-9.

**Vieland VJ**. **2001.** The replication requirement. *Nat.Genet.* **29:** 244-245.

**Villard E, Tiret L, Visvikis S, Rakotovao R, Cambien F, Soubrier F**. **1996.** Identification of new polymorphisms of the angiotensin I-converting enzyme (ACE) gene, and study of their relationship to plasma ACE levels by two-QTL segregation-linkage analysis. *Am.J.Hum.Genet.* **58:** 1268-1278.

**Vineis P, McMichael AJ**. **1998.** Bias and confounding in molecular epidemiological studies: special considerations. *Carcinogenesis* **19:** 2063-2067.

**Visscher P, Haley C**. **2001.** True and false positive peaks in genomewide scans: The long and the short of it. *Genet.Epidemiol.* **20:** 409-414.

**Vogt IR, Shimron-Abarbanell D, Neidt H, Erdmann J, Cichon S, Schulze TG, Muller DJ, Maier W, Albus M, Borrmann-Hassenbach M, Knapp M, Rietschel M, Propping P, Nothen MM**. **2000.** Investigation of the human serotonin 6 [5-HT6] receptor gene in bipolar affective disorder and schizophrenia. *Am.J.Med.Genet.* **96:** 217-221.

**Volavka J, Neziroglu F, Yaryura-Tobias JA**. **1985.** Clomipramine and imipramine in obsessive-compulsive disorder. *Psychiatry Res.* **14:** 85-93.

**von Bartheld CS, Byers MR, Williams R, Bothwell M**. **1996.** Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. *Nature* **379:** 830-833.

**Wachtel SR, Brooderson RJ, White FJ**. **1992.** Parametric and pharmacological analyses of the enhanced grooming response elicited by the D1 dopamine receptor agonist SKF 38393 in the rat. *Psychopharmacology (Berl)* **109:** 41-48.

**Walitza S, Wewetzer C, Warnke A, Gerlach M, Geller F, Gerber G, Gorg T, Herpertz-Dahlmann B, Schulz E, Remschmidt H, Hebebrand J, Hinney A**. **2002.** 5-HT2A promoter polymorphism -1438G/A in children and adolescents with obsessive-compulsive disorders. *Mol.Psychiatry* **7:** 1054-1057.

**Walitza S, Wewetzer C, Gerlach M, Klampfl K, Geller F, Barth N, Hahn F, Herpertz-Dahlmann B, Gossler M, Fleischhaker C, Schulz E, Hebebrand J, Warnke A, Hinney A**. **2004.** Transmission disequilibrium studies in children and adolescents with obsessive-compulsive disorders pertaining to polymorphisms of genes of the serotonergic pathway. *J.Neural Transm.* **111:** 817-825.

**Wall JD**. **2001.** Insights from linked single nucleotide polymorphisms: what we can learn from linkage disequilibrium. *Curr.Opin.Genet.Dev.* **11:** 647-651.

**Wall JD, Pritchard JK**. **2003.** Assessing the performance of the haplotype block model of linkage disequilibrium. *Am.J.Hum.Genet.* **73:** 502-515.

**Wallach MB**. **1974.** Proceedings: Drug-induced stereotyped behavior: similarities and differences. *Psychopharmacol Bull.* **10(3)**: 12-13.

**Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, Ryder OA, Spence MA, Swanson JM, Moyzis RK**. **2004.** The genetic architecture of selection at the human dopamine receptor D4 (DRD4) gene locus. *Am.J.Hum.Genet.* **74:** 931-944.

**Ward RP, Hamblin MW, Lachowicz JE, Hoffman BJ, Sibley DR, Dorsa DM**. **1995.** Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. *Neuroscience* **64:** 1105-1111.

**Warren JT, Jr., Peacock ML, Rodriguez LC, Fink JK**. **1993.** An MspI polymorphism in the serotonin receptor gene (HTR2): detection by DGGE and RFLP analysis. *Hum.Mol.Genet.* **2:** 338.

**Watkins WS, Ricker CE, Bamshad MJ, Carroll ML, Nguyen SV, Batzer MA, Harpending HC, Rogers AR, Jorde LB**. **2001.** Patterns of ancestral human diversity: an analysis of Alu-insertion and restriction-site polymorphisms. *Am.J.Hum.Genet.* **68:** 738-752.

**Weiss KM, Clark AG**. **2002.** Linkage disequilibrium and the mapping of complex human traits. *Trends Genet.* **18:** 19-24.

**Weilburg JB, Mesulam MM, Weintraub S, et al. 1989.** Focal striatal abnormalities in a patient with obsessive-compulsive disorder. Arch Neurology. **46:** 233-235.

**Von Economo C**. **1932**. (Newman KO trans.), Encephalitis lethargica: Its sequelae and treatment Oxford University Press, London.

**Weissman MM, Bland RC, Canino GJ, Greenwald S, Hwu HG, Lee CK, Newman SC, Oakley-Browne MA, Rubio-Stipec M, Wickramaratne PJ, . 1994.** The cross national epidemiology of obsessive compulsive disorder. The Cross National Collaborative Group. *J.Clin.Psychiatry* **55 Suppl:** 5-10.

**Wenthold RJ, Prybylowski K, Standley S, Sans N, Petralia RS**. **2003.** Trafficking of NMDA receptors. *Annu.Rev.Pharmacol.Toxicol.* **43:** 335-358.

**Williams J, McGuffin P, Nothen M, Owen MJ**. **1997.** Meta-analysis of association between the 5-HT2a receptor T102C polymorphism and schizophrenia. EMASS Collaborative Group. European Multicentre Association Study of Schizophrenia. *Lancet* **349:** 1221.

**Williams SM, Haines JL, Moore JH**. **2004.** The use of animal models in the study of complex disease: all else is never equal or why do so many human studies fail to replicate animal findings? *Bioessays* **26:** 170-179.

**Willour VL, Yao SY, Samuels J, Grados M, Cullen B, Bienvenu OJ, III, Wang Y, Liang KY, Valle D, Hoehn-Saric R, Riddle M, Nestadt G**. **2004.** Replication study supports evidence for linkage to 9p24 in obsessive-compulsive disorder. *Am.J.Hum.Genet.* **75:** 508-513.

**Wilson JF, Goldstein DB**. **2000.** Consistent long-range linkage disequilibrium generated by admixture in a Bantu-Semitic hybrid population. *Am.J.Hum.Genet.* **67:** 926-935.

**Wilson P. 1934.** A study of twins with special reference to hereditary as a factor determining the difference in environment. *Human Biology.* **6:** 768-773

**Winqvist R, Lundstrom K, Salminen M, Laatikainen M, Ulmanen I**. **1992.** The human catechol-O-methyltransferase (COMT) gene maps to band q11.2 of chromosome 22 and shows a frequent RFLP with BglI. *Cytogenet.Cell Genet.* **59:** 253-257.

**Winsberg ME, Cassic KS, Koran LM**. **1999.** Hoarding in obsessive-compulsive disorder: a report of 20 cases. *J.Clin.Psychiatry* **60:** 591-597.

**Witzig R**. **1996.** The medicalization of race: scientific legitimization of a flawed social construct. *Ann.Intern.Med.* **125:** 675-679.

**Woodruff R, Pitts FN, Jr. 1964.** Monozygotic twins with obsessional illness. *Am.J.Psychiatry* **120:** 1075-1080.

**Woolf B**. **1955.** On estimating the relation between blood group and disease. *Ann.Hum.Genet.* **19:** 251-253.

**Wright AF, Carothers AD, Pirastu M**. **1999.** Population choice in mapping genes for complex diseases. *Nat.Genet.* **23:** 397-404.

**Wright AF, Hastie ND**. **2001.** Complex genetic diseases: controversy over the Croesus code. *Genome Biol.* **2:** 2007.

**Wyszynski M, Lin J, Rao A, Nigh E, Beggs AH, Craig AM, Sheng M**. **1997.** Competitive binding of alpha-actinin and calmodulin to the NMDA receptor. *Nature* **385:** 439-442.

**Xie E, Zhu L, Zhao L, Chang LS**. **1996.** The human serotonin 5-HT2C receptor: complete cDNA, genomic structure, and alternatively spliced variant. *Genomics* **35:** 551-561.

**Xie W, Hong H, Yang NN, Lin RJ, Simon CM, Stallcup MR, Evans RM**. **1999.** Constitutive activation of transcription and binding of coactivator by estrogen-related receptors 1 and 2. *Mol.Endocrinol.* **13:** 2151-2162.

**Xu CF, Lewis K, Cantone KL, Khan P, Donnelly C, White N, Crocker N, Boyd PR, Zaykin DV, Purvis IJ**. **2002.** Effectiveness of computational methods in haplotype prediction. *Hum.Genet.* **110:** 148-156.

**Yamada K, Nabeshima T**. **2003.** Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J.Pharmacol.Sci.* **91:** 267-270.

**Yaich, L., W. D. Dupont, D. R. Cavener, and F. F. Parl**. **1992**. Analysis of the PvuII restriction fragment-length polymorphism and exon structure of the estrogen receptor gene in breast cancer and peripheral blood. *Cancer Res*. **52**:77-83.

**Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR**. **2002.** Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus:

requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. *J.Neurosci.* **22:** 1532-1540.

**York JD, Veile RA, Donis-Keller H, Majerus PW**. **1993.** Cloning, heterologous expression, and chromosomal localization of human inositol polyphosphate 1-phosphatase. *Proc.Natl.Acad.Sci.U.S.A* **90:** 5833-5837.

**Yu YW, Tsai SJ, Lin CH, Hsu CP, Yang KH, Hong CJ**. **1999.** Serotonin-6 receptor variant (C267T) and clinical response to clozapine. *Neuroreport* **10:** 1231-1233.

**Yuen EC, Mobley WC**. **1999.** Early BDNF, NT-3, and NT-4 signaling events. *Exp.Neurol.* **159:** 297-308.

**Zahn TP, Kruesi MJ, Swedo SE, Leonard HL, Rapoport JL**. **1996.** Autonomic activity in relation to cerebrospinal fluid neurochemistry in obsessive and disruptive children and adolescents. *Psychophysiology* **33:** 731-739.

**Zhang H, Leckman JF, Pauls DL, Tsai CP, Kidd KK, Campos MR**. **2002.** Genomewide scan of hoarding in sib pairs in which both sibs have Gilles de la Tourette syndrome. *Am.J.Hum.Genet.* **70:** 896-904.

**Zhang S, Pakstis AJ, Kidd KK, Zhao H**. **2001.** Comparisons of two methods for haplotype reconstruction and haplotype frequency estimation from population data. *Am.J.Hum.Genet.* **69:** 906-914.

**Zhao H, Pfeiffer R, Gail MH**. **2003.** Haplotype analysis in population genetics and association studies. *Pharmacogenomics.* **4:** 171-178.

**Zhu QS, Chen K, Shih JC**. **1995.** Characterization of the human 5-HT2A receptor gene promoter. *J.Neurosci.* **15:** 4885-4895.

**Ziv E, Burchard EG**. **2003.** Human population structure and genetic association studies. *Pharmacogenomics.* **4:** 431-441.

**Zohar J, Mueller EA, Insel TR, Zohar-Kadouch RC, Murphy DL**. **1987.** Serotonergic responsivity in obsessive-compulsive disorder. Comparison of patients and healthy controls. *Arch.Gen.Psychiatry* **44:** 946-951.

**Zohar J, Insel TR**. **1987.** Drug treatment of obsessive-compulsive disorder. *J.Affect.Disord.* **13:** 193-202.

**Zohar, J. and S. Kindler**. 1992. Serotonergic probes in obsessive compulsive disorder. Int.Clin.Psychopharmacol. **7 Suppl 1**:39-40.

**Zohar AH, Pauls DL, Ratzoni G, Apter A, Dycian A, Binder M, King R, Leckman JF, Kron S, Cohen DJ**. **1997.** Obsessive-compulsive disorder with and without tics in an epidemiological sample of adolescents. *Am.J.Psychiatry* **154:** 274-276.

**Zollner, S. and J. K. Pritchard**. **2005**. Coalescent-based association mapping and fine mapping of complex trait loci. Genetics **169**:1071-1092.

**Zondervan KT, Cardon LR**. **2004.** The complex interplay among factors that influence allelic association. *Nat.Rev.Genet.* **5:** 89-100.