CHROMOSOMAL ABERRATIONS IN THE XHOSA SCHIZOPHRENIA POPULATION

by

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 10 November 2008

STUDIE OPSOMMING

AGTERGROND: Skisofrenie is ‘n heterogene siekte wat ontstaan vanuit ingewikkelde geen-omgewing interaksie. Die meerderheid van bestaande molekulêre genetiese studies het Kaukasiers betrek en resultate vanuit hierdie en Asiatiese populasies wys dat 2-32% van lyers kromosomale afwykings toon. Tot dusver ontwyk die ontdekking van ‘n spesifieke vatbaarheidsmeganisme of geen ons, maar die gebruik van endofenotipes word aanbeveel as bruikbaar vir hierdie soektog. Soortgelyke sitogenetiese studies is nog nie in enige inheemse Afrika populasie beskryf nie.

DOELWIT: Die doel van die studie was om genotipiese en fenotipiese data, soos gestruktureerd versamel in ‘n homogene populasie, te kombineer, met die hoop om ‘n endofenotipe te beskryf wat gebruik sou kon word vir meer akkurate identifikasie van individue met moontlike kromosomale afwykings.

METODOLOGIE: Xhosa skisofrenie pasiënte (n=112) is onderwerp aan ‘n gestruktureerde kliniese onderhoud. (Diagnostic Interview for Genetic Studies, insluitend Schedules for the Assessment of Negative and Positive Symptoms.) Bloedmonsters (kariotipering en/of FISH analise) sowel as urinemonsters (dwelmsifting) is versamel en nege kop en gesigsafmetings is geneem. Beskrywende statistieke met verwysing na demografiese, kliniese en morfologiese veranderlikes is bereken. Vergelykings tussen gemiddelde verskille vir hierdie veranderlikes is gedoen.

RESULTATE: FISH analise is gedoen op die monsters van 110 deelnemers en geen kromosoom 22q11 mikrodelesies is waargeneem nie. Monsters van 50

deelnemers is gekariotipeer en kromosomale afwykings is in vyf deelnemers (10%) gevind. Hierdie afwykings was: [46,XY,22pss]; [46,XY,1qh+]; [46,XY/47,XXY/47,XX+asentriese fragment] en twee gevalle van [46,XY,inv(9)]. Geen beduidende verskillende kon gedemonstreer word tussen die kariotiperingsubgroep (KSG) en die totale studiegroep vir enige van die veranderlikes nie. ‘n Beduidende verskil vir een morfologiese afmeting, d.i. glabella na subnasale (p=0.036), kon gedemonstreer word tussen die KSG en die vyf deelnemers met afwykings.

BESPREKING & AFLEIDINGS: Die kromosomale afwykings in ons studiegroep is almal as normale variante gerapporteer. Bloot omdat spesifieke afwykings nog nie met skisofrenie geassosieer is kan ons hulle egter nie sondermeer as nie-beduidend afmaak nie. Indien ons werklik enige moontlike skakel met psigiatriese siekte sou wou uitsluit, is dit nodig dat groepe individue met hierdie spesifieke afwykings omvattende gestuktureerde psigiatriese evaluasies ondergaan. Opsporingstegnieke is vinnig besig om meer gesofistikeerd te raak en, veral belangrik, meer nie-Kaukasiese groepe word gewerf. Ons deelnemers met kromosomale afwykings is bevind om, in vergelyking, beduidend langer neuse te hê. Kraniofasiale dismorfologie is reeds voorheen bewys om in die breë geassosieer te wees met afwykings wat beskou word om op skisofrenie-breinpatologie te dui. So, ‘n verlenging van die neus verteenwoordig moontlik ‘n onderbreking in ontwikkeling van die frontonasale prominensie, ‘n struktuur wat ‘n intieme embriologiese verhouding met die ontwikkeling van die anterior brein geniet. Dus, in opsomming, ondersteun ons bevindinge, in teenstelling met die

gerapporteer in byvoorbeeld Turkse en Afrikaner populasies, nie ‘n beduidende rol vir kromosomale afwykings in die vatbaarheid vir die ontwikkeling van skisofrenie in hierdie populasie nie. Ons kon egter etno-spesifiek morfologiese eienskappe in ons studiegroep aantoon. Te same gesien, onderstreep ons resultate dus die belang van meer nie-Kaukasiese studies in skisofrenie, met morfologiese en genetiese resultate wat duidelik nie bloot oor studiegroepe heen geekstrapoleer kan word nie.

STUDY SYNOPSIS

BACKGROUND: Schizophrenia is a heterogeneous illness resulting from complex gene-environment interplay. The majority of molecular genetic work done has involved Caucasian populations, with studies in these and Asian populations showing 2-32% of sufferers to have chromosomal aberrations. So far the discovery of a specific susceptibility mechanism or gene still eludes us, but the use of endophenotypes is advocated as a useful tool in this search. No cytogenetic studies of this nature have been reported in any African schizophrenia population.

AIM: The aim of the study was to combine genotypic and phenotypic data, collected in a homogenous population in a structured manner, with the hope of characterising an endophenotype that could be used for more accurate identification of individuals with possible chromosomal abnormalities.

METHODOLOGY: A structured clinical interview was conducted on 112 Xhosa schizophrenia patients. (Diagnostic Interview for Genetic Studies, including Schedules for the Assessment of Negative and Positive Symptoms.) Blood samples (karyotyping and/or FISH analysis) as well as urine samples (drug screening) were obtained and nine head and facial measurements were performed. Descriptive statistics were compiled with reference to demographic, clinical and morphological variables. Comparisons between mean differences for these variables were made.

RESULTS: FISH analysis was performed on 110 participant samples, with no chromosome 22q11 microdeletions detected. Fifty participant samples were karyotyped revealing chromosomal aberrations in five (10%). These were: [46,XY,22pss]; [46,XY,1qh+]; [46,XY/47,XXY/47,XX+acentric fragment] and two cases of [46,XY,inv(9)]. No significant differences could be demonstrated between the karyotyping subgroup (KSG) and the sample as a whole for any of the variables reported on. A significant difference for only one morphological measurement, ie glabella to subnasale ($p=0.036$), could be demonstrated between the KSG and the five participants with aberrations.

DISCUSSION & CONCLUSION: The chromosomal aberrations detected in our group were all reported as normal variants. However, the fact that particular aberrations have not yet been linked to schizophrenia does not conclusively mean that they can be disregarded as non-significant. In order to truly exclude a possible link to psychiatric illness, groups of individuals with such variants need to undergo comprehensive psychiatric evaluation in a structured manner. Detection techniques are rapidly becoming more sophisticated and, very importantly, more non-Caucasian samples are being recruited. Our participants with chromosomal aberrations were demonstrated to have, comparatively, significantly longer noses. Craniofacial dysmorphology has been shown to be associated in general terms with abnormalities found to evidence brain pathology in schizophrenia. The lengthening of the nose possibly represents a disruption in the development of the frontonasal prominence, a structure which enjoys the most intimate embryologic relationship with the development of the anterior brain.

In summary, our findings, in contrast to those reported in e.g. Turkish and Afrikaner populations, do not support a significant role for chromosomal aberrations in the susceptibility to develop schizophrenia in this population. However, ethno-specific morphological characteristics could be demonstrated in our sample. Taken together, these results highlight the need for more non-Caucasian studies in schizophrenia, as clearly, morphological and genetic results cannot just be extrapolated across samples.

DEDICATION

I dedicate this thesis to my parents and thank them for their love and encouragement

I would also like to thank my friends for their support not only in this but also at so many other moments over time

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CHAPTER 1

INTRODUCTION – RATIONALE BEHIND STUDY CONCEPT

CONTENTS

1. WHY IS SCHIZOPHRENIA RESEARCH NEEDED?

Schizophrenia is a severe chronic psychiatric disorder with the main clinical features of delusions, hallucinations, disorganised thinking, disorganised behaviour and negative symptoms such as affective flattening, alogia and avolition.[1] It has a prevalence rate of about 1% and although outcomes are variable, the typical course is one of relapses followed by only partial remission. It leads to significant impairment in functioning and social isolation for sufferers and creates a substantial financial burden to society, both directly and in loss of productivity.[2] In spite of the rapid expansion of knowledge achieved in recent years in the fields of neuroscience and behavioural science, many issues surrounding the pathophysiology of schizophrenia remain unresolved. Continued prioritisation of schizophrenia research therefore remains imperative if we hope to not only effectively manage this illness but also, ultimately, to prevent it.

2. SEARCHING FOR SCHIZOPHRENIA GENES

Numerous family, twin and adoption studies have shown in a conclusive manner that schizophrenia has a high familial heritability risk and that genes have a major contributory role in the aetiology of the disorder.[3] In fact, schizophrenia has one of the highest heritabilities (approaching 80%) among the complex genetic disorders, similar to that of type I diabetes mellitus (72-88%) and greater than that of breast cancer (30%) and heart disease in males (57%).[4-6] Specific genes that have been associated with schizophrenia risk in a number of populations

around the world include: catechol-O-methyltransferase (chromosome 22q), dysbindin-1 (chromosome 6p), neuregulin 1 (chromosome 8p), metabotropic glutamate receptor 3 (chromosome 7q), glutamate decarboxylase 1 (chromosome 2q), and disrupted-in-schizophrenia 1 (chromosome 1q).[7]

Studies in Caucasian and Asian populations have revealed that between 2% and 32% of schizophrenia subjects have major chromosomal abnormalities such as translocations, deletions and inversions.[8;9] Indeed, research on this type of developmental insult has yielded some of the strongest risk factors for the development of schizophrenia to date. Specifically, the chromosome 22q11 deletion and the balanced chromosomal translocation [t(1,11)(q42.1;q14.3)] disrupting two genes on chromosome 1 (*DISC1* and *DISC2*) have both been demonstrated to increase the risk for schizophrenia.[10;11]

A microdeletion at chromosome 22q11 is the most frequently known interstitial deletion found in humans, occurring in approximately one of every 4 000 live births, and its occurrence is associated with a characteristic facial dysmorphology, a range of congenital abnormalities and schizophrenia.[12] In fact, the prevalence of psychosis in those with 22q11 deletion syndrome is high (30%), suggesting that haploinsufficiency of a gene or genes in this region may confer a substantially increased susceptibility risk.[13]

Unfortunately, the search for chromosomal loci and genes has been a slow and tedious process, most probably because there are multiple susceptibility genes, each with small effect, which act in conjunction with epigenetic processes and environmental factors.[6] Currently, the number of susceptibility loci, the disease risk conferred by each locus, the extent of genetic heterogeneity and the degree of interaction among all the loci, remain unknown quantities.

Furthermore, schizophrenia shows considerable clinical heterogeneity as reflected by an early description of this disease: "Dementia praecox, a number of disease entities".[14] This clinical heterogeneity arguably reflects the heterogeneous nature of susceptibility factors for schizophrenia. Not only do we find multiple combinations of symptoms existing in individuals but also both disease course and outcome display considerable heterogeneity. Currently, we cannot be certain whether this is a single disorder with different clinical manifestations or in fact a group of syndromes, each with unique or overlapping pathophysiology.

3. THE USE OF ENDOPHENOTYPES IN LIMITING HETEROGENEITY

Considerable attempts have been made to elucidate the heterogeneity of the schizophrenia phenotype by exploring the relationships between the various symptom dimensions and possible subtypes. In the search for susceptibility genes it has became apparent that one possible method would be to consider a

role for endophenotypes. Several approaches have been advocated for endophenotype analysis in schizophrenia. These include demographic variables, clinical symptoms, physical characteristics and neurodevelopmental insults.[15]

Previous work from our research group has led to the publication of both an exploratory and subsequent confirmatory factor analysis and concordance analysis on affected sibling pairs of Xhosa patients with schizophrenia.[16;17] Not only did this reveal a five factor symptom solution similar to that found in Caucasian samples, but specific symptoms (e.g. delusions of control) were delineated. These findings may serve as good markers for developing exploratory clinical subtypes for genetic studies.

Furthermore, one such possible clinical subtype may be linked to the early developmental model for schizophrenia.[18] This model implies that peri-natal insults (including chromosomal rearrangements) will predispose the individual to the later development of schizophrenia. The quantative and qualitative measurement of anthropometric proportions to demonstrate the presence or absence of dysmorphic features (so-called minor physical anomalies (MPAs)) represents one endophenotype that can be used. The presence of MPAs act as biologically-timed markers of developmental disturbance within a foetus and as such craniofacial anomalies are of special interest as the brain and the overlying face both develop from common embryonic ectoderm.[19]

Although some of the current evidence is contradictory and more research is needed, the presence of an excess of MPAs in schizophrenia subjects, in comparison to normal controls, has repeatedly been demonstrated.[20] The use of MPAs as endophenotype fits in well with the neurodevelopmental hypothesis of schizophrenia and seems to be particularly appropriate in our setting as these measurements can not only be done inexpensively, but observers can be relatively easily trained to perform these measurements.

Indeed, our findings of increased concordance of specific morphological abnormalities (linked to crucial brain developmental phases) in affected sibling pairs, make it imperative for us to explore chromosomal aberrations (e.g. breakpoints) that could account for these abnormalities and possibly predispose the individual to the development of schizophrenia.[21] These aberrations may provide candidate loci and unique phenotypes linked to genetic aberrations in the affected region.

4. WHY USE AN AFRICAN POPULATION?

To date, by far the majority of molecular genetic work done in schizophrenia has involved Caucasian populations. In fact, no cytogenetic studies of this nature have been reported in any African schizophrenia population. There is a general paucity of data on schizophrenia, as well as other mental illness, in this group. Taking into account the evidence suggesting ethno-specific loci as well as apparent ethno-specific pharmacological responses to atypical antipsychotic

treatment in African-American and African samples, it seems clear that indigenous African populations also need to be investigated.[22-25]

The Xhosa people are the second largest and southernmost indigenous African grouping within South Africa and belong to the Nguni linguistic grouping.[26] Given the historical and geographical influences that formed this group, the Xhosa population can be regarded to represent a culturally homogenous grouping with an active traditional belief system. Therefore, taking into account the seemingly uniform core symptom profile reported in both Caucasian and African groups (including the Xhosas), as well as the marked paucity of clinical and susceptibility data for Xhosa-speaking schizophrenia patients, this group would seem to present us with an invaluable opportunity for molecular genetic research.[16;25;27-31]

5. SUMMARY

We therefore believe that by combining genotypic with phenotypic data collected in comprehensive clinical interviews, our study could lead to the discovery of unique characteristics that may be used to assist in the more accurate identification of individuals with possible chromosomal abnormalities. Such a discovery would not only help mental health care workers to better understand and care for patients with schizophrenia, but should also lead to the referral of the most appropriate candidates for genetic research in particular. This will increase the future chances of identifying chromosomal breakpoints and other aberrations that could provide candidate regions associated with genetic

susceptibility to schizophrenia in this population. Ultimately, breakthroughs such as these will contribute to more accurate diagnosis of the illness as well as the development of more effective treatment.

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CHAPTER 2

SCHIZOPHRENIA – AN AETIOLOGICALLY HETEROGENOUS SYNDROME

CONTENTS

1. INTRODUCTION

Symptom descriptions suggestive of a diagnosis of schizophrenia date back to pre-classical cultures. However, with the exception of the ancient Greek and later Arabic physicians, societies believed these symptoms could mostly be attributed to supernatural forces. It was only in the late 17th and 18th centuries that the concept of organic etiologies for mental illness was more broadly adopted, leading to the establishment of the modern concept of schizophrenia in the early 19th century. Ultimately, Emil Kraepelin and Eugen Bleuler (who coined the term schizophrenia in 1911), can be credited with finally delineating schizophrenia from other psychoses.[1]

Furthermore, although the 20th century has seen the introduction of the concepts of schizophreniform (1933) and schizo-affective (1939) disorders, after Kraepelin and Bleuler there have been relatively few modifications to the central schizophrenia concept.[1]

Schizophrenia is a severe chronic psychiatric disorder with the main clinical features of delusions, hallucinations, disorganised thinking, disorganised behaviour, and negative symptoms such as affective flattening, alogia and avolition.[2] Current thinking holds schizophrenia to be a syndrome with characteristic symptoms and impairments resulting from multiple causal pathways involving disorders of normal brain function.

Research has revealed a number of well-established predictors of outcome, which include: family history of affective disorder, good premorbid adjustment, affective symptoms, acute onset, early treatment, good response to treatment (positive) and family history of schizophrenia, long duration of untreated psychosis, substance misuse, bizarre delusions, negative symptoms, schizoid traits (negative).[3;4]

Although outcomes are variable, the typical course is one of relapses followed only by partial remission and resulting in significant impairment in social functioning and social isolation.[5] In fact, current evidence would seem to support the notion that less than half of patients with a diagnosis of schizophrenia or schizophrenia spectrum disorders show any substantial functional improvement at five to six year follow-up.[6;7]

2. EPIDEMIOLOGY

Schizophrenia exerts a significant economic burden, both directly and indirectly, (reported to be $1.2 trillion in the USA alone) and it also places a severe emotional burden on both sufferers and their family members.[8;9]

Research by the World Health Organisation (WHO) has shown that narrowly-defined schizophrenia exists in all cultures around the world.[10] However, contrary to the popular belief (also supported by the initial report of the WHO 10-country study), that the illness has a uniformity of incidence, a recent systematic review

by McGrath et al. has concluded that the incidence of schizophrenia in fact shows clear worldwide variation.[11;12] This review included data from 158 studies drawn from 32 countries and showed that, based on conservative estimates, rates for the incidence of schizophrenia fell within a range of 7.7 to 43.0 per 100000, in other words, over a five-fold difference.[11]

This data is further supported by recently published findings of the AESOP study supporting significant and independent variation of incidence of schizophrenia in terms of sex, age, ethnicity and place.[13] Developing an understanding of the factors that contribute to these observed variations in incidence will no doubt be of great value in the ongoing process of discovery surrounding this disabling illness.

3. AETIOLOGY

One of the many challenging aspects of schizophrenia lies in the heterogeneity observed in this illness. The clearly observed variability in symptomatology, course and outcome has long led researchers to suspect an underlying etiological heterogeneity. Broadly, the main developmental models for schizophrenia have been divided into two categories – neurodegenerative and neurodevelopmental.[14]

3.1 NEURODEGENERATIVE

With no direct evidence for neurotoxicity having yet been demonstrated post-mortem, the concept of a neurodegenerative process contributing to schizophrenia remains a controversial topic.

To date, numerous magnetic resonance studies have shown abnormalities in the brain structure of schizophrenia patients.[15] These include decreased grey matter volume and increased ventricular size. This then gives rise to the question as to when these changes first occur? Grey matter changes have been demonstrated in vulnerable individuals prior to onset of psychosis.[16;17] Also, progressive loss of brain volume has been shown in first-episode patients, this feature being particularly prominent in those with a poorer clinical outcome.[18-21]

The cognitive decline associated with schizophrenia has also been suggested to represent a neurodegenerative process with some evidence supporting the duration of initial untreated psychoses to be associated with this decline.[22] However, due to the fact that other factors influencing prognosis can also be linked to speed of and access to treatment, this finding remains debatable.[23] Cognitive testing, although clearly showing abnormalities, has also not tended to support a decline during the first few years following onset of psychosis.[24]

Interestingly, a correlation between the severity of tardive dyskinesia (TD) and that of cognitive impairment has also been reported.[25] Although this could lend

support to the presence of a shared neurotoxic process contributing to both, it could also just indicate poor cognition to be a risk factor for TD. Furthermore, antioxidants, such as vitamin E, have been studied as a potential treatment for TD, but results have been inconsistent.[26-28] A potential role for antioxidants in slowing a proposed neurotoxic component to the illness has not been shown.

Thus, the definitive discovery of a mechanism for a putative neurodegenerative process in the developments of schizophrenia continues to elude us.

3.2 NEURODEVELOPMENTAL

In contrast to the neurodegenerative model, considerably more evidence has consistently been put forward in support of the neurodevelopmental etiology of schizophrenia. These include many risk factors, both environmental and genetic, and at least some of them could offer us pointers toward developing rational preventions for the illness. The remaining part of the chapter will be dedicated to a summary of the salient points with regard to each of the risk factors that are currently regarded to show most promise for inclusion in a neurodevelopmental model.

3.2.1 PRENATAL

3.2.1.1 NUTRITIONAL DEFICIENCY

Some evidence suggests that nutritional deficiency during pregnancy may play a role in the origin of some cases of schizophrenia.[29] Due to the existence of comprehensive records, interesting data was obtained from the so-called Dutch Famine period (1944-1945) at the end of World War II. For this cohort, early prenatal famine could specifically be associated with each of three conditions: (1) congenital anomalies of the central nervous system, (2) schizophrenia, and (3) schizophrenia spectrum personality disorders.[30] In fact, the risk for schizophrenia was demonstrated to increase two-fold in offspring exposed to famine.

Similar results have been demonstrated in a Chinese cohort exposed to a massive famine period from 1959 to 1961. Among births that occurred during the famine years, the adjusted risk of developing schizophrenia in later life increased significantly, from 0.84% (1959) to 2.15% (1960) and 1.81% (1961).[31]

Possible mechanism 1: Vitamin D (Vit D) insufficiency has been proposed as one possible explanation for this phenomenon. Recently some data have suggested a neuroprotective role for Vit D.[32] The most active metabolite of Vit D3, 1,25-dihydroxy-vitamin D ($1,25(OH)_2D$) is a hormone with multiple roles, in particular genomic stability, and there is growing evidence that low prenatal levels of it can influence critical components of brain development.[33] Furthermore, it has been

demonstrated that supplementation with Vit D in the first year of life could be associated with a greatly diminished risk for schizophrenia in males.[34]

Possible mechanism 2: Another hypothesis is that of DNA hypomethylation in which either stress and/or nutritional factors are postulated to modify DNA transcription.[35;36] DNA methylation is an essential epigenetic mechanism, modulating gene expression during cellular differentiation as well as being essential for the expression of imprinted genes. In humans, DNA methylation involves mainly the addition of a methyl group to the 5' position of cytosine within Cytosine-phosphodiester-Guanine dinucleotides.[35] These methyl groups are supplied either from diet (e.g. folic acid or Vitamin B12) or synthesised from one-carbon metabolism. It is therefore suggested that the folate deficiency that has been reported in patients with schizophrenia could possibly be responsible for an increased risk via this mechanism.[37-39] As pregnancy burdens maternal folate reserves, it could also possibly account for the suggested association between shorter birth intervals and increased schizophrenia in offspring.[40]

3.2.1.2 VIRAL INFECTIONS

It has long been debated whether there is a possible role for certain viral infections contributing to the risk of developing schizophrenia. Whilst a number of studies have shown increased rates of schizophrenia after maternal exposure to influenza, other infections including rubella, the poliovirus and the herpes simplex virus, have shown less robust associations.[41-46]

However, the mechanisms by which these infections might lead to schizophrenia have yet to be well-delineated.[42] Potential mechanisms include teratogenic effects of maternal antibodies on neurodevelopment and a surge in circulating cytokines. Interestingly, recent animal models have suggested that influenza and immune activation do have effects on the foetal brain that appear to be concordant with findings observed in schizophrenia.[47]

To date, no consistent evidence of viral markers in the cells of schizophrenia sufferers have been identified. Therefore, the evidence remains circumstantial. Still, could the link be proven, data from, for example, Brown et al., would suggest that as many as 14% of schizophrenia cases would not have occurred if influenza infection during early to mid-gestation had been prevented.[41] This may have significant implications, given the numerous preventive strategies available for influenza and other infections, including vaccination, antibiotics, and simple hygienic measures.

3.2.2 OBSTETRIC-RELATED EVENTS

A significant body of literature exists to support a link between obstetric complications/events and an increased risk for the development of schizophrenia. Currently, the pooled odds ratio of this effect is estimated to be about 2.0.[48] In their comprehensive meta-analysis Cannon et al. separated obstetric complications into three categories that could be correlated to increased

risk for development of schizophrenia – complications of pregnancy, complications of delivery and abnormal foetal development.[49]

3.2.2.1 COMPLICATIONS OF PREGNANCY

Definite increased vulnerability for psychosis has been demonstrated for pre-eclampsia, diabetes, rhesus incompatibility and severe bleeding. Although the mechanisms by which these factors influence susceptibility remain unclear, some theories have received strong support. These include: abnormal foetal blood-flow with resulting hypoxia (pre-eclampsia, bleeding), abnormal glucose metabolism (diabetes), autoimmune process (diabetes, rhesus) and even the so-called uterine rejection of the “schizophrenia-vulnerable” foetus (bleeding).

3.2.2.2 COMPLICATIONS OF DELIVERY

Associations have been demonstrated for asphyxia, emergency caesarean sections and the atonic uterus. As such, neurotoxic effects as a consequence of foetal hypoxia are thought to be the possible common mechanism by which increased vulnerability to schizophrenia is conferred by these complications. With regard to this category it is of course important to remember that delivery complications could in fact be due to pre-existing problems during pregnancy.[50]

3.2.2.3 ABNORMAL FOETAL DEVELOPMENT

A fairly consistent association with low birth weight has been demonstrated and as there is no clear association between this and prematurity, intrauterine growth retardation has been postulated as a contributing factor. However, as women with schizophrenia tend to exhibit an increase in other behaviours associated with adverse effects in the foetus (e.g. smoking), this theory remains controversial. An association with reduced head circumference at birth, once again independent of prematurity, has also been demonstrated but until now no clear evidence has emerged to support either a genetic component or early somatic trauma in the development thereof.

Interestingly, there is some evidence from animal studies to show that caesarean section and perinatal hippocampal damage (such as associated with hypoxia and prematurity) can facilitate the development of dopamine sensitisation once the animal matures, therefore providing a possible link to the development of schizophrenia.[51;52]

In summary, as it is well-known that obstetric complications occur commonly in the general population and the vast majority do not lead to the development of schizophrenia, it would be fair to say that these complications are neither necessary nor sufficient causal factors for schizophrenia. However, as obstetric complications remain one of the best-replicated ‘‘environmental’’ risk factors for schizophrenia it is clear that they form a part of the causal pathway for at least

some individuals and as such should remain strongly represented in our quest to elucidate the causal mechanisms and gene-environment interactions leading to this complex disorder.

3.2.3 SUBSTANCE USE

The possible link between substance use and increased schizophrenia risk has long been acknowledged.[53] The majority of the research has focused on attempting to delineate the relationship between cannabis, psychosis and schizophrenia. Multiple explanations have been offered for the observed increased rates of cannabis use in schizophrenia. These range from simple self-medication to dull early symptoms to postulating a possible role for cannabis as trigger mechanism for illness onset in vulnerable individuals.[54] Interestingly, there is also some evidence showing that in experiments where cannabis was administered to healthy volunteers under controlled laboratory circumstances, a broad range of transient symptoms, behaviours and cognitive deficits resembling some aspects of schizophrenia could be produced.[55]

With a number of cohort studies, such as the Dunedin study, now having shown that cannabis use usually predates psychosis, there is consistent evidence to support a role for an association with cannabis.[56] In fact, the Dunedin study, even after controlling for many potential confounding factors, showed a four-fold increase in rates of schizophreniform disorder by age 26 for individuals using cannabis at age 15.[57] Furthermore, a meta-analysis by Henquet et al. revealed

that the use of cannabis could be associated with nearly a doubling in risk of schizophrenia.[58]

However, as the majority of cannabis users do not later develop a psychotic disorder it remains patently clear that the relationship is not a straightforward one. In fact it seems likely that other risk factors interacting or acting with cannabis may be necessary for the final outcome. One possibility could be that there is a great variation in individual sensitivity for the psychosis-inducing effects of cannabis. At least two independent studies have supported this theory by using subtle measures to determine levels of expression of psychosis proneness.[58;59]

Caspi et al., also studying the Dunedin birth cohort, presented the first evidence of a gene by environmental interaction predisposing schizophrenia.[60] They demonstrated that a functional polymorphism in the catechol-O-methyltransferase (COMT) gene (enzyme essential in breakdown of dopamine in prefrontal cortex) moderates the influence of adolescent cannabis use. Users homozygous for the high activity Val allele showed an at least five-fold increased risk of developing schizophreniform disorder in comparison to those with Met/Met (odds ratio 1.1) and Val/Met (odds ratio 2.5) status. PET and post-mortem studies have indicated that the Val allele is associated with markedly increased dopamine synthesis in midbrain neurones projecting to the ventral striatum.[61] Furthermore, it has been shown that cannabis markedly increases dopaminergic

neuronal firing and that cannabinoid receptor (CB1) agonists increase the release of dopamine at terminal fields in the striatum and prefrontal cortex.[62] Taking these two pieces of evidence into account, this could in fact explain why Val/Val individuals are more susceptible to the psychotogenic effects of exogenous cannabis.

The psychostimulant methamphetamine has also received some attention. Not only is it well-known that use of this substance can induce a syndrome in many ways identical to schizophrenia, but an interaction between methamphetamine abuse and premorbid schizotypal personality traits as a measure of personal vulnerability for psychosis, has also been reported.[63] Furthermore, with some preliminary data seeming to suggest that schizophrenia risk may be increased with use, this area clearly warrants further study.[64]

3.2.4 DEMOGRAPHIC FACTORS

3.2.4.1 FAMILY HISTORY

Numerous family, twin and adoption studies conclusively support the notion that genetic factors play an important role in influencing susceptibility to many adult psychiatric disorders. General estimates of heritability can be calculated from twin studies and these have been shown to be high, comparable to (e.g. diabetes) or higher than (e.g. heart disease in males) that of other complex

disorders.[65] See chart 1 for a comparison of some of these heritability percentages.

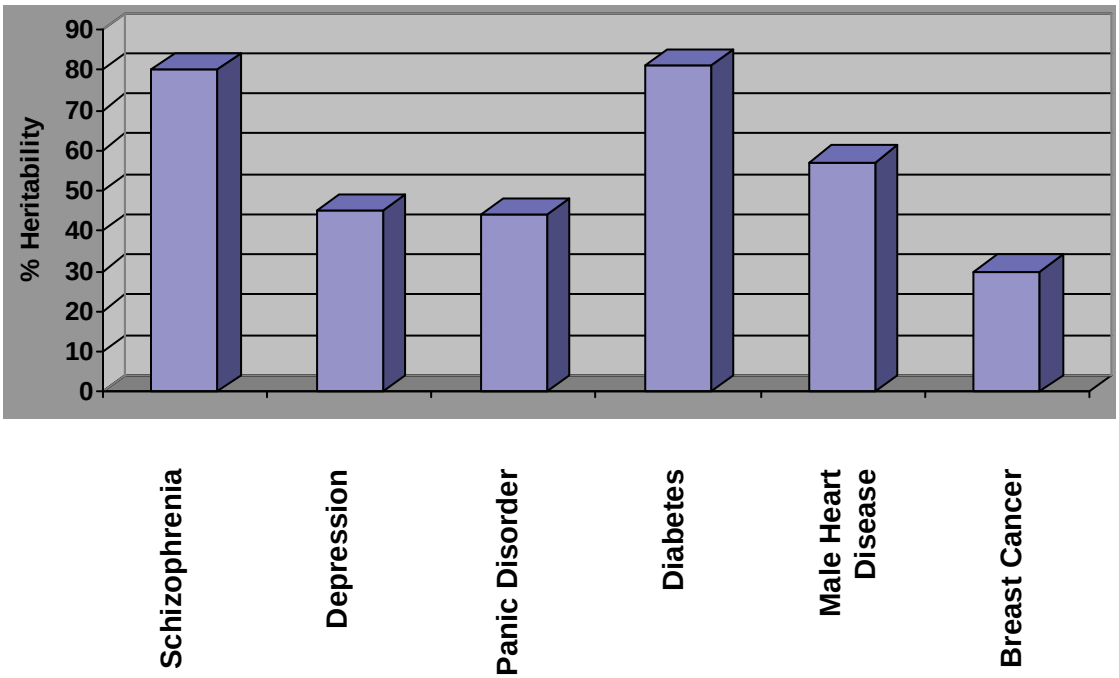

CHART 1: COMPARISON OF HERITABILITIES BETWEEN SOME PSYCHIATRIC AND COMPLEX MEDICAL DISORDERS

Schizophrenia has one of the highest heritabilities with a 10-fold increase in risk to siblings of probands. However, as heritability is not 100% and an estimated 85% of schizophrenia sufferers have no first-degree relative with the illness, we are left with the suggestion that at the heart of this illness there are probably several susceptibility genes working in conjunction with epigenetic and environmental factors.[66] The reader is referred to the chapter on chromosomal aberrations where the topic of genetic vulnerability to schizophrenia is discussed in more detail.

3.2.4.2 SEASON OF BIRTH

Nearly 300 studies have looked at season of birth as a risk factor for the development of schizophrenia.[67] The majority of the data comes from the northern hemisphere (NH) and systematic meta-analysis of NH data supports an association between excess winter/spring births and an increased risk for schizophrenia in later life.[67;68] Although the seasonality effect has not been so clearly validated by Southern Hemisphere data, season of birth has also been associated with different subtypes of schizophrenia, differences in prognosis, demographic factors and clinical presentation.[69-71] In a study from our own group on a previous Xhosa schizophrenia sample, we demonstrated a spring excess of 4% in birth rate compared to the general Xhosa population.[72] Furthermore, patients born in autumn/winter were more likely to have avolition/apathy than those born in summer/spring.

Several possible reasons for the seasonality of schizophrenia births have been proposed, including, but not limited to, light variations, nutrition, infective processes, genetic factors and environmental toxins.[68;73] Although seasonality confers only a small increased risk and the mechanism for this is currently still unknown, evidence seems to suggest that this observation is unlikely to be due to chance.

3.2.4.3 URBANICITY

As far back as 1939, Farris and Dunham published data showing that the rate of schizophrenia was higher in urbanised areas.[74] Since then, a number of well-designed studies have supported the notion that those born or brought up in cities have an increased risk (possibly close to twofold) of developing schizophrenia in comparison to those born and/or brought up in rural regions.[11;75;76] Evidence supporting an especially greater risk for those having lived for a greater number of years in an area with a higher degree of urbanisation has also been presented.[77] Studies have suggested that the increased risk is not confined to a diagnosis of schizophrenia alone but that it can also be observed for subtle psychosis-like phenomena.[78;79] This effect was demonstrated to be independent of numerous important variables, including, but not limited to, the rate of psychotic disorders, ethnic groupings and drug use.

Currently the mechanism for the increased risk linked to urbanicity is not known. Numerous hypotheses such as greater facilitation of the transmission of infections, diet toxin exposure, pollutants, increased health risk behaviours (e.g. drinking, other substance abuse) and obstetric complications have been put forward, but none been conclusively proven.[80] However, there is some consensus that the kind of geographical variation in incidences associated with urbanization, is in support of an environmental effect that has its impact through continuous or repeated exposure.[77;81] Furthermore, it seems most likely that this effect is exerted during childhood and adolescence and not in adulthood.[76;82]

With only a small minority of those living in urban areas developing schizophrenia, it would however seem that any environmental effect would need to be conditional on another factor. With some data in support of a link between urbanicity and pre-existing indicators of genetic risk for psychosis, it could ultimately be that this factor boils down to a gene-environment interaction.[83;84]

3.2.4.4 GENDER

Some controversy still exists regarding the possible influence of gender in the development of schizophrenia. For some time the view most commonly held, was that there was either no gender difference in terms of lifetime risk for developing the disorder or that the evidence was inconclusive.[85] However, two recent independent large systematic reviews, each using a different summary method, have both reached the conclusion that the incidence of schizophrenia is significantly higher in men than in women.[11;86]

Aleman et al. included studies published between 1980 and 2001 whilst McGrath et al. included the date range from 1965 to 2001.[11;86] Both reviews found that the overall male:female risk ratio could be demonstrated to be 1.4 and that the difference could not be accounted for by methodological factors related to age range or diagnostic criteria.

A substantial body of literature exists to support gender differences in the way schizophrenia presents.[87] Of these, the finding of an earlier age of onset for

males is probably the most commonly known. Evidence shows this finding to exist irrespective of culture, definition of onset or definition of illness.[88-91] Furthermore, women have been shown to demonstrate a relative risk of late onset schizophrenia two to three times greater than that of men.[92;93] Interestingly, the age of onset difference appears to apply only to sporadic and not to familial cases of schizophrenia.[94]

Considerable support has also been shown for numerous other gender differences, including, but not limited to, poorer premorbid functioning, more negative symptoms and cognitive deficits and greater structural brain and neurophysiological abnormalities (males) and more affective symptoms, a more favourable short and middle-term course of illness and less smoking and substance abuse (females). As a comprehensive overview of these is beyond the scope of this chapter, the reader is referred to an excellent review by Leung and Chue for more on this topic.[87]

Interestingly, no clear sex differences in family history, obstetric complications, minor physical anomalies or neurological soft signs have yet been demonstrated.[87] Taking this into account, it can be postulated that the neurodevelopmental model of schizophrenia most likely accounts for the majority of cases in both males and females. With males seemingly more prone to this model, estrogen may play a prominent role in the explanation of some of the gender differences.[95;96] Estrogen has been shown to have neuroprotective

effects and protects against necrotic neuronal death.[97;98] Since most schizophrenic illnesses manifest themselves after adolescence, the neural processes which occur during puberty, including intensive synaptic pruning, may play a significant role. Estrogen affects synaptic pruning, which occurs intensively during adolescence, in a sex-specific manner and may contribute to less aberrant pruning in females versus males.[99]

Recently, it has also been demonstrated that normal males respond to an amphetamine challenge by releasing more striatal dopamine than normal females.[100] Possibly this greater sensitivity of the dopamine system in males renders them more likely to develop the striatal hyperdopaminergia that underlies psychosis. Further studies in the area of gender differences are necessary, with the hope of developing individualized preventative and treatment strategies for both males and females with schizophrenia.

3.2.4.5 MIGRANT STATUS

Ödegaard first reported in 1932 on the observed phenomenon of an increased prevalence of psychosis in Scandinavian immigrants to the USA.[101] Since then much data has emerged, confirming the increased incidence of schizophrenia in immigrants. In their 2005 meta-analysis, Cantor-Graae and Selton included 18 rigorously selected studies, including both first and second-generation migrants and calculated that overall, immigrants were 2.9 times more at risk for developing schizophrenia than the host population.[102] This increase in risk was similar in

both genders but was significantly increased for migrants from countries were the majority of the population was black.

Furthermore, the increased risk is not restricted only to young adults; it has also been observed both for very late onset schizophrenia-like psychosis and in the 6-18 years age group.[103;104] Although much less studied, literature also seems to support increased rates of mania and bipolar disorders, as well as discrete psychotic symptoms (i.e. hallucinations) in migrant populations.[105-107] The increased risk has also been noted in second generation and very young immigrants.[102;108] Taking all of this into account, as well as the reported normal incidence rates for schizophrenia at the migrants' points of origin, it becomes clear that Ödegaard's initial suggestion of selective migration as a causal link cannot be regarded as the sole explanation for these findings.[101;109;110]

Other possible suggestions have been urbanisation, social defeat and adversity as well as stressful life-events.[111-114] Although some of these in themselves are associated with an increased risk for developing schizophrenia (see elsewhere in this chapter), current available evidence cannot support any of these to fully explain the magnitude of the observed immigration effect.

Interestingly, Vitamin D insufficiency (see elsewhere in chapter), has also been suggested to be the culprit in the increased psychosis risk observed in especially dark-skinned immigrants.[37] Vitamin D is mainly synthesized in the skin by the

ultra-violet component of sunlight and fortified by milk or margarine. It is therefore hypothesized that due to the fact that individuals with darker skins require longer sun exposure – as well as the evidence that subjects of African descent are often found to be lactose intolerant – such individuals are more prone to develop a Vitamin D insufficiency.[115] In support of this a number of studies have been published providing proof of Vitamin D insufficiency, particularly in African-American women.[116-118]

However, the increased rate of psychosis is not only observed in dark-skinned immigrants. Dealberto has therefore postulated a possible role for DNA methylation, (see elsewhere in chapter) specifically in light-skinned populations.[37] One possible mechanism for DNA hypomethylation is that of impaired one-carbon metabolism. The enzyme mehtylenetetrahydrofolate reductase (MTHFR) has been implicated in this. Two recent meta-analyses suggested that the TT genotype of MTHFR inferred a greater risk for developing schizophrenia than the CC genotype.[119;120] Those studies included in the meta-analyses, reporting on ethnicity, observed that this variant of MTHFR was very infrequent in subjects of African ancestry, leading DeAlberto to hypothesize the link to the increased risk for psychosis observed in light-skinned populations.[37]

3.2.4.6 SOCIO-ECONOMIC STATUS

An association between lower socio-economic status (SES) and schizophrenia has been suggested by a number of studies connecting the illness with being

born into, or raised in, an impoverished environment, with a recent meta-analysis reporting this effect to be at least modest in size.[121] In fact, research seems to suggest that this is true not only for schizophrenia but for other severe mental illnesses as well.[122]

A longstanding debate exists as to whether this phenomenon is indicative of "social stress" or "social drift". In other words, whether lower SES precedes schizophrenia or whether lower SES leads to schizophrenia. Studies have found both these theories to demonstrate some validity, but in different populations. [123]

Focusing on the "social stress" theory, likelihood of exposure to a number of other risk factors that are associated with schizophrenia (as discussed elsewhere in this chapter), such as poor nutrition, poor prenatal health care and stressors such as inadequate housing and limited job prospects, is definitely increased in lower SES groupings.

3.2.5 EXPOSURE TO STRESS

Stress is widely accepted to be a contributor to the pathogenesis of psychosis and schizophrenia in particular.[113;124] Animal studies have supported a role for social adversity as a risk factor with data showing, amongst other findings, that a submissive position in social hierarchy increases the reactiveness of the mesolimbic dopamine system and that housing a mouse with a large "bully"

mouse, can induce brain-derived neurotrophic factor (which controls the dopamine system) in the former.[125;126]

However, despite the prominence of the stress-diatheses model, life events literature has provided inconsistent conclusions in relation to schizophrenia, with no particular pattern or type of event being consistently related to subsequent illness development. A detailed discussion of this literature is well beyond the scope of this chapter and the reader is referred to an excellent review by Gispen-De Wied.[127]

In terms of a possible causal link, some authors have suggested that previous exposure to major life events modified subsequent emotional reactions to minor stress, thus cumulatively increasing the risk of emotional dysfunction, which in turn may than open up direct emotional pathways to psychotic experiences.[128-130]

As stated elsewhere in this chapter, DNA hypomethylation, in which stress or nutritional factors are postulated to modify DNA transcription, has also been put forward as a possible hypothesis.[35;36] Currently however, this theory is not yet backed by any evidence directly linking stress to impaired DNA methylation.

3.2.6 AUTOIMMUNE DISEASE

Associations with either higher or lower than expected prevalence have been reported for both schizophrenia patients and their relatives for a number of

autoimmune disorders, including rheumatoid arthritis, type 1 diabetes, thyroid disorders, and celiac disease.[131-134] To date, the most consistent finding in the area of schizophrenia and autoimmune diseases is that of the negative relationship with rheumatoid arthritis.[133;135;136]

Recently, Eaton et al. used the Danish Psychiatric Register to gather data on 7704 schizophrenia patients.[137] They found that a history of any autoimmune disease was associated with a 45% increase in risk for schizophrenia. In particular, thyrotoxicosis, celiac disease, acquired hemolytic anemia, interstitial cystitis, and Sjögren's syndrome had higher prevalence rates among patients with schizophrenia and their family members in comparison to controls and their family members, respectively.

One of the possible hypotheses for these findings is that schizophrenia shares a genetic diathesis with the family of autoimmune diseases, For example, association studies have highlighted the role of HLA genes for certain autoimmune diseases.[138;139] The epidemiologic association between these disorders could therefore be a result of 1) direct involvement of HLA antigens or 2) physical closeness between loci for the autoimmune disorders and schizophrenia loci in HLA regions. Outside the HLA region, the search for variants for common autoimmune diseases has not, as yet, suggested many clusters related to schizophrenia.[140] However, there is some possible evidence such as linkage studies suggesting that schizophrenia and celiac disease may

have genes that are close to each other or identical and separate association studies have connected the methylenetetrahydrofolate reductase (MTHFR) gene to schizophrenia and to rheumatoid arthritis.[141-144]

4. SUMMARY

The aim of this chapter was to provide a brief overview on each of the topics discussed therein in order to introduce the reader to the difficulties inherent to studying a complex heterogenous disorder such as schizophrenia. It was not meant as a comprehensive summary and for further reading the reader is referred to the various texts referenced on each of the individual topics.

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CHAPTER 3

USING THE INVALUABLE RESEARCH OPPORTUNITIES INHERENT TO A CULTURALLY HOMOGENOUS POPULATION

CONTENTS

1. INTRODUCTION TO CULTURE AND MENTAL HEALTH

Western medicine is rapidly expanding and the scientific treatment of mental illness has shown great advances in recent decades. Even so, interest in non-western or indigenous forms of healing is probably higher than ever with calls for the integration of the best of both worlds into health services. In the field of mental health, such views are in part driven by the belief (supported by a large body of literature) that the way mental illness is experienced, understood and managed, finds different expression in different cultures.

Webster's Dictionary broadly defines culture as "the concepts, habits, skills, art, instruments, institutions etc. of a given people in a given period; civilisation."[1] However, ultimately, culture cannot be static and Helman's view of culture as "a set of inherited guidelines used to view and emotionally experience the world and shape behaviour towards it and others in it", probably lends itself more to defining our understanding of the concept within the context of mental illness.[2]

It is not within the scope of this chapter to provide a detailed discussion on the topic of cross-cultural psychiatry and for such an analysis it is suggested that the reader refer to the text, "Culture and mental health: a Southern African view" by Leslie Swartz.[3] Although standardised "western" criteria such as the DSM-IV-TR has no doubt brought improved consistency to psychiatric patient management, a blanket application of such a theoretical framework may downplay socio-cultural influences on nosology.[4;5] Suffice to say, that in a multi-cultural society such as

our own, all mental health care practitioners aiming to provide comprehensive care, need, on the most basic level, to at least cultivate an awareness of the concept and its potential impact.

2. THE XHOSA – CONTEXTUAL HISTORY

A considerable amount of research has been conducted into the origins and subsequent dispersal of African-language peoples across Sub-Saharan Africa and, in fact, numerous different models describing African migration routes exist.[6] However, there are some points of consensus. Most likely, from a nexus in West Africa (between Nigeria and Cameroon), a series of migrations occurred starting conservatively around 5,000 years ago. This ultimately resulted in the formation of an Eastern African core around 3,000 years ago, from which further expansions saw the initial colonisation of southern Africa during the first millennium AD.[7] Since then, as productive subsistence economies fuelled population growth, a considerable number of subgroups, under different leaders, spread throughout southern Africa.[7;8] Today these subgroups can be seen as local populations linked together based on linguistic, cultural, and historical evidence, e.g., Natal Nguni (Zulu and Swazi), Cape Nguni (Xhosa), and Sotho (Southern Sotho and Tswana).

The Xhosa people are the second largest African culture group within South Africa and belong to the Nguni linguistic grouping.[9] They are the southernmost indigenous African people and as a homogenous population with an apparently

common ancestry, they offer a unique opportunity for the study of an illness such as schizophrenia.

Archaeological evidence indicates a possible Xhosa presence in the Eastern Cape as far back as the seventh and eight centuries. However, it was only in the last quarter of the eighteenth century that the settler ("trekboer") advance into the South African interior began approaching the outermost fringes of the Xhosa settlements, leading to first large-scale contact with western civilisation.[9]

Genealogical records take us back to the fifteenth century after which internal revolutions led to the Xhosa kingdom's political fragmentation. However, cultural norms have remained largely intact as evidenced by comparisons between more current descriptions and those from early shipwreck survivors.

The Eastern Cape was the region of the first continuous contact between the colonists and the Xhosa. The protracted so-called Xhosa wars that followed had tremendous longterm social and political impact in the shaping of the transformed South African political landscape.

Prior to democracy, the nature of the socio-political situation in South Africa was in many ways responsible for in the Xhosa people's relative geographic isolation in homelands in the Eastern Cape and townships in major cities. Ultimately, this

has ensured that the population displays a relatively homogenous cultural constitution.

3. SCHIZOPHRENIA AND THE XHOSA CULTURE

3.1 BACKGROUND

From the outset, descriptions of the Xhosa people refer to a hospitable society, disposed to peace rather than war, exhibiting a fierce loyalty to their chiefs. The hospitable responses were strongly affected by the common emphasis placed on group rather than individual interests ("ubuntu"). "Ubuntu" underlined the basis of social law with the primary object being the preservation of tribal equilibrium.[9] Another aspect underlying their unique cultural norms, centres on a strong belief in supernatural powers and specifically ancestor reverence, which directly influences how illness (physical as well as mental) is viewed.[10] It was the pioneering work of Vera Buhrmann that first emphasised the importance of ancestral reverence as part of the Xhosa cultural belief system.[11] Work like hers finally allowed the broader society some insight into the significance of ancestral appeasement rituals and how misunderstanding of them could lead to the loss of ancestral protection and ultimately even precipitate mental illness.

These cultural norms greatly influenced the experience and interpretation of symptoms as well as health-seeking pathways. Clearly this will impact on data gathering and results of studies conducted within a unique cultural grouping such

as the Xhosa. As previously mentioned, a full discussion on this falls outside the bounds of this chapter and for our purpose we will therefore focus on these influences specifically within the context of managing schizophrenia in the Xhosa population.

3.2 XHOSA CULTURAL BELIEFS WITH REGARD TO SCHIZOPHRENIA

As part of a world-wide move to a better understanding of the causes and presentation of mental illness, the concept of "culture-bound syndromes" was incorporated into the broader discussion. The term refers to any one of a number of recurrent, locality-specific patterns of aberrant behaviour and experiences that appear to fall outside of the conventional Western psychiatric diagnostic categories.[5] Within a local context, these illness patterns are considered to represent specific diagnostic entities and most have local names.

None of the culture-bound syndromes described within the South African context have been included in the DSM-IV-TR, although the so-called "brain fag" (first described in West African literature) has some overlap with "isimnyama eskolweni".[3] Nonetheless, the two Xhosa illness-terms often used to describe behaviour that correlates to or can be viewed as schizophrenia-like symptoms, "amafufunyana" and "thwasa", are considered to be well-recognised cultural phenomena found in the indigenous African Xhosa population.[3;12;13]

"Amafufunyana" is broadly viewed as a negative possession state that is commonly associated with mental illness. Original descriptions attempted to outline a list of typical symptoms with emphasis placed on the hysterical, strange and unpredictable behaviour of the sufferer, also characterised by him/her speaking in a strange muffled voice in an incomprehensible language. Although overlap apparently existed with schizophrenia, it was viewed as a condition without a clear Western equivalent that could not be fitted into recognised Western classification systems.[11;14]

"Thwasa" is regarded as a more positive state and within this context Buhrman clearly distinguished it from bewitchment.[14] The condition is commonly described to consist of irritability, social withdrawal and auditory hallucinations and within the cultural context is regarded as the state of emotional turmoil a person experiences when called to become an indigenous healer. The belief holds that resisting such a calling from the ancestors may lead to illness whereas answering the call leads to receiving "special powers".[3]

In order to improve our understanding of cultural attitudes to health seeking behaviour, it would be invaluable to have insight into how these traditional illness models ("amafufunyana" and "thwasa") are viewed and applied. Traditional healers play an important role in many cultures (including the Xhosa) and it is not uncommon in many societies for patients and/or families to first seek help from them prior to making contact with "Western" medicine.[15;16]

Niehaus et al. were the first to attempt to systematically study the frequency by which “amafufunyana” and “thwasa” are used by traditional healers to explain schizophrenia in the Xhosa population.[17] The authors recruited 247 subjects (62 female and 185 male) with a DSM IV-diagnosis of schizophrenia who were then subdivided into 3 groups, i.e. an "amafufunyana"-group, a "thwasa"-group, and a group of patients with diagnoses other than "amafufunyana"/"thwasa". The division was done on the basis of a structured interview regards the use of traditional treatment and diagnostic methods. Two-hundred (80.97%) of the participants had used such services and could be included in the analysis. Of these 106 were diagnosed with “amafufunyana” and only 9 with “thwasa”.

The comparison between the two groups yielded no significant differences for either demographic characteristics or for the presence/absence of any of the core symptoms of schizophrenia. Interestingly, however, both a family history of schizophrenia ($p=0.004$) and of other psychiatric disorders ($p=0.008$) was significantly more common in the “thwasa” group. See table 1 for a comparison of some of the phenomenological characteristics of the two groups.

As previously stated, a positive view is more often attached to “thwasa” in comparison to “amafufunyana” within the cultural context. Taking this into account, the authors concluded that one possible explanation for their finding of family history being more significantly present in “thwasa” patients, was that psychotic symptoms could in their case be seen as an inherited “giftedness”

rather than a sporadic illness. It would be important to note that although “thwasa” is not regarded as an illness in the Xhosa culture, in the case of this dataset, no difference could be shown between the two groups with regard to the core symptoms of schizophrenia.

TABLE 1: COMPARISON BETWEEN "AMAFUFUNYANA" AND "THWASA" GROUPS

PHENOMENOLOGICAL CHARACTERISTICS	"AMAFUFUNYANA" GROUP (n=106)	"THWASA" GROUP (n=9)	GROUP DIFFERENCES (STATISTICAL SIGNIFICANCE)
Family history of schizophrenia	50.9*	100	χ^2=8.059; p=0.004
Family history of other psychiatric disorders	14.2	55.6	χ^2=9.899; p=0.008
Bizarre or aggressive behaviour	82	88.9	NS
Affective changes:			
Restricted	47.2	77.8	NS
Blunting	65.1	55.6	NS
Inappropriate	16.9	44.4	NS
Persecutory delusions	94.3	77.8	NS
Bizarre delusions	37.7	33.3	NS
Hallucinations (any)	80.2	88.9	NS

NS - No significant difference detected; significance at p<0.05

* - All values are percentages

Interestingly, in comparison to the “amafufunyana”/”thwasa” groups those in the group that had received neither of these diagnoses were significantly more likely to be married (p=0.004), live in rural environment (p=0.007), have a history of an identifiable stressor prior to onset of illness (p=0.026) as well as a history of

cannabis abuse seeming to predate onset of psychotic symptoms (p=0.015). The authors suggest that these differences could possibly be explained by the presence of another more apparent reason (e.g. stressor, substance) for the illness or that a traditional diagnosis was possibly less likely in patients with higher premorbid functioning (e.g. married).

International literature has shown that lay public misconceptions regarding the causes and treatment of schizophrenia exist worldwide.[18-21] However, these seem to differ among different cultural groupings. Whilst studies in Germany have found that the public believes schizophrenia to be mainly caused by intra-psychic, biological or psychosocial stressors, Mbanga et al. demonstrated dissimilar findings in their study conducted amongst the Xhosa population.[19;22;23]

For the purposes of the above study, a trained psychiatric nurse interviewed 100 caregivers or close family members of Xhosa schizophrenia patients, administering a structured questionnaire focusing on participants' views regards the causes, treatment options and course of illness. The mean age of the participants was 61.1 (±13.0) years, 76% were female, 59.2% mothers, 21.4% fathers and the mean years of schooling 6 (±3.5). Respondents attributed the development of schizophrenia to varied number of causes (see table 2).

Although some of the so-called more "biological" or "Western" causes such as "brain disease" (46%) or "stressful life-events" (38%) were also endorsed,

witchcraft or evil spirits (67%) were most commonly reported. With many respondents also endorsing more than one trigger, the results suggest a complex explanatory model for schizophrenia in the Xhosa population, with several of the suggested causes linking to cultural beliefs such as the phenomena of witchcraft as well as not breaking with traditional values.

TABLE 2: PERCEIVED CAUSES OF SCHIZOPHRENIA AS REPORTED BY 100 CAREGIVERS OR CLOSE FAMILY MEMBERS OF XHOSA SCHIZOPHRENIA PATIENTS

	Yes	No	Unsure
Family relationship problems	12*	46	42
Work difficulties	13	68	19
Stressful events	**38**	40	22
Brain disease	**46**	41	13
Heredity	34	50	16
Lack of will power	10	72	18
Expecting too much of oneself	14	82	4
Unconscious conflicts	3	84	3
Being brought up in a broken home	25	59	16
Lack of parental affection	31	54	15
Over-protective parents	19	75	6
Loss of traditional values	29	56	15
Loss of a natural way of life	3	84	13
Will of God	31	49	20
Witchcraft, evil spirits	**67**	18	15
Being poisoned	**37**	48	15
Signs of the Zodiac	2	52	46

*** - All values are percentages**

Another aspect central to Xhosa culture, the traditional initiation ceremony ("umkhwetha"), also seems to influence the onset and course of schizophrenia in some cases. "Umkhwetha" represents a rite of passage for boys entering manhood and the aim of this essential process is to learn of the responsibilities and expectations of adulthood.[10;24;25] Recently, Le Roux et al. interviewed 75 Xhosa males diagnosed with schizophrenia to examine their perceptions of the role of initiation in the onset and course of their illness. In all, 10.7% perceived the initiation rites as a stressful event that had triggered the onset of a psychotic episode, and 8% felt it precipitated a relapse. These findings once again underline the importance of understanding the cultural background of patients. [26]

A further topic of considerable importance concerns the actual use of traditional treatment methods. Bhat and Jacobs conducted an intensive survey on the general use of traditional treatment methods amongst the Xhosa people in the Eastern Cape over a two-year period.[27] They concluded that use of traditional medical practices could be divided into four categories: (1) Use of common remedies not followed by rituals, mostly practiced by common villagers; (2) Use of remedies considered to be family secrets handed down from one generation to the other (herbalists); (3) Use of remedies by traditional doctors (known as "amagqirha" in Xhosa) who keep in contact with their ancestors and who divine the cause of disease as misfortunes or who acquire the knowledge of medicinal plants and their application from the ancestors in their dreams and; (4) Use of remedies by traditional doctors ("amaxwhele" in Xhosa) who physically diagnose,

prescribe and sell the medicines for various ailments and who do not divine the causes of the illness.

Furthermore, findings indicate that traditional treatments are purchased and consumed by a cross-section of the African population. In their recent study conducted in the Eastern Cape, Cocks et al. clearly showed that contrary to some assumptions, traditional medicines were not only purchased by those groups stereotypically associated with being steeped in traditional customs and beliefs such as rural people, the poorly educated, the elderly and religious traditionalists.[25] It was also apparent that allegiance to Christianity did not prevent patronage. In fact, Du Toit and Abdalla note that for the majority of Christian Africans no contradiction exists between readily accepting both Christian dogma and church rituals while simultaneously practicing ancestor reverence.[28] In the Cocks study, patrons were from social groupings representing all levels of education, religious affiliations and income groups.[25] These findings on the social background of customers are consistent with that of Hirst's study of the clientele of traditional healers operating in Grahamstown, a university town some 90km from one of the Cocks study sites.[29]

Use of traditional treatment methods is also not limited to only one population or illness grouping. A study of 300 physiotherapy patients in KwaZulu-Natal showed that 70% would choose to consult a traditional healer as their first choice.[30] Also in KwaZulu-Natal Mkize et al. conducted a survey to investigate pathways to care

and found that traditional healers and faith healers were often the first point of contact.[31] Of 134 patients presenting for the first time at a psychiatric clinic in Malaysia, 69% said that they had visited a traditional healer (*bomoh*) before consulting a psychiatrist.[32] In Nigeria, a sample of 80 members of the general population showed a generally favourable attitude to traditional healers being involved in the treatment of psychiatric illness.[15]

With regards to schizophrenia, Koen et al. reported on a sample of 236 Xhosa schizophrenia sufferers who were interviewed regards their use of traditional treatment.[16] Of the 236 participants, 198 (84%) said that they had visited a traditional healer at some stage of their illness and had adhered to the treatment as prescribed by the healer. Treatments prescribed showed great variation and most often were: taking of an oral solution (n=109), taking of an oral solution/tablet to induce vomiting (n=89), washing (n=61), enema (n=33), use of snuff (n=23), slaughter of cattle (n=2), steam (n=24), wearing of beads (n=7) and cutting of own skin with a sharp instrument (n=14). Interestingly, ancestral appeasement methods, e.g. slaughter of cattle, were not commonly prescribed. The mean age of the group was 36.25 years (SD ± 9.41), with males constituting 75% of the group. The mean number of treatments was 1.87 (SD ± 1.43) and 60% of the group were urban dwellers. No statistically significant difference could be noted for any of the treatment choices when male and female or urban and rural groups were compared to each other.

Interestingly, in the previously mentioned study by Mbanga et al. 88% of family members and caregivers interviewed, considered psychotropic medication to be a recommended form of treatment for schizophrenia.[23] However, the vast majority supported this only in conjunction with the simultaneous use of traditional treatment methods. Whilst many felt that traditional healers' methods were essential to protect sufferers from invasion by "bad spirits", they regarded Western treatment to be necessary to prevent symptoms from worsening. This endorsement of psychotropic medication in fact contradicts the reportedly poor support for Western medicine as claimed in German literature.[33;34]

Unfortunately, despite the apparent support for psychotropic medication, the world-wide significant public stigma attached to the illness also seems to exist in Xhosa communities. In her study, Mbanga et al. reported that more than 40% of respondents had, in comparison to the average person, a generally negative view of the probands.[23] Reporting on stigma, as perceived by a mixed sample of 100 South African schizophrenia sufferers, Botha et al.'s results seemed to suggest that especially Xhosa-speaking patients reported experiencing a higher incidence of abuse as a form of stigmatisation.[35] One possible explanation for this high perceived degree of stigma could in fact be a lack of knowledge regards schizophrenia and the possible belief that witchcraft or evil spirits play a significant role in its causation

It is necessary to keep some important limitations in mind when generally interpreting the results of the studies conducted amongst the Xhosa schizophrenia population and their caregivers or family members reported on here. Interviewers were trained mental health practitioners which could have led to bias towards endorsing “Western” over traditional methods. Participants were often older with poorer education levels and broad generalisations to the results to other groupings should be done with caution.

However, the data show that only partial support exists for the biomedical model of schizophrenia in this population and culture-specific beliefs often play a significant role in how the illness is viewed. These include, but are not confined to, the acceptance of cultural diagnoses such as “amafufunyana” and “thwasa”, the acceptance of cultural beliefs in the causation of the illness, as well as the involvement of traditional healers and treatment practices in health seeking pathways. Whether this reflects a more holistic view of the causation of mental illness, or whether it comments on the reality of a country with 11 official languages, with practitioners unable to communicate in a patient’s home language, is an issue that warrants further scrutiny. Regardless, it is clear that a more proactive stance needs to be taken to further co-operation with reputable traditional healers in order to ensure a more integrated westernised/traditional approach in promoting health for all.

3.3 RESEARCH ETHICS – GENETICS, CULTURE AND PSYCHIATRY

Putting safeguards into place to ensure ethical standards in all aspects of research has become a standard part of the agenda of research funding agencies and relevant governmental and non-governmental organizations. Long gone is the time when the discipline of psychiatry found it adequate to merely reach a consensus for research standards and then have the text translated into many languages and distributed. In fact, since then psychiatry has moved from mostly focusing on treatment and rehabilitation to also include promotion of mental health and therefore prevention strategies as well as the involvement of the patient, family, broader community and the industry in such. This change is also reflected in the increased debate surrounding ethical issues.

Biomedical research is usually guided by the Nuremburg paradigm and the status and integrity of ethical guidelines based and the Nuremburg (1947) and Helsinki (1964) codes (and later revisions thereof) are well established. However, as researchers in the field of genetics have shown growing interest in samples from homogenous populations some sensitive ethical issues have come to the fore highlighting that strict adherence to the Nuremburg paradigm may not at all times be practical or even appropriate. For example, as described in this chapter, the Xhosa population place great value on communal interests rather than those of the individual (“ubuntu”). Therefore, the current western emphasis on the right of the individual to autonomy may not find favour in such a society. As stated, the

care of the Xhosa psychiatric patient is more often than not the concern of not only the mental health professional but also the family and traditional healers.

Other issues associated with the Nuremburg paradigm regarding its possible limitations for psychiatric general and genetic research have also come to the fore. Not least of these are the inherent difficulties in obtaining informed consent from someone who suffers from a psychotic illness such as schizophrenia. At any given time when conducting research within a schizophrenia population some of the patients may be suffering an acute exacerbation of illness and this needs to be taken into account during the ongoing consent process. True informed consent implies that the choice is freely and intentionally made with rational understanding of that which is chosen. Clearly, in order for such to be valid the information needed to make the decision must be presented in a way that is appropriate for the particular patient (e.g. taking into account language, intellectual abilities, culture etc.).

Once again cultural beliefs may play a role as some non-western societies adhere to the principal of substituted decision making with the head of a group (family) or even the whole group responsible for providing consent. However it has also been made clear that broad generalisation within a particular cultural grouping cannot solely dictate research practices (such as obtaining informed consent) within that grouping. At any give time specific individuals within the culture may have reached different levels of acculturation in various aspects of

their functioning. Furthermore, our beliefs about certain cultures are often based on outdated anthropological data. When all of this is taken into account it is clear that researchers need to factor cultural diversity into their decision making process.

With specific reference to genetic research one must also not lose sight of the fact that the cultural meanings people ultimately attach to their symptoms can shape the final presentation of a particular disorder. Furthermore, information about, and even involvement of biological family members is pivotal to genetic research. In a worst case scenario, this could lead to revealing sensitive information in a way that could cause significant distress and possibly even precipitate relapse.

With specific reference to genetic research one must also not lose sight of the fact that the cultural meanings people ultimately attach to their symptoms can shape the final presentation of a particular disorder.[36] Furthermore, information about, and even involvement of biological family members is pivotal to genetic research.[37] In a worst case scenario, this could lead to revealing sensitive information in a way that could cause significant distress and possibly even precipitate relapse.

4. SUMMARY

Given the historical and geographical influences that formed this group, the Xhosa population can be regarded as a culturally homogenous grouping with an

active traditional belief system. Although this system seems to raise some questions with regard to a population-specific course of schizophrenia, it does not seem to influence the presentation of the core symptoms of schizophrenia. The Xhosa schizophrenia population therefore presents us with an invaluable research opportunity to further knowledge with regard to this debilitating illness, all the while ensuring that possible ethical issues receive due consideration.

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CHAPTER 4

CHROMOSOMAL ABERRATIONS IN SCHIZOPHRENIA

CONTENTS

1. BASIC INTRODUCTION TO THE CONCEPT OF CHROMOSOMAL ABERRATIONS

The discovery of the principles of heredity by Gregor Mendel in 1865 went largely unnoticed until William Bateson, who first coined the term genetics, translated his paper from German to English in 1900. This led to increased interest and the seminal work on alkaptonuria by Archibald Garrod, who together with Francis Galton are regarded as the founders of medical genetics.[1] In medical practice, genetics still finds its chief significance in its role in the etiology of a large number of disorders.[2] Three main types of conditions secondary to faulty genetic information are recognised – (1) single gene disorders caused by mutant genes, (2) chromosomal disorders where aberrations affect development and (3) multifactorial inheritance – a combination of several genes each having a smaller or larger contribution (in concert with environmental effects) that together produce a serious defect.[3]

In 1956, Tjio and Leven as well as Ford and Hamerton conclusively demonstrated (independently of each other) that the number of chromosomes in the normal human was 46.[4;5] These chromosomes are arranged in 23 pairs and are made up of the two sex chromosomes (X,Y) and the so-called autosomes.[6] One copy of a pair is inherited from each parent. A chromosome is a singular piece of DNA, which contains many genes, regulatory elements and other nucleotide sequences. They also contain DNA-bound proteins, which serve to package the DNA and control its functions. Each chromosome has a

constriction point, called the centromere, which divides the chromosome into the so-called short “p” and long “q” arms.[2] (See diagram 1)

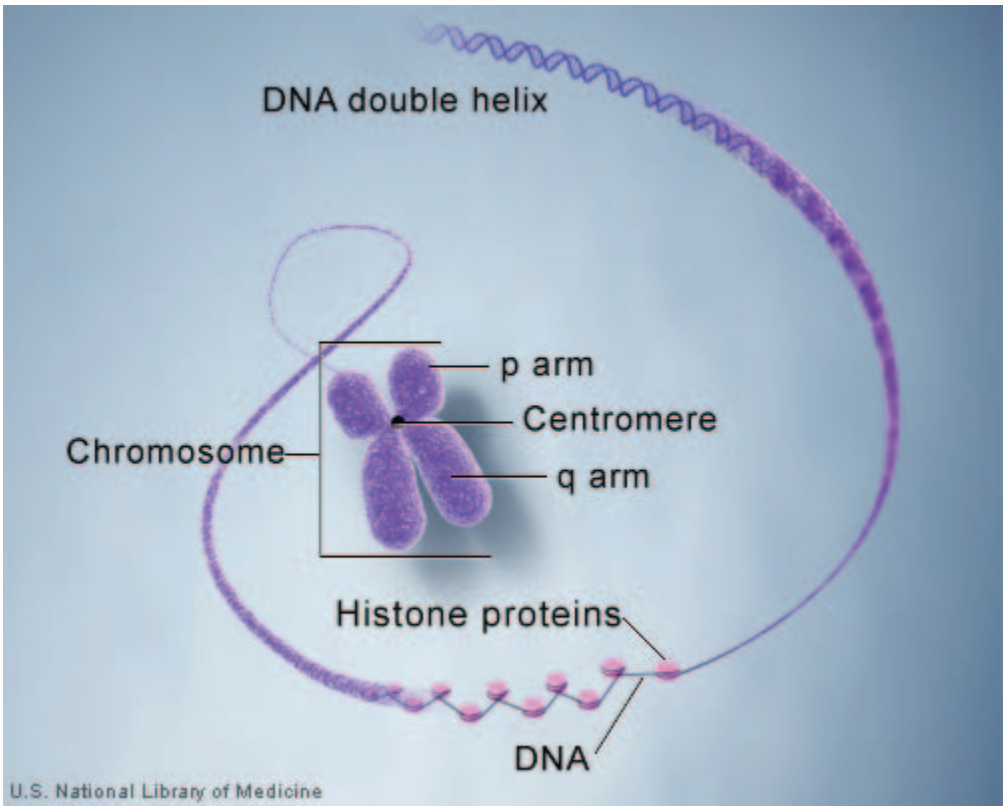

DIAGRAM 1: REPRESENTATION OF A CHROMOSOME

The discovery by Lejuene and colleagues in 1959 that children with Down syndrome have 47 chromosomes instead of the normal 46 paved the way to a new era in medical genetics.[7] Since then chromosomal aberrations have become well-defined causes of maldevelopment shown to be more frequent and varied than originally anticipated. They are regarded to be a significant cause of birth defects and foetal loss, present in an estimated 0.7 percent of live births and nearly half of all spontaneous first-trimester abortions.[8;9]

On a basic level, chromosomal aberrations can be divided into structural or numerical abnormalities.[2] Numerical abnormalities involve the loss or gain of one or two chromosomes (aneuploidy) or the gain of a whole chromosome set (polyploidy). Examples of aneuploidy would include the well-known Down Syndrome (trisomy 21), Edwards Syndrome (trisomy 18) and Patau Syndrome (trisomy 13). True polyploidy rarely occurs in humans; although it is seen in some tissues (especially in the liver).[10]

Structural abnormalities result from chromosomal breakage, followed by reconstitution in an abnormal combination. These breaks usually occur spontaneously at a low frequency, but can also be induced by a variety of agents such as radiation, viral infections and many chemicals. The changes in chromosomal structure resulting from breakage may be either stable (e.g. deletions, duplications, inversions, translocations, insertions or isochromosomes) or unstable (e.g. dicentrics, acentrics and rings).[6]

Chromosomal inversion involves fragmentation of a chromosome by two breaks, followed by reconstitution with inversion of the section of the chromosome between the breaks (e.g. AAAbcdEEE becomes AAAdcbEEE). Inversions are described as either paracentric (centromere outside the inversion) or pericentric (inversion spanning the centromere). As such, inversions alone do not appear to lead to an abnormal phenotype in man, although in theory they could if the break was within a gene or its regulatory sequences. Rather, the medical significance

of inversions lies in the fact that the subsequent generation is exposed to the consequences of a crossing over between a normal and an inverted chromosome. For example, in 1975 a large pedigree from Newfoundland was described in which there were several carriers of an inversion of the long arm of chromosome 3, leading to children being born with a syndrome of congenital defects including hirsutism, micrognathia and mental retardation.[11]

Two main types of chromosomal translocation have been described – reciprocal and Robertsonian. Reciprocal translocation is the exchange of blocks of chromatin between two non-homologous chromosomes, a process that requires breakages in both chromosomes with repair in an abnormal arrangement. Robertsonian translocation involves two acrocentric chromosomes which fuse at the centromere region and lose their heterochromatic short arms.[2]

Chromosomal deletion refers to loss of a part of the chromosome, usually interstitially between two breaks, but sometimes terminally, following a single break and leading to the lack of the genetic information that was present in the lost fragment. A common example of deletion in humans is the cri-du-chat syndrome in which part of the short arm of chromosome 5 is deleted.[2]

Two other significant concepts are that of genomic imprinting and the contiguous gene syndrome. Genomic imprinting refers to certain genes being active only on specifically either the inherited paternal or maternal copy of the chromosome.

Contiguous gene syndrome refers to disorders that are caused by the deletion of multiple gene loci that are adjacent to one another. These syndromes are characterised by multiple, apparently unrelated, clinical features caused by deletion of the multiple adjacent genes. Each of the individual genes within a contiguous region, when mutated, gives rise to a distinct feature. An example where both these concepts can be illustrated is via two syndromes caused by deletions in the chromosome 15q11-13 region.[12]

Angelman syndrome (marked by mental retardation, movement or balance disorder, characteristic abnormal behaviours, and severe limitations in speech and language), is caused when the deletion results in the loss or mutation of the *UBE3A* gene, although possible associations with other genes have also been suggested.[13-15] Normally people would inherit one copy of the gene from each parent but in the brain only the maternal copy is active.[16] In most cases, this condition therefore results from either a deletion in or a non-transmission of the maternal copy, resulting in no working copies of the *UBE3A* gene in the brain, which likely causes the characteristic features of the syndrome.

Prader-Willi syndrome (PWS) (marked by obesity, muscular hypotonia, mental retardation, short stature, hypogonadotropic hypogonadism, and small hands and feet), on the other hand, is caused in most cases when the paternal copy of chromosome 15 is partly or entirely missing as the genes necessary to prevent the syndrome are active only on the paternal copy.[13;17] Currently, the precise

genes that cause this syndrome have not been identified but numerous protein coding genes and non-coding transcripts have been isolated from the PWS candidate region.[18-20]

The aim of this introduction was to provide a very basic overview on some topics relevant to the concept of chromosomal aberrations. It was not meant as a comprehensive summary and for such the reader is referred to the various texts available on this subject.

2. BACKGROUND TO GENETICS OF SCHIZOPHRENIA

The current model of liability to schizophrenia holds that both genetic and environmental risk factors contribute to the development of the illness. Although, to date, no risk factor of either type has yet been unambiguously identified, recent progress in this field would suggest that we are closer to understanding specific genetic influences than we are to characterising specific environmental ones. For example, even though epidemiological studies have identified a number of possible contributors such as obstetric complications, immigration, season of birth and use of illegal drugs (especially cannabis); the effect sizes are small and the direction of causation remains uncertain in most cases.[21]

To genetically study any complex trait, including schizophrenia, there are two very important areas of inquiry. Firstly, it must be determined whether there is an additional risk for the trait in relatives of cases and, if so, whether the risk is

attributable to shared genetic rather than shared environmental factors. Secondly, linkage and association studies are needed. Linkage studies are used to demonstrate a relationship between a trait and a specific region of the genome, while association studies are more focused and used to establish whether a relationship exists between a trait and specific genes.

Numerous family, twin and adoption studies have shown in a conclusive manner that schizophrenia has a high familial heritability risk and that genes have a major contributory role in the aetiology of the disorder.[22] In fact, schizophrenia has one of the highest heritabilities (approaching 80%) among the complex genetic disorders, similar to that of type I diabetes mellitus (72-88%) and greater than that of breast cancer (30%) and heart disease in males (57%).[21;23;24] In real terms, this can be interpreted to mean that a close relative of a schizophrenia patient has, on average, ten times the baseline population risk of developing the disorder.

However, it is important to remember that since the heritability estimates are less than 100% and the concordance for monozygotic twins is approximately 50%, it is clear that environmental factors must play some role. Furthermore, the risks as a result of some gene-environment interactions are probably attributed to genes in most genetic epidemiology studies.[25]

The genetic heterogeneity is mirrored by clinical heterogeneity, which arguably reflects the heterogeneous nature of susceptibility factors for schizophrenia. Not only do we find multiple combinations of symptoms existing in individuals but also both disease course and outcome display considerable heterogeneity. Therefore, as previously stated, we currently cannot be certain whether this is a single disorder with different clinical manifestations or in fact a group of syndromes, each with unique or overlapping pathophysiology.

3. THE SEARCH FOR GENES IN SCHIZOPHRENIA

Considerable attempts have been made to elucidate the heterogeneity of the schizophrenia phenotype by exploring the relationships between the various symptom dimensions and possible subtypes. Some researchers have felt that genetic studies in schizophrenia using phenotypes would profit from the use of symptoms that are closer to their etiology than the end-stage clinical symptoms that are currently used by the DSM-IV-TR and the ICD10 to define schizophrenia. They therefore advocate the use of a system such as Leonhard's classification of psychoses, which claims to define real diseases on the basis of differentiated and operationalised psychopathological syndromes, rather than DSM-IV-TR or ICD10 to define the phenotype for such linkage studies.[26]

Clearly the identification of susceptibility genes would be of great benefit to further explore the validity of our current nosological categories. This should help

illuminate heterogeneity within the current schizophrenia concept and further the process of understanding its relationship to other diagnostic groups.

Unfortunately, the search for chromosomal loci and genes has been a slow and tedious process, most probably because there are multiple susceptibility genes, each with small effect, which act in conjunction with epigenetic processes and environmental factors. Currently, the number of susceptibility loci, the disease risk conferred by each locus, the extent of genetic heterogeneity and the degree of interaction among all the loci, remain unknown quantities.

To date, three main approaches have been used to seek genes for schizophrenia (1) molecular genetic studies, (2) studies of functional candidate genes and (3) identification of chromosomal aberrations associated with schizophrenia.

3.1 MOLECULAR GENETIC STUDIES - LINKAGE ANALYSIS

In contrast with several other complex disorders such as Parkinson's disease and epilepsy, no forms of schizophrenia following Mendelian inheritance patterns have yet been discovered. Indeed, until recently results of linkage studies have been meagre as most studies neither achieved stringent "genome-wide" levels of significance nor replicated pre-existing findings.[27] Small sample sizes, small genetic effects and the use of marker maps with insufficient density have probably all contributed to these disappointing findings. It is also important to remember that in human populations strict replication studies are virtually

impossible as samples will almost certainly differ with regards to genetic architecture due to ethnicity, ascertainment, variation in exposure to environmental risks and, where there are multiple disease loci, sampling variance.[28;29]

In an attempt to address some of these issues two large meta-analysis of schizophrenia linkage have recently been undertaken. Badner et al. performed a medline search to identify all published genome scans for schizophrenia. Of these they included only studies where all regions and/or markers with p-values <.05 were reported and the specific location was given.[30] Follow-up papers were excluded on the basis of being subject to possible publication bias (i.e. less likely to be published if negative). Lewis et al. included all published and presented (unpublished) data that represented whole genome scans of schizophrenia, excluding partial scans and candidate region studies.[31] Ultimately the meta-analyses reported on eighteen and twenty studies respectively of which eleven were reported on by both.[30;31]

The regions (presented in descending order of significance) supported by Badner et al. were 22q, 8p, 13p and by Lewis et al. 2p, 5q, 3p, 11q, 6p, 1q, 22q, 8p, 20q, 14p.[30;31] Therefore only 8p and 22q were supported by both analyses. When taking into account how the meta-analyses were performed, the differing results are not surprising. Also, the results are in keeping with Risch's calculations that existing data for recurrence risk in relatives is incompatible with the existence of

a single locus in schizophrenia, conferring a relative risk in siblings of greater than three and that models with two or three loci carrying a relative risk of ≤2 are more plausible.[32]

Taking the phenotypic approach, Stober et al. used periodic catatonia, a clinical entity derived form Leonhard's classification of schizophrenias, to conduct a genome-wide linkage analysis.[26;33] In periodic catatonia, the nuclear syndrome consists of distinct hyperkinetic and/or akinetic disturbances of psychomotor behaviour. These qualitative psychomotor symptoms are present in both the acute psychotic episode and the residual state. On the basis of their non-parametric and parametric linkage analysis Stober et al. identified two schizophrenia susceptibility loci.[33] One, 22q13, was supported mainly on data for a single large family, but the other, 15q15 (LOD score 3.57; p<.000026), met Lander's criteria for linkage and was regarded as significant.[34] Interestingly, on chromosome 15q15 the candidate segment overlaps with a putative schizophrenia locus defined by a neurophysiological deficit of the P50-auditory-evoked-response inhibition in patients with schizophrenia.[35]

3.2 CANDIDATE GENES

3.2.1 POSITIONAL CANDIDATE GENES

The convergence of the positive linkage findings has led to several detailed mapping studies of linked regions and some of these have implicated specific

genes. Based on current published data the most convincing of these loci seem to be dystrobrevin-binding protein 1 (*DTNBP1*) (6p22), neuregulin 1 (*NRG1*) (8p12-21), D-amino acid oxidase (*DAO*), D-amino acid oxidase activator (*DAOA*) (both 13q22-34), and regulator of protein signaling 4 (*RGS4*) (1q21-22).[36]

3.2.2 FUNCTIONAL CANDIDATE GENES

Schizophrenia candidate-gene literature reveals a large number of positive and negative findings and many of the initial positive associations have yet to be replicated. Two genes that have been the focus of several replication attempts are V-AKT murine thymoma viral oncogene homolog 1 (*AKT1*) (14q22-32) and glutamate receptor metabotropic 3 (*GRM3*) (7q21-22).[36]

3.3 CHROMOSOMAL ABERRATIONS

Studies in Caucasian and Asian populations have revealed that between 2% and 32% of schizophrenia subjects have chromosomal aberrations such as translocations, deletions and inversions.[37;38] However, though there have been numerous reports of associations between schizophrenia and chromosomal abnormalities, none, with the exception of two, can be regarded to have provided any convincing evidence for the location of a susceptibility gene.[39] The two in question are the deletions on chromosome 22q11 and the balanced chromosomal translocation (1;11)(q42;q14.3) disrupting two genes on chromosome 1 (Disruption in schizophrenia 1 (*DISC 1*) and 2 (*DISC2*)).

3.3.1 DISRUPTION IN SCHIZOPHRENIA 1

DISC1 was first identified in an extended Scottish pedigree (see diagram 2) when a balanced chromosomal translocation disrupting two genes (*DISC1* & *DISC2*) on chromosome 1 was discovered and then demonstrated to show linkage to a fairly broad phenotype, consisting of schizophrenia, bipolar disorder and recurrent major depression.[Blackwood 2001] Interestingly, both these genes are located close to the chromosome markers implicated in two Finnish linkage studies.[40;41]

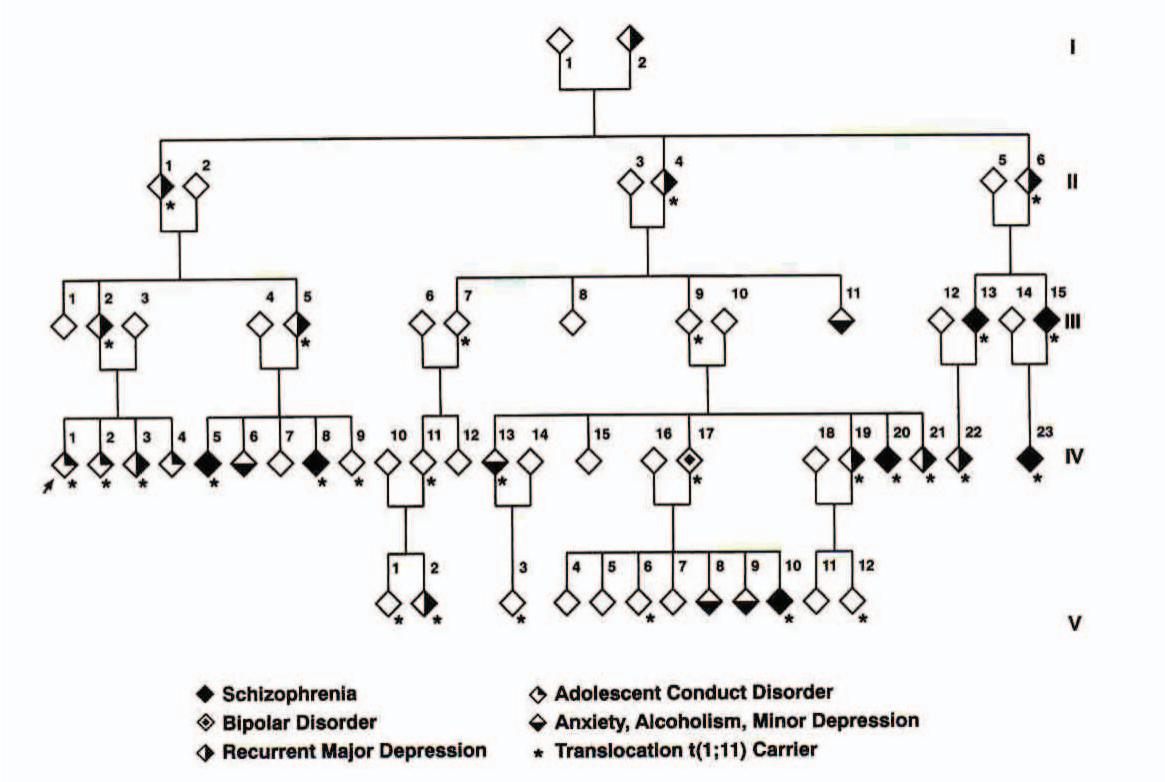

DIAGRAM 2: PART OF THE SCOTTISH PEDIGREE WITH (1;11)(q42;q14.3) TRANSLOCATION. SHOWN ARE THE 58 FAMILY MEMBERS FOR WHOM CARRIER STATUS AND PSYCHIATRIC PHENOTYPE HAD BEEN DETERMINED[42]

Currently the functions of *DISC1* are not understood well and therefore those ascribed to it are inferences from the known functions of the proteins that interact with it.[43] It has been suggested that *DISC1* and a number of interacting proteins may play a role in neuronal migration, cortical layering during foetal brain development, hippocampal formation and probably adult neurogenesis in the dentate gyrus all of which is consistent with the neurodevelopmental hypothesis of schizophrenia.[44-46] Whereas *DISC1* encodes a novel multifunctional scaffold protein, *DISC2* is a putative noncoding RNA gene antisense to *DISC1* which may regulate *DISC1* expression.[47] If future investigations can elucidate the molecular mechanism by which *DISC1* regulates the activity of these protein-binding partners it will ultimately lead to a better understanding of the role of *DISC1* at the level of brain function.

However, it is important to remember that translocations can exert effects on genes other than those directly disrupted. Thus, to implicate *DISC1* and/or *DISC2* conclusively in the pathogenesis of schizophrenia it would be necessary to identify mutations or polymorphisms that are associated with schizophrenia in another population and to show that those associations cannot be attributed to linkage disequilibrium with neighbouring genes. To date, four published studies have attempted to find such evidence. Two, including one by the group who originally identified *DISC1* and *DISC2*, have been negative but positive findings have been reported in a large Finnish and an US sample.[48-51] All in all, *DISC1* is

currently regarded to be one of the best replicated genetic findings with biological plausibility for major mental illness.[52]

3.3.2 CHROMOSOME 22q11 DELETIONS

Chromosome 22 is the second smallest of the human autosomes comprising 1.6-1.8% of the genomic DNA.[53] It was the first chromosome of which an operationally complete sequence of the euchromatic portion was finalized and as such represents a sequencing landmark in the human genome project.[54] Due to the extensive body of literature available on this chromosome and related topics, a separate chapter is devoted specifically to it.

4. SUMMARY

Genes are the first objective clues to the primary causes of mental disorders and to the mechanisms of cellular pathogenesis. Although they are not the only etiologic factors in the pathogenesis of schizophrenia (both environment and epigenetic processes may also be important), the major benefit for genes is that they are bounded and objective and their biological effects can be more readily identified and quantitated. Therefore, taking all evidence into account it would be difficult to not conclude that genetic research about schizophrenia has, to date, generated a credible list of potential susceptibility genes, thereby starting to give us a glimpse into potential mechanisms by which these genes influence the risk

of disease. Ultimately, this will lead to our understanding of this disorder being enhanced dramatically.

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CHAPTER 5

CHROMOSOME 22q11 DELETION SYNDROME AS AN ENDOPHENOTYPE IN SCHIZOPHRENIA

CONTENTS

1. INTRODUCTION

The so-called 22q11 deletion syndrome (22q11DS) is the second most common human chromosomal anomaly (after trisomy 21) and the most frequently known interstitial deletion found in humans, occurring in approximately one of every 4000 live births.[1] It has been reported in association with more than 120 different birth defects and usually occurs as a sporadic mutation. Autosomal dominant inheritance is observed in 15% or less of cases.[2;3] As noted in the previous chapter, the microdeletion at chromosome 22q11 remains one of only two (the other being a balanced chromosomal translocation [t(1;11)(q42.1;q14.3)]) reported associations between schizophrenia and chromosomal abnormalities that currently show any convincing evidence for the location of a susceptibility gene.[4]

2. 22q11 ABERRATIONS IN HUMANS

Genomic disorders are caused by an alteration of the genome that might lead to the complete loss or gain of a gene(s) sensitive to a dosage effect or, alternatively, might disrupt the structural integrity of a gene. Examples are the disorders caused by DNA rearrangements owing to homologous recombination involving region-specific low-copy repeats.[5] The 22q11 region can be regarded as a model for these genomic disorders as it is seems particularly susceptible to such chromosomal rearrangements, with three different congenital malformation syndromes having been described thus far. These are the cat-eye syndrome

(CES), derivative (22) syndrome (der(22)) and what is collectively referred to in the literature as 22q11 deletion syndrome (22q11DS). (Originally CATCH 22 was suggested as an all encompassing term with the acronym representing Cardiac defects, Abnormal facies, Thymic hypoplasia, Cleft palate and Hypocalcaemia.[6] However, due to the emergence of more divergent clinical presentations as well as objections from some patient groups, this term is no longer in common use.[7]) The three disorders are most often identified by intellectual disability and/or congenital malformations and of the three, 22q11DS is by far the most common.

2.1 22q11 DELETION SYNDROME (22q11DS)

The 22q11DS is associated with a variable phenotype that can present with characteristic facial dysmorphology, a range of congenital abnormalities, and emotional (including psychosis), behavioural and cognitive deficits. Due to the frequency of the occurrence of certain sub-clusters of these symptoms in the presence of the characteristic facial dysmorphology, 22q11DS is regarded to encompass several distinct genetic "entities" including DiGeorge (DGS), conotruncal anomaly face (CAFS), and velocardiofacial (VCFS) syndromes as well as, in some cases, Cayler and Opitz GBBB syndromes.[8-13] Numerous combinations of anomalies are found in sufferers of each of these syndromes but there are some classical findings which are summarised in table 1.

The syndrome was first described in the 1950s and further delineated by Shprintzen et al.[13;14] The first clue to the aetiology came when De la Chapelle et

al. reported a family with a chromosomal translocation t(20;22)(q11;q11) in which four subjects with unbalanced products had DGS.[15] However, as routine karyotype analysis cannot be used to detect the deletion, it only became more generally identified with the availability of specialised chromosomal studies in the 1990s.[16]

TABLE 1: BRIEF SUMMARY OF THE CLASSICAL FEATURES FOUND IN EACH OF THE COMMONLY KNOWN DISTINCT ENTITIES THAT ENCOMPASS 22q11DS[8-13]

SYNDROME	CLASSICAL FEATURES
DiGeorge	▪ Interrupted aortic arch ▪ Tetralogy of Fallot ▪ Truncus arteriosus ▪ Parathyroid & thymic hypoplasia
Velocardiofacial	▪ Velopharyngeal insufficiency ▪ Learning disorder ▪ Conotruncal abnormalities
Conotruncal anomaly face	▪ Velopharyngeal insufficiency ▪ Learning disorders ▪ Ventricular septal defect
Cayler	▪ Unilateral facial palsy (congenital hypoplasia of depressor anguli oris muscle) ▪ Ventricular septal defect
Opitz GBBB	▪ Hypertelorism ▪ Hypospadia ▪ Swallowing difficulties ▪ Cleft of lip, palate & uvula ▪ Laryngotracheo-esophageal cleft

Currently the deletions can be readily detected using standard fluorescence in situ hybridization (FISH) analysis techniques. By far the majority of patients with 22q11DS have either a common recurrent deletion of approximately 3Mb (87% of cases) or a smaller, less common, 1.5Mb (8% of cases) deletion. The 1.5Mb deletion involves the same proximal breakpoint but a nested distal deletion endpoint.[17] Both deletions apparently occur as a result of homologous

recombination between nonallelic flanking low-copy repeat (LCR) sequences located in 22q11.2.[18;19] Although eight different LCRs are located in proximal 22q, only a few cases of atypical deletions using alternative LCRs have been described.[20]

2.2 CAT-EYE SYNDROME (CES)

CES presents with borderline-to-moderate mental retardation as well as clinical features distinct from those found in 22q11DS that mostly show variable expressivity and penetrance. These include ocular colobomata, anal atresia, microphthalmia, congenital heart defects (most typically total anomalous pulmonary venous return (TAPVR)), renal malformations, craniofacial anomalies (e.g. preauricular skin tags and pits), male genital anomalies and skeletal defects.[21] CES patients have a small supernumerary bi-satellited marker chromosome 22pter-q11 resulting from an inverted duplication of the proximal 22q11 region. The additional CES chromosome generally arises de novo from either of the parents and currently no data is available on sibling recurrence risk or population incidence estimates. The CES chromosome contains two 22q11 breakpoints, but the location of the individual breakpoints may differ.[22] Both these regions occur in the same intervals as do the proximal and distal breakpoints of the 3Mb 22q11DS deletion.[18]

2.3 DERIVATIVE (22) SYNDROME (Der(22))

Der(22), also known as Emanuel syndrome, shares, with the distinct exception of the colobomata, microphthalmia and TAPVR, the features of CES as described above.[23] In addition, mental retardation, cleft palate and hypotonia are much more common in der(22) than in CES.[21] The syndrome is caused by a chromosomal translocation t(11;22) that currently represents the most common recurrent non-Robertsonian translocation reported in humans.[24] Normal balanced t(11;22) translocation carriers are at risk of giving birth to offspring with der(22) syndrome, resulting from a 3:1 meiotic non-disjunction.[25] In der(22) the t(11;22) breakpoint occurs in the same interval as does the nested distal 1.5Mb 22q11DS breakpoint.[26] To date, no studies have reported any association between either CES or der(22) and neuropsychiatric manifestations.

3. THE 22q11DS PHENOTYPE

3.1 GENERAL FEATURES

Initial descriptions of the 22q11DS phenotype focused mainly on the cardiac defects, characteristic physiognomy, palate abnormalities and intellectual deficits. However, it soon became clear that a number of later onset conditions such as hypocalcaemia, thrombocytopenia and neuropsychiatric manifestations were also associated with 22q11DS. In fact, the phenotype has been shown to vary considerably between families as well as among members of the same family.[27]

The physical structures that are primarily affected in 22q11DS are all derivatives of the branchial arch/pharyngeal pouch system and also all receive a contribution from the rostral neural crest during embryogenesis. Neural-crest cells migrate from a position adjacent to the neural tube and participate in the formation of both pharyngeal arches and their derivatives. Experiments that have been designed to interfere with crest functioning have led to the production of good phenocopies of the main features of 22q11DS.[28;29] Furthermore, natural or induced mutations of genes thought to be involved in the control of neural crest development can also lead to malformations suggestive of 22q11DS.[30-33] These findings form the basis of the hypothesis that the deletion of 22q11 disrupts the neural crest cells, or the cells with which it interacts, at a critical phase of organogenesis.

As mentioned, the distinct clinical features of 22q11DS show variable expressivity and incomplete penetrance and there seems to be no apparent correlation between the severity of the pattern of the expressed phenotype and the extent of the deletion for 22q11DS.[34] It is also important to note that profound neurodevelopmental, cognitive, behavioural and psychiatric symptoms have been shown to occur in the absence of any major physical anomalies. In fact, the prevalence of psychosis in those with 22q11DS is high (30%), suggesting that haploinsufficiency of a gene or genes in this region may confer this substantially increased susceptibility.[35]

Furthermore, in a fair percentage (<10%) of patients with syndromal features the microdeletion cannot be demonstrated indicating possible point mutations in the 22q11 region, that have not yet been identified or possible involvement of other chromosomal aberrations.[36-39] In particular, deletions of the short arm of chromosome 10 (10p) can often be detected although these are much less common than those of 22q11 occurring at an estimated frequency of only 1 in 200000 live births.[40] Interestingly, in comparison, 10p patients seem to present with a higher frequency of renal abnormalities and growth retardation as well as more severe intellectual disabilities but with less cardiac defects than 22q11DS patients.[41]

Non-genetic causes such as exposure to teratogens during pregnancy could also play a role. Examples include the offspring of diabetic mothers and prenatal exposure to retinoids or alcohol that have been associated with similar-looking phenotypes, possibly through exerting effects on neural crest cell migration during embryonic development.[42-45]

3.2 FACIAL DYSMORPHOLOGY

Facial dysmorphology can be quite variable and is most pronounced during the childhood period.[46] Features most helpful to assist in the diagnosis are regarded to be the ear shape, prominent nasal root and in the younger child, the mouth. In infancy, micrognathia may be present. The ears are typically low set and deficient in the vertical diameter with abnormal folding of the pinna. The face

appears long and narrow with flat cheeks. Palpebral fissures are often short and sometimes narrow and the root and bridge of the nose wide and prominent. The philtrum is often short and poorly modelled with the mouth small, the lips thin and the chin retracted.[6;46-48]

3.3 CARDIAC ABNORMALITIES

The congenital heart defects (CHD) found in a large number of patients, are the main cause of morbidity and mortality in 22q11DS. The type of CHD is variable but very often involves the outflow tract of the heart and the derivatives of the branchial arch arteries. Malformations include, among others, interrupted aortic arch (IAA), pulmonary atresia with a ventricular septal defect, tetralogy of Fallot and truncus arteriosus.[49] Interestingly, it has been reported that the likelihood of finding a deletion is greater in cases with IAA type B (interruption between the left carotid and subclavian arteries) compared to those with IAA type A (interruption just beyond left subclavian artery).[50]

3.4 IMMUNE SYSTEM

Significant rates of immunodeficiency have been reported in 22q11DS samples, but the degree of severity varies considerably.[51;52] Most have a mild form characterised by low absolute T-cell numbers and children can be susceptible to upper respiratory infections. However, at the other end of the spectrum the

immunodeficiency may be very severe, as seen in patients presenting with thymic aplasia or hypoplasia and absent T-cells[6;53]

3.5 ENDOCRINE SYSTEM

Hypocalcemic hypoparathyroidism as a result of absent or small parathyroid glands has been reported to varying degrees (40-60%) in different samples.[6;46;47;51] This can probably be readily explained by the fact that neonatal presentation can resolve spontaneously and then sometimes reoccur later in life, or the condition could be missed as it can be latent at times.[54;55] Patients are often short of stature and this can sometimes be linked to growth hormone deficiency which in some cases has been shown to be due to pituitary abnormalities as diagnosed on MRI.[51;56] Both hyper- and hypothyroidism have also been reported but this phenomenon is not well characterised.[47;51]

3.6 OTOLARYNGEAL

Palate abnormalities are commonly reported with the velopharyngeal insufficiency and hypoplasia of the nasopharynx probably contributing to speech delays and the conductive hearing loss that is frequently seen.[46;51] A wide spectrum of speech abnormalities occur, with hypernasal speech often a prominent feature. Expressive language skills are more impaired than receptive, with no correlation found between language impairment and IQ.[57]

3.7 COGNITIVE AND BEHAVIOURAL

Learning disabilities were part of the original VCFS phenotype described by Shprintzen et al. in 1978.[13] Over time a behavioural phenotype with a characteristic pattern of motor, cognitive, linguistic and social deficiencies, has become apparent. Delayed developmental milestones can be prominent.[58] The majority of 22q11DS sufferers have mild to moderate intellectual impairment and verbal IQ scores tend to be higher than non-verbal. Intellectual disability is higher in familial deletions and more severe in the phenotype associated with the 10p deletion.[41;46-48;58-61] Behavioural and psychiatric disorders have also been shown to be prevalent in the 22q11DS populations. (Discussed separately in this chapter)

3.8 MISCELLANEOUS

Structural renal abnormalities, renal insufficiency and renal tubular acidosis have all been reported as well as a wide range of neurological abnormalities including structural brain abnormalities, 7th cranial nerve palsy, hemipareses and recurrent seizures. Minor abnormalities of the skeletal system such as talipes equinovarus, polydactyly, syndactyly and abnormal vertebrae (kyphosis/scoliosis) are also seen.[46-48;51] A number of other less common, but not isolated, abnormalities also occur in numerous systems. The interested reader is referred to Basset et al. for a more comprehensive list.[47]

3.9 VARIED FREQUENCY OF PHYSICAL ANOMALIES

Available literature contains data on numerous 22q11DS patient populations with descriptions ranging from case reports to fairly large samples. In spite of this, the true frequency of individual features remains somewhat uncertain as rates vary with developmental stage, ascertainment and completeness of assessment.[48;62] Two of the largest groups studied to date are that of Cohen et al. who documented data from a sample of 126 adults (76 transmitting parents of affected offspring) collected via English language MEDLINE search (Jan 1966 to July 1998) and that of Ryan et al., whose sample was collected by submitting data questionnaires to 23 European centres, and consisted of 425 children, 72 adolescents and 61 adults (27 transmitting parents of affected offspring).[46;48] (See table 2 for a comparison of major clinical findings and sex distribution in these two studies.)

In comparison to Ryan et al., Cohen et al. reported a significantly lower rate of major cardiac anomalies ($p<0.0001$) but a significantly higher rate of a wide range of palate abnormalities ($p<0.0001$), cognitive difficulties ($p<0.0008$) and psychiatric conditions ($p<0.02$).[46;48] Ascertainment biases and age required to identify manifestations not obvious at birth would most likely explain these differences.[48] Furthermore, cardiac anomalies contribute most significantly to morbidity in 22q11DS patient population and this could also provide an explanation as to the differing rates for this group of anomalies in differing age groups. Interestingly, in the Cohen et al. sample, transmitting parents had

consistently lower levels of cardiac anomalies (p<0.0001), cleft palate (p<0.005) and reported psychiatric conditions (p<0.0001) than the rest of the sample.[48] This would be in keeping with the notion that these patients represent a milder phenotype.[46;63]

TABLE 2: COMPARISON OF SEX DISTRIBUTION AND MAJOR CLINICAL FINDINGS IN 22q11DS BETWEEN THE SAMPLES REPORTED ON BY RYAN et al. & COHEN et al.[46;48]

	Cases reporting data on the absence or presence of a particular finding						Cases with finding present					
	Cohen		Ryan		Difference		Cohen		Ryan		Difference	
Clinical findings	N	(%)	N	(%)	X^{2*}	p	N	(%)	N	(%)	X^{2*}	p
Female sex	123	98	399	72	38.02	<.0001	83	67	202	51	10.97	<.0015
CHD	120	95	545	98	2.00	NS	36	30	409	75	88.65	<.0001
All palatal anomalies	105	83	496	89	2.79	NS	92	88	233	47	56.95	<.0001
Cleft palate							43	41	72	15	37.45	<.0001
VPI							49	47	161	32	7.77	<.0004
LD/MR	111	88	338	61	34.84	<.0001	104	94	268	79	12.13	<.0008
Psychiatric disorders (adults)[a]	46	37	61	-	-	-	45	36	11	18	5.71	<.02

*df = 1
CHD = congenital heart defects, major anomalies only (see text)
[a]Includes any motor, language, or speech delay.
NS = non-significant
VPI = velopharyngeal insufficiency.
LD/MR = learning difficulties or mental retardation.

The expected sex ratio for an autosomal dominant condition is 1:1 and it is therefore of interest that a number of 22q11DS samples including that of Cohen et al. report a female excess.[48] One possible explanation for this could be preferential maternal transmission of the deletion as has been suggested by some authors.[6;64] In turn, the reason for this phenomenon could be impaired

reproductivity of males or possibly decreased survival of males into adulthood.[27;64]

In the largest sample of 22q11DS adults (n=78) where patients were all systematically assessed by the same clinicians, Bassett et al. documented all features that appeared in at least 5% of the sample. The group identified 43 lifetime features (median of nine per patient (range 3-22)) of which 12 could be further subdivided.[47] Five of these, learning disabilities (92.3%), hypocalcaemia (64.1%), palatal abnormalities (42.3%), cardiovascular anomalies (52.6%) and thymic hypoplasia (10.3%) are characteristic features of 22q11DS. Other, less commonly appreciated features that were demonstrated in a significant number of participants were obesity (35%), hypothyroidism (20.5%), hearing deficits (28%), cholelithiasis (19%), dermatological abnormalities (28%) and scoliosis (47%).

The sample was primarily collected from a congenital cardiac centre (n=35) and psychiatric referral sources (n=39).[47] Marked ascertainment bias effects could be directly related to this, as, in comparison to the whole sample, each ascertainment subgroup had less than half of the other's highlighted anomaly. To assess other possible effects of major ascertainment biases the results from two non-overlapping subgroups (31 with Tetralogy of Fallot; 31 with schizophrenia) were also determined. No other significant differences could be demonstrated

between the two, however due to its size, the sample would have had limited power to detect differences for features with lower frequencies.

Better delineation of the 22q11DS phenotype is of great importance as it not only helps clinicians with early detection and appropriate treatment of the associated medical features, but also aids counselling, especially in terms of common later onset conditions, including psychiatric disorders. Genetic counselling is also crucial in families with an affected parent as the sibling recurrence risk is 50% and offspring are often more severely affected. Furthermore, an awareness of suggestive phenotypic features could help a wide variety of clinicians such as endocrinologists, hematologists and psychiatrists to identify 22q11DS in their patients.

4. 22q13 ABERRATIONS

For the sake of completeness it is necessary to mention the syndrome associated with the 22q13 terminal microdeletion that has been described in the literature. Patients share a common phenotype, including generalised developmental delay, hypotonia, compromised language development, autistic-like behaviours, normal to accelerated growth and mild dysmorphic facial features. Other features that have been described with regularity are, partial agenesis of the corpus callosum, bilateral ureteropelvic stricture, gastroesophageal reflux, seizures, increased tolerance to pain and hearing loss.[65] Males and females seem to be equally affected but currently the

prevalence of this condition is unknown and in all likelihood it is under-diagnosed. The deletion is usually a de novo finding; however, approximately 20% of reported cases are familial.[66]

Using mostly FISH analyses it has been shown that the deletion varies considerably with sizes from 130kb to 9Mb reported.[67] Statistically significant correlations between the size of the deletion and the degree of developmental delay, as well as the increased severity or higher incidence of clinical features such as hypotonia, have been demonstrated.[66] The breakpoint has been demonstrated within exon 21 of the *PSAP2* (*SHANK3*) gene. As the *SHANK3* gene encodes a structural protein of the postsynaptic density, it has been proposed that haploinsufficiency of this gene is a major causative factor in the neurologic symptoms of this syndrome.[68] Due to the autistic-like behaviours that have been reported it has been suggested as a potential cause for this disorder.[69] No other psychiatric associations have been reported and although the 22q13 area has been studied in both schizophrenia and bipolar disorder, no conclusive linkage has been demonstrated.[70;71]

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5. 22q11DS AND NEUROPSYCHIATRY

Although few studies initially placed any focus on the presence of psychiatric disorders in 22q11DS, high rates of neuropsychiatric disorders were often reported in such samples.[72-76] Interestingly, one of the first clues that psychiatric symptomatology may be associated with the microdeletion was when children

with 22q11DS were demonstrated to show affective flattening and monotonous speech as part of the phenotype although they did not meet criteria for a specific psychotic illness.[77] Specifically in children, attention-deficit hyperactivity disorder, primarily the inattentive subtype, is one of the most common psychiatric problems associated with 22q11DS, with reported rates of 37-41%.[78;79] Autistic spectrum disorders (ASD) also seem to appear commonly, with for example, Fine et al. reporting approximately 11% of children in their sample (n=98) to be autistic, and showing greater developmental delays than those without ASD.[80] Both bipolar disorder and depression have also been reported to be common in children with 22q11DS.[81] It has also been suggested that more careful scrutiny of pre-adolescents reveals psychotic manifestations to be present earlier than typically reported, possibly accompanied by manifestations such as reduced verbal IQ performance and decreased adaptative social skills.[82] In their study, Gothelf et al. compared a group of 31 adolescents with 22q11DS with a control group (n=29) at baseline and five-year follow-up.[83] Although baseline neuropsychiatric profiles had been similar, at follow-up, 32.1% of the 22q11DS subjects had developed psychotic disorders in comparison to only 4.3% of the control subjects.

In adult samples, between 22% and 31% have been shown to meet criteria for schizophrenia or schizoaffective disorder.[47;74;84] Murphy et al. reported on an adult sample of 50 VCFS patients showing a prevalence of 24% for schizophrenia, whilst Bassett et al. reported a prevalence of 22.6% in their

sample of 78 22q11DS patients.[47;84] This finding would be in keeping with the fact that the mean age of onset for these disorders is early adulthood in the general population. It would also fit with the estimation that 1 in 4 children with 22q11DS will develop schizophrenia in their lifetime.[85]

In addition to schizophrenia, high rates of obsessive compulsive disorder (OCD) and bipolar disorder have also been reported. Gothelf et al. showed a lifetime prevalence for OCD 32.6% of participants (n=43) and Papolos et al. a rate of more than 40% for bipolar-spectrum disorders (n=25).[73;79] These findings stand in contrast to the only mildly elevated (3%) reported rates of schizophrenia co-morbid with mental retardation.[86] Also, co-morbid primary psychiatric illness (and especially psychosis), is rarely, if ever, reported in other genetic syndromes with behavioural phenotypes (e.g. Fragile X, Rett's and Turner Syndrome).[87] Also neither Bassett et al. nor Pulver et al. could demonstrate a relationship between the high rate of schizophrenia and the presence of a major psychotic illness in first or second degree relatives of 22q11DS patients.[74;88]

6. 22q11DS AS AN ENDOPHENOTYPE IN SCHIZOPHRENIA

Viewed from a schizophrenia population group perspective, there seems to be a clear increased risk for a 22q11 deletion (with reported rates of 0.3-2%) in comparison to that of the general population (0.016-0.025%).[2;17;89;90] Furthermore, this risk also seems to increase with earlier age of onset

(childhood-onset of schizophrenia (COS)) (5-6% in cases with onset before the age of 13 years).[90;91] It has also been suggested that differing prevalence rates are seen in diverse population groups.[17;89;92;93] Importantly, seen together, these studies suggest an approximate 25-fold risk for schizophrenia in patients with 22q11 microdeletion, therefore representing the highest known genetic risk factor for this illness (comparable to having two parents with schizophrenia and second only to having an affected monozygotic twin).

As such, 22q11DS has in fact been proposed as a disease model for a genetically mediated subtype of schizophrenia. In order for such a model to be of value to researchers and clinicians it would not only be important to demonstrate that the schizophrenia phenotype is similar to that of other forms of schizophrenia but also that specific features can be identified which will help us find patients with the 22q11DS subtype of the illness. In a COS sample, Sporn et al., found no differences between those with or without 22q11DS in terms of age of onset, IQ, premorbid functioning or severity of psychosis, however, the 22q11DS group did exhibit significantly less negative symptoms.[90] Furthermore, both groups had similar total cerebral grey matter, lateral ventricle volumes and rate of progressive reduction of grey matter.

In their large adult group comparison, Bassett et al., compared 16 schizophrenia patients (without concurrent mental retardation), known with 22q11DS, to a group of 46 schizophrenia controls.[94] No differences could be demonstrated in terms of

age of onset, lifetime or cross-sectional core positive and negative symptoms of schizophrenia or global functioning between the two groups. This was in keeping with previous studies with the exception of Murphy et al. who found a relatively later age of onset and fewer negative symptoms to be associated with 22q11DS schizophrenia.[84;88;95;96] One possible reason for this difference may be found in the fact that a much larger percentage of this group were parents (54% compared to the 18.8% of Bassett et al.) possibly indicating a group with a milder form of schizophrenia.[84;88]

Furthermore, although their sample seemed to have a lower lifetime incidence of co-morbid substance use as well higher excitement factor scores, Bassett et al. could not demonstrate the presence of any specific features of this schizophrenia subtype that could be used to help identify patients with 22q11DS. The current set of screening criteria, based mostly on characteristic physical phenotypic features (as suggested by a number of studies) is therefore still the most reliable method to identify these patients.[88;97-99] (See table 3.) In the absence of these, clinicians therefore need to focus on careful history-taking and searching for subtle physical and cognitive features in order to identify such individuals.

It is also important to be aware that some of the phenotypic features may be influenced by age or the parkinsonistic side-effects of antipsychotic medications (e.g. hypotonia may be obscured and kyphosis/scoliosis may be worsened).[100] As with other genetic syndromes, developmental delays, suggestive medical and

family history as well as some laboratory results (e.g. low platelet count, low calcium) may, when present as a pattern of several features, help increase suspicion for the presence of 22q11DS.[97]

TABLE 3: PROPOSED SCREENING CRITERIA TO IDENTIFY PATIENTS WITH SCHIZOPHRENIA AT INCREASED RISK FOR 22q11DS[97]

Presence of two or more of the following features:	
1	Hypernasal speech, history of speech therapy, velopharyngeal incompetence, cleft palate (usually submucosal)
2	Characteristic facial features: e.g., long, narrow face, narrow palpebral fissures, flat cheeks, prominent nose tip, small ears, small mouth, retruded chin
3	Learning difficulties, history of special education, mental retardation (borderline to mild)
4	Congenital heart defect: e g., ventricular septal defect, tetralogy of Fallot, right aortic arch, double aortic arch
5	Other significant congenital anomaly: e.g., talipes (club foot), polydactyly (extra finger or toe), kyphosis/scholiosis, renal anomaly, hypospadias
6	History of hypocalcemia (neonatal, childhood, adolescence or adult onset) and/or hypoparathyroidism
7	History of athymia (absent thymus gland) or severe immune deficiency in infancy

Neurocognitive dysfunction has long been regarded as a core component of schizophrenia.[101] Chow et al. described the neurocognitive profiles of patients with 22q11DS with or without schizophrenia demonstrating no significant difference in mean IQ (71.6 versus 74.8) or academic achievement.[102] However, the schizophrenia group performed significantly worse on tests of motor skills, verbal learning and social cognition whilst attentional dysfunction seemed to be a more general feature of 22q11DS. Thus, the differences between the groups mimicked characteristics found between schizophrenia and non-schizophrenia

control populations, further supporting 22q11DS as a model for neurodevelopmental investigations of schizophrenia.

Some other hypotheses also strengthen the argument that 22q11DS-related schizophrenia is relevant to identifying a susceptibility locus. Firstly, we find evidence from research on animal models – please see elsewhere in this chapter. Secondly, results from a number of structural neuro-imaging studies have indicated that similar brain abnormalities, such as enlarged ventricles, reduced total brain volume and increased prevalence of midline brain abnormalities, exist in both schizophrenia and 22q11DS.[103-105] Also, cortical grey matter loss such as seen in COS has been demonstrated in brain imaging studies of non-psychotic children with 22q11DS.[106]

Thirdly, non-22q11DS-related schizophrenia has been suggested to have neurodevelopmental origins as evidenced by the increased prevalence of minor physical anomalies, as well as the presence of developmental delays, minor co-ordination deficits, blunted affect and other abnormalities of early neurodevelopment.[107;108] In comparison 22q11DS, in addition to major features, has a number of minor dysmorphic features that overlap with those found in schizophrenia. Intellectual deficits can often be demonstrated in 22q11DS and the midfacial and cardiac abnormalities found might result from disturbed development and migration of neural crest cells. In combination these could point

to common developmental mechanisms based partly on disturbed cellular migration.

Due to the considerable heterogeneity displayed by the illness, identifying a 22q11DS subtype of schizophrenia could have several advantages. Firstly, it could serve as a neurodevelopmental model of the illness and lead to a better understanding of the pathogenesis, including an improved determination of possible environmental risk factors that interact with genetic risk. Secondly, identifying such a subtype would be a very important step towards reducing the heterogeneity associated with the disorder. And thirdly, finding the location of a gene for even a rare subtype of the illness would provide us with a vital piece of the puzzle in our search to determine the genetic aetiology of schizophrenia.

7. POSSIBLE SCHIZOPHRENIA CANDIDATE GENES IN THE 22q11 REGION

With at least one schizophrenia patient having been described as carrying the smaller 22q11 deletion, the 1.5Mb deletion has been defined as the "schizophrenia critical region".[17] The relatively small size of the implicated region may be considered to be an advantage and as the majority of genes in this region are known, this locus is amenable to molecular genetic analysis. This area most likely contributes via hemizygous deletion (30 genes are located in area) to the development of schizophrenia in approximately 2% of cases, while non-deletion variants in one or more of the individual genes from this locus may

make a larger contribution to susceptibility to schizophrenia in the wider population and could explain the increased risk for schizophrenia associated with this locus.[85]

Eight genes from the regions deleted in 22q11DS have been reported as candidate genes in association with schizophrenia. These are *COMT* (currently most widely studied), *PRODH, ZDHHC8, CLDN5, DGCR14, DGCR2, TBX1* and *GNB1L.*

7.1 *COMT*

COMT encodes catechol-*O*-methyl-transferase, an enzyme that is critical for dopamine catabolism. As far back as 1976, Groshong et al. reported an association between higher COMT enzyme activity in the blood of schizophrenia patients and dopaminergic dysfunction.[109] It has been shown that dopamine levels in the prefrontal cortex (PFC) are critical for modulating cognitive function and that *COMT*, in turn, is important for modulating PFC dopamine levels, particularly under conditions that pertain during the performance of PFC-dependant tasks.[110;111] Stable deficits in cognitive functions referable to the PFC and cortical physiological abnormalities during performance of such tasks, have been consistently reported in studies of schizophrenia.[112;113]

A common *COMT* polymorphism creates a valine-to-methionine (Val158/108Met) substitution at codon 108 or 158. The Val and Met isoforms represent high- and

low-activity enzymes, respectively.[114] When specifically looking at PFC functions, individuals homozygous for the high-activity Val allele perform worse in tests of cognition and working memory.[115;116] In 22q11DS patients similar results were found, with Met-polymorphism-hemizygous individuals shown to perform better on a composite measure of executive functioning.[117] However, it was also associated with cognitive decline and increased severity of psychosis in patients with 22q11DS.[118]

Unfortunately, association studies between the functional *COMT* polymorphism and schizophrenia have, to date, generated mostly conflicting results.[119] The major evidence for a positive association between the high activity Val allele and schizophrenia is derived from a large population-based study of Ashkenazi Jews of European ancestry.[120] However, although one recent meta-analysis also supported the fact that the Val allele may be a weak risk factor for schizophrenia, at least in those of European ancestry, another meta-analysis concluded that available evidence did not support a single locus effect for the Val/Met polymorphism on schizophrenia risk.[121;122]

Therefore, the current direction of research in this area centres around the premise that other functional alleles exist in COMT beyond the Val/Met. Investigators have expanded their analysis to additional markers and haplotypes and although the largest study to date did not find any evidence in favor of association, other studies have supported an association for COMT that appears

stronger with haplotypes than with the Val/Met locus alone.[123-127] Clearly, the relationship is a complex one and currently a definitive answer as to whether or not *COMT* can be regarded to be a susceptibility gene for schizophrenia, still lies beyond our grasp.

7.2 *PRODH*

PRODH encodes proline dehydrogenase (POX), a mitochondrial enzyme involved in proline catabolism and glutamate biosynthesis. Using a relatively dense genetic map of 72 single nucleotide polymorphisms (SNPs) distributed across the entire 1.5Mb region of 22q11 associated with susceptibility to schizophrenia, Liu et al. identified two independent peaks of association, one at the proximal and one at the distal end of the locus.[128] The proximal peak of association was due to overtransmission of a haplotypic variant of *PRODH*. Li et al. analyzed the *PRODH* gene in patients with schizophrenia and their families in China and found an association with two haplotypes.[129] Some patients with 22q11DS have been shown to have elevated levels of plasma proline, a finding that has also been demonstrated in schizo-affective and bipolar disorder, but not in schizophrenia.[130-132] However, Jacquet et al. identified a family of two schizophrenia subjects with a heterozygous deletion of the entire *PRODH* gene and consequently hyperprolinemia.[131] One could hypothesize that there are at least two ways in which decreased *PRODH* activity could possibly contribute to schizophrenia susceptibility. Firstly, L-proline itself may serve as a direct

modulator of glutaminergic transmission in the brain and secondly, *PRODH* has been implicated in the initiation of apoptosis.[133]

7.3 *ZDHHC8*

As discussed in the previous paragraph, the Liu et al. study also revealed a distal independent peak of association.[128] Statistical evidence strongly implicates the *ZDHHC8* gene as the prime candidate for this region. With at least two yeast *ZDHHC* proteins, ERF2 and AKR1, being transmembrane palmitotyltransferases (PMT), it is regarded as likely that *ZDHHC8* is also a PMT with a yet unidentified substrate. Palmitoylation is a posttranslational modification of proteins with the lipid palmitate and recent work has shown that palmitate reversibly modifies numerous classes of neuronal proteins, including those important for neuronal development, neurotransmitter receptors and synaptic scaffolding indicating that palmitoylation may play an important role in synaptic transmission.[134] Through screening for association with schizophrenia one SNP (rs175174A/G), associated with a relative abundance of unspliced transcripts, localised to intron 4 of *ZDHHC8* was identified. Mukai et al. demonstrated an association between the A allele and schizophrenia in women, but other studies could either not confirm the association of rs175174 with schizophrenia, or could confirm it with rs175174G, but not the A allele.[127;135-139] Currently, the role of *ZDHHC8* in schizophrenia remains unclear.

7.4 *CLDN5 & DGCR14*

A weak association between schizophrenia and SNPs in *CLDN5*, which encodes claudin-5, has been reported.[140;141] Claudin proteins are a component of tight junctions that play crucial roles in response to changing natural, physiologic and pathologic conditions. Associations of promoter polymorphisms of *DGCR14* have also been reported, but the relation between this or claudin-5 and schizophrenia is not yet known.[142]

7.5 *DGCR2*

DGCR2 encodes a putative adhesion receptor protein and association between its polymorphisms and schizophrenia have been noted, with the expression of *DGCR2* found to be elevated in the dorsolateral prefrontal cortex of schizophrenica patients relative to matched controls. A possible explanation for this increase could be patient exposure to antipsychotic drugs, as significantly elevated levels of *DGCR2* transcripts were found in rats exposed to such medication.[120]

7.6 *TBX1 & GNB1L*

TBX1 is a member of the T-box transcription factor family and *GNB1L* encodes an evolutionarily conserved peptide of unknown function and is essential to embryonic development. Both genes have been shown to play a role in prepulse inhibition and as *GNB1L* and *TBX1* lie only 17 kb apart, it is strongly considered

that the engineered mutation of either gene may affect expression of its neighbour. Importantly, *TBX1* point mutations were identified in five individuals with classic 22q11DS but without the common chromosomal deletion, suggesting *TBX1* as a major contributor to 22q11DS.[143] A recent comparison between a large Caucasian control (n=436) and patient (n=446; psychotic and affective disorders) sample demonstrated no evidence for significant group differences in *TBX1* variation.[144] Similarly, no significant difference in the genotype or allele distributions could be found between 328 Chinese schizophrenia patients and 288 controls for any of the *TBX1* polymorphisms, nor was there any haplotype association.[145] However, Williams et al. recently found evidence for a male-specific genotypic association for *TBX1/GNB1L* in 662 schizophrenia cases and 1416 controls from the UK and replicated this finding in two independent case-control samples.[146]

8. CHROMOSOME 22: ANIMAL MODELS

The usefulness of an animal model in the study of a human illness is directly related to its ability to mimic the aetiology, underlying pathophysiological mechanisms and responsiveness to treatment of the illness as well as, in the case of neuropsychiatric disorders, its psychological constructs.[147] Specifically in the case of schizophrenia, the clearly observed heterogeneity in symptoms, course and aetiology presents a particular challenge. Furthermore, the development of a valid animal model could be confounded by the uniquely human nature of the illness which includes defects of perception, thinking and

emotion.[108;148] Initially, animal models in schizophrenia have had either a pharmacological or developmental focus. (See summary in table 4.)

TABLE 4: SUMMARY OF WELL-CHARACTERISED PHARMACOLOGICAL AND DEVELOPMENT MODELS OF SCHIZOPHRENIA AND RELATIONSHIP TO SCHIZOPHRENIA-RELATED BEHAVIOURS[151]

Pharmacological models	Hyperactivity	Social interaction deficits	Disruption of prepulse inhibition	Disruption of latent inhibition	Cognitive deficits	Increased sensitivity to DA agonists	Increased sensitivity to NMDA agonists
Treatment with DA agonists	Yes	Yes	Yes	Yes	Yes	-	-
Treatment with NMDA agonists	Yes	Yes	Yes	No	Yes	-	-
Developmental models							
Neonatal hippo-campal insult	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Social isolation	Yes	Yes	Yes	Yes	No	Yes	Yes
Prenatal MAM administration	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Prenatal immunological activation	Yes	Yes	Yes	Yes	Yes	Yes	Yes

MAM = Methylazoxymethanol acetate
NMDA = N-methyl-D-aspartate

The dopamine (DA) hyperfunction hypothesis of schizophrenia is supported by the fact that DA agonists can both exacerbate psychotic symptoms in patients with schizophrenia and also induce them in normal controls.[149] In rodents, DA-releasing drugs have been shown to produce disruption of prepulse inhibition (PPI) and latent inhibition as well as cognitive deficit models of schizophrenia. Hyperactivity has also been seen, which has been likened to the psychomotor disturbance of schizophrenia, but this model has been criticised as not representing a core feature of the illness. Due to the simpatomimetic effects of N-methyl-D-aspartate (NMDA) receptor antagonists dysregulation of glutaminergic transmission has also been hypothesized in schizophrenia.[150] In rodents, NMDA

antagonists have been shown to not only produce hyperactivity, PPI and cognitive deficit symptoms of schizophrenia, but also social interaction deficits.

Targeted brain lesions have been used in attempts to model neurodevelopmental aspects of schizophrenia. Although an in-depth discussion of the literature on animal adult and neonatal lesion studies is not within the scope of this chapter (see full review Lipska[148]), numerous interesting results have been obtained, for example, that rodents with neonatal hippocampal lesions demonstrate delayed onset of hyperdopaminergic behaviour, disrupted PPI and social interaction.

The construction of mice with targeted mutations has specifically enhanced our ability to uniquely identify the functional significance of the targeted genes and their encoded proteins. Due to their high fertility rate and short life span, mice are regarded as particularly appropriate animal models. These mouse models for schizophrenia susceptibility genes are not only useful in helping us to understand how these genes contribute to the pathophysiology of the illness, but they may also help us to address potential interactions between these genes. Although complex disease manifestations such as delusions and hallucinations can not be modelled in mice, certain endophenotypes which can be modelled relatively accurately in mice, could be of particular value. Examples of these would be sensory gating, working memory and attention, all of which several studies have suggested could contribute to schizophrenia susceptibility.[152-154] They are often present in childhood, prior to the full phenotypic expression of the illness and can

also be seen in first-degree, clinically unaffected relatives of schizophrenia patients.[153;155] Mouse models afford us an opportunity to conduct thorough and detailed evaluation of cellular and molecular neuropathology as well as the possibility to quantify brain mRNA transcripts using microarray technology.[156] (See table 5 for summary of behaviours exhibited in some schizophrenia susceptibility genes' mutant mice models.)

Therefore, taking into account the robust association between the 22q11 microdeletion and the risk of developing schizophrenia coupled with our precise knowledge of the human/mouse sequence and chromosomal synteny at this locus, we are presented with a unique opportunity to use mouse models to understand the biological basis of the increased psychosis risk associated with this genetic lesion.

The syntenic region of the human 22q11 locus lies on mouse chromosome 16. With the exception of *CLTCL1*, all the human genes are represented albeit with some minor changes in gene order. In order to help narrow the search for causative genes that can be associated with psychiatric disorders, Maynard et al. undertook a comprehensive expression profile of 22q11 orthologues in the mouse.[157] They determined the CNS expression of 32 of these orthologues of which 25 are mapped to the proximal 1.5Mb regions consistently deleted in 22q11DS. None of these were found to be uniquely expressed in the developing or adult mouse brain. However, of the 32, 27 are localised in the embryonic

forebrain as well as other sites phenotypically associated with 22q11DS (i.e. aortic and branchial arches and limb buds). These are expressed at apparently constant levels in the foetal, postnatal and adult brains except for three – *TBX1, ProDH2, T10* – which increase in adolescence and decline in maturity. Whereas the others are present fairly constantly throughout the critical processes of neuronal proliferation, migration and circuit differentiation these three could be particularly important during the refinement and stabilisation of synaptic connections.[158] Previous reports have indicated that synaptic refinement is most likely compromised in patients with schizophrenia.[159;160]

By using gene targeting and chromosomal engineering the 22q11 deletion (so-called D*f*1) has been modelled in mice.[161] Mice heterozygous for the deleted region (D*f*1/+) developed identical 22q11DS cardiovascular abnormalities and in certain genetic backgrounds thymic and parathymic defects.[162] Interestingly, the conotruncal defects of mice hemizygous for the 1.5Mb deletion can be partially rescued by a human BAC containing *TBX1* (a gene highly expressed in the pharyngeal arches during mouse embryonic development), whilst mice heterozygous for the null mutation in *TBX1* develop conotruncal defects.[163;164] This suggests that *TBX1* could be responsible for some (or many) of the physical features associated with the deletion.

However, due to the variable expressivity of these features, one has to conclude that additional modifiers are involved. *VEGF* which encodes vascular endothelial

growth has been reported as a modifier of cardiovascular birth defects in 22q11DS.[165] The *Vegf164* isoform in mice causes birth defects similar to that of 22q11DS and knocked-down *VEGF* levels enhanced pharyngeal arch artery defects induced by *TBX1* knockdown in zebrafish. *FGF8* that encodes fibroblast growth factor 8 has been shown as a potential modifier of 22q11DS with *Fgf8* mutants displaying the complete array of cardiovascular, glandular and craniofacial phenotypes seen in human 22q11DS.

The prepulse inhibition (PPI) of the acoustic startle reflex is considered an intermediate phenotypic marker of sensorimotor gating in schizophrenia. In a longitudinal study of 22q11DS children and their unaffected siblings, the 22q11DS group showed significantly less PPI with analyses suggesting that this deficit did not reflect developmental delay.[166] Unfortunately, specificity of reduced PPI to individual psychiatric disorders is low as it is associated with schizophrenia, OCD, Asperger syndrome and others.[152] Paylor et al. mapped PPI deficits in a panel of mouse mutants and revealed that either *TBX1+/-* or *GNB1L+/-* mice had reduced PPI.[167] Expression of *TBX1+/-* is limited to the brain vasculature of fullterm mice embryos or adult mice. Hanson and Gottesman have proposed a role for microvasculature in the pathophysiology of schizophrenia as such damage could satisfy both the developmental and degenerative models of the illness.[168] Furthermore, *TBX1+/-* mice, in response to amphetamine, did not display behavioural differences in comparison to wild-type littermates, suggesting the possibility that the haploinsufficiency of *TBX1* does

not contribute to the behavioural abnormalities associated with 22q11DS other than PPI deficits in mice.[169]

Individuals with reciprocal duplication of the chromosome region deleted in 22q11DS have been identified and display a phenotype ranging from mild to severe, sharing a tendency for velopharyngeal insufficiency with DGS/VCFS, whilst displaying other distinctive characteristics.[18;170-172] Although their neuropsychiatric disorders have not been fully characterised they display impulsivity, aggression, social immaturity, attention span and cognitive deficits. Mice that overexpress a 200kb region of human 22q11.2 showed spontaneous sensitisation of hyperactivity and a lack of habituation that could be modified by antipsychotic drugs.[169]

Mouse models for individual candidate genes of the 22q11 region could also help to increase the understanding of these genes and more specifically their impact on schizophrenia. Mice homozygous to a mutation in the mouse orthologue of the human *PRODH* gene have been shown to have hyperprolenaemia comparable to that seen in some individuals who carry the 22q11 microdeletion, providing a accurate model of not only a susceptibility gene but also an susceptibility allele.[173] These mice also exhibit deficient PPI, although not to the same degree as mice that carry long-range deletions who have normal proline levels.[174]

TABLE 5:

SCHIZOPHRENIA-RELATED BEHAVIOURAL PHENOTYPES AS DEMONSTRATED IN MICE MUTANT FOR SCHIZOPHRENIA SUSCEPTIBILITY GENES[151]

Human Gene	Chromosome Location	Function in Humans	Mutant mouse model	Evidence for a schizophrenia-related behavioural phenotype
Neuregulin – 1 (*NRG1*)	8p21-p22	Synapse formation; neuronal migration, synaptic plasticity	Heterozygous *NRG-1* KO; EGF-like domain; mixed 129/SvEv-C57BL6 background	Open-field hyperactivity
		Regulation of NMDA & AMPA receptor function and NMDA & GABA receptor submit expression	Heterozygous *NRG-1* KO (TM domain); C57BL6 background	Open-field hyperactivity, impaired exploration and habituation to novel environment; disrupted PPI, reduced forebrain NMDA receptor density
			Heterozygous *NRG-1 K*O (IG domain); C57BL6 background	Disruption of latent inhibition
			Heterozygous *Erb2, ErbB3, ErbB4* KO; mixed 129/SvEv C57BL6 background	Open-filed hyperactivity in ErbB4 mutants
			Heterozygous *ErbB4* KO; C57BL6 background	Open-field hyperactivity; PPI disruption
			Homozygous CNS-specific *ErbB4* KO;C57BL6 background	Disruption of spatial learning and memory in water maze in heterozygous males
			FVB Background	No evidence
Regulator of G protein signalling 4 (*RGS4*)	1q21-q22	Regulation of G protein coupled receptors	Homozygous *RGS4* KO; mixed 129-B6D2 background, Backcrossed C57B16 three times	No evidence: intact PPI, spatial learning and memory
Dysbindin (*DTNBP1*)	6p22.3	Regulation of synaptic structure and signalling	Homozygous *DTNBP1* KO (sdy mutant); mixed DBA/21; PWK background	No evidence: intact PPI
Catechol-O-methyl transferase (*COMT*)	22q11	Involved in DA metabolism, particularly in prefrontal cortex	Homozygous *COMT* KO: mixed 129J-C57B16 background	No evidence
Proline dehydrogenase (*PRODH*)	22q11	Regulation of cortical Ach function	Homozygous *PRODH* KO; 129SvEv background	PPI disruption
		Metabolic precursor or glutamate in subpopulation of glutamate neurons		Altered glutaminergic neurotransmission in hippocampus; Evidence for epistatic interaction between PRODH and COMT at behavioural and transcriptional level
Disrupted in schizophrenia 1 (*DISC-1*)	1q42	Neuronal migration, neuronal differentiation	Conditional fore-brain restricted inducible mutant *DISC1* transgenic mouse; strain unknown	Increased locomotion in novel environment impaired spatial learning and memory; disrupted PPI
			Conditional inducible mutant *DISC1* transgenic mouse; strain unknown	Reduced sociability; impaired latent inhibition; disruption of spatial working memory
			DISC1 transgenic mouse; strain unknown	Enlargement of lateral ventricles

Small-scale transcriptional profiling (which could help provide an unbiased evaluation of the transcriptional programmes affected by the disruption of a gene) that was done in the cortex of *PRODH*-deficient mice showed fewer than 10

genes as differentially expressed.[85] Interestingly, *COMT* was listed as one of the upregulated genes and follow-up behavioural analysis of these mice revealed a pronounced hyperresponsivity to dopamine. This could be particularly important in the light of the fact that dopaminergic hypersensitivity in schizophrenia is a well-established concept.[175] The transcription therefore revealed a previously unsuspected interaction between *PRODH* and *COMT*, implying that if COMT upregulation is one of the mechanisms employed to control cortical dopaminergic hypersensitivity then schizophrenia patients with a 22q11 microdeletion are at a particular disadvantage because they are deficient in both genes.

In a knockout mouse model of *ZDHHC8,* female mutant mice displayed significantly lower PPI as well as profound deficits in indices of fearfulness in comparison to wild-type littermate controls.[127] Furthermore, these mice appeared to be less sensitive to stimulation of locomotor activity induced by a psychomimetic suggesting that *ZDHHC8* could, at least in part, be affecting behaviour through interference with glutamatergic transmission.

9. SUMMARY

The 22q11 deletion syndrome is a multi-system disorder that currently represents the most common microdeletion found in humans. It presents with a variable phenotype that includes specific congenital heart and velopharyngeal defects, cognitive deficits and/or behavioural abnormalities coupled with facial dysmorphologies. Significant rates of co-morbid neuropsychiatric disorders have

been reported including attention-deficit/hyperactivity disorder, autism, obsessive compulsive disorder and in 30% of cases, schizophrenia. On the other hand schizophrenia samples have shown reported rates of 0.3-2% for 22q11DS (with ethnic differences possibly accounting for the variation), suggesting that a deletion of 22q11 may represent one of the highest known risk factors for this illness. As such, 22q11DS has been proposed as a disease model for schizophrenia with support being found in a) neuro-imaging studies, b) evidence suggesting common neurodevelopmental mechanisms and c) animal models. Identifying a 22q11DS subtype of schizophrenia could lead to a breakthrough in the search for schizophrenia genes. Eight genes (*COMT, PRODH, ZDHHC8, CLDN5, DGCR14, DGCR2, TBX1, GNB1L)* from the region deleted in 22q11DS have been reported as candidate genes for association with schizophrenia. Of these, *COMT* has been by far the most studied but, to date, the complex relationship between *COMT* and schizophrenia has not yet been untangled. Thus, although analyses of 22q11DS have contributed to our understanding of the genetics and molecular pathogenesis of schizophrenia, more studies are needed if critical questions relating to this devastating illness are to be answered.

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CHAPTER 6

USING DYSMORPIC FEATURES AS AN ENDOPHENOTYPE IN SCHIZOPHRENIA

CONTENTS

1. THE USE OF ENDOPHENOTYPES IN SCHIZOPHRENIA RESEARCH

Current knowledge strongly supports the notion that schizophrenia seems to be a heterogeneous illness resulting from a complex interplay between genetic and environmental risk factors. However, as delineated in a previous chapter, despite the apparent genetic contribution, the specific mechanism or gene has yet to be found.

Furthermore, schizophrenia shows considerable clinical heterogeneity as reflected by an early description of this disease entity: "Dementia praecox, a number of disease entities".[1] This clinical heterogeneity arguably reflects the heterogeneous nature of susceptibility factors for schizophrenia. Not only do we find multiple combinations of symptoms existing in individuals, but also both disease course and outcome, display considerable heterogeneity. As has been explained before, we can't currently be certain whether this is a single disorder with different clinical manifestations or in fact a group of syndromes, each with unique or overlapping pathophysiology.

Considerable attempts have been made to elucidate the heterogeneity of the schizophrenia phenotype by exploring the relationships between the various symptom dimensions and possible subtypes. In the search for the susceptibility genes it has become apparent that one possible method would be to consider a role for endophenotypes.

Whereas genotypes can be measured with techniques of molecular biology, phenotypes in fact represent observable characteristics of an organism which are the joint product of both genotypic and environmental influences.[2] However, as psychiatric illnesses such as schizophrenia have complex genetic underpinnings, Gottesman and Shields suggest he use of “endophenotypes”, described as internal phenotypes discoverable by a biochemical test or microscopic examination, to provide more appropriate clinical phenotypes for use in psychiatric genetic studies.[3]

Despite the clear advantages that the endophenotype concept held, it was not immediately actively used. In fact, it is only now, with the arrival of the 21st century, that endophenotypes are increasingly being seen as a perhaps necessary mechanism for overcoming the barriers to progress in the genetic analysis of a complex disorder such as schizophrenia.[4] Several approaches have been advocated for endophenotype analysis in schizophrenia. These include demographic variables, clinical symptoms, physical characteristics and neurodevelopmental insults.[5]

The neurodevelopmental hypothesis of schizophrenia holds that the etiological origins of the disease can be traced back to disturbed early prenatal development.[6-8] Currently, numerous diverse lines of evidence support this hypothesis. These include the presence of obstetric complications, epidemiological data (i.e. in utero disease and stress exposure), the presence of

pre-morbid behavioral and neuromotor deficits, the observed pattern of developmental brain anomalies, higher frequency of neurological soft signs and the increased rate of dysmorphic features (often referred to as minor physical anomalies) that tend to accompany the disease.[9-16]

The specific criteria that have been suggested to try and ensure maximum outcomes for the use of markers in psychiatric genetics can also be applied to endophenotypes.[17] These are that the endophenotype: (1) should be associated with illness in the population; (2) should be heritable; (3) should be primarily state-independent (i.e. manifesting itself in the individual whether or not the illness is active); (4) should co-segregate with the illness within families; and (5) should be found in non-affected family members at a higher rate than in the general population. Taking this into account, one possible candidate endophenotype that suggests itself in schizophrenia, could be the presence, or absence of observable differences in anthropometric proportions, the so-called minor physical anomalies.

2. ANTHROPOMETRY – A BRIEF INTRODUCTION

Anthroposcopy, derived from the greek *anthropos* (human) and *skopein* (examine), refers to the practice of judging the body's build by inspection. However, such mere visual assessment, one of the oldest methods of examination still used in medicine today, is not wholly reliable because it is highly subjective. Examiners are very much influenced by their own aesthetic

perceptions and a great deal of experience is essential to relatively compare features that are not objectively measured. Therefore, in order to be able to effectively use data collected from bodily observations the biological science of anthropometry (greek: *anthropos* + *metron* (measure)), the measuring of size, weight and proportions of the human body, was developed.[18]

Measurements of the human face have been performed since ancient Greece and quite a number of aspects of ancient measurements can be found in modern clinical anthropometry.[18] In fact, Egyptian artisans seemed to have been the first to use a crude system of measurement to depict human figures. However, as no well-defined landmarks can be found throughout known examples of Egyptian art, the existence of standards, as such, has not been proven.[19] The Greek sculptor, Polycleitus (c.450-c.420 BC) seems to have been the first to document and use defined measurements. He reported the height of the face to be one-tenth of the length of the body, the whole head one-eight of it and the head and neck together to be one-sixth of the length of the whole athlete.[19] For his part, Aristotle (384-322 BC), not only emphasised the proportions of aesthetics, but tried to use his observations on human structure to prove that certain groups of people were superior to others, also publishing on this topic.[20] These include *Physiognomica*, describing the science of reading one's character from one's bodily features, and *Historia Animalium* with its descriptions of features and judgments regarding the alleged qualities of people with these features.

The so-called neoclassical canons of facial proportion were derived by artists and anatomists such as Da Vinci and Dürer.[21] Leonardo da Vinci (1452-1519) extensively reported on ideal proportions for bodies and faces equating a well-proportioned face to one where (a) the size of the mouth equaled the distance between the parting of the lips and the edge of the chin, (b) the distance from chin to nostrils, nostrils to eyebrows and eyebrows to hairline are all equal, and (c) the height of the ear equals the length of the nose.[22] Albert Dürer (1471-1528) supported Da Vinci in believing that the face could be divided into three equal lengths – forehead, nose, mouth + chin – with further division of the latter into four equal parts.[23] Even though these artists' (and others') canons correlate and are still used in facial analysis, studies using anthropometry have in fact revealed little applicability for these neoclassical canons to Caucasian, Asian, Caribbean or African-American populations.[21;24-27]

Physical anthropology has its roots in the 18th and 19th centuries, with most of the so-called facial measurements being taken directly from skulls and only very few soft-tissue measurements performed. As in the case of Aristotle, the measurements were used predominantly to prove that certain groups of people were superior to others. For example, Cesare Lombroso (1836-1909) described how gangsters, murderers, alcoholics, epileptics and dwarfs could be distinguished from "normal" people by using anthropometric assessments.[28] Unfortunately, because little quantitative data were available, investigators of this era often manipulated results to endorse their own stereotyped hypotheses.[29]

During the first decades of the 20th century cephalometric radiology, a technique using oriented radiographs for the purpose of head measurements was developed.[30] This technique became a vital research tool, used in the pioneering Bolton Study, which pursued dentofacial roentgenographic studies of healthy children from birth to adulthood, collecting 22 800 recordings of 5 400 children over a period of 36 years.[31]

Ultimately, the honour of being the father of modern facial soft-tissue anthropometry lies with Leslie Farkas. By measuring and comparing more than hundred dimensions and proportions in hundreds of people, he defined the standards for almost every soft-tissue measurement in the head and face.[18] He clearly showed that the historical tendency to divide facial measurements into perfect proportions merely reflected the search for perfection as in reality these proportions are only present incidentally and by no means reflect the norm.[21]

3. USING CLINICAL ANTHROPOMETRY

The development of the field of clinical anthropometry has been of particular value to two groups; craniofacial surgeons and clinical geneticists. These groups have widely divergent goals, with the surgeons hoping to restore normality to the abnormal face whilst the geneticists aim to diagnose a wide range of conditions. However, both groups need a consistent system of measurements in order to use collected data as by far the majority of observed anomalies are quantative

traits (i.e. graded on a continuum) with only a few being qualitative traits (e.g. preacuricular skin tags).

At present, various methods of medical craniofacial anthropology exist, including direct anthropometry (in which measurements are taken directly from the subject) and three indirect methods; photogrammetry, soft-tissue facial profile cephalometry and computer-imaged three-dimensional craniofacial surface scans. All these methods share three basic elements of examination (1) location of craniofacial surface landmarks, (2) execution of measurements, and (3) evaluation of the findings using normative data.[32]

With regard to craniofacial landmarks, forty-seven have been described.[18] (Please see appendix A for a full description of these.) Most can be identified visually or their location can be defined with respect to the lateral view of the face or the base view of the nose or at underlying bony areas identified by palpation. At present, 132 measurements are listed in direct anthropometry and the reliability of the measurements depend on technical precision in locating landmarks, maintaining required craniofacial orientation and patient compliance. Ultimately the interpretation of group results relies heavily on the availability of proper population norms for both individual measurements as well as their mutual relationships. Particularly, it has been shown that significant racial and ethnic morphometric differences exist, but some norms are available for North

American Caucasians, young adult African-Americans as well as Chinese subjects.[18;33]

Both the direct and the indirect measurement methods have their pros and cons with all the indirect methods involving a substantial cost investment.[32] Advantages of direct methods include access to measurements of areas covered by hair (e.g. circumference, width, length and height of head) or areas that would be distorted by indirect anthropometry (e.g. the depth of the face in photogrammetry); measurements that require special positions of the head (e.g. base view when measuring the soft nose structures and the nasal root depth) and those that require a special technique such as pressing the tip of the instrument to the underlying bone surface when a measurement is made between bony landmarks (e.g. the width of the face between the zygions). Disadvantages of the direct methods include the prolonged time needed to perform the examination, requirement of a certain level of skill in performing the measurements and a dependence on the co-operation of the examinee. Currently however, of the methods available, only direct anthropometry can be done in the absence of highly specialised and expensive equipment and is therefore readily available to researchers in general.

4. THE USE OF DYSMORPHIC FEATURES AS AN ENDOPHENOTYPE

Minor physical anomalies (MPAs) may be regarded as a collective term for the mild dysmorphic features representing subtle alterations in the developments of various bodily structures in the mouth, eye, ear, global head, hand and feet areas.[34] Once formed, MPAs persist into adult life and are detected easily by simple visual examination and in themselves are considered to be of little functional or cosmetic consequence. As such their presence is regarded as being on a spectrum from normal to indicating major congenital disorder. Although hypotheses still differ as to the exact timing of MPA development, it is still most commonly believed that they are formed during periods of abnormal ectodermal development (due to genetics or a teratogen such as maternal alcohol abuse and/or smoking or influenza during pregnancy).[35]

The brain and the face both develop from common embryonic ectoderm during the first or early second trimester and their morphogenesis is interlinked.[36] Furthermore, although the relationship between prenatal development of the brain and craniofacial structure is not fully understood, these processes appear to occur in tandem.[37] Therefore, with the structures involved in their expression typically sharing their embryonic origin with that of the brain, MPAs are regarded to be potentially valuable indices of disturbances in early neurodevelopment.[38] They may therefore represent risk markers for underlying disease susceptibility timed according to the normal developmental chronology of the embryo.[39] This

could be especially true when multiple MPAs occur together in a given individual and/or when the individual is already at high risk. (e.g. first degree relative of an affected individual).[40]

In fact, MPAs have been shown to provide important clues in specific malformation diagnosis, brain pathology and timing of adversity and therefore have increasingly been the target of studies in a range of mental, behavioural and physical disorders.[40;41] An excess of MPAs have been demonstrated in autism, hyperactivity, epilepsy, mental retardation, poor motor co-ordination, attention deficit disorder, foetal alcohol syndrome, cerebral palsy and schizophrenia.[42;43]

5. MINOR PHYSICAL ANOMALIES IN SCHIZOPHRENIA

The presence of an increased rate of dysmorphic features in some individuals with psychoses in comparison to normal individuals was already documented long before modern conceptions of schizophrenia started to form.[1;44] More recently, numerous studies have confirmed physical deviations in schizophrenia populations in comparison to healthy controls, patients with other DSM Axis I diagnoses and non-schizophrenic twins from disconcordant twin pairs.[16;43;45;46]

5.1 MEASUREMENT OF DYSMORPHIC FEATURES

5.1.1 SCALES AND TECHNIQUES

As previously mentioned, direct measurement techniques are most readily available to general researchers. To date, the Waldrop Physical Anomaly Scale has been the most frequently used instrument in the assessment of dysmorphic features as a sign of neurodysontogenesis and the vast majority of schizophrenia studies have also used this scale (or a modification thereof).[47-50] The original scale was developed for use in Down's Syndrome and consisted of 18 items (see table 1) from six body regions (head, eyes, ears, mouth, hands, feet) mostly scored qualitatively as present (1) or absent (0), but in some instances in a graded manner according to severity (1) or (2).[49]

Although widely used, the Waldrop has been criticised for inherent limitations involving both content and form.[14;15;51;52] Trixler et al. specifically fault the Waldrop for not making a distinction between minor malformations (MM), which arise during organogenesis, and phenogenetic variants (PV), which appear after organogenesis.[52] In other words, MM are always abnormal and can point to problems during organogenesis, but PV are developmentally identical to normal variants. However, other authors argue that more knowledge about the timing and exact nature of the development of MPAs will not necessarily lead to improved knowledge regarding the influence of it on the later development of schizophrenia.[53]

A further common criticism concerning the Waldrop is the fact that it has low internal consistency, probably due to heterogeneity of the anomalies in terms of location, character, period of prenatal origin, time and adversity-specific vulnerability, phase specificity of risk factors, developmental heterochronia and induction processes.[54] However, from an embryological point of view, many of the Waldrop items (especially the craniofacial features) are closely related to underlying brain development, lending some legitimacy to the use of this tool in psychiatric research.[37;55]

In order to address some of the issues, specifically with regards to schizophrenia research, attempts have been made to expand the Waldrop. Ismail et al. added 23 items to the original 18, focusing on documented MPAs that belonged to the same body regions as those measured in the original in order to gain more data without having the subject remove further clothing during the examination.[50] Interestingly, they note that although both the original and their new items independently show significantly higher rates of MPAs in schizophrenia patients in comparison to control subjects, the original Waldrop appears to function well in identifying minor physical anomalies that are especially strongly associated with schizophrenia.[50]

In their modification, Gourion et al. placed focus on craniofacial asymmetry.[47] This addition was based on recent findings indicating significantly decreased brain asymmetry and increased prevalence of atypical handedness in

schizophrenia as well as a relationship between cerebral structural measures and dermatoglyphic abnormalities.[47;56;57] (See table 1 for clarification of specific items used on the original and expanded versions.)

TABLE 1: MINOR PHYSICAL ANOMALIES EXAMINED BY THE ORIGINAL WALDROP AND MODIFIED BY ISMAIL ET AL. AND GOURION ET AL. [47;50]

WALDROP	ISMAIL	GOURION
Head		
Head circumference abnormal Fine, electric hair Hair whorls abnormal	Eyebrows fused Frontal bossing Nostrils anteverted Micrognathia	Widening nose basis Facial symmetry
Eyes		
Epicanthus Telecanthus	Heterochromia Ptosis Colobomata	
Ears		
Low-seated ears Adherent earlobes Malformed ears Asymmetric ears Soft, pliable ears	Pre-auricular skin tag Pre-auricular sinus Ear lobe skin tag	
Mouth		
High/steepled palate Furrowed tongue Tongue with texture spots	Cleft lip Cleft uvula Thin upper lip	
Hands		
Curved fifth finger Abnormal palmar crease	Clinodactyly Tapered fingers Overlapping fingers Small fingernails Hyperconvex fingernails Single crease 5th finger	Asymmetric hands
Feet		
3rd toe longer than 2cnd Partial syndactyly Gap between 1st & 2cnd toe	Overlapping toes Absent 4th or 5th toe Deep creases on soles Hyperconvex toenails	Asymmetric feet

In spite of modifications, some authors still contend that a detailed anthropometric scale is of greater value than a scale such as the Waldrop as it provides more information regards the location and exact nature of MPAs in schizophrenia.[51] Such a scale should therefore not only include qualitative measurements but also quantitative measurements obtained using direct measurement techniques (see earlier in chapter for a more detailed discussion on the different techniques used to obtain quantitative anthropometric measurements). An example is the Lane dysmorphology scale, consisting of 70 items that include both linear and angular craniofacial measurements as well as scoring of individual dysmorphic features.[51] This scale has been used successfully to readily distinguish schizophrenia patients from controls.[51;58]

Unfortunately, one major problem for general researchers using either the Waldrop (or modification thereof) or a comprehensive scale such as the Lane, is that these scales require either well-trained examiners of significant periods of face-time per subject, making them potentially difficult-to-use tools, both time and labour-wise. A possible solution to this dilemma can be found in limiting the examination to a small number of pre-determined quantitative head/facial measurements. To optimise reliability, measurements should be chosen that (1) have reliable landmarks, (2) can be conducted using easily available tools such as a cloth tape measure and calipers and (3) can readily be taught to examiners.

Working from this premise, a number of studies have been conducted that included only a select group of quantitative facial measurements. It has been demonstrated that this method can be used to successfully differentiate between schizophrenia subjects and non-psychiatric controls.[59] Importantly, this differentiation has not only been demonstrated in Caucasian populations but also in a Mestizo sample as well as an predominantly African-American sample.[60;61]

5.1.2 INABILITY TO BLIND RATERS

A major criticism of investigations of MPAs in adults with schizophrenia has always been that such assessments can never be conducted in a truly blinded fashion due to the face-to-face approach needed. In order to try and minimise such bias different samples have employed different methods. These include inter-rater reliability assessments, using assessors unable to communicate in the participants' language and using assessors blinded to any other part of the interview.[58;60;62]

5.2 RELATIONSHIP BETWEEN SCHIZOPHRENIA AND MINOR PHYSICAL ANOMALIES

To date, numerous studies have examined the relationship between MPAs and schizophrenia.[8] In most cases the studies fall into one of two categories; either they report on a comparison of the frequency of MPAs between schizophrenia or at-risk cases and healthy controls or they report on an exploration of the

relationship between MPAs and other putative indices (e.g. cognitive, morphology).

5.2.1 INCREASED FREQUENCY OF MPAs IN SCHIZOPHRENIA SUBJECTS

In the case-control studies (for which mean total MPA scores can be calculated) that used the Waldrop to report on differences between schizophrenia subjects and healthy controls, schizophrenia subjects have been consistently observed to have more MPAs than controls.[8] In fact, the magnitude of the case-control difference has been shown to be quite large, providing support for MPAs as a reliable discriminator between schizophrenia cases and controls.

In one such study, Sivkov and Akabaliev specifically investigated a role for MPAs as a potential discriminator between schizophrenia patients and normal subjects.[63] They found that a total MPA-score (on a 19-item Waldrop) higher than 4 could be regarded as the cut-off score that optimally discriminates between the two groups with a sensitivity of 76.3% and a specificity of 72%. Furthermore, they also demonstrated that the schizophrenia group showed a significantly higher percentage of subjects with large total MPA scores, possibly supporting the hypothesis that MPAs might reflect extragenetic stressful events.

Moving beyond the Waldrop, other researchers have focused also on quantitative measurements. Various studies, using both direct and indirect anthropometric measurement techniques, have demonstrated that these techniques can also be

used to successfully differentiate between schizophrenia subjects and non-psychiatric controls.[51;58-61;64;65] A number of studies have also supported the contention that head and face MPAs can best be used to discriminate between schizophrenia patients and controls.[39;53;66;67] Compared to controls, patients have been shown to have more brachycephalic skulls (wider skull base, shorter lower facial heights, longer facial depths).[51;59] Recent research has shown that middle cranial fossa size and shape influence skull base shape and facial measures.[68] Interestingly, the middle cranial fossa cradles the anterior portion of the temporal lobe, an area of interest in schizophrenia with research consistently identifying a decrease in temporal lobe volume in patients versus controls.[69]

5.2.2 RELATIONSHIP BETWEEN MPAs AND OTHER INDICES IN SCHIZOPHRENIA SUBJECTS

5.2.2.1 FAMILY MEMBERS OF SCHIZOPHRENIA PATIENTS

Findings from studies of MPAs in relatives of schizophrenia patients are mixed. Whilst some studies report that relatives have no MPA elevations, showing frequencies similar to healthy controls, others reveal that relatives have clearly increased rates of MPAs in comparison to controls, although not to the same degree as patients.[47;50;53;70-72]

In their comparison of 21 normal siblings to 60 adult schizophrenia patients as well as 75 normal no-related subjects Ismail et al. demonstrated that although the siblings also had significantly higher rates of MPAs (schizophrenia > sibs >

controls), no correlation was found for the level or type of MPAs within the same family.[50] One possible interpretation could be that schizophrenia subjects and their family members are subjected to one or more genetic or shared environmental factors that increase the risk for developing both MPAs and schizophrenia but that the ultimately increased rate of MPAs in patients (versus siblings), could signal an accentuated effect of such factor(s) in those who later develop schizophrenia.

This evidence was further supported by that of Gourion et al. who investigated 18 trios (two non-psychotic parents and schizophrenia proband) and found no direct evidence for an intra-familial transmission of level or type of MPAs.[47] Furthermore, Cantor-Graae et al. demonstrated increased MPAs in monozygotic twins discordant for schizophrenia specifically showing that although both twins had increased rates in comparison to normal subjects there was a trend toward higher MPA rates in the ill comparatively to the well co-twin.[45] Interestingly, data from Griffiths et al. seem to indicate sporadic cases of schizophrenia may manifest more MPAs than those from multiplex families.[73] This supports the notion that an abnormality of prenatal development seems to be particularly implicated in sporadic schizophrenia.

5.2.2.2 GENDER

Research has shown the male foetus to have a higher sensitivity to adversities of the ectodermal activity and a greater overall vulnerability.[74] Within this context

there seems to be increasing evidence for sex differences in the epidemiology of schizophrenia, as suggested by associations such as earlier onset, poorer premorbid function and poorer outcome in males that hint to a greater vulnerability of the male brain for the neurodevelopmental disruption leading to schizophrenia.[75;76]

A possible role for gender in MPAs has also been investigated but to date results remain inconclusive with a number of schizophrenia studies reporting no sex differences, others an excess in MPAs in females and yet more others an excess in males.[42;45;47;63;71-73;77-80] However, most of these studies just focused on mean total MPA score rather than investigating a possible relationship for MPAs from specific regions and/or individual MPAs.

In this regard Akabaliev and Sivkov reported results not only showing that male schizophrenics had significantly higher total MPA scores (compared to females and controls), but also that females scored significantly higher for ear and mouth anomalies.[77] Interestingly these are structures that undergo complex morphogenesis. The ear derives from six rudiments, the palate requires exact spatial relationships for its proper formation and the tongue originates from derivatives of the three distinct embryonal layers—ectoderm, mesoderm, and endoderm.[35] Due to their complex development, these structures would be more prone to malformations due to prenatal adverse events and the higher scores in females suggest either more severe or different in nature extragenetic effects.

The head and eye regions were more dysmorphic in male schizophrenics, which might reflect the inexorably intertwined development of brain and skull and greater susceptibility of the male brain to neurodevelopmental disruptions. Akabaliev and Sivkov therefore contended that in the total etiological panorama of schizophrenia, gender could conceivably still provide insight into some of the unanswered questions within the multifactorial-polygenetic threshold model of the disorder. [77]

Clear gender differences have also been demonstrated in samples using quantitative rather than qualitative techniques. Using 3D craniofacial morphometrics, Henessey et al. demonstrated gender-specific differences in directional asymmetry in male and female schizophrenia subjects, both in relation to controls but also in relation to each other.[65] Compton et al. conducted direct craniofacial measurements on a predominantly African-American sample revealing gender-specific differences in comparison to controls and each other for both male and female subjects.[60]

5.2.2.3 BRAIN CORRELATES

Reports that morphological abnormalities of the brain are already present in the early stages of schizophrenia have lent support to the neurodevelopmental hypothesis.[81] A number of attempts have been made to demonstrate correlations between MPAs and morphological abnormalities of the brain. Initially no significant relationship could be demonstrated for either CT or MRI for general

schizophrenia groups.[42;82] However, Hata et al. theorised that they would be more likely to demonstrate this difference in a subgroup of schizophrenia patients with early (before age of 18) onset schizophrenia (EOS).[83] They performed MRIs on 27 EOS patients and demonstrated a significant correlation between total MPAs and ventricular enlargement.

Furthermore, in a recent MRI study by Dean et al., the authors demonstrated that total MPA score in group of first episode psychosis patients was significantly associated with a reduction of grey matter volume in the prefrontal cortex and precuneus and with a grey matter excess in the basal ganglia, thalamus and lingual gyrus.[84] Importantly, the areas highlighted have previously been implicated in the pathogenesis of psychosis.[85-88]

5.2.2.4 SYMPTOM DIMENSIONS

Both schizophrenia sufferers and their non-psychotic relatives have been shown to have significantly increased rates of cognitive dysfunction on formal testing.[89;90] These findings on cognitive dysfunction were supported by Ismail et al. but they could not demonstrate any significant correlation between MPAs and these in either schizophrenics or their non-psychotic siblings.[53] Their findings are in keeping with most previous studies, supporting the notion that MPAs as a group should preferably be considered as markers of generalised early neuromaldevelopment rather than markers of specific dysfunction.[38;78;91] This

hypothesis is further supported by several studies reporting no association between MPAs and positive, negative, or disorganised symptoms.[42;43;92]

5.2.2.5 ETHNICITY

The distribution of MPAs as a marker of disturbed neurodevelopment across ethnic groups has received very little attention. An excess of psychosis has been demonstrated in particular ethnic groups but published data has been unable to link this to neurodevelopmental abnormalities.[93;94] One could hypothesize that MPA rates in such groups would therefore possibly be lower than in other groups. However, in their recent study comparing four ethnically diverse (Caucasian, African Carribean, Black African, Other) groups of first episode psychosis patients, Dean et al. showed that the rate of MPAs (using an abridged version of the Lane scale) was consistently higher amongst those with psychosis than amongst controls and that this association existed irrespective of ethnicity.[95]

Another recent US study compared an ethnically-mixed sample of Caucasian and African-American schizophrenia patients with controls and found MPAs (using only quantitative measurements) to be elevated in the patient group as a whole.[96] However, no attempt was made to ethnically stratify the sample in order to make inter-group comparisons. Quantitative measurements were also used to demonstrate increased rates of dysmorphic features for schizophrenia subjects in comparison to controls in a small Mestizo (admixture Spanish, Indian, Black) sample and a larger predominantly African-American sample.[60;61]

5.2.2.6 AGE

Studies broadly examining a relationship between age of onset and MPAs have mostly yielded negative results, however studies focusing specifically on a comparion between MPAs in early onset (under age of 18) schizophrenia (EOS) versus later onset have shown an increased rate in EOS.[42;43;82;97;98] Such results would possibly support the hypothesis that EOS represents a subset of patients in which neuromaldevelopment is significantly involved.

No studies have specifically focused on MPAs in schizophrenia patients above the age of 60. Lloyd et al. used the Lane dysmorphology scale to compare rates of MPAs between healthy older (>60) and younger individuals and found the older group to significantly differ on four items i.e. higher frequency of absent trichions due to alopecia, shorter and broader palates and greater right and left ear protrusions.[99] The authors concluded that changes associated with normal ageing (hair loss and loss or extraction of teeth followed by longstanding pressure from dentures leading to the observed palate shape) made at least two of these items invalid comparisons and that a separate scale needs to be validated for the elderly. However, Henriksson et al. recently did a statistical analysis using a comparative age-breakdown of participants in a number of large studies reporting significant differences in MPA rates between controls and schizophrenia subjects.[100] They demonstrated that no statistically significant differences could be observed between MPA rates and age for any of the study

groups and concluded that current data did not support increased age to be a confounding factor in the study of MPA rates in schizophrenia.

The question has also been posed as to whether MPAs present at an early age could in fact predict future psychiatric illness. Schiffman et al. assessed subjects at age 11-13 years, before onset of mental illness, collecting follow-up data on their adult psychiatric outcome 20 years later.[101] Presenting with a higher number of MPAs in childhood significantly differentiated between those who developed schizophrenia spectrum disorders (SSD) and those with no mental illness. Furthermore, for those individuals with a parent suffering from schizophrenia, a higher number of MPAs in childhood significantly differentiated between those who developed SSD and those with no mental illness as well as those with other psychopathology. The results seem to support a neurodevelopmental hypothesis for SSD. For high-risk individuals, MPAs seem to either signify a stressor relevant to those at genetic risk to develop schizophrenia or that high-risk status does in fact increase the risk for neuro-maldevelopment.

5.2.2.7 NEUROLOGICAL SOFT SIGNS

Neurological “subtle” or “soft” signs (NSS) are abnormalities that illustrate generalised and nonspecific neurological pathology. As is the case for MPAs, several studies have reported an increased prevalence of NSS in patients suffering from schizophrenia.[102-104] The presence of MPAs and NSS have also both separately been found to be predictive of future schizophrenia.[101;105]

Gourion et al. examined a group of schizophrenia subjects, their non-psychotic parents and normal controls for both MPAs and NSS.[72] Total MPA and NSS scores could be positively correlated; these results being in keeping with the expectation that these two variables could reflect an early developmental insult. These findings were supported by that of John et al. who demonstrated in a small sample that schizophrenia and control subjects were most accurately classified (82.9%) when MPAs and NSSs were considered jointly rather than independently[106] Both groups of authors therefore concluded that the co-assessment of these markers could be useful as a composite phenotype for genetic studies.

6. SUMMARY

Due to the complex genetic and clinical heterogeneity exhibited in schizophrenia, the use of endophenotypes has long been considered a viable option in the search for susceptibility genes. The quantitative and qualitative measurement of anthropometric proportions to demonstrate the presence or absence of dysmorphic features (so-called minor physical anomalies (MPAs)) represents one such endophenotype. The presence of MPAs act as biologically-timed markers of developmental disturbance within a foetus and craniofacial anomalies are of special interest as the brain and the overlying face both develop from common embryonic ectoderm. Although some of the current evidence is contradictory and more research is needed, the presence of an excess of MPAs in schizophrenia subjects, in comparison to normal controls, has repeatedly been

demonstrated. The use of MPAs as endophenotype fits in well with the neurodevelopmental hypothesis of schizophrenia and seems to be particularly appropriate in our setting as not only can these measurements be done inexpensively, but observers can be relatively easily trained to perform these measurements.

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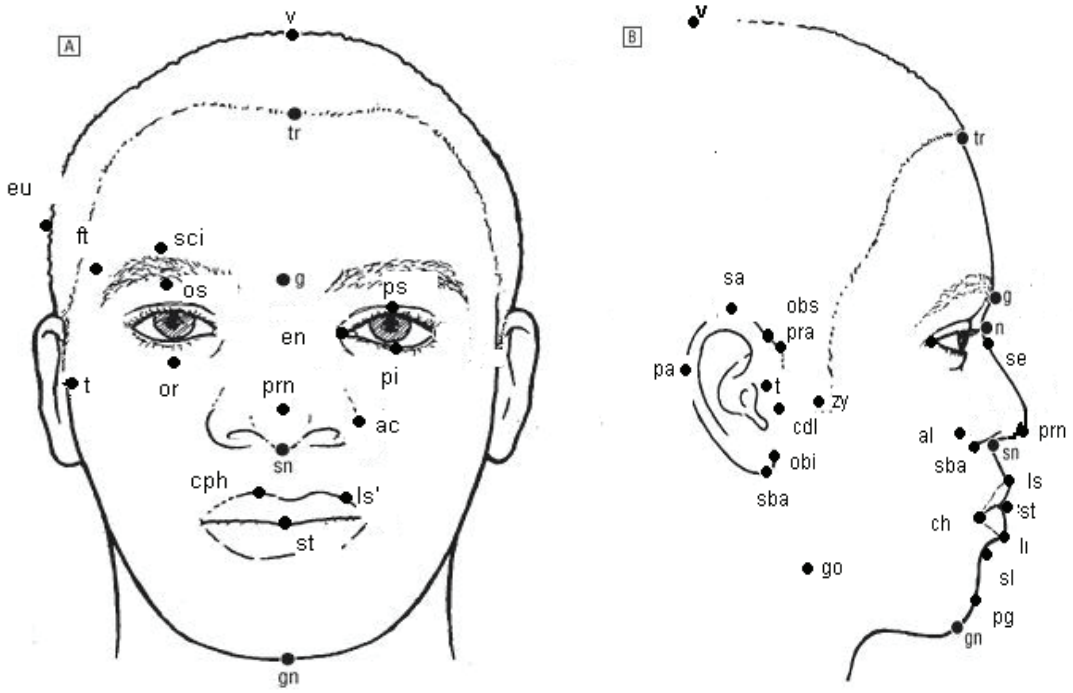
APPENDIX A: LANDMARKS FOR HEAD AND FACIAL MEASUREMENTS

In direct measurements of the head and face, 47 landmarks are used: 6 on the head, 6 on the face, 8 on the orbits, 11 on the nose, 6 on the lips and mouth, and 10 on the ears.



HEAD

Vertex: (v) Highest point of the head when normally oriented. The vertex is not identical to the bregma, the bony landmark in the middle of the top of the skull, where the coronal and sagittal sutures cross.

Glabella: (g) Most prominent midline point between the eyebrows and is identical to the bony glabella on the frontal bone.

Opisthocranion: (op) Point situated in the occipital region of the head and is most distant from the glabella, that is, it is the most posterior point of the line of greatest head length.

Eurion: (eu) Most prominent lateral point on each side of the skull in the area of the pariental and temporal bones.

Frontotemporale: (ft) Point on each side of the forehead, laterally from the elevation of the linea temporalis.

Trichion: (tr) is the point on the hairline in the midline of the forehead. It cannot be determined on a balding head.

FACE

Zygion: (zy) Most lateral point of each zygomatic arch and is identified by trial measurement, not by anatomical relationship. It is identical to the bony zygion of the malar bones.

Gonion: (go) Most lateral point on the mandibular angle close to the body gonion. It is identified by palpation.

Sublabiale: (sl) Determines the lower border of the lower lip or the upper border of the chin. It corresponds with the mentolabial ridge of anatomists, a point marked as inferior labial point, supramentale, submental point or labiomentale.

Pogonion: (pg) Most anterior midpoint of the chin, located on the skin surface in front of the identical bony landmark of the mandible.

Menton/Gnathion: (gn) Lowest median landmark on the lower border of the mandible. It is identified by palpation and is identical to by bony gnathion. This landmark is the lowest point used in measuring facial height.

Concylion laterale: (cdl) Most lateral point on the surface of the condyle of the mandible. It is identified by palpation at each temporomandibular joint when the jaw is open.

ORBITS

Endocanthion: (en) Point at the inner commissure of the eye fissure. The soft endocanthion is located lateral to the bony landmark that is used in cephalometry.

Exocanthion: (ex) Point at the outer commissure of the eye fissure. The soft exocanthion is slightly medial to the bony exocanthion.

Center point of the pupil: (p) Determined when the head is in the rest position and the eye is looking straight forward. Identification is easiest when the patient is reclining, the eye fissures are horizontal and the eyes are gazing straight forward.

Orbitale: (or) Lowest point on the lower margin of each orbit. It is identified by palpation and is identical to the bony orbitale.

Palpebrale superius: (ps) Highest point in the midportion of the free margin of each upper eyelid.

Palpebrale inferius: (pi) Lowest point in the midportion of the free margin of each lower eyelid.

Orbitale superius: (os) Highest point on the lower border of the eyebrow, close to the highest bony point of the upper margin of each orbit, where the bony supraorbitale landmark is located.

Superciliare: (sci) is the highest point on the upper borderline in the midportion of each eyebrow.

NOSE

Nasion: (n) Point in the midine of both the nasal root and the nasofrontal suture. The slight ridge on which it is situated can be felt by the observer's fingernail. This point always is above the line that connects the two inner canthi.

Sellion: (se) Deepest landmark located on the bottom of the nasofrontal angle. The point usually occurs somewhere between the level of the supratarsal fold and eyelash.

Mazillofrontale: (mf) Base of the nasal root medially from each endocanthion, close to the bony maxillofrontale of the medial margin of each orbit, where the mazillofrontal and nasofrontal sutures meet.

Alare: (al) Most lateral point on each alar contour.

Pronasale: (prn) Most protruded point of the apex nasi, identified in lateral view of the rest position of the head. In the case of the bifid nose, the more protruding tip is chosen.

Subnasale: (sn) Midpoint of the angle at the columella base where the lower border of the nasal septum and the surface of the upper lip meet. The landmark is identified in base view of the nose, or from the side.

Subalare: (sbal) Point at the lower limit of each alar base, where the alar base disappears into the skin of the upper lip. The landmarks indicate the labial insertion of the alar base.

Alar curvature: (ac) Most lateral point in the curved base line of each ala, indication the facial insertion of the nasal wingbase.

Highest point of the columella: (c') is the point of each comumella crest, level with the top of the corresponding nostril.

Alare': (al') Marking level at the midportion of the alae where the thickness of each ala is measured.

Subnasale': (sn') Midpoint of the columella crest where the thickness of the columella is measured. When measuring the width of the nostril floor between the subalare and the crest of the columella, the sn' point designates the columella crest at the bottom line.

LIPS AND MOUTH

Crista philtri: (cph) Point on each elevated margin of the philtrum just above the vermilion line.

Labiale superius: (ls) Midpoint of the upper vermilion line.

Labiale superius': (ls')Laterally from the midpoint, also located on the upper vermilion line vertically beneath the right and left subalare points.

Labiale inferius: (li) Midpoint of the lower vermilion line.

Stomion: (st) Imaginary point at the crossing of the vertical facial midline and the horizontal labial fissure between gently closed lips, with teeth shut in the natural position.

Cheilion: (ch) Point located at each labial commissure.

EARS

Superaurale: (sa) Highest point on the free margin of the auricle.

Subaurale: (sba) Lowest point on the free margin of the ear lobe.

Preaurale: (pra) Most anterior point of the ear, located just in front of the helix attatchment to the head.

Postaurale: (pa) Most posterior point on the free margin of the ear.

Otrobasion superius: (obs) Point of attachment of the helix in the temporal region. It determines the upper border of the ear insertion.

Otobasion inferius: (obi) Point of attachment of the ear lobe to the cheek. It determines the lower border of the ear insertion.

Porion: (po) Highest point on the upper margin of the cutaneous auditory meatus. This point is a few millimeters medial to the bony portion.

Tragion: (t) Notch on the upper margin of the tragus.

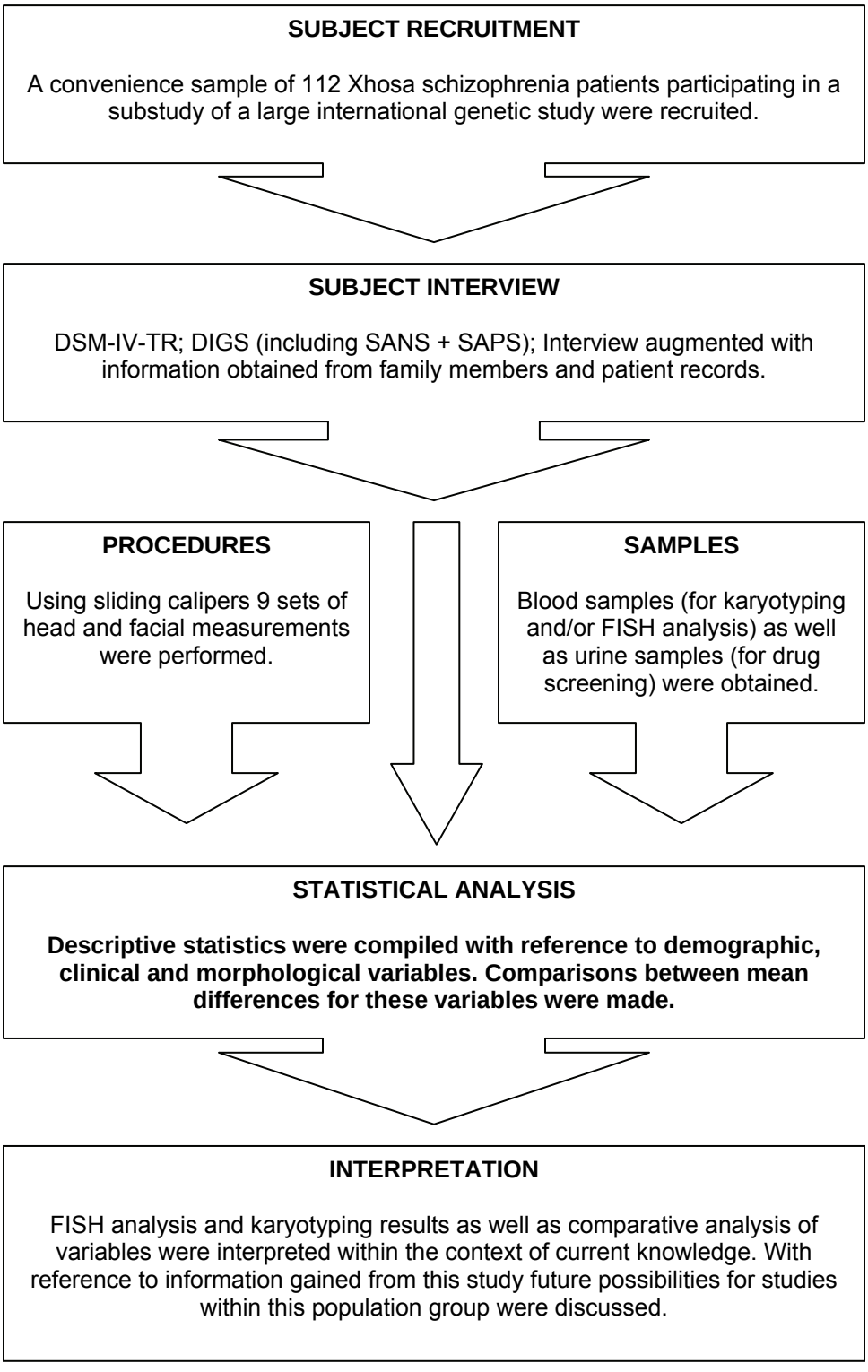
Points establishing the medial longitudinal axis: (1) uppermost point is determined by halving the upper portion of the ear (i.e., the portion above the upper insertion point of the ear), and (2) the lowest point is the middle of the free border of the ear lobe.

CHAPTER 7

METHODOLOGY

CONTENTS

1. VISUAL METHODOLOGICAL OVERVIEW



2. STUDY SUBJECTS

2.1 OVERVIEW

Over a period of 24 months, a group of potential participants, all of Xhosa (African population of the Nguni language group) ethnicity and known to have a diagnosis of schizophrenia, were recruited from in and outpatient hospital services and community clinics in the Western Cape Province of South Africa for participation in an international genetic study. Participants were initially identified by hospital or community mental health workers and were at various stages of illness and treatment. Subjects who met inclusion and exclusion criteria and were able to give informed consent (consent obtained from caregivers in the event of diminished capacity) were included in the study group.

During the course of the above-mentioned study a number of subprojects were also initiated concurrently, using convenience samples from the larger study population. All of the subjects who presented for assessment for one such study (over a six month period) were approached in consecutive order by a Xhosa-speaking social scientist to discuss possible participation in this study. Of the 112 patients approached, all agreed to participate. Once again subjects were screened as to meeting inclusion and exclusion criteria and informed consent was obtained prior to any study procedures being initiated. Blood samples of fifty consecutively recruited participants from the total study population of 112 were selected for karyotyping.

2.2 INCLUSION CRITERIA

(a) Adult male and female subjects known with a diagnosis of schizophrenia according to DSM-IV-TR criteria[1];

(b) Known Xhosa ethnicity (this was defined as all four grandparents being of Xhosa origin).

(c) Current stage of illness did not exclude a subject from participation.

2.3 EXCLUSION CRITERIA

(a) Known organic aetiology that could significantly impact on diagnosis.

3. SUBJECT ASSESSMENT

3.1 STRUCTURED ASSESSMENT TOOLS

Once informed consent was obtained, a trained clinician administered a standardised interview (DIGS – Diagnostic Interview for Genetic Studies; Version 2.0) in either English or Xhosa (with the help of an experienced Xhosa-speaking social scientist).[2] The DIGS is a comprehensive clinical assessment interview specially developed for diagnosing major mood and psychotic spectrum disorders and also includes the SANS and SAPS (Schedules for the Assessment of Positive and Negative Symptoms).[3;4] The SANS and SAPS each assess symptom complexes to obtain clinical ratings of negative and positive symptoms respectively in patients with schizophrenia. They are – SANS – (1) affective

blunting; (2) alogia (impoverished thinking); (3) avolition/apathy; (4) anhedonia/asociality; and (5) disturbance of attention; and SAPS – (1) hallucinations; (2) delusions; (3) inappropriate behaviour; (4) thought disorder. Ratings are made on each symptom cluster, after consideration has been given to each of the items contained within the various complex scores. Anchor points are given for each item. (0 = not at all; 1 = questionable; 2 = mild; 3 = moderate; 4 = marked; 5 = severe). The DIGS (including the SANS and SAPS) has previously been used as interview tool for studies in the Xhosa population and it has been shown that the core symptoms of schizophrenia were found to be similar to those of Caucasian samples.[5-7] The interview was augmented with information gathered from family members of participants as well as that obtained from hospital records.

3.2 FOCUS OF ASSESSMENT

Information with reference to relevant demographic characteristics, previous and current medical history and history of substance use/abuse was obtained. In terms of psychiatric history, full data with regard to current and previous episodes of illness, number of episodes and hospitalisations (as well as duration), “trigger” factors, current and previous medications, age of onset, family history, presence of previous and current risk behaviours (e.g. suicidality) and co-morbid diagnosis was collected.

3.3 SAMPLE COLLECTION

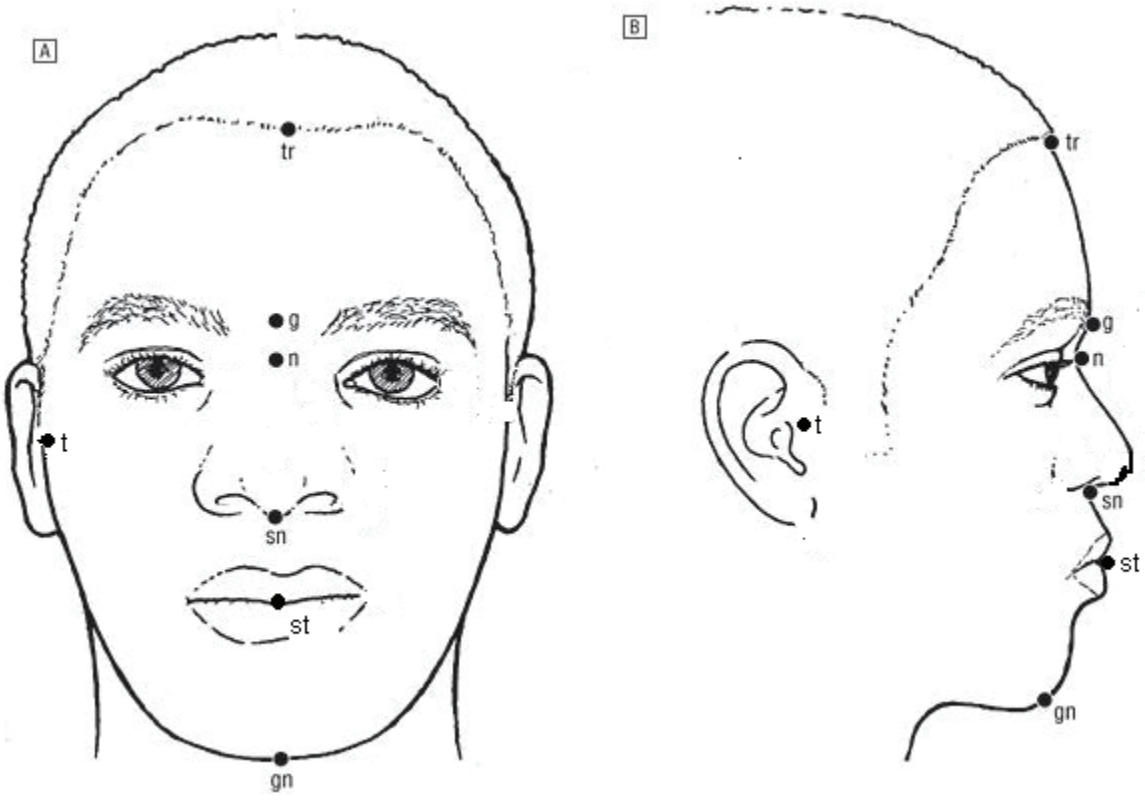
Blood samples (for karyotyping and/or FISH analysis) as well as urine samples (for drug screening *(cannabis, opioids, cocaine, metaqualone, methamphe-tamine))* were obtained from each participant.

3.4 MORPHOLOGICAL ASSESSMENT

In a previous study conducted by our group digital still images were captured and then evaluated by an experienced clinical geneticist who performed a Modified Waldrop Scale (MWS) assessment on each.[8] However, one of the objectives of the current study was to obtain information that could contribute to the development of a simple, user-friendly, "risk profile" screening tool for use by community mental health workers. As such, we decided that due (1) to the complexity of training lay individuals to use the MWS and, (2) the cost in providing a digital camera for many different sites the previous procedure would not be feasible for the current project.

Therefore, a decision was taken to rather perform head and facial measurements using sliding calipers. Nine sets of measurements were captured; please see diagram for the landmarks used. In the case of depth measurements only the right side of the face was used.

The nine sets of measurements used were: (1) Head circumference (2) Trichion (tr) to Glabella (g); (3) g to Nasion (n); (4) g to Subnasale (sn); (5) g to Stomion (st); (6) g to gnathion (gn); (7) Tragion (t) to tr; (8) t to sn; and (9) t to gn. Please see diagram below identifying these landmarks. To optimize quality, we selected measurements with reliable landmarks. These measurements form part of those suggested by Lane et al and have previously been used for measurements in schizophrenia subjects.[9;10]



4. ANALYSIS

4.1 KARYOTYPING AND FISH

Fluorescence In Situ Hybridisation (FISH) analysis was done using a DiGeorge/VCFS region probe (LSI TUPLE 1 SpectrumOrange/ARSA (22q13.3)). Karyotyping (resolution of appropriate 500 Giemsa-band stage) was performed according to standardised UNISTEL laboratory protocol. Please see Appendix A for these protocols.

4.2 STATISTICAL ANALYSIS

Descriptive statistics were compiled for each of the subgroups (total sample, sample karyotyped, participants with chromosomal aberrations) with reference to demographic, clinical and morphological variables using the Software Package for Social Sciences (SPSS) (V10.0). Comparisons between mean differences for demographic and clinical variables including living arrangements, marital status, employment status, level of qualification, number of admissions, duration of illness, substance use, lifetime illness features as well as the nine morphological measurements were made. Student T-Tests were used as appropriate.

5. ETHICAL CONSIDERATIONS

This study formed part of a large multi-national effort to study genetic risk factors in schizophrenia and as such was approved by the Committee for Human Research of the University of Stellenbosch. (97/005).

All study procedures and aims were explained in lay terms to subjects in their home language (via an interpreter when necessary) and if appropriate to their caregivers/legal guardians. Informed consent was only accepted if the patient was able to understand and communicate this understanding to the researchers. Legal guardians/caregivers gave consent, in addition to the patient's consent, if any doubt existed as to his/her ability to offer informed consent. Participation did not lead to any costs for the participants.

All participation was voluntary and refusal to participate or withdrawal would in no way affect a participant’s right or access to future treatment. Any request for withdrawal of participation would immediately be implemented. The study findings would be available to all participating individuals, if requested.

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APPENDIX A: UNISTEL STANDARD OPERATING PROTOCOLS

UNISTEL STANDARD OPERATING PROTOCOL: KARYOTYPING

Chromosome preparation from peripheral blood lymphocytes:

Culture Procedure:

Set up cultures twice a week – Tuesdays and Fridays.

1. Place PBMax in incubator (37°C) at least 2 hours prior to setting up of cultures (8ml/blood specimen)
2. Switch on the Laminar flow cabinet 20 minutes before setting up of cultures and clean the Laminar flow cabinet with ethanol.
3. Take blood specimens, PHA and enough syringes and needles and wipe it with ethanol before putting it in the flow cabinet.
4. Prepare the culture tubes by writing the patient lab number and the culture number on both tubes (A and B cultures). Write the numbers on the cap of the tube as well.
5. Place 4ml of PBMax medium in every tube. Add 50 µl PHA to culture A and 100µl PHA to culture B.
6. Add 0.5ml blood to each prepared culture tubes in the case of an adult and 0.3ml if it is a baby or a cordocentesis.
7. Mix the contents of each culture tube gently by inverting it a few times.
8. Incubate the cultures at 37°C in a slanting position. (48 hours for cultures set up on Tuesday and 72 hours for cultures set up on Fridays.)

Cell-Syncronisation:

1. Clean the Laminar flow cabinet with ethanol and take the MTX out of the freezer and let it thaw. Everything that goes in to the flow cabinet must be cleaned with ethanol.
2. Add 25µl MTX sterile to the cultures at 15H00 on Mondays (cultures set up on Fridays) and Thursdays (cultures set up on Tuesdays.
3. Mix the cultures and incubate it for a further 16-18 hours.
4. Wash out the MTX block on Tuesday (cultures set up on Fridays) and Friday (cultures set up on Tuesdays) mornings.
5. Place PBMax medium in incubator at 37°C and thaw the thymidine.
6. Clean the Laminar flow cabinet with ethanol.
7. Centrifuge the cultures at 1500 rpm for 8min. Discard the supernatant and add a mixture of medium and thymidine (3 ml PBMax and 30µl thymidine per culture).
8. Incubate the cultures for 4¾ hours in a slanting position at 37°C in the incubator in the harvesting laboratory.

Preparations for Harvesting:

1. Place KCI in the incubator (37°C) at least 1 hour before harvesting. (8ml per culture)
2. Make up die fixative at least half an hour before harvesting and put in freezer:
   1 Glacial Acetic Acid: 3 Methanol (20ml per tube + 20ml)
   1 Glacial Acetic Acid: 6 Methanol (10 ml per tube)

Harvesting:

1. Add 100µl Colcemid to each culture tube and mix by gently inverting a few times and incubate at 37°C for 30 minutes in a slanting position.
2. Check to see if the number on the tube correlates with the number on the cap of the tube.
3. Centrifuge the culture at 1200 rpm for 10 min and remove the supernatant with an aspiration pump until ± 2 ml of fluid remains. Mix up cell pellet gently.
4. Add 8ml KCI, mix well and place in the incubator (37°C) for 15 min.
5. Add 5 drops of 1:3 fixative to each tube and mix gently.
6. Centrifuge at 1200 rpm for 10 min. Aspirate supernatant and mix up cell pellet.
7. Gradually add 5 ml 1:3 fixative to cultures and mix thoroughly.
8. Centrifuge at 1200 rpm for 10 min. Aspirate supernatant and mix up cell pellet.
9. Repeat this procedure 4 times. After the fourth centrifuge and aspiration of the supernatant 1:6 fixative must be added to the cultures.
10. Centrifuge at 1200 rpm for 10 min. Aspirate supernatant and mix up cell pellet.
11. Gradually add 5 ml 1:6 fixative to cultures and mix thoroughly.
12. Centrifuge at 1200 rpm for 10min. Aspirate the supernatant and remove as much of it as possible.
13. Place in freezer for slide preparation the next day or for at least 30 minutes.

UNISTEL STANDARD OPERATING PROTOCOL: FISH

PROCEDURE: FISH (Fluorescent in situ hybridization) – FISH procedure

1. **SCOPE**
   This SOP is applicable to all staff of the FISH Division, the QA manager and all staff of UML allowed to give out and discuss the results of this test with either the referring clinician or the patient.

2. **PURPOSE**
   This SOP describes the complete procedure for testing human DNA for the presence/absence of specific genes, whole chromosomes, translocations, inversion or duplications.

3. **FREQUENCY**
   This SOP is used as often as required for routine testing of referred samples, as often as required for testing new probes/other chemicals/equipment used in this procedure, and as often as it is required for evaluating and validating (a) any new variations in this procedure or (b) any other procedure developed to replace or complement this procedure.

4. **BACKGROUND**
   FISH (Fluorescent in situ hybridization) is a cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence similarity. Fluorescence microscopy can be used to find out where the fluorescent probe bound to the chromosome. FISH is often used for finding specific features in DNA. The features can be used in genetic counseling, medicine and species identification.

5. **PROCEDURE**

5.1 Materials required

All chemicals are to be made up fresh on the day of preparation:

1. Sterile distilled deionised (SDD) water prepared according to UnistelSOP.
2. Distilled deionised (DD) water prepared according to UnistelSOP.
3. Patient DNA, Prepared according to UnistelSOP.
4. 70% ethanol, 90% ethanol and 100% ethanol
5. HCI
6. 20xSSC (Nacl, Tri-NaSitrate and distilled water) made up according to UnistelSOP.
7. Phosphate buffer (PBS)
8. $MgCl^2$
9. PFA

10. Pepsin
11. Methanol
12. Acetic acid
13. KCL

5.2 Equipment required

1. Incubator 37°C
2. Water bath 73°C
3. Yellow 20µl Gilson pipette tips
4. Blue 1000µl Gilson pipette tips
5. Transparent 20µl Gilson pipette tips
6. 20 clean Hellendahl containers (individually marked for dedicated chemicals used)
7. Four heat resistant plastic containers
8. 100µl Gilson Pipette
9. 2µl Gilson Pipette
10. 20µl Gilson Pipette
11. 1000µl Gilson Pipette
12. Microscope cover glasses 24x50mm
13. Microscope cover glasses 22x22mm
14. Minute/second count down timer
15. Plastic tweezers
16. Fluorescent microscope
17. Fluorescent microscope filters (red, gold, aqua, green, and dapi)
18. Fan
19. Eppendorphs
20. Clean glass slides
21. Warm fan
22. Light microscope

5.3 Preparation of samples

The following samples can be prepared for the FISH technique:

- EDTA or heparinised blood
- EDTA or heparinised bone marrow
- Amniotic fluid
- Products of conception
- Semen (sperm)

5.4.1 Preparation of FISH samples

Place KCI in the incubator (8ml per tube) at least 1 hour before harvesting
Make up fixative at least half an hour before harvesting and put in the freezer.
1 Glacial Acetic Acid: 3 Methanol (Make up 10ml per tube + 20ml)

1. Check to see that the number on the tube correlates with the number on the cap of the tube.
2. Centrifuge the amniotic fluid, culture at 1200 rpm for 10 min and remove the supernatant with an aspiration pump until ± 2 ml of fluid remains. If there are signs of blood in the pellet, it must be documented.
3. For the blood and bone marrow, mix well and decant 2 ml into a clean dry tube.
4. The products of conception must be biopsied according to UnistelSOP and stored in culture medium.
5. Then the samples are ready for preparation.
6. Add warm (37°C) 8 ml KCI, mix well and place in incubator (37°C) for 20 min.
7. Remove tubes from the incubator
8. Add 5 drops of 1:3 fixative to each tube and mix gently.
9. Centrifuge at 1200rpm for 10 min. Aspirate the supernatant and mix up cell pellet.
10. Add 5ml 1:3 fixative and mix thoroughly. Place cultures overnight in refrigerator.

Next day:

Make up fresh fixative at least half an hour before harvesting and put in freezer.
1 Glacial Acetic Acid: 3 Methanol (15ml/tube)

1. Centrifuge cultures at 1200 rpm for 10 min.
2. Aspirate supernatant and mix up pellet.
3. Add 5 ml fixative and mix thoroughly
4. Repeat this 3 times. After the third 5 ml fixative is added, the cultures are centrifuged and the supernatant is aspirated (as much as possible) depending on the size of the pellet.
5. Samples are now ready for slide preparation.

5.5 Making of the slides

1. Place all sample tubes in a Perspex container.
2. Reconcile all samples with their relevant request forms.
3. Ensure that lab numbers on the sample tubes correspond to the lab numbers on the patient forms.
4. Use the slides that have been cleaned in an ethanol ether solution. As per UnistelSOP.
5. Only make on slide at a time
6. Write the corresponding lab number (e.g. BF11111) on the frosted side of the slide in pencil

7. Using a 50µl pipette and designated pipette tips draw up a well suspended pipette tip full of sample.
8. For bone marrow and blood samples distribute sample evenly over the slide.
9. For amniotic fluid samples make two designated concentrated areas of cells on either side of the slide
10. Place slides on a flat surface in front of a warm dryer to dry.
11. Evaluate the dry slide under the phase contrast microscope. If more cells are needed place more sample drops on the slide and let dry.
12. Store the remaining sample in the fridge for at least 6 months.

5.6 Preparation of solutions to be used for one FISH procedure

Always make up fresh solutions to be used on the same day for the FISH slide treatment procedure.

1. Mark and fill three Hellendahl containers respectively 10% methanol, 70% acetic acid: methanol (70 ml acetic acid + 30 ml Methanol) and 100% methanol.
2. Mark one glass staining dish Pepsin, and fill with a solution consisting of 100 ml phosphate buffer (PBS) 100µl HCR and 18µl Pepsin (to be added later). Place container with solution in a 37°C incubator to warm up.
3. Mark and fill four Hellendahl containers respectively.
   Two containers marked PBS (phosphate buffer prepared according to UnistelSOP)
   PBS + $MgCl^2$ (95ml PBS and 5 ml $MgCl^2$ prepared according to UnistelSOP)
   PFA paraformimide prepared according to UnistelSOP) 50 ml PFA + 45 ml PBS and 5 ml $MgCl^2$)
4. Mark and fill three Hellendahl containers respectively 100% ethanol, 90% ethanol (90 ml ethanol + 10 ml distilled water) and 70% ethanol (70 ml ethanol + 30 ml distilled water)
5. Discard all solutions after use, rinse containers under tap and let dry overnight.
6. Remove the aliquoted Pepsin, 20X SSC and FA from the freezer to defrost.

5.7 FISH Procedure

1. Place cooled down, dry slides in 100% Methanol container for 10 min
2. Move slides to the 70% acetic acid: methanol solution for 1 min 30 seconds
3. Move slides to a 100% methanol container for 2 min
4. Remove slides and let dry in front of a fan for 10 min
5. After 10 min in front of fan, add 18µl defrosted and well mixed Pepsin to the PBS/HCI staining container, add slides to the solution and place at 37°C for 15 min
6. After 15 min, remove slides and place in PBS for 5 min.
7. Move slides to PBS/MgCl solution for 5 min
8. Move slides to PFA solution for 5 min
9. Move slides to PBS solution for 5 min
10. Remove slides from the PBS solution

11. Place them in the 70% ethanol solution (70 ml ethanol + 30 ml dH^2O) for 3 min
12. Move slides to the 90% ethanol solution (90 ml ethanol + 10 ml dH^2O) for 3 min
13. Move slides to the 10% ethanol solution for 3 min
14. Remove slides from the 100% ethanol and place in front of a fan for 10 min to dry
15. The slides are now ready for denaturation and probing.

5.7.1 Denaturation

The probes are light sensitive! When working with the probes, ensure that the light is turned OFF at all times.
There are two different types of probes that require two different types of denaturation procedures.
Some probes can be co-denatured with DNA but others (most of the probes) need to be denatured separately from the DNA.

5.7.2 Separate denaturation technique

1. Turn main lights off
2. Turn on the water bath and set to 73°c
3. Make up the Hybridization solution according to the following formula
4. 70µl deonised Formamide + 20µl sterile dH^2O + 10µl 20 X SSC
5. (This is enough solution to denature one slide of DNS, make up enough for all the slides to be denatured)
6. Mix the denatured solution well
7. Place 10µl denaration solution on every slide, cover with a long cover slip
8. Place the slide on a hot plate 75°C for 3 min
9. After 3 min remove the cover slips and place the slides immediately in 4°C 70% ethanol solution for 3 min.
10. Move slides to the 90% ethanol solution (90 ml ethanol + 10 ml dH^2O) for 3 min
11. Move slides to the 100% ethanol solution for 3 min
12. Remove slides from the 100% ethanol and place in front of a fan for 10 min to dry
13. Slides are now ready for probing

5.8 Probing

Remove all the required probes from the freezer and allow to reach room temperature.
Probes will be applied and handled in two different ways.

5.8.1 Probes for separate enaturation

This method is used for the single copy probes

1. Ensure the lights are turned off at all times when working with the probe
2. Ensure probes and hybridization buffer are at room temperature
3. Centrifuge the probe and the hybridization buffer to ensure it is mixed well
4. Mark eppendorf's with the corresponding probe name
5. Prepare the probe mix according to the following formula:

- 7,0 µl hybridization buffer
- 2 µl sterile distilled water
- 0,6 µl desired probe

6. Mix well and denature the probe by floating the eppendorf containing the probe mix in a water bath at 73°C for 5 min
7. After 5 min remove the eppendorf and place the denature probe on the corresponding already denatured slide, cover with a 21mm x 50mm cover slip.
8. Remove most of the air bubbles and place in a humidity camber in an incubator at 37°C
9. Allow to re-anneal overnight in a dark incubator at 37°C

5.8.2 Probes for co-denaturation

This method is used for the multiple copy probes

1. Ensure the lights are turned off at all times when working with the probe
2. Ensure probes are at room temperature
3. Centrifuge the probe to ensure it is mixed well
4. Measure 5µl of required probe and place in the middle of a 24mm x 24mm cover slip.
5. Invert the already prepared Fish slide onto the cover slip with the probe.
6. Ensure there is not air bubbles trapped under the cover slip.
7. Seal all 4 sides with glue
8. Place on a hotplate at 75°C for 5 min to denature
9. After 5 min remove slides immediately and place in a humidity camber in a 37°C incubator
10. Allow to re-anneal overnight in the dark.

6. Post-hybridization washes.

There are two types of post-hybridization washes, a different wash for both the single copy probes (a less stringent wash) and the multiple copy probes (a more stringent wash).

6.1 More stringent wash for the multiple copy probe.

1. Label three plastic coplin jars respectively
    - 0,4 x SSC/0,3% Tween
    - 2 x SSC/),1% Tween
    - PBS
2. In the 0,4 x SSC/0.3% Tween container make a solution containing:

2.1 53.9 ml dH^2O + 14 ml 2 x SSC + 2.1 ml 10% Tween
2.2 Place in a water bath at 68°C
2.3 Allow 20 min to warm up

3. In the 2 x SSC/),1% Tween container make a solution containing:
3.1 69.3 ml 2 x SSC + 700 µl 10% Tween
3.2 Leave at room temperature

4. Fill the PBS container with PBS and leave at room temperature
5. Once the 0,4 x SSC/0,3% Tween solution reached 68°C
6. remove the slides from the humidity camber in the incubator
7. Carefully remove the glue without disturbing the cover slips
8. Carefully remove the coverslips by lifting them off with a razor blade
9. Once all the cover slips have been removed, place the slides back to back and into the warmed up 0,4 x SSC/0,3% Tween solution for 3 min
10. After 3 min remove the slides and place it into the 2 x SSC/0,1% Tween solution at room temperature for 3 min
11. After 3 min remove the slides and place it into the PBS for 3 min
12. Slides are now ready for mounting.

6.2 Less stringent wash for the single copy probe

1. Label 7 Hellendahl containers respectively
    - Mark three Hellendah containers 50% FA/SSC
    - Mark two containers 2 X SSC
    - Mark one container 1 X SSC 50µl 10% Tween
    - PBS

2. In the three containers marked 50% FA/SSC make a solution containing 50% Formamide and 50% 2 X SSC
    2.1 Measure 150 ml Formamide and 150 ml 2 X SSC mix well and divide between the three containers.
    2.2 Place containers in a water bath at 42°C and allow to warm up

3. Fill the two containers marked 2 X SSC with 2 X SSC and place in the water bath at 42°C and allow to warm up.
4. In the container marked 1 X SSC/500µl 10% Tween make a solution containing:
    4.1 100 ml 2 X SSC and 500µl 10% Tween
    4.2 Place in the water bath to warm up
5. Once the solutions have reached 42°C the wash can begin
6. Remove the slides from the humidity camber in the incubator
7. Carefully remove the cover lips by lifting them off with a razor blade
8. Once all the cover slips have been removed, place the slides back to back and into the warmed up 50% FA/SSC solution for 5 min
9. After 5 min move the slides to the next 50% FA/SSC solution for 5 min
10. After 5 min move the slides to the next 50% FA/SSC solution for 5 min
11. After 5 min remove the slides and place it into the 2 X SSC solution for 5 min
12. After 5 min move the slides to the next 2 X SSC solution for 5 min
13. After 5 min remove the slides and place it into the 2 X SSC and 500µl 10% Tween solution for 5 min

14. After 5 min move the slides to PBS at room temperature for 3 min
15. Slides are now ready for mounting

7. Counter staining and mounting of the slides

The counter stain contains Dapi which stains the DNA blue and is extremely poisonous, gloves must always be warn when working with Dapi.

1. The Dapi/Antifade is made up according to UnistelSOP and stored in eppendorphs in the dark at 4°C
2. Remove the Dapi/Antifade solution and allow it to reach room temperature
3. After moving slides from the PBS during the post-hybridzation washes, allow most of the PBS to run of the slide by blotting it on absorbent paper
4. Drop 2 drops of Dapi/Antifade solution on the slide and cover with a 24mm X 50mm cover slip
5. Invert slide cover slip at the bottom onto an absorbent paper to remove excess Dapi/Antifade solution
6. Ensure all air bubbles are removed
7. Seal the sides of the cover slip onto the slide with clear cutex
8. Place in front of a fan and allow cutex to dry
9. Analyze

8. Analysis of the results

There are different FISH probe manufacturers, this results in different color combinations. There are also different types of probes such as break apart and dual fusion dual color probes. Therefore it is not possible to write a standard operating procedure for the analysis of the FISH probes. Always consult the probe description insert to ensure that the correct signal pattern is observed during analysis.

LSI DiGeorge/VCFS Region Probe
(LSI TUPLE 1 Spectrum Orange/ARSA(22q13.3))
(SpectrumGreen Control Probe)
32-191028-20 Assays

The LSI DiGeorge/VCFS Region Probe may be used to identify deletions of band 22q11.2 found in DiGeorge and velocardiofacial (VCFS) syndromes. Deletions of 22q11.2 are commonly referred to as the CATCH-22 region because of their association with syndromes of various phenotypes. The Vysis LSI DiGeorge-BCFS Region Dual Color Probe is a two color probe mixture that contains the SpectrumOrange TUPLE 1 (HIRA) probe (3 non-coding region of TUPLE 1, D22S553, D22S609 AND D22S942) and the SpectrumGreen LSI ARSA (Arylsulfatase A) gene control probe that maps very close to the telomeric end of 22q at 22q13.3. The 110kb TUPLE 1 probe does not contain the more telomeric loci D22S941 and D22S943. It is not known if the TUPLE 1 probe contains the gene DVL22.

CHAPTER 8

RESULTS

CONTENTS

1. DEMOGRAPHIC CHARACTERISTICS

Our sample comprised of 112 Xhosa participants with a diagnosis of schizophrenia. All patients were recruited from the Greater Cape Town area in the Western Cape, South Africa. Twenty one subjects were female and ninety-one male with the mean age at interview being 36.2 years (SD 11.85; range 17-64). The majority were single at the time of interview (n=86; 76.8%) with only 15.2% (n=17) married and the remainder being either divorced (n=2), separated (n=3) or widowed (n=4).

Only one participant was employed and of the remaining one hundred and eleven, 75.9 % (n=85) were receiving a disability grant. Most of the participants (n=70; 62.5%) had not progressed beyond a primary school level of education. See chart 1 for full description. Hundred and one of the subjects were right-handed.

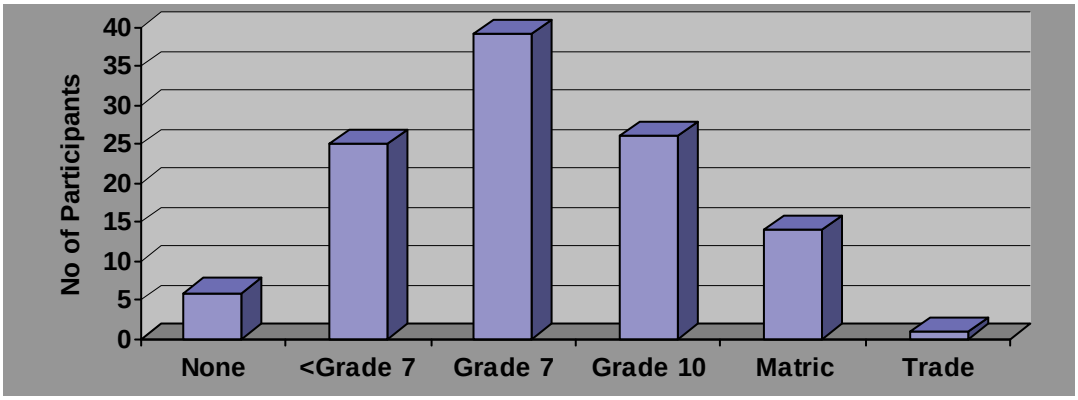


CHART 1: NUMBER OF PARTICIPANTS REACHING EACH LEVEL OF EDUCATION

2. GENERAL ILLNESS FEATURES

The mean age of onset for the illness was 23.38 years (SD 7.17; range 12-55). The number of hospitalisations ranged from 0-13 with 2.94 (SD 2.52) being the mean. As to number of episodes the mean was 3.19 (SD 2.24; range 0-10). Sixty-seven (59.8%) reported no family history of psychiatric illness. History regarding the duration of the prodromal period was available for only fifty-one of the subjects with the reported time varying from an acute onset of symptoms to up to three hundred weeks.

Thirty (26.78%) of the participants reported one or more possible stressor as precipitating events prior to the onset of the illness. Of these the death or serious illness of a close family member (n=8), participation in an initiation ceremony (n=5) and general family hardship (financial and other) (n=6) were most often cited. Two subjects also related it to giving birth, two to their participation in events relating to the political struggle and two to academic demands.

3. LIFETIME CLINICAL FEATURES

3.1 RESIDUAL SYMPTOMS

Only two of the participants denied experiencing any residual symptoms. For the remainder it was difficult to accurately determine the duration of such symptoms as sixty-six were unable to venture any clear guestimate as to the length of the

period. Three of the participants were acutely psychotic at the time of the interview.

3.1.1 HALLUCINATIONS

Hallucinations of an auditory nature were by far the most common with 110 (98.2%) subjects admitting to experiencing such over the lifetime of their illness. Seventy-eight (69.6%) heard two or more voices and conversing voices (n=67; 59.8%) and those of a commentary nature (n=63; 56.3%) were also reported to occur by the majority of participants. A significant number of participants experienced auditory hallucinations of a threatening nature (n=44; 39.3%) and noises (n=22; 19.6%) and command hallucinations (n=57; 50.9%) were also found to be quite frequent. A fair percentage of participants also reported disturbances in one or more of the other sensory modalities. See chart 2 for a full breakdown.

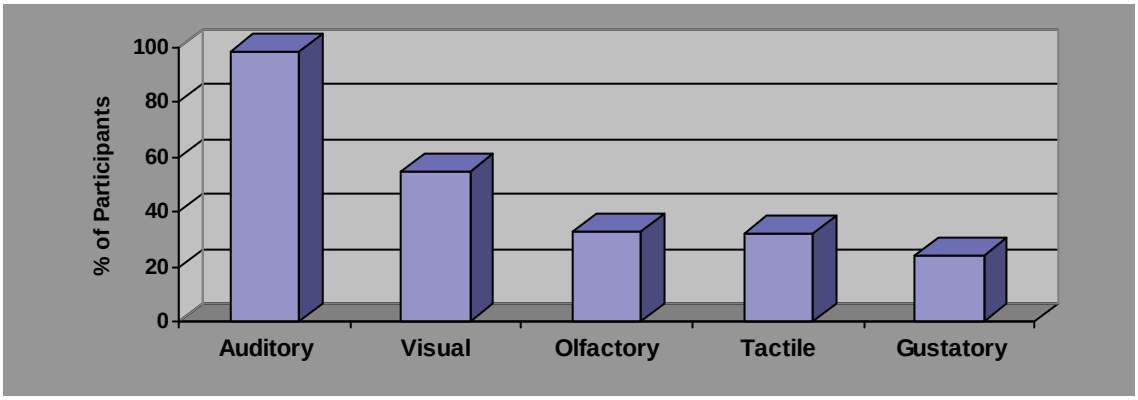


CHART 2: PERCENTAGE OF PARTICIPANTS WITH LIFETIME HISTORY OF A PARTICULAR PERCEPTUAL DISTURBANCE

3.1.2 DELUSIONS

Persecutory delusions were by far the most common, with 90.2% (n=101) of participants reporting a lifetime history of these. Delusions of reference (n=64; 57.1%), mind-reading (n=46; 41.1%) and those with a religious (n=41; 36.6%) or grandiose (n=47; 42%) content were also reported to occur frequently. Interestingly, delusions of a bizarre nature were only reported by 7 participants (6.3%). See chart 3 for a full breakdown.

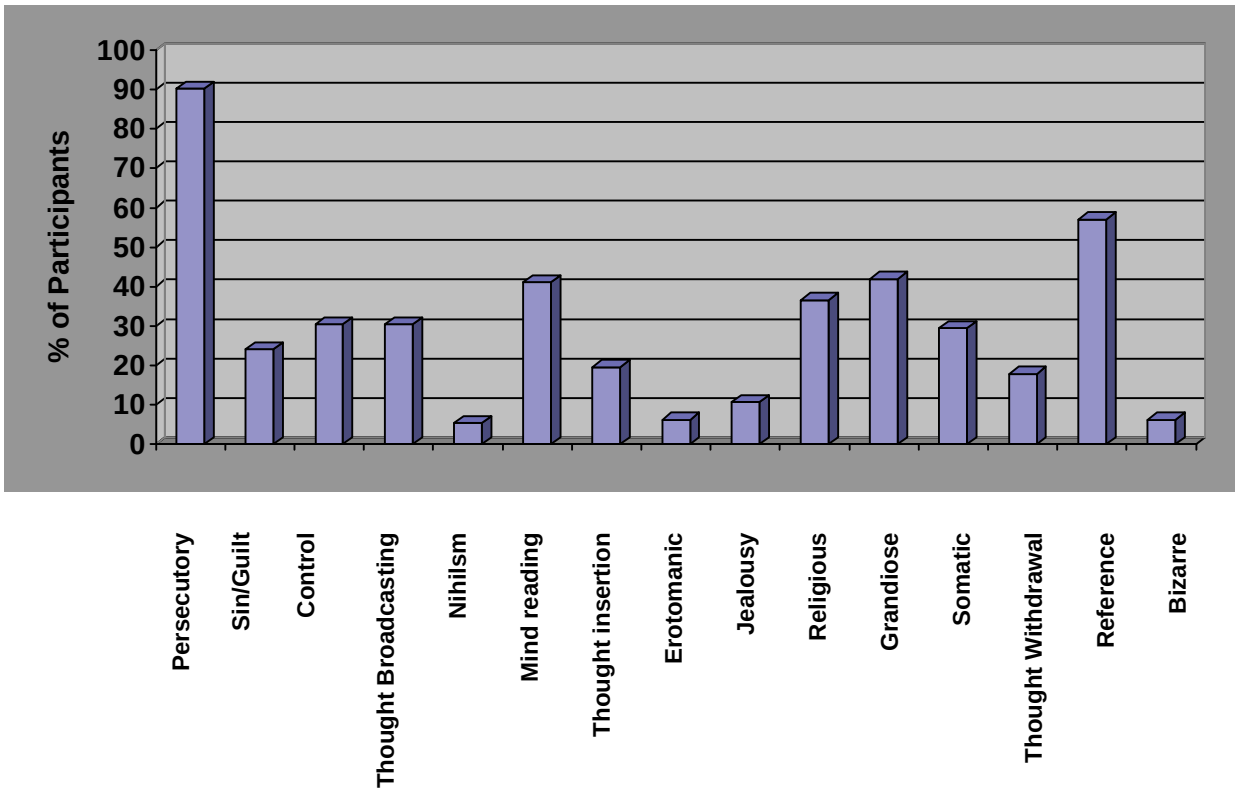


CHART 3: PERCENTAGE OF PARTICIPANTS WITH LIFETIME HISTORY OF A PARTICULAR TYPE OF DELUSION

3.1.3 BEHAVIOUR

A lifetime history of some form of inappropriate behaviour was reported for 90.2% (n=101) of the participants. Incidents of verbal or physical aggression were by far the most common (n=85; 75.9%). Sexually disinhibited behaviour was reported for 11.7% (n=13) of the sample. Catatonic symptoms (chart 4) were reported in only 9 (8.1%) of the participants.

CHART 4: PERCENTAGE OF PARTICIPANTS WITH LIFETIME HISTORY OF A PARTICULAR TYPE OF CATATONIC BEHAVIOUR

Eleven participants admitted to having made one or more suicide attempts during the course of their illness. Methods varied from ingesting different substances (pills (n=2), paraffin, washing powder, bleach) to the more violent (cutting own throat (n=2), hanging (n=2), throwing himself in front of a train). One participant was reported by family to have made a number of attempts by unknown method.

3.1.4 THOUGHT DISORDER

A lifetime history of thought disorder could be documented for seventy-six (67.9%) of the participants. Incoherence was the specific form of thought disorder most commonly documented. (n=41; 36.6%). See chart 5 for a full breakdown.

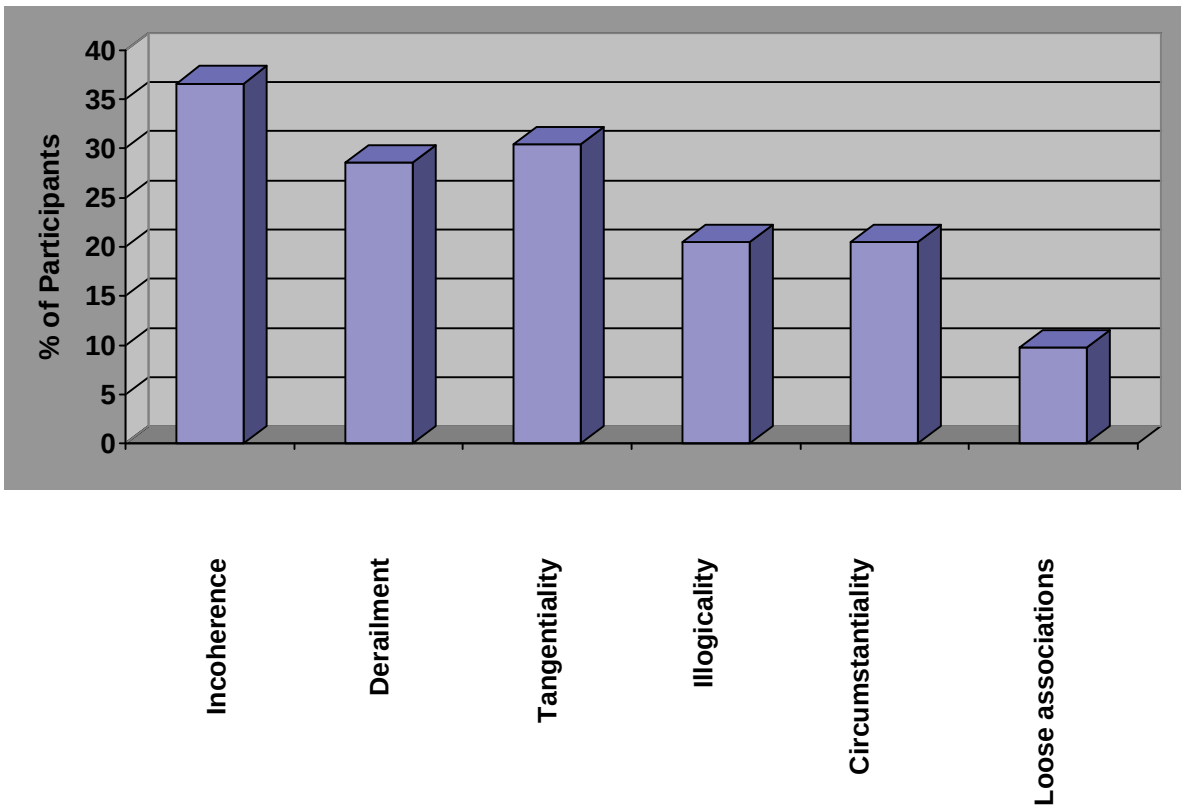


CHART 5: PERCENTAGE OF PARTICIPANTS WITH LIFETIME HISTORY OF A PARTICULAR TYPE OF THOUGHT DISORDER

3.2 COMORBID FEATURES

3.2.1 DEVELOPMENTAL AND MEDICAL HISTORY

Eighty (72%) participants reported no significant co-morbid medical illness. For the remainder a history of pulmonary tuberculosis (n=13; 11.7%) was by far the most common. Others included, head injury (n=2), orthopaedic trauma (n=2), hypertension (n=2), known to be HIV-positive (n=2), meningitis (n=1), epilepsy (n=1) and a visual impairment (n=1). None of the illnesses had a temporal relationship to the onset of schizophrenia.

One case of prolonged labour was reported whilst generally poor school performance was reported in two cases. It was also noted that one participant only started speaking at the age of five.

3.2.2 SUBSTANCE USE

Seventy-seven (68.8%) of the participants admitted to smoking regular tobacco frequently. Twenty participants (17.9%) met lifetime criteria for alcohol abuse and a further two (1.8%) that for dependency. With regard to cannabis, forty-six (41.1%) met lifetime criteria for abuse and nine (8%) for dependence. Only one (0.9%) met criteria for both alcohol and cannabis dependence with 13 meeting criteria for both alcohol and cannabis abuse (11.6%). Twenty-two (19.8%) participants tested positive for cannabis on a urinary drug screen performed on the day of the interview. Previous mandrax abuse (in conjunction with cannabis

abuse) was reported for two participants and mandrax and cocaine abuse (in conjunction with alcohol abuse) for one. No tests for other drugs of abuse were found to be positive on the drug screen.

3.2.3 COMORBID MOOD AND ANXIETY DISORDERS

Twelve participants (10.7%) could be diagnosed with a suspected co-morbid mood disorder at some time during their illness. Of these, major depressive disorder was the most prevalent, occurring in eight of the participants with adjustment disorder with depressed mood (n=2) and mania (n=2) representing the other diagnoses.

Nine participants (8%) were suspected to have a co-morbid anxiety disorder at some stage. Probable post traumatic stress disorder and panic disorder occurred in three participants each, with a further two admitting to criteria for obsessive compulsive disorder and another exhibiting an array of mixed anxiety symptoms.

3.2.4 ABNORMAL INVOLUNTARY MOVEMENTS

The Abnormal Involuntary Movement Scale (AIMS) was completed for ninety-six (86.4%) of the participants. The mean score was only 0.71 (SD 2.48; range 0-17). Clear tardive dyskinesia could be documented for seven (6.25%) of the participants.

4. TREATMENT

At the time of interview, information for only three (2.67%) of the participants was not available with regard to at least the name of the primary antipsychotic drug they were being treated with. All participants professed to be treatment-compliant. Besides the three, no information was available for a further three as to the name of a second drug they were on and for a further two as to the name of a third drug. A second drug could be documented for 76 (67.85%) and a third for 31 (27.9%) of the participants. Thirty-six participants were receiving two different antipsychotics and three were on a combination of three different antipsychotics. See chart 6 for a full breakdown.

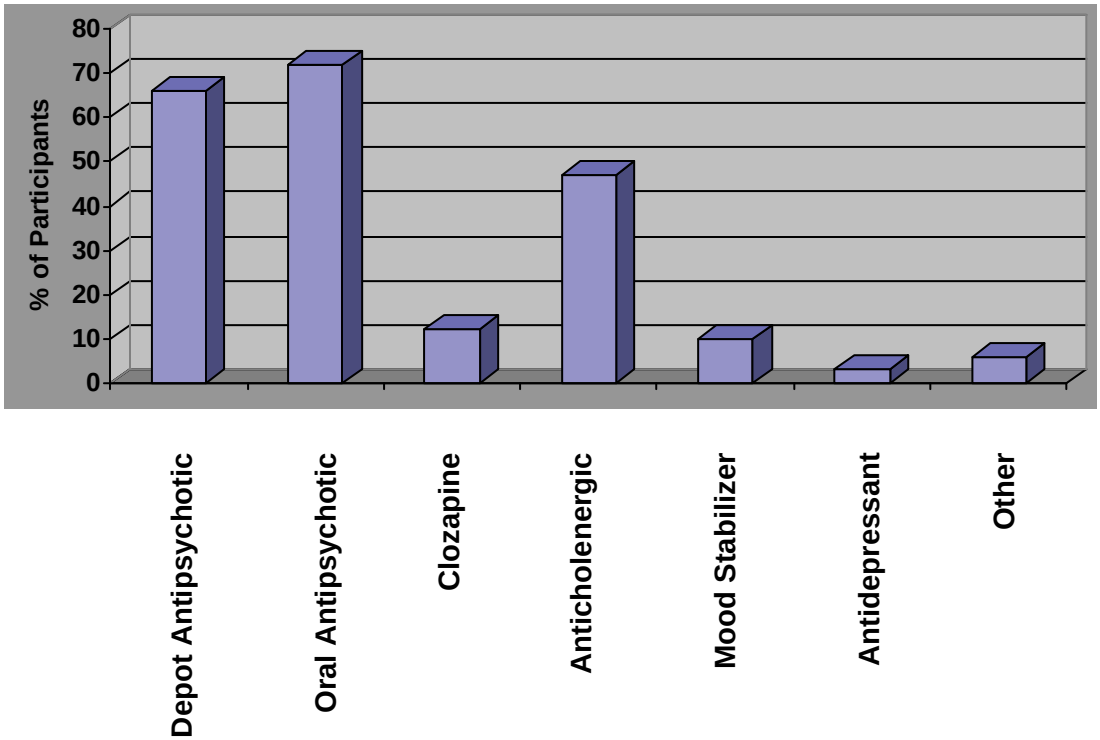


CHART 6: NUMBER OF PARTICIPANTS REPORTING CURRENT USE OF EACH TYPE OF MEDICATION

With the exception of clozapine (n=12; 10.9%) none of the subjects in this sample were on an atypical antipsychotic. Interestingly, three of the patients were still on thioridazine which had already been withdrawn from the market at the time of the interviews. Other than antipsychotics the only medications reported by participants were anticholinergics (n=47; 42.3%), mood stabilisers (n=10; 8.92%), antidepressants (n=3), thiamine (n=3) and reserpine, phenytoin, lorasepam (n=1 each).

5. HEAD AND FACIAL MEASUREMENTS

In accordance with the previously described procedure (see methodology) a total of nine head and facial measurements were taken for 69 (61.6%) of the participants. Please see table 1 for full results (divided into male and female groups) as well as the diagram below for orientation to the landmarks used.

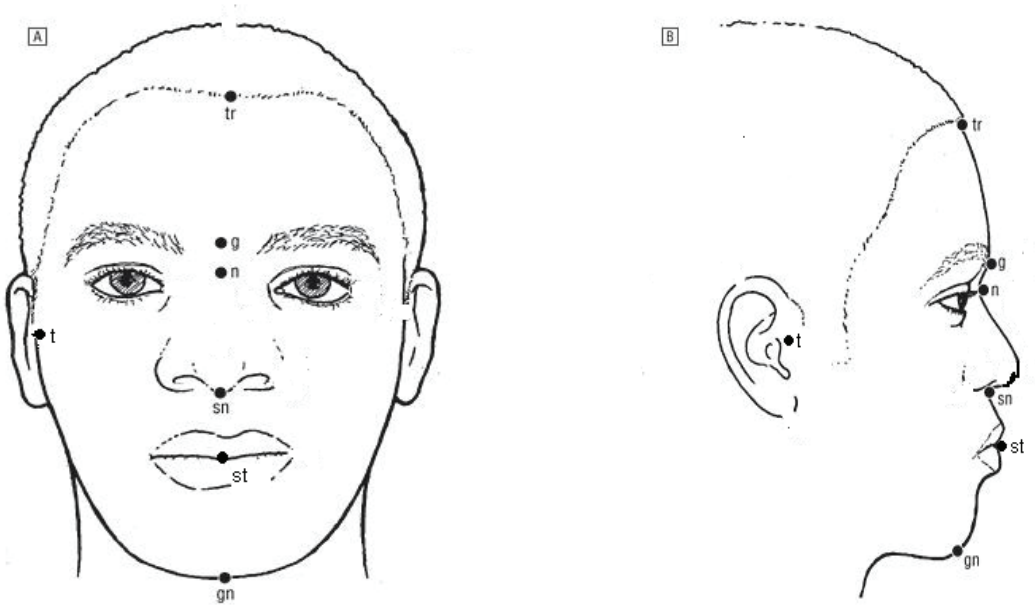


TABLE 1: SUMMARY OF HEAD AND FACIAL MEASUREMENTS TAKEN (REPORTED IN MILLIMETRE)

	MALES (n=53)		FEMALES (n=16)	
ITEM MEASURED	**MEAN**	**STD DEV**	**MEAN**	**STD DEV**
Head circumference	578.19	19.00	578.66	19.46
Trichion(tr) to Glabella (g)	57.55	10.49	56.09	6.90
Glabella to Nasion (n)	20.50	9.20	21.84	9.99
Glabella to Subnasale (sn)	58.03	5.64	55.62	5.08
Glabella to Stomion (st)	83.93	5.75	78.24	8.65
Glabella to Gnathion (gn)	129.48	10.47	119.47	9.25
Tragion (t) to Trichion	139.26	8.10	135.77	8.18
Tragion to Subnasale	132.55	7.44	126.17	4.61
Tragion to Gnathion	145.63	9.13	139.34	5.01

6. CLINICAL FEATURES ON INTERVIEW

As part of the interview, Schedules for the Assessment of Positive and Negative Symptoms (SANS; SAPS) were also performed on each participant. Whereas the rest of the interview provided information on lifetime symptomatology and history, the SANS and the SAPS were used to elicit a snapshot view of the participants as they presented on interview.

6.1 SANS

The calculated SANS-total mean score was 8.93 (SD 3.71; range 2-20). Participants achieved scores in all of the subscales (affective flattening; alogia; avolition/apathy; anhedonia/asociality; attention) but to differing degrees.

On interview, global affective flattening could be observed for 100 (89.2%) of the participants. However, it was regarded to be only mild in 63 of the cases, with 22 rated as moderate, 13 as marked and 2 as severe. Please see table 2 for a full results breakdown.

TABLE 2: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL AFFECTIVE FLATTENING SUBSCALE ITEMS OF THE SANS

AFFECTIVE FLATTENING ITEMS	MILD	MODERATE	MARKED	SEVERE
Unchanging facial expression	55	27	13	1
Decreased spontaneous movement	46	19	3	1
Paucity of expressive gestures	50	20	4	1
Poor eye contact	46	20	4	1
Affective non-responsivity	43	17	4	2
Inappropriate affect	4	6	3	1
Lack of vocal inflections	43	19	6	1

Global alogia could be documented for 70 (62.5%) of the participants. Once again, mild cases made up the largest part of the group comprising 42 (60.0%) of the cases with only 12 (17.1%) reported to be either marked or severe. Please see table 3 for a full results breakdown.

TABLE 3: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL ALOGIA SUBSCALE ITEMS OF THE SANS

ALOGIA ITEMS	MILD	MODERATE	MARKED	SEVERE
Poverty of speech	44	11	7	2
Poverty of content of speech	21	34	9	7
Blocking	1	0	1	0
Increased latency of response	7	0	2	1

Ninety-nine (88.4%) of the participants received a definite score on the global avolition/apathy subscale, with 44 rated mild, 38 moderate, 14 marked and 1 severe. Please see table 4 for a full results breakdown.

Ninety-five percent (n=107) of the participants were regarded to definitely qualify for a score on the global anhedonia/asociality subscale. Mild symptoms were documented in 50.5% (n=54) of these cases and marked or severe in only 12.1% (n=14). Please see table 5 for a full results breakdown.

TABLE 4: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL AVOLITON/APATHY SUBSCALE ITEMS OF THE SANS

AVOLITION/APATHY ITEMS	MILD	MODERATE	MARKED	SEVERE
Grooming and hygiene	45	21	2	1
Inpersistance at work or school	34	39	14	4
Physical anergia	31	34	15	1

TABLE 5: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL ANHEDONIA/ ASOCIALITY SUBSCALE ITEMS OF THE SANS

ANHEDONIA/ASOCIALITY ITEMS	MILD	MODERATE	MARKED	SEVERE
Recreational interests and activities	53	36	11	1
Sexual activity	7	6	3	0
Ability to feel intimacy + closeness	42	41	12	1
Relationship with friends + peers	49	41	12	1

Only 14 (12.5%) participants were regarded to definitely exhibit a disturbance of attention making this the lowest, by far, of all five the global SANS subscales. For 9, the disturbance was regarded to be mild, 2 moderate, 1 marked and 2 severe. Please see table 6 for a full results breakdown.

TABLE 6: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL ATTENTION SUBSCALE ITEMS OF THE SANS

ATTENTION ITEMS	MILD	MODERATE	MARKED	SEVERE
Social inattentiveness	10	2	1	2
Inattentiveness during mental status testing	1	1	0	0

6.2 SAPS

The calculated SAPS-total mean score was 4.77 (range 0-15; SD 4.39). Participants achieved scores in all of the subscales (hallucinations; delusions; bizarre behaviour; positive formal thought disorder) but to differing degrees.

One or more form of current perceptual disturbances were reported or could be observed for nearly half of all participants (n=55; 49.1%) on interview. Presence of and/or impairment due to these were regarded to be mild in 22.3% (n=25) of cases, moderate in 14.3% (n=16), marked in 9.8% (n=11) and severe in 1.8% (n=2). Of these, auditory hallucinations were by far the most common (n=50; 44.6%) with both voices providing running commentary (n=35; 31.25%) and two or more voices conversing (n=37; 33.0%) occurring in a significant percentage of participants. Please see table 7 for a full results breakdown.

TABLE 7: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL HALLUCINATION SUBSCALE ITEMS OF THE SAPS

HALLUCINATION ITEMS	MILD	MODERATE	MARKED	SEVERE
Auditory hallucinations	19	14	13	4
Voices commenting	19	9	6	1
Voices conversing	17	8	10	2
Somatic/Tactile hallucinations	9	3	0	1
Olfactory hallucinations	17	3	0	0
Visual hallucinations	8	7	2	0

Fifty-six (50.0%) participants were documented to report or observed to have the presence of one or more type of delusion on interview. The effect of these or presence thereof was regarded to be severe for 2 (1.8%) participants, marked for 9 (8%), moderate for 24 (21.4%) and mild for 21 (17.9%). Although many different types of delusions could be documented persecutory delusions (n=42; 37.5%) were the most frequent with delusions of reference (n=26; 23.2%), the only other type occurring in more than 15% of participants. Please see table 8 for a full results breakdown.

Some form of inappropriate behaviour was reported or observed on interview for nearly a third (n=34; 30.3%) of participants. Of these aggressive or agitated behaviour (n=26; 23.2%) was most common with repetitive or stereotypical behaviour (n=5; 4.5%) least common. See table 9 for a full results breakdown.

The presence of some degree of formal thought disorder on interview could be documented for forty (35.7%) participants. Mostly this was regarded to be mild (n=23; 20.5%), but for 13 (11.6%) participants it could be recorded to be moderate and for 4 (3.6%) marked. Tangentiality (n=37; 33.0%) and derailment (n=33; 29.5%) were most often observed and clanging in only 1 participant. Please see table 10 for a full results breakdown.

TABLE 8: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL DELUSION SUBSCALE ITEMS OF THE SAPS

DELUSION ITEMS	MILD	MODERATE	MARKED	SEVERE
Persecutory delusions	15	17	8	2
Delusions of jealousy	1	1	0	0
Delusions of guilt or sin	3	2	0	0
Grandiose delusions	7	9	4	0
Religious delusions	8	5	2	0
Somatic delusions	4	4	0	0
Delusions of reference	5	17	3	1
Delusions of being controlled	5	6	3	1
Delusions of mind reading	9	6	2	0
Thought broadcasting	5	3	1	0
Thought insertion	4	6	0	0
Thought withdrawal	2	5	0	0

TABLE 9: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL BIZARRE BEHAVIOUR SUBSCALE ITEMS OF THE SAPS

BIZARRE BEHAVIOUR ITEMS	MILD	MODERATE	MARKED	SEVERE
Clothing and appearance	11	3	2	1
Social/Sexual	9	3	6	0
Aggressive/Agitated	9	11	5	1
Repetitive/Stereotyped	2	1	0	2

TABLE 10: NUMBER OF PARTICIPANTS EXHIBITING PRESENCE OF MILD TO SEVERE SYMPTOMS FOR EACH OF THE INDIVIDUAL THOUGHT DISORDER SUBSCALE ITEMS OF THE SAPS

THOUGHT DISORDER ITEMS	MILD	MODERATE	MARKED	SEVERE
Derailment	21	9	3	0
Tangentiality	25	9	3	0
Incoherence	11	3	2	1
Illogically	10	4	1	0
Circumstantiality	14	7	4	1
Pressure of speech	3	2	1	0
Distractible speech	4	1	0	0
Clanging	1	0	0	0

7. FISH ANALYSIS

Of the 112 participants 110 had FISH analysis performed on their blood samples. For the remaining two, the blood samples yielded insufficient metaphases for analysis and we were unable to locate either participant (due to both having moved) in order to collect a second sample. No chromosome 22 microdeletions could be detected.

8. KARYOTYPING SUBGROUP

Fifty participant samples were karyotyped. When compared in terms of demographic and clinical variables, no statistically significant differences could be demonstrated between the karyotyping subgroup (KSG) and the sample as a whole for any of the demographic, clinical or morphological measurement variables reported on.

8.1 KSG DEMOGRAPHICS AND CLINICAL FEATURES

The majority were male (n=42; 84%) and single (n=37; 73%). All were unemployed with thirty-one (62%) participants receiving a disability grant. In terms of education level only four (8%) had progressed beyond primary school. The mean age of onset for the illness was 23.04 years (SD 8.12; range 12-55). The number of hospitalisations ranged from 0 to 10 with 2.88 (SD 2.14) being the mean. As to number of episodes, the mean was 3.17 (SD 2.09; range 1-10). Eighteen (36%) reported a family history of psychiatric illness. Twelve (24%) of

the participants reported one or more possible stressor as precipitating events prior to the onset of the illness. Of these, death or serious illness of a close family member (n=6) was most often reported.

Reported lifetime history of psychotic symptoms did not differ significantly between the KSG and the full sample. As with the total group, auditory hallucinations (n=49; 98%) were by far the most common type of perceptual disturbance reported. See table 11 for a full breakdown.

TABLE 11: NUMBER OF PARTICIPANTS IN KSG REPORTING A LIFETIME HISTORY FOR ANY TYPE OF HALLUCINATION

TYPE OF HALLUCINATION	n	%
All auditory hallucinations	49	98
Visual hallucinations	31	62
Olfactory hallucinations	9	18
Tactile hallucinations	11	22
Gustatory hallucinations	6	12

Persecutory delusions (n=44; 88%) were most commonly experienced; see chart 7 for full breakdown. Where inappropriate behaviour was reported incidents of physical or verbal aggression (n=40; 80%) were the most common with sexual disinhibition (n=5; 10%) and catatonic symptoms (n=4; 8%) also reported.

Incoherence (n=17; 34%) was the type of thought disorder most often reported. See chart 8 for full breakdown.

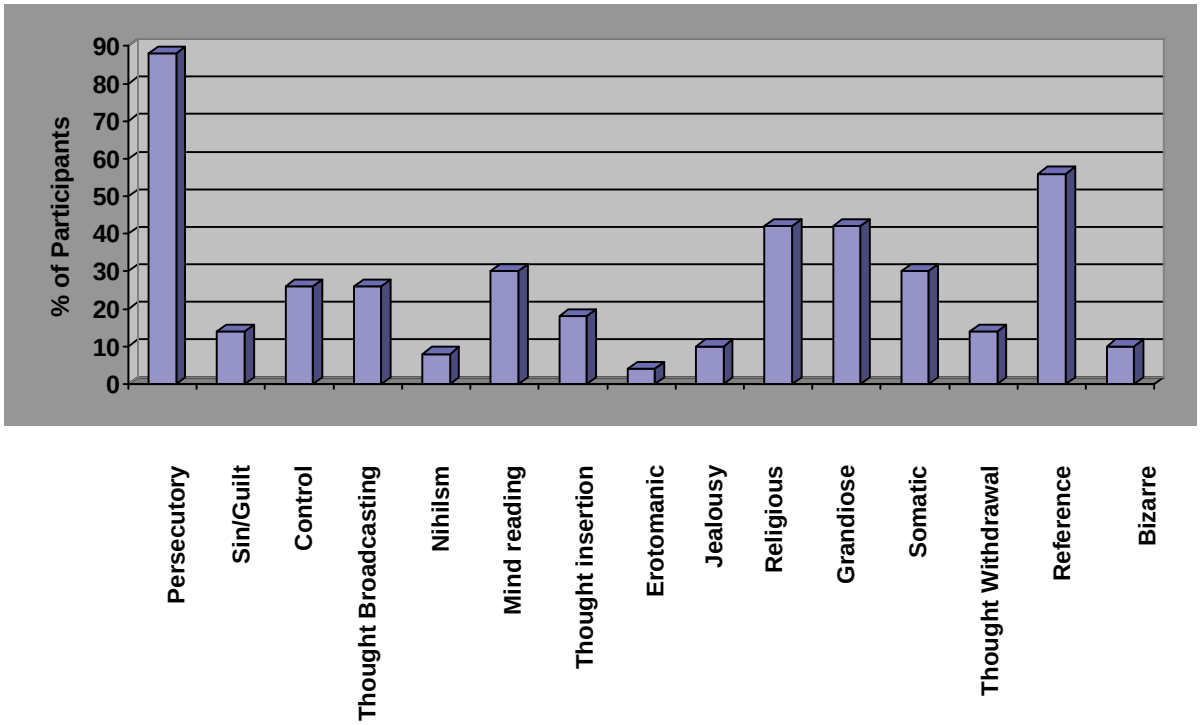


CHART 7: PERCENTAGE OF KSG MEMBERS WITH LIFETIME HISTORY OF A PARTICULAR TYPE OF DELUSION

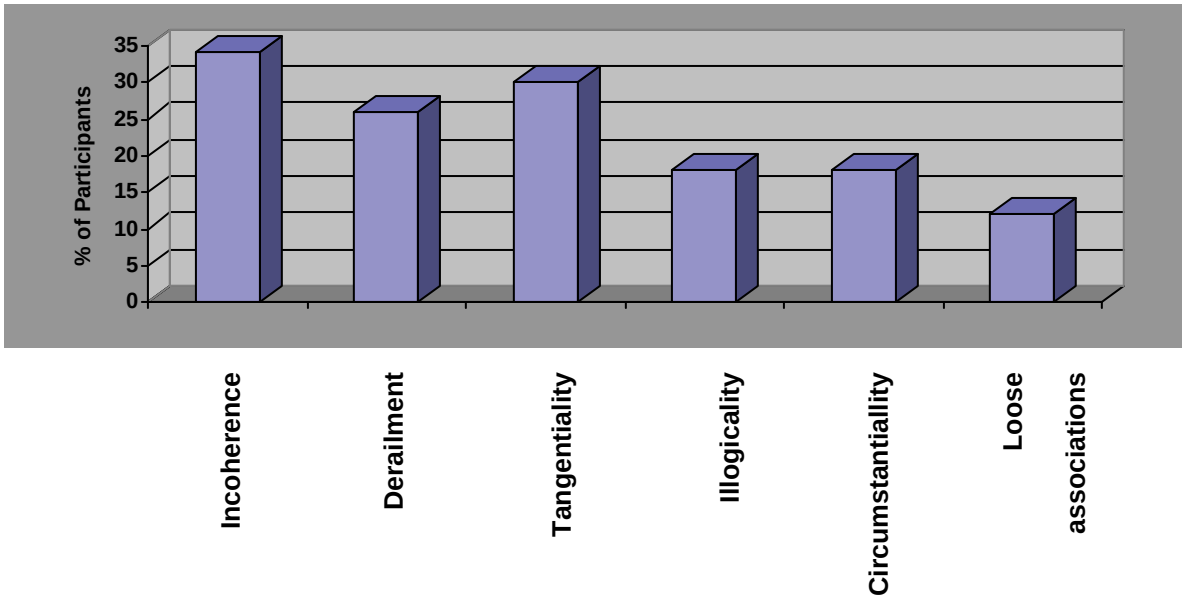


CHART 8: PERCENTAGE OF KSG MEMBERS WITH LIFETIME HISTORY OF A PARTICULAR TYPE OF THOUGHT DISORDER

Twenty (40%) of the KSG members reported a history of a significant medical illness. Of these a history of pulmonary tuberculosis (n=10; 20%) was by far the most common. Thirteen (26%) met criteria for lifetime alcohol abuse and one (2%) for dependency. Thirty-seven (73%) smoked regular tobacco and twenty-one (44%) met lifetime criteria for cannabis abuse and four (8%) for dependency. Eight (16%) participants tested positive for cannabis on an urinary drug screen performed on the day of the interview.

Thirteen (26%) of the group were suspected to have had a co-morbid mood or anxiety disorder at some time during their illness. Six (12%) participants admitted to having made one or more suicide attempts during the course of their illness. Three attempts were of a more violent nature (cutting own throat (n=2) and hanging (n=1)). The AIMS could be completed for forty-five (90%) of the group. Clear tardive dyskinesia could be documented for five (10%) of the participants.

At the time of interview, information as to at least the name of the primary antipsychotic drug they were being treated with was available for all KSG members with the exception of one. Twelve (24%) participants were receiving two different antipsychotics and one was on a combination of three different antipsychotics. Twenty-three (46%) of the participants were receiving a depot antipsychotic and only 5 (10%) were on an atypical (clozapine). Other than antipsychotics the only medication reported by participants were anticholinergics

(n=20; 40%), mood stabilisers (n=4; 8%), antidepressants (n=2), thiamine (n=2) and phenytoin, lorasepam (n=1 each).

8.2 KSG HEAD AND FACIAL MEASUREMENTS

Head and facial measurements as previously described were done on all 50 KSG members. Please see diagram and table 12 for full breakdown.

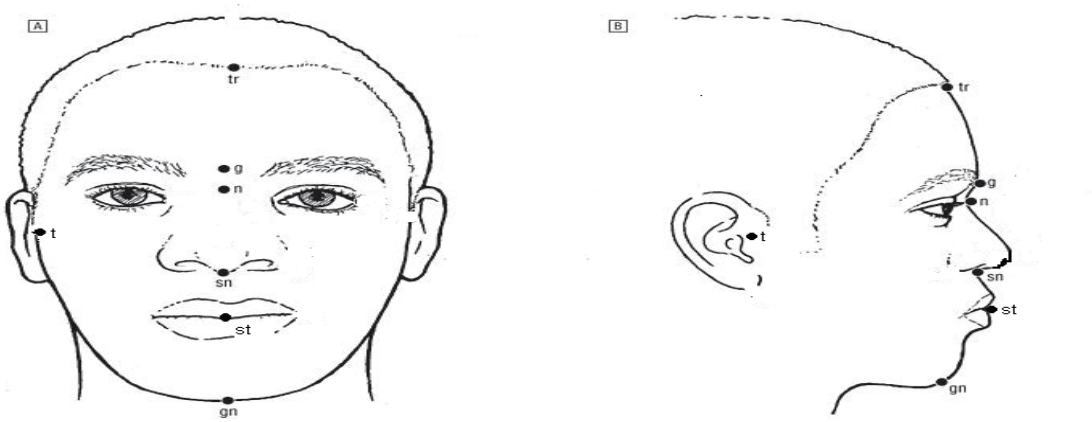


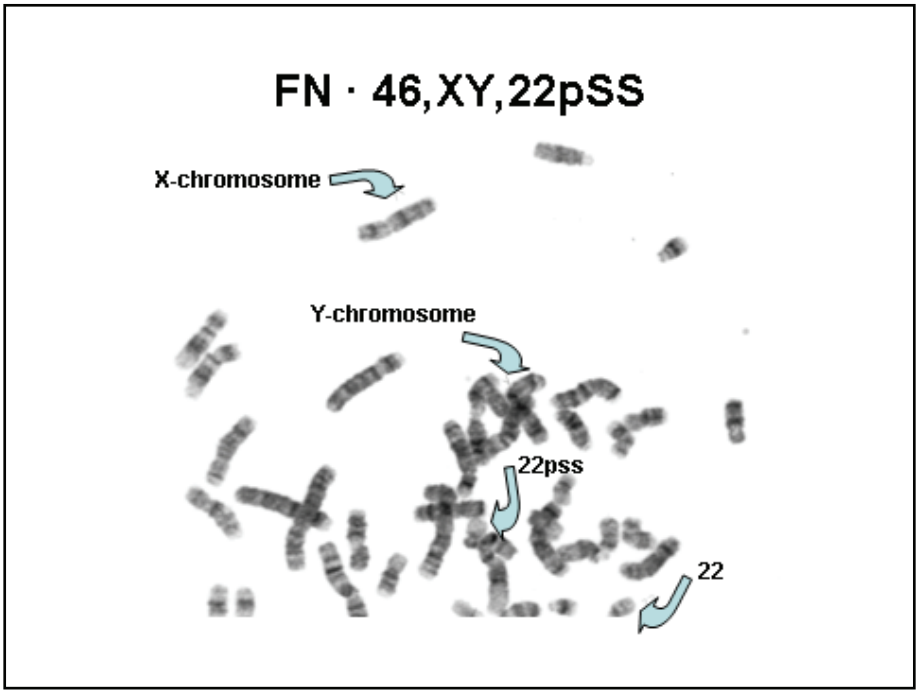
TABLE 12: SUMMARY OF HEAD AND FACIAL MEASUREMENTS TAKEN ON KSG MEMBERS (REPORTED IN MILLIMETRE)

	MALES (n=42)		FEMALES (n=8)	
ITEM MEASURED	**MEAN**	**STD DEV**	**MEAN**	**STD DEV**
Head circumference	580.45	20.01	588.88	20.53
Trichion(tr) to Glabella (g)	56.84	10.36	57.01	5.78
Glabella to Nasion (n)	21.25	9.74	27.31	8.74
Glabella to Subnasale (sn)	57.68	5.39	54.51	4.70
Glabella to Stomion (st)	84.22	5.06	75.83	9.77
Glabella to Gnathion (gn)	128.88	10.50	115.96	10.52
Tragion (t) to Trichion	139.26	7.99	135.26	9.92
Tragion to Subnasale	132.85	7.66	126.63	6.04
Tragion to Gnathion	146.25	9.64	137.89	5.54

8.3 KARYOTYPING RESULTS

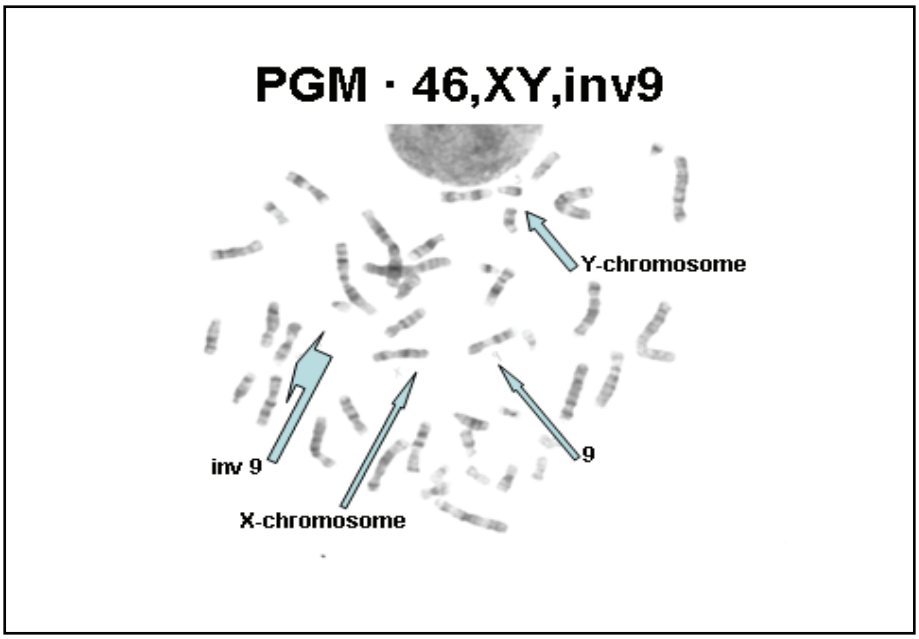
Of the fifty participants who were karyotyped, chromosomal aberrations were identified in five (10%). All of these participants were male. FISH analysis had revealed no chromosome 22 microdeletions and the aberrations were reported as follows:

Participant FN – Male chromosome complement with double satellites was observed on chromosome 22. [46,XY,22pss]

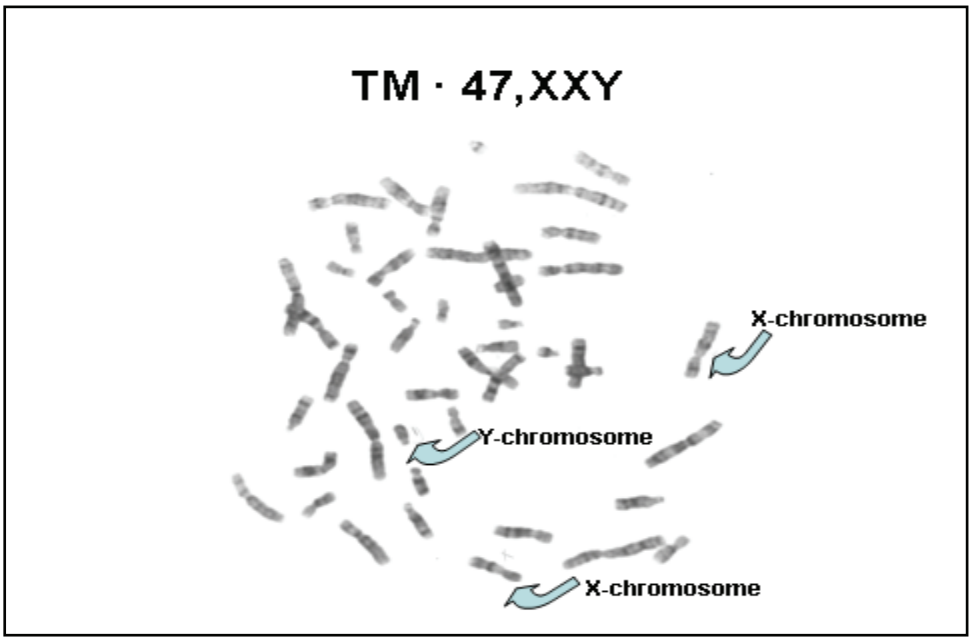


Participant TN – Male chromosome complement with an inversion of the heterochromatic area of chromosome 9. [46,XY,inv(9)]

Participant PGM – Male chromosome complement with an inversion of the heterochromatic area of chromosome 9. [46,XY,inv(9)]



Participant TM – Male chromosome complement consisting of 46,XY (18 metaphases); 47,XXY (2 metaphases); 47,XX,+acentric fragment (1 metaphase). The combination could point to a low-grade mosaic. The acentric fragment could be from the long arms of the X-chromosome.



Participant SK – Male chromosome complement with a variant of the heterochromatic portion of chromosome 1. [46, XY,1qh+]

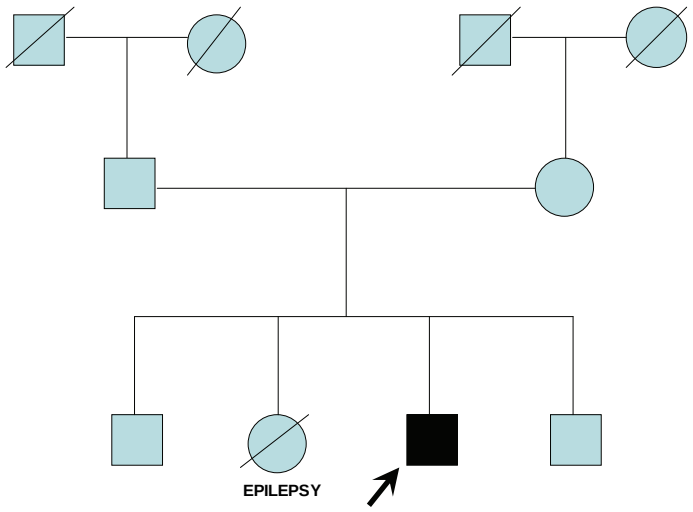
A descriptive analysis of each of these five participants follows:

8.3.1 PARTICIPANT FN

FN was a single (never married) male participant. At the time of the interview he was 35 years old and was an outpatient, with his last discharge from a psychiatric hospital having been approximately eight months earlier. He had a Grade 10 education and was unemployed, receiving a disability grant due to his schizophrenic illness. He had never been employed and was still living in the family home. Information was obtained from the patient, his father and the available clinic files.

There was no specific history of pregnancy complications and he was a NVD at full-term, the third child out of a total of four. Please see pedigree.

His first episode of illness occurred at the age of 19 but the duration of the prodromal period was unknown. No clear stressor could be associated with his first psychotic episode. He was treated as an outpatient at the time. He had had a total of four acute psychotic breakdowns including the above-mentioned and for the other three he needed psychiatric hospitalisation. No family history of any psychiatric illness was reported.



Prior to the onset of psychotic illness he had been fully treated for pulmonary tuberculosis and some years after the onset he was also diagnosed with epilepsy. At the time of the interview no convulsions had been observed for a period of at least two years. His sister was also known to suffer from epilepsy but there was no family history for any other significant medical illness.

He admitted to alcohol use and smoking regular tobacco. No history (lifetime or current) was obtained for any substance use, abuse or dependence and his urinary drug screen was negative. There was no history of any co-morbid mood or anxiety disorder or any suicide attempts. His AIMS score was zero.

At the time of the interview he was being treated with a monthly antipsychotic depot (zuclopenthixol) and sodiumvalproate (for his epilepsy). Exact duration was unknown but he had been on the combination for some time.

A lifetime history of auditory hallucinations (including running commentary and voices conversing) was reported. The participant also had a lifetime history of tactile hallucinations which he described as a cramping sensation that went from his arms to throat whereafter he then experienced a sensation of feeling strangled. A lifetime history of persecutory delusions as well as delusions of reference, guilt and jealousy could be documented. Numerous incidents of physical and verbal aggression during periods of acute illness were reported with agitation and unpredictability in between. No history of bizarre behaviour, catatonic symptoms or symptoms of thought disorder was reported.

At the time of the interview no hallucinations, delusions or symptoms of formal thought disorder were reported or observed with the patient scoring zero in each of these sections of the SAPS. Please see table 13 for a summary of the items in each section of the SANS and SAPS for which the patient achieved a score. (Scoring key 1 = questionable, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

TABLE 13: SUMMARY OF SANS AND SAPS ITEMS ON WHICH PARTICIPANT FN ACHIEVED ANY SCORE

ITEM	SCORE	ITEM	SCORE
AFFECTIVE FLATTENING – GLOBAL SCORE	4	**AVOLITION / APATHY – GLOBAL SCORE**	3
Unchanging facial expression	4	Grooming and hygiene	3
Decreased spontaneous movement	3	Inpersistance at work or school	3
Paucity of expressive gestures	4	Physical anergia	3
Poor eye contact	3	**ANHEDONIA / ASOCIALITY – GLOBAL SCORE**	3
Affective non-responsivity	4	Recreational interests and activities	3
Lack of vocal inflections	4	Ability to feel intimacy and closeness	3
ALOGIA – GLOBAL SCORE	3	Relationship with friends and peers	3
Poverty of speech	3	**BIZARRE BEHAVIOUR – GLOBAL SCORE**	2
Poverty of content of speech	3	Clothing and appearance	2
		Aggressive and agitated behaviour	3

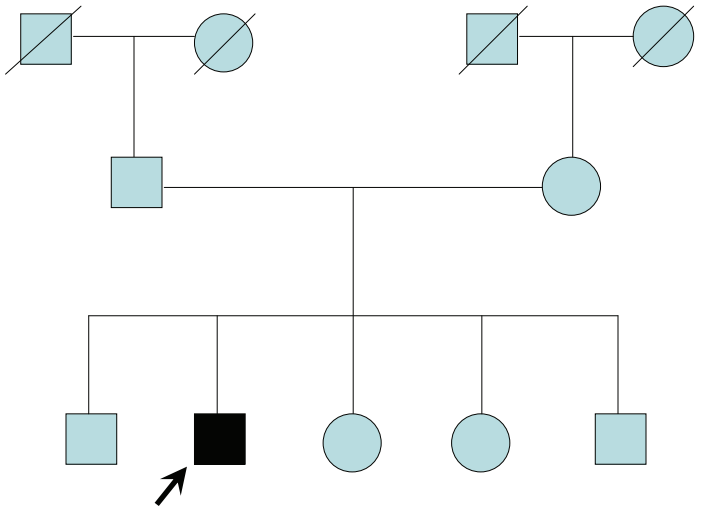
TABLE 14: SUMMARY OF HEAD AND FACIAL MEASUREMENTS TAKEN ON PARTICPANT FN (REPORTED IN MILLIMETRE)

ITEM MEASURED		ITEM MEASURED	
Head circumference	570	Glabella to Gnathion	136.2
Trichion to Glabella	68.3	Tragion to Trichion	143.1
Glabella to Nasion	20.2	Tragion to Subnasale	133.8
Glabella to Subnasale	67.1	Tragion to Gnathion	142.8
Glabella to Stomion	90.6		

8.3.2 PARTICIPANT TWO – TN

TN was a single (never married) male participant. At the time of the interview he was 24 years old and was an inpatient, having been in hospital for approximately three weeks. He had a Grade 9 education and was unemployed, receiving a disability grant due to his schizophrenia. He had never been employed and had been living in a shack with four relatives in the ten months preceding his admission. Information was obtained from the patient, his mother and the available clinic files.

There was no specific history of pregnancy complications and he was a NVD at unknown gestation, the second child out of a total of five. Please see pedigree.



His first episode of illness occurred at the age of 15 but the duration of the prodromal period was unknown. No clear stressor could be associated with his first psychotic episode. He reported a sexual assault in the same period but maintained that it had only happened after he had become ill. He was treated as an inpatient at the time. The patient's mother reported that he had had numerous psychotic breakdowns since becoming ill, needing repeated hospitalisation but the exact number of episodes was unknown. No family history of any psychiatric illness was reported.

With the exception of receiving treatment after the sexual assault, the patient had no medical history and there was also no family history of significant medical illness.

He admitted to smoking regular tobacco and using alcohol but did not meet current or lifetime criteria for either alcohol abuse or dependence. He also admitted to using cannabis very regularly between 1998 and 2001 (when he stopped to please his mother) and met criteria for lifetime abuse (not dependence). His urinary drug screen was negative. There was no history of any co-morbid mood or anxiety disorder or any suicide attempts. His AIMS score was zero.

At the time of the interview he was being treated with a monthly antipsychotic depot (zuclopenthixol) and haloperidol. He was non-compliant at the time of his

admission and had therefore been on this combination for approximately three weeks. He was known to have been previously treated with “an injection” as well but the name, dose and duration was unknown.

A lifetime history of visual and auditory hallucinations (running commentary) as well as delusions (persecutory, somatic, reference) was reported. No bizarre behaviour but numerous incidents of physical and verbal aggression during periods of acute illness was reported. No history of catatonic symptoms was reported but a lifetime history of thought disorder (tangentiality) could be documented.

At the time of the interview no hallucinations, delusions or symptoms of formal thought disorder were reported or observed with the patient scoring zero in each of these sections of the SAPS. Please see table 15 for a summary of the items in each section of the SANS and SAPS for which the patient achieved a score. (Scoring key 1 = questionable, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

TABLE 15: SUMMARY OF SANS AND SAPS ITEMS ON WHICH PARTICIPANT TN ACHIEVED ANY SCORE

ITEM	SCORE	ITEM	SCORE
AFFECTIVE FLATTENING – GLOBAL SCORE	3	**ATTENTION – GLOBAL SCORE**	2
Unchanging facial expression	2	Social inattentiveness	2
Decreased spontaneous movement	2	**HALLUCINATIONS – GLOBAL SCORE**	3
Paucity of expressive gestures	2	Auditory hallucinations	3

Poor eye contact	3	Voices commenting	3
Affective non-responsivity	3	Voices conversing	3
Inappropriate affect	3	Visual hallucinations	3
Lack of vocal inflections	3	**DELUSIONS – GLOBAL SCORE**	3
ALOGIA – GLOBAL SCORE	2	Delusions of reference	3
Poverty of speech	1	**BIZARRE BEHAVIOUR – GLOBAL SCORE**	3
Poverty of content of speech	2	Clothing and appearance	2
AVOLITION / APATHY – GLOBAL SCORE	3	Social and sexual behaviour	2
Grooming and hygiene	3	Aggressive and agitated behaviour	3
Inpersistance at work or school	3	**FORMAL THOUGHT DISORDER – GLOBAL SCORE**	2
Physical anergia	3	Derailment	2
ANHEDONIA / ASOCIALITY – GLOBAL SCORE	3	Tangentiality	2
Recreational interests and activities	3		
Sexual Activity	3		
Ability to feel intimacy and closeness	3		
Relationship with friends and peers	3		

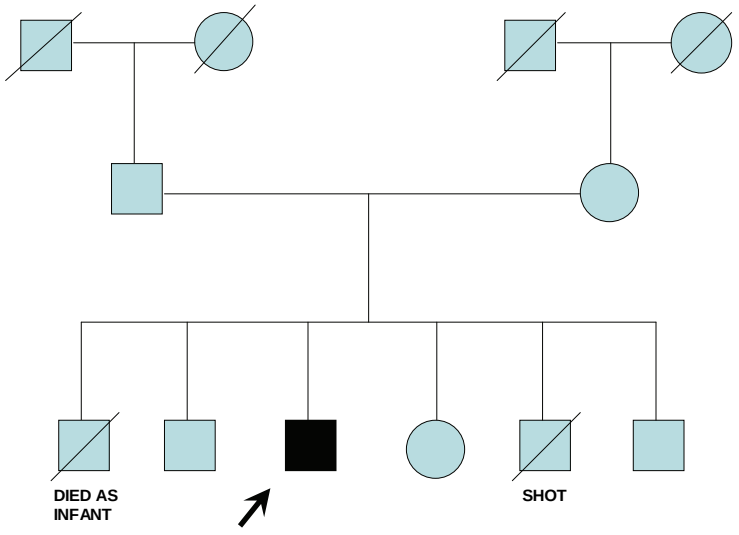
TABLE 16: SUMMARY OF HEAD AND FACIAL MEASUREMENTS TAKEN ON PARTICPANT TN (REPORTED IN MILLIMETRE)

ITEM MEASURED		ITEM MEASURED	
Head circumference	585	Glabella to Gnathion	137.6
Trichion to Glabella	50.6	Tragion to Trichion	137.5
Glabella to Nasion	15.5	Tragion to Subnasale	133.1
Glabella to Subnasale	61.9	Tragion to Gnathion	150.3
Glabella to Stomion	87.5		

8.3.3 PARTICIPANT THREE – PGM

PGM was a single (never married) male participant. At the time of the interview he was 36 years old and was an inpatient, having been admitted to hospital six months earlier. He had a Grade 7 education and was unemployed, receiving a disability grant due to his schizophrenic illness. He had previously been employed in a low-level watchman position but it had been more than a year since his last employment. Information was obtained from the patient, ward staff and the available ward files.

There was no specific history of pregnancy complications and he was a NVD (gestation unknown), the third child out of a total of six. Please see pedigree.



His first episode of illness occurred at the age of 29 but the duration of the prodromal period was unknown. No clear stressor could be associated with his first psychotic episode and he was hospitalised at the time. Including that episode he had had a total of five acute psychotic breakdowns needing psychiatric hospitalization for all. A family history of schizophrenia was reported for one of his cousins on his mother's side. The patient had no personal or family history of significant medical illness.

He admitted to smoking regular tobacco and met criteria for a lifetime (not current) history of alcohol abuse (not dependence). He also admitted to using cannabis up until two years prior to the time of the interview and met criteria for lifetime abuse (not dependence) and his urinary drug screen was negative.

Previous symptoms suggesting mania were found documented in the patient's file (euphoria, disinhibition, overfamiliarity, restlessness, lability). On the interview there were no symptoms suggesting a current mood episode. His AIMS score was zero.

At the time of the interview he was being treated with clozapine and sodiumvalproate and he was regarded as treatment-resistant according to his clinical notes. The treatment had been started during his current period of hospitalisation and he had been on the combination for some weeks already.

A lifetime history of auditory hallucinations (two or more voices, voices conversing, noises and those of a command, commentary and threatening nature) as well as visual hallucinations was reported. With regards to thought content a lifetime history of thought withdrawal and delusions of a persecutory, religious, grandiose, nihilistic, reference and control nature was reported. There was no history of catatonic symptoms but a lifetime history of thought disorder (tangetiality, circumstantiality, derailment, illogicality) and inappropriate behaviour (verbal aggression; non-bizarre) could be documented.

At the time of the interview the patient still exhibited numerous positive and negative symptoms. Please see table 17 for a summary of the items in each section of the SANS and SAPS for which the patient achieved a score. (Scoring key 1 = questionable, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

TABLE 17: SUMMARY OF SANS AND SAPS ITEMS ON WHICH PARTICIPANT PGM ACHIEVED ANY SCORE

ITEM	SCORE	ITEM	SCORE
AFFECTIVE FLATTENING – GLOBAL SCORE	2	**DELUSIONS – GLOBAL SCORE**	4
Unchanging facial expression	2	Persecutory delusions	4
Decreased spontaneous movement	1	Grandiose delusions	3
Paucity of expressive gestures	2	Religious delusions	4
Poor eye contact	2	Delusions of control	4
Affective non-responsivity	1	Delusions of reference	4
Inappropriate affect	3	Thought withdrawal	3
Lack of vocal inflections	1	**BIZARRE BEHAVIOUR – GLOBAL SCORE**	3

ALOGIA – GLOBAL SCORE	1	Social and sexual behaviour	4
Poverty of content of speech	2	Aggressive and agitated behaviour	2
Increased latency of response	1	Repetitive or stereotyped behaviour	1
AVOLITION / APATHY – GLOBAL SCORE	4	**FORMAL THOUGHT DISORDER – GLOBAL SCORE**	3
Grooming and hygiene	3	Derailment	2
Inpersistance at work or school	5	Tangentiality	3
Physical anergia	1	Illogicality	1
ANHEDONIA / ASOCIALITY – GLOBAL SCORE	2	Circumstantiality	4
Recreational interests and activities	3	Pressure of speech	3
Ability to feel intimacy and closeness	2	Distractible speech	1
Relationship with friends and peers	2		
HALLUCINATIONS – GLOBAL SCORE	4		
Auditory hallucinations	4		
Voices commenting	4		
Voices conversing	4		
Visual hallucinations	1		

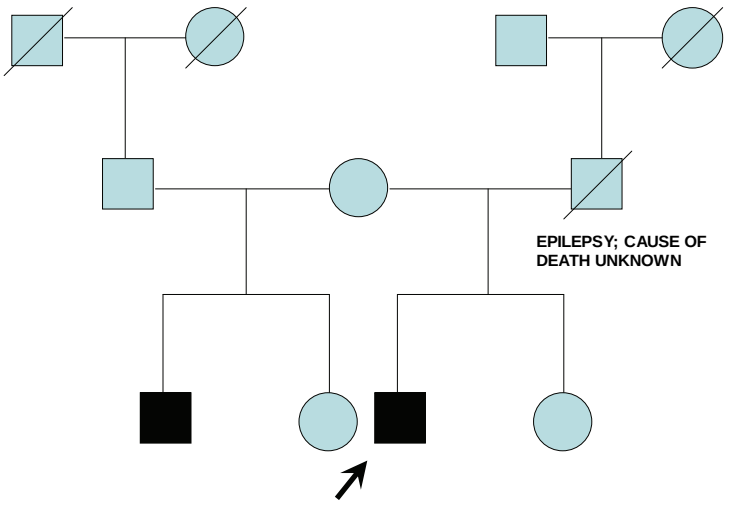
TABLE 18: SUMMARY OF HEAD AND FACIAL MEASUREMENTS TAKEN ON PARTICPANT PGM (REPORTED IN MILLIMETRE)

ITEM MEASURED		ITEM MEASURED	
Head circumference	570	Glabella to Gnathion	121.0
Trichion to Glabella	60.0	Tragion to Trichion	132.0
Glabella to Nasion	33.0	Tragion to Subnasale	140.0
Glabella to Subnasale	62.0	Tragion to Gnathion	142.0
Glabella to Stomion	94.0		

8.3.4 PARTICIPANT FOUR – TM

TM was a single (never married) male participant. At the time of the interview he was 39 years old and was an outpatient with his last discharge from a psychiatric hospital having been many years ago. He had a Grade 7 education and was unemployed, receiving a disability grant due to his schizophrenia. He had previously been employed as a miner but it had been many years since he had last worked. He had been living in a squatter camp with six relatives for the previous 18 years. Information was obtained from the patient, his mother and the available clinic files.

There was no specific history of pregnancy complications and he was a NVD at fullterm, the eldest of two siblings and he also had two older half-siblings, sharing the same mother. Please see pedigree.



His first episode of illness occurred at the age of 19 but the duration of the prodromal period was unknown. No clear stressor could be associated with his first psychotic episode and he not was hospitalised at the time. Including that episode he had had a total of at least three acute breakdowns but was only hospitalised for one. The patient's halfbrother was reported to also suffer from a mental illness (diagnosis unknown). The patient had no personal history of significant medical illness but his father who passed away when the patient was five, was known to have had epilepsy.

He admitted to smoking regular tobacco and using alcohol but did not meet current or lifetime criteria for either alcohol abuse or dependence. He also admitted to using cannabis since his teens and met criteria for both lifetime abuse and dependence and his urinary drug screen was positive. There was no history of any co-morbid mood or anxiety disorder or any suicide attempts. His AIMS score was zero.

At the time of the interview he was being treated with a monthly antipsychotic depot (flupentixol) and had been on it for an extended period.

A lifetime history of auditory hallucinations (including running commentary, threatening voices and voices conversing) was reported. A lifetime history of persecutory delusions as well as delusions of reference, religion and jealousy could be documented. Numerous incidents of physical and verbal aggression

during periods of acute illness were reported as well as episodes of bizarre behaviour specifically centered around attempts to steal washing from clotheslines. No catatonic symptoms were reported but the participant was known to display thought disorder (derailment and tangentiality) during acute episodes.

At the time of the interview, the participant displayed some mild negative symptoms but with the exception of a mildly bizarre appearance as well as the possibility of some thought disorder, the participant scored zero in each of the sections of the SAPS. Please see table 19 for a summary of the items in each section of the SANS and SAPS for which the patient achieved a score. (Scoring key 1 = questionable, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

TABLE 19: SUMMARY OF SANS AND SAPS ITEMS ON WHICH PARTICIPANT TM ACHIEVED ANY SCORE

ITEM	SCORE	ITEM	SCORE
AFFECTIVE FLATTENING – GLOBAL SCORE	2	**AVOLITION / APATHY – GLOBAL SCORE**	4
Unchanging facial expression	2	Grooming and hygiene	4
Decreased spontaneous movement	2	**ANHEDONIA / ASOCIALITY – GLOBAL SCORE**	2
Paucity of expressive gestures	2	Recreational interests and activities	2
Poor eye contact	2	Ability to feel intimacy and closeness	2
Affective non-responsivity	2	Relationship with friends and peers	2
Lack of vocal inflections	2	**BIZARRE BEHAVIOUR – GLOBAL SCORE**	2
ALOGIA – GLOBAL SCORE	2	Clothing and appearance	2

Poverty of speech	2	**FORMAL THOUGHT DISORDER – GLOBAL SCORE**	1
Poverty of content of speech	2	Derailment	1
Increased latency of response	1	Tangentiality	1

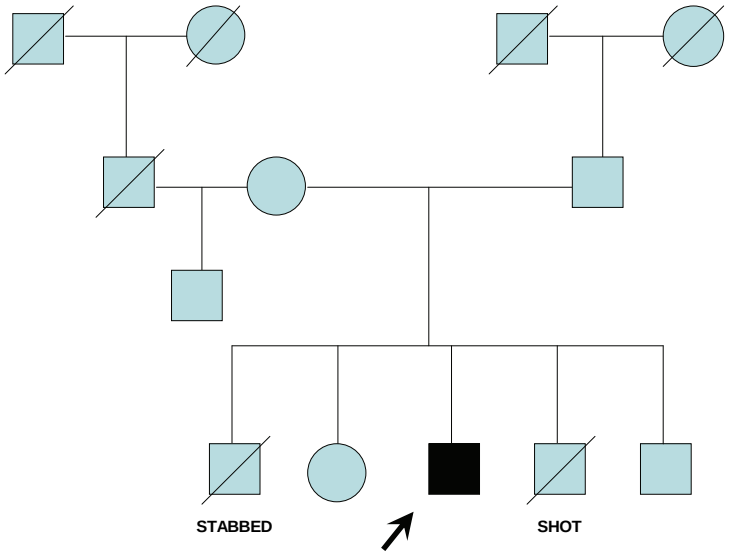
TABLE 20: SUMMARY OF HEAD AND FACIAL MEASUREMENTS TAKEN ON PARTICPANT TM (REPORTED IN MILLIMETRE)

ITEM MEASURED		ITEM MEASURED	
Head circumference	610	Glabella to Gnathion	116.5
Trichion to Glabella	62.0	Tragion to Trichion	132.0
Glabella to Nasion	44.1	Tragion to Subnasale	146.5
Glabella to Subnasale	57.6	Tragion to Gnathion	151.7
Glabella to Stomion	84.0		

8.3.5 PARTICIPANT FIVE – SK

SK was a single (never married) male participant. At the time of the interview he was 22 years old and was an outpatient, having never been hospitalised for psychiatric illness. He had a Grade 11 education and was unemployed and was not receiving a disability grant. He had never been employed and had been living on his own in a shack for four years. Information was obtained from the patient and the available clinic files.

There was no specific history of pregnancy complications and he was a NVD at fullterm, the third child out of a total of five; also having one older halfsibling sharing a mother. Please see pedigree.



His first episode of illness occurred at the age of 21 but the duration of the prodromal period was unknown. Death of a family member was associated with his first psychotic episode. Initially his family took him to a traditional healer who diagnosed amafufunyana and he received treatment. When he did not respond after two months he was taken to the clinic where an antipsychotic was started. The patient had not yet had a second acute episode at the time of the interview and according to the clinic notes, his psychotic symptoms had resolved completely approximately four months after treatment was started. According the patient he had a cousin who was also “mad”.

The patient reported a previous history of “asthma” and ten years prior to the onset of psychiatric illness he was fully treated for pulmonary tuberculosis. He was not receiving any current treatment for medical illness and there was no family history of significant medical illness.

He admitted to smoking regular tobacco and using alcohol but did not meet current or lifetime criteria for either alcohol abuse or dependence. The patient admitted to using cannabis (but was vague as to the duration). He met criteria for lifetime abuse (not dependence) and his urinary drug screen was positive. As an adolescent the patient reportedly suffered two episodes of depression both related to the death of close family members. He received no treatment for these and had no history of any suicide attempts. No mood symptoms were associated with his psychotic breakdown and he has experienced none since. His AIMS score was two with some minimal tardive diskinesia of upper limbs observed.

At the time of the interview he was being treated with haloperidol and orphenadrine. He had been on this combination for approximately 12 months and regularly attended clinic visits.

A lifetime history of visual and auditory hallucinations (two voices, conversing, threatening in nature) as well as delusions (persecutory, grandiose, religious, bizarre) was reported. No bizarre behaviour but verbal aggression during his period of acute illness was reported. No history of catatonic symptoms was

reported but a lifetime history of thought disorder (incoherence) could be documented.

At the time of the interview no hallucinations, delusions or symptoms of formal thought disorder were reported or observed with the patient scoring zero in all sections of the SAPS. Please see table 21 for a summary of the items in each section of the SANS and SAPS for which the patient achieved a score. (Scoring key 1 = questionable, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

TABLE 21: SUMMARY OF SANS AND SAPS ITEMS ON WHICH PARTICIPANT SK ACHIEVED A SCORE

ITEM	SCORE	ITEM	SCORE
AFFECTIVE FLATTENING – GLOBAL SCORE	1	**AVOLITION / APATHY – GLOBAL SCORE**	2
Unchanging facial expression	1	Grooming and hygiene	2
Decreased spontaneous movement	1	Inpersistance at work or school	2
Poor eye contact	1	**ANHEDONIA / ASOCIALITY – GLOBAL SCORE**	2
Affective non-responsivity	1	Recreational interests and activities	2
AVOLITION / APATHY – GLOBAL SCORE	2	Sexual Activity	2
Grooming and Hygiene	2	Ability to feel intimacy and closeness	2
Inpersistance at work or school	2	Relationship with friends and peers	2

TABLE 22: SUMMARY OF HEAD AND FACIAL MEASUREMENTS TAKEN ON PARTICPANT SK (REPORTED IN MILLIMETRE)

ITEM MEASURED		ITEM MEASURED	
Head circumference	545	Glabella to Gnathion	121.8
Trichion to Glabella	42.2	Tragion to Trichion	138.3
Glabella to Nasion	12.4	Tragion to Subnasale	124.7
Glabella to Subnasale	60.2	Tragion to Gnathion	139.7
Glabella to Stomion	81.6		

8.4 COMPARISON

No significant differences could be demonstrated for any of the demographic or clinical variables for the group of five when compared to the rest of the KSG group. See table 23-25 for comparison of selected variables.

TABLE 23: COMPARISON OF SELECTED DEMOGRAPHIC VARIABLES BETWEEN KSG GROUP AND THE FIVE PARTICPANTS REPORTED WITH CHROMOSOMAL ABERRATIONS (5CA)

	FN	TN	PGM	TM	SK	KSG
Chromosomal aberration	46,XY,22pss	46,XY,inv(9)	46,XY,inv(9)	46,XY/47,XXY/ 47,XX+acentric fragment	46,XY,1qh+	
Age illness onset	19	15	29	19	21	23.04
No of hospitalisations	3	Numerous	5	1	0	2.88
No of episodes	4	Numerous	5	3	1	3.17
Family history	No	No	No	Yes	Yes	36%
Stressor or precipitating event	No	No	No	No	Yes	24%

TABLE 24: COMPARISON OF SELECTED CINICAL VARIABLES BETWEEN KSG GROUP AND THE 5CA GROUP

	FN	TN	PGM	TM	SK	KSG
Chromosomal aberration	46,XY,22pss	46,XY,inv(9)	46,XY,inv(9)	46,XY/47,XXY/ 47,XX+acentric fragment	46,XY,1qh+	
Auditory hallucinations	Yes	Yes	Yes	Yes	Yes	98%
Visual hallucinations	No	Yes	Yes	No	No	62%
Olfactory hallucinations	No	No	No	No	No	18%
Tactile hallucinations	Yes	No	No	No	No	22%
Persecutory delusions	Yes	Yes	Yes	Yes	Yes	88%
Sin/Guilt delusions	Yes	No	No	No	No	14%
Control delusions	No	No	Yes	No	No	26%
Thought broadcasting	No	No	No	No	No	26%
Nihilistic delusions	No	No	Yes	No	No	8%
Mind reading	No	No	No	No	No	30%
Thought insertion	No	No	No	No	No	18%
Erotomanic delusions	No	No	No	No	No	4%
Jealousy delusions	Yes	No	No	Yes	No	10%
Religious delusions	No	No	Yes	Yes	Yes	42%
Grandiose delusions	No	No	Yes	No	Yes	42%
Somatic delusions	No	Yes	No	No	No	30%
Thought withdrawal	No	No	Yes	No	No	14%
Delusions of reference	Yes	Yes	Yes	Yes	No	56%
Bizarre delusions	No	No	No	Yes	Yes	10%
Physical/Verbal aggression	Yes	Yes	Yes	Yes	Yes	80%
Sexual disinhibition	No	No	Yes	No	No	10%
Catatonic symptoms	No	No	No	No	No	8%

TABLE 25: COMPARISON OF SELECTED CO-MORBIDITY VARIABLES BETWEEN KSG GROUP AND THE 5CA GROUP

	FN	TN	PGM	TM	SK	KSG
Chromosomal aberration	46,XY,22pss	46,XY,inv(9)	46,XY,inv(9)	46,XY/47,XXY/ 47,XX+acentric fragment	46,XY,1qh+	
Medical illness	Yes	No	No	No	Yes	40%
Alcohol abuse	No	No	Yes	No	No	26%
Alcohol dependence	No	No	No	No	No	2%
Regular tobacco	Yes	Yes	Yes	Yes	Yes	73%
Cannabis abuse	No	Yes	Yes	Yes	Yes	44%
Cannabis dependence	No	No	No	Yes	No	8%
Co-morbid mood/anxiety disorder	No	No	Yes	No	Yes	26%
Any suicide attempts	No	No	No	No	No	12%

However, when the morphological measurement variables were compared between groups, a significant difference was observed for the measurement between the glabella and the subnasale. (p=0.036) Comparison only done for male participants as all of the participants with chromosomal aberrations were male. See table 26.

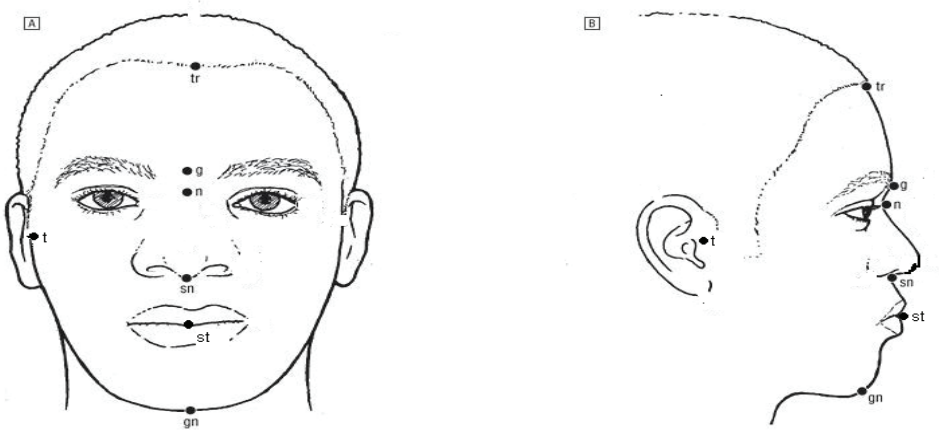


TABLE 26: COMPARISON BETWEEN HEAD AND FACIAL MEASUREMENTS TAKEN ON MALES OF KSG GROUP AND THE 5CA GROUP (REPORTED IN MILLIMETRE)

MEASUREMENT	OTHER MALES KSG GROUP (n=37)	CHROMOSOMAL ABERRATIONS (n=5)	p-VALUE
Head circumference	581.05	576.00	0.670
Trichion to Glabella	56.86	56.62	0.962
Glabella to Nasion	21.01	23.06	0.684
Glabella to Subnasale	57.13	61.76	**0.036***
Glabella to Stomion	83.77	87.54	0.171
Glabella to Gnathion	129.19	126.62	0.602
Tragion to Trichion	139.62	136.60	0.263
Tragion to Subnasale	132.48	135.62	0.452
Tragion to Gnathion	146.38	145.30	0.719

* significance at $p<0.05$

9. SUMMARY

In our study, FISH analysis of 110 participants yielded no chromosome 22 microdeletions. Of the fifty participants who were karyotyped, chromosomal aberrations were identified in five (10%). These were: two cases of inversion of chromosome 9 [46,XY,inv(9)]; and one each of double satellites on chromosome 22 [46,XY,22pss]; a variant of the heterochromatic portion of chromosome 1 [46, XY,1qh+] and a low-grade mosaic [46,XY / 47,XXY / 47,XX, +acentric fragment].

No significant differences could be demonstrated for any of the demographic, clinical or morphological measurement variables for the karyotyping subgroup (n=50) in comparison to the total study group (n=112). Furthermore, no significant differences could be demonstrated for any of the demographic or clinical variables for the karyotyping subgroup in comparison to the five participants reported with chromosomal aberrations. However, one morphological measurement variable (glabella to subnasale) showed a significant difference (p=0.036) between these two groups.

CHAPTER 9

DISCUSSION

CONTENTS

1. INTRODUCTION

More than one hundred years ago, Emil Kraepelin, who first described dementia praecox (the disease concept ultimately known as schizophrenia), and thereafter Eugene Bleuler, who introduced the term schizophrenia, both already agreed that the entity they were dealing with was a familial illness. However, as families not only share genes but often also common environments, it was only since the early seventies that conclusive data from adoption, twin and family studies started showing a consistent pattern of results favouring an important genetic contribution in the development of schizophrenia.

Unfortunately, in spite of compelling evidence in support of a significant role for genetic factors in the development of the disorder, to date, in spite of intensive investigation, the nature of the genetic transmission remains unknown. A simple single-gene explanation is regarded as highly implausible and there is no doubt that the demonstration of modes of transmission by a few genes of moderate effect (oligogenic) or many genes of small effect (polygenic) will be much more difficult. Nonetheless, the molecular basis of other common complex disorders (such as non-insulin dependent diabetes) have been demonstrated by using molecular genetic approaches and this remains one of the most promising techniques in the investigation of the biological basis of schizophrenia.

Current literature shows that by far the majority of molecular genetic work done in schizophrenia has involved Caucasian populations. Taking into account the evidence suggesting ethno-specific loci as well as apparent ethno-specific pharmacological responses to atypical antipsychotic treatment in African-American and African samples it seems clear that indigenous African populations also need to be investigated.[1-4] The Xhosa people are a linguistically distinct population grouping that diverged within the last 2 000 years. Therefore taking into account the seemingly uniform core symptom profile reported in both Caucasian and African groups (including the Xhosas), as well as the marked paucity of clinical and susceptibility data for Xhosa-speaking schizophrenia patients, this group would seem to present an invaluable opportunity for molecular genetic research.[4-10]

2. CHROMOSOMAL ABERRATIONS

Chromosomal abnormalities in those with mental illness are a valuable resource in as much as they can help us redefine phenotypes, identify candidate genes and refine areas of linkage.[11] Literature reports on chromosomal analysis in schizophrenia started consistently appearing in the early 1960s. Initially these were mostly case reports or cytogenetic studies focussing primarily on the sex chromosomes, paying relatively little attention to potential autosomal abnormalities.[12-15]

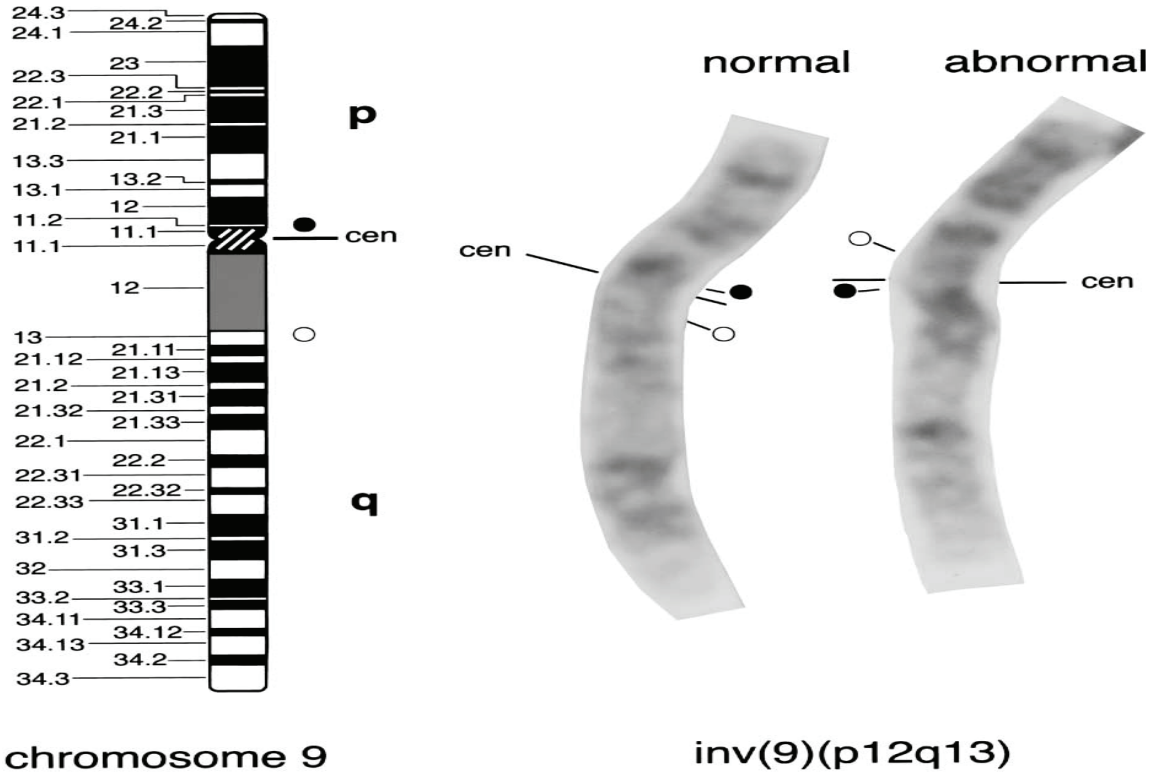
The first large study reporting on full chromosomal analyses was that of Judd and Brandkamp published in 1967.[16] Their study group consisted of a group of 40 schizophrenia patients and they could only demonstrate an aberration (sex chromosome mosaic) in one patient. They concluded that cytogenetic methods available at that point in time were not sufficient to allow for the detection of possible genetic factors in the aetiology of schizophrenia.

Fortunately, since then techniques have improved dramatically and more recent studies in Caucasian and Asian populations have revealed significant percentages of schizophrenia subjects with chromosomal abnormalities. These have ranged between 2% (2/100) and 32% (43/134) with others in between – for example 6.2% (9/134) and 13% (21/161).[17-20] In our study, of the fifty participants who were karyotyped, chromosomal aberrations were identified in five (10%). These were: two cases of inversion of chromosome 9 [46,XY,inv(9)]; and one each of double satellites on chromosome 22 [46,XY,22pss]; a variant of the heterochromatic portion of chromosome 1 [46, XY,1qh+] and a possible low-grade mosaic [46,XY / 47,XXY / 47,XX, + acentric fragment].

2.1 CHROMOSOME 9

Chromosome 9 displays the highest degree of structural variability of all human chromosomes.[21] In fact, the most common inversion seen in human chromosomes is the pericentric inversion of chromosome 9 [inv(9)] and as such it

is the most common reciprocal translocation found in humans.[22] Please see diagram for an ideogram of normal chromosome 9 and high-resolution CTG-banded photographs of a normal chromosome 9 and an inverted chromosome 9.



Inv(9) is transmitted to offspring in a dominant fashion and the prevalence varies with ethnicity. Hsu et al. examined the frequency in four major ethnic groups reporting an incidence of 0.73% (17/2334) in Caucasians, 2.42% (42/1737) in Hispanics, 0.26% (1/384) in Asians and 3.57% (64/1795) in African-Americans.[23] Another study, by Serra et al., examining 7613 newborns estimated the prevalence of inv(9) to be 0.85% in European newborns.[24] Later studies showing incidences of 1.2% (16/1350) in Taiwanese foetuses and 1.65% (25/1513) in

Japanese newborns, indicated that the incidence in Asian populations could probably be estimated at approximately 1.5%.[25;26]

In addition to inv(9) occurring commonly, no specific phenotype has been demonstrated to be associated with this particular chromosomal rearrangement. This has lead to inv(9) generally being regarded to be a normal variant rather than an abnormal karyotype and, in fact, in our sample it was reported as such by Unistel.[27] However, Kiss and Osztovics found this aberration to be present in 1.9% of a group of dysmorphic children (younger than one year) and postulated that it was in fact not a normal variant, but rather that it might play an important role in the aetiology of unspecified dysmorphic syndromes.[28]

There is also some evidence linking inv(9) to subfertility and recurrent abortions and a number of cases have been documented where patients with inv(9) exhibited psychiatric symptoms.[29;30] These include Kumar et al. reporting on a patient with a personality disorder (diagnosed with a hysterical, immature and psychopathic personality) and Miyaoka et al. reporting on a case of a patient diagnosed with psychosis not otherwise specified.[31;32] Furthermore, an unusually increased prevalence (9.7%) of inv(9) in a sample of male patients with “paranoid psychosis” was already reported more than 20 years ago.[33]

Our study shows an incidence of 4% (2/50) for inv(9). This is a similar percentage to that reported in a number of other schizophrenia studies such as

Nanko et al. 3.4% (4/116); Kunugi et al. 4.5% (6/134) and Demirhan et al. 5.2% (7/134).[17;18;34] In contrast, both Gorwood et al. (0/25) and DeLisi et al. (0/46) reported no incidences of inv(9) in their samples.[35;36] Interestingly, an international two-stage genome scan for linkage with schizophrenia performed by Moises et al. obtained a positive result (p=0.009) for a marker (D9S175) on the pericentric region of chromosome 9.[37] Also, Levinson et al. conducted a genome scan and found a suggested linkage (p<0.05) between schizophrenia and a marker (D9S257) located 20cM from the centromere of chromosome 9.[38]

Therefore, though current evidence seems to indicate that inv(9) is unlikely to have a major effect on the development of schizophrenia, it may be an risk-increasing factor. Inv(9) has been characterised using fluorescence in situ hybridisation (FISH) analyses, indicating that there are several different forms of inv(9) which have differential breakpoints.[39] Localisation of these breakpoints on chromosome 9 may ultimately lead to the cloning of schizophrenia-susceptibility genes.

2.2 X CHROMOSOME ANEUPLOIDES

The occurrence of sex chromosome aneuploides are well documented and a great variety of mosaics have been described in humans.[40] Incidences at birth of the various sex chromosome abnormalities have been reported at between 0.1

and 1.3%. Well-known examples of these include both Klinefelter and Turner syndromes.

Several lines of evidence seem to suggest that the sex chromosomes have a role in the expression of schizophrenia with an apparent excess of sex chromosome aneuploidies (XXY, XXX, and possibly XYY) having been reported in schizophrenia populations.[41] This hypothesis seems to find further support in the gender differences in response to treatment, age at onset of illness and prognosis reported in schizophrenia.[42] Also, some data seem to support an increased concordance for sex in sibling pairs with schizophrenia although results reported so far have been inconclusive.[43]

It has long been observed that the risk for schizophrenia does not follow the simple dominant or recessive form of Mendelian inheritance.[44] As such, risk among relatives of probands is shown to be greatly reduced in second- and third-degree relatives in comparison to siblings. Furthermore, in contrast to the accepted Mendelian pattern of transmission, there is an unequal distribution of risk between categories of first degree relatives (e.g. greater risk to children than siblings) and the persistence of a high prevalence in the face of a substantial fecundity disadvantage.[45] Thus, though schizophrenia has been considered a "complex genetic disorder" with heterogeneity and environmental or epigenetic factors contributing to its origin, no robust genetic locus or clear environmental factor has yet been identified.

After taking all the evidence into account, a pseudoautosomal location at the telomeric extremity of the short arms of the X and Y chromosomes was first proposed for a schizophrenia susceptibility locus by Crow in 1988.[46] However, consensus was soon reached that linkage within the short arm pseudoautosomal region could be excluded.[41] On the other hand, a number of regions of homology between the X an Y chromosomes outside the pseudoautosomal regions have been described and these were next considered as a possible susceptibility locus.[47] A number of studies followed but even at best the evidence remains weak.[48]

Our study showed one X-chromosome low-grade mosaic [46,XY/47,XXY/ 47,XX,+acentric fragment] translating into a 2% prevalence. Other schizophrenia samples have shown percentages of 1.7% (2/113), 2% (5/250) and 8.6% (14/161).[18;20;41] Numbers drop when persons with mosaics of the sex chromosomes are excluded; these authors argue that this is appropriate as we are dealing with a very heterogeneous group with varying degrees of mosaicism and therefore the abnormal cell line could be present to varying degrees in different tissues, including the brain. Unfortunately, large-scale studies have failed to show any evidence of consistent linkage between sex chromosome aneuploidies or particular regions on the X chromosome and risk for schizophrenia.[49;50]

However, there is also the possibility of finding a susceptibility gene that is more strongly linked to an intermediate phenotype predisposing to schizophrenia susceptibility. With regard to the X chromosome, one such phenotype is reduced cerebral asymmetry, for which a multipoint QTL analysis for degree of handedness produced a LOD of approximately 3.0 within the Xq21.3 region.[51] Also, evidence from studies of the parental origin of Turner syndrome (XO), shows that a gene on the X chromosome relates to social ability and is subject to differential imprinting.[52] Such a gene, accounting for the cerebral anomalies present in the sex chromosome aneuploidies, could also relate to psychosis. Thus, while no variant in DNA sequence on the X chromosome is currently linked to schizophrenia, the possibility cannot be excluded that an important mechanism on the X chromosome related to its susceptibility could still be found.

2.3 OTHER AUTOSOMAL ABNORMALITIES

As stated in a previous chapter, in spite of numerous reports of possible associations for chromosomal aberrations with schizophrenia, to date, bar the exception of two, none can be regarded to have provided convincing evidence for the location of a susceptibility gene.[53] The two being (a) the chromosome 22q11 microdeletions and (b) the balanced chromosomal translocation (1;11)(q42;q14.3) disrupting two genes on chromosome 1 (Disruption in

schizophrenia 1 (*DISC 1*) and 2 (*DISC2*)). Please review the chapters on aberrations and chromosome 22 for a summary of evidence currently available.

2.3.1 CHROMOSOME 22

In our sample karyotyping revealed one chromosome 22 complement with double satellites [46,XY,22pss] that was reported as a normal variant. FISH analysis revealed no chromosome 22q11 deletions for any of the participants in our study. At first glance, this seems to stand in contrast to previous findings suggesting 22q11 deletion syndrome to be the highest known genetic risk factor for schizophrenia. However, available data lends support to a notion that differing prevalence rates are seen in differing population groups. In strong contrast to reported Caucasian sample prevalence rates of ±2%, Arinami et al. could only demonstrate a 0.3% prevalence in their large Japanese case sample (300 unrelated patients and controls each).[19;54;55] Furthermore, in the only other sample to date reporting on a black African population, Riley et al. failed to find evidence to support the linkage of chromosome 22 markers to schizophrenia in their sample (22 families (Xhosa, Zulu, Sotho and Tswana)).[4] This data were further supported by the findings of Takahashi et al. who demonstrated significant linkage for chromosome 22 markers with schizophrenia in their family-based association study for both European-American (34 pedigrees) and Chinese (52 pedigrees) samples but not for an African-American sample (29 pedigrees).[56]

Interestingly, one of the reported Caucasian samples consists of an Afrikaner (population group that cohabitates with the Xhosa population in South Africa) schizophrenia group. This sample showed a prevalence of 2.4% (2/85) for chromosome 22q11 deletions.[55] The same research group has reported on de novo copy number mutations (CN) in an Afrikaner cohort consisting of 152 sporadic schizophrenia or schizo-affective disorder patients and 159 controls.[57] Their results showed that confirmed de novo (CN) mutations could significantly be associated with schizophrenia (p=0.00078) and were collectively ≈8 times more frequent in sporadic (but not familial) cases with schizophrenia than in unaffected controls (15/152 vs 2/159). Of particular note was that 3 of the 15 were de novo 22q11.2 microdeletions (1.8% rate) with sizes consistent to the two predominant sizes (3 Mb or 1.5 Mb) reported in the literature. They concluded that these results once again confirmed the importance of microdeletions in the 22q11.2 locus as the only known recurrent CN mutation associated with schizophrenia.[19]

Furthermore, the prevalence of obsessive-compulsive symptomatology (OCD) in an Afrikaner schizophrenia sample (which included the above 85) was reported to be 13.25% (53/400), which also seems distinctly different to that of the 1.7% OCD prevalence seen in our study sample.[58] Other independent samples of Xhosa schizophrenia subjects also reported very low prevalences of OCD (0.5%; 3/509) and hoarding behaviour (3.9%; 4/102) respectively.[59;60] This is of special

interest as OCD has been associated with 22q11-13 deletions.[61] Taken together, our findings therefore support available data that seem to suggest that current evidence does not support the linkage of markers on chromosome 22 to susceptibility to schizophrenia in black African populations.

2.3.2 CHROMOSOME 1

A recent large meta-analysis of schizophrenia linkage by Lewis et al. showed support for chromosome 1q as an area of interest.[62] By far the most attention has focused on the balanced translocation (1;11)(q42.1;q14.3) first identified in a large Scottish family and referred to as "Disruption in Schizophrenia 1" (*DISC1*).[63] In fact, it generated enough interest to be included as part of Science magazine's nominations for the "Scientific Breakthroughs of the Year, 2005".[64] To date, it has been implicated through genetic analysis in not only schizophrenia but also schizo-affective and bipolar disorders as well as major depression.[65] Furthermore, causal relationships between *DISC1* and measurable trait variables as well as *DISC1*-binding with a number of proteins known to be involved in essential processes of neuronal function have been demonstrated.[65;66]

One participant in our study was reported to demonstrate a normal variant of the heterochromatic portion of chromosome 1 [46, XY,1qh+]. Since this variant was only identified in one patient and literature does not show a previously reported

association, it would be difficult to establish current relevance for it in schizophrenia.

2.4 SIGNIFICANCE OF FINDINGS

The chromosomal aberrations detected in our sample were all reported as normal variants by UNISTEL. Normal variants are wide-spread in human populations and have no apparent effect on phenotype. These variants are mostly found in some specific chromosomal regions including the highly variable regions on chromosomes 1, 9 and 16, the distal two thirds of the long arm of the Y chromosome and the short arms and satellites of the acrocentric chromosomes.[23]

One could therefore ask what the relevance of our findings are? Firstly, it would be important to reiterate that although the findings are reported as normal variants, they may in fact not be normal, but rather have, as yet undetected, significance. In order to truly exclude a possible link to psychiatric illness, groups of individuals with such variants need to undergo proper psychiatric evaluation in a structured manner.

There can be no doubt that the search for schizophrenia susceptibility genes has been, and remains, a difficult task. It has been less than a decade since linkage and association studies have started to produce replicated data for linkage

regions and genes. Detection techniques are rapidly becoming more sophisticated and, very importantly, more non-Caucasian samples are being recruited. As such, the fact that a particular chromosomal aberration has not yet been linked to schizophrenia cannot be interpreted to conclusively mean that the aberration is a normal variant with no likelihood for association.

Therefore, although chromosomal aberrations in schizophrenia may be due to chance, the alternatives have to be considered. Aberrations may be involved with causing schizophrenia by disrupting an important locus, they may represent linkage to a susceptibility locus or they may provide a permissive genetic environment for mutations elsewhere in the genome to become expressed as psychotic illness.[67] In a genetically complex illness such as schizophrenia, it is much more difficult to either confirm or disprove these possibilities, than in a single gene disorder (e.g. Huntington's Chorea).

Furthermore, even if none of our detected aberrations can be shown to individually have a major effect on the development of schizophrenia, they may still be risk-increasing factors. An example of a mechanism that could encompass several chromosomal associations, is the disruption of transcription factors that interact with genes important in brain development. Retinoic acid signaling could be involved in such a process, because it seems to play an important role in forebrain, limb, face development as well as the development and functioning of the dopamine system.[68;69]

Previous samples of chromosomal aberrations in schizophrenia populations reported on in the literature have included all aberrations found in their results.[17;18;20] To our mind, it would therefore be premature to dismiss any of our findings as non-significant and therefore we have included all five of the patients in our karyotyping subgroup.

3. MORPHOLOGY

3.1 COMPARITIVE DISCUSSION

Our dataset reporting on selected craniofacial measurements represents the first in an African schizophrenia population. As described in the methods section, nine sets of measurements (from those suggested by Lane et al. and previously chosen in schizophrenia subjects) were used: (1) Head circumference (2) Trichion (tr) to Glabella (g); (3) g to Nasion (n); (4) g to Subnasale (sn); (5) g to Stomion (st); (6) g to gnathion (gn); (7) Tragion (t) to tr; (8) t to sn; and (9) t to gn.[70;71] Please see diagram for location of the landmarks used.

The anthropometric study of facial morphology in groups differing in ethnicity and race has revealed that significant inter-group differences exist.[72] Most of the normative data of facial measures currently available, comprise of studies done on Caucasian, and more specifically North American, groups. However, some African-American data on normal controls are available.[73-75] Please see table 1

for data comparison between some of the larger studies and our dataset. As there was no significant difference between the karyotyping subgroup (KSG) and the whole group, we will use the KSG dataset for comparison purposes.

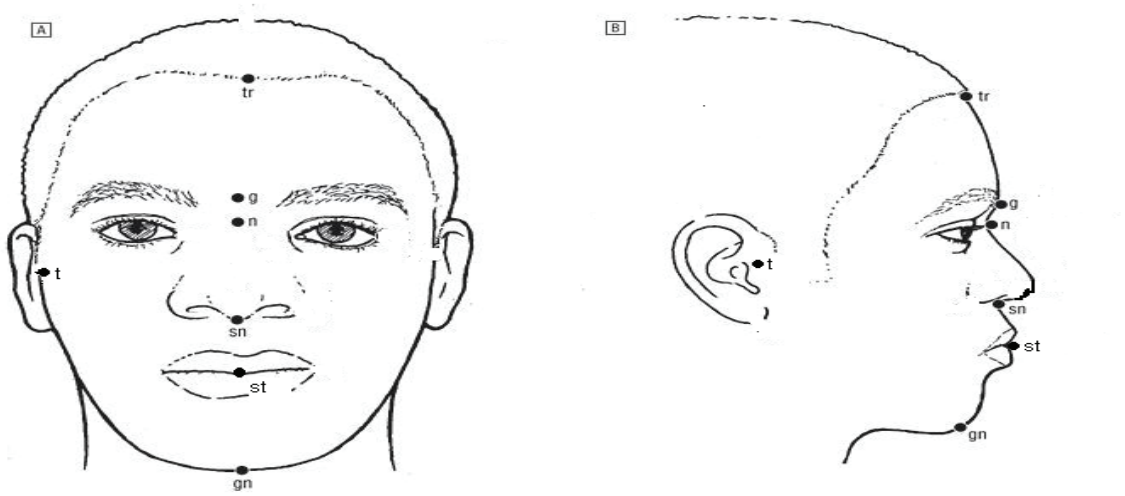


TABLE 1: COMPARITIVE MEASUREMENTS BETWEEN OUR SAMPLE AND THOSE REPORTED FOR THREE AFRICAN-AMERICAN CONTROL SAMPLES (REPORTED IN MILLIMETRE(mm))

	OWN DATASET		FARKAS[73]		PORTER[75]	PORTER[74]
	MALE n = 42	FEMALE n = 8	MALE n = 50	FEMALE n = 50	MALE n = 109	FEMALE n = 108
HEAD CIRC	580.45	578.88	573.6	547.0	--	--
G – TR	56.84	57.01	61.8	55.7	60.2	55.7
G – N	21.25	27.31	11.8	11.4	11.1	13.2
G – SN	57.68	54.51	68.6	64.6	62.4	62.0
G – ST	84.22	75.83	89.8.	84.1	--	--
G – GN	128.88	115.96	137.7	127.9	136.5	129
T – SN	132.85	126.63	132.6	125.4	--	--
T – GN	146.25	137.89	149.5	138.1	--	--

From the comparison it is clear that although both male and female participants have similar face width (tragion to subnasale; tragion to gnathion) as reported in the African-American datasets they clearly have decreased anterior facial height (AFH) measurements, i.e. glabella to gnathion. Although females comparatively show a slightly increased glabella to trichion measurement (upper portion of face) their overall AFH is still decreased and for males all three parts of the face show decreased measurements. Comparatively, both males and females demonstrate an increased measurement from the glabella to the nasion but the overall distance between glabella and subnasale (middle portion of the face) is still significantly shorter.

At first glance, the glabella to nasion measurement could give cause for concern as to a possible technique error, as our value is nearly double that reported in the comparative datasets. However, another African-American dataset reports measurements closer to our own for both males (15.7) (n=33) and females (19.2) (n=32) and as in our dataset the female measurements are, in fact, the longer of the two.[76] (Please see table 2.)

As before, our sample still shows decreased AFH whilst the width measurement is similar. Such inter-ethnic differences in craniofacial measurement between African-American samples have previously been reported, with the various racial admixtures given as a possible explanation.[77] These differences have also been demonstrated in Caucasian samples comprised of differing European ethnicity.[72]

TABLE 2: COMPARITIVE MEASUREMENTS (mm) BETWEEN OUR SAMPLE AND AN AFRICAN-AMERICAN (A-A) CONTROL SAMPLE[76]

	G – TR	G – N	G – SN	G – GN	T – SU
XH MALE	56.84	21.25	57.68	128.88	132.85
A-A MALE	64.6	15.7	73.6	137	133.6
XH FEMALE	57.01	27.31	54.51	115.96	126.63
A-A FEMALE	59.5	19.2	63.3	127.2	123.3

It would be interesting to speculate why there is such a clear difference between our sample and the known African-American samples. One possibility that must be considered is technique error. It has been suggested that inexperienced anthropometrists often prefer to use rulers rather than calipers.[78] However, rulers often cannot be placed directly on the facial landmarks, whereas callipers can. Caliper-derived data is believed to be more accurate and allows easier comparison to the existing sets of normative data.[78] Our sets of measurements were specifically chosen for ease of landmark identification and ultimately the data obtained follows the same general pattern as that reported in previous samples. Therefore, whilst technique error as a possibility cannot be completely excluded, we probably need to look elsewhere for an answer.

Another possibility to consider is that African-American population groupings in fact represent an admixture of Caucasian, Native American and African groups.[79;80] Their craniofacial morphology may therefore not resemble that of

native African people (e.g. the Xhosa) who have remained fairly unmixed and might exhibit unique features.

Although no studies are available for normal controls in the Xhosa population reporting on facial measurements using the same methods we employed, there are some data in African populations that was acquired using cephalometric techniques. Specifically, a recent study evaluated cephalograms of 101 adult ethnic Shona subjects and compared their findings with existing norms in African-American and North American Caucasian populations.[81] The Shona are the native people of Zimbabwe and as a group contributed, via migration, to the ultimate formation of some of the latterday South African ethnic groupings, including the Xhosa. Both the Shona and the Xhosa are descended from the Eastern Bantu language migration grouping.[82] Using markers that can be roughly correlated to the nasion, gnathion and subnasale, Dandajena et al. demonstrated that both total AFH and lower AFH were significantly shorter for Shona males and females in comparison to African-American norms. Thus, their results supported the trend exhibited in our own. [81]

It would also be important to remember that the comparative samples reported on here are those of normal controls. Numerous studies have shown differences do exist between schizophrenia and control samples with regard to the presence of minor physical anomalies.[83] Although fewer studies are available that report specifically on quantitative facial measurements in schizophrenia subjects, some

have been conducted, with majority of the available data being for Caucasian patients. Importantly, existing data has specifically reported decreased glabella to subnasale measurement in schizophrenia subjects in comparison to normal controls.[71] The general premise of shorter lower facial heights was also supported by a small sample (n=20) consisting of Mestizo (admixture of Spanish, Indian and Black genetic heritage) males.[84]

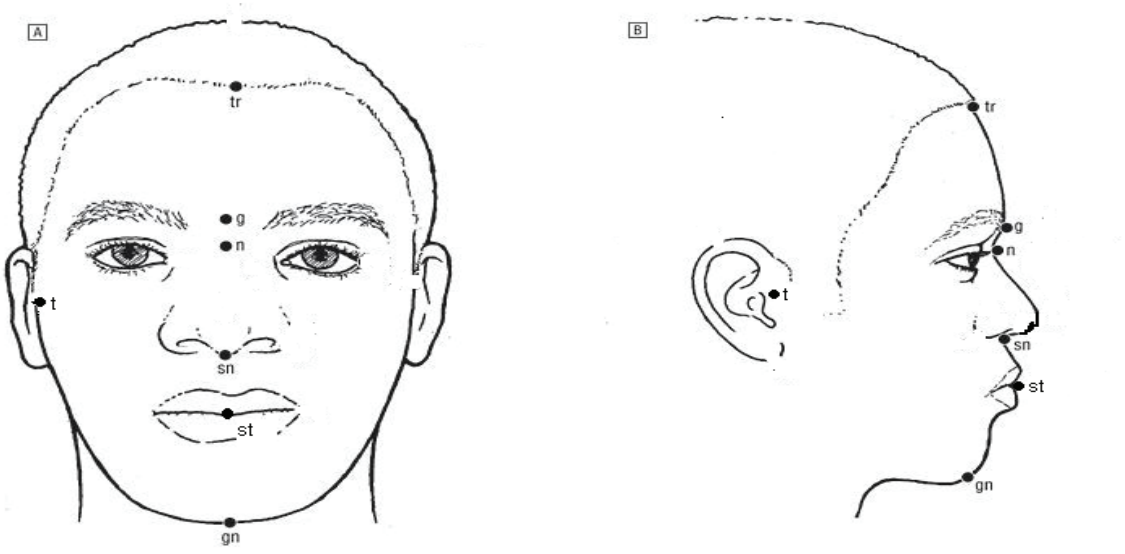
Furthermore, in the only predominantly African-American schizophrenia sample reported on to date (using similar facial measurements and differentiating between genders), although the difference is much less pronounced, the same trend for a comparatively decreased AFH is demonstrated.[76] Also, gender-specific significant differences could be demonstrated in the Compton et al. sample between schizophrenia subjects and controls.[76] (See table 3)

TABLE 3: COMPARITIVE MEASUREMENTS (mm) BETWEEN OUR SAMPLE AND AN AFRICAN-AMERICAN (A-A) SCHIZOPHRENIA SAMPLE[76]

	G – TR	G – N	G – SN	G – GN	T – SU
XH MALE	56.84	21.25	57.68	128.88	132.85
A-A MALE	61.6	18.6	71.2	136.1	134.5
XH FEMALE	57.01	27.31	54.51	115.96	126.63
A-A FEMALE	59.0	20.0	68.4	128.2	127.5

As discussed in previous chapters, the neurodevelopmental model of schizophrenia is increasingly being supported by new evidence. Amongst other data we find support for this model, which proposes that early brain insults eventually cause dysfunction and predisposition to schizophrenia, in the fact that some patients with schizophrenia exhibit subtle developmental abnormalities that presumably occur during embryogenesis.[85] These gross somatic abnormalities (including minor physical anomalies such as facial dysmorphology) that tend to accompany the disease, have been proposed as one possible marker that could be used as an endophenotype in our search for schizophrenia susceptibility genes.

Interestingly, when comparing the facial measurements of the five patients with documented chromosomal aberrations with the other males (n=37) who were karyotyped, a statistically significant difference could be observed for one measurement. Please see table 4 for comparison of all measurements.



The significant difference was observed for the measurement between the glabella and the subnasale. However, there was no significant difference for the measurement between the glabella and the nasion. Therefore it can be deduced that the significant difference actually occurs in the measurement stretching between the nasion and the subnasale, and that the five participants with chromosomal abnormalities had significantly longer noses in comparison to the rest of the group.

TABLE 4: COMPARITIVE MEASUREMENTS (mm) BETWEEN ALL MALES OF KSG GROUP AND THOSE REPORTED WITH A CHROMOSOMAL ABERRATION

MEASUREMENT	OTHER MALES KSG GROUP (n=37)	CHROMOSOMAL ABERRATIONS (n=5)	p-VALUE
Head circumference	581.05	576.00	0.670
Trichion to Glabella	56.86	56.62	0.962
Glabella to Nasion	21.01	23.06	0.684
Glabella to Subnasale	57.13	61.76	**0.036***
Glabella to Stomion	83.77	87.54	0.171
Glabella to Gnathion	129.19	126.62	0.602
Tragion to Trichion	139.62	136.60	0.263
Tragion to Subnasale	132.48	135.62	0.452
Tragion to Gnathion	146.38	145.30	0.719

* significance at $p<0.05$

3.2 SIGNIFICANCE OF FINDINGS

It has previously been suggested that if minor physical anomalies were to be used to search for an endophenotype, facial measurements may be of particular interest given that the face and some of the regions of the brain develop in concert from the same ectodermal tissue.[70;86;87] In fact, craniofacial morphogenesis proceeds intimately with cerebral morphogenesis, in accordance with a unitary embryological origin. This is clearly illustrated in classical neurodevelopmental disorders (e.g. Down Syndrome, foetal alcohol syndrome, velocardiofacial syndrome) which are characterised by dysmorphic features involving different regions of the body but the craniofacies in particular. Furthermore, individuals with facial dysmorphogenesis such as cleft lip/palate have been reported to show abnormalities of brain structure in association with cognitive deficits.[88;89]

There is a clear timeline to embryological events with the diverse but interdependent sequelae of craniofacial and cerebral morphogenesis evolving and attaining their basic structures at particular gestational intervals.[90] In accordance with this, craniofacial dysmorphology has been shown to be associated in general terms with abnormalities found to evidence brain pathology in schizophrenia.[91] For example, palatal morphology originates at 6-9 weeks and achieves its essential postnatal form by 16-17 weeks of gestation.[92-94] The hippocampal formation is one of the first cortical areas to differentiate at

approximately 9-10 weeks, undergoing further tremendous differentiation, particularly up to weeks 15-19 of gestation, illustrating some temporal contiguity between the developmental trajectories of the aforementioned.[95;96] Abnormalities of hippocampal structure have been demonstrated in schizophrenia whilst palatal anomalies are one of the most consistent minor physical anomalies reported in schizophrenia populations.[87;97-100]

Therefore, when one tries to make sense of our finding of a significantly increased nasion to subnasale measurement it would be important to first have an understanding of foetal embryology. Development of the face and some facial proportioning occurs mainly between the sixth and the twelfth weeks of gestation with the frontonasal prominence forming the apex of the nose and the sides (alae) derived from the lateral nasal prominences.[90] Morphogenesis of the frontonasal prominence, forebrain, and anterior midline cerebral regions are known to be intimately regulated via epithelial–mesenchymal signaling interactions.[94;101-103]

Anterior facial dysmorphogenesis could thus be linked to several aspects of structural brain pathology as reported in adult schizophrenia, such as abnormalities of temporal lobe structures that over early foetal life emerge in the forebrain before rotating to their final location, together with thalamic and other anterior midline structures.[94;95;104] Dysmorphogenesis along the anterior midline would be expected to disrupt neuroectodermal patterning and the critical

repulsive and attractive guidance cues which regulate neuronal connectivity.[105] This would be in line with models of neuronal network disconnectivity described in schizophrenia, particularly in a fronto–striato–pallido–thalamo–cortical-temporal network.[106;107]

One could argue that lengthening of the nose possibly represents a disruption in the development of the frontonasal prominence, a structure which enjoys the most intimate embryologic relationship with the development of the anterior brain.[103;108] Importantly, the frontonasal prominence also contributes to development of the forehead, philtrum of upper lip, and primary palate, keeping in mind that the palate is a midline structure noted consistently to be dysmorphic in schizophrenia, as previously mentioned.[87;98-100;103;108] Morphogenesis in other domains of facial development (such as the lateral nasal prominences forming the sides of the nose and the maxillary prominences contributing to the sides of the face and lips) also appear to be at least in part orchestrated by the frontonasal prominence.[103;108] This suggests that understanding the genetic and epigenetic regulation of midline morphogenesis involving the frontonasal prominence may inform importantly on early developmental perturbation in schizophrenia.

4. CLINICAL

4.1 GROUP DEMOGRAPHICS

The total study group consisted of 112 participants, FISH analysis for chromosome 22 was conducted on samples from 110 participants and samples of 50 were karyotyped (KSG). The mean age at interview for the two groups (total 112 always reported first) was 36.2 and 35.82 years respectively and the majority of participants in each group were male (81.25%; 84%). Means for age of onset of illness (23.38; 23.04), number of hospitalisations (2.77; 2.65) and number of episodes (3; 2.86) were also calculated. Slightly more than a third of the sample reported a family history of psychiatric illness (40.2%; 36%). This would be in keeping with the fact that although schizophrenia has one of the highest heritabilities it does not equal 100% and in fact it is estimated that 85% of sufferers do not have a first-degree relative with schizophrenia.[109]

At the time of the interview only one participant was employed. This stands in stark contrast to the fact that more than a third of the sample (35.7%; 36%) reported having previously been gainfully employed. This downward shift in occupational status has previously been reported in the Xhosa schizophrenia population and our findings, once again, highlight the need for proactive engagement in work rehabilitation as part of the management of this illness.[110]

With no significant differences between the demographics of the two groups, the data supports the supposition that the KSG can be regarded as representative of the total sample. Furthermore, the composition of the group as a whole is similar to that of the largest Xhosa schizophrenia sample previously reported on clinically.[111]

4.2 PRODROMAL PERIOD AND STRESSORS PRIOR TO ILLNESS ONSET

For the majority of the sample information regarding the prodromal period was not available. Difficulties in retrospectively assessing the prodromal period have been highlighted by a number of authors.[112;113] However, available research on the prodrome has shown some support that the treatment of such could delay onset of psychosis and that prodrome could possibly contribute to elucidating the pathophysiology of psychosis.[114;115] Therefore, important consideration should be given to designing studies in the South African population that pay particular attention to overcoming barriers in illness mapping, such as selecting optimal sampling strategies and/or integrating procedures and data across prodromal and first episode research projects.[112]

Approximately a quarter of participants related one or more stressors as possible precipitating events prior to onset of illness. Much literature has been devoted to the hypothesis that psychosis develops as a result of the interaction between genetic vulnerability and environmental stressors.[116] Indeed, a previous study

focusing specifically on black South African women showed that stressful life events influenced the course of their mental health.[117]

Of particular interest was the fact that five participants reported an association with their participation in an initiation ceremony. As previously stated, the traditional initiation ceremony (umkhwetha), represents a rite of passage for Xhosa boys entering manhood and has been reported as a stressor related to both initial onset and relapse precipitation in this population.[118-121] Taking into account the cultural significance of this ceremony and the fact that those who do not participate are often regarded as being “less than men”, the importance of understanding and incorporating the cultural background of patients into the broader management of their illness is once again underlined.[122]

4.3 POSITIVE SYMPTOMS

A lifetime history of auditory hallucinations was by far the most common symptom reported. (97.3%; 98%) Perceptual disturbances in all other modalities were also reported although to a much lesser degree; visual (54.5%; 62%), olfactory (33%; 18%), tactile (32.1%; 22%), gustatory (24.1%; 12%). None of the differences between the two groups were significant.

Although the prevalence of the different types of hallucinations tends to vary widely across different studies the rank order of frequency (auditory most

prominent, then visual and others less prevalent) remains the same.[123] Our findings were therefore in keeping with previously reported studies in international, South African and more specifically Xhosa samples.[111;124-127]

Interestingly, all three of the usually less prevalent perceptual disturbances (tactile, olfactory and gustatory) were reported by a fair number of participants. Classically gustatory and olfactory hallucinations tend to be associated with organic illness. However in our sample, there was low rate of co-morbid neurological (and other medical illness) as well as developmental difficulties making any association unlikely. Some literature has also suggested that olfactory or gustatory hallucinations are in fact harbingers of severe delusions.[128] This opens up a myriad of possibilities because, if in fact we are able to link perceptual disturbances to a measurable characteristic this could ultimately lead to an endophenotype that could prove useful in the search for schizophrenia genes.

With regard to lifetime delusions, the persecutory type was by far the most common. (89.3%; 88%) Other types of delusions commonly reported were: reference (57.1%; 56%), grandiose (41.1%; 42%), religious (36.6%; 42%) and mind-reading (42%; 30%). Once again the general pattern was in keeping with that of the broad spectrum of previously reported studies.[111;124-127]

A lifetime history of delusions has been used as a marker in both schizophrenia and bipolar disorder to search to possible genotype-phenotype associations. Working from the basis of the purported relationship between positive symptoms and hippocampal volume and the reported effects of DISC1 genotype on hippocampal volume, DeRosse et al. detected significant associations between a DISC1 haplotype containing Ser704Cys and lifetime severity of delusions in schizophrenia in a Caucasian population.[129] The D-amino acid oxidase activator (DAOA/G30) locus found on chromosome 13q34, has been shown to be associated with both schizophrenia and bipolar affective disorder.[130;131] Schulze et al. demonstrated in a Caucasian population that the association between bipolar affective disorder and DAOA/G30 was only seen when case definition was restricted to cases with persecutory delusions.[132] Their data thus suggest that bipolar disorder with persecutory delusions constitutes a distinct subgroup of bipolar affective disorder that overlaps with schizophrenia.

Behavioural disturbances as a core feature of the acute phase of illness was commonly reported with some form of verbal or physical aggression (75.9%; 80%) by far the most prevalent. Although this may seem disproportionately high, it is important to note that the DIGS questionnaire only records qualitative statements regarding behaviour and therefore any incident (independent of severity) is noted.[133] However, in schizophrenia, behavioural disturbances remain an important area of study as this illness seems to be overrepresented in

involvement with violent incidents during hospitalisation, having been shown to be the diagnosis in 30-45% of all such cases.[134;135]

The contribution of genetic factors to the manifestation of aggressive behaviour associated with schizophrenia has been investigated in several studies, with data suggesting that a moderate to substantial influence can be attributed to genetic factors.[136;137] However, to date the only such study done on a South African schizophrenia population investigated a role for functional variants in the catecholamine-O-methyl transferase gene (*COMT*) and the monoamine oxidase-A gene (*MOA-A*), showing negative results, rather supporting non-genetic markers such as substance abuse and the presence of specific delusions as risk factors for violent behaviour.[126]

The lifetime presence of catatonic symptoms was low (8.1%; 8%), which is in keeping with reports from previous studies.[111;125;126] This area holds particular interest as previous data suggest that association with major disease loci can be demonstrated for periodic catatonia as a subtype of schizophrenia.[138;139]

Lifetime presence of thought disorder was commonly reported (67.9%; 60%). Incoherence was most prevalent (36.6%; 34%) with loose associations least prevalent (9.8%; 12%). Although thought disorder is a common manifestation in schizophrenia, literature suggests that as such it plays no particular role in the

decreased quality of life experienced by schizophrenia patients in comparison to healthy subjects.[140]

4.4 NEGATIVE SYMPTOMS

Negative symptoms were commonly observed with especially affective flattening, anhedonia and avolition/apathy being very prevalent. However, those regarded to be markedly or severely affected never constituted more than 14% of the total sample of participants. The concept of remission in a psychiatric disorder (major depression) was first proposed 15 years ago. When we consider this concept for patients with schizophrenia, especially negative symptoms remain a challenge as we accept that remission needs to be thought of as being distinct from recovery. The proposal of the first set of standardised remission criteria for schizophrenia once again highlighted the importance of achieving resolution in all symptomatic domains of schizophrenia.[141] Unfortunately, as a recent review underlines, in spite of numerous efforts no current treatment exists that effectively and consistently addresses this enduring and debilitating component of the psychopathology of schizophrenia.[142]

4.5 TREATMENT

Information was available for all except three participants, regarding the primary antipsychotic drug they were using at the time of the interview. As the sample consisted of in- and outpatients recruited either from the hospitals or psychiatric

community services, the high compliance rates could be due to selection bias. However, similar findings have been reported in other Xhosa schizophrenia samples.[111;143]

Depot preparations were commonly prescribed, with a large portion of the participants using these (58.9%; 46%). These rates compared favourably to that previously reported in a Xhosa sample.[111] Since the development of depot antipsychotic preparations in the 1960s, their advantages in relapse prevention due to (amongst others) stable plasma levels, a pattern of regular contact with the health care system and guaranteed drug delivery has repeatedly been demonstrated.[111;144;145] However, as a recent survey of international psychiatrists' prescribing habits showed, long-acting formulations, even after the arrival of a second generation preparation, are still usually reserved for patients with chronic schizophrenia who are at high-risk of non-compliance.[146]

As our sample consisted primarily of multi-episode patients who had been diagnosed with the illness for an extended period of time, this could account for the high rate of depot prescriptions in comparison to international literature.[147;148] On the other hand, a number of previous studies have shown that African-American patients are more likely to be prescribed depot antipsychotic preparations than Caucasian patients, possibly due to the perception that non-compliance is more prevalent in this population.[147;149] As no South African data

exist to either support or disprove this notion it would be interesting to do a comparative prescription analysis on an ethnically diverse sample.

A trend that was observed in the sample was the high rate of antipsychotic polypharmacy in the form of two (32.1%; 24%) and in a few cases, even three (2.6%; 2%), antipsychotics being prescribed. This stands in sharp contrast to a previously reported Xhosa sample as well as international literature.[111;150] This practice is specifically worrying if one takes into account the increased potential for adverse drug effects (notably extrapyramidal side-effects) and unwanted pharmacokinetic drug interactions.[151] More than two thirds (42.3%; 40%) of the sample were using anticholinergic drugs. It has also been shown that exposure to high doses of antipsychotic medication could be a risk factor for the development of tardive dyskinesia.

In contrast, the sample had quite a low rate of clozapine prescription (10.9%; 10%) in comparison to that reported in international literature.[152] The low frequency of clozapine use may merely reflect practical implications such as severity of side-effect profile, the requirement of regular leukocyte counts to monitor the risk of agranulocytosis and difficulties related to re-introduction of clozapine after discontinuation for longer than 48 hours. If so, this would be unfortunate as on interview a fair portion of the sample still had significant ongoing positive symptoms. Furthermore, at the time the data were collected, clozapine was the only atypical antipsychotic available in the state sector in the

Western Cape and, at the time of writing, still remains the only one that is readily available.

The general impression created by the prescription data was that the medication requirements of the sample as a whole could stand reassessment. Unfortunately, many of the trends observed can probably be explained by the understaffed community psychiatric services where stable patients are evaluated by a psychiatric registrar only once in six months (if that often). Placing dedicated psychiatric medical officers at these clinics would no doubt lead to an improvement in prescription practices and consequently, patient care. Also, as it is true that many patients are discharged from hospital prior to being fully stabilised, it could be that ultimate medication goals, as planned, are never reached. Finally, as a fair portion of the sample were still inpatients at the time of interview, medication changes could still have been made prior to discharge.

4.6 CO-MORBID CONDITIONS AND SYMPTOMS

Internationally, high rates for the presence of co-morbid mood and anxiety disorders have been repeatedly reported for both first and multi-episode as well as childhood-onset schizophrenia.[153-157] However, a more general overview shows there to be quite a bit of variation in different studies. For example in Braga et al.'s review, co-morbidity rates for the prevalence of the different anxiety

disorders ranged from 0-35% for obsessive compulsive disorder; 3.3-43% for panic disorder; 8.2-36.3% for social phobia; 1.3-51% for post traumatic stress disorder; 2.5-16.7% for generalised anxiety disorder; and 2.5-13.6% for specific phobia.[158] Be that as it may, in comparison, the rates of suspected co-morbid mood (10.7%; 18%) and anxiety disorders (8%; 8%) reported for our study group still seem to cluster at the lower end of the spectrum. Although this could possibly be a function of the main assessment tool (DIGS) used (which relies heavily on self-report or the availability of collateral) or the fact that the DIGS needed to be translated orally into Xhosa; other factors could also very well be involved.

Many of the previous studies (specifically with regards to multi-episode populations) focused on Caucasian samples. It could be that cultural factors influence the expression of mood and anxiety symptom profiles in patients with schizophrenia. In support of this notion, we find data from cross-national comparative studies that suggest a wide variation in rates of, for example major depression and social phobia, with especially Asian countries exhibiting much lower rates.[159;160] Furthermore, not only do our rates compare well to that of a previous Xhosa sample, but they also compare favourably to rates found in a previous mixed-ethnicity South African sample.[111;126] Interestingly, Emsley et al. also reported a generally low prevalence of anxiety and depressive symptoms in a South African schizophrenia sample.[161]

Therefore, if the data with regard to the low prevalence of mood and anxiety disorders could be replicated in a Xhosa or other South African sample by using more diagnostically specific (and if possible culturally sensitive) screening tools, it could support a possible protective role for cultural or genetic factors in the development of co-morbid disorders in schizophrenia. While it is recognised that specific biological mechanisms play an important role in the pathogenesis of mood and anxiety disorders, ethnic variations in these underlying factors could in fact be protective factors in certain groups. Further comparative studies would clearly be of value to investigate such a hypothesis.

We relied on self-report and collateral information to collect data with regard to substance use disorders. However, within the confines of our setting we regard this as a reliable method as a previous study by our group in a Xhosa schizophrenia sample showed a low rate of inaccurate self-report.[111] Regular tobacco smoking was reported by the majority of the participants (68.8%; 73%). This is in keeping with international data for both schizophrenia and mental illness in general that has shown people suffering from mental illness to be twice as likely to smoke than members of the general population.[162]

A co-morbid substance use disorder could be documented for a significant portion of the sample (48.2%; 60%) with most participants using either alcohol (17.85%; 28%) or cannabis (40.1%; 50%) and only three reporting other substances. This compares with international literature that suggests that nearly

50% of schizophrenia patients have a co-morbid substance disorder (a rate of three times as high as that of the general population).[163] Disturbingly, very high substance co-morbidity rates has also been documented for first episode schizophrenia (34.7%).[164] It has been shown that these co-occurring substance use disorders contribute substantially to the financial and emotional burden of the illness – for patients, families and the mental health care system.[165] Taking into account the already extremely constrained state sector in our setting, it would be in our best interest to develop a more integrated approach to the management of these disorders in our schizophrenia patients.

A total of eleven participants admitted to having made one or more suicide attempts during their lifetime. This is quite low in comparison to previous data on a Xhosa sample (19.8%).[166] International data also suggest that 20-40% of schizophrenia patients attempt suicide, with approximately 10% of patients with the diagnosis having suicide as cause of death.[167] This has been supported by two recent national co-morbidity surveys, one in the UK and one in the US, both showing significantly excess co-morbidity for a diagnosis of schizophrenia.[168;169] Of note, however, was that nearly one third of the attempts in our sample were characterize by a more violent mode which was also reflected by the UK sample with over a quarter of the schizophrenia patients (27%) choosing a violent method in comparison to only 10% of the remaining sample.

Interestingly, a recent study aiming to investigate possible transcultural differences (comparing North America, Europe, Eastern Europe, South America and South Africa) between schizophrenia spectrum disorder patients who did or did not attempt suicide, showed that for all regions, except South Africa, the presence of comorbid substance abuse disorder and smoking could be significantly associated with suicide attempts.[170] Our own previously reported sample revealed that earlier age of onset of illness as well as not being part of a sibship significantly increased the risk for suicide attempts.[166]

Patients with co-morbid medical and mental illness are known to have higher mortality rates than those with medical illness alone. A recent literature review revealed that people with schizophrenia have higher prevalences of HIV infection and hepatitis, osteoporosis, altered pain sensitivity, sexual dysfunction, obstetric complications, cardiovascular diseases, being overweight, diabetes, dental problems, and polydipsia than the general population.[171] In fact, in comparison to the general population patients with schizophrenia have been reported to have a 40% increased risk of death from medical causes.[172] Although, medical co-morbidity could be documented for a fair percentage of our sample (28.5%; 40%), pulmonary tuberculosis was the only diagnosis that was commonly reported. However, it is important to note that we relied on self-report and psychiatric folders alone and it is possible that all co-morbidity was not documented. Taking into account the significant role for co-morbid medical illness, we could once again argue that the presence of dedicated psychiatric

medical officers in the community setting would lead to improved care as earlier identification of such illness and initiation of appropriate treatment would be more likely.

4.7 SIGNIFICANCE OF FINDINGS

In general terms the demographic and clinical findings presented here correlate with those of the only other large Xhosa sample reported on in the literature. As noted in the results chapter, no significant differences could be demonstrated for any of the demographic or clinical variables for the group of five subjects with chromosomal aberrations, when compared to the rest of the KSG group.

5. CONCLUSION

Our findings, in contrast with those reported in e.g. the Turkish and Afrikaner populations, do not support a significant role for chromosomal aberrations in the susceptibility to develop schizophrenia in this population. However, ethno-specific morphological characteristics could be demonstrated for our sample. Taken together, these results highlight the need for more non-Caucasian population-specific studies in schizophrenia, as clearly, morphological and genetic results cannot just be extrapolated across samples.

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APPENDIX A: GLOSSARY

Allele: One of the variant forms of a gene at a particular locus, or location, on a chromosome. Different alleles produce variation in inherited characteristics such as hair color or blood type. In an individual, one form of the allele (the dominant one) may be expressed more than another form (the recessive one).

Autosome: Any chromosome other than the sex chromosomes. Man has 22 pairs of autosomes.

Contiguous gene syndrome: A characteristic complex phenotype produced by deletion of a short chromosome segment, resulting from haplo-insufficiency of several genes in the deleted segment.

Cytogenetic: The study of the structure, function, and abnormalities of human chromosomes.

Deletion: Absence of a segment of DNA; may be as small as a single base or large enough to encompass one or more entire genes. Large deletions involving a whole segment of a chromosome may be detected by routine examination of the chromosomes; intermediate deletions involving a few genes may be detected by using FISH; smaller deletions involving a portion of a gene may only be detected by analyzing the DNA.

Epigenetic: Refers to heritable factors affecting the development or function of an organism that are not associated with its DNA sequence; relating to, being, or involving changes in gene function that do not involve changes in DNA sequence

Fluorescent in situ hybridization: (FISH) A technique in which a deoxyribonucleic acid (DNA) probe is labeled with a fluorescent dye (that can be visualized under a fluorescent microscope) and then hybridized with target DNA, usually chromosome preparations on a microscopic slide. It is used to precisely map genes to a specific region of a chromosome in prepared karyotype, or can enumerate chromosomes, or can detect chromosomal deletions, translocations, or gene amplifications in cancer cells. Abbreviated FISH.

Gene: A hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism.

Genome: The total genetic content contained in a haploid set of chromosomes in eukaryotes, in a single chromosome in bacteria, or in the DNA or RNA of viruses; an organism's genetic material.

Genomic imprinting: A genetic phenomenon by which certain genes are expressed in a parent of origin-specific manner.

Genotype: The genetic makeup, as distinguished from the physical appearance, of an organism or a group of organisms.

Haploinsufficiency: The situation in which an individual who is heterozygous for a certain gene mutation or hemizygous at a particular locus, often due to a deletion of the corresponding allele, is clinically affected because a single copy of the normal gene is incapable of providing sufficient protein production as to assure normal function.

Heritability: The contribution of genetic as opposed to environmental factors to phenotypic variance; i.e. the proportion of phenotypic variance attributable to variance in genotypes.

Heterochronia: The origin or development of tissues or organs at an unusual time or out of the regular sequence.

Heterogeneity: Variance in characteristics within a defined illness.

Heterozygous: Possessing two different forms of a particular gene, one inherited from each parent.

Homozygous: Possessing two identical forms of a particular gene, one inherited from each parent.

Inversion: A chromosomal rearrangement in which a segment of genetic material is broken away from the chromosome, inverted from end to end, and re-inserted into the chromosome at the same breakage site. Balanced inversions (no net loss or gain of genetic material) are usually not associated with phenotypic abnormalities, although in some cases gene disruptions at the

breakpoints can cause adverse phenotypic effects, including some known genetic diseases; unbalanced inversions (loss or gain of chromosome material) nearly always yield an abnormal phenotype.

Karyotype: A photographic representation of the chromosomes of a single cell, cut and arranged in pairs based on their banding pattern and size according to a standard classification

Mendelian inheritance: Better known as Mendel's laws or mendelian laws, which are the basic principles of genetics based on the experiments of Gregor Mendel in the nineteenth century. Two basic genetic principles were established: the law of segregation and the law of independent assortment. According to the law of segregation, the genetic characteristics of a species are represented in the somatic cells by a pair of units called genes that separate during meiosis so that each gamete receives only one gene for each trait. According to the law of independent assortment, the members of a gene pair on different chromosomes segregate independently from other pairs during meiosis, so that the gametes offer all possible combinations of factors.

Metaphase: The stage of mitosis and meiosis, following prophase and preceding anaphase, during which the chromosomes are aligned along the metaphase plate. This is the stage at which chromosomes are most easily studied.

Microdeletion: The loss of a tiny piece of a chromosome, a piece so small its absence is not apparent on ordinary examination (using a regular light microscope to look at chromosomes prepared in the usual fashion). The detection of microdeletions requires special techniques such as high-resolution chromosome banding, molecular chromosome analysis (with FISH), or DNA analysis.

Mosaic: When an individual has two or more cell populations with a different chromosomal makeup, this situation is called chromosomal mosaicism.

Orthologues: Sequences or genes in different organisms that are direct evolutionary counterparts; that is, they are related by descent from a common ancestor. Orthologous genes normally have the same cellular function.

Paracentric: An inversion in which the breakpoints are confined to one arm of a chromosome; the inverted segment does not span the centromere

Pericentric: An inversion in which the breakpoints occur on both arms of a chromosome. The inverted segment spans the centromere.

Phenotype: The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences.

Pseudoautosomal: Pertaining to segments of the X and Y chromosomes that undergo obligatory crossing-over so that they show on autosomal pattern of inheritance instead of the typical x- or y-linked pattern.

Syndrome: A group of symptoms that collectively indicate or characterize a disease, psychological disorder, or other abnormal condition.

Syntenic region: A genomic region found in two organisms with a co-linear order of genes (or nucleotides).

Translocation: A chromosome alteration in which a whole chromosome or segment of a chromosome becomes attached to or interchanged with another whole chromosome or segment, the resulting hybrid segregating together at meiosis; balanced translocations (in which there is no net loss or gain of chromosome material) are usually not associated with phenotypic abnormalities, although gene disruptions at the breakpoints of the translocation can, in some cases, cause adverse effects, including some known genetic disorders; unbalanced translocations (in which there is loss or gain of chromosome material) nearly always yield an abnormal phenotype.