Mutation screening of pre-eclampsia candidate genes, *LEP* (*ob)* and *LEPR* (*obR*).

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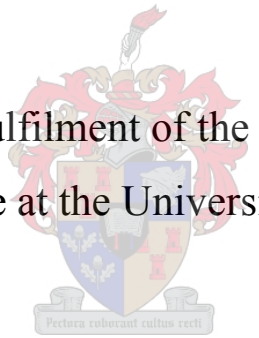

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Declaration

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

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Abstract

Pre-eclampsia is a multisystemic disorder with an incidence of ~6-8% in non-Caucasian women in the Western Cape. Trophoblast invasion is vital for adequate anchorage of the placenta to the uterine wall as well as for the optimisation of utero-placental blood flow in uncomplicated pregnancies. This process is facilitated by the fetal trophoblast cells that digest the extracellular matrix of the uterus by secreting various molecules, including the metalloproteinases (MMP), of which MMP-9 has an increased production during the first trimester. Leptin, an autocrine regulator of MMP-9 secretion, functions via the leptin receptor to prevent over-invasion of maternal tissues.

The aim of this study was to investigate the role of the leptin (*ob*) and leptin receptor (*obR*) genes in predisposition to pre-eclampsia and involved screening the genes in South African non-Caucasian cohorts and performing statistical analysis to determine whether any variants contributed to the disease profile.

Fifty-two mother/cord blood patient combination and forty-one matched control samples were screened using a combination of Multiphor SSCP/HD analysis, restriction enzyme analyses and sequencing techniques.

Two novel variants were identified in this study: a coding 107a/g (Lys36Arg) in the *ob* gene and an A→G transition 34 bp downstream of *obR* exon 14.

An additional five documented sequence variants were identified in the *ob* gene including a C→A transversion 188 bp upstream of untranslated exon 1, a G→A transition 19 bp into exon 1, a C→T transition 50 bp 3'to the start of exon 3, and the 280a/g (Val94Met) and 309c/t (Asn103Asn) variants in exon 3. No significant association was demonstrated between the control and patient genotypes, although in some cases (-188c/a and +19g/a) frequencies approached significance, but Hardy-Weinberg equilibrium deviations limited interpretation.

No associations were demonstrated for the *obR* Lys109Arg variant in exon 4, Gln223Arg variant in exon 6, Ser343Ser variant in exon 9 and the G→C loci 15 bp 3' of exon 18. However, an association

was demonstrated (p = 0.05) at the 1986c/g (Lys656Asn) exon 14 locus between mother and infant genotypes aligned in the recessive model (C/C and C/G vs. G/G).

Genotype-phenotype comparisons were performed. The *obR* exon 14 Lys656Asn, exon 6 Gln223Arg and exon 9 Ser343Ser variants demonstrated weak association with poor clinical outcome in infants of pre-eclamptic pregnancies.

While no single *ob* and *obR* sequence variants could be shown to significantly increase susceptibility to pre-eclampsia in mothers or their infants, these genes may harbour variants which modulate the clinical severity of pre-eclampsia.

Opsomming

Pre-eklampsie is ‘n multisistemiese siekte met ‘n voorkoms van ~6-8% in nie-Kaukasiese vroue in die Wes-Kaap. Trofoblastiese indringing is noodsaaklik vir geskikte verankering van die plasenta aan die baarmoederwand en vir die optimalisering van die utero-plasentale bloedvloei in ongekompliseerde swangerskappe. Hierdie proses word bemiddel deur fetale trofoblastiese selle wat die ektrasellulêre matriks van die baarmoeder verteer deur verskeie molekules af te skei, insluitend die metalloproteïnases (MMPs), waarvan MMP-9 ‘n verhoogte produksie in die eerste trimester van swangerskap het. Leptin, ‘n outokriene reguleerder van MMP 9-sekresie, funksioneer via die leptinreseptor om oormatige indringing van die moederweefsel te verhoed.

Die doel van hierdie studie was om die rol van leptin (*ob)* en die leptinreseptorgene (*obR*) in die vatbaarheid vir pre-eklampsie na te vors. Dit het die sifting van die gene in Suid-Afrikaanse nie-Kaukasiese groepe en die uitvoering van statistiese analises ingesluit om te bepaal of enige variante tot die siekteprofiel bygedra het.

Twee-en-vyftig moeder/naelstringbloed pasiëntkombinasies en een-en-veertig ooreenstemmende kontrolemonsters is gesif deur gebruik te maak van die Multiphor ESKP/HD- (enkelstring konformasie polimorfisme/heterodupleks) analise, restriksie-ensiemverteringanalise en volgorde-bepalingtegnieke.

Twee nuwe variante is geïdentifiseer in die studie: ’n koderende 107a/g (Lys36Arg) in die *ob*-geen en ‘n A→G transisie 34 bp stroom-af van die *obR*-ekson 14.

‘n Bykomende vyf gedokumenteerde volgordevariante is geïdentifiseer in die *ob*-geen, insluitende ‘n C→A vervanging 188 bp stroom-op van die onvertaalde ekson 1, ‘n G→A transisie 19 bp tot in ekson 1, ‘n C→T vervanging 50 bp 3’ van die begin van ekson 3, en die 280a/g (Val94Met) en 309c/t (Asn103Asn) variante in ekson 3. Geen betekenisvolle verband is tussen die kontrole- en pasiëntgenotipes vertoon nie, alhoewel die frekwensies in sommige gevalle (-188c/a en +19a/g) betekenisvol begin raak het, maar Hardy-Weinberg ekwilibriumafwykings het die verklaring daarvan beperk.

Geen assosiasies is vertoon vir die *obR* Lys109Arg-variant in ekson 4, Gln223Arg variant in ekson 6, Ser343Ser variant in ekson 9 en die G→C lokus 15 bp 3' van ekson 18 nie. Nogtans is 'n assosiasie vertoon (p = 0.05) vir die 1986c/g (Lys656Asn) ekson 14-lokus tussen moeder en baba genotipes wat in die ressesiewe model (C/C and C/G vs. G/G) inskakel.

Genotipe-fenotipe-vergelykings is uitgevoer. Die *obR*-ekson 14 Lys656Asn, ekson 6 Gln223Arg en ekson 9 Ser343Ser-variante het weinig assosiasie met swak kliniese uitkomste in babas van pre-eklamptiese swangerskappe vertoon.

Alhoewel geen enkele *ob*- en *obR*-volgordevariante gewys kon word om die vatbaarheid vir pre-eklampsie betekenisvol in moeders en hul babas te verhoog nie, kan die gene dalk variante huisves wat die kliniese graad van erns van pre-eklampsie moduleer.

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List of Abbreviations

~	approximately
%	percentage
°C	degrees Celsius
3’	3’prime
5’	5’prime
µl	microlitre
µmol/l	micromole per litre
@	at
A	adenosine
AGT	angiotensinogen gene
AIDS	Acquired Immune Deficiency Syndrome
Ala	alanine
APS	ammonium persulphate
Arg	arginine
Asn	asparagine
Asp	aspartic acid
AV	anchoring villi
BLAST	Basic Local Alignment of Sequences Tool
BMD	bone mineral densitometry
BMI	body mass index
bp	base-pair
BSA	bovine serum albumin
C	cytosine
cAMP	3’, 5’ cyclic AMP
CAMs	cell adhesion molecules
CBS	cystathionine β- synthetase gene
C/EBPα	CCAAT/enhancer-binding protein alpha
dbSNP: rs	database single nucleotide polymorphism: reference sequence
ddH_2O	double distilled water
dH_2O	distilled water

dHPLC	denaturing High Performance Liquid Chromatography
DNA	deoxyribonucleic acid
dNTPs	2'-deoxy-nucleotide-5'-triphosphates
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
ET-1	endothelin-1
ex	exon
F	forward primer
F2	prothrombin gene
FV	floating villi
FVL	Factor V Leiden variant
g	gram
G	guanosine
G-CSF	granulocyte-colony stimulating factor
g/dl	grams per decilitre
GH	growth hormone
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GP-M	glycogen phosphorylase muscle isoform
GST	glutathionine -S- transferase gene
h	hour
hCG	human chorionic gonadotrophin
HD	heteroduplex analysis
HDL	high density lipoprotein
HELLP	haemolysis, elevated liver enzymes and low platelets syndrome
HLA-G	human leukocyte antigen-g
IDT	Integrated DNA Technologies
ILs	interleukins
ISSHP	International Society for the Study of Hypertension in Pregnancy
IUGR	intrauterine growth restriction
JAK-STAT	janus kinase protein-signal transducer and activator of transcription protein

l	litre
LDL	low density lipoprotein
Leu	leucine
LPL	lipoprotein lipase gene
Lys	lysine
kb	kilobases
kDA	kilodalton
M	moles
Met	methionine
MFMN	maternal fetal medicine network
mg	milligram
mg/ml	milligram per millilitre
min	minutes
ml	millilitre
mm	millimetre
mM	milli-molar
mmHg	millimetre of mercury
mmol/l	milli-moles per litre
MMPs	metalloproteinases
MOUs	Midwife Obstetric Units
mRNA	messenger ribonucleic acid
MTHFR	methylenetetrahydrofolate reductase
n/a	not applicable
NCBI	National Centre for Biotechnology Information
ng	nanogram
NICU	Neonatal Intensive Care Unit
NIDDM	non-insulin-dependent diabetes mellitus
N^{o}	number
NOS3	nitric oxide synthase
NPY	neuropeptide Y
ob	leptin gene
obR	leptin receptor gene
p	short arm of chromosome

PAs	plasminogen activators
PAGE	polyacrylamide gel electrophoresis system
PAIs	plasminogen activator inhibitors
PBS	phosphate buffered saline
PCOS	polycystic ovary syndrome
PCR	polymerase chain reaction
PDA	piperizine diacrylamide
PE	pre-eclampsia
pH	potential of hydrogen
Phe	phenylalanine
PIH	pregnancy-induced hypertension
Pro	proline
q	long arm of chromosome
R	reverse primer
RBC	red blood cells
RE	restriction enzymes
REA	restriction enzyme analysis
rpm	revolutions per minute
s	seconds
Ser	serine
SNPs	single nucleotide polymorphisms
SOCS-3	suppressor of cytokine signalling-3
SSCP	single strand conformation polymorphism
T	thymine
T_a	annealing temperature
Taq	*Thermus aquaticus*
TBE	tris-borate/EDTA
TDT	transmission disequilibrium test
TE	tris/EDTA
TEMED	N, N, N' N', -tetramethylethylenediamine
THBD	thrombomodulin
Thr	threonine
TIMPs	tissue inhibitors of metalloproteinases

T_m	melting temperature
TM	transmembrane domain
TNF	tumour necrosis factor
TPR	total peripheral resistance
Trp	tryptophan
U	units
UTR	untranslated region
UV	ultraviolet
V	volts
Val	valine
WCC	white cell count