

**ENDOTHELIAL FUNCTION &
GENETIC POLYMORPHISMS IN
CEREBRAL SMALL VESSEL DISEASE**

**A study investigating the relationships between endothelial
function, genetic polymorphisms and cerebral small vessel disease**

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TABLE OF CONTENTS:

THESIS ABSTRACT	IV
DECLARATION	VI
ACKNOWLEDGEMENTS	VII
CONFERENCE PRESENTATIONS.....	VIII
PUBLICATIONS	IX
LIST OF FIGURES	X
LIST OF TABLES	XI
INDEX OF ABBREVIATIONS	XIII
INTRODUCTION	1
CHAPTER 1 CEREBRAL SMALL VESSEL DISEASE.....	2
1.1 LACUNAR INFARCTION	3
1.2 LEUKOARAIOSIS.....	4
1.3 METHODS TO CATEGORISE SVD	6
1.4 RISK FACTORS OF SVD.....	10
1.5 GENETICS IN SVD.....	12
1.6 SUMMARY.....	17
CHAPTER 2 ENDOTHELIAL FUNCTION	18
2.1 THE ENDOTHELIUM.....	18
2.2 ENDOTHELIAL DYSFUNCTION	24
2.3 METHODS TO MEASURE EF	28
2.4 INFLUENTIAL FACTORS OF EF	36
2.5 CLINICAL IMPLICATIONS OF ED	44
2.6 SUMMARY.....	50

CHAPTER 3 GENETIC POLYMORPHISMS	51
3.1 SELECTION OF CANDIDATE GENETIC POLYMORPHISMS	51
3.2 INTERLEUKIN-6 (IL-6) -174G/C	52
3.3 NADH/NADPH-OXIDASE (N-Ox) P22 PHOX 242 C/T	54
3.4 TISSUE PLASMINOGEN ACTIVATOR (tPA).....	57
3.5 ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS)	60
3.6 ENDOTHELIN-1 (ET-1).....	61
3.7 PARAOXONASE (PON1).....	63
3.8 HAPLOTYPE STUDIES	64
3.9 SAMPLE SIZE CALCULATIONS	65
3.10 SUMMARY.....	66
CHAPTER 4 STUDY AIMS AND RATIONALE	67
4.1 STUDY AIMS & HYPOTHESES.....	67
4.2 STUDY DESIGN	67
4.3 JUSTIFICATION OF STUDY	67
CHAPTER 5 RESEARCH METHODS	69
5.1 CLINICAL METHODS	69
5.2 LABORATORY METHODS	75
5.3 STATISTICAL METHODS	82
CHAPTER 6 RESULTS	85
6.1 STUDY SAMPLE.....	85
6.2 ASSOCIATION BETWEEN CEREBRAL SMALL VESSEL DISEASE AND ENDOTHELIAL FUNCTION	90
6.3 ASSOCIATION BETWEEN GENETIC POLYMORPHISMS AND ENDOTHELIAL FUNCTION	95
6.4 ASSOCIATION BETWEEN GENETIC POLYMORPHISMS AND CEREBRAL SMALL VESSEL DISEASE	100
6.5 RESULTS SUMMARY.....	106

CHAPTER 7 DISCUSSION	109
7.1 INTRODUCTION	109
7.2 ASSOCIATION BETWEEN CEREBRAL SMALL VESSEL DISEASE AND ENDOTHELIAL FUNCTION	110
7.3 ASSOCIATION BETWEEN GENETIC POLYMORPHISMS AND ENDOTHELIAL FUNCTION	113
7.4 ASSOCIATION BETWEEN GENETIC POLYMORPHISMS AND CEREBRAL SMALL VESSEL DISEASE	120
7.5 STUDY LIMITATIONS	130
7.6 CONCLUSION	137
CHAPTER 8 FUTURE DIRECTIONS.....	138
8.1 THE IDEAL STUDY	138
8.2 GENETICS.....	142
8.3 IMAGING	143
8.4 OTHER RELATED STUDIES	144
FINAL CONSIDERATIONS.....	145
BIBLIOGRAPHY	146
APPENDIX 1: PUBLICATION: CEREBRAL SMALL VESSEL DISEASE – GENETIC RISK ASSESSMENT FOR PREVENTION AND TREATMENT.....	192
APPENDIX 2: DNA EXTRACTION PROTOCOL.....	206
APPENDIX 3: LOGISTIC REGRESSION MODEL ADJUSTMENT (LA).....	207
APPENDIX 4: LOGISTIC REGRESSION MODEL ADJUSTMENT (LI).....	211

Thesis Abstract

Background

The pathogenesis of cerebral small vessel disease (SVD), encompassing lacunar infarction (LI) and leukoaraiosis (LA), is heterogeneous, with impaired endothelial function (EF) and altered fibrinolysis proposed as important contributors. Genetic factors are involved and may exert their influence via the above mechanisms.

The aim of this study was to explore the relationship between EF and SVD, and to examine the role of candidate polymorphisms in both EF and SVD.

Methods

The study cohort consisted of patients who had undergone a brain magnetic resonance image (MRI) scan for non-vascular indications. Vascular risk factors were collected by interviewing participants. SVD was classified using a modified Fazekas rating scale, where SVD burden was divided into three categories: absent/mild, moderate and severe. LI was graded separately.

EF was assessed using applanation tonometry (ApT) and the radial pulsewave. A global EF score that accounts for both endothelium-dependant and –independent vasodilation was used as the index for comparison. A higher global EF score indicated better EF.

Participants were genotyped using the sequence-specific polymerase chain reaction (PCR-SS) for eight candidate polymorphisms chosen based on biological plausibility and/or previous study evidence: interleukin-6 (IL-6) -174 G/C, NADPH oxidase p22 phox 242 C/T, tissue plasminogen activator (tPA) 20324 C/T, tPA -4360 G/C, tPA -7351 C/T, endothelial nitric oxide synthase (eNOS) -786 T/C, endothelin-1 (ET-1) 138 D/I and paraoxonase-1 (PON1) -107 C/T.

Statistical analyses were performed using Intercooled Stata 9.2, GraphPad Prism and the SNPstats. Regression models were adjusted for the appropriate variables.

Results

A total of 132 participants were assessed. All participants were genotyped and 84 of these 132 participants also had their EF assessed using ApT, but only 72 participants were successful.

Participants were graded separately for LI and LA. LA controls (n=119) were defined as participants with absent/mild LA, and LA cases (n=13) were participants with moderate or severe LA. LI controls (n=126) were participants without a radiologically defined LI and LI cases (n=6) were participants with radiologically defined LI.

The results of the study can be summarised as follows:

1. there was no significant difference between the EF of cases and controls. Subgroup analyses showed that the risk of LA decreased as the global EF values increased after adjusting for confounding influences, but the relationship was not significant ($p=0.23$);
2. there were no significant differences in EF between the genotypes of the eight candidate polymorphisms, except for the tPA 20324 C/T, where the TT genotype was associated with higher EF compared to the CC/CT genotypes ($p=0.02$);
3. the tPA 20324 TT genotype was significantly associated with an increased risk of LI compared to the CC/CT genotypes ($p=0.03$), although the association is under powered. No other significant associations were found.

Although the intent was to achieve a pre-determined sample size, the methodology, and in particular the exclusion criteria, restricted recruitment and consequently the study was under powered to achieve its goals. The study could therefore be considered a pilot study and any conclusions forthwith require validation in a larger sample.

Conclusion

The tPA 20324 TT genotype was significantly associated with LI, while also being significantly associated with better EF. This result may be a Type I error reflective of the small sample size. However, the result does support the hypothesis that impaired fibrinolysis has an important pathogenic role in LI. This study does not support impaired EF as a significant pathogenic contributor to SVD.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Ada Lam and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Lam AK et al; Cerebral small vessel disease – Genetic risk assessment for prevention and treatment; *Molecular Diagnosis and Therapy* 2008; 12(3): 145-156 [Wolters Kluwer Health | Adis]

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Conference Presentations

Poster Presentation:

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Poster Presentation:

“Endothelial Function in Cerebral Small Vessel Disease – A Pilot Study”. The Queen Elizabeth Hospital Research Day, Adelaide, Australia, October 2008

Platform Presentation:

“Endothelial Function in Cerebral Small Vessel Disease”. 6th Asia Pacific Conference Against Stroke and 20th Stroke Society of Australasia ASM, Cairns, Australia, September 2009

Platform Presentation:

“Endothelial Function in Cerebral Small Vessel Disease”. The Queen Elizabeth Hospital Research Day, Adelaide, Australia, October 2009

Publications

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McLennan SN, **Lam AK**, Mathias JL, Koblar SA, Hamilton-Bruce MA, Jannes J; Vasodilation reponse and cognition; *Cerebrovascular Diseases* 2010; in submission.

Chen CS, Rudkin AK, Lee AW, **Lam AK**, Patel S, Khoo E, Hamilton-Bruce MA, Jannes J, Koblar SA; Association of retinal nerve fibre layer brain volume change in leukoaraiosis; *Journal of Neurology, Neurosurgery and Psychiatry* 2010; in submission.

LIST OF FIGURES

FIGURE 1.1 – CLASSIFICATION OF ISCHAEMIC CVD.....	2
FIGURE 1.2 – T ₂ MRI OF THE BRAIN SHOWING A LACUNAR INFARCTION, CONFIRMED BY THE FLAIR.....	3
FIGURE 1.5 – T ₂ MRI OF THE BRAIN SHOWING SEVERE LA CONFIRMED BY THE FLAIR.	5
FIGURE 2.1 – THE MAJOR MODULATORS AND PATHWAYS INVOLVED IN EF & DYSFUNCTION	19
FIGURE 2.2 – PERIPHERAL & ARTERIAL WAVEFORM	34
FIGURE 2.3 – THE VARIOUS RISK FACTORS FOR ENDOTHELIAL DYSFUNCTION.....	37
FIGURE 2.4 – PROPOSED PATHWAYS OF EF IN SVD	48
FIGURE 3.1 – GENE STRUCTURE OF IL-6.	53
FIGURE 3.2 – GENE STRUCTURE OF N-OX P22 PHOX.....	55
FIGURE 3.3 – STRUCTURE OF THE eNOS GENE	60
FIGURE 3.4 – THE GENE STRUCTURE OF PREPROENDOTHELIN	62
FIGURE 3.5 – GENE STRUCTURE OF PON1	64
FIGURE 5.1 – RECRUITMENT PATHWAY	70
FIGURE 5.2 – DIGITALLY ENHANCED PHOTOGRAPH OF THE GEL UNDER UV LIGHT	80
FIGURE 5.3 – CHROMATOGRAM SHOWING THE DNA SEQUENCE OF THE TPA 20324 C/T POLYMORPHISM USING THE CONSENSUS PRIMER.....	82
FIGURE 6.1 – BREAKDOWN OF THE RECRUITMENT PROCESS	85
FIGURE 6.2 – BOX AND WHISKERS PLOTS SHOWING THE DISTRIBUTION OF GLOBAL EF AAIx ...	91

LIST OF TABLES

TABLE 1.1 – A SUMMARY OF DIFFERENT VISUAL RATING SCALES	8
TABLE 1.2 – MULTIVARIATE ODDS RATIO AND 95% CIs FOR A FAMILY HISTORY OF STROKE	14
TABLE 1.3 – RELATIONSHIP BETWEEN AGE OF STROKE AND POSITIVE FAMILY HISTORY OF STROKE	15
TABLE 2.1 – CEREBRAL EF AND SYSTEMIC EF IN LI PATIENTS, CONTROLS WITH SIMILAR RISK FACTORS AND HEALTHY CONTROLS	49
TABLE 3.1 – EFFECT OF THE N-Ox p22 PHOX 242 C/T POLYMORPHISM ON SVD.....	57
TABLE 3.2 – SAMPLE SIZE ESTIMATES FOR CANDIDATE POLYMORPHISMS.....	66
TABLE 5.1 – MRI DETAILS	72
TABLE 5.2 – OLIGONUCLEOTIDE PRIMER SEQUENCES	77
TABLE 5.3 – FINAL CONCENTRATIONS OF PRIMERS	78
TABLE 6.1 – REASONS FOR EXCLUSION AFTER SCREENING #1	86
TABLE 6.2 – REASONS WHY THE INVITEES DECLINED TO PARTICIPATE.	86
TABLE 6.3 – REASONS FOR EXCLUSION AFTER SCREENING #2	87
TABLE 6.4 – DESCRIPTIVE STATISTICS OF ALL 132 PARTICIPANTS BASED ON THE LA CLASSIFICATION.	88
TABLE 6.5 – DESCRIPTIVE STATISTICS OF ALL 132 PARTICIPANTS BASED ON THE LI CLASSIFICATION.	89
TABLE 6.6 – UNIVARIATE ANALYSES OF KNOWN CEREBROVASCULAR RISK FACTORS WITH LA.	90
TABLE 6.7 – UNIVARIATE ANALYSES OF KNOWN CEREBROVASCULAR RISK FACTORS WITH LI. .	90
TABLE 6.8 – DESCRIPTIVE STATISTICS OF ALL THE PARTICIPANTS WHO UNDERWENT APT BASED ON THE LA CLASSIFICATION.	92
TABLE 6.9 – DETERMINATION OF WHICH VARIABLES SHOULD BE INCLUDED IN THE MULTIPLE LOGISTIC REGRESSION MODEL FOR LA AND ED.....	93
TABLE 6.10 – DESCRIPTIVE STATISTICS OF PARTICIPANTS WHO UNDERWENT APT BASED ON THE LI CLASSIFICATION.	94
TABLE 6.11 – GENOTYPE DISTRIBUTION FOR ALL APT PARTICIPANTS (N=72)	95
TABLE 6.12 – GENOTYPE DISTRIBUTION FOR SVD-FREE CONTROLS (N=65).....	95
TABLE 6.13 – UNADJUSTED LINEAR REGRESSION OF INDIVIDUAL GENOTYPES WITH GLOBAL EF (N=72)	96
TABLE 6.14 – UNADJUSTED LINEAR REGRESSION OF DOMINANT AND RECESSIVE MODELS WITH GLOBAL EF (N=72).....	97

TABLE 6.15 – ADJUSTED VALUES OF THE TPA 20324 C/T RECESSIVE AND N-Ox p22 242 C/T RECESSIVE LINEAR REGRESSION MODELS (N=72).....	98
TABLE 6.16 – UNADJUSTED LINEAR REGRESSION OF DOMINANT AND RECESSIVE MODELS WITH GLOBAL EF IN SVD-FREE CONTROLS (N=65)	99
TABLE 6.17 – ADJUSTED VALUES OF THE TPA 20324 C/T RECESSIVE LINEAR REGRESSION MODEL (N=65)	100
TABLE 6.18 – SUBJECT NUMBERS (TOTAL N=132) AND P-VALUES FOR HARDY-WEINBERG CALCULATIONS FOR EACH POLYMORPHISM.....	100
TABLE 6.19 – GENOTYPE DISTRIBUTION BASED ON LA.	101
TABLE 6.20 – UNIVARIATE ANALYSES FOR ALL POLYMORPHISMS WITH LA	102
TABLE 6.21 – MULTIVARIATE ANALYSES FOR EACH POLYMORPHISM WITH LA.....	103
TABLE 6.22 – GENOTYPE DISTRIBUTION BASED ON LI.	104
TABLE 6.23 – UNIVARIATE ANALYSES FOR ALL POLYMORPHISMS WITH LI.....	105
TABLE 6.24 – MULTIVARIATE ANALYSES FOR EACH POLYMORPHISM WITH LI.	105
TABLE 6.25 – POST-HOC STATISTICAL POWER CALCULATIONS AND ESTIMATED SAMPLE SIZES REQUIRED FOR EACH CANDIDATE POLYMORPHISM.....	106
TABLE 8.1 – SUMMARY OF THE FEATURES OF THE IDEAL SVD STUDY.....	142

Index of Abbreviations

ACE	angiotensin converting enzyme
ACEI	angiotensin converting enzyme inhibitor
ADMA	asymmetric dimethylarginine
AGE	advanced glycation end products
AIx	augmentation index
AngII	angiotensin II
ApT	applanation tonometry
ARB	angiotensin receptor blocker
ATP	adenosine triphosphate
ATR	angiotensin receptor
BH4	tetrahydrobiopterin
Ca ²⁺	calcium ions
CAD	coronary artery disease
	cerebral autosomal dominant arteriopathy stroke and ischaemic
CADASIL	leukoencephalopathy
CarVD	cardiovascular disease
CF-PWV	carotid-femoral pulsewave velocity
cGMP	cyclic guanosine monophosphate
CRP	C-reactive protein
DAG	1,2-diacylglycerol
DDAH	dimethylarginine dimethylaminohydrolase
DWM	deep white matter
ECE	endothelin converting enzyme
ED	endothelial dysfunction
EDCF	endothelium derived contracting factor
EF	endothelial function
eNOS	endothelial nitric oxide
ET-1	endothelin-1
ET _A	endothelin receptor type A
ET _B	endothelin receptor type B
FLAIR	fluid attenuated inversion recovery
FMC	Flinders Medical Centre, Bedford Park, Adelaide, SA
FMD	flow-mediated dilation

GTN	glyceryl trinitrate
GTP	guanosine triphosphate
HDL	high density lipoprotein
HUVEC	human umbilical vein endothelial cell
ICAM-1	intercellular adhesion molecule-1
LA	leukoaraiosis
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
LDL	low density lipoproteins
LMH	Lyell McEwin Hospital, Elizabeth Vale, Adelaide, SA
LSM	lymphocyte separation medium
MCP-1	monocyte chemoattractant protein-1
MI	myocardial infarction
MMP	metalloproteinase
MRI	magnetic resonance imaging
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NF- κ B	nuclear factor- κ B
NO	nitric oxide
N-Ox	NADPH oxidase
NSF	N-ethylmaleimide-sensitive factor
nNOS	neuronal nitric oxide synthase
OCSP	Oxfordshire Community Stroke Project
PAI-1	plasminogen activator inhibitor-1
PBS	Dulbecco's Phosphate Buffered Solution (Calcium and Magnesium free)
PCR-SS	polymerase chain reaction (sequence specific)
PGI ₂	prostacyclin
PKC	protein kinase C
PLC	phospholipase C
PON-1	paraoxonase-1
PV	periventricular
PWA	pulse-wave analysis
RAH	Royal Adelaide Hospital, Adelaide, SA
RAS	renin-angiotensin system
ROS	reactive oxygen species

SGP	strain gauge plethysmography
SM	smooth muscle
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
SVD	small vessel disease (cerebral)
TIA	transient ischaemic attack
TNF- α	tumour necrosis factor- α
TOAST	Trial of Org 10172 in Acute Stroke Treatment
tPA	tissue plasminogen activator (protein)
TQEH	The Queen Elizabeth Hospital, Woodville South, Adelaide, SA
VCAM-1	vascular adhesion molecule-1
vWF	von Willebrand factor
WMH	white matter hyperintensity