ENDOTHELIAL FUNCTION & GENETIC POLYMORPHISMS IN CEREBRAL SMALL VESSEL DISEASE

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

ADA KARMAN LAM

Discipline of Medicine
THE UNIVERSITY OF ADELAIDE
SOUTH AUSTRALIA

A thesis submitted in fulfilment of the requirements
for the degree of PhD in Medicine
March 2010

TABLE OF CONTENTS:

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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works:

> 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]

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository. The Library catalogue, and the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed:……...…….. Date:……..…….

Acknowledgements

I would like to sincerely thank my principal supervisor, Dr Jim Jannes, for the time, effort, support and encouragement he has given me throughout my candidature. His belief in my abilities and his guidance has enabled me to mature as a scientific researcher.

I also thank my other supervisors Dr Anne Hamilton-Bruce and Dr Simon Koblar for their input throughout all stages of my PhD.

My gratitude extends to Ms Skye McLennan for helping me recruit and interview participants where possible, Dr Sandy Patel and Dr EeWin Khoo for their radiological input, Mr Austin Milton for his scientific support and training in laboratory techniques, Mr John Fields for his statistical advice and Ms Sally Michail and Ms Helve Doecke for identifying grammatical errors.

Lastly, I thank my parents for their never-ending love and support throughout the entire process.

Conference Presentations

Poster Presentation:
"Endothelial Function in Cerebral Small Vessel Disease – A Pilot Study". 6th World Stroke Congress, Vienna, Austria, September 2008

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

Lam A, Hamilton-Bruce M, Koblar S, Khoo EW, Patel S, Jannes J; Endothelial function in cerebral small vessel disease; *International Journal of Stroke* 2009; 4(S1):2.

Lam A, Hamilton-Bruce MA, Jannes J, Koblar SA; Cerebral small vessel disease: Genetic risk assessment for treatment and prevention; *Molecular Diagnosis and Therapy* 2008; 12(3): 145-156.

Lam AK, Hamilton-Bruce MA, Khoo E, Patel S, Koblar SA, Jannes J; Endothelial function in cerebral small vessel disease (CSVD): A pilot study; *International Journal of Stroke* 2008; 3(S1):374.

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

LIST OF TABLES

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	tetrahydrobipterin
Ca^{2+}	calcium ions
CAD	coronary artery disease
CADASIL	cerebral autosomal dominant arteriopathy stroke and ischaemic 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	intercelllar 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 resonace imaging
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor-κB
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