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


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.
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<tbody>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
</tr>
<tr>
<td>ACEI</td>
<td>angiotensin converting enzyme inhibitor</td>
</tr>
<tr>
<td>ADMA</td>
<td>asymmetric dimethylarginine</td>
</tr>
<tr>
<td>AGE</td>
<td>advanced glycation end products</td>
</tr>
<tr>
<td>AIx</td>
<td>augmentation index</td>
</tr>
<tr>
<td>AngII</td>
<td>angiotensin II</td>
</tr>
<tr>
<td>ApT</td>
<td>applanation tonometry</td>
</tr>
<tr>
<td>ARB</td>
<td>angiotensin receptor blocker</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATR</td>
<td>angiotensin receptor</td>
</tr>
<tr>
<td>BH4</td>
<td>tetrahydrobiopterin</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>calcium ions</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CADASIL</td>
<td>cerebral autosomal dominant arteriopathy stroke and ischaemic</td>
</tr>
<tr>
<td>CarVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>CF-PWV</td>
<td>carotid-femoral pulsewave velocity</td>
</tr>
<tr>
<td>cGMP</td>
<td>cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DAG</td>
<td>1,2-diacylglycerol</td>
</tr>
<tr>
<td>DDAH</td>
<td>dimethylarginine dimethylaminohydrolase</td>
</tr>
<tr>
<td>DWM</td>
<td>deep white matter</td>
</tr>
<tr>
<td>ECE</td>
<td>endothelin converting enzyme</td>
</tr>
<tr>
<td>ED</td>
<td>endothelial dysfunction</td>
</tr>
<tr>
<td>EDCF</td>
<td>endothelium derived contracting factor</td>
</tr>
<tr>
<td>EF</td>
<td>endothelial function</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial nitric oxide</td>
</tr>
<tr>
<td>ET-1</td>
<td>endothelin-1</td>
</tr>
<tr>
<td>ETₐ</td>
<td>endothelin receptor type A</td>
</tr>
<tr>
<td>ETₐ</td>
<td>endothelin receptor type B</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid attenuated inversion recovery</td>
</tr>
<tr>
<td>FMC</td>
<td>Flinders Medical Centre, Bedford Park, Adelaide, SA</td>
</tr>
<tr>
<td>FMD</td>
<td>flow-mediated dilation</td>
</tr>
</tbody>
</table>
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-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)
PGI2 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