

**Proteomic Investigations and Biomarker Discovery in
Transient Ischaemic Attack**

A thesis by

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*To my wife and family
whose love and support
made this thesis possible*

“We are all visitors to this time, this place. We are just passing through. Our purpose here is to observe, to learn, to grow, to love... and then we return home.”

Australian Aboriginal Proverb

DECLARATION

I certify that 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 Michael Djukic 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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SUMMARY

Between 15-26% of ischaemic strokes are preceded by transient ischaemic attack (TIA) making accurate and timely diagnosis of TIA important for stroke prevention. However, TIA diagnoses are highly reliant on subjective history gathering and clinical assessments to differentially diagnose true TIA conditions from mimic presentations. Unfortunately, the subjective nature of TIA diagnosis has created a surprisingly high amount of variability between diagnoses made by physicians and specialist neurologists. Use of biomarker tests could offer an objective quantitative measuring tool that reduces inter-observer variation through the establishment of standardised quantitative measures and improved reproducibility. When used in combination with comprehensive clinical assessments and neurological imaging, biomarkers may offer a useful adjunct to assist a treating clinician to accurately and reliably interpret the clinical finding and confidently diagnose and treat a TIA or mimic condition. This thesis proposes a framework for undertaking an exploration of the human plasma proteome, and performs the very first proteomic pilot study that identifies candidate plasma protein biomarkers associated with TIA, which could also be used to distinguish from mimic presentations.

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LIST OF ABBREVIATIONS AND ACRONYMS

%	percentage
°C	degrees Celsius
x g	x gravity
π	<i>pi</i>
Δ	change
μg	microgram
μL	microliter
μm	micrometre
2DE	two dimensional electrophoresis
ABCD2	age, blood pressure, clinical features, duration, diabetes
ACE	angiotensin converting enzyme
AngII	angiotensin II
ANOVA	analysis of variance
APOA	apolipoprotein A
APOB	apolipoprotein B
APOE	apolipoprotein E
AWGPN	Adelaide Western General Practice Network
BD	Becton Dickinson
xx	

BH ₄	tetrahydrobiopterin
BP	band pass
BVA	biological variation analysis
C4A	complement component C4-A
CBF	cerebral blood flow
CBV	cerebral blood volume
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate detergent
CI	confidence interval
CID	collision induced dissociation
cm	centremetre
CNS	central nervous system
COMBAT	community-based rapid access TIA clinic
COX	cyclooxygenase
CSF	cerebrospinal fluid
CT	computed tomography
CTA	computed tomography angiography
CTP	computed tomography perfusion
CyDye	cyanine dye
Da	dalton
DALYs	disability adjusted life years
dH ₂ O	deionised water
DIA	differential in-gel analysis
DIGE	differential in-gel electrophoresis
DMF	dimethylformamide
DNA	deoxyribonucleic acid
DSA	digital subtraction angiography
DTT	dithiothreitol

DWI	diffusion weighted imaging
EBT	erichrome black T
ECG	electrocardiogram
eNOS	endothelial nitric oxide synthase
ESI	electrospray ionisation
EtOH	ethanol
FGA	fibrinogen alpha-chain
FGB	fibrinogen beta-chain
FIBG	fibrinogen gamma chain
FLAIR	fluid-attenuated inversion recovery
fMRI	functional magnetic resonance imaging
FPLC	fast protein liquid chromatography
FWHM	full width at half-maximum
GE	General Electric
GFAP	glial fibrillary acidic protein
GP	general practitioner
GP-SIS	general practitioner with a special interest in stroke
GRE	gradient-recalled echo magnetic resonance imaging
GS	gelsolin
H ₂ O	water
HbA1C	glycated haemoglobin
HCl	hydrochloric acid
HCV	healthy control volunteer
HDL-C	high density lipoprotein cholesterol
HEMO	hemopexin
HPLC	high performance liquid chromatography
HPPP	human plasma proteome project

HREC	human research ethics committee
hsCRP	high-sensitivity C-reactive protein
HUPO	human proteome organisation
ICH	intracranial haemorrhage
ICP	intracranial pressure
IEF	isoelectric focusing
IFN- γ	interferon gamma
IL	interleukin
INR	international normalised ratio
IPG	immobilised pH gradient
I.S.	internal standard
iTRAQ	isobaric tagging technology for relative and absolute quantitation
K ₂ EDTA	dipotassium ethylenediaminetetraacetic acid
kDa	kilodalton
KR	Keil rule- trypsin cleaves next to arginine or lysine but not before proline
LC	liquid chromatography
LDL-C	low density lipoprotein cholesterol
Lp-PLA2	lipoprotein-associated phospholipase A2
M	molar
MARS-Hu6	multiple affinity removal system – human 6 immunodepletion column
MBP	myelin basic protein
MCA	middle cerebral artery
mg	milligram
mL	millilitre
mm	millimetre
mM	millimolar
mmHg	millimetre of mercury

MPa	megapascal
mRNA	messenger ribonucleic acid
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTT	mean transit time
MW	molecular weight
m/z	mass-to-charge ratio
<i>n</i>	sample size
NCCT	non-contrast heat computed tomography
NGS	next generation sequencing
NSE	neuron specific enolase
NHS	N-hydroxyl succinimidyl
nL	nanolitre
NL	non linear
nm	nanometre
nmol	nanomole
NSAID	non-steroidal anti-inflammatory drug
PAGE	polyacrylamide gel electrophoresis
PAI-1	plasminogen activator inhibitor 1
PBP	platelet basic protein
PCA	principal component analysis
PEEK	polyetheretherketone
PET	positron emission tomography
pH	hydrogen ion concentration
pI	isoelectric point
pmol	picomole

PMT	photo multiplier tube
ppm	parts per million
psi	pound-force per square inch
PWI	perfusion weighted imaging
<i>r</i>	radius
RAC	rapid assessment clinic
rt-PA	recombinant tissue plasminogen activator
S-100B	S100 calcium binding protein B
SAMP	serum amyloid P-component
SD	standard deviation
SDS	sodium dodecyl sulfate
SILAC	stable isotope labelling by amino acids in cell culture
SNP	single nucleotide polymorphism
TC	total cholesterol
TIA	transient ischaemic attack
TNF	tumour necrosis factor
TOF	time of flight
TOF-MRA	time of flight magnetic resonance angiography
TQEH	The Queen Elizabeth Hospital, Woodville South, Adelaide, SA
Trig	triglyceride
TUC	thiourea, urea, CHAPS, tris lysis buffer
UV	ultraviolet
V	volts
Vhrs	volt hours
v/v	volume per volume
w/v	weight per volume
ZA2G	zinc-alpha-2-glycoprotein

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