DNA damage and nutrient status as risk factors for mild cognitive impairment and Alzheimer's disease

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This dissertation is dedicated to my beloved

Mom and Dad:
Thank you for your selfless devotion
and blessings in making my life meaningful

Siblings:
Thank you for being my special cheer squad

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Abstract

The incidence of neurodegenerative diseases such as Alzheimer's disease (AD), which has an Australian prevalence of over 320,000, is expected to increase 3 fold in the next 30 years as a result of Australia's ageing population. Currently, 24.3 million people worldwide are diagnosed as having AD, with 4.6 million new cases being clinically diagnosed each year. Alzheimer's disease (AD) is associated with the abnormal increased accumulation of extracellular beta amyloid peptide 42 (Aβ42), which creates neurotoxic plaques in the brains of AD patients who have also been found to have increased rates of DNA damage events in different tissues, such as brain, buccal cells and peripheral blood lymphocytes. Previous studies have shown that mild cognitive impairment (MCI) may reflect the early stages of more pronounced neurodegenerative disorders such as AD. To date, conclusive diagnostic tests and the impact of dietary status for neurodegenerative disease risk have not been clearly defined. Therefore, it is important to identify potential DNA damage biomarkers and plasma micronutrient profiles associated with MCI and AD that may aid in understanding the biology of the disease and inform the design of potential dietary preventative measures.

The content of this thesis comprises 2 distinct parts.

For the *in vitro* study, the aim was:

(i) To determine whether extracellular Aβ40 or Aβ42 induces chromosomal DNA damage and cell death in human peripheral lymphocytes

(ii) To investigate whether there is an interactive effect between extracellular Aβ and folic acid status

For the *in vivo* cross-sectional study, the aim was:

(i) To investigate whether human peripheral blood lymphocytes of newly diagnosed MCI and AD cases have a different spontaneous chromosomal DNA damage biomarker profile relative to healthy age- and gender-matched controls in an Australian population

(ii) To identify an array of important micronutrients, organ function and stress markers in plasma associated with MCI or AD diagnosis prior to medication and to compare these profiles to those observed in healthy age- and gender- matched controls

(iii) To determine lipid status, including plasma cholesterol and triglycerides, and red blood cell fatty acid profiles of newly diagnosed MCI or AD individuals relative to healthy age- and gender- matched controls

The cytokinesis-block micronucleus cytome (CBMN-Cyt) assay was the main assay used in these experiments as previous studies conducted at CSIRO (Nutrigenomics and Genome Health Laboratory) have shown that lymphocytes are an ideal tool for measuring the effect of genome stability (e.g. micronuclei, nucleoplasmic bridges, and nuclear buds) in cells exposed to endogenous or exogenous genotoxins or under conditions of micronutrient deficiency.

Findings from the *in vitro* study showed that (i) extracellular Aβ40 is not genotoxic or cytotoxic, (ii) extracellular Aβ42 reduces nuclear division capacity and induces significantly elevated rates of necrosis in human peripheral lymphocytes, but does not appear to have a strong effect on chromosomal DNA damage markers in the CBMN-Cyt assay and (iii) no interactive effect was found between folic acid and extracellular Aβ42.

Results from the *in vivo* study showed that (i) the genome instability biomarker, nuclear buds, is significantly elevated in South Australians with MCI and, together with micronuclei and nucleoplasmic bridges, is associated with poor global cognitive

function scores as measured using the Mini-Mental State Examination, (ii) micronutrients, organ function and stress biomarker profiles in plasma are significantly altered in newly diagnosed MCI and AD individuals compared to healthy controls, and (iii) lipid profiles are also significantly altered in newly diagnosed MCI and AD cases compared to healthy controls. These findings shed some light on the etiopathogenesis of MCI and AD, which may be relevant in identifying those at increased risk of these neurodegenerative diseases and in determining the design and assessing the efficacy of future intervention studies.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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 any 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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Sau Lai Lee (……2014)

Acknowledgements

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Abbreviations

5,10-MeTHF	5,10-methylenetetrahydrofolate
5-MeTHF	5-methyltetrahydrofolate
8-OHdG	8-hydroxydeoxyguanosine
ABCA7	ATP-binding cassette, sub-family A (ABC1), member 7
AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive
ADNI	Alzheimer's Disease Neuroimaging Initiative
AIBL	Australian Imaging, Biomarker & Lifestyle Study of Ageing
AICD	Amyloid Precursor Protein Intracellular Domain
ApoAI	Apolipoprotein AI
ApoB	Apolipoprotein B
APOE	Apolipoprotein gene
ApoE	Apolipoprotein protein
APOEε4	Apolipoprotein E allele 4
Apop	Apoptosis
APP	Amyloid Precursor Protein
APP-CTF83	Amyloid Precursor Protein 83 amino acid C-terminal Fragment
APP-CTF99	Amyloid Precursor Protein 99 amino acid C-terminal Fragment
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
Aβ40	Beta amyloid 40
Aβ42	Beta amyloid 42
B2M	Beta-2 Microglobulin
BACE	Beta site of Amyloid protein Cleaving Enzyme
BDNF	Brain-Derived Neurotrophic Factor
BER	Base Excision Repair
BFB	Breakage-Fusion-Bridge Cycle
BIN1	Bridging Integrator 1
BM-Cyt	Buccal Micronucleus Cytome Assay
BN	Binucleated cell
Ca	Calcium
CBMN-Cyt	Cytokinesis-Block Micronucleus Cytome assay
CD2AP	CD2-associated protein
CD33	CD33 molecule
CDR	Clinical Dementia Rating scale
CHOL	Cholesterol
CI	Confidence Interval
CIR	Circular cell

CLU	Clusterin
Co (I)/(III)	Cobalamin monovalent state/ trivalent state
CON-AD	Age- and gender- matched controls for Alzheimer's Disease
CON-MCI	Age- and gender- matched controls for Mild Cognitive Disease
COX-2	Cyclooxygenase-2
CR1	Complement component receptor 1
CSF	Cerebrospinal Fluid
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CT	Computed Tomography
Cu	Copper
CV	Coefficient of Variation
Cyto-B	Cytochalasin-B
DHA	Docosahexaenoic Acid
DHF	Dihydrofolate
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dTMP	Deoxythymidine Monophosphate
dUMP	Deoxyuridine Monophosphate
ECLIA	Electro-Chemiluminescence Immunoassay
EDTA	Ethylenediaminetetraacetic Acid
eGFR	estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked Immunosorbent Assay
EPHA1	Ephrin type-A receptor 1
FA	Fatty Acids (Chapter 7)
FA	Folic Acid (Chapter 4)
FADs	Fatty Acid Desaturase gene
FAM	Folic Acid replete RPMI-1640
FBS	Foetal Bovine Serum
FDM	Folate-Deficient RPMI-1640
Fe	Iron
FISH	Fluorescence *in situ* hybridisation
fMRI	functional Magnetic Resonance Imaging
GC-MS	Gas Chromatography-Mass Spectrometry
HBSS	Hanks Balanced Salt Solution
Hcy	Homocysteine
HDL-CHOL	High Density Lipoprotein Cholesterol
HF	High Folate
HNE	4-Hydroxynonenal
HPLC	High-Performance Liquid Chromatography
HS	Horse-Shoe cell
ICP-OES	Inductively Coupled Plasma Atomic Emission Spectroscopy

IL-2	Interleukin 2
IMVS	Institute of Medical and Veterinary Science
K	Potassium
K-EDTA	Potassium-Ethylenediaminetetraacetic Acid
LCPUFA	Long Chain Polyunsaturated Fatty Acids
LDL/HDL	Low Density Lipoprotein Cholesterol/High Density Lipoprotein Cholesterol Ratio
LDL-CHOL	Low Density Lipoprotein Cholesterol
LF	Low Folate
Lith/Hep	Lithium/Heparin
LTL	Leukocyte Telomere Length
MCI	Mild Cognitive Impairment
MD	Mixed Dementia
MDRD	Modification of Diet in Renal Disease
Mg	Magnesium
MMA	Methylmalonic Acid
MMCoA MUTASE	Methyl-Malonyl CoA Mutase
MMSE	Mini–Mental State Examination
MN	Micronuclei/ Micronucleus
MRI	Magnetic Resonance Imaging
MS4A4/MS4A6E	Membrane-spanning 4-domains, subfamily A, member 4/6E
MTHFR	Methylenetetrahydrofolate Reductase
MTR	Methionine Synthase
MTRR	Methionine Synthase Reductase
MUFA	Monounsaturated Fatty Acids
Na	Sodium
NADPH-oxidase	Nicotinamide Adenine Dinucleotide Phosphate-Oxidase
NBUD	Nuclear Buds
NDI	Nuclear Division Index
Necro	Necrosis
NF-κB	Nuclear-transcription Factor Kappa B
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NPB	Nucleoplasmic Bridges
NS	Not Significant
OGG1	Oxoguanine Glycosylase
OR	Odd Ratio
OS	Oxidative Stress
P	Phosphorus
PARP	Poly ADP Ribose Polymerase
PBL	Peripheral Blood Lymphocytes/ Peripheral Blood Leukocytes

PCR	Polymerase Chain Reaction
PD	Parkinson's Disease
PET	Positron Emission Tomography
PHA	Phytohaemagglutinin
PiB	Pittsburgh compound B
PICALM	Phosphatidylinositol binding clathrin assembly protein
PSEN1	Presenilin 1
PSEN2	Presenilin 2
PUFA	Polyunsaturated Fatty Acids
Q-FISH	Quantitative Fluorescent *In Situ* Hybridization
RBC	Red Blood Cell
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
RT	Room Temperature
RT-PCR	Real Time-Polymerase Chain Reaction
S	Sulphur
S.E.M	Standard Error of the Mean
SAH	S-Adenosyl-L-homocysteine
SAM	S-adenosylmethionine
SAND	South Australian Neurodegeneration, Nutrition and DNA damage
sAPP-α	Secreted Amyloid Precursor Protein-α
sAPP-β	Secreted Amyloid Precursor Protein-β
SatFA	Saturated Fatty Acids
SCR	Serum Creatinine concentration
SHMT1	Serine Hydroxymethyltransferase
SOD	Superoxide Dismutase
SPECT	Single Photon Emission Computed Tomography
TAU	TAU gene
tCholesterol	Total Cholesterol
TG	Triglycerides
THF	Tetrahydrofolate
TL	Telomere Length
TS	Thymidylate Synthase
VD	Vascular Dementia
Vit B12	Vitamin B12
Vit B6	Vitamin B6
WAS	Waite Analytical Services laboratory
Zn	Zinc

Publications Arising From This Thesis

1. **Lee SL**, Thomas P, Fenech M. 2014. Genome instability biomarkers and blood micronutrient risk profiles associated with mild cognitive impairment and Alzheimer's disease. Mutat Res-FUND MOL M. Invited Special Issue: DNA damage and human diseases. ***In Press***. DOI 10.1016/j.mrfmmm.2014.12.012.
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2. **Lee SL**, Thomas P, Fenech M. 2013. Extracellular amyloid beta 42 causes necrosis, inhibition of nuclear division, and mitotic disruption under both folate deficient and folate replete conditions as measured by the cytokinesis-block micronucleus cytome assay. Environ Mol Mutagen **55**(1):1-14.
   Impact factor: 2.55

3. **Lee SL**, Thomas P, Hecker J, Faunt J, Fenech M. 2014. Chromosomal DNA damage measured using the cytokinesis-block micronucleus cytome assay is significantly associated with cognitive impairment in South Australians. Environ Mol Mutagen **56**(1):32-40.
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Awards Arising From This Thesis

Best Poster Presentation

The effect of folate and Aβ42 on genome stability and cytotoxicity in human lymphocytes measured using cytokinesis-block micronucleus assay. Faculty of Health Sciences Postgraduate Research Conference, 31st August 2012, Adelaide, Australia.

Best Oral Presentation

The effect of folate and Aβ42 on genome stability and cytotoxicity in human lymphocytes measured using cytokinesis-block micronucleus assay. Nutrition Society of Australia Student Presentation Night, 20th November 2012, Adelaide, Australia.

Presentations Arising From This Thesis

International

Year	Presentation
2012	24th Alzheimer's Association International Conference, Vancouver, Canada, 13th-17th July 2012 **Poster presentation:** "Nutrigenomic biomarkers for increased risk of mild cognitive impairment and Alzheimer's disease"
2012	24th Alzheimer's Association International Conference, Vancouver, Canada, 13th-17th July 2012 **Poster presentation:** "The effect of folate and Aβ42 on genome stability and cytotoxicity in human lymphocytes measured using cytokinesis-block micronucleus assay"

National

Year	Presentation
2014	8th Congress International Society of Nutrigenetics/ Nutrigenomics, Gold Coast, 2nd-3rd May 2014 **Early Career Scientist Session Oral Presentation**: "Extracellular Amyloid Beta 42 Causes Necrosis, Inhibition of Nuclear Division, and Mitotic Disruption Under Both Folate Deficient and Folate Replete Conditions as Measured by the Cytokinesis –Block Micronucleus Cytome Assay"
2013	Commonwealth Scientific and Industrial Research Organisation (CSIRO, CAFHS) PhD Post-doctoral and PhD student workshop, Werribee, Australia, 1st – 2nd October 2013 **Oral presentation**: "Nutrition and genome stability profiles of mild cognitive impairment and Alzheimer's disease"
2012	2nd The Science of Nutrition in Medicine and Healthcare, Melbourne, Australia, 4th-6th May 2012 **Oral presentation:** "The effect of folate and Aβ42 on genome stability and cytotoxicity in human lymphocytes measured using cytokinesis-block micronucleus assay"

2012 Nutrition Society of Australia Annual Scientific Meeting, Wollongong, Sydney 27th-30th November 2012
Oral presentation: "The effect of folate and Aβ42 on genome stability and cytotoxicity in human lymphocytes measured using cytokinesis-block micronucleus assay"

2012 Australian Society for Medical Research 2012, Adelaide, Australia, 6th June 2012
Poster presentation: "The effect of folate and Aβ42 on genome stability and cytotoxicity in human lymphocytes measured using cytokinesis-block micronucleus assay"

2012 Faculty of Health Sciences Postgraduate Research Conference 2012, Adelaide, Australia, 31st August 2012
Poster presentation: "The effect of folate and Aβ42 on genome stability and cytotoxicity in human lymphocytes measured using cytokinesis-block micronucleus assay"

2009 1st Nutritional Genomics - The Ageing Brain Symposium, Adelaide 2nd June 2009
Poster presentation: "Nutrigenomic biomarkers for increased risk of mild cognitive impairment and Alzheimer's disease

Statement of Authorship

Publication:

Lee SL, Thomas P, Fenech M. Genome instability biomarkers and blood micronutrient risk profiles associated with mild cognitive impairment and Alzheimer's disease. Mutat Res-FUND MOL M. Invited Special Issue: DNA damage and human diseases. ***In Press***. DOI 10.1016/j.mrfmmm.2014.12.012.

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Wrote manuscript and contributed to planning of article

Signed …… Date ……

Philip Thomas

Contributed to planning of article and provided critical evaluation of the manuscript

Michael Fenech

Contributed to planning of article and provided critical evaluation of the manuscript