DNA Damage, Micronutrient and Lipid Profiles of Human *APOE* ε4 Carriers

A thesis submitted to the University of Adelaide
for the degree of Doctor of Philosophy

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Abstract

Alzheimer's disease (AD) is an increasing global health problem that is expected to affect 65.7 million people by 2030. The major susceptibility gene, the Apolipoprotein (*APOE*) ε4 allele is associated with a 4-fold increased risk for AD if one ε4 allele is present and can advance the age-of-onset of AD by 7-9 years. Carriers of the ε4 allele are also associated with poorer cognitive performance, increased amyloid plaque and neurofibrillary tangle burden and greater levels of neuronal cell death. Previous studies have demonstrated that AD individuals possess elevated levels of genomic instability and an altered lipid and nutritional profile. At present the mechanism by which *APOE* ε4 may accelerate the onset of AD is unknown but may include genomic instability in AD individuals via promoting the intracellular accumulation of the neurotoxic amyloid beta 42 peptide ($A\beta_{42}$) or directly altering the homeostasis of important lipids such as Docosahexaenoic acid (DHA) and cholesterol, which are crucial for optimal brain function. To date, there is limited information regarding the genomic instability profile of APOE ε4 carriers and their nutritional status.

The primary aims of the thesis were to investigate the following hypothesis:

(i) *APOE* ε4 carriers without cognitive impairment have greater levels of chromosomal DNA damage and cell death as measured in human peripheral lymphocytes compared to non-ε4 carriers.

(ii) *APOE* ε4 carriers without cognitive impairment have an altered lipid and nutritional profile compared to non-ε4 carriers.

The thesis comprised of two distinct studies:

(i) An *in vitro* study which investigated whether long term supplementation of two fatty acids present in fish oil, i.e. Docosahexaenoic acid (DHA) and Furan Fatty Acids (FFA) can prevent oxidative stress-induced cell death and DNA damage in an astrocytoma cell lines with or without the *APOE* ε4 allele.

(ii) An *in vivo* study investigating the primary hypothesis that *APOE* ε4 carriers that were not cognitively impaired exhibit greater levels of

chromosomal DNA damage and an altered lipid and nutritional profile compared to non-ε4 carriers.

The Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay was the primary genome instability assay used in both studies, as it is a comprehensive technique that allows chromosomal DNA damage to be measured visually in once-divided cells that are recognised by their binucleate appearance. Examples of chromosomal DNA damage scored include micronuclei (MNi, chromosome breakage and/or loss), nuclear buds (NBUDs, DNA amplification and or removal of DNA repair complexes) and nucleoplasmic bridges (NPBs, DNA misrepair and/or telomere end fusions).

Previous studies have demonstrated that DHA promotes antioxidant defences in the brain, while FFA is emerging as a potential antioxidant and believed to contribute to the benefits of fish oil. The cytotoxic and genotoxic effects of DHA and FFA was investigated in *in vitro* cultures of U87MG (*APOE* ε3/ε3) and U118MG (*APOE* ε2/ε4) astrocytoma cell lines with and without a hydrogen peroxide (H_2O_2, 100 μM) challenge. The *APOE* ε4 cell line was found to be more sensitive to the cytostatic ($P < 0.001$), cytotoxic (i.e., apoptosis, $P < 0.001$) and DNA damaging effects (i.e. MNi, $P < 0.001$; NPBs, $P < 0.001$ and NBUDs, $P < 0.01$) of H_2O_2 when compared to the non-ε4 cell line. DHA at 100 μg/mL significantly affected cytostasis ($P < 0.05$) and increased DNA damage in the form of NPBs and MNi ($P < 0.05$) in both cell lines, whereas it decreased necrosis ($P = 0.0251$) in the non-ε4 cell line. FFA had no effect on chromosomal DNA damage in both cell lines investigated.

Findings from the *in vivo* study suggest that *APOE* ε4 carriers do not experience significantly different rates of cytostasis, cytotoxicity and/or chromosomal DNA damage (MNi, NPBs and NBUDs) as measured by the CBMN-Cyt assay, when compared to non-*APOE* ε4 carriers after correcting for age and gender. However, there was a trend for increased NPBs and NBUDs in homozygous ε4 carriers compared to non-carriers. Analysis of plasma nutrients and lipids showed no significant differences between ε4 and non-ε4 carriers with the exception of Phosphorus ($P = 0.042$), total plasma cholesterol ($P < 0.0001$) and LDL-cholesterol ($P < 0.0001$) which were higher in *APOE* ε4 carriers.

This study was the first to characterise the CBMN-Cyt assay chromosomal DNA damage profile of *APOE* ε4 carriers. Although no statistically significant differences for any of the cytome biomarkers in our cohort was reported, the limited trend for increased NPBs (r = 0.118, $P = 0.06$) suggests the possibility of the loss of systemic genome integrity as measured in surrogate tissues such as lymphocytes but may not adequately explain why *APOE* ε4 carriers are at higher risk of developing AD.

The results of this study are not generalisable to other cohorts with poor lifestyle habits or to non-healthy or older *APOE* ε4 carriers in which the effects of *APOE* ε4 may be more evident (i.e. smokers and obese individuals). In addition, whilst there were no substantial differences in chromosomal DNA damage biomarkers in the form of MNi, NPBs and/or NBUDs in peripheral lymphocytes, DNA damage studies using a large cohort to increase statistical power and other biomarkers such as the buccal micronucleus cytome assay, telomere length and integrity, comet assay and γH2AX would allow a more complete characterisation of the DNA damage profile of *APOE* ε4 carriers and a more definitive assessment of the role of DNA damage in *APOE* ε4-related pathology.

Declaration

I, Ann Chua, the author certify that this work contains no material that has been accepted for the award of any other degree or diploma 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.

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Signed: …… Date: ……

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Presentations and Publications Arising From Thesis

PUBLICATIONS

1. *Effect of Docosahexaenoic Acid and Furan Fatty Acids on Cytokinesis block Micronucleus Cytome Assay biomarkers in Astrocytoma Cell lines Under Conditions of Oxidative Stress.*

   Ann Chua, Philip Thomas, Chakra Wijesundera, Peter Clifton, Michael Fenech

   Environmental and Molecular Mutagenesis. 2014, 55:573-590, **Impact Factor: 2.553**

2. *Chromosomal DNA Damage in APOE ε4 Carriers and Non carriers Does Not Appear to Be Different*

   Ann Chua, Philip Thomas, Peter Clifton, Michael Fenech.

   Environmental and Molecular Mutagenesis, 2015, DOI 10.1002/em.21949, **Impact Factor: 2.553**

PRESENTATIONS

Oral

1. CSIRO, Animal Food and Health Sciences (CAFHS) Post-doc and Student Workshop (2013), *"DNA Damage and Nutritional Risk Factors of APEOε4 Carriers"*

   Chua A, Thomas P, Clifton P, Fenech M

2. CSIRO Preventative Health Flagship Seminar (2011),*"Does APOE ε4 Genotype Predispose to DNA Damage Depending on Nutrition"*

   Chua A, Thomas P, Clifton P, Wijesundra C, Fenech M

3. PHD 2nd Year Progress Review (2011), "DNA Damage in *APOE ε4* carriers and Prevention by Nutritional Supplementation with Antioxidants and Omega-3 Fatty Acids"

   Chua A, Thomas P, Clifton P, Fenech M

Poster

1. **Chua A,** Thomas P, Clifton P, Wijesundra C, Fenech M. (2013) *Effect of Furan Fatty Acid Supplementation in Relation to Oxidative Stress in Astrocytoma Cell Lines*. Nutritional Society of Australia conference, Brisbane, University of Adelaide

2. **Chua A,** Thomas P, Clifton P, Wijesundra C, Fenech M. (2013) *Effect of Furan Fatty Acid Supplementation in Relation to Oxidative Stress in Astrocytoma Cell Lines*. Faculty of Health Sciences Postgraduate Research conference, University of Adelaide

3. **Chua A,** Thomas P, Clifton P, Wijesundra C, Fenech M. (2013) *Effect of Furan Fatty Acid Supplementation in Relation to Oxidative Stress in Astrocytoma Cell Lines*. Australian Society for Medical Research (ASMR) conference, SA

4. **Chua A,** Thomas P, Fenech M. (2010) *DNA Damage in Cells of Human APOE ε4 Carriers and Prevention by Nutritional Supplementation with Antioxidants and Fatty Acids*. Faculty of Health Sciences Postgraduate Research conference, University of Adelaide,

5. **Chua A,** Thomas P, Fenech M. (2010) *DNA Damage in Cells of Human APOE ε4 Carriers and Prevention by Nutritional Supplementation with Antioxidants and Fatty Acids*. Australian Society for Medical Research (ASMR) conference, SA,

Abbreviations

A	
ABCA1	ATP-Binding Casette Transport Protein Family A Member 1
AD	Alzheimer's Disease
Al	Aluminium
AICD	Amyloid Precursor Protein (APP) Intracellular Domain
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APOE	Apolipoprotein gene
APOE	Apolipoprotein
APOE ε2	Apolipoprotein gene variant epsilon 2
APOE ε3	Apolipoprotein gene variant epsilon 3
APOE ε4	Apolipoprotein gene variant epsilon 4
APOE -/-	Apolipoprotein gene Knockout
APOE2	Apolipoprotein E2
APOE3	Apolipoprotein E3
APOE4	Apolipoprotein E4
APOE4-165	Apolipoprotein E4 C-Terminal Fragment with deleted 166-299 amino acids
APOE4-271	Apolipoprotein E4 C-Terminal Fragment with deleted 272-299 amino acids
APP	Amyloid Precursor Protein
ATCC	American Type Culture Collection
Aβ	Amyloid Beta
$A\beta_{40}$	Amyloid Beta 40 peptide
$A\beta_{42}$	Amyloid Beta 42 peptide
B	
BBB	Blood Brain Barrier
BC	Buccal Cells
BFB	Breakage Fusion Bridge
BH	Bad Habit
BN	Binucleate
BN with MNi	Binucleate with Micronuclei
BN with NBUD	Binucleate with Nuclear Buds
BN with NPB	Binucleate with Nucleoplasmic Bridges
BSA	Bovine Serum Albumin
C	
Ca	Calcium
CAFHS	CSIRO Animal, Food and Health Sciences
CBMN-Cyt	Cytokinesis blocked micronucleus cytome assay
CBS	Cystathione Beta Synthase
CCD	Charged Couple Device
Cd	Cadmium
CHO	Chinese Hamster Ovary
CNS	Central Nervous System
Co	Cobalt
COX	Cytochrome-*c* oxidase
CSIRO	Commonwealth Scientific & Industrial Research Organisation
CSF	Cerebrospinal Fluid
CTFα	C-Terminal Membrane Fragment produced by α-secretase
	C-Terminal Membrane Fragment produced by β-secretase

CTFβ	Copper
Cu	Cytochalasin B
CytoB	Myristic Acid
C14:0	Pentadecylic Acid
C15:0	Palmitic Acid
C16:0	Palmitoleic Acid
C16:1 n9	Stearic Acid
C18:0	Oleic Acid
C18:1	Linoleic Acid
C18:2 n6	α-Linolenic Acid
C18:3 n3	γ-Linolenic Acid
C18:3 n6	Arachidic Acid
C20:0	Di-homo-γ-linolenic Acid
C20:3 n6	Arachidonic Acid
C20:4	Eicosapentanoic Acid (EPA)
C20:5 n3	Behenic Acid
C22:0 n6	Erucic Acid
C22:1 n9	Docosapentaenoic Acid
C22:5 n3	Docosahexaenoic Acid (DHA)
C22:6 n3	Tricosylic Acid
C23:0	Lignoceric Acid
C24:0	Nervonic Acid
C24:1	Oleic Acid
$C_{18}H_{34}O_2$	Arachidic Acid
$C_{20}H_{40}O_2$	Lignoceric Acid
$C_{24}H_{48}O_2$	

D

DDR	DNA Damage Responses
DHA	Docosahexaenoic Acid
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DSBs	Double Strand Breaks

E

EMEM	Minimum Essential Medium Eagle
EOAD	Early Onset Alzheimer's Disease
EPA	Eicosapentanoic Acid
ER	Endoplasmic Reticulum
ETC	Electron Transport Chain

F

FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FBS	Fetal Bovine Serum
Fe	Iron
FFA	Furan Fatty Acid

G

GH	Good Habit
GPx	Glutathione Peroxidase
GR	Glutathione Reductase
GSH	Glutathione
GSSG	Glutathione Disulphide

H

HBSS	Hank's Banks Salt Solution
Hcy	Homocysteine
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein-Cholesterol
HNE	4-hydroxy-2-nonenal
H_2O	Water
H_2O_2	Hydrogen Peroxide
HPA	Hypothalamic-Pituitary-Adrenal
HPI	Health Practice Index

I

ICPAES	Inductively Coupled Plasma Atomic Emission Spectrometry
IMVS	Institute of Medical and Veteran Services

K

K	Potassium

L

LDL	Low Density Lipoprotein
LDL-C	Low Density Lipoprotein-Cholesterol
LDLR	Low Density Lipoprotein Lipid Receptor
LH	Lithium-Heparin
LOAD	Late Onset Alzheimer's Disease
LPC	Lysophosphatidylcholine
LRP1	Low Density Lipoprotein Receptor Related Protein
LSC	Laser Scanning Cytometry

M

MCI	Mild Cognitive Impaired
MDA	Malondialdehyde
Mfsd2a	Major Facilitator Superfamily
Mg	Magnesium
mg/L	Milligram per Litre
mmol/L	Millimole per Litre
µmol/L	Micromole per Litre
MMSE	Mini Mental State Examination
MNi	Micronuclei
MONO	Mononucleate
Mo	Molybdenum
MRI	Magnetic Resonance Imaging
mtDNA	Mitochondrial DNA
mtΔ4977	4977 base pair Mitochondrial DNA deletion
MTHFD1	5-10-methylene tetrahydrofolate dehydrogenase 1
MTR	Methionine Synthase
MTRR	Methionine Synthase Reductase
MUFA	Monounsaturated Fatty Acid
MULT	Multinucleate

N

Na	Sodium
NBUDs	Nuclear Buds
NDI	Nuclear Division Index
nDNA	Nuclear DNA
NFQ	Neurofibrillary Tangles
NFTs	Non-fluorescent quencher

NHEJ	Non-Homologous End Joining
Ni	Nickel
nmol/L	Nanomole per Litre
NPB	Nucleoplasmic Bridges
n-3	Omega-3
n-6	Omega-6

O

OH^-	Hydroxyl Radicals
OR	Odds Ratio
OS	Oxidative Stress
$^{\bullet}O_{2-}$	Superoxides

P

P	Phosphorus
Pb	Lead
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
PHA	Phytohaemagglutinin
PHF	Paired Helical Filaments
PLOOH	Phospholipid Hydroperoxides
pmol/L	Picomole per Litre
PSEN-1	Presenilin-1 gene
PSEN-2	Presenilin-2 gene
PUFA	Polyunsaturated Fatty Acids
PUFA (n-3)	Omega-3 Polyunsaturated Fatty Acids
PUFA (n-6)	Omega-6 Polyunsaturated Fatty Acids

R

RAGE	Receptor for advanced glycation end products
RBC	Red Blood Cell
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species

S

S	Sulphur
sAPPα	Soluble N-Terminal APP fragment produced by α secretase
sAPPβ	Soluble N-Terminal APP fragment produced by β-secretase
SD	Standard Deviation
Se	Selenium
SEM	Standard Error Mean
SFA	Saturated Fatty Acid
SNP	Single Nucleotide Polymorphism
SOD	Superoxide Dismutase
SorLA	Sortilin-Related Receptor
SSBs	Single Strand Breaks

T

Tau-P	Phosphorylated Tau
TBARs	Thiobarbitituric Acid-Reactive Substances
TBE	Tris-Borate EDTA
TC	Total Cholesterol
TG	Triglycerides
TGN	Trans Golgi Network

THF	Tetrahydrofolate
TL	Telomere Length

U

UCB	Unconjugated Bilirubin

V

VaD	Vascular Dementia
Vit B_{12}	Vitamin B_{12}
VLDL	Very Low Density Lipoprotein
VLDLR	Very Low Density Lipoprotein Receptor

Z

Zn	Zinc

8-OHdG	8-hydroxy-2-deoxy-guanosine
8-OHG	8-hydroxyguanine
5-MTHF	5-methyl tetrahydrofolate
[13C]-DHA	Isotopically Labelled DHA
24-OHC	24-hydroxycholesterol
24S-OHC	24S-hydroxycholesterol