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Genetic determinants of rates of cognitive decline in preclinical Alzheimer's Disease

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Genetic determinants of rates of cognitive decline in preclinical Alzheimer's Disease

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Declaration

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Abstract

Background

In 2015 the number of people worldwide living with Dementia was 46.8 million, with approximately 50-75% of these cases being clinically defined as Alzheimer's disease (AD). Despite extensive efforts, clinical trials have so far failed to yield a treatment that successfully addresses the underlying cause of AD. This lack of treatment has been suggested, in part, to be a result of late stage of intervention in current clinical trial design. For this reason, greater focus has been placed on preclinical trials and in turn both the identification of individuals at-risk for AD and, amongst these, those that are expected to decline over the course of a trial. While brain imaging to determine Aβ-amyloid burden has utility in identifying individuals with preclinical AD, further work needs to be conducted to determine what influences rates of change during these early disease stages. Of particular focus is the rate of decline in cognitive performance, as it is the primary outcome measure of efficacy in clinical trials. A number of genetic variants have been associated with cognitive performance, however additional research needs to be conducted to accurately understand the influence that genetic variation has on cognition in preclinical AD.

Aims

Initially the aim of this thesis was to assess the combined genetic influence of established AD risk genetic variants on preclinical cognitive performance, specifically using AD-risk effect-size weighted polygenic risk scores (PRSs) (Chapter 2). It was then aimed to evaluate the effects on cognitive rates of change in preclinical AD of genes with *a priori* evidence for association with cognition, both individually (Chapter

3) and then when combined (Chapter 4). The results of the preceding chapters informed the final aim which was to determine a novel method of weighting individual variants in genes associated with AD-risk and/or cognition, for use in a genetic risk score that would improve the prediction of preclinical cognitive rates of change (Chapter 5).

Methods

All studies presented in this thesis utilised data from the highly characterised Australian Imaging, Biomarkers and Lifestyle Study of Aging (AIBL). The AIBL study is a longitudinal cohort study collecting data at 18-monthly intervals, currently consisting of 7.5 years of follow up. Individuals investigated in this thesis had been Positron Emission Tomography (PET) imaged to determine neocortical amyloid burden. Further, all individuals were classified as $A\beta^{high}$ or $A\beta^{low}$ based on tracer specific cut offs. In addition, a subset of these samples underwent lumbar puncture for CSF collection at the study baseline, and $A\beta_{42}$, total-tau and phospho-tau were quantified. Finally, based on the AIBL neuropsychological test battery, three cognitive composites previously developed were calculated for all participants. The cognitive composites investigated were; verbal episodic memory, a statistically driven global cognition composite, and the Pre-Alzheimer's Cognitive Composite.

The AD-risk weighted PRS (Chapter 2) consisted of 22 genetic variants associated with AD classification, and was calculated by weighting individual variants based on their previously published associations with risk for AD. A statistically derived Cognitive Genetic Risk Profile (*Cog-GRP*), specifically driven by verbal episodic memory, was developed using a decision tree analysis (Chapter 4). Finally, a 27 genetic variant cognition weighted PRS (*cw*PRS), was developed and tested in a preclinical AD sample

(Chapter 5). For the *cw*PRS, effect sizes for decline in a verbal episodic memory were determined individually for all variants in a reference sample. The resulting effect sizes were then used to calculate the *cw*PRS for each participant in a test sample (Chapter 5). For both the AD-risk weighted PRS (Chapter 2) and the *cw*PRS (Chapter 5), PRS calculations were conducted with both the inclusion and exclusion of the major genetic risk factor for, Apolipoprotein E (*APOE*).

In all studies, linear mixed models were used to investigate associations between genetic factors, independent or in combination, and longitudinal rates of cognitive performance.

Results

In CN older adults the AD-risk weighted PRS, both including and excluding *APOE,* was positively correlated with brain and blood biomarkers, specifically; brain Aβ burden, CSF total-tau and phospho-tau (Chapter 2). When investigating cognitive performance, specifically in CN $A\beta^{high}$ participants, significant associations with baseline and longitudinal cognition were only observed in the AD-risk weighted PRS with *APOE* (Chapter 2).

When investigating gene variants previously reported to influence cognition, in CN $A\beta^{high}$ participants, no independent associations were observed for any variant (Chapter 3). However, in the same sample, after interaction with *APOE* ε4, significant associations were observed for variants in the Kidney Brain Expressed Protein (*KIBRA*) and Spondin-1 (*SPON1*) genes (Chapter 3). The combination of variants investigated in Chapter 3, with additional variants, resulted in the development of the *Cog-GRP*

(Chapter 4). The *Cog-GRP* was able to delineate four groups: *APOE* ε4+ Risk, *APOE* ε4+ Resilient, *APOE* ε4- Risk, *APOE* ε4- Resilient, with the ε4+ Risk group reporting significantly faster decline in cognition than all other groups (Chapter 4).

Finally, a PRS encompassing a combination of AD-risk genes (Chapter 2) and cognitive-risk genes (Chapters 3 and 4), weighted by episodic memory (*cw*PRS), was reported to be associated with preclinical longitudinal cognitive performance (Chapter 5). Further, these associations were observed irrespective of the presence or absence of *APOE* in the calculation of the *cw*PRS (Chapter 5).

Conclusions

The work presented in this thesis provides an in depth investigation of genetic influences in preclinical AD, particularly on cognitive performance. Importantly, it supports the hypothesis that there is are differences between the genetic architectures of AD-risk and AD progression. The results presented here support the use of combinatory approaches when investigating genetic influence. Finally, reported here is a novel method for PRS weighting, with the ability to predict preclinical cognitive performance in the presence and absence of *APOE*. Further investigation is required in cohorts with comparable data to the AIBL study, to validate the methods explored in this thesis, allowing for their eventual use in a clinical setting.

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Statement of Contribution of others

The thesis *"Genetic determinants of rates of cognitive decline in preclinical Alzheimer's Disease"* is submitted as a series of publications for the Degree of Doctor of Philosophy, by Tenielle Porter, School of Medical and Health Sciences, Edith Cowan University, 2018. Below is a statement of Contribution of others for the publications included.

Publication Reference	**Contribution by Tenielle Porter (%)**
Chapter 1	
Porter, T., Gozt, A.K., Mastaglia, F.L., Laws S.M. Genetics of Neurodegenerative D*isease:* Role of genetics in Alzheimer's and Parkinson's Disease. In: Martins, RN and Brenan, C (eds) Factors that Modify Risk of Alzheimer's Disease: The role of Genes, Diabetes, Diet, Exercise and Hormones (2018) Wiley, UK. *Submitted 21.03.2018*	*TP 51%* (literature review, chapter preparation)
Chapter 2	
Porter T, Burnham SC, Milicic L, Savage G, Maruff P, Lim YY, Li QX, Ames D, Masters CL, Rainey-Smith SR, Rowe CC, Salvado O, Groth D, Verdile G, Villemagne V, Laws SM., for the AIBL research group. Utility of an Alzheimer's risk-weighted polygenic risk score for predicting rates of cognitive decline in preclinical Alzheimer's disease: a prospective longitudinal study. *Submitted 03.04.2018; Alzheimer's Research and Therapy*	*TP 60%* (literature review, study design, genetic data collection, data analysis, manuscript preparation)
Chapter 3	
Porter T, Burnham SC, Dore V, Savage G, Bourgeat P, Begemann K, Milicic L, Ames D, Bush AI, Maruff P, Masters CL, Rowe CC, Rainey-Smith SR, Martins RN, Groth D, Verdile G, Villemagne V, Laws SM. *KIBRA* is associated with altered rates of Aβ-amyloid and *APOE* ε4 related cognitive decline and neurodegeneration in cognitively normal adults *Sci Rep. 2018 Feb 1;8(1):2034. doi: 10.1038/s41598-018-20513-y. PMID: 29391469*	*TP 60%* (literature review, study design, genetic data collection, data analysis, manuscript preparation)

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Porter T, Burnham SC, Milicic L, Savage G, Maruff P, Lim YY, Ames D, Masters CL, Rainey-Smith SR, Rowe CC, Salvado O, Groth D, Verdile G, Villemagne V, Laws SM., for the AIBL research group. *COMT* val158met is not associated with Aβ-amyloid and *APOE* ε4 related cognitive decline in cognitively normal older adults. *Final draft for submission; Neurobiology of Aging (Genetic Report)**	*TP 65%* (literature review, study design, genetic data collection, data analysis, manuscript preparation)
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Chapter 4

Porter T, Villemagne V, Savage G, Milicic L, Lim YY, Maruff P, Masters CL, Ames D, Bush AI, Rainey-Smith SR, Rowe CC, Groth D, Taddei K, Martins RN, Verdile G, Burnham SC, Laws SM., for the AIBL research group. Cognitive Gene Risk Profile for the Prediction of Cognitive Decline in Presymptomatic Alzheimer's Disease. *Personalized Medicine in Psychiatry March 2018 7-8:14-20. doi:10.1016/j.pmip.2018.03.001*	*TP 60%* (literature review, study design, genetic data collection, data analysis, manuscript preparation)

Chapter 5

Porter T, Burnham SC, Savage G, Maruff P, Lim YY, Milicic L, Peretti M, Ames D, Masters CL, Martins RN, Rainey-Smith SR, Rowe CC, Salvado O, Taddei K, Groth D, Verdile G, Villemagne V, Laws SM. A polygenic risk score derived from episodic memory weighted genetic variants is associated with cognitive decline in preclinical Alzheimer's disease. *Submitted 01.05.2018; JAMA Neurology*	*TP 65%* (literature review, study design, genetic data collection, data analysis, manuscript preparation)

I, *Tenielle Porter,* contributed to the above listed publications at the stated level.

Signed: Date: 30/04/2018

* At time of submission of the thesis these manuscripts were not yet submitted, but are hereby certified as being at a stage approved by co-authors to be ready for submission for peer-review

_____ **Date:** _____

Assoc. Prof. Simon M Laws *(Principle Supervisor and Corresponding Author)*

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

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Publications

Accepted publications contributing to the thesis

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Porter T, Villemagne V, Savage G, Milicic L, Lim YY, Maruff P, Masters CL, Ames D, Bush AI, Rainey-Smith SR, Rowe CC, Groth D, Taddei K, Martins RN, Verdile G, Burnham SC, Laws SM., for the AIBL research group. Cognitive Gene Risk Profile for the Prediction of Cognitive Decline in Presymptomatic Alzheimer's Disease. *Personalized Medicine in Psychiatry 2018 7-8:14-20. doi:10.1016/j.pmip.2018.03.001*

Publications accepted through the course of the PhD but not contributing to the thesis

Porter T, Bharadwaj P, Groth D, Paxman A, Laws SM, Martins RN, Verdile G. The effects of latrepirdine on amyloid beta aggregation and toxicity. *J Alzheimers Dis 2016 50(3): 895-905. doi: 10.3233/JAD-150790. PMID: 26836170*

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List of Abbreviations

AA	Alzheimer's Assocation
Aβ	Amyloid β
ABCA7	ATP Binding Cassette Subfamily A Member 7 Gene
AD	Alzheimer's Disease
ADAD	Autosomal Dominant AD
ADPD	Autosomal Dominant PD
ADAM10	A Disintegrin And Metalloprotease 10 Gene
ADAS-COG	Alzheimer's Disease Assessment Scale – Cognitive Section
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADRDA	Alzheimer's Disease and Related Disorders Association
AIBL	Australian Imaging, Biomarkers and Lifestyle
AICD	APP Intracellular Domain
ANOVA	Analysis of Variance
APOE	Apolipoprotein E Gene
ApoE	Apolipoprotein E Protein
ApoJ	Apolipoprotein J Protein/Clusterin Protein
AP2	Adaptor Protein Complex 2
APP	Amyloid Precursor Protein Gene
APP	Amyloid Precursor Protein
ATP	Adenosine Triphosphate
ATP13A2	Probable Cation-Transporting ATPase 13A2 Gene
AUC	Area Under the Curve
BACE-1	β-Site APP Cleaving Enzyme

BDNF	Brain Derived Neurotropic Factor Gene
BeCKeT	Before the Centiloid Kernel Transformation
BIN1	Bridging Integrator 1 Gene
Bin1	Bridging Integrator 1 Protein
BNT	Boston Naming Test
BST1	Bone Marrow Stromal Cell Antigen 1
C99	C-Terminal of APP
CAA	Cerebral Amyloid Angiopathy
CASS4	Cas Scaffolding Protein Family Member 4 Gene
CD2AP	CD2-Associated Protein Gene
CD33	Myeloid Cell Surface Antigen CD33 Gene
CELF1	CUGBP, Elav-Like Family Member 1 Gene
CDR	Clinical Dementia Rating
CDR_{SB}	CDR Sum of Boxes
CHMP2B	Charged Multivesicular Body Protein 2B Gene
CI	Confidence Interval
CLU	Clusterin Gene
CLU	Clusterin Protein
CN	Cognitively Normal
CNS	Central Nervous System
Cog-GRP	Cognitive Genetic Risk Profile
COMT	Catechol-O-Methyltransferase Gene
COWA	Controlled Oral Word Association Test
CR1	Complement Receptor 1 Gene
CSF	Cerebrospinal Fluid

CSMD1	CUB and Sushi Multiple Domains 1
CVLT-II	California Verbal Learning Test-Second Edition
$CVLT_{FP}$/$CVLT_{LDFR}$	CVLT False Positives and Long Delay Free Recall
*cw*PRS	Cognitive Weighted PRS
DGKQ	Diacylglycerol Kinase Theta Gene
DJ-1	Protein Deglycase DJ-1 Gene
D-KEFS	Delis-Kaplan Executive Function System
DNA	Deoxyribonucleic Acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5th Edition
ECM	Extracellular Matrix
EEG	Electroencephalography
EIF4G1	Eukaryotic Translation Initiation Factor 4 Gamma 1 Gene
ELISA	Enzyme-Linked Immunosorbent Assays
EOAD	Early Onset AD
EOFAD	Early Onset Familial AD
EOPD	Early Onset PD
EPHA1	EPH Receptor A1 Gene
FBXO7	F-Box Protein 7 Gene
FDG	^{18}F-Fluorodeoxyglucose
FDR	False Discovery Rate
FERMT2	Fermitin Family Member 2 Gene
fMRI	Functional MRI
FSIQ	WAIS-III Full Scale Intelligence Quotient
FTLD	Frontotemporal Lobar Degeneration

GAK	Cyclin G-Associated Kinase Gene
GBA	Glucocerebroside Gene
GERARD1	Genetic and Environment Risk in AD Consortium 1
GDS	Geriatric Depression Scale
GIGYF2	PERQ Amino Acid-Rich with GYF Domain-Containing Protein 2 Gene
GSK3	Glycogen Synthase Kinase 3
GWA	Genome Wide Association
GWAS	Genome-Wide Association Study
HABS	Harvard Aging Brain Study
HLA	Human Leukocyte Antigen
HLA-DRA	HLA Class II Histocompatibility Antigen, DR Alpha Chain Gene
HLA-DRB1	Major Histocompatibility Complex Class II, DR Beta 1 Gene
HLA- DRB5	Major Histocompatibility Complex Class II, DR Beta 5 Gene
HLA-DQB1	Major Histocompatibility Complex, Class II, DQ Beta 1 Gene
HTRA2	HtrA Serine Peptidase 2 Gene
IGAP	International Genomics of Alzheimer's Project
IGF	Insulin-Like Growth Factor
IL-1β	Interleukin 1 β
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-18	Interleukin 18
INPP5D	Inositol Polyphosphate-5-Phosphatase Gene

IQ	Intelligence Quotient
JPD	Juvenile PD
KIBRA	Kidney Brain Expressed Protein Gene
KIBRA	Kidney Brain Expressed Protein
KL	Klotho Gene
LDL	Low Density Lipoprotein
LDLR	Low Density Lipoprotein Receptor
LME	Linear Mixed Effects
LMI/LMII	Logical Memory I and II
LOAD	Late Onset AD
LOPD	Late Onset PD
LRP1	Low Density Lipoprotein Receptor-Related Protein-1
LRRK2	Leucine-Rich Repeat Kinase 2 Gene
MAF	Minor Allele Frequency
MAPK	Mitogen-Activated Protein Kinase
MAPT	Microtubule-Associated Protein Tau Gene
MCI	Mild Cognitive Impairment
MeDi	Mediterranean Diet
MEF2C	Myocyte Enhancer Factor 2C Gene
MHC	Major Histocompatibility Complex
MMSE	Mini-Mental State Examination
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MS4A	Membrane Spanning 4 Domains, Subfamily A

MS4A4A	Membrane Spanning 4 Domains, A4A Gene
MS4A6A	Membrane Spanning 4 Domains, A6A Gene
mtDNA	Mitochondrial DNA
NADH	Nicotinamide Adenine Dinucleotide
NART	North American National Adult Reading Test
NCD	Neurocognitive Disorder
ND3	NADH Dehydrogenase 3
NDPK	Nucleoside Diphosphate Kinase
NFT	Neurofibrillary Tangles
NGS	Next Generation Sequencing
NIA	National Institute on Aging
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
NME8	NME/NM23 Family Member 8 Gene
OR	Odd Ratio
PACC	Pre-Alzheimer's Cognitive Composite
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PD	Parkinson's Disease
PiB	^{11}C-Pittsburgh Compound B
PICALM	Phosphatidylinositol Binding Clathrin Assembly Lymphoid Myeloid Protein Gene
PINK-1	PTEN-Induced Putative Kinase 1 Gene
PKCζ	Protein Kinase C Isoform ζ
PKMζ	Protein Kinase M Isoform ζ

PLA2G6	Phospholipase A2 Group 6 Gene
PLD3	Phospholipase D3 Gene
PLOSL	Polycystic Lipomembranous Osteodysplasia with Sclerosing Leukoencephalopathy
PSEN1	Presenilin 1 Gene
PSEN2	Presenilin 2 Gene
PRKN	Parkin Gene
PRS	Polygenic Risk Score
PTC	Premature Termination Codon
PTK2B	Protein Tyrosine Kinase 2β Gene
RA	Rheumatoid Arthritis
RAVLT	Rey Auditory Verbal Learning Test
RCFT	Rey Complex Figure Test
RIN3-	Rab Interactor 3 Gene
RIT2	Ras Like Without CAAX 2 Gene
ROC	Receiver Operating Characteristic Curve
ROCF	Rey-Osterrieth Complex Figure Test
SAD	Sporadic AD
SETD1A	SET Domain Containing 1A
SD	Standard Deviation
SLC24A4	Solute Carrier Family 24 Sodium/Potassium/Calcium Exchanger, Member 4 Gene
SLE	Systemic Lupus Erythematosus
SNARE	SNAP (Soluble NSF(N-ethylmaleimide-sensitive factor) Attachment Protein) Receptor

SNCA	α-Synuclein Gene
SNP	Single Nucleotide Polymorphism
SORL1	Sortilin-Related Receptor LDLR Class A Repeats Containing Gene
SPON1	Spondin 1 Gene
SUVR	Standardized Uptake Value
SUVR	Standardized Uptake Value Ratios
TNFα	Tumor Necrosis Factor α
TREM2	Triggering Receptor Expressed on Myeloid Cells 2 Gene
UCHL1	Ubiquitin Carboxy-Terminal Hydrolase L1 Gene
UPS	Ubiquitin Proteasome System
USD	United States Dollar
VAMP2	Vesicle Associated Membrane Protein 2
VLDL	Very Low Density Lipoprotein
VPS35	Vacuolar Protein Sorting 35 Gene
WTAR	Wechsler Test of Adult Reading
WAIS-III	Wechsler Adult Intelligence Scale-Third Edition
WMS	Wechsler Memory Scale
WWC1	WW Domain-Containing Protein 1
ZCWPW1	Zinc Finger, CW Type With PWWP Domain 1 Gene

Chapter 1

Please note that this online copy of the thesis does not contain the complete version of Chapter 1

CHAPTER 1: General Introduction

1.1 Dementia and Alzheimer's Disease

In 2015 there were almost 900 million people over the age of 60 [1]. As the global population continues to age there is a growing focus on age-associated diseases such as Dementia. Dementia is described generally as progressive decline in a patient's cognitive functioning greater than what is expected to occur in normal aging. The term "Dementia" does not define a single disease, but describes several diseases typified by the detrimental changes in brain function. Domains in which these changes occur include language, memory, perception, personality and cognitive skills [2].

In 2015 46.8 million people globally were living with dementia, with this number expected to double every 20 years [1]. It was estimated that there are 9.9 million new cases a year, or one new case every 3.2 seconds [1]. In Australia alone there are approximately 410,000 people with Dementia [3]. Further, in 2015 dementia was the second leading cause of death in Australia [4].

In addition to the human cost of dementia there is a significant economic cost. The global cost of dementia increased by USD$214 billion dollars between 2010 and 2015, rising from USD$604 to USD$818 billion [1]. These costs include social care, professional and volunteer, and medical care [1]. It has been estimated that dementia will cost USD$1 trillion by 2018 [1]. It has been predicted that by delaying the onset of dementia by 5 years, the number of people with the condition in Australia could be

reduced by around one third [5]. Additionally, in America, a study was conducted that reported delaying the onset of Alzheimer's disease (AD) by 5 years would result in an economic saving of USD$935 billion over 10 years [6].

It has been reported that 50-75% of dementia cases are clinically defined as Alzheimer's Disease (AD) [2, 7]. AD is characterised by neuronal loss, abnormal protein deposition in the brain, and the deterioration of both cognitive function and the ability to perform activities of daily living.

1.1.1 Pathological Features

The pathological features of AD can be divided into macroscopic structural changes to the brain, and the presence in increased concentrations of extra- and intracellular fibrous protein deposits. Importantly, it is generally increased concentrations of these proteins, due to inefficient clearance or over production, which results in pathogenicity [8]. It has previously been observed that low concentrations have a non-pathogenic effect in cognitively normal older adults (CN) [9].

1.1.1.1 Cerebral Atrophy

The major macroscopic hallmark of AD is the progressive loss of brain volume, termed cerebral atrophy (Figure 1.1.1). The brain regions most significantly affected by atrophy have been reported to change as the disease develops [10, 11]. It has been observed that hippocampal atrophy occurs earliest in the disease process, followed by atrophy of the temporal parietal lobes, and in the late disease stages the frontal lobe [10]. Significant atrophy of the medial parietal lobe has been observed throughout the disease [10, 12]. A number of studies have reported hippocampal volume significantly

reduced in Mild Cognitive Impairment (MCI) [13] and AD [13] when compared to cognitively normal (CN) individuals. Additionally, longitudinal rates of hippocampal atrophy have been associated with progression to AD (from MCI) [14], and classification of AD [15].

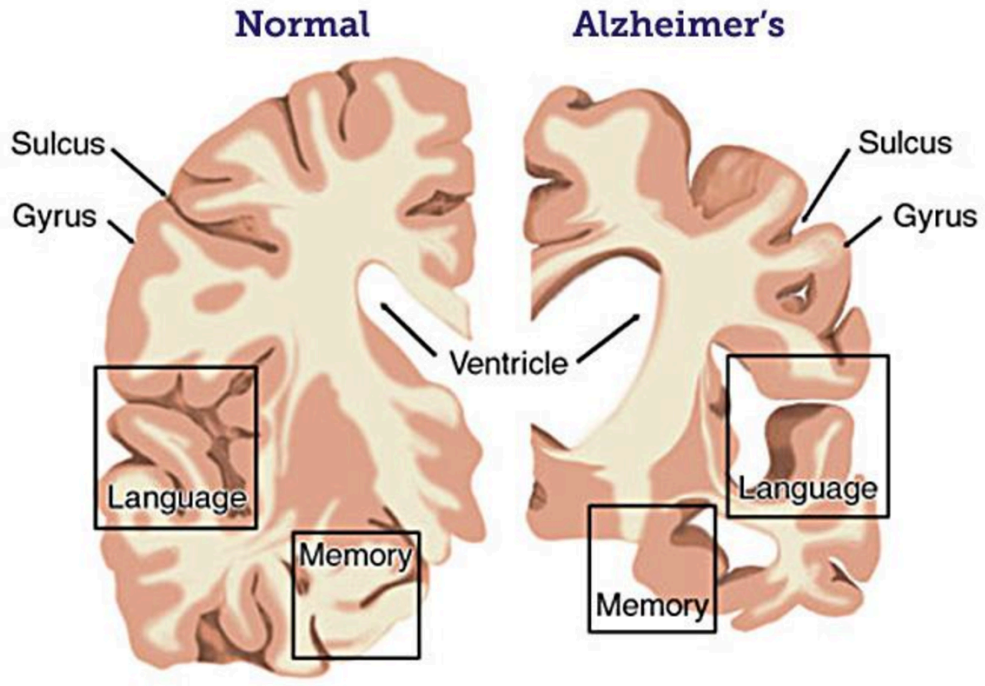

Figure 1.1.1. Cerebral Atrophy

Representation of the brain of a cognitively normal older adult when compared to that of an Alzheimer's patient, displaying the gross structural changes associated with the disease. Image sourced from the BrightFocus Foundation (2000).

1.1.1.2 Cerebral Amyloid Angiopathy

The accumulation of Amyloid Beta (Aβ-amyloid; hereby abbreviated to Aβ) in the cerebral and meningeal blood vessels, Cerebral Amyloid Angiopathy (CAA), is a prominent hallmark in AD (Figure 1.1.2, [16]). The most common, and most severely affected, brain region affected by CAA is the occipital lobe, followed by the parietal, frontal and temporal [17-19]. Sporadic age-related CAA has been observed in the brains of healthy elderly individuals [20, 21], however it is more prevalent in those of AD patients [21, 22]. Further, CAA has been reported occurring more frequently in pathology confirmed AD than in other diseases leading to dementia [18, 21].

1.1.1.3 Senile Amyloid Plaques

Senile amyloid plaques, or neuritic plaques, are the most commonly associated pathological hallmark of AD (Figure 1.1.3). Senile amyloid plaques have been used in the post-mortem diagnosis of AD, due to their occurrence being uncommon in other neurodegenerative disorders. The initial deposition of amyloid plaques occurs through the frontal, parietal, temporal, or occipital neocortex [24]. These areas are followed by the entorhinal region and addition subcortical regions, and eventually the cerebellum and brainstem [24]. A number of protein components make up amyloid plaques including; a core of insoluble Aβ [25, 26], surrounded by apolipoprotein E (ApoE), α2-macroglobulin, interleukins, α2-macroglobulin receptor, and low-density lipoprotein receptor-related protein [27-30].

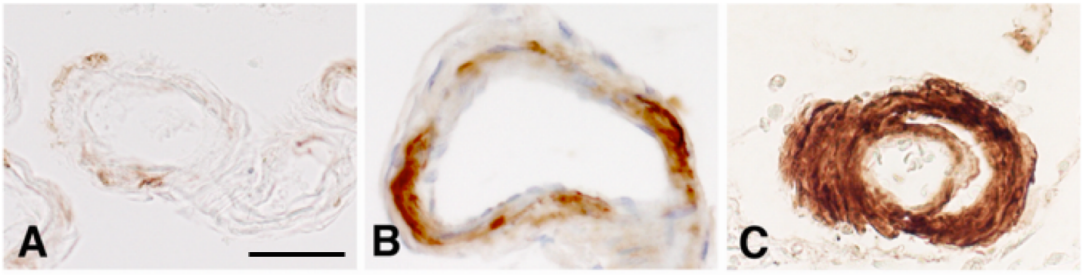

Figure 1.1.2. Cerebral Amyloid Angiopathy

(A) Mild, (B) moderate, and (C) severe cerebral amyloid angiopathy (CAA) in the meningeal vessels of the temporal lobe, immunohistochemistry with anti-amyloid antibody 4G8, scale bar: 50 μm. Image sourced from [23].

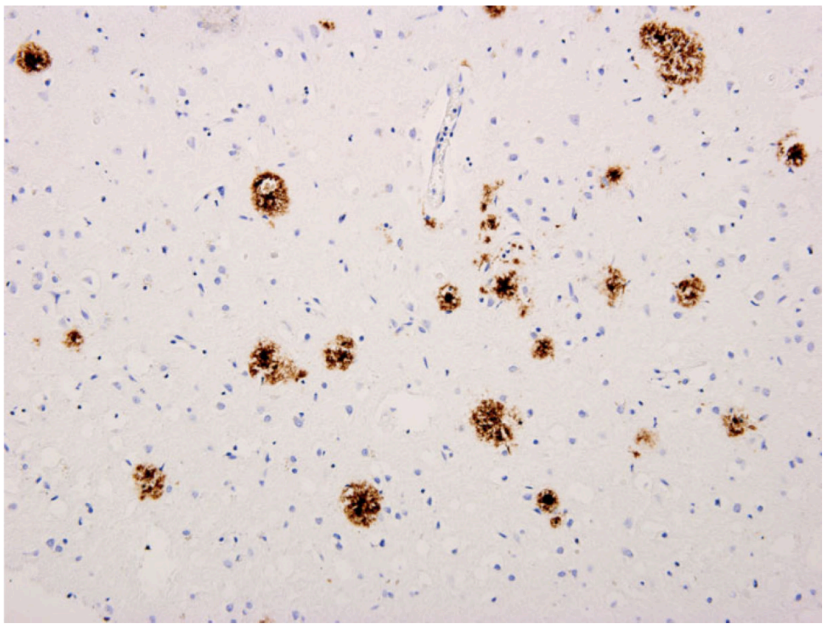

Figure 1.1.3. Senile Amyloid Plaques

Advanced stage AD patient's temporal cortex with senile amyloid plaques evident (brown), immunochemistry with anti-amyloid antibody 4G8, 100× magnification. Image sourced from [31].

1.1.1.3.1 Amyloid Beta

Aβ, the major component of senile amyloid plaques, is a small 4-5kDa protein found in the brain, CSF and blood of CN older adults and AD patients [26]. Proteolytic cleavage of the amyloid precursor protein (APP), a 110-135kDa transmembrane glycoprotein, results in the generation of Aβ and other peptides. The cleavage of APP to produce Aβ is via the amyloidogenic pathway (Figure 1.1.4A, E-G), which occurs in healthy CN individuals but is favoured in AD. APP is first cleaved at the N-terminus of the Aβ domain by β-site APP cleaving enzyme (BACE-1) (Figure 1.1.4E), producing soluble APPβ and the C-terminal of APP (C99; Figure 1.1.4F). C99 is then cleaved within the transmembrane domain by γ-secretase, releasing Aβ and the APP intracellular domain (AICD; Figure 1.1.4F, G). Cleavage of APP via the non-amyloidogenic pathway involves cleavage within the Aβ domain by α-secretase, precluding Aβ production (Figure 1.1.4A-D). Due to differences in cleavage sites several Aβ isoforms are produced including; $A\beta_{1\text{-}42}$, $A\beta_{4\text{-}42}$, $A\beta_{1\text{-}40}$ and $pGluA\beta_{3\text{-}42}$. It has been shown that in the AD brain, there are higher concentrations of $A\beta_{1\text{-}42}$, the more toxic Aβ isoform. This toxicity is due to its increased ability to aggregate, which results from two additional hydrophobic amino acids at the C terminus of the peptide.

Aβ accumulation occurs by the aggregation of monomeric Aβ into soluble aggregates (dimers, trimers and tetramers), collectively termed oligomers. Evidence suggests these soluble oligomers are the toxic species associated with AD [32-35]. Oligomers further aggregate into protofibrils and fibrils, with the aggregation of fibrils leading to the senile plaque formation. Proposed mechanisms of oligomer toxicity include; mitochondrial dysfunction [36, 37], synaptic toxicity [38], membrane depolarisation [39], oxidative stress [40], and inhibition of long-term potentiation [41, 42].

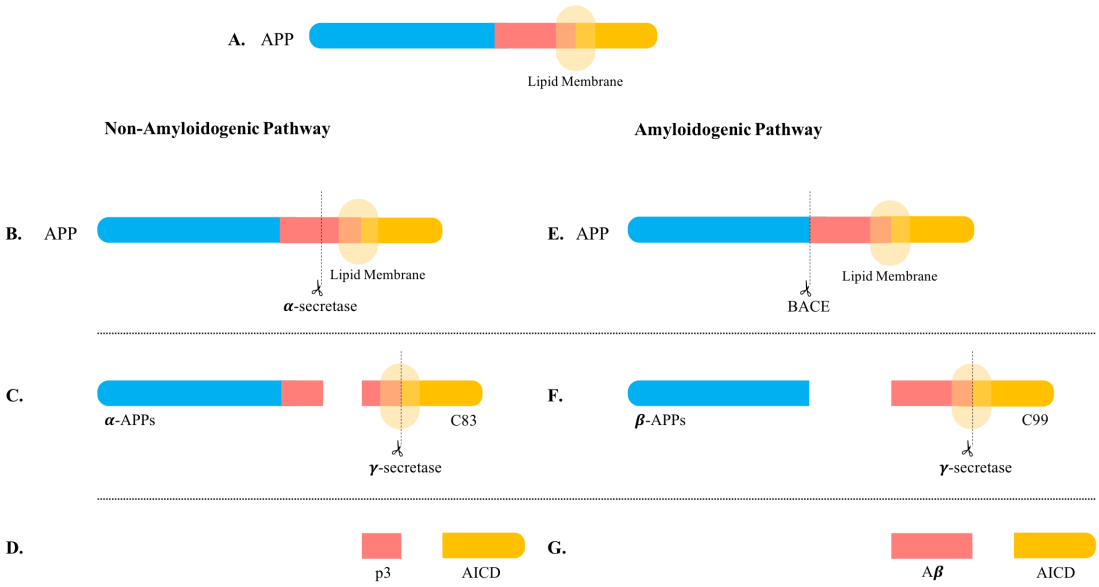

Figure 1.1.4. APP Processing

Non-amyloidogenic (A, B, C, D) and amyloidogenic (A, E, F, G) Amyloid Precursor Protein (APP) processing. In the non-amyloidogenic pathway, APP (A) is cleaved by α-secretase within the Aβ domain (B), releasing α-APPs and C83 (C). C83 is then cleaved by γ-secretase within the transmembrane or lipid membrane (C), releasing p3 and the APP intracellular domain (AICD; D). The amyloidogenic pathway involves APP (A) being cleaved by β-site APP Cleaving Enzyme (BACE-1) within at the N-terminus of the Aβ domain (E), releasing α-APPs and C99 (F). C99 is then cleaved by γ-secretase within the lipid membrane (F), releasing Aβ and AICD (G).

1.1.1.4 Neurofibrillary Tangles

Neurofibrillary tangles (NFT) are described as bundles of paired, helically wound filaments present in the cytoplasm of neurons (Figure 1.1.5, [43]). It has been observed that the density of NFTs is correlated with the severity of AD [44]. NFTs have been shown to be concentrated in the entorhinal cortex, hippocampus, amygdala, and frontal, temporal, and parietal lobes [45]. The main component of NFTs is aggregated hyperphosphorylated insoluble microtubule-associated protein tau (MAPT, Tau) [46-48].

1.1.1.4.1 Tau

Tau, encoded by the *MAPT* gene located on chromosome 17, is expressed in 6 isoforms ranging from 45-65kDa, which are produced by the alternative splicing of the mRNA. The main function of the tau protein is the stabilisation of microtubules which constitute the neuronal cytoskeleton [50]. Phosphorylation of tau occurs normally as a method of microtubule binding regulation [51]. However, hyperphosphorylation is proposed to be, in some cases, a result of up-regulation of kinases that interact with proteins involved in APP processing [52]. The hyperphosphorylated tau is hypothesised to alter the binding of microtubules and result in aggregation [51]. This aggregation leads to the reduction in tau's ability to stabilise dendrite and axon branches, leading to synaptic loss.

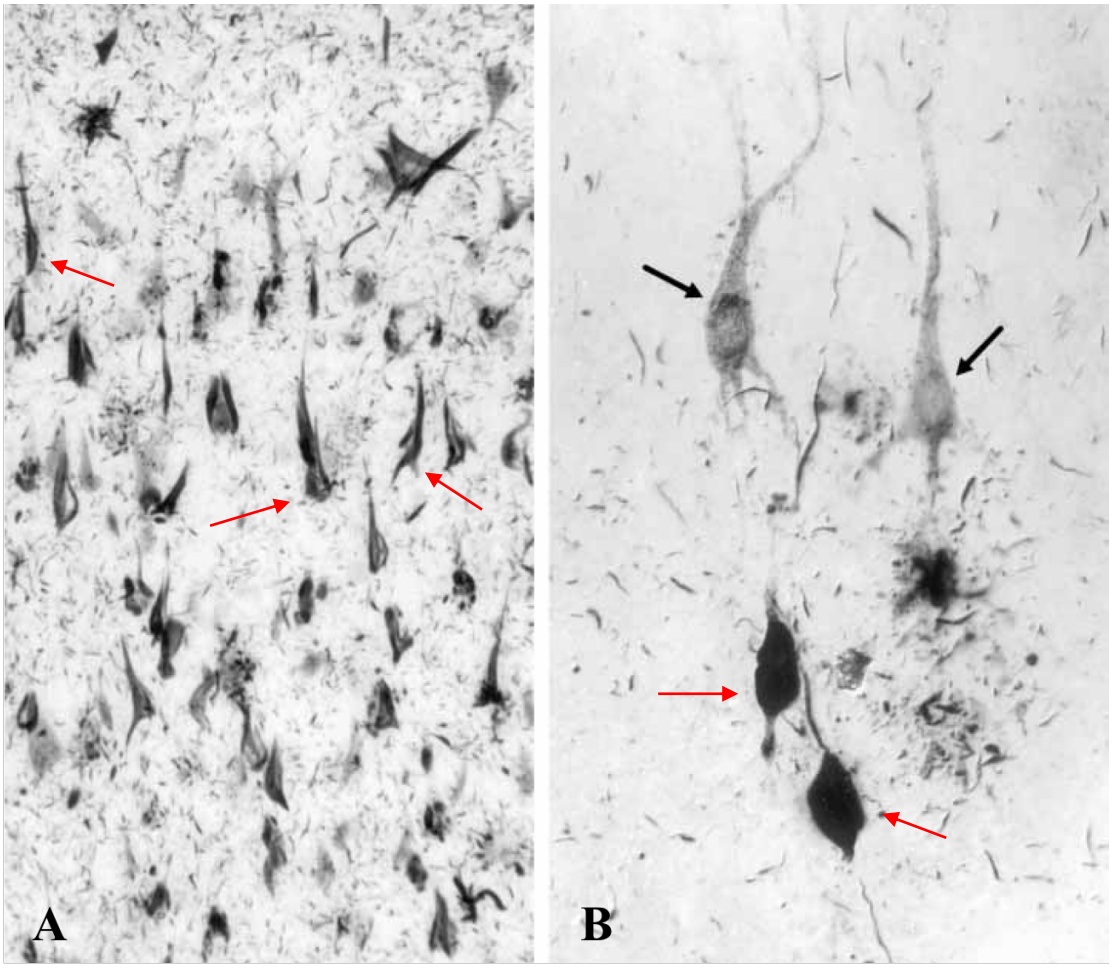

Figure 1.1.5. Neurofibrillary Tangles

Sections of an Alzheimer's disease patient's hippocampus, immunochemistry with anti-tau antibody. (A) A number of neurofibrillary tangles (NFT) are observed with examples highlighted by red arrows (180× magnification). (B) Two NFTs present (red arrows), as well as two neurons with low levels of tau immunoreactivity in the 'pre-tangle' stage (black arrows; 360× magnification). Image sourced from [49].

1.1.2 Clinical Features

The deterioration of memory, cognition and the ability to perform functions required for daily living characterises the clinical presentation of AD [53]. Initially, patients find difficulty in learning and retaining new information with little impact on older memories. A patient in the early disease stages will also begin to struggle when organising complex tasks of daily living [53]. Further, neuropsychological tests will be able to observe in some patients subtle decline in vocabulary and speech fluency [54]. Finally, patients can experience some disorientation and issues with navigation during the early disease stages [55].

This progresses gradually to the loss of recent memories, obvious difficulties when verbally expressing themselves, and the inability to perform functions of daily life without supervision [53]. Additionally, disease progression is accompanied by the deterioration of facets of visual processing including; disorientation, impaired recognition of known faces and delusions [56].

Most cognitive functions are impacted in severely demented AD patients. In particular, loss of early memories and the ability to verbally communicate characterise this stage [54]. Additionally, misunderstandings of carer's actions can lead to aggressive behaviour by patients [57, 58]. During the late disease stage, impairment of daily living tasks becomes so severe it results in a significant reduction in life expectancy [59].

1.1.3 Diagnosis and Monitoring

Definitive AD diagnosis requires post-mortem identification of neuropathological hallmarks in the brain of the patient [60]. However, clinical assessment, cerebrospinal fluid (CSF) and blood biomarkers, and brain imaging can provide tentative diagnoses. Further, these techniques, in particular brain imaging, have the ability to monitor disease progression from preclinical to symptomatic AD.

1.1.3.1 Neuropsychological Testing

In recent years there have been revisions of the criteria previously used in the diagnosis of AD to reflect the increased understanding of the disease. The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria published in 1984, initially characterised a patient's likelihood of having AD (definite, probable, possible, unlikely) based on the number of cognitive domains (memory, language, perceptual skills, attention, constructive abilities, orientation, problem solving, functional abilities) impaired [61]. The National Institute on Aging/Alzheimer's Association (NIA/AA) updated guidelines included the addition of measurements of changes occurring in the brain as measured by biomarkers [62].

The Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) 2013 update included the renaming of Dementia to 'major neurocognitive disorder' (NCD) and the recognition of early cognitive decline (mild NCD) [63]. The DSM-5 diagnoses NCD by the observing cognitive impairment as the defining feature of a patient's impairment. Further, the cognitive symptoms observed must impair the patient's ability to function in daily life [63]. The cognitive domains affected as presented in the DSM-

5 include; complex attention, executive function, learning and memory, language, perceptual-motor function and social cognition. Mild NCD is diagnosed by the modest impairment of the previously mentioned cognitive domains from previous performance, while not interfering with independence in daily activity. The diagnosis of NCD or mild NCD is followed by the identification of the causative disorder (e.g. AD) based on the domains affected and the order in which they are affected [63].

There are a number of neuropsychological assessments used to evaluate cognitive function for the diagnostic criteria above. These assessments have also been used to monitor disease progression and symptoms in patients and decline in at risk or healthy individuals. Table 1.1.1 lists the commonly utilised cognitive assessments in AD as well as the cognitive domains they aim to measure. The combination of results from these assessments into domain specific composite scores has been shown to improve the measurement of subtle preclinical cognitive decline [64-67].

1.1.3.2 Neuroimaging

Neuropsychological testing alone is not accurate in diagnosing early stages of AD, where clinical features are not pronounced. For this reason, much research focus has been on the observation of biological changes occurring as the disease progresses. The ability to monitor pathological changes early is favourable due to the extensive preclinical stage in AD. Brain imaging techniques have proven successful in evaluating these changes, allowing for the observation of gross structural changes and accumulation of disease associated proteins.

Neuropsychological Assessments	Cognitive Domain	Reference
Alzheimer's Disease Assessment Scale – Cognitive section (ADAS-COG)	Memory, Language, Attention	[68]
Boston Naming Test (BNT)	Language	[69]
Brief Visuospatial Memory Test—Revised	Memory, Visuospatial Ability	[70]
California Verbal Learning Test (CVLT)	Memory	[71]
Clinical Dementia Rating (CDR)	Clinical Progression of Dementia	[72]
Clock Test	Visuospatial Ability	[73]
Controlled Oral Word Association Test (COWA)	Language	[74]
Delis-Kaplan Executive Function System (D-KEFS)	Executive Function	[75]
Mini-Mental State Examination (MMSE)	General Cognition	[76]
North American National Adult Reading Test (NART)	Intelligence	[77]
Rey Auditory Verbal Learning Test (RAVLT)	Memory	[78]
Rey Complex Figure Test And Recognition Trial (RCFT)	Visuospatial Ability	[79]
Rey-Osterrieth Complex Figure Test (ROCF)	Memory	[80, 81]
Stroop Task	Executive Function	[82]
Trail Making Test	Attention, Problem-Solving	[83]
Wechsler Adult Intelligence Scale (WAIS)	Intelligence	[84]
Wechsler Memory Scale (WMS)	Memory, Attention	[85]
Wechsler Test of Adult Reading (WTAR)	Intelligence	[86]

Table 1.1.1. Neuropsychological Assessments in AD

Neuropsychological assessments used to diagnose and monitor progression and risk for AD, including the cognitive domains they aim to measure.

1.1.3.2.1 Magnetic Resonance Imaging

The most widely utilised Magnetic Resonance Imaging (MRI) technique facilitates the observation of structural brain changes, in particular brain atrophy, and is considered important in AD diagnosis. In addition to allowing for the investigation of both the whole brain structure and specific areas, MRI is widely available, relatively inexpensive and non-invasive.

MRI global and regional brain volume measures have previously been shown to have the ability to discriminate between AD, MCI and CN classifications (Figure 1.1.6, [13, 87-90]). Cross-sectional and longitudinal MRI measures have been associated with conversion from MCI to AD [14, 88, 89, 91-95], and the severity of disease [96]. Further, in studies investigating ongoing disease progression and cognitive decline, the addition of MRI measures significantly improved the prediction power of the models containing age, gender and baseline memory scores [97-99]. More recently, measures of regional brain volume by MRI have been used in healthy elderly populations as a way of predicting the development of AD before clinical symptomology [100].

1.1.3.2.2 Positron Emission Tomography

Positron emission tomography (PET) is an imaging technique based on the detection of positron-emitting radioisotopes. Appropriate ligands and radiolabelled isotopes constitute imaging agents, or tracers, in PET scanning. Commonly used ligands in AD studies utilise the structure of the hallmark protein aggregates for binding and detection. Aggregated Aβ and Tau form β-sheet secondary structures [102, 103] within which the aromatic tracers bind [104].

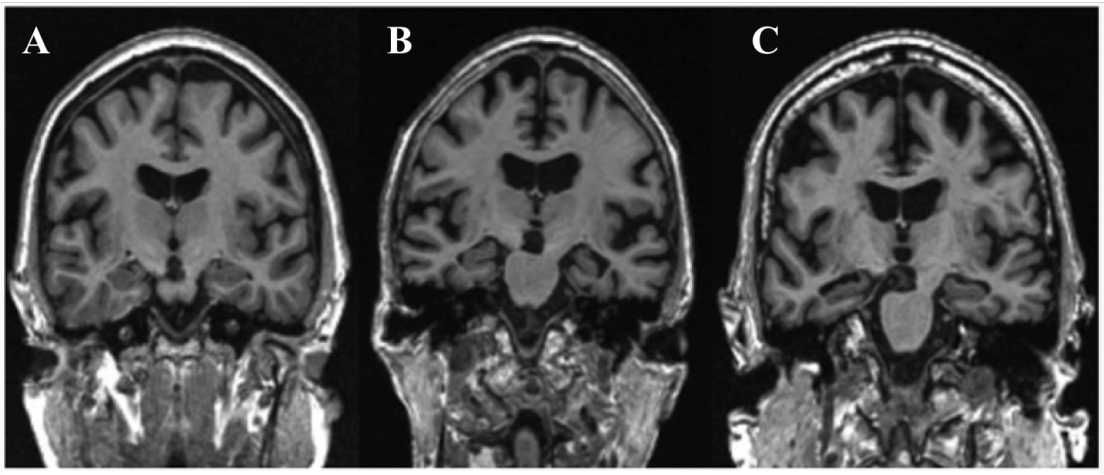

Figure 1.1.6. Magnetic Resonance Imaging

T1-weighted volume Magnetic Resonance Images (MRIs), in (A) cognitively normal, (B) mildly cognitively impaired, and (C) Alzheimer's disease older adults (≥ 70 years old). Image sourced from [101].

Aβ, as discussed previously, is the main component of one of the defining hallmarks of AD. Additionally, it is relatively abundant in the diseased brain [105], has regional distribution specific to AD, and begins accumulation well before clinical diagnosis [106]. For these reasons there has been much research into the development and use of Aβ specific PET imaging agents. The first Aβ tracer developed, Pittsburgh Compound B (PiB), consisted of modified thioflavin-T and a carbon-11 (^{11}C) label [107]. Due to the short half-life of ^{11}C (20 minutes) research has since focused on tracers labelled with fluorine-18 (^{18}F, 110 minutes, [108]). ^{18}F tracers approved for clinical use include; florbetapir [109], flutemetamol [110] and florbetaben [111].

Neocortical Aβ-amyloid burden as measured by PET has been consistently correlated with both post-mortem brain Aβ burden [112-117] and CSF $A\beta_{42}$ [118-123]. Further, brain regions previously associated with increase Aβ plaque load (by autopsy or biopsy), were replicated in PET studies by levels of tracer retention [117, 124-130]. The extent of neocortical Aβ burden has been shown to be significantly different between clinical classifications of CN, MCI and AD (Figure 1.1.7, [110, 131-133]). Longitudinal studies have reported rates of Aβ accumulation from CN or MCI to AD [134, 135] consistent with the accepted timelines of AD development [106]. Increased amounts of neocortical Aβ has been associated with cognitive decline and brain atrophy in CN and MCI [136-141].

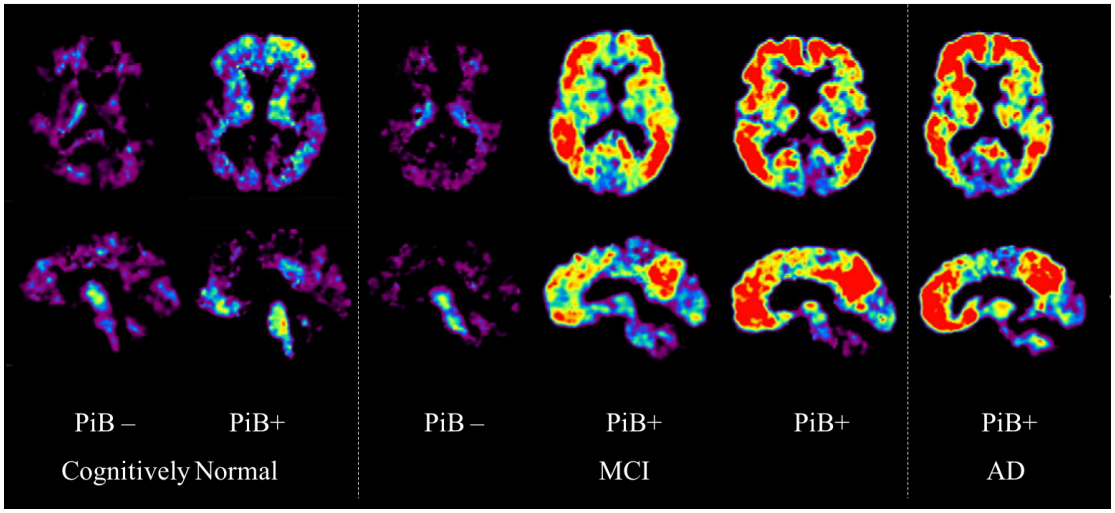

Figure 1.1.7. Positron Emission Tomography

Positron Emission Tomography (PET) images utilising a ^{11}C Pittsburgh Compound B (PiB) tracer in cognitively normal, mildly cognitively impaired (MCI), and Alzheimer's disease participants. Colour intensity is correlated with the concentration of deposited amyloid. Image sourced from [142].

While major research initially focused on the development of tracers for Aβ detection, there has been increased interest in the development of Tau specific tracers. There are numerous difficulties associated with the development of tracers for Tau including; the location of Tau aggregation requiring the tracer to cross the blood brain barrier, the low concentration of Tau aggregates, and Tau aggregation not being specific to AD [143]. Despite these difficulties, a number of Tau tracers have been developed and used in human trials [144-146]. These tracers have demonstrated the ability to bind to hyperphosphorylated Tau in the brains of patients with AD [147, 148], and have been associated with levels of cognitive impairment [147]. Further, there are a number of tracers in development currently [149, 150]. Like Aβ tracers, those used for Tau detection use both ^{11}C [146] and ^{18}F [144, 145, 149, 150] isotope tags.

1.1.4 Preclinical Disease

The notion of the preclinical AD stage has been investigated since the 1970s when, through autopsy, neuropathological changes were observed in the brains of asymptomatic individuals [151, 152]. Broadly, preclinical AD is the long period in which abnormal neuropathological features accrue while the individual is considered cognitively normal. In 2013, Villemagne *et al.* reported that the deposition of Aβ occurs for ~20 years before the clinical diagnosis of AD (Figure 1.1.8, [106]). Further, it has been observed in both the Harvard Aging Brain Study (HABS) and the Australian Imaging Biomarker and Lifestyle Study of Aging (AIBL), that ~30% of CN individuals have significant Aβ deposition [153, 154].

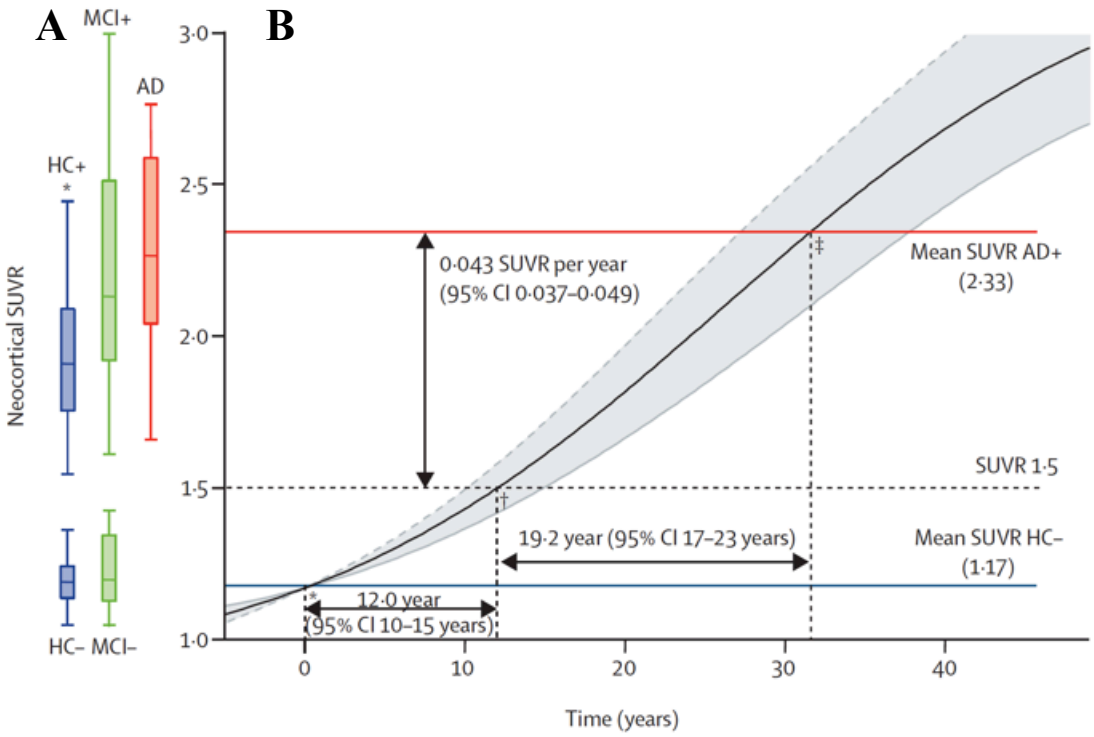

Figure 1.1.8. Aβ Deposition in Alzheimer's Disease

(A) The distribution of Aβ-amyloid (Aβ) burden based on clinical classification and (B) the timeline of Aβ deposition. Image sourced from [106]. Healthy control (HC), Mild cognitive impairment (MCI), Alzheimer's disease (AD).

Clinically normal individuals with high Aβ burden are more likely to present with neurodegeneration as measured by hippocampal volume or glucose metabolism [106, 139, 155]. Further, individuals with high Aβ burden and/or neurodegeneration are reported to have impaired cross-sectional and longitudinal cognitive performance [106, 155-157]. A meta-analysis of 38 studies investigating relationships between Aβ levels (CSF or neocortical) and cognition found Aβ-related impairment in global cognition, visuospatial function, processing speed, episodic memory, and executive function [158]. Additionally, this meta-analysis reported observable decline in global cognition, semantic memory, visuospatial function, and episodic memory related to Aβ burden [158].

Finally, there have been a number of genetic factors, in particular Apolipoprotein E (*APOE*) and Brain Derived Neurotropic Factor (*BDNF*; both discussed in detail below), shown to influence preclinical decline. *APOE* and *BDNF* have been reported to increase the rates of cognitive decline and hippocampal atrophy in CN older adults with high levels of neocortical Aβ, both individually (*APOE* or *BDNF*; [159-164]) and in combination (*APOE*×*BDNF*; [165]).

Due to these developments in the understanding of preclinical disease, in addition to the updated diagnostic guidelines, the NIA/AA also published recommended criteria for different stages of preclinical AD (Figure 1.1.9, [166]). These stages precede MCI and AD and aim to represent the progression of asymptomatic individuals. Stage 1 includes individuals that demonstrate Aβ-accumulation (amyloidosis), as measured by

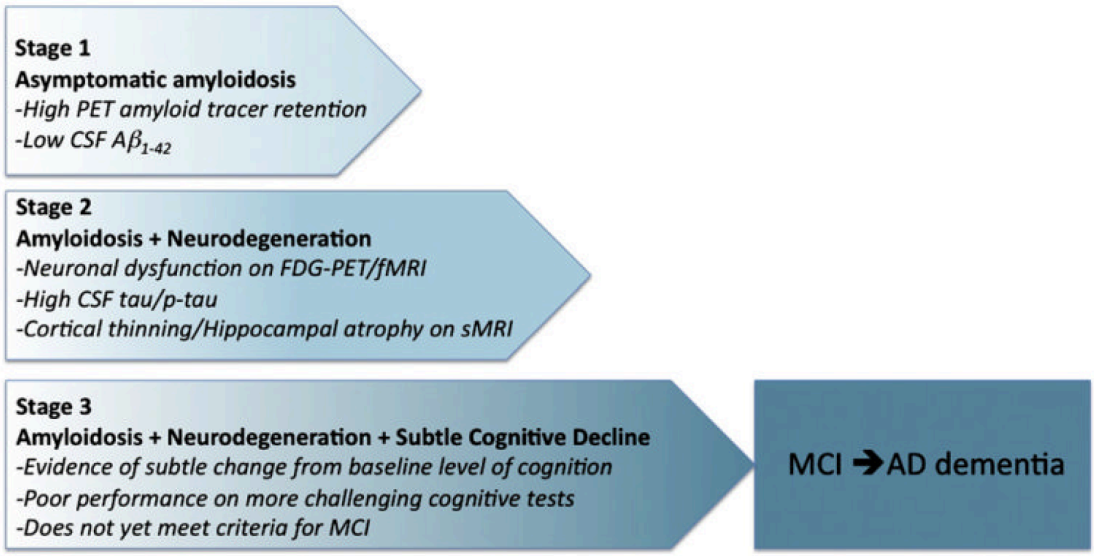

Figure 1.1.9. Stages of Preclinical AD

Preclinical AD stages represented graphically. Not all individuals once entering a preclinical AD stage are expected to progress to the following stage. Imaged sourced from [166].

CSF biomarkers or PET, in the absence of any additional change in neuropathological or cognitive changes [166]. Stage 2 involves amyloidosis and measures of neurodegeneration, including; structural MRI, ^{18}F-fluorodeoxyglucose (FDG) PET (a measure of glucose metabolism), or elevated levels of CSF tau or phospho-tau [166]. Finally, stage 3 consists of amyloidosis, neurodegeneration, and subtle cognitive decline that does not yet meet criteria for the diagnosis of MCI [166].

1.1.5 Risk Factors

Risk factors associated with the development of AD can be separated into 2 categories. Autosomal Dominant AD (ADAD), is characterised by the inheritance of autosomal dominant mutations, and accounts for ~1% of all AD cases. ADAD commonly presents a more aggressive course of disease, and has an early age of onset, usually younger than 65 years. Alternatively, Sporadic AD (SAD), with an age at onset generally older than 65 years, is a complex disease believed to result from the combination of genetic (non-modifiable), environment and lifestyle (modifiable) factors.

1.1.5.1 Lifestyle

There has been a wide range of modifiable lifestyle risk factors that have been associated with the development of SAD. These factors include; smoking, diet, sleep and physical activity. It has previously been reported that smoking is associated with increased risk of AD [167], and results in increased rates of brain atrophy [168, 169] and cognitive decline [168]. A risk of AD [170-172] and increased rates of cognitive decline [172, 173] have been associated with poor adherence to healthy dietary patterns, a principle example of which is the Mediterranean Diet (MeDi). Briefly, the MeDi can be characterised by an increased consumption of fruits and vegetables, legumes and cereals, fish, and unsaturated fatty acids, and decreased consumption of dairy, meat and poultry, and saturated fatty acids. Further, moderate but regular alcohol consumption, mostly in the form of wine, also typifies the MeDi. Sleep disturbance has been observed to increase the risk of AD development and cognitive decline in CN [174, 175]. Finally, higher levels of physical activity have been associated with a reduction in risk for AD [176-178], as well as improved longitudinal and cross-sectional cognitive function [179-181], memory [179] and attention [182].

1.1.5.2 Genetics

The causative genes implicated in ADAD encode proteins involved in APP processing, and are associated with alterations in Aβ production and aggregation. Alternatively, genes associated with increased risk of developing SAD have been generally implicated in a number of biological pathways involved in Aβ clearance and processing. The following book chapter provides in depth information into the genes that have been implicated in ADAD, SAD and Parkinson's disease (PD).

1.2 Genetics of Neurodegenerative Disease: *Role of genetics in Alzheimer's and Parkinson's Disease*

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Martins, RN and Brenan, C (eds)

**The Parkinson's disease component of this chapter was contributed by Francis L Mastaglia*

1.3 Polygenic Risk Scores

As discussed in the preceding book chapter, there have been a number of GWAS identifying variants associated with AD [192, 200, 609-611]. These studies have reported that individually, the variants identified have small effect sizes for the risk of developing the late-onset form of AD when compared to the major genetic risk factor, *APOE*. To account for their nominal effects, the combination of these gene variants into a polygenic risk score (PRS) has been employed in various forms.

In general, PRS are calculated by the sum of the risk alleles for all variants, or the sum of risk alleles weighted by odds ratios or effect sizes [612]. A popular approach in AD research is the calculation of PRS using the gene variants identified in the aforementioned GWAS [192, 200, 609-611]. These PRS contain between 3 and 23 variants and have been associated with a range of cognitive and biological disease markers. Studies investigating un-weighted PRS have evaluated baseline and longitudinal cognitive change in cognitively normal older adults, and have failed to identify any significant associations [613-616]. In contrast, when weighting risk alleles by previously published odds ratios, or beta coefficients, a number of associations with overall disease risk have been observed. In cohorts investigating CN to AD participants, PRS weighted by GWAS reported odds ratios, were associated with; incidence of dementia [443], age of disease onset [617], age related structural brain changes [618], memory decline [619], and measures of CSF $A\beta_{42}$ [617, 620]. Additionally, in a CN to AD cohort evaluating a PRS weighted by beta coefficients, associations with diagnostic status and the severity of MRI measures were identified [324]. Significant associations between PRS and disease measures have also been identified in preclinical and

cognitively normal cohorts. In MCI cohorts, PRS weighted by odds ratios were associated with cognitive decline [621], measures of CSF Tau and phosphorylated Tau [621], and accelerated progression to AD [622]. Finally, when investigating CN participants, odds ratio weighted PRS were associated with baseline and longitudinal cognition [613, 623], hippocampal volume [390, 624], and cortex thickness [625].

In addition to the PRS reported above, others have been reported with altered methods of variant inclusion and weightings. While focusing on AD-risk genome-wide significant SNPs is important, it has two potential limitations. The first of these is the resultant loss of much genetic variance, i.e. the exclusion of genetic variants that are statistically significant but fail to reach stringent genome-wide significance cut-offs. To address this first potential limitation, PRS have been developed using an expanded selection of SNPs that are identified through decreasing the stringency of genetic association with AD risk. In a study by Mormino *et al.* an AD-risk weighted PRS, which set the criteria for inclusion at p=0.01 resulted in the inclusion of ~16,000 SNPs. This "conservative" PRS, was reported to be associated with baseline and longitudinal memory and executive function, baseline hippocampal volume, progression to MCI or AD, and neocortical Aβ [626]. Lupton *et al.* also reported an association between hippocampal volume and an odds ratio weighted PRS employing a less stringent AD-risk association threshold ($p<1\times10^{-4}$; n(SNPs)=~1000; [627]). Finally, Escott-Price *et al.* reported a PRS consisting of ~200,000 SNPs ($p\leq0.5$) with an 84% prediction accuracy for pathology confirmed AD [628]. Further, different methods of risk evaluation and weighting in PRS have been investigated for the prediction of AD phenotypes. A PRS of AD-associated variants, weighted by a combination of AD risk

and population-based rates of AD, has been associated with individuals age of AD onset [629].

The second potential limitation of focusing on AD-risk genetic variants is the exclusion of SNPs that are more associated with pathological or symptomatic (i.e. cognition) changes than AD-risk. This potential limitation has been far-less explored to date. However, the combination of two genes previously associated with cognitive decline has been investigated, namely *APOE* and Brain Derived Neurotrophic Factor (*BDNF*). While *APOE* is the main genetic risk factor in AD, it is also significantly associated with rates of cognitive decline [159, 162]. On the other hand, whilst *BDNF* has been associated with cognition [161] the AlzGene meta-analysis suggests that it is not risk factor for AD [630]. The combination of these genetic variants has been shown to significantly impact Aβ-induced cognitive decline. In a cohort of CN older adults with high levels of brain Aβ, those carrying both an *APOE* ε4 and the *BDNF* met allele (rs6265) declined cognitively significantly faster when compared to all other groups of allele combinations [165]. These studies highlight the potential importance of expanding the genes included in AD PRS from only those associated with AD risk to those gene variants that have been previously associated with disease phenotypes. This notion provides the principle theoretical framework for the research undertaken through the course of this doctoral thesis.

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1.4 Theoretical Framework

In 2014 it was reported by Cummings *et al.* that of the 413 AD clinical trials performed between 2002 and 2012, there was only a 0.4% overall success rate [631]. It has been reported that these failures are due partly to the late disease stage of trial implementation. This, in addition to the increasing understanding of the extended preclinical stage of AD, has resulted in greater focus placed on clinical trials being conducted in the earlier pre-symptomatic disease stages. These trials involve the identification of at-risk individuals who are expected to decline over the course of the trial based on brain imaging and CSF biomarkers. However, these biomarkers do not inform on rates of cognitive change, which remains in most cases the primary outcome evaluated in trials. It has been observed that in biomarker-positive AD at-risk individuals there is much variability between individuals in longitudinal measures of cognition. This results in uncertainty as to whether at-risk individuals selected for trial inclusion will decline cognitively at rates appropriate for short clinical trials.

In addition to the genetic influence on AD risk outlined in the review above, cognition has been reported to be highly heritable and polygenic. Combinations of genes associated with AD-risk based on the large GWAS studies have been associated with cognitive decline. Further, combinations of genes associated with cognition, namely *APOE* and *BDNF,* have been reported to be associated with longitudinal cognition in CN at-risk cohorts. As such, genetics could be utilised, in combination with brain imaging and CSF biomarkers, for the identification of individuals appropriate for enrolment in clinical trials

The aim of this thesis was to confirm previously reported associations between AD-risk genes and cognition associated genes with sensitive cognitive composite scores in a preclinical subset of a highly characterised longitudinal cohort. Further it was aimed to combine the effects of AD-risk genes and genes associated with cognition in a novel method that had the ability to appropriately weight genes based on their influence on the preclinical endophenotypes being tested rather than late stage measures of disease risk.

1.5 Hypothesis and Aims

The overarching hypothesis of this thesis is that *genetic factors in combination influence cognitive rates of change in preclinical Alzheimer's disease*.

Aim 1 (Chapter 2):

Assess the impact of genes previously associated with AD risk on measures of cognition at a preclinical stage.

Aim 2 (Chapter 3):

Assess the effects of genes with *a priori* evidence for association with cognition on cognitive rates of change in preclinical AD.

Aim 3 (Chapter 4):

Investigate whether there is a synergistic effect of genes previously associated with cognition, and further what the best combination of these genes would be.

Aim 4 (Chapter 5):

Determine a method of weighting genes associated with both AD-risk and cognition, for use in a genetic risk score to improve the prediction of preclinical cognitive rates of change.

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Chapter 2

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CHAPTER 2: Association of a priori candidate, AD-risk associated, genes with cognitive rates of change in preclinical AD

2.1 Prologue

As discussed in Chapter 1 (Section 1.3), there have been numerous Polygenic Risk Scores (PRSs) calculated and subsequently associated with Alzheimer's disease (AD) related phenotypes, including AD-related imaging and fluid biomarkers and cognition. In most such studies, the PRSs that have been calculated have combined AD risk associated genetic variants, previously identified through genome wide association (GWA) studies (GWAS). These individual variants are invariably weighted by a measure of AD risk, typically the respective odds ratios or effect sizes generated from large GWAS, the most common being the International Genomics of Alzheimer's Project (IGAP) meta-analysis of GWA data [1]. Presented in this chapter is a replication of this form of PRS in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study of Aging. As no AIBL samples were included in the IGAP meta-analysis, presented here is an independent validation of previous studies. Specifically, it investigates cognitively normal (CN) individuals in the preclinical stages of AD.

Previous studies investigating associations between PRS and measures of cognitive decline have produced varying results, particularly in CN cohorts. The lack of PRS-cognition

associations reported by some studies could be attributed to sample heterogeneity within the CN cohort, or the use of cognitive measures not sensitive enough for early in the disease process. The ensuing study attempts to overcome these limitations, by focusing specifically on CN older adults who are biomarker positive based on Aβ-amyloid (Aβ) imaging. Further, three cognitive composite scores are utilised that measure the first cognitive changes occurring in AD. Through this approach, the first aim of this thesis, to *assess the impact of genes previously associated with AD risk on measures of cognition at a preclinical stage*, is addressed.

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Chapter 3

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CHAPTER 3: Association of *a priori* candidate, cognition associated, genes with cognitive rates of change in preclinical AD

3.1 Prologue

The results presented in Chapter 2 evidence the potential lack of utility of Alzheimer's disease (AD) risk weightings when investigating the association of polygeneic risk scores (PRSs) with preclinical disease outcomes, such as decline in cognition. When investigating the combinatory influence of gene variants most commonly associated with the development of AD, significant associations with rates of cognitive decline in a preclinical cognitively normal population were only observed with the inclusion of *APOE* ε4 weighting.

While it was observed that the PRS both with and without *APOE* were associated with neocortical amyloid beta (Aβ) burden and CSF-tau, the same was not seen when investigating longitudinal cognition. This discrepancy in results between disease biomarkers and cognition could be due to the disease-risk weighting applied to the genetic variants differing from the actual influence that these variants have on cognitive performance. Further, limiting the inclusion of single nucleotide polymorphisms (SNPs) in the PRS to those with association with clinical diagnosis of AD is biased against those variants which may influence the rate of decline in the preclinical stages of the disease. For example, the non-synonymous rs6265 (Val66Met) SNP in brain derived neurotropic factor (*BDNF*) has been reported by the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study of Aging and others, to be associated with altered rates of cognitive decline [1-4] but is not amongst the leading AD genetic risk factors and thus has

been excluded from PRS calculations. A number of genes have previously been associated with cognitive performance in both AD and CN populations. The studies presented in this chapter aimed to *assess the effects of genes with a priori evidence for association with cognition on cognitive rates of change in preclinical AD*, and determine their potential viability for inclusion into a polygenic measure.

A review of the literature was conducted to identify genes and genetic variants that have previously been associated with cognitive performance. The genes that were selected for inclusion are described below:

- *KIBRA*: Kidney and Brain expressed protein, variants within this gene have been associated with memory performance
- *SPON1*: Spondin1, expression of this gene has been associated with improved learning and cognition
- *COMT*: Catechol-O-methyltransferase, non-synonymous variants involved in the expression of this gene are associated with cognition
- *KL*: Klotho, variants within this gene controlling it's expression have been associated with aging phenotypes and cognitive performance

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3.2 *KIBRA* is associated with accelerated cognitive decline and hippocampal atrophy in *APOE* ε4-positive cognitively normal adults with high Aβ-amyloid burden

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3.2.1 Abstract

A single nucleotide polymorphism, rs17070145, in the KIdney and BRAin expressed protein (*KIBRA*) gene has been associated with cognition and hippocampal volume in cognitively normal (CN) individuals. However, the impact of rs17070145 on longitudinal cognitive decline and hippocampal atrophy in CN adults at greatest risk of developing Alzheimer's disease is unknown. We investigated the impact rs17070145 has on the rate of cognitive decline and hippocampal atrophy over six years in 602 CN adults, with known brain Aβ-amyloid levels and whether there is an interactive effect with *APOE* genotype. We reveal that whilst limited independent effects of *KIBRA* genotype were observed, there was an interaction with *APOE* in CN adults who presented with high Aβ-amyloid levels across study duration. In comparison to *APOE* ε4-ve individuals carrying the rs17070145-T allele, significantly faster rates of cognitive decline (global, *p=0.006*; verbal episodic memory, *p=0.004*;), and hippocampal atrophy (*p=0.04*) were observed in individuals who were *APOE* ε4+ve and did not carry the rs17070145-T allele. The observation of *APOE* effects in only non-carriers of the rs17070145-T allele, in the presence of high Aβ-amyloid suggest that carriers of the rs17070145-T allele are conferred a level of resilience to the detrimental effects of high Aβ-amyloid and *APOE* ε4.

3.2.2 Introduction

In cognitively normal older individuals, high levels of neocortical amyloid-β (Aβ-amyloid) are associated with subtle but detectable cognitive decline [1] and hippocampal atrophy [2]. This observation is consistent with models of Alzheimer's disease (AD) which propose a protracted preclinical phase that could take up to 20 years [3]. This provides a period of opportunity for understanding, and even interfering with, AD pathogenesis and thus the identification of biological factors, or trait characteristics, that themselves can influence AD progression has become of increased importance.

Several genes have been associated with cognitive performance, particularly episodic memory, and hippocampal atrophy. Previous studies have associated genetic polymorphisms, in particular apolipoprotein E (*APOE*) ε2/ε3/ε4 genotype (see review [4, 5]) and the non-synonymous rs6265 (Val66Met) SNP in brain derived neurotropic factor (*BDNF*) [6-9], with altered rates of episodic memory decline and hippocampal atrophy. Decline in measures of episodic memory, modified by genetic variation, have been reported in both the healthy elderly [10] and those predicted to be in the early stages of AD based on neocortical Aβ-amyloid imaging [6, 7, 11]. These findings raise the potential that other genetic factors may also moderate the toxic effects of Aβ-amyloid early in AD and contribute to altered rates of cognitive decline and hippocampal atrophy.

One such candidate is the gene encoding the KIdney and BRAin expressed protein (*KIBRA*; sometimes referred to as WW domain-containing protein 1 (*WWC1*)) [12]. KIBRA is a cytoplasmic, signal transducer protein expressed mainly in the kidney and brain [13] and *in vitro* experiments suggest that, through reduction in postsynaptic levels, it mediates tau induced memory loss and disruption of synaptic plasticity [14]. This *in vitro* data is supported through

genetic studies that report the association of allelic variation in the *KIBRA* gene with memory performance, hippocampal atrophy and measurable differences in brain activation. Specifically, a substitution of C for T in the 9th intron (rs17070145), was initially identified through a GWAS of verbal episodic memory performance and replicated in two additional independent cohorts [12]. Episodic memory is one of the earliest cognitive domains to decline, with previous studies observing decline 4-8 years prior to executive function and up to 7-10 years prior to other cognitive domains [15-17].

However, there is a lack of consensus in subsequent studies that attempted to replicate these genetic associations with memory performance. Cross-sectional studies of cognitively normal (CN) older adults, carriage of the rs17070145-T allele has been associated with better performance in episodic memory [18-22], delayed recall [23-25] and spatial learning [26] and increased hippocampal volume [20] and activity [19, 24]. Conversely, several studies have either associated the absence of rs17070145-T with better semantic [27] and long-term [28] memory, executive function [29] and overall cognitive performance [30] or were unable to show any association of the SNP with cross sectional episodic memory [29, 31-33] and hippocampal volume [31] or longitudinal decline in episodic memory and hippocampal volume [31]. However, common to all these studies is the lack of inclusion of Aβ-amyloid imaging, which may contribute to the lack of consensus due to the impact of underlying Aβ-amyloid burden on cognition not being considered [1, 6, 7, 11].

To address this conjecture requires the availability of comprehensive longitudinal data from the prospective cohort studies of AD, such as the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study, which offers the opportunity to retrospectively evaluate candidate biological factors (e.g. genetic variation) to determine the impact on progression of AD related

phenotypes, such as cognitive decline and hippocampal atrophy. The AIBL Study has now more than six years of serial cognitive and neuroimaging assessments, including Aβ-amyloid and structural imaging, in a group of CN adults collected at 18-month intervals. Therefore, the aim of this study was to characterise, through reporting on 6-years of longitudinal data, the role of *KIBRA* rs17070145 allelic variation in this highly characterised CN adult sample and examine the extent to which this allelic variation is associated with Aβ-amyloid related cognitive decline and atrophy of the hippocampus. The hypothesis was that CN adults who carry the rs17070145-T allele would show a slower rate of memory decline and hippocampal atrophy than those not carrying this allele, though this relationship would be dependent on the presence of a high brain Aβ-amyloid burden and interact with *APOE* genotype.

3.2.3 Methods

3.2.3.1 Participants

This study included 602 CN Caucasian adults enrolled in the AIBL Study, a prospective longitudinal study of ageing. Information regarding the AIBL Study's design, enrolment process, neuropsychological assessments, and diagnostic criteria has been previously described [34]. The clinical classification of CN, MCI or AD was determined, after clinical review, by a panel of old age psychiatrists, geriatricians, neurologists, and neuropsychologists who were blinded to Aβ-amyloid status. Individuals were classified as CN if they did not meet the clinical criteria for diagnosis of MCI [35] or dementia [36], as described previously [34]. Approval of the AIBL Study has been granted by each of the ethics committees of each of the member institutions; Austin Health, St Vincent's Health, Hollywood Private Hospital, and Edith Cowan University, and informed written consent was given by all volunteers. All clinical investigations were conducted in accord with the principles expressed in the Declaration of Helsinki 1975. All participants were assessed every 18-months. Cognitive, neuroimaging and laboratory assessment were acquired within 3-months of each other.

3.2.3.2 Cognitive Measures

The neuropsychological test battery administered in the AIBL study has been described in detail previously [34]. Briefly, it incorporates at each 18-month follow-up, the Mini-Mental State Examination (MMSE), Clock Drawing Test, California Verbal Learning Test-Second edition (CVLT-II), Logical Memory I and II (LMI; LMII; Story A only), D-KEFS verbal fluency, a 30-item version of the Boston Naming Test (BNT), Wechsler Test of Adult Reading (WTAR) for premorbid IQ, Digit Span and Digit Symbol-Coding subtests of the Wechsler Adult Intelligence Scale-Third edition (WAIS-III), the Stroop task (Victoria version), and the Rey Complex Figure Test (RCFT). Resultant data from this battery, in addition to the Clinical

Dementia Rating (CDR), have been previously used to statistically derive cognitive composites as previously described [37]. In this study, a verbal episodic memory composite (CDR sum of boxes (CDR_{SB}), LMII, CVLT false positives ($CVLT_{FP}$) and long delay free recall ($CVLT_{LDFR}$)), and a statistically driven global composite (CDR_{SB}, MMSE, LMII, $CVLT_{FP}$ and Clock), aimed as a sensitive measure for longitudinal decline in individuals predisposed to AD [37], were investigated across five study time points: baseline, 18, 36, 54 and 72 months. A correction for age, gender, years of education, WTAR-estimated premorbid IQ (WAIS-III Full Scale Intelligence Quotient (FSIQ)) and depressive symptoms (Geriatric Depression Scale (GDS)) was incorporated in the calculation of the cognitive composites [38].

3.2.3.3 Brain Imaging

The 602 CN adults included in this study had undergone Aβ-amyloid imaging, at varying time points, with PET using ^{11}C-Pittsburgh Compound B (PiB), ^{18}F-florbetapir or ^{18}F-flutemetamol as previously described [39-41]. PET standardized uptake value (SUV) ratio (SUVR) data was determined for all tracers using using CapAIBL, a web based freely availably MR-less methodology [42]. Briefly, SUVs were summed and normalized to either the cerebellar cortex SUV (PiB), whole cerebellum SUV (florbetapir) or pons SUV (flutemetamol) to yield the target-region to reference-region SUVR. These SUVRs were then classified as either low ($A\beta^{low}$) or high ($A\beta^{high}$) Aβ-amyloid burden, based on a tracer-specific SUVR threshold; ≥1.5, ≥1.10 and ≥0.62 for PiB, florbetapir and flutemetamol, respectively, as previously described [43]. Of these 602 participants, 548 also underwent clinical magnetic resonance imaging (MRI) for clinical screening and co-registration with PET images. MRI parameters have been described in detail previously [44]. Briefly, a 3T T1-weighted MRI was performed using the ADNI magnetization-prepared rapid gradient echo protocol, with an in-plane resolution of 1×1 mm and a slice thickness of 1.2 mm. Hippocampal volume was calculated after correcting for

age in years and intracranial volume, defined as the sum of grey matter, white matter and cerebrospinal fluid volumes, as previously described [45].

3.2.3.4 Genotyping

DNA extraction from 5mL of whole blood was performed using QIAamp DNA Blood Maxi Kits (Qiagen, Hilden, Germany) according to manufacturer's instructions. TaqMan® genotyping assays were used to determine *APOE* (rs7412, assay ID: C____904973_10; rs429358, assay ID: C___3084793_20) and *KIBRA* (rs17070145, assay ID: C__33286269_10) genotypes (Life Technologies, Carlsbad, CA). All TaqMan® genotyping assays were performed on a QuantStudio 12K Flex™ Real-Time-PCR systems (Applied Biosystems, Foster City, CA) using the TaqMan® GTXpress™ Master Mix (Life Technologies) methodology as per manufacturer instructions. *KIBRA* genotype was observed not depart from Hardy-Weinberg equilibrium. For the purpose of this study *APOE* carrier status is defined by the presence (1 or 2 copies) or absence (0 copies) of the *APOE* ε4 allele, henceforth referred to as *APOE* ε4+ve or *APOE* ε4-ve, respectively.

3.2.3.5 Statistical Analyses

All statistical analyses were performed using Rstudio (Rstudio Team 2015) Version 0.98.1103 for Macintosh [46]. All analyses were performed based on a dominant model for the *KIBRA* rs17070145-T (minor) allele, i.e. T carrier (i.e. C_T and T_T) compared with non-T carrier (i.e. C_C), as per previous studies [12, 18-21, 24]. Baseline demographic data analyses provided means, standard deviations, and percentages across the entire PET imaged cognitively normal sample and stratified by *KIBRA* rs17070145-T allele carrier (*KIBRA*-T*)* and non-carrier (*KIBRA* non-T) status. ANOVA (age, premorbid IQ, depressive symptoms) and chi-squared

tests (gender, years of education, *APOE* ε4+ve, high Aβ-amyloid burden) were used to determine the significance of differences between allelic groups. To determine differences in rates of cognitive change and hippocampal atrophy random intercepts linear mixed-effects (LME) models were performed using the "nlme" package in R. LMEs were performed due to their ability to model fixed and random effects, and their robustness when dealing with missing data [47].

After the inclusion of main effects within the model, i.e. *KIBRA* genotype, interaction terms and covariates were included and modelled as described here. Specifically, to investigate the effect of *KIBRA* on the rate of cognitive decline and hippocampal atrophy, initially a *KIBRA*×Time interaction was modelled across the entire sample, covarying for *APOE* ε4 carrier and Aβ-amyloid status, with the cognitive composites and hippocampal volume as the dependent variables. The effect of Aβ status in combination with *KIBRA* was then investigated by separately modelling an Aβ×*KIBRA*×Time interaction, co-varying for *APOE* ε4 carrier status. The third analysis focused on only $A\beta^{high}$ participants, with *APOE* included within an *APOE*×*KIBRA*×Time interaction. In addition, all analyses for hippocampal atrophy co-varied for gender. Graphical representations of all models are presented with time dependent standard error. Further, for all analyses correction for the False Discovery Rate (FDR) using Q-Value (bootstrap method) was performed [48]. Finally, chi-squared analyses were performed between groups to ascertain that group differences in rates of decline were not due to disproportionate rates of clinical conversion over the course of the study.

3.2.4 Results

3.2.4.1 The effect of KIBRA on cognition and hippocampal atrophy in cognitively normal adults

A total of 602 CN older adults, defined through the AIBL battery of clinical and neuropsychological assessments [34] were included in this study. As shown in Table 3.2.1 there were no significant differences or trends between rs17070145 (henceforth referred to simply as *KIBRA*) T carriers and non-T carriers at baseline with respect to demographic variables, premorbid intellect, depressive symptoms, or genotype. In the initial analysis, co-varied for *APOE* ε4 carrier and Aβ-amyloid status (classified by being above ($A\beta^{high}$) or below ($A\beta^{low}$) Positron Emission Tomography (PET) Aβ-amyloid tracer-specific thresholds) there were no significant differences in the trajectories between T carriers and non-carriers for measures of global cognition or episodic memory amongst CN adults (Supplementary Data; Figure S3.2.3, Table S3.2.4). However, there was a trend towards T-carriers having a mild improvement (0.028 standard deviations (SD)/year) in both global cognition (non-T carriers, -0.025SD/year; p=0.051) and verbal episodic memory (non-T carriers, -0.019SD/year; p=0.085), likely due to a practice effect. When evaluating the effect of *KIBRA* on hippocampal atrophy in all cases, and co-varying for *APOE* ε4 carrier and Aβ-amyloid status, no significant difference (p=0.242) was observed between T carriers (-0.017 cm^3/year), and non-T carriers (-0.026 cm^3/year) over six years (Supplementary Data; Figure S3.2.3, Table S3.2.4). Further, no significant differences were observed at baseline in any measures of cognition or hippocampal volume.

		Overall n = 602	*KIBRA* T carrier n = 335	*KIBRA* non-T carrier n = 267	p
Age (years)		70.79 (6.55)	70.73 (6.49)	70.72 (6.41)	0.9788
Female (%)		334 (55.48)	188 (56.12)	146 (54.68)	0.7871
Years of Education	**0-8**	48 (8.00)	27 (8.08)	21 (7.89)	0.9419
	9-12	222 (37.00)	127 (38.02)	95 (35.71)	
	13-15	126 (21.00)	69 (20.66)	57 (21.43)	
	15+	204 (34.00)	111 (33.23)	93 (34.96)	
Premorbid IQ (FSIQ)		107.86 (7.23)	107.66 (7.28)	108.14 (7.30)	0.4311
Depressive Symptoms (GDS)		1.05 (1.28)	1.05 (1.35)	1.04 (1.18)	0.9156
***APOE* ε4 carriage (%)**		165 (27.97)	84 (25.53)	81 (31.03)	0.1655
High Aβ-amyloid burden (%)		145 (24.09)	76 (22.69)	69 (25.84)	0.4215
MRI (n)		548	301	247	NA

Table 3.2.1 Demographic Information

Baseline demographic and clinical characteristics of all imaged cognitively normal adults in the AIBL study, and based on *KIBRA* rs17070145 T carriage (T_T and C_T) and non-carriage (C_C). p values represent statistical significance when comparing T carriage and non-carriage. GDS, Geriatric Depression Scale; FSIQ, Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) Full Scale Intelligence Quotient.

No significant differences were observed at baseline in either measure of cognition or hippocampal volume when investigating the Aβ×*KIBRA*×Time interaction. Relative to $A\beta^{low}$/*KIBRA* T carriers, the $A\beta^{high}$/*KIBRA* non-T carrier group showed a significantly greater rate of decline in global cognition (0.037 SD/year; -0.085 SD/year; p=0.008, q=0.036), and the verbal episodic memory (0.033 SD/year; -0.080SD/year; p=0.012, q=0.042) (Figure 3.2.1, Table 3.2.2). However, no statistical difference was seen between $A\beta^{high}$/*KIBRA* T carriers and $A\beta^{low}$/*KIBRA* non-T carriers. Analysis of hippocampal atrophy revealed that relative to $A\beta^{low}$/*KIBRA* T carriers (-0.015 cm^3/year), the $A\beta^{high}$/*KIBRA* non-T carrier group (-0.055 cm^3/year) showed a significantly greater rate of hippocampal atrophy (p=0.002, q=0.034) over six years (Figure 3.2.1, Table 3.2.2). Likewise, this trajectory of hippocampal atrophy was also significantly different (p=0.009, q=0.034) relative to $A\beta^{low}$/*KIBRA* non-T carriers (-0.017 cm^3/year). In contrast, $A\beta^{high}$/*KIBRA* T carriers' rate of atrophy did not differ from the $A\beta^{low}$ groups.

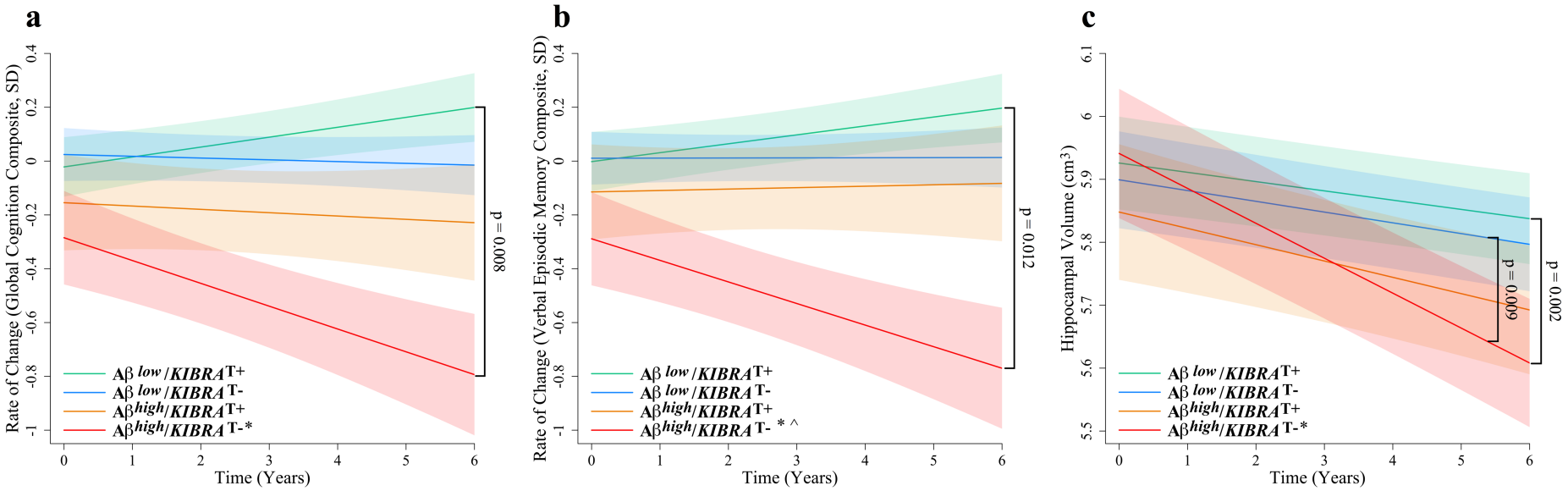

Figure 3.2.1 Rates of change in cognitively normal adults based on KIBRA T carriage and Aβ-amyloid status.

Rates of change are presented for (a) a statistically driven global composite, (b) a verbal episodic memory composite, and (c) hippocampal atrophy (n=548) in cognitively normal adults (n=602 unless otherwise stated). $A\beta^{low}$, low Aβ-amyloid burden; $A\beta^{high}$, high Aβ-amyloid burden. $A\beta^{low}$/*KIBRA* T carriers (green), $A\beta^{low}$/*KIBRA* non-T carriers (blue), $A\beta^{high}$/*KIBRA* T carriers (orange), $A\beta^{high}$/*KIBRA* non-T carriers (red), controlling for *APOE* ε4 carrier status. Hippocampal atrophy analysis also controlled for gender (shading represents time dependent standard error, *p<0.05 when comparing to the $A\beta^{low}$/*KIBRA* T carrier group, ^p<0.05 when comparing to the $A\beta^{low}$/*KIBRA* non-T carrier group, φ p<0.05 when comparing to the $A\beta^{high}$/*KIBRA* T carrier).

	Aβ^{low} *KIBRA* T carrier n=259	**Aβ^{low} *KIBRA* non-T carrier n=198**	**Aβ^{high} *KIBRA* T carrier n=76**	**Aβ^{high} *KIBRA* non-T carrier n=69**
	β	**β**	**β**	**β**
Global	0.037	-0.006	-0.012	**-0.085***
Verbal Episodic Memory	0.033	0.0004	0.005	**-0.080***
Hippocampal Atrophy	-0.015	-0.017	-0.026	**-0.055*^**

Table 3.2.2 Group slopes for cognitive composites and hippocampal atrophy in all imaged cognitively normal participants by KIBRA carrier and Aβ-amyloid status

Group slopes for cognitive composites (presented in SD/year; n=602) and hippocampal atrophy (presented in cm^3/year; n=548) in all imaged cognitively normal participants, controlling for *APOE* ε4 carrier status. Aβ^{low}, low Aβ-amyloid burden; Aβ^{high}, high Aβ-amyloid burden. *p<0.05 when comparing to the Aβ^{low}/*KIBRA* T carrier (T_T and C_T) group, ^p<0.05 when comparing to the Aβ^{low}/*KIBRA* non-T carrier group, **φ** p<0.05 when comparing to the Aβ^{high}/*KIBRA* T carrier

3.2.4.2 The effect of KIBRA on cognition and hippocampal atrophy in cognitively normal adults with high Aß-amyloid

No significant differences were observed in $A\beta^{high}$ CN adults at baseline in either measure of cognition or hippocampal volume when investigating the *APOE*×*KIBRA*×Time interaction. Relative to *APOE* ε4-ve/*KIBRA* T carriers, the *APOE* ε4+ve/*KIBRA* non-T carrier group showed a significantly greater rate of decline in global cognition (p=0.006, q=0.034) and verbal episodic memory (p=0.004, q=0.034) over six years (Figure 3.2.2, Table 3.2.3). Further, relative to *APOE* ε4+ve/*KIBRA* T carriers, the *APOE* ε4+ve/*KIBRA* non-T carrier group showed a nominally significantly greater rate of decline on the verbal episodic memory composite, however after FDR correction this remained only a strong trend (p=0.018, q=0.055) over six years (Figure 3.2.2, Table 3.2.3). Hippocampal atrophy analysis revealed that relative to *APOE* ε4-ve/*KIBRA* T carriers (-0.016 cm^3/year), the *APOE* ε4+ve/*KIBRA* non-T carrier group (-0.067 cm^3/year) had nominally significantly different rates of hippocampal atrophy however did not survive correction for multiple testing (p=0.040, q=0.107) over six years (Figure 3.2.2, Table 3.2.3). This trajectory of hippocampal atrophy was suggestive of being different to *APOE* ε4-ve/*KIBRA* non-T carriers (-0.006 cm^3/year), however this did not reach significance (p=0.125), even though this trajectory showed negligible difference to *APOE* ε4-ve/*KIBRA* T carriers. *APOE* ε4+ve/*KIBRA* T carriers' rate of atrophy did not differ from the *APOE* ε4-ve groups. To ascertain that these differences in rates of decline were not due to disproportionate rates of clinical conversion, the frequency of individuals who converted to Mild Cognitive Impairment (MCI) or AD over the course of the study was investigated. Within the *APOE* ε4+ve group there was no significant difference (p=0.43) between *KIBRA* non-T carriers (0.294, 15 out of 41) and *KIBRA* T carriers (0.294, 10 out of 34) in terms of clinical conversion.

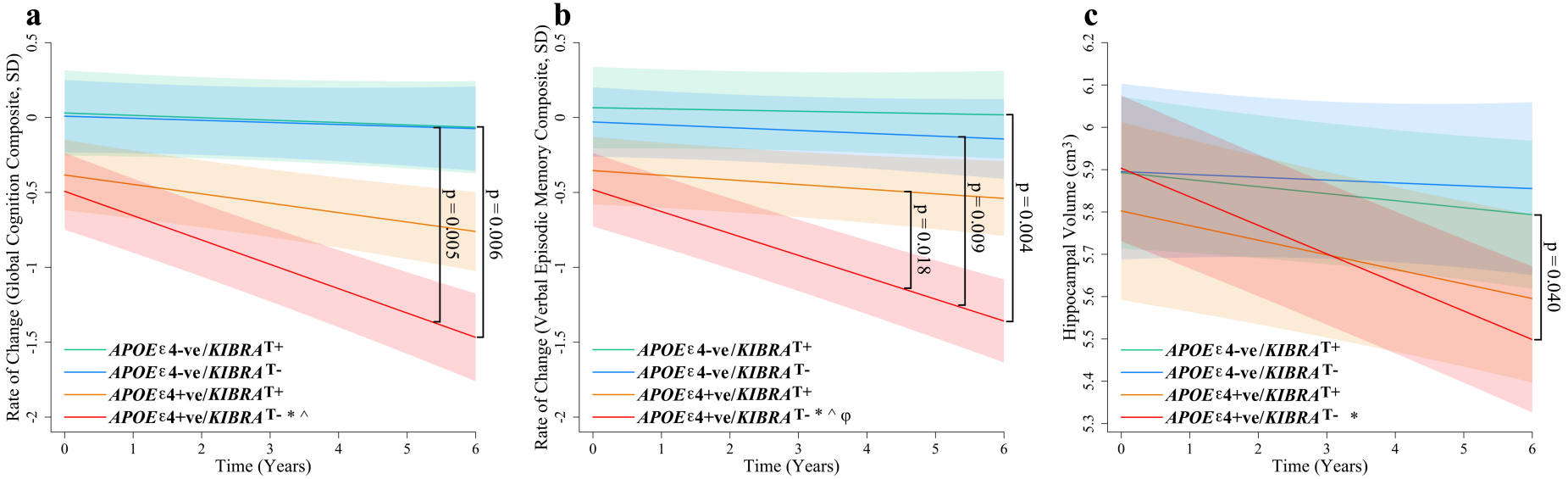

Figure 3.2.2 Rates of change in cognitively normal adults with high Aβ-amyloid burden

Rates of change are presented for (a) a statistically driven global composite, (b) a verbal episodic memory composite, (c) hippocampal atrophy in cognitively normal adults with high Aβ-amyloid (n=145). *APOE* ε4-negative/*KIBRA* T carriers (green), *APOE* ε4-ve/*KIBRA* non-T carriers (blue), *APOE* ε4+ve/*KIBRA* T carriers (orange), *APOE* ε4+ve/*KIBRA* non-T carriers (red). Hippocampal atrophy analysis controlled for gender (shading represents time dependent standard error, *p<0.05 when comparing to the *APOE* ε4-ve/*KIBRA* T carrier group, ^p<0.05 when comparing to the *APOE* ε4-ve/*KIBRA* non-T carrier group, φ p<0.05 when comparing to the *APOE* ε4+ve/*KIBRA* T carrier).

	***APOE* ε4-ve *KIBRA* T carrier n=38** β	***APOE* ε4-ve *KIBRA* non-T carrier n=27** β	***APOE* ε4+ve *KIBRA* T carrier n=34** β	***APOE* ε4+ve *KIBRA* non-T carrier n=40** β
Global	-0.016	-0.014	-0.063	**-0.163*^†**
Verbal Episodic Memory	-0.008	-0.019	-0.031	**-0.146*^φ†**
Hippocampal Atrophy	-0.016	-0.006	-0.034	**-0.067***

Table 3.2.3 Group slopes for cognitive composites and hippocampal atrophy in imaged cognitively normal adults with high Aβ-amyloid.

Group slopes for cognitive composites (presented in SD/year) and hippocampal atrophy (presented in cm^3/year) in imaged cognitively normal adults with high Aβ-amyloid (n=145). *p<0.05 when comparing to the *APOE* ε4-ve/*KIBRA* T carrier group, ^p<0.05 when comparing to the *APOE* ε4-ve/KIBRA non-T carrier group, φ p<0.05 when comparing to the *APOE* ε4+ve/*KIBRA* T carrier. †q<0.05 for those reporting nominal significance at p<0.05.

3.2.5 Discussion

The data reported here support the hypothesis that *KIBRA* genotype, in combination with *APOE* ε4 and Aβ-amyloid, affects rates of memory decline and hippocampal atrophy in cognitively normal adults. In those CN adults with high Aβ-amyloid burden at baseline, *KIBRA* non-T carriers showed significantly faster decline in the statistically driven global composite, and verbal episodic memory when compared to T carriers with low Aβ-amyloid burden. Within the subset of CN adults with high Aβ-amyloid burden, we showed that those who are *APOE* ε4+ve and *KIBRA* non-T carriers had significantly faster rates of decline in verbal episodic memory over 6 years, compared to *APOE* ε4+ve/*KIBRA* T carrier and both *APOE* ε4-ve groups. Importantly, minimal decline was also observed in the *APOE* ε4+ve/*KIBRA* T carrier group, suggesting that carriage of the *KIBRA* T allele imparts a level of resilience to negative effects of *APOE* ε4 and Aβ-amyloid on memory performance. Further, between group comparisons of the rates of clinical conversion (CN>MCI/AD) over the course of the study revealed no significant differences, suggesting that the faster rates of decline were not due to a higher rate of clinical conversion.

This is further supported by the observations that rates of hippocampal atrophy in this study also differ based on *KIBRA* genotype. In CN adults Aβ-amyloid has been previously reported to be associated with increased hippocampal atrophy [2, 45, 49], however in this study this was only observed in those individuals who did not possess the *KIBRA* T-allele, whilst in contrast *KIBRA* T-carriers' rate of atrophy did not significantly differ from the $A\beta^{low}$ groups. In a meta-analysis of *APOE* neuroimaging studies, hippocampal atrophy has been shown to be increased in *APOE* ε4 carriers [5]. Here we report that this association, in a group of $A\beta^{high}$ CN individuals, was again only observed in those individuals who did not possess the *KIBRA* T-allele, whilst in contrast *APOE* ε4+ve/*KIBRA* T-carriers' rate of atrophy did not differ from the

APOE ε4-ve groups. Taken together, we propose that the *KIBRA* T allele affords carriers a level of resilience to the detrimental effects of Aβ-amyloid and *APOE* ε4 allele on neurodegeneration, specifically hippocampal atrophy.

The findings presented herein are in line with the original study [12] and subsequent reports linking the *KIBRA* T allele with resilience in episodic memory performance [18-21, 24]. The absence of replication by other studies [27-29, 31-33] may be in part due to the lack of consistency in the measures of memory decline, whereby varying single neuropsychological tests, aiming to measure a certain feature of memory or cognition, were used. The use in this current study of a combination of global and episodic memory composite scores, which encompass several different tests best associated with a cognitive construct, could also have contributed to the ability to detect associations with the *KIBRA* genotype. However, the lack of inclusion of an assessment of underlying Aβ-amyloid burden in the previous studies may in fact be the more telling contributor to the lack of consensus on the association of *KIBRA* with cognitive performance. The level of neocortical Aβ-amyloid is associated with differential rates of cognitive decline [1, 50], and this is further altered by genetic factors, in particular *APOE* [10, 11] and *BDNF* [6, 7]. Accounting for the underlying Aβ-amyloid burden in the current study may have further contributed to the detection of differences in rates of cognitive decline and hippocampal atrophy reported with *APOE* ε4 and *KIBRA*.

Whilst the incorporation of cognitive composites and accounting for underlying Aβ-amyloid burden is considered a strength of this study, the following limitations of the study are acknowledged. Firstly, the use of different cognitive tests individually or in combination for the calculation of domain composites, then those specifically described in this study and using the methodology described herein, may yield different results. Second, this study included 6-

years of longitudinal follow-up and validation in other longitudinal cohorts, not undertaken herein, over longer durations of follow-up, may result in different findings. Third, the cognitively normal participants in this study were volunteers and not selected at random from the community, they were generally well educated and performed well on cognitive assessments and as such the findings presented herein may be applicable only to similar cohorts. Fourth, there is an overlap between those who are $A\beta^{high}$ and those who are *APOE* ε4+ve, which could confound the results when looking at their interaction. Finally, the *KIBRA* T-allele's previously reported association with altered brain activation using functional MRI (fMRI) [12, 19] could not be tested due to the lack of fMRI data, under a non-resting state, in the AIBL Study.

Studies have previously demonstrated the main areas of *KIBRA* expression in the brain are those also that are implicated in memory function, the hippocampus and temporal cortex [12, 51]. Furthermore, increased *KIBRA* gene expression in the temporal cortex [52] and hippocampus [22] has been associated with late onset AD. However, in a recent post-mortem brain transcriptomic study in neuropathogically normal individuals by Piras and colleagues a trend towards increased *KIBRA* gene expression was observed in *KIBRA* T homozygotes [53]. Further quantitative PCR analysis reported an over-expression in T-homozygotes compared to C-homozygotes in the hippocampus [53]. Further, the transcriptomic analysis revealed differential activation of the mitogen-activated protein kinase (MAPK) pathway [53], a pathway important in learning and memory processes, suggesting a potential mechanism underpinning a decline in memory performance reported in this study. It has also been shown that there is increased hippocampal activity in episodic memory performance tasks in *KIBRA* T carriers when compared with non-T carriers [19], consistent with the notion of protection from memory decline. *KIBRA* T allele carriers have also been shown to have a decreased levels

of brain activation compared to non-T allele carriers in several hippocampal regions activated during memory retrieval [12]. The authors hypothesised that individuals who do not carry the T allele require a greater level of hippocampal activation for memory retrieval [12].

In addition to the association studies described above, recent *in vivo* evidence provides molecular insights into mechanisms by which *KIBRA* is involved in memory performance. Synaptic plasticity, which is altered in AD, is modulated by dendrin, which in turn binds to the protein that *KIBRA* encodes (KIBRA; see review [54]). Further, KIBRA protein contains a protein kinase C (isoform ζ; PKCζ) binding domain [55] and has been reported to co-localise with protein kinase M (isoform ζ; PKMζ) [56], a brain specific variant of PKCζ, which plays important roles in memory formation and long-term potentiation. Johannsen et al have shown the function of the KIBRA protein to be regulated by its C2 domain [51], which is required for Ca^{2+} binding and is therefore involved in signal transduction in the neurons. This regulation is hypothesised to mediate the effect of the KIBRA protein on memory formation [51]. In a recent study, Tracy and colleagues have proposed a novel mechanism by which acetylated *tau* associated memory loss and disruption of synaptic plasticity is mediated by a reduction in postsynaptic KIBRA protein [14]. This finding links the previous reports of reduced *KIBRA* gene expression in AD with a biological mechanism mediated by acetylated *tau*. Whether the *KIBRA* T allele affords a level of resilience to this loss of synaptic plasticity remains to be determined.

Our findings indicate that *KIBRA* rs17070145 genotype, when combined with high brain Aβ-amyloid burden and *APOE* ε4 carriage, modifies longitudinal rates of decline in verbal episodic memory, a global cognitive composite and hippocampal volume. We propose that early in the disease process of AD, carriers of the *KIBRA* T-allele are conferred a level of resilience to Aβ-

amyloid and *APOE* ε4 driven decline. The potential mechanisms by which *KIBRA* contributes to synaptic plasticity, and AD progression warrant further investigation, including the potential impact on Aβ-amyloid accumulation, and may reveal novel pathways contributing to neuroprotection/neurodegeneration. Our results also highlight the potential application of genetics for risk stratification when designing clinical trials, particularly those that employ Aβ-amyloid imaging for screening. The nature of the effects of genetic variations, specifically assessing the combined effect(s) of additional genes affecting cognitive performance would have merit in such settings and requires further investigation.

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3.2.7 Competing Financial Interests

CLM is an advisor to Prana Biotechnology Ltd and a consultant to Eli Lilly. PM is a full-time employee of Cogstate Ltd. DA has served on scientific advisory boards for Novartis, Eli Lilly, Janssen, and Pfizer Inc. RNM is a consultant to Alzhyme. SML has previously been a paid consultant to Alzhyme. CCR has served on scientific advisory boards for Bayer Pharma, Elan Corporation, GE Healthcare and AstraZeneca; has received speaker honoraria from Bayer Pharma and GE Healthcare; and has received research support from Bayer Pharma, GE Healthcare, Piramal Lifesciences and Avid Radiopharmaceuticals. VLV served as a consultant for Bayer Pharma; and received research support from a NEDO grant from Japan. All other authors have nothing to disclose.

3.2.8 Author Contribution Statement

TP contributed to acquisition of genetic data, statistical analysis, interpretation of findings, drafting the manuscript. SCB contributed to specific study concept and design, study supervision, statistical analysis, interpretation of findings, and drafting of the manuscript. VD, PB contributed to acquisition and analysis of imaging data and revising the manuscript. GS contributed to AIBL study design, obtaining funding, interpretation of findings. KB, LM contributed to acquisition of genetic data. DA, AIB, CLM, CCR, RNM contributed to AIBL study design, obtaining funding and revising the manuscript. PM contributed to AIBL study design, obtaining funding, interpretation of findings, revising the manuscript. SRS contributed to revising the manuscript. DG, GV contributed to study supervision and revising the manuscript. VLV contributed to current study concept and design, obtaining funding, study supervision, acquisition of data, interpretation of findings and drafting of the manuscript. SML contributed to current study concept and design, obtaining funding, study supervision, acquisition of data, interpretation of findings and drafting of the manuscript. All authors read and approved the final manuscript.

3.2.9 Data Availability

All data and samples used in this study are derived from the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study of Ageing. All AIBL data, and that specific to this study, is publically accessible to all interested parties through an Expression of Interest procedure and is governed by the AIBL Data Use Agreement, for more information please see https://aibl.csiro.au/awd/.

3.2.11 Supplementary Data

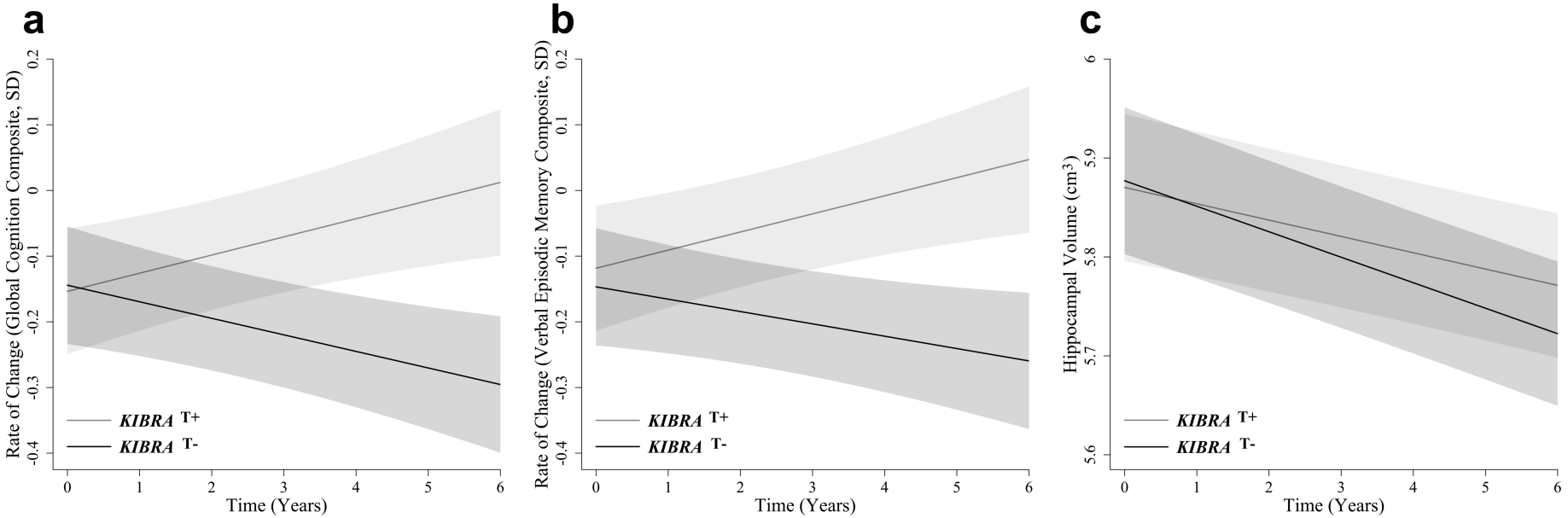

Figure S3.2.3 Rates of change in cognitively normal adults based on KIBRA T carriage.

Rates of change are presented for (a) a statistically driven global composite, (b) a verbal episodic memory composite, (c) hippocampal atrophy (n=548) in cognitively normal adults (n=602 unless otherwise stated). *KIBRA* T carriers (grey) and non-T carriers (black), controlling for *APOE* ε4 carrier and Aβ-amyloid status. Hippocampal atrophy analysis also controlled for gender (shading represents time dependent standard error, * $p<0.05$).

	KIBRA T carrier	*KIBRA* non-T carrier	
	β	β	p
Global	0.028	-0.025	0.051
Verbal Episodic Memory	0.028	-0.019	0.085
Hippocampal Atrophy	-0.017	-0.026	0.242

Table S3.2.4 Group slopes for cognitive composites and hippocampal atrophy in all imaged cognitively normal participants by KIBRA carrier status

Group slopes for cognitive composites (presented in SD/year; n=602) and hippocampal atrophy (presented in cm^3/year; n=548) in all imaged cognitively normal participants, controlling for *APOE* ε4 carrier and Aβ-amyloid status. *Represents a nominally statistically significant difference in slope of the *KIBRA* non-T carrier (C_C) group when compared to the *KIBRA* T carrier (T_T and C_T) group.

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3.3 *SPON1* rs11023139 is associated with Aβ-amyloid and *APOE* ε4 related cognitive decline in cognitively normal adults.

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3.4 *COMT* val158met is not associated with Aβ-amyloid and *APOE* ε4 related cognitive decline in cognitively normal older adults

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3.5 Klotho allele status is not associated with Aβ and *APOE* ε4 related cognitive decline in preclinical Alzheimer's disease

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3.6 Epilogue

The preceding studies presented in this chapter aimed to investigate the effect of variants within a targeted set of genes, with *a priori* evidence of association with cognitive performance, on cognition in a preclinical AD cohort. When investigating the *KIBRA* and *SPON1* variations, no independent associations were observed with cognitive composite measures. However, after interaction with *APOE* ε4 in individuals with high levels of neocortical amyloid beta (Aβ) significant associations were observed. No significant associations were observed, independently or with interaction, when investigating *KL* or *COMT*. Taken together the studies presented in this chapter, along with previous associations of *BDNF* with preclinical cognitive decline, provide strong evidence to support the inclusion of genetic variants over and above those associated with the clinical diagnosis of AD in polygenic risk scores (PRSs). Further, the biased inclusion of AD risk variants may potentially be at the detriment of the performance of the PRS, particularly in the prediction of cognitive decline in preclinical AD.

Although significant associations were observed for a subset of variants studied, these analyses were in isolation and as such does not completely discount the influence of these variants. Specifically, the contribution of a genetic variant to influencing preclinical cognitive decline may be apparent when considered in combination with other variants rather than as an independent effect. The next chapters explore different methods of combining these variants. In Chapter 4, genetic variants associated with cognitive decline are combined. Then, in Chapter 5, an approach is taken to combine both cognitive performance and AD risk associated variants such that it would allow for the accurate prediction of cognitive decline in preclinical AD.

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CHAPTER 4: Assessing the utility of combining a priori candidate, cognition associated, genes for predicting cognitive decline in preclinical AD.

4.1 Prologue

The studies presented in Chapter 3 provided the characterisation of a targeted selection of genes with *a priori* evidence of association with cognitive performance. Specifically, the independent and interactional effects (with *APOE* ε4) of the cognitive gene variants on rates of cognitive decline were explored in cognitively normal older adults in the Australian Imaging Biomarkers and Lifestyle (AIBL) Study of Aging. These studies were the first to investigate the influence of these genetic variants on cognition in a preclinical Alzheimer's disease (AD) cohort, defined using Aβ-Amyloid (Aβ) brain imaging.

Of the four candidates investigated in Chapter 3, two reported to have significant associations with longitudinal cognitive performance. That is, in cognitively normal individuals with high levels of neocortical Aβ and carrying at least one copy of the *APOE* ε4 allele, variants within *SPON1* and *KIBRA* were associated with significantly difference rates of cognitive decline. Conversely, when investigated *KL* and *COMT* gene variants were not associated with differences in cognitive performance, even after interaction with Aβ burden and *APOE* ε4 carriage. Whilst the preceding studies did not present significant associations across all genes investigated, it is still plausible that individual genes may have subtle effects on cognitive decline in the preclinical stages of AD that are more apparent when studied in combination with other genes.

The ensuing study presented in this chapter hypothesized that cognitive genes could have an additive influence that is obviously not observed when investigating variants independently. To address this hypothesis, the study aimed to address the third aim of the thesis, being to *investigate whether there is a synergistic effect of genes previously associated with cognition, and further what the best combination of these genes would be.*

To achieve this aim it was proposed to combine genes associated with cognitive performance using a method that would be simple to use and replicate, allowing for ease of use clinically. In addition to the genes investigated in Chapter 3, the Val66Met variant in *BDNF,* previously studied in the AIBL cohort [1, 2], and a variant within the "CUB and Sushi Multiple Domains 1" (*CSMD1*) gene were included. *CSMD1* is involved in complement regulation and variants within it have been associated with cognitive performance in healthy individuals [3, 4]. A decision tree approach was undertaken to derive groups based on rates of cognitive decline, specifically decline in a composite measure of verbal episodic memory in cognitively normal individuals with high levels of brain Aβ.

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4.2 Cognitive Gene Risk Profile for the Prediction of Cognitive Decline in Presymptomatic Alzheimer's Disease

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4.2.1 Abstract

INTRODUCTION: In cognitively normal (CN) older adults, high levels of Aβ-amyloid are associated with significant decline in cognition, especially episodic memory. Several genes have previously been associated with cognition, including *APOE, KIBRA, KLOTHO, BDNF, COMT, SPON1* and *CSMD1*. While some of this variation has been attributed to some of these genes individually, the combined effects of these genes on rates of cognitive decline, particularly in preclinical Alzheimer's Disease remain largely unknown.

METHODS: To elucidate if risk alleles within these genes can be suitably combined to predict cognitive decline 127 CN older adults with elevated PET-ascertained Aβ-amyloid were included in a decision tree analysis to define a "cognitive gene risk profile" for decline in a verbal episodic memory composite.

RESULTS: The episodic memory-derived cognitive gene risk profile defined four groups: *APOE* ε4+ Risk, ε4+ Resilient, ε4- Risk, ε4- Resilient, with the ε4+ Risk group declining significantly faster than all other groups (ε4+ Resilient, p=0.0008; ε4- Risk, p=0.025; ε4- Resilient, p=0.0006). The ε4+ Risk group also declined significantly faster than all other groups on Global, Clinical Progression and Pre-Alzheimer's cognitive composites.

DISCUSSION: The defined cognitive gene risk profile has potential utility in participant selection/stratification for preclinical AD trials that incorporate Aβ-amyloid and where decline in cognition is essential to determine therapeutic effectiveness.

KEY WORDS: genetic risk profiles**,** cognitive decline, Alzheimer's disease, episodic memory, Aβ-amyloid

NON-STANDARD ABBREVIATIONS: AIBL, Australian Imaging Biomarkers and Lifestyle study of Ageing; CN, Cognitive Normal; Cog-GRP, Cognitive Gene Risk Profile; PACC, Pre-Alzheimer's Cognitive Composite

4.2.2 Introduction

Evidence from prospective longitudinal cohort studies suggests that the pathological changes in Alzheimer's Disease (AD) commence decades before the onset of clinical symptomology [1]. Further, it has been established that higher levels of Aβ-amyloid (Aβ) in cognitively normal (CN) older adults is associated with accelerated decline in cognition [2]. As such, cerebrospinal fluid (CSF) and imaging biomarkers of Aβ are used to define the preclinical stage of AD [3, 4]. However, at the preclinical stage of AD there is considerable interpersonal variability in the rate of cognitive decline, suggesting that while Aβ is a necessary condition for AD, other factors influence the relationship between this biomarker and clinical disease progression. Cognition has been shown to be both highly heritable and highly polygenic [5] and allelic variation in several genes associated with cognition has been shown to explain some variation in cognitive function in older adults and in Aβ related cognitive decline in early AD [6-8]. Thus suggesting that genetics could help inform and predict rates of cognitive decline, and identify groups of CN older adults that are at a higher risk of a more rapid decline in cognition.

There have been several individual genes associated with cognitive performance and decline. The major genetic risk factor for AD, the ε4 allele of apolipoprotein E (*APOE*) [9], has been consistently associated with accelerated rates of episodic memory decline and hippocampal atrophy (reviewed in [10]). The non-synonymous rs6265 (Val66Met) single nucleotide polymorphism (SNP) in the brain derived neurotropic factor (*BDNF*), has been linked with altered rates of decline in several cognitive domains, and hippocampal atrophy [7, 8]. A further non-synonymous SNP that regulates dopamine availability in the central nervous system, rs4680 (Val158Met) within Catechol-O-methyltransferase (*COMT*), has also been associated with cognitive performance [11]. The Klotho gene (*KL*), initially discovered in transgenic mice

with a phenotype resembling human aging [12], has a functional variant, *KL*-VS that has been associated with life expectancy [13], global cognition [14], processing speed [14], and brain volume [15].

A further gene, *KIBRA*, that encodes the KIdney and BRAin expressed protein has recently been shown to be involved in the mediation of tau-induced memory loss and synaptic plasticity [16]. Allelic variation in the *KIBRA* gene, specifically a substitution of C for T in the 9th intron (rs17070145), has been reported to be associated with memory performance [17], hippocampal atrophy [18] and measurable differences in brain activation [17]. We have described recently how this gene contributes to moderating Aβ driven cognitive decline [19]. Additionally, several SNPs in the *CSMD1* (CUB and Sushi Multiple Domains 1) gene, involved in the regulation of complement and inflammation [20], have been associated with episodic memory and general cognition in a cognitively normal sample [21]. Finally, multiple SNPs within the Spondin 1 (*SPON1*) gene, involved in the processing of amyloid precursor protein (APP) [22], have been associated with disease severity [23] and rates of cognitive decline [24], though only in AD individuals.

Several studies have investigated the extent to which combinations of genes can influence cognitive decline and clinical progression in AD [25-28]. However, most of these studies focused on genes shown previously to be associated with risk for AD, with gene weighting based on AD risk [25, 26]. Thus these polygenic approaches may have resulted in exclusion of genes associated with cognitive performance, or if included, their influence diluted due to a disease risk based weighting [26]. Further, few studies have taken brain Aβ burden into consideration and investigated combining genes associated with cognitive performance in preclinical AD [8, 29].

This study hypothesised that combining genes shown to be associated with cognition would explain variance in Aβ related cognitive decline in preclinical AD. This study aimed to combine these genes into a straightforward profile able to discriminate individuals based on cognition, and particularly episodic memory, which is one of the earliest cognitive domains to decline [30]. The profile was created in CN older adults, signified at risk of cognitive decline based on brain imaging biomarkers, enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study. Extensive 18-monthly assessment, including cognitive and neuroimaging, within the AIBL Study allows for the longitudinal evaluation of this profile. Such a genetic profile could be easily implemented for the identification of individuals with accelerated rates of cognitive decline, which could have utilisation for clinical trial design, leading to more efficient clinical trials and secondary prevention studies.

4.2.3 Materials and Methods

4.2.3.1 Study Participants

One hundred and thirty-three CN biomarker positive (based on brain imaging) older adults enrolled in the AIBL Study, a prospective longitudinal study of ageing, were included in this study. The study design, enrolment process, neuropsychological assessments, and diagnostic criteria of the AIBL Study have been previously described [31]. Approval of the AIBL Study has been granted by each of the ethics committees of each of the member institutions: Austin Health, St Vincent's Health, Hollywood Private Hospital, and Edith Cowan University, and all volunteers gave informed written consent. Assessments occurred every 18 months, with cognitive, neuroimaging and laboratory assessment achieved within 3 months of each other.

4.2.3.2 Cognitive Measures

Burnham *et al*. previously calculated cognitive composite scores using the AIBL neuropsychological test battery and the Clinical Dementia Rating (CDR) scale [32]. These composite scores were used in this study to assess cognitive performance. The AIBL neuropsychological test battery consists of Mini-Mental State Examination (MMSE), Clock Drawing Test, California Verbal Learning Test-Second edition (CVLT-II), Logical Memory I and II (LMI; LMII; Story A only), D-KEFS verbal fluency, a 30-item version of the Boston Naming Test (BNT), Wechsler Test of Adult Reading (WTAR), Digit Span and Digit Symbol-Coding subtests of the Wechsler Adult Intelligence Scale-Third edition (WAIS-III), the Stroop task (Victoria version), and the Rey Complex Figure Test (RCFT) [31]. Briefly, a verbal episodic memory composite (CDR sum of boxes (CDR_{SB}), LMII, CVLT-II recognition false positives ($CVLT_{FP}$) and long delay free recall ($CVLT_{LDFR}$)) was used as the primary cognitive measure for defining groups with different rates of decline. Groups defined by decline in episodic memory were also assessed against a global cognition composite (CDR_{SB}, MMSE,

LMII, $CVLT_{FP}$ and Clock), and a composite measure of clinical progression (CDR_{SB}, MMSE) [32]. In addition, the Pre-Alzheimer's cognitive composite (PACC) previously calculated by Donohue *et al.* was also investigated [33]. In the calculation of the statistically driven composites there were corrections for age, sex, years of education, premorbid IQ (WTAR-estimated WAIS-III Full Scale Intelligence Quotient (FSIQ)) and depressive symptoms (Geriatric Depression Scale (GDS)) [34]. Five cognitive assessment time points were used: baseline, 18, 36, 54 and 72 months.

4.2.3.3 Brain Imaging

Aβ imaging with positron emission tomography (PET) using ^{11}C-Pittsburgh Compound B (PiB), ^{18}F-florbetapir or ^{18}F-flutemetamol was performed on the 133 cognitively normal adults included in this study as previously described [35-37]. The same region of interest template was used to determine PET standardized uptake value (SUV) ratio (SUVR) data for all tracers [38]. Briefly, SUVs were summed and scaled based on tracers PiB, florbetapir, and flutemetamol, to the cerebellar cortex, whole cerebellum or pons, respectively, to yield the target-region to reference-region SUVR. This study classified participants as high ($A\beta^{high}$) Aβ burden, based on a tracer-specific SUVR threshold; ≥1.5, ≥1.10 and ≥0.62 for PiB, florbetapir and flutemetamol, respectively, as previously described [39]. For cross-sectional comparison of Aβ burden with multiple tracers a linear regression transformation was applied to ^{18}F-labbelled tracers to generate PiB-like SUVR units termed the "Before the Centiloid Kernel Transformation" (BeCKeT) scale [40].

4.2.3.4 Genotyping

We have previously described methods of DNA extraction and SNP genotyping [41]. Briefly, manufacturer's instructions were followed to extract DNA from 5mL of whole blood using QIAamp DNA Blood Maxi Kits (Qiagen, Hilden, Germany). TaqMan® genotyping assays were used to determine *APOE* (rs7412, assay ID: C____904973_10; rs429358, assay ID: C___3084793_20), *BDNF* (rs6265, assay ID: C__11592758_10), *KIBRA* (rs17070145, assay ID: C__33286269_10), *COMT* (rs4680, assay ID: C__25746809_50*), KL* (*KL*-VS; rs9536314, assay ID: C___2983037_20; rs9527025, assay ID: C___2983036_20), *SPON1* (rs11023139, assay ID: C_____55174_30), and *CSMD1* (rs2740931, custom designed assay) genotypes (Life Technologies, Carlsbad, CA). TaqMan® genotyping assays were performed on a QuantStudio 12K Flex™ Real-Time-PCR systems (Applied Biosystems, Foster City, CA) using the TaqMan® GTXpress™ Master Mix (Life Technologies) as per manufacturer instructions. *APOE* carrier status is defined by the presence (1 or 2 copies) or absence (0 copies) of the *APOE* ε4 allele, henceforth referred to as *APOE* ε4+ve or *APOE* ε4-ve, respectively. Further *KL*-VS homozygotes (n=6) were excluded from all analyses resulting in the inclusion of 127 CN adults.

4.2.3.5 Statistical Analysis

All statistical analyses were performed using Rstudio (Rstudio Team 2015) Version 0.98.1103 for Macintosh [42]. Baseline demographic data analyses, using the generic functions of the R "base" package, provided means, standard deviations, and percentages across the cognitively normal sample. The first stage of analysis was the definition of the individual slopes for verbal episodic memory decline in the $A\beta^{high}$ sample (n=127), which would then be included in the subsequent decision tree analysis. These individual slopes were created using the "nlme" package in R using random intercepts linear mixed-effects (LME) models, which model fixed

and random effects, and deal with missing data robustly [43]. In this analysis, a verbal episodic memory × time interaction was modelled to generate per person β values (slopes). As the calculation of the verbal episodic memory composite is controlled for age, sex, years of education, premorbid IQ and depressive symptoms, no further covariates were included in the LME models. The second stage of analysis utilised these slopes (dependent variables) in combination with the seven genes of interest (*APOE*, *BDNF*, *KIBRA*, *KL*, *COMT*, *SPON1* and *CSMDI;* independent variables), in a decision tree model using the "rpart" package in R, to define the "Cognitive Gene Risk Profile" (*Cog-GRP*) groups to be used in subsequent analyses. The final stage of analysis was to assess the performance of the defined *Cog-GRP* groups. To achieve this differences in rates of cognitive change between these groups were assessed using random intercepts LME models, using the "nlme" package in R. Specifically, a *Cog-GRP* group × Time interaction was modelled across the entire sample, with the cognitive composites as the dependent variables. With the exception of analyses for the AIBL-PACC, which co-varied for age, no additional covariates were included due to their inclusion in the generation of the cognitive composites. All LME models were presented graphically with time point dependent standard error. Effect sizes were calculated based on cognitive performance at the sixth year of follow-up using the "effsize" package in R.

4.2.4 Results

4.2.4.1 Aβ^{high} cognitively normal adults baseline demographics, genotype frequencies and cognitive slopes

Table 4.2.1 shows the demographics, genotype frequencies and cognitive slopes of the 127 Aβ^{high} CN older adults included in the study. The statistically driven global composite (-0.0901 SD/year), clinical progression (-0.0484 SD/year), and verbal episodic (-0.0774 SD/year) composites all presented with a negative rate of change when investigating Aβ^{high} CN older adults.

4.2.4.2 Defining the Cognitive Gene Risk Profile (Cog-GRP) and group stratification

The "Rpart" package in R was used to calculate the decision tree that defined the *Cog-GRP*. The decision tree was constructed using 7 gene variants (*APOE* ε4+/ε4-, *BDNF* Met+/Val/Val, *KIBRA* T-/T+, *COMT* Val+/Met/Met, *KLOTHO* VS-/VS+, *SPON1* A-/A+, and *CSMD1* G-/G+) against a composite score of verbal episodic memory in an Aβ^{high} sample. The analysis resulted in the selection of six of the seven genes (*COMT* falling out of the analysis, Figure 4.2.1a), which were used to classify the participants into 8 groups. Due to small sample sizes in the resultant 8 groups, groups were collapsed at the end of the respective ε4+ and ε4- branches. This was based on their same directions, and similar rates, of change (SD/year) in verbal episodic memory over the assessed 6-years. The resulting 4 groups were then classified as "at risk" or "resilient" based on carriage of the ε4 allele and differences in decline on the verbal episodic composite for these collapsed groups were reconfirmed (Figure 4.2.1b). The ε4+ Risk (-0.1891 SD/year) group had a significantly faster rate of decline than ε4+ Resilient (0.0014 SD/year; p=0.0008; At 6th year: Cohen's *d*=1.48, 95% CI 0.91-2.02), ε4- Risk (-0.0749 SD/year; p=0.025; At 6th year: Cohen's *d*=1.14, 95% CI 0.67-1.59) and ε4- Resilient (0.0097 SD/year; p=0.0006; At 6th year: Cohen's *d*=2.37, 95% CI 1.63-3.04) groups and reached

clinically significant thresholds of cognitive impairment (performance at 1.5 standard deviations below controls, dashed line Figure 4.2.1b) after 5 years. For comparison purposes, Figure 4.2.1b, shows that this threshold is crossed at approximately 9.6 years when only carriage of the *APOE* ε4 allele is considered, which had a decline of -0.110 SD/year.

		Aβ^{high} CN older adults
Age (years)		73.17 (6.50)
Female (%)		66 (51.97)
Years of Education (%)	**0-8**	11 (8.66)
	9-12	51 (40.16)
	13-15	31 (24.41)
	15+	34 (26.77)
Premorbid IQ (FSIQ)		108.54 (6.87)
Depressive Symptoms (GDS)		1 (1.26)
***APOE* (% ε4+ve)**		66 (51.97)
***BDNF* (% Met+ve)**		43 (33.86)
***KIBRA* (% T–ve)**		62 (48.82)
***KL* (%VS–ve)**		99 (77.95)
***COMT* (% Val+ve)**		103 (81.10)
***CSMD1* (% G–ve)**		42 (33.07)
***SPON1* (% A–ve)**		112 (88.19)
Global Cognition*		-0.0901
Clinical Progression*		-0.0484
Verbal Episodic Memory*		-0.0774
AIBL-PACC†		-0.1144

Table 4.2.1 Baseline demographic information

Baseline demographic, genotype frequencies and cognitive composites slopes for cognitively normal (CN) older adults with high Aβ-amyloid (Aβ^{high}) in the AIBL Study (n=127). *Cognitive composites presented in SD/year, and the †Pre-Alzheimer's Cognitive Composite (AIBL-PACC) presented in 4×SD/year. AIBL-PACC controlled for age. *KL-VS* homozygotes excluded (n=6). GDS, Geriatric Depression Scale; FSIQ, WTAR-estimated FSIQ.

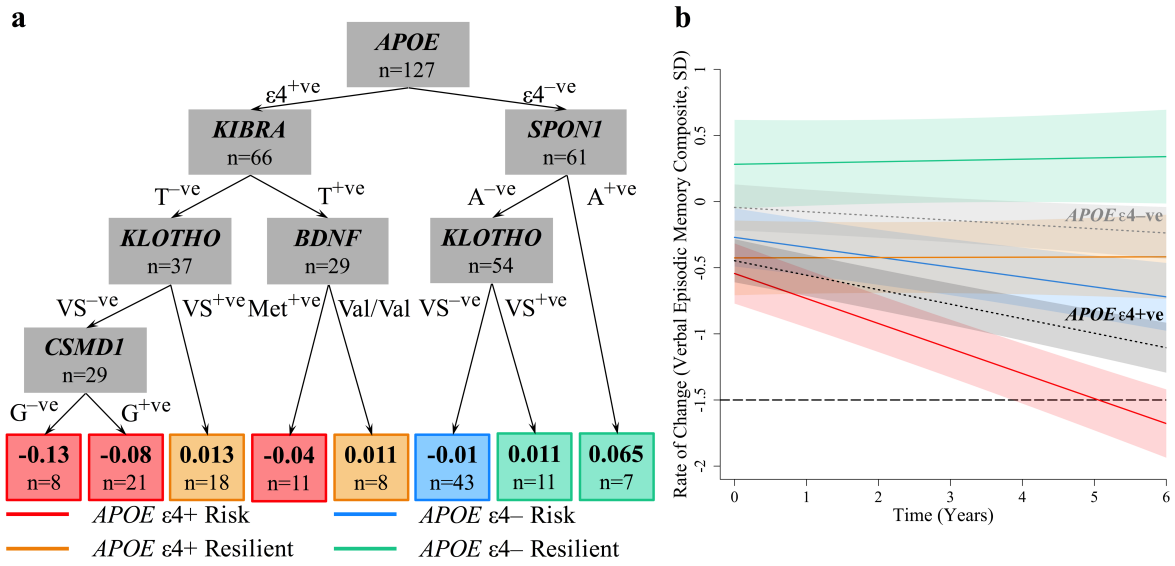

Figure 4.2.1 Cognitive Gene Risk Profile (Cog-GRP)

(a) Derivation of the cognitive gene risk profile (*Cog-GRP*) within cognitively normal adults high Aβ-amyloid ($A\beta^{high}$; n=127) using decision tree analysis. Defining four groups ε4+ Risk (red, n=40), ε4+ Resilient (orange, n=26), ε4– Risk (blue, n=43), ε4– Resilient (green, n=18). Values represent SD/year change in the verbal episodic memory composite. All analyses were corrected for age, sex, years of education, premorbid IQ and depressive symptoms. (b) Confirmation of performance of collapsed groups identified by the *Cog-GRP* in $A\beta^{high}$ cognitively normal adults (n=127) and comparative performance of *APOE* when considered independently. ε4– Resilient group (green line), ε4– Risk group (blue line), ε4+ Resilient group (orange line), ε4+ Risk group (red line), *APOE* ε4-ve (grey dotted line), *APOE* ε4+ve (black dotted line). Broken black line represents 1.5 SD of decline. Error bars represent time dependent standard error, *p<0.05 when comparing to the ε4+ Risk group

4.2.4.3 Performance of Cog-GRP groups on cognition in cognitively normal adults

When investigating the association between *Cog-GRP* groups and cognition in $A\beta^{high}$ CN older adults, no significant differences were observed at baseline in any composite measures. The ε4+ Risk group showed a significantly greater rate of decline on the global composite (p=0.00009), clinical progression composite (p=0.0003) and AIBL-PACC (p=0.0022), over six years when compared to ε4- Resilient group (Table 4.2.2, Figure 4.2.2). At the 6th year of follow-up there was a large effect observed for the global (Cohen's *d*=2.57, 95% CI 1.82-3.26) and clinical progression composites (Cohen's *d*=1.87, 95% CI 1.20-2.50) and AIBL-PACC (Cohen's *d*=2.32, 95% CI 1.60-2.99). Further, relative to the ε4- Risk and ε4+ Resilient groups, the ε4+ Risk group also showed a significantly greater rate of decline on the global composite (p=0.020, p=0.001), and clinical progression (p=0.023, p=0.015) over six years (Table 4.2.2, Figure 4.2.2). Large effects were again observed at the 6th year of follow-up for both the global (ε4- Risk: Cohen's *d*=1.18, 95% CI 0.70-1.63; ε4+ Resilient: Cohen's *d*=1.52, 95% CI 0.94-2.06) and clinical progression composites (ε4- Risk: Cohen's *d*=0.95, 95% CI 0.48-1.39; ε4+ Resilient: Cohen's *d*=1.27, 95% CI 0.72-1.79). The ε4+ Risk group also declined significantly faster on the AIBL-PACC (p=0.040) when compared to the ε4– Risk group (at 6th year, Cohen's *d*=1.18, 95% CI 0.70-1.63), though only a trend toward significance, (p=0.073), when comparing to the ε4+ Resilient group. Across all cognitive composites there was no significant difference between groups in terms of baseline cognitive performance, with the exception of the extremes of ε4+ Risk compared to ε4- Resilient groups in the Global cognitive composite (Table 4.2.2). Finally, mean Aβ burden was observed to be significantly different between *Cog-GRP* groups (ε4+ Risk, 2.02±0.35; ε4+ Resilient, 1.85±0.21; ε4- Risk, 1.82±0.24; ε4- Resilient 1.97±0.46; F= 3.41, p=0.020); though this was driven only by a difference between ε4+ Risk and ε4- Risk groups (Post-hoc Bonferroni, p=0.026). Finally, when analyses were repeated in 397 $A\beta^{low}$ CN older adults from the AIBL study, to determine whether the defined Cog-GRP

had utility in defining cognitive decline in in biomarker negative CN older adults, no significant differences at baseline or between slopes in any composite measures were observed (see Section 4.2.8 Supplementary Data, for full sample demographics and analysis outcome measures in $A\beta^{low}$ CN older adults; Tables S4.2.3 and S4.2.4).

	ε4+ Risk group		ε4+ Resilient group		ε4– Risk group		ε4– Resilient group	
	α	β	α	β	α	β	α	β
Global	-0.578	-0.218	-0.385	**-0.019***	-0.284	**-0.089***	**0.291***	**0.030***
Clinical Progression	-0.087	-0.100	0.048	**-0.032***	-0.029	**-0.042***	0.079	**0.006***
AIBL-PACC	7.168	-0.334	8.349	-0.072	7.982	**-0.066***	8.603	**0.126***

Table 4.2.2 Group intercepts and slopes for cognitive composites in $A\beta^{high}$ CN older adults

Group intercepts (α; as SD) and slopes (β; as SD/year) for cognitive composites (presented in SD/year) and AIBL-PACC (presented in SD×4/year) in imaged cognitively normal (CN) older adults with high Aβ-amyloid ($A\beta^{high}$; n=127). *p<0.05 when comparing to the ε4+ at Risk group.

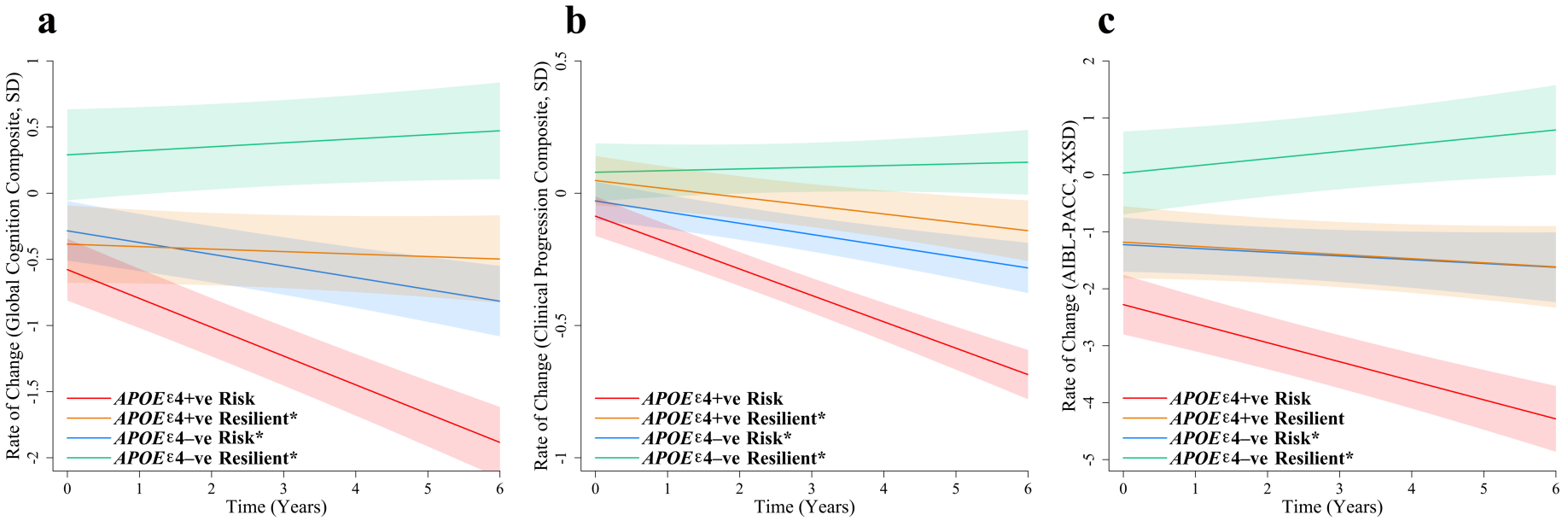

Figure 4.2.2 Cognitive rates of change in Aβ^{high} CN older adults

Cognitive rates of change are presented for a (a) global composite, (b) clinical progression composite, and (c) Pre-Alzheimer's Cognitive Composite (AIBL-PACC) in cognitively normal (CN) older adults with high Aβ-amyloid (Aβ^{high}; n=127). ε4– Resilient group (green line), ε4– Risk group (blue line), ε4+ Resilient group (orange line), ε4+ Risk group (red). AIBL-PACC controlled for age. Error bars represent time dependent standard error, *p<0.05 when comparing to the ε4+ Risk group.

4.2.5 Discussion

Results from this study support the hypothesis that combining genes previously associated with cognitive performance allows for the identification of groups of individuals with accelerated rates of cognitive decline. In CN older adults with high Aβ burden at baseline a decision tree was created driven by decline in a composite score of verbal episodic memory to define a *Cog-GRP*. This profile combined the effects of *APOE, BDNF, KIBRA, KLOTHO, SPON1* and *CSMD1*. *COMT* dropped out of the model due to lack of influence in discriminating the cognitive change within the sample. There is no association between *COMT* individually and rates of cognitive decline in this population (data not shown), so it is unsurprising that it has not contributed to the genetic risk profile created. Due to the lack of significant difference in decline and the small sample size between the groups at either extreme of the *Cog-GRP* these groups were collapsed to 4 overall classifications. These classifications were described based on carriage of the *APOE* ε4 allele; ε4+ Risk, ε4+ Resilient, ε4- Risk and ε4- Resilient. The ε4+ Risk group showed significantly faster decline in the global composite and the composite of clinical progression when compared to all other groups. Further, the ε4+ Risk group showed significantly faster decline in the AIBL-PACC when compared to the ε4- Risk and ε4- Resilient groups, however the difference between the ε4+ Resilient group only trended towards significance. Finally, we report that the defined *Cog-GRP* has no utility in defining cognitive decline in $A\beta^{low}$ CN older adults.

In $A\beta^{high}$ CN older adults, where the *Cog-GRP* was able to differentiate rates of cognitive decline, no significant differences in baseline cognition was apparent, whilst Aβ burden was only observed to be different between the two "at risk" groups. Suggesting that the observed cognitive outcomes were more dependent upon the *Cog-*

GRP than driven by either of these factors. However, the lack of observable impact of the *Cog-GRP* in in $A\beta^{low}$ CN older adults suggests that above threshold levels of brain Aβ burden is required for observable cognitive decline. This has been reported previously, where it is suggested that the absence of above threshold level of Aβ burden, even in the presence of neurodegeneration, does not confer an increased risk for cognitive decline [44]. Taken together these observations have potential implication for the design of clinical trials. Specifically, trials would likely benefit from the inclusion of both a measure of Aβ burden and risk stratification through, for example, a genetic risk profile as presented in this study.

We have previously reported the ability of genetic factors to discriminate individuals with accelerated rates of cognitive decline in the AIBL Study, above and beyond the effects of *APOE* alone [8]. In the present study, the defined *Cog-GRP* polygenic approach, showed that the ε4+ Risk group would reach clinically significant thresholds of cognitive impairment in episodic memory (performance at 1.5 standard deviations below controls, illustrated by the dashed line in Figure 4.2.1b) after 5 years, compared to approximately 14 years in the ε4- Risk group and indeterminate years for the remaining groups (due to positive slopes), including the ε4+ Resilient group. A slightly shorter period of approximately 4 years was estimated for a clinically significant decline in the global composite. The period of time to cross threshold in the ε4+ Risk group is almost twice as fast as that when considering *APOE* alone (ε4+ Risk, 5 years; ε4+ only, 9.6 years) whilst the ε4+ Resilient group shows no decline, suggesting that the additional genes affected trajectories of cognitive decline above and beyond the effects of *APOE* alone and are able to clearly define ε4 who decline or remain stable . This is further emphasised by the negligible differences in cognitive performance at

baseline in these groups. Finally, the defined ε4+ Risk group captures twice the number of individuals (n=40) as *APOE* and *BDNF* alone (n=20). Taken together, these finding suggest that broadening the scope in terms of genetic variants may provide more clinical utility for implementation in clinical trials where cognitive decline is a primary endpoint.

APOE, BDNF, KIBRA, KL, SPON1 and *CSMD1* have all previously been associated with both cross-sectional and longitudinal measures of cognitive change [7, 14, 17, 21], whereby the independent influences of these genes have been investigated thoroughly. In contrast, there is minimal research focused on the combined effects of these genes, with the research that has been conducted focusing on combination effects with either *APOE* or *BDNF* [8, 29]. In AD, polygenic investigations of disease progression and cognition have focused broadly on those genes identified in case-control GWAS studies [26-28]. While there have been a number of genetic risk scores that have been developed and are associated with longitudinal and cross-sectional cognition, these scores have had limited validation in at-risk preclinical AD cohorts. The decision tree derived *Cog-GRP* reported in this study is novel in its use of cognitively associated genes to predict decline in preclinical disease. Whilst the significant differences of large effect observed at the 6th year of follow-up, which would likely be considered to be clinically meaningful [45] (Cohen's $d > 1.0$ across all cognitive composites), suggests the strong potential for translation into clinical practice. Decision trees have been widely investigated in neurodegenerative disease research, typically for diagnosis of disease or disease stage. Investigators have used a range of approaches to achieve this: neuropsychologically-framed interview questions to discriminate dementia, MCI and controls [46], or between neurodegenerative disorders [46, 47], gene expression to

diagnose AD [48], demographic variables to determine cognitive and functional change [49], fMRI, behavioural and demographic information for diagnosis of AD [50], MMSE, neurofibrillary tangles and gene expression to classify disease stages [51]. Similarly, the decision tree reported in this study was developed for possible clinical use making the method's ease of utility favourable.

The combination of genes associated with cognitive decline is a strength of this study as it allows for the discrimination of rates of cognitive decline at preclinical disease stages, however, the authors do acknowledge the limitations in the study. The decision tree created within this study was statistically driven based on an episodic memory composite derived from specific cognitive assessments, and the use of different neuropsychological tests to create these composite scores could result in the creation of a different genetic risk profile. Secondly, participants in the AIBL Study are not randomly selected: they volunteer for involvement, likely accounting for a typically slightly higher than average level of performance in cognitive assessments, which may not represent the general population and might complicate replication in other cohorts. In addition to these limitations, the small sample sizes of the groups after discrimination by the *Cog-GRP* created could influence the results, and it will be important to replicate these findings in other cohorts that are conducive to cross validation of comparable cognitive endpoints.

Overall, this study reports a genetic risk profile, derived from *a priori* gene candidates previously associated with cognitive performance, that can partition CN older adults into groups that differ significantly in rates of cognitive decline. With the later disease stage intervention strategy of previous AD clinical trials generally considered to have

contributed to their lack of success and decline on clinical endpoints (cognitive tests) still essential to assess efficacy, there is a now a focus on preclinical AD trials and the appropriate means to select participants. The ease of clinical utility of the presented *Cog-GRP* would not only readily allow its employment in clinical trial design for group stratification but also for use in the retrospective analysis of prior clinical trial data. Furthermore, *Cog-GRP* also supports the investigation of additional genes beyond those associated with AD risk in GWAS, for defining polygenic risk scores for cognitive decline in presymptomatic biomarker positive individuals.

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4.2.7 Competing Financial Interests

CLM is an advisor to Prana Biotechnology Ltd and a consultant to Eli Lilly. PM is a full-time employee of Cogstate Ltd. DA has served on scientific advisory boards for Novartis, Eli Lilly, Janssen, and Pfizer Inc. RNM is a consultant to Alzhyme. SML has previously been a paid consultant to Alzhyme. CCR has served on scientific advisory boards for Bayer Pharma, Elan Corporation, GE Healthcare and AstraZeneca; has received speaker honoraria from Bayer Pharma and GE Healthcare; and has received research support from Bayer Pharma, GE Healthcare, Piramal Lifesciences and Avid Radiopharmaceuticals. VLV served as a consultant for Bayer Pharma; and received research support from a NEDO grant from Japan. All other authors have nothing to disclose.

4.2.8 Supplementary Data

4.2.8.1 Supplementary Materials and Methods

4.2.8.1.1 Study Participants

In addition to the 133 biomarker positive individuals, 406 CN biomarker negative older adults enrolled in the AIBL Study, a prospective longitudinal study of ageing, were investigated. Participants were classified as low ($A\beta^{low}$; n=406) Aβ burden, based on a tracer-specific SUVR threshold; <1.5, <1.10 and <0.62 for PiB, florbetapir and flutemetamol, respectively, as previously described [39]. Nine individuals were excluded from further analysis based on *KL*-VS homozygosity resulting in the inclusion 397 $A\beta^{low}$ CN adults.

4.2.8.1.1 Statistical Analysis

All statistical analyses were performed using Rstudio (Rstudio Team 2015) Version 0.98.1103 for Macintosh [42]. Baseline demographic data analyses provided means, standard deviations, and percentages across the cognitively normal sample. Differences in rates of cognitive change between the groups defined by the *Cog-GRP* were assessed in $A\beta^{low}$ group using random intercepts linear mixed-effects (LME) models and were performed using the "nlme" package in R. A *Cog-GRP* group × Time interaction was modelled across the entire sample, with the cognitive composites as the dependent variables. All analyses for the AIBL-PACC co-varied for age.

4.2.8.2 Supplementary Results

4.2.8.2.1 $A\beta^{low}$ cognitively normal adults baseline demographics, genotype frequencies and cognitive slopes

Table S4.2.3 shows the demographics, genotype frequencies and cognitive slopes of the 406 $A\beta^{low}$ CN older adults included in the study. All composites presented with positive rates of change in $A\beta^{low}$ CN adults, likely reflecting a practice effect in these participants.

4.2.8.2.2 Performance of Cog-GRP groups on cognition in cognitively normal Aβlow adults

No significant differences at baseline or between slopes in any composite measures were observed when investigating cognitively normal $A\beta^{low}$ older adults (Table S4.2.4).

		Aβ^{low} CN older adults
Age (years)		69.81 (6.20)
Female (%)		224 (56.42)
Years of Education (%)	30 (7.56)	11 (8.66)
	150 (37.78)	51 (40.16)
	72 (18.14)	31 (24.41)
	145 (36.52)	34 (26.77)
Premorbid IQ (FSIQ)		107.80 (7.09)
Depressive Symptoms (GDS)		1 (1.21)
***APOE* (% ε4+ve)**		79 (19.90)
***BDNF* (% Met+ve)**		144 (36.27)
***KIBRA* (% T–ve)**		172 (43.32)
***KL* (%VS–ve)**		293 (73.80)
***COMT* (% Val+ve)**		302 (76.07)
***CSMD1* (% G–ve)**		115 (28.97)
***SPON1* (% A–ve)**		358 (90.18)
Global Cognition*		0.0218
Clinical Progression*		0.0010
Verbal Episodic Memory*		0.0248
AIBL-PACC†		0.0213

Table S4.2.3 Baseline demographic information for Aβ^{low} CN older adults

Baseline demographic, genotype frequencies and cognitive composites slopes for cognitively normal (CN) older adults with low Aβ-amyloid (Aβ^{low}) in the AIBL Study (n=397). *Cognitive composites presented in SD/year, and the †Pre-Alzheimer's Cognitive Composite (AIBL-PACC) presented in 4×SD/year. AIBL-PACC controlled for age. *KL-VS* homozygotes excluded (n=9). GDS, Geriatric Depression Scale; FSIQ, WTAR-estimated FSIQ.

	ε4+ Risk group β	ε4+ Resilient group β	ε4– Risk group β	ε4– Resilient group β
Global	0.014	0.033	0.015	0.034
Clinical Progression	0.004	0.012	0.003	-0.008
Verbal Episodic Memory	0.026	0.028	0.017	0.038
AIBL-PACC	0.028	0.083	0.009	0.020

Table S4.2.4 Mean slopes for cognitive composites in $A\beta^{low}$ CN older adults

Mean slopes for cognitive composites (presented in SD/year) and AIBL-PACC (presented in SD×4/year) in imaged cognitively normal (CN) older adults with low Aβ ($A\beta^{low}$ n=397). *p<0.05 when comparing to the ε4+ at Risk group

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4.3 Epilogue

The study presented in this chapter aimed to investigate whether there is a synergistic effect of genes previously associated with cognition, and further what the best combination of these genes would be. To successfully achieve this aim, a decision tree approach was implemented utilising genetic variants in seven genes, *APOE, KIBRA, KLOTHO, BDNF, COMT, SPON1* and *CSMD1*. The resultant episodic memory-derived cognitive gene risk profile included all variants apart from *COMT* and defined four groups: *APOE* ε4+ Risk, ε4+ Resilient, ε4- Risk, ε4- Resilient. In measures of verbal episodic memory, global cognition, and clinical progression the *APOE* ε4+ Risk group declined significantly faster that all other groups. This defined cognitive gene risk profile supports the notion that combining genetic variants associated with cognition has utility for prediction of cognitive decline at the preclinical stages of AD, even if independently they do not. It also provides further weight for such variants to be considered for inclusion, along with AD risk associated variants, in polygenic risk score PRS development.

With this view, and considering the lack of utility of AD risk weighted PRS and the overriding impact that *APOE* ε4 has, the study presented in Chapter 5 attempts to undertake a novel approach to combine both cognitive performance and AD risk associated variants into a single PRS weighted by a phenotype more suited to the desired outcome, being the prediction of cognitive decline in preclinical AD. Employing such a phenotype specific approach to PRS weighting is hypothesised to yield a PRS that would allow for a more accurate prediction of cognitive decline in preclinical AD.

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Chapter 5

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CHAPTER 5: Assessing the utility of a novel method of weighting a priori candidate, cognition and AD-risk associated, genes for predicting cognitive decline in preclinical AD.

5.1 Prologue

The preceding chapters in this thesis have explored several approaches for the prediction of cognitive decline in the preclinical stages of Alzheimer's disease (AD). In Chapter 2 a polygenic risk score (PRS) weighted by AD risk was analyzed that as a whole was significantly associated with cognitive decline. However, after the removal of Apolipoprotein E (*APOE*) this association was lost, suggesting it had negligible utility above and beyond *APOE.* The following chapters aimed to broaden the scope for selection of genetic variants to be included in polygenic approaches to predict longitudinal cognitive performance. The studies presented in Chapter 3 provided evidence that a targeted selection of genes with *a priori* evidence of association with cognitive performance had utility. Whilst Chapter 4 suggested that a genetic risk profile combining the effects of the cognitive risk genes examined in Chapter 3 could be used to define a population of individuals declining at significantly accelerated rates. Chapters 3 and 4 thus suggest that broadening the scope of genetic variants included in a PRS may provide increased utility. However, as suggested by the study presented in Chapter 2, the choice of weighting to apply to such a PRS needs to be carefully considered.

These preceding chapters have therefore laid the foundation and provided the supporting evidence for the study presented in this chapter, which hypothesizes that through the use of an endophenotype weighting of genetic variants previously associated with AD risk and cognitive decline, improved prediction of preclinical rates of cognitive decline would be possible. To address this hypothesis, this study will address the fourth and final aim of the thesis, being to *determine a method of weighting genes associated with both AD-risk and cognition, for use in a genetic risk score to improve the prediction of preclinical cognitive rates of change.*

To achieve this aim, it was proposed to combine genes and variants studied in this thesis, that have previously been associated with either an increased risk for AD (Chapter 2) or associated with cognitive performance (Chapter 3 and 4). These variants would be weighted by effect sizes for decline in verbal episodic memory, one of the earliest cognitive domains to decline, [1-3], in a reference sample of cognitively normal individuals with high brain Aβ-burden. The resultant effect sizes allowed for the calculation of a cognitively weighted PRS (*cw*PRS), the performance of which was then assessed in a further test sample with respect to decline in performance across multiple cognitive composites.

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5.2 A polygenic risk score derived from episodic memory weighted genetic variants is associated with cognitive decline in preclinical Alzheimer's disease

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CHAPTER 6: Overall Discussion

The limited success of clinical trials for effective disease modifying treatments for Alzheimer's disease (AD) has, in part, been attributed to targeting the disease at symptomatic clinical stages where underlying neurodegeneration is well established [1]. In recognition of this there has been an increased research focus on characterising the preclinical disease stages of AD thereby enabling the implementation of disease modifying interventions at stages where the disease is pre-symptomatic [2]. To achieve this requires the accurate identification of pre-symptomatic individuals who will progress to develop AD (i.e. preclinical AD) and subsequently those who are most likely to present with the fastest rates of change in clinical end-points. Together, the identification of these individuals is critical for the design of preventative or early intervention clinical trials.

The accuracy of identification of individuals at the preclinical stages of AD has improved greatly through the advances in brain imaging, i.e. Aβ-amyloid (Aβ). This is reflected in both the current diagnostic criteria, which incorporates high brain Aβ burden [2], and the use of Aβ imaging in pre-trial screening of participants. The major limitation of imaging techniques is the high cost involved in serial imaging, deceasing its feasibility as a primary outcome measure in large clinical trials. At present, the efficacy of clinical trials is predominantly determined through the use of a primary end-point of arresting decline in cognitive performance. This places importance on the ability to identify those individuals whose cognition declines at an observable rate within the trial time frames (typically up to 2 and 4 years for Phase 2 and 3, respectively [3]). A major difficulty with this approach is the variability known to exist in rates of

cognitive performance at these earliest stages of the disease process between individuals [4].

As outlined in detail in Chapter 1, while the imaging of Aβ has increased the accuracy of preclinical AD identification, and thus identifying those who will eventually exhibit cognitive decline, it is less sensitive at predicting the rate of this decline. This suggests that, while Aβ is a necessary condition for AD, other factors (e.g. imaging, fluid biomarkers or genetics) influence the relationship between this biomarker and clinical disease progression. Recently, there has been an increased focus on tau imaging, the results showing it is associated with cognitive performance [5]. Cerebrospinal Fluid (CSF) biomarkers have been shown to predict risk of AD and cognitive performance. The collection of CSF biomarkers is invasive and so, as with imaging, does not present a viable option for serial collections. At present, there is limited information regarding the genetic factors defining the rates of cognitive performance in the preclinical stages of AD. However, if genetic factors could be established, a combinatory approach with genetics augmenting preclinical AD identification (through Aβ imaging), could present a practical method to predict the rates of cognitive decline at the early stages of the disease.

Cognitive performance has been observed to be heritable and highly polygenic [6]. In addition to genetic variants identified as conferring risk for AD (as reviewed in [7]), a number of single nucleotide polymorphisms (SNPs) have been associated with cognitive performance independent of an association with AD risk [8-16]. Several methods are currently used for the investigation of combined genetic effects on disease risk and phenotypes [17]. In AD, polygenic influences have been reported for disease

risk [18, 19], pathological biomarkers [19-22], and cognitive performance [23]. However, studies reporting on polygenic effects in cognitively normal elderly, particularly on cognitive performance, have had varying outcomes [23-32]. The discrepancy in outcomes could be partly due to a lack of knowledge regarding participants' Aβ status and thus whether they are truly representative of the preclinical stages of AD. This thesis aimed to provide clarity concerning the inconsistent results previously reported, and further, to improve the methods by which combinations of genetic variants are investigated, particularly with respect to preclinical cognitive performance in AD. The overarching hypothesis of this thesis was that *a combination of genetic factors will influence cognitive rates of change in preclinical Alzheimer's disease*.

To clearly define factors, genetic or otherwise, that contribute to changes in cognition at the earliest pre-clinical stages of AD, highly characterised longitudinal cohorts are required. This thesis benefits from access to a world leading longitudinal cohort, the Australian Imaging, Biomarkers and Lifestyle Study of Aging (AIBL). The AIBL study has collected extensive data from participants at 18-monthly intervals including cognitive, neuroimaging and biological assessments. Currently, the study consists of 7.5 years of longitudinal follow-up, allowing for a detailed analysis of preclinical performance.

Initially, I implemented established strategies to assess combined genetic influence of established AD risk genetic variants on preclinical cognitive performance (Chapter 2). AD-risk effect-size weightings, derived from large AD-risk GWAS [33], were applied and resultant PRSs assessed with respect to cognitive performance. In order to address

the potential biased selection of genetic variants and thus encompass a wider range of gene variants, I then investigated genetic variants previously associated with cognitive performance and assessed their influence in preclinical AD, both independently (Chapter 3) and in combination (Chapter 4). Finally, a novel method of phenotype derived effect-size weighting was applied to both AD-risk and cognition associated gene variants, which allowed for them to be appropriately combined into a PRS and used to predict preclinical cognitive performance (Chapter 5).

Polygenic risk scores weighted by a measure of AD-risk have limited utility for the prediction of preclinical cognitive performance.

The most common approach currently utilised for investigating the influence of genetic variant combinations in AD, is through the calculation of AD-risk weighted Polygenic Risk Scores (PRSs). Using the calculation methods previously described [19], I *assessed the impact of genetic variants previously associated with AD risk on measures of cognition at a preclinical stage* (Chapter 2). In addition to this aim, associations between an AD-risk weighted PRS, and AD fluid and imaging biomarkers were also investigated. Further, the PRS was investigated both with ($PRS^{\bar{c}APOE}$) and without ($PRS^{\bar{s}APOE}$) Apolipoprotein E (*APOE*), to understand the dependence of these associations on carriage of the *APOE* ε4 allele. The results presented in this chapter confirmed previous reports of significant associations between $PRS^{\bar{c}APOE}$ and pathological biomarkers, particularly increased neocortical Aβ [30], and reduced levels of CSF Aβ [19, 21, 28] and Aβ:total-tau ratios. The $PRS^{\bar{s}APOE}$ was significantly associated with increased levels of CSF total-tau [27] and phospho-tau [27]. It could be speculated that the contrasting results when including or excluding *APOE* could be explained by differences in genetic associations with specifically different aspects of

AD pathology. It is currently well accepted that *APOE* is strongly associated with brain Aβ burden [34], and it can be hypothesised that the additional genes included in the PRS calculation are more closely associated with Tau. Several studies have observed an influence of a number of these genes on *in vivo* pathological changes in Tau and its propagation [35-37].

As previously established, the use of brain imaging data available within the AIBL study allows for the true identification of individuals in the preclinical stages of AD. When specifically investigating the association between PRSs and cognitive performance in a preclinical AD sample (defined as cognitively normal older adults with high neocortical Aβ) the following findings were reported. Significant associations were reported between $PRS^{\bar{c}APOE}$ and composite measures of cognition, specifically global cognition, verbal episodic memory, and the AIBL Pre Alzheimer's Cognitive Composite (AIBL-PACC). These associations were observed at both study baseline and in terms of longitudinal change. However, after the removal of *APOE* from the calculation of the $PRS^{\bar{s}APOE}$ no significant associations were recorded.

These results confirm previous findings reported in the literature, particularly the loss of association after removal of *APOE* [24, 25]. Additional investigations found that individuals within the upper quartile of $PRS^{\bar{c}APOE}$ scores declined cognitively at rates significantly faster than individuals within all other quartiles. However, closer examination of the distribution of *APOE* ε4 carriage amongst these quartiles revealed that this association is most likely driven by the influence of this distribution. It was observed that the upper quartile included all of the *APOE* ε4 homozygote individuals and a significant proportion of *APOE* ε4 heterozygotes. Further, the proportion of

APOE ε4 heterozygotes within quartiles declined with reducing risk. The chapter described above represents one of a small number of studies investigating the utility of AD-risk weighted PRSs for the prediction of preclinical cognitive performance in AD. To the best of the author's knowledge it is the first to thoroughly investigate the influence of *APOE* within a PRS. Specifically, the saturating effect of *APOE*, and the spread of ε4 alleles over the range of the PRS, particularly influencing investigations concerning cognitive measures.

Results from this study suggest that while PRSs calculated in this manner can be utilised for the prediction of AD and pathological AD biomarkers, they lack utility when predicting rates of preclinical cognitive decline. Particularly, this is supported by the observed *APOE* dependent cognitive association. These results, combined with the hypothesis that the genetic architecture of AD-risk likely differs from that of AD progression, informed the decision to investigate genes with *a priori* evidence of influence over broad cognitive performance (Chapter 3).

Genetic variants previously associated with broad cognitive performance influence rates of cognitive decline in a preclinical AD sample.

A perceived weakness of previous efforts that have investigated polygenic risk in AD is the biased selection of genetic variants which have focused largely on those associated with AD diagnosis. A number of genes and gene variants have been associated with cognitive performance and have been speculated to influence decline in preclinical AD. However, due to a lack of association with clinical AD diagnosis have been largely overlooked or excluded [24] from previous polygenic approaches.

One such example is the non-synonymous variant (rs6265; Val66Met) within brain derived neurotropic factor (*BDNF*). Our group has extensively reported its influence on rates of cognitive performance [9, 10], yet it has negligible influence on AD risk [38]. In previous studies in AIBL, the effect of *BDNF* Val66Met on longitudinal cognitive performance is seen above that of *APOE* ε4 in preclinical AD, specifically in cognitively normal older adults having increased neocortical Aβ burden [9, 10]. In addition to *BDNF*, a number of other genetic variants have been associated with cognitive performance that are independent of AD risk. For these reasons, it was the aim of Chapter 3 to *assess the effects of genes with a priori evidence for association with cognition on cognitive rates of change in preclinical AD.*

Previous studies have shown associations between the gene variants investigated in Chapter 3 (Kidney Brain expressed protein, *KIBRA* [39]; F-Spondin, *SPON1* [16]; Catechol-O-methyltransferase, *COMT* [40]; Klotho, *KL* [41]) and cognitive performance. No such associations were observed here when assessing the independent influence of each variant in the current studies. However, in the presence of an elevated brain Aβ burden and *APOE* ε4 carriage a number of significant associations are found. It should be noted that the studies presented in this thesis are the first to investigating the interactional effects of the gene variants of interest with Aβ burden and *APOE* ε4 carriage.

Investigation of *KIBRA* rs17070145, in combination with Aβ burden and *APOE* ε4 carriage, showed an influence of the gene over memory performance and hippocampal atrophy[14]. Specifically, individuals who had high Aβ burden, carried an *APOE* ε4

allele, and homozygote for the *KIBRA* C allele declined significantly faster than those carrying at least one copy of the *KIBRA* T allele [14].

The findings reported in Chapter 3 support those from previous studies reporting a protective influence of the *KIBRA* T allele over cognitive performance [13, 39, 42-45]. Gene expression [39, 46-48], brain activity [13, 39], and functional studies [46, 49, 50] investigating *KIBRA* support the hypothesis that the *KIBRA* T allele promotes resilience to cognitive decline. *KIBRA* expression occurs mainly in those areas responsible for memory function [39, 46], with those carrying a T allele reporting increased hippocampal activity during memory tasks [13, 39]. More recently, the postsynaptic KIBRA protein has been reported to mediate *tau* associated memory decline [50].

Assessment of *SPON1* rs11023139 resulted in trends towards significance when investigating the independent effect of this variant on cognitive performance, particularly in measures of global cognition and verbal episodic memory. As in the analysis of *KIBRA*, after interaction with Aβ burden and *APOE* ε4 carriage, significant associations between SPON1 rs11023139 and cognitive performance were observed. High Aβ burden, carriage of an *APOE* ε4 allele, and a *SPON1* A allele resulted in cognitive decline at a significantly faster rate compared to those with the same Aβ burden and *APOE* ε4 carriage carrying no *SPON1* A alleles. These findings related to this variant are the first to build upon those initially reported in the Alzheimer's disease neuroimaging initiative (ADNI) [16].

Biologically, functions of the protein (Spondin-1) encoded by *SPON1* support the genetic variant findings described above. Specifically, Spondin-1 is involved in

neuronal development in embryos [51], regeneration of axons [52], and regulation of amyloid precursor protein (APP) cleavage by beta-secretase [53]. The specific effects of identified genetic variation within *SPON1* have not been assessed, and further such investigation will be valuable.

In contrast with the analysis of the two previous gene variants, analysis of *COMT* Val158Met and *KL*-VS variations revealed no significant associations with cognitive performance were observed, even after interaction with Aβ burden and *APOE* ε4 carriage. While the current study assessing *COMT* Val158Met was unable to replicate previous reported associations with cognitive performance [54-58], it is not the first to report no effect of the variant [59-61]. Likewise, previous reports investigating the influence of *KL*-VS have differed with a number reporting that heterozygosity was associated with improved cognitive performance or reduced decline [12, 41, 62, 63], and a further study similarly reporting no associations [62].

While significant associations were not observed for all variants investigated here, it cannot be assumed that they confer no influence over preclinical cognitive decline. As observed in *KIBRA* and *SPON1*, where significant associations were only observed after interaction with *APOE*, it could be hypothesised that variants within *KL* and *COMT* require combination with additional genetic factors before their impact is observable. As such, studies outlined in Chapters 4 and 5 were undertaken that aimed to determine the influence on cognitive performance of the combination of the cognition associated genes studied in Chapter 3 (Chapter 4) and then the combination of both AD-risk and cognition associated genes (Chapter 5).

Combining genes previously associated with cognition can define profiles of risk for, and resilience to, preclinical decline in cognition.

The Initial aim was to focus on those genes previously associated with cognitive performance, specifically, *investigate whether there is a synergistic effect of genes previously associated with cognition, and further what the best combination of these genes would be* (Chapter 4). Investigating a reduced number of genes known to influence cognition was proposed to be optimal for use in a simple method for routine clinical use.

Clinically, decision tree based risk profiles have been utilised for risk triage and disease differentiation in neurodegenerative disorders [64-68]. Thus, a cognitive genetic risk profile (*Cog-GRP*) was developed using a statistically calculated decision tree driven by longitudinal change in verbal episodic memory. This profile was developed in cognitively normal individuals with high neocortical Aβ burden. In addition to the genes investigated in the previous chapter (*KIBRA*, *SPON1*, *COMT*, *KL*), *APOE* [8], *BDNF* [9, 10] and *CSMDI* (CUB and Sushi Multiple Domains 1) [15, 69] were also included in the definition of the *Cog-GRP* based on their associations with cognitive performance in other studies.

Calculation of the *Cog-GRP* resulted in the utilisation of all genes with the exception of *COMT*. No significant associations were observed independently between *COMT* and cognition, as presented in Chapter 3. This finding supports the results of a number of previous studies including a large meta-analysis [70]. Four classifications were determined based on the *Cog-GRP* after the groups were collapsed based on sample size and similarities in rates of cognitive change; ε4+ Risk, ε4+ Resilient, ε4- Risk and

ε4- Resilient. The most at risk group, ε4+ Risk, was reported to decline significantly faster on additional measures of cognition including; global cognition and clinical progression, compared to all other groups. Further, it was observed that when comparing to *APOE* alone, those individuals in the ε4+ Risk group, declined to a clinically significant threshold (1.5 standard deviations lower in cognitive performance than controls) twice as fast, 5 years compared to 9.6 years. While the decision tree method is not novel, the combination of the genes presented here in the *Cog-GRP* is the first to the author's knowledge to be published.

A phenotypically relevant weighting of genetic variants can define a polygenic risk score for preclinical cognitive performance in the presence and absence of APOE.

The $PRS^{\bar{c}APOE}$ and $PRS^{\bar{s}APOE}$ described in Chapter 2 were calculated through applying an AD-risk weighting. The admixture of additional genetic variants was observed to have no effect above that of *APOE* alone, despite these genes having been previously associated with AD-risk and cognitive performance [24, 71-76]. In additional, despite being previously associated with cognition, the genes presented in Chapters 3 and 4 have been, at best, weakly associated with AD risk. For these reasons, when combining AD-risk and cognitive-risk genes the current most utilised method of polygenic risk score calculation, AD-risk weighting, was deemed inappropriate. As such, it was aimed to *determine a method of weighting genes associated with both AD-risk and cognition, for use in a genetic risk score to improve the prediction of preclinical cognitive rates of change* (Chapter 5).

While previously published PRSs, with conservative SNP inclusions, have largely not included variants associated with cognitive performance, or have excluded them based

on lack of influence [24], results from the aforementioned *Cog-GRP* provides evidence for their inclusion. To accurately account for the impact of the included genetic variants on preclinical cognitive performance, each was weighted by an effect size associated with verbal episodic memory performance over 7.5 years. As in the development and testing of previous genetic associations, these weightings were created and tested in a preclinical AD sample as defined by cognitive normality and high Aβ burden. The resulting PRS (*cw*PRS) was tested in an additional sample and found to be associated with cognitive performance, specifically, verbal episodic memory, global cognition and the AIBL-PACC. The *cw*PRS was also associated with cognitive performance after the exclusion of *APOE* from score calculation. This thesis therefore presents, to the best of this researcher's knowledge, the first cognitively weighted PRS developed in preclinical AD. It is also one of a small number of PRSs with the ability to predict longitudinal cognitive performance in a cognitively normal sample.

6.1 Limitations

Despite attempts to overcome weaknesses within the studies presented, the following limitations of the results reported in this thesis are acknowledged. Limitations exist which are specifically related to the cohort utilised in the studies. The Australian Imaging, Biomarkers and Lifestyle Study of Aging (AIBL) cohort, data from which was utilised in all studies presented here, represents a Caucasian population which is not representative of the wider community in Australia or globally. The voluntary recruitment of AIBL participants has led to high levels of education observed in the cohort which results in cognitive performances above expectations [77]. As of 2018 the AIBL Study has an extensive 7.5-year follow-up period. Whilst this is valuable when investigating the AIBL study independently, it has the potential to hinder the ability to

validate these results in similar studies with reduced follow-up periods. Despite AIBL being a relatively large longitudinal cohort, at times reduced sample sizes are reported due to genetic stratification. Some studies presented in this thesis and therefore the reference profiles and measures developed may be difficult to replicate when investigated in smaller or less comprehensive cohorts.

This thesis focused on rates of cognitive performance in preclinical AD. It aimed to measure performance in cognitive domains known to be impacted in the early stages of AD (particularly verbal episodic memory). For this reason, statistically derived cognitive composites previously developed in the AIBL study were utilised. This is considered a limitation as the cognitive composite scores were developed based on the AIBL neuropsychological test battery, which differs from batteries administered in other studies. The absence of similar scores for the precise measurement of cognitive performance could impact on the ability to validate the results in other cohorts. At the time of completing this thesis there was a concerted effort underway amongst the cognitive arms of large prospective longitudinal cohorts to address this and define cognitive measures which would allow for future ease of cross-validation.

Finally, it is widely accepted that the *APOE* ε4 allele is the strongest genetic risk factor for AD, with carriage of one increasing an individual's risk for AD by four times and carriage of two by twenty times [78]. Within the studies presented here, and in the wider community, there is an overlap observed between increased neocortical Aβ-amyloid burden and carriage of an *APOE* ε4 allele. This could confound the investigations of genetic interactions in those with high Aβ burden. That being said, in Chapter 5, this thesis presents a novel PRS that shows utility independent of *APOE*. There was an

observable reduction in association with the exclusion of *APOE* and so further studies are recommended to address this potential confounder.

6.2 Future Directions

Published studies investigating polygenic risk in AD currently focus on AD-risk as a weighting measure [18-28, 31, 32, 79-87]. Only recently have studies been presented taking into account addition phenotypic weighting factors [88]. Further, even in these recent studies, a lack of understanding of polygenic risk in the prediction of preclinical cognitive performance in AD remains.

The research presented here confirms the *APOE* dependent nature of associations between AD-risk weighted PRSs and cognition [24, 25], and further describes the influence of specific genetic variants on cognitive performance in preclinical AD. To build upon these findings, a phenotype weighted PRS was developed and found to be associated with cognitive decline over 7.5 years. Further validation of the methods investigated in this thesis is required in other independent longitudinal studies with similar phenotypic information. This will increase the ability to assess clinical utility of the work presented. Outlined here are future directions of the work currently presented, including those which would possibly allow for its transition into a clinical setting.

Presented in all studies in the current thesis are results based on the assessment of previously developed cognitive composite scores [89]. These scores were statistically driven to best represent the verbal episodic memory domain, global cognition, and changes that occur in preclinical AD (AIBL-PACC) [90]. As described in the limitations above, while these composite scores strengthen the study by specifically

measuring domains impacted early in AD, there is a lack of comparable scores in the other large cohort studies. A number of these studies exist, most namely ADNI, however differences in the cognitive battery undertaken present difficulties for replication. In order to replicate the results presented in this thesis, the development of comparable cognitive composite scores in additional large cohort studies would be required, followed by development of reference measures for the weighting of genetic variants. Presented here are studies reporting on weighting of genetic variants in relatively small sample sizes (~150-600 participants). Currently the most utilised reference measure for the weighting of PRSs, is the odds ratio for AD-risk as calculated in a meta-analysis from the International Genomics of Alzheimer's Project (IGAP) consisting of >50,000 participants [33]. For wide scale utility of the methods discussed in this thesis, larger reference cohorts would be required to ensure wider validations.

Once the results presented in the current study are adequately validated, the aim would be for the methods and scores described to transition from research use into a clinical setting, with particular utility in patient selection for clinical trials. Clinical trials utilising genetics are currently occurring in a number of diseases including in AD. The TOMORROW Study aimed to focus drug treatment on individuals based on their *APOE* genotype and Translocase of Outer Mitochondrial Membrane 40 (*TOMM40*) repeat status, although it has recently failed due to lack of treatment effect.

As discussed previously, neocortical amyloid imaging alone is unable to predict individuals decline. The polygenic approaches developed here are targeted at individuals with high Aβ which needs to be identified through amyloid imaging. The recent development of plasma Aβ biomarkers being reported to predict brain Aβ

burden, could mean this can be utilised in the identification of individuals at risk of decline [91]. The combination of plasma biomarkers and genetic testing would represent an inexpensive and relatively non-invasive screening method for clinical trials, as a single blood sample would be sufficient for both tests.

Multimodal approaches for the development of risk profiles in preclinical AD are valuable. In addition to genetic variation, amyloid imaging and CSF biomarkers, recent developments in the mapping of brain iron have been shown to predict cognitive performance [92]. The use of multiple methods to predict cognitive performance will increase the likelihood of selecting appropriate clinical trial participants. Genetic testing would not only allow for the selection of appropriate trial cohorts after the identification of those with high brain Aβ burden, but could also reduce the number of individuals initially requiring amyloid imaging for confirmation of their preclinical AD status.

Due to the biological actions of drug candidates for AD treatments, many of these result in adverse side effects [93]. The polygenic approaches described here could assist in the movement towards personalised medicine [94], wherein individuals are prescribed medications, including adjusted doses, dependent on their expected rate of preclinical decline. Individuals expected to progress towards disease at an increased rate could require more aggressive levels of treatment to halt decline. Conversely, tailoring treatments based on rates of preclinical decline could also reduce side effects in those patients not declining at accelerated rates, by possibly lowering required dosages.

Finally, while outcomes from the current study aim to play a role in the enrolment of appropriate individuals for clinical trials going forward, they could also have utility in the analysis of historical clinical trial data. Particularly in AD, there is a wealth of information from clinical trials which is being further investigated to better understand the reasons for their failures [95]. Improving the understanding of previous clinical trials and the reasons for their failures is important in moving forward with new treatments and targets. Retrospective trial analyses and a more complete understanding of the natural history of AD have resulted in the current changes to focus clinical trials on the preclinical disease phase. Having a better understanding of individuals' genetic composition could also assist in understanding the possible reasons for the prior trial failures. This could include determining whether, based on the time frames and individuals investigated, any change in cognitive performance could have been expected.

6.3 General Conclusion

This thesis provided a thorough investigation of genetic influence over rates of cognitive performance in preclinical AD. The work highlights the importance of polygenic approaches in association studies and the limitations of the current methods, particularly in preclinical disease. The findings provide evidence that cognitive performance in preclinical AD is genetically influenced and that the genetic architecture of cognitive decline does not mirror that of AD-risk. This understanding of the genetic influences over rates of preclinical cognitive performance has significant implications in clinical trial design. Combined genetic approaches may assist the selection of those individuals that are likely to show rapid cognitive decline for inclusion in preclinical AD trials, allowing these trials to be conducted in feasible time

frames. Whilst further study is required to validate and build on the results presented here for their transition into a clinical setting, appropriate participant inclusion in AD preclinical trials would improve the likelihood of identifying an appropriate treatment for AD, reducing the enormous global impact of the disease.

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Appendices