Predicting Cognitive Decline: Genetic, Environmental and Lifestyle Risk Factors

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Declaration

This work was conducted from February 2013 to August 2016 at the Genome Diversity and Health Group, John Curtin School of Medical Research, The Australian National University, Canberra, ACT.

This thesis by compilation consists of five original publications describing the analyses I have performed during my candidature investigating the role of genetic, environmental and lifestyle risk factors in normal cognitive aging. All five publications have been published in Q1 ranked journals according to the SCImago Journal & Country Rankings in the fields of neurology, genetics, psychiatry and mental health, and geriatric and gerontology. My specific contribution to each manuscript is detailed in the subsequent pages in the form of a statement signed by the senior author of each publication.

This document has not been submitted for qualifications at any other academic institution.

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April 2017

Published Papers

Andrews, SJ, Das, D., Anstey, KJ., Easteal, S. (2015). **Interactive effect of APOE genotype and blood pressure on cognitive decline: The PATH through life project.** Journal of Alzheimer's Disease. 44(4): 1087-98. DOI: 10.3233/JAD-140630

For this publication, I designed and performed all statistical analyses and wrote the manuscript. A slightly modified version of this paper is presented in **Chapter 3.**

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Andrews SJ, Das D, Cherbuin N, Anstey KJ, Easteal S. (2016). **Association of genetic risk factors with cognitive decline: The PATH through life project.** Neurobiology of Aging. 41: 150-158. DOI:10.1016/j.neurobiolaging.2016.02.016

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For this publication, I designed and performed all statistical analyses and wrote the manuscript. A slightly modified version of this paper is presented in **Chapter 7**.

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Kaarin J. Anstey

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Abstract

With advancing age individuals experience a deterioration in cognitive abilities that is characterized by substantial inter-individual variation in the observed trajectories of cognitive decline. Late onset Alzheimer's disease (LOAD) susceptibility genes and environmental risk factors are good candidates for association with cognitive decline, as the pathological features of LOAD progress to varying degrees in individuals without dementia or cognitive impairment and are associated with nonclinical cognitive decline.

This thesis investigates whether Alzheimer's disease risk factors and genetic variants previously associated with cognitive function are also associated with cognitive decline. Data collected from the 60+ cohort of the Personality and Total Health (PATH) through life project was used, in which 2,551 participants were assessed at 4-year intervals for a total of 12 years on a comprehensive battery of cognitive tests.

The publications in this thesis investigate the following. First, whether *APOE*ε4* moderates the association between high blood pressure and cognitive function in late life. It was observed that a *APOE*–hypertension interaction was associated with a small but statistically significant increase in the rate of decline of episodic memory, verbal ability and global cognition. In contrast, the interaction between *APOE* and mean arterial pressure interaction had no effect on rate of decline.

Second, the role of 25 LOAD risk loci in non-linear cognitive change was examined, both individually and collectively as a genetic risk score (GRS). Twelve LOAD risk loci were associated with baseline cognitive performance (*ABCA7, MS4A4E, SORL1*), linear rate of change (*APOE, ABCA7, EPHA1, INPP5D, ZCWPW1, CELF1*) or quadratic rate of change (*APOE, CLU, FERMT2*). In addition, a weighted GRS was associated with linear rate of change in episodic memory and information processing speed.

Third, the role of 9 single nucleotide polymorphisms that have been previously associated with cognitive performance was further examined, with 6 SNPs observed to be associated with baseline cognitive performance (BDNF, *PDE7A, AKAP6*), linear rate of change (*COMT*, *CTNNBL1, PDE7A*) or quadratic rate of change (*MIR2113*).

Finally, it was examined whether a risk score comprised of lifestyle, medical and demographic factors (the Australian National University Alzheimer's disease Risk Index; ANU-ADRI) and a LOAD GRS were predictors of progression to Mild Cognitive Impairment (MCI). A higher ANU-ADRI score was associated with a higher probability of transitioning from normal cognition to cognitive impairment, while the GRS was associated with an increased risk of transitioning from normal cognition to dementia.

These results suggest that a subset of LOAD related SNPs may be associated with cognitive decline. However, the effect size of each locus is small and when demographic and lifestyle factors are taken into account, neither individual SNPs nor GRS explain a significant proportion of the variance in cognitive decline in our sample. Further research is required to verify these results and to examine the effect of preclinical LOAD in genetic association studies of cognitive decline. The identification of LOAD risk loci associated with cognitive performance may help in screening for individuals at greater risk of cognitive decline.

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Abbreviations

3MS	Modified Mini-Mental State
Aβ	Amyloid beta
ACR	Annualized conversation rate
AD	Alzheimer's disease
aMCI	Amnestic mild cognitive impairment
ANU-ADRI	Australian National University Alzheimer's disease risk index
BBB	Blood brain barrier
CN	Cognitively normal
CSF	Cerebroespinal fluid
fAD	Familial Alzheimer's disease
FDG-PET	Fludeoxyglucose F 18 Positron emission tomography
EV-GRS	Explained variance genetic risk score
GPS	Genome-wide polygenic score
GRS	Genetic risk score
GWAS	Genome-wide association study
LD	Linkage disequilibrium
LOAD	Late onset Alzheimer's disease
LMM	Linear mixed effect model
OR-GRS	Odds ratio genetic risks score
MAF	Minor allele frequency
MAP	Mean arterial pressure
MCI	Mild cognitive impairment
MCI-TB	Test based mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
MSM	Multi-state models
naMCI	Non-amnestic mild cognitive impairment
NFT	Neurofibrillary tangle
SC-GRS	Simple count genetic risks score
SNP	Single nucleotide polymorphism
VaD	Vascular dementia

Chapter 1: Introduction

With advancing age individuals experience a deterioration in cognitive abilities that is characterized by substantial inter-individual variation in the observed trajectories of cognitive decline, reflecting a broad spectrum of cognitive change (Figure 1.1). This continuum consists of individuals at one end whose cognitive abilities are preserved or decline at a rate that is considered within the bounds of normal cognitive aging, to those who exceed expected rates of decline and see their cognitive and functional abilities severely impaired, and who are often diagnosed with dementia. In between these two extremes, there are various stages of cognitive impairment.

1.1 The Continuum of Cognitive Change

1.1.1 Cognitive Aging

Inter-individual differences in cognitive abilities follow a hierarchical structure, with individuals differing in their general cognitive ability ('*g*'), their cognitive ability in broad cognitive domains, and their ability in relation to specific cognitive tests. The concept of general cognitive ability was initially proposed by Spearman, who observed that an individual's abilities on specific cognitive tests were highly correlated and that *g* explained about 40% of total variance in cognitive test performance [1]. Furthermore, it was observed that specific cognitive tasks with similar content correlated more strongly than those with different content, implying that specific tests were associated with different cognitive domains [2]. Individual cognitive tests can be broadly categorized as measuring either 1) *crystallized cognitive abilities* related to verbal and numerical abilities and general knowledge, which are assessed by tests of stored knowledge, or 2) *fluid cognitive abilities* related to memory, executive functions, reasoning and processing speed, which are assessed by tests of on-the-spot processing. However, these cognitive domains explain a relatively small proportion of individual cognitive variation. Most is explained by either general cognitive ability (g) or specific tests.

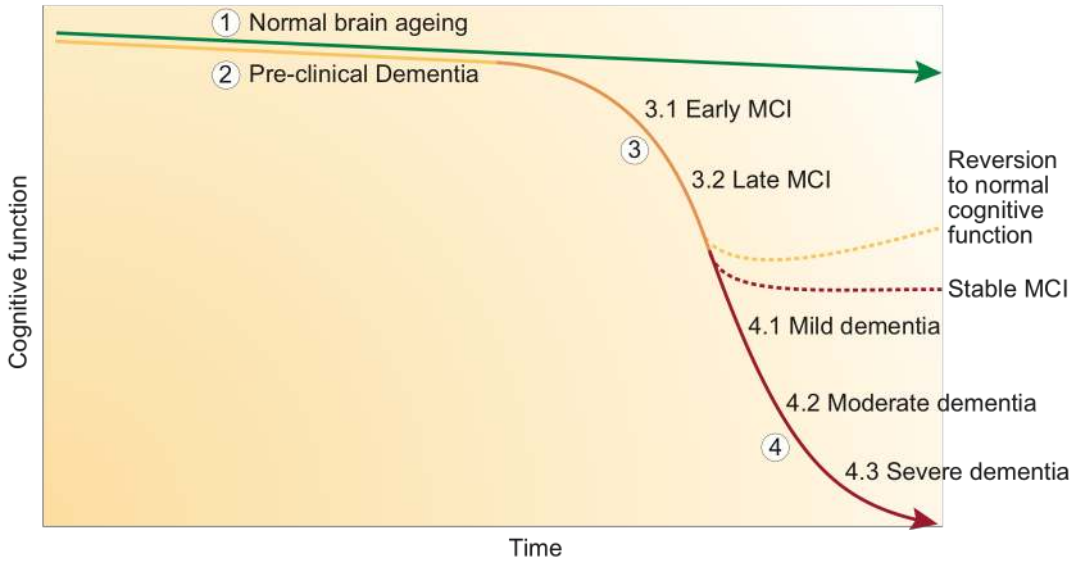

Figure 1.1: *The Hypothetical trajectory of normal and pathological cognitive decline. Individuals who undergo pathological decline pass through several stages including Pre-clinical dementia (see subsection 1.2.4), mild cognitive impairment (see subsection 1.1.2) and dementia (see subsection 1.1.3). (Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Neurology, Hampel and Lista 2016* [3]*, copyright 2016).*

Cognitive domains are affected differently by age. Ageing has the greatest adverse impact on domains related to fluid intelligence, with ability peaking around the third decade of life and then declining at a rate of -0.02 SD per year thereafter [4]. Conversely aging has positive effects on cognitive domains related to crystallized intelligence, resulting in a gradual improvement in crystallized abilities at a rate of 0.02 SD per year through to the sixth or seventh decades of life before gradually declining [4].

Age-associated cognitive decline is associated with increased difficulties in performing tasks involving memory or rapid information processing. This is particularly important in daily living, as many activities in contemporary life require these cognitive abilities. Declines in cognitive performance have been associated with poor decision making [5], difficulties with instrumental activities of daily living [6,7] and, poor health literacy [8]. Even though no single aspect of daily life is critically impaired, the slight reduction across multiple aspects can accumulate over one's life and have a significant impact on quality of life, even in the absence of dementia. Furthermore, a faster rate of

decline is associated with life outcomes, particularly with the development of dementia [9,10] and mortality [11,12].

1.1.2 Mild Cognitive Impairment

Mild Cognitive Impairment (MCI) refers to self-reported decline in cognitive function that is corroborated by an informant or clinician, and for which there is objective evidence of cognitive impairment via neuropsychological testing. The cognitive deficits does not impair global cognitive function or basic activities of daily living and the individual does not meet the clinical criteria for dementia [13]. MCI can be stratified into amnestic (aMCI) and non-amnestic (naMCI) subtypes, with aMCI characterized by specific cognitive impairment in memory and naMCI characterized by cognitive impairment in non-memory domains [14]. Both aMCI and naMCI can further be classified into single or multiple domain MCI depending on the number of cognitive domains that are impaired [14].

The global prevalence of MCI is estimated be 15-20% in individuals aged over 65, with the rates heavily dependent on age group [15]. Incidence rates of MCI range between 51 and 76.8 per 1,000 person-years for all MCI subtypes; 9.9 and 40.6 per 1,000 person-years for aMCI; and 28 and 36.3 per 1,000 person-years for naMCI [16].

Individuals who develop MCI are more likely to further progress to AD. However, the clinical course of MCI is heterogeneous, as many individuals will revert to normal cognition, remain cognitively stable and not progress further or progress towards non-AD dementia. A meta-analysis found that the annualized conversation rate (ACR) from MCI to AD was 8.1% [17], which is similar to a more recent systematic review which found that it ranged from 5.4% to 16.5% per year [18]. The risk of conversion to dementia is heavily dependent on age, with an ACR of 4.6 and 20.8 in individuals aged 50-59 years and 90+ years respectively. Furthermore, there is a higher rate of progression to AD in individuals with aMCI than with naMCI, while conversely, individuals with naMCI are more likely to progress to other types of dementia [19,20].

A recent meta-analysis found that 25% of individuals who are classified as MCI will at a subsequent interview revert to normal cognition [21], though these individuals still have a higher risk of progressing to MCI or dementia at a later date [22]. This instability in the classification of MCI has lead to the US National

Institute of Aging and the Alzheimer's Association proposing a definition of 'MCI due to AD' whereby individuals meet the clinical criteria for MCI and have varying levels of AD biomarkers that are consistent with an early diagnosis of AD [23].

1.1.3 Alzheimer's Disease

At the extreme end of the continuum of cognitive decline is 'pathological cognitive decline' which leads to the development of dementia. In 2015 it was estimated that there were 46.8 million people worldwide living with dementia and an estimated incidence of 9.9 million new cases each year [24]. The number of new cases doubles with every 6.3-year increase in age, from 3.9 per 1000 person years at age 60-64 to 104.8 per 1000 person years at age 90+ [24]. The projected prevalence of dementia is expected to double every 20 years, to 74.7 million in 2030 and 131.5 million in 2050 [24]. This will have major implications for national health and social services, with the cost of caring for afflicted individuals expected to rise from USD $818 billion in 2015 to USD $2 trillion in 2030 [24].

Alzheimer's disease is the most common cause of dementia, estimated to account for 60-80% of cases [25]. The clinical symptoms of AD typically begin with a gradual deterioration of episodic memory (84-96% of cases) with other cognitive dysfunctions becoming apparent as the disease progresses resulting in impairments in visual-spatial abilities, reasoning, executive function, language ability and changes in personality and behavior [26,27]. Accompanying the loss of cognitive function is a decline in functional abilities that begins with impairment in basic activities of daily living, eventually resulting in complete loss of independent living, necessitating full-time care, and it is ultimately fatal.

AD is best conceptualized as a spectrum in which the underlying pathophysiological process begins decades before the onset of clinical symptoms [26,27]. The preclinical stage of AD is characterized by the gradual accumulation of AD pathology, which can be objectively measured using biomarkers. Critically, based on cognitive measures, individuals with preclinical AD do not meet the clinical criteria for either AD or for MCI due to AD. As a result, they are indistinguishable from individuals who are cognitively normal.

1.2 Neuropathology of Alzheimer's Disease

The aggregation and accumulation of extracellular amyloid-β peptides into amyloid plaques and the accumulation of intraneuronal hyperphosphorylated and misfolded tau into neurofibrillary tangles are the characteristic neuropathological hallmarks of AD. The accumulation of amyloid plaques and neurofibrillary tangles prompts the pathogenesis of AD by promoting alterations in lipid metabolism, neuro-inflammation, endocytosis and synaptic dysfunction and loss that ultimately leads to neuronal cell death [28,29].

1.2.1 β-Amyloid

Aβ peptides are produced by the cleavage of the transmembrane amyloid precursor protein (APP), which can occur via either the amyloidgenic or non-amyloidogenic proteolytic cleavage pathways (Figure 1.2). The majority of APP processing occurs via the non-amyloidogenic pathway which is initiated by cleavage of APP in the Aβ domain by α-secretase, releasing two molecules, soluble APPα and C83 [30]. Amyloidogenic processing of APP is initiated by β-secretase cleavage of APP, which releases two molecules, soluble APPβ, and C99, containing the Aβ domain [31]. C99 is further cleaved by γ-secretase releasing Aβ peptides that are 34-50 amino acids in length. The major form of Aβ is $A\beta_{40}$ (90%), while a smaller faction is $A\beta_{42\text{-}43}$ (5%) which is the more neurotoxic form as it is more prone to oligomerization and the formation of amyloid plaques [32]. Non-amyloidogenic processing primarily occurs at the cell surface where α-secretase is present, while amyloidogenic processing occurs after APP has been internalized and trafficked through endocytic and recycling organelles where it encounters β- and γ-secretase [33].

Increased accumulation of Aβ peptides can be attributed to increased production of Aβ, particularly in familial Alzheimer's disease as a result of increased γ-secretase activity. However, in sporadic Alzheimer's disease perturbations in Aβ clearance and degradation pathways play a larger role in Aβ accumulation [33]. The principal clearance pathway of Aβ is transcytosis across the blood-brain barrier mediated by low-density lipoprotein-receptor related protein 1 (LRP1) [34]. LRP1 regulates receptor-mediated endocytosis of cellular Aβ uptake that delivers Aβ to lysosomes for degradation [35]. Proteolytic

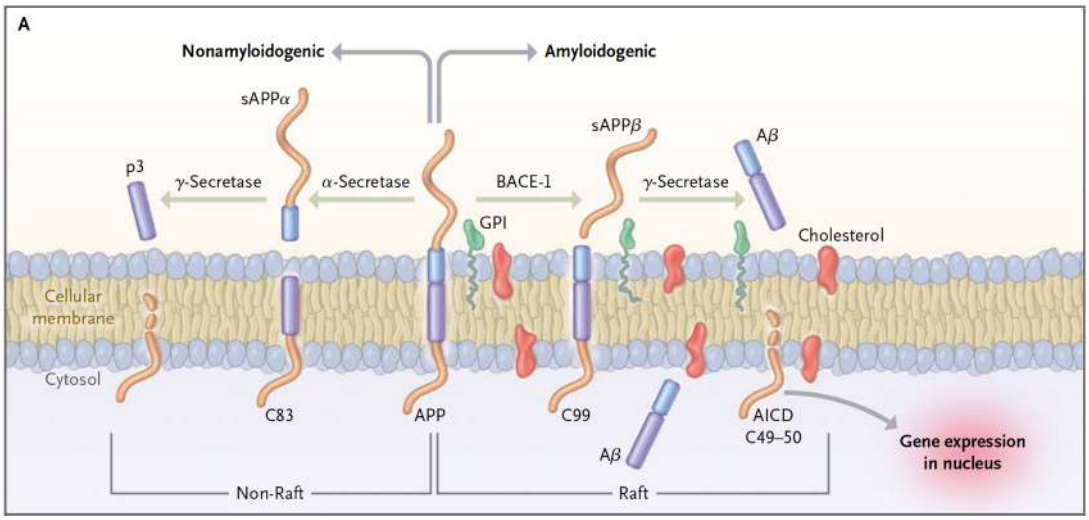

Figure 1.2: *Processing of APP via the nonamyloidgenic and amyloidgenic pathways (Reproduced with permission from Querfurth and Laferla 2012* [45]*, Copyright Massachusetts Medical Society).*

degradation also plays a major role in the removal of Aβ by cleaving Aβ peptides into shorter soluble molecules with less neurotoxic effects. Insulin-degrading enzyme and neprilysin are the principal enzymes involved in extracellular and intracellular degradation of Aβ [36,37], with other proteases including cathepsin B [38], matrix metalloprotein-9 [39], angiotensin-converting enzyme [40], endothelin-converting enzyme [41] and plasmin [42] also playing roles.

In Alzheimer's disease Aβ plaque deposits are initially observed in the isocortical regions of the brain, subsequently progressing to other regions of the allocortex (including the hippocampus, amygdala, entorhinal region and cingulate gyrus) and in the latter stages of AD progressing into subcortical regions (basal ganglia, diencephalon midbrain, medulla oblongata pons and the cerebellum) [43] (Figure 1.3).

1.2.2 Tau

Tau is encoded by the microtubule-associated protein tau (*MAPT)* gene and is predominantly expressed in neurons where it is mainly located in axons and binds to tubulin, promoting the assembly and stabilization of microtubules in the cytoskeleton [44,45]. Binding of tau to microtubules is regulated by phosphorylation of tau via kinases, and dephosphorylation via phosphatases [44,45]. An imbalance in the activities of kinases and phosphatases results in the

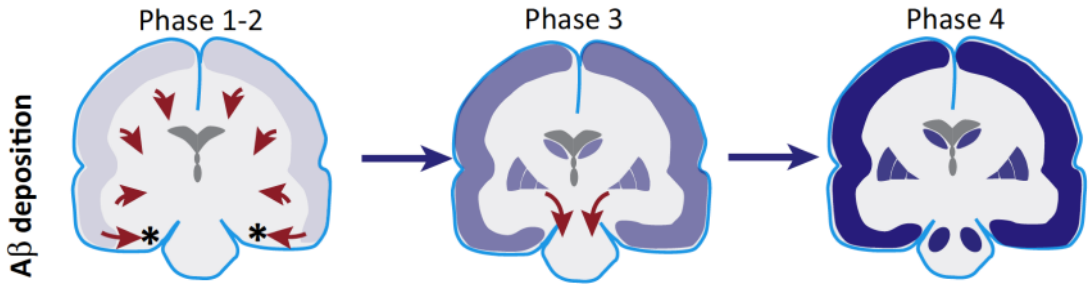

Figure 1.3: *Progression of the disposition of Aβ in the brain. (Reprinted from Trends in Neurosciences 38/10, Mhatre et al, Microglial Malfunction: The Third Rail in the Development of Alzheimer's Disease, 621-636, Copyright 2015, with permission from Elsevier* [56]*)*

aberrant hyperphosphorylation of tau, leading to the detachment of tau from microtubules where it is prone to self-aggregate into paired helical filaments (PHFs) that further aggregate into neurofibrillary tangles [44,45]. The sequestering of tau into PHFs and NFTs disrupts normal microtubule dynamics, which are essential for normal cell morphology, trafficking, functions and viability [44,45].

There are three morphological stages in the development of intracellular tau pathology [46]. First abnormally phosphorylated tau protein is observed in the axon, soma, and dendrites of otherwise morphologically normal neurons. Subsequently, mature NFTs develop in the cytoplasm, gradually expanding and displacing the nucleus to the periphery of the soma. Finally, after neuronal death, the NFT remains as an extracellular 'ghost' NFT.

Tau pathology can spread to surrounding cells in a prion-like fashion [47]. Disruption of normal microtubule dynamics induces zeiosis, releasing vesicles containing hyperphosphorylated tau into the surrounding extracellular space, which is taken up into the surrounding neurons via endocytosis [48-50]. The hyperphosphorylated tau interacts with the healthy tau in the recipient neuron, sequestering the healthy tau in a new NFT and causing the healthy tau to become pathogenic [51].

In AD the progression of tau pathology across the brain mostly goes in the opposite direction to the spread of Aβ plaques. The Braak staging scheme is used to describe the spread of tau pathology, with NFT first observed in the entorhinal cortex and hippocampus (stages I-II), progressing to the limbic structures (stages III-IV) and finally to the isocortex (stages V-VI) [52] (Figure 1.4).

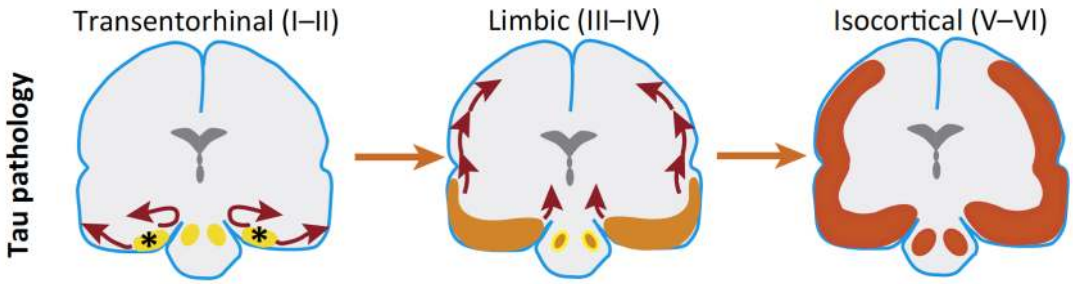

Figure 1.4: *Progression of the disposition of Tau pathology in the brain. (Reprinted from Trends in Neurosciences 38/10, Mhatre et al, Microglial Malfunction: The Third Rail in the Development of Alzheimer's Disease, 621-636, Copyright 2015, with permission from Elsevier* [56]*).*

1.2.3 Amyloid Cascade Hypothesis

The dominant model of AD pathogenesis is the amyloid cascade hypothesis, which states that the aggregation of Aβ peptides is the first step in a chain of pathological events that results in AD [53]. Translation of the amyloid cascade hypothesis into a model that can be objectively tested using contemporary techniques led to the most influential heuristic model for biomarkers in AD pathogenesis [54]. In this model (Figure 1.5) biomarkers become abnormal in a temporal order beginning with amyloid deposition (CSF Aβ and amyloid PET), progressing to markers of neurodegeneration (CSF Tau and FDG-PET) and ending with neuroanatomical atrophy (structural MRI). The gradual accumulation of abnormal levels of these biomarkers is associated with the development of the clinical symptoms of AD, though risk factors for AD can moderate the levels required for the expression of clinical symptoms.

The strongest evidence for the amyloid cascade hypothesis comes from the underlying genetic pathways of autosomal dominant familial AD (fAD). Mutations and three genes, *APP, PSEN1,* and *PSEN2* are linked to the development of fAD [60]. These genes are involved in the production of Aβ peptides (see section 1.2.1), with *APP* the precursor protein for Aβ peptides and *PSEN1* and *PSEN2* encoding the proteins Presenilin 1 and 2, which are catalytic subunits of the γ-secretase complex involved in the cleavage of Aβ peptides. Mutations in these genes favour increased production of the amyloidogenic $A\beta_{42}$ peptide [55,56]. This evidence is consistent with the amyloid cascade hypothesis because the accumulation of abnormal levels of Aβ plaques in fAD occurs prior to tau pathology, neurodegeneration, and

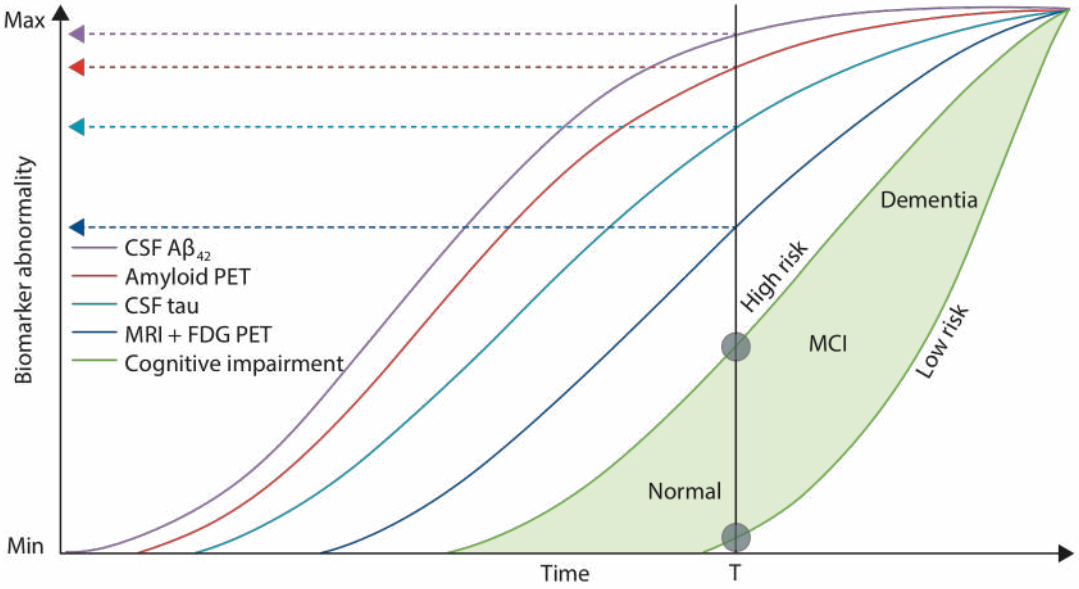

Figure 1.5: *The amyloid cascade model proposed by Jack et al 2013* [54]. *As time progresses AD biomarkers become progressively abnormal with accumulation of Amyloid followed by increased Tau pathology and neurodegeneration. At a given time (T) the level of cognitive impairment is indicated by the biomarker profile, although clinically it may be moderated by risk and protective factors. (Reprinted from The Lancet Neurology, 12/2, Jack et al, "Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers", 207-216, Copyright 2013, with permission from Elsevier* [54]*).*

dementia [57]. Evidence of this temporal ordering also comes from mutations in *MAPT* which are associated with neurodegenerative disease but do not induce Aβ pathology [58,59]. This observation has been confirmed in mouse models in which human *APP* and *MAPT* are co-expressed and the presence of Aβ peptides accelerates the formation of NFT, but not vice versa [60].

The disposition of Aβ peptides into senile plaques is necessary but not sufficient for the development of the clinical symptoms of AD. Aβ burden does not correlate well with the severity or the duration of AD [61-66], with the burden of Aβ plaques reaching a plateau shortly after the development of clinical symptoms [67], or anatomically, with neurodegeneration first observed to occur in the hippocampus and entorhinal cortex [68,69]. Conversely, tau pathology is strongly correlated with the severity and duration and the temporal and spatial development of AD [61-66]. Nevertheless, the neurodegenerative effects of tau require the presence of Aβ to be triggered, with the spread of tau pathology

beyond the limbic system and the neuronal loss associated with tau pathology only observed in individuals with coexistent Aβ pathology [70-73]. It is unclear exactly how Aβ triggers tau toxicity, although there is suggestive evidence that Aβ stimulates protein kinases and phosphatases that regulate tau phosphorylation [74].

The Aβ cascade does not operate in isolation and a complex network of cellular mechanisms may either moderate or exacerbate the Aβ cascade [53]. These include cerebrovascular disease [75], oxidative and nitrative stress [76,77], mitochondrial dysfunction [78] and inflammation [79].

To summarize the amyloid cascade hypothesis: In the normal brain there is homeostasis between the production, degradation and clearance of Aβ peptides. An imbalance in Aβ production and/or clearance results in the gradual accumulation and aggregation of pathological Aβ species. The aggregation of Aβ triggers acceleration in the formation of NFT, possibly by modulating protein kinases and phosphatases that regulate tau phosphorylation. Genetic variation, environmental stressors, other cellular mechanisms or their interactions can either moderate or exacerbate the accumulation of abnormal Aβ and tau. Formation of NFTs disrupts normal microtubule dynamics, cell morphology, trafficking and synaptic function, and promotes neuronal death. The gradual loss of neurons as a result of the spread of NFT throughout the brain underlies the clinical symptoms of AD. Additional comorbid pathologies can influence the rate of neuronal loss, thereby accelerating the appearance of clinical symptoms.

1.2.4 AD pathology in Normal Cognitive Decline

Post-mortem analysis has shown that at 40 years of age, most individuals contain evidence of pre-tangle tau pathology, and a small percentage of individuals have evidence of early stage NFT and amyloid plaques [80]. With increasing age, the prevalence of individuals with early or late stage NFT and amyloid plaques increases, with NFT Braak stage III-VI (see subsection 1.2.2) and amyloid plaques observed in 80% and 50% of individuals aged 80, respectively [80] (Figure 1.6). This pattern has been verified in studies assessing AD pathology using biomarkers

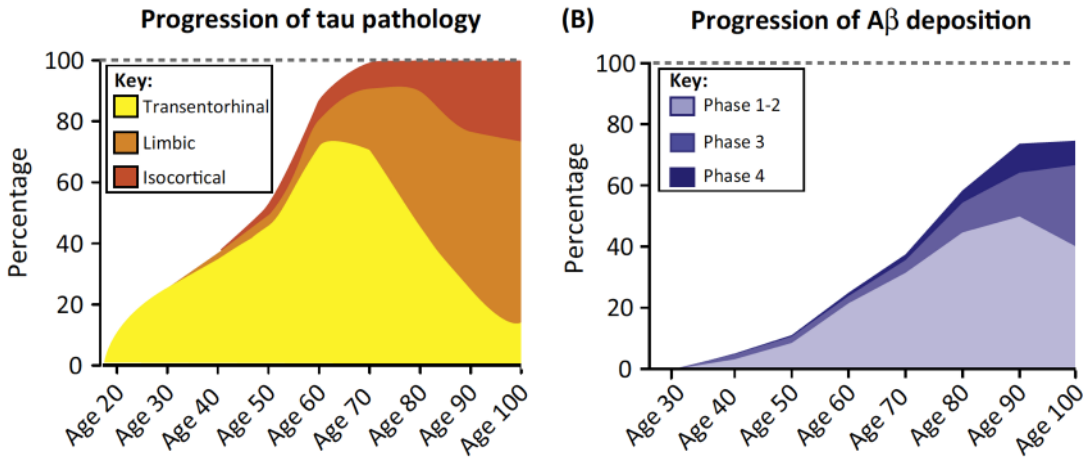

Figure 1.6: *Distribution of (A) Tau and (B) Aβ pathology by age(Reprinted from Trends in Neurosciences 38/10, Mhatre et al, Microglial Malfunction: The Third Rail in the Development of Alzheimer's Disease, 621-636, Copyright 2015, with permission from Elsevier* [56]*).*

in cerebrospinal fluid (CSF) and positron emission tomography (PET). A meta-analysis of forty-one studies evaluating the prevalence of amyloid pathology in participants with normal cognition showed that prevalence increases from 10% to 44% from the ages of 50 to 90 [81]. Tau pathology is mostly limited to early Braak stages (0-IV) in cognitively normal individuals, with pathology steadily increasing with advancing age [82,83].

This raises the question of whether all individuals with evidence of AD pathology will eventfully develop AD if they live long enough, or if the accumulation of AD pathology is a process associated with healthy aging. This prompted the NIA-AA [84] and International Working Group 2 (IWG-2) [26] to propose a framework for the preclinical stage of AD consisting of cognitively normal individuals (i.e., the absence of the clinical symptoms of AD) with biomarker evidence of AD pathology. Emerging evidence from longitudinal studies indicates that using biomarkers to classify individuals in preclinical stages of AD is predictive of future progression towards dementia. Abnormal levels of amyloid pathology in cognitively normal individuals does increase the risk of progression to dementia, with a recent meta-analysis showing an approximate ~fourfold increased risk of progression over a period of 2-7 years [85]. The co-occurrence of both Aβ and tau pathology substantially increases the risk of progressing towards dementia, with risk increasing exponentially with age [86,87]. As such, preclinical AD can be further subdivided based on low/high-risk of future progression

towards AD. Individuals with a single pathophysiological marker (either Aβ or tau) are at low risk and those with co-occurrence of both markers are at high risk [88].

Nevertheless, not all individuals with abnormal levels of AD pathology will manifest clinical symptoms of AD in their lifetime. Post-mortem analysis of individuals without a clinical diagnosis of dementia has shown that upwards of 30% of non-demented individuals at death meet the pathological criteria for AD [89]. Under the current model of AD pathogenesis this is expected because of the 15-20 year lag between the initial accumulation of AD pathology and the development of clinical symptoms, which results in many individuals with AD pathology dying before they show clinical symptoms [88,90]. From a practical point of view, however, Aβ and tau pathology are associated with subtle changes in cognitive performance in individuals even if they die before they manifest clinical symptoms of AD.

Neurotic plaques and NFT have been estimated to explain 30% of the variation in cognitive decline [91], with pathway analysis indicating that the age effect on cognitive decline is predominantly mediated via direct effects on tau pathology, in addition to small indirect effects of Aβ on cognitive decline via tau pathology [92]. Furthermore, the effect of age on cognitive performance is severely attenuated when individuals in the preclinical stages of AD are excluded from the analysis [93] or after controlling for AD pathology [94].

Increased amyloid burden was correlated with decreased episodic memory, executive function and global cognition in a meta-analysis of 16 cross-sectional datasets that used Pittsburgh compound B (PiB) PET imaging and, in an expanded analysis of 34 datasets that included additional methods of assessing amyloid burden [95]. In retrospective longitudinal post-mortem studies, individuals in the preclinical stages of AD have a faster rate of decline in global cognition compared to cognitively normal individuals [96]. Increased amyloid and tau pathology are also associated with increased cognitive decline in working and episodic memory, respectively, in addition to global cognition [97]. These results are well supported by prospective longitudinal biomarker studies where individuals with abnormal levels of amyloid pathology have a faster rate of decline in episodic memory, executive function, attention, visuospatial skills and global cognition [82,98,99]. Furthermore, individuals that accumulate Aβ at a faster rate have an increased rate of cognitive decline [67]. Increased Tau pathology in the entorhinal cortex (Braak

Stage I/II) is associated with increased rate of decline in episodic memory, and increased tau pathology in the entorhinal cortex, the limbic system (Braak stages III/IV), and isocortical structures (Braak stages V/VI) are associated with an increased rate of decline in global cognition [82]. Co-occurrence of both amyloid pathology and neurodegenerative biomarkers is associated with accelerated decline in composites of neuropsychological tests assessing global, memory and non-memory cognition, suggesting that Aβ and tau interact synergistically [100,101].

In summary, the influence of age on cognitive performance is heavily influenced by AD pathology. This raises the possibility that genetic, environmental and lifestyle factors that mediate the accumulation of LOAD pathology may also affect the rate of cognitive change in normal aging.

1.3 Genetic Risk Factors

1.3.1 Genetics of Cognition

Genetic factors contribute to the inter-individual variability observed in cognitive decline, with common genetic variants estimated to account for 40-50% of the variance in general cognitive functioning in later life and 24% of the variance in lifetime cognitive change [102,103]. To date, the majority of genetic research on cognitive decline has focused on candidate genes that have been previously associated with age-related disease, traits or mechanisms [104,105], and particularly with genes related to neurotransmitters, neurotrophins, cognitive function and neurodegenerative disease. Despite the publication of numerous genetic associations with cognitive decline, the variants identified typically explain a very small fraction of the phenotypic variance, and many remain to be replicated. Furthermore, failure to reproduce an initial positive result is common due to differences in participant characteristics (e.g. baseline education, mean age, gender, and ethnicity) and methodologies (e.g. sample size, duration of the study, number of follow-ups, population stratification, variation in classification and cognitive measures) [104].

1.3.2 The role of Alzheimer's Disease Risk Loci in Cognitive Aging

Late-onset Alzheimer's disease (LOAD) susceptibility genes are good candidates for association with cognitive decline because, as described above, pathological features of LOAD progress to varying degrees in individuals without dementia or cognitive impairment and are associated with non-clinical cognitive decline.

Genetic variance accounts for 53% of the total phenotypic variance of LAOD [106]. The *Apolipoprotein E (APOE)* epsilon (*ε4) allele was the first common genetic variant to be associated with LOAD and it remains the strongest genetic predictor of LOAD [107]. In addition to *APOE*, recent GWAS of LOAD have identified single nucleotide polymorphisms (SNPs) at 23 loci associated with LOAD (Figure 1.7; Table 1.1). GWAS performed separately by four LOAD genetic consortia initially identified 11 loci (*ABCA7, BIN1, CD2AP, CD33, CLU, CR1, EPHA1, MS4A4A, MS4A4E, MS4A6A, and PICALM* [108-112]). A further 12 loci (*HLA-DRB5, PTK2B, SORL1, SLC24A4-RIN3, DSG2, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERMT2,* and *CASS4* [113]) were identified in a meta-analysis by the International Genomics of Alzheimer's Project (IGAP).

Previous studies of associations between the initial GWAS LOAD risk loci and cognitive performance are characterized by a lack of consistent findings. The limited number of studies that have examined the role of the IGAP LOAD risk loci in cognitive performance have also produced mixed results [114-133]**.** The characteristics for these studies are listed in Table 1.2.

The *APOE* gene contains three common alleles, *ε2, *ε3, and *ε4, with the *ε4 allele conferring the largest known genetic risk for LOAD, approximately 2-3 fold and 10-12 fold increased risk for heterozygotes and homozygotes, respectively [134]. Conversely, the *ε2 allele is associated with reduced risk [134]. *APOE* is involved in lipid homeostasis, mediating transport of cholesterols and other lipids from one tissue or cell type to another [135]. Within the brain *APOE* binds to soluble Aβ and influences the aggregation, deposition and clearance of Aβ in an isoform-dependent manner, with *ε4 being the least efficient [135]. Neuropathological and neuroimaging studies have shown that the *ε4 allele is associated with more abundant Aβ disposition. In biomarker studies it is associated with CSF $A\beta_{42}$ and tau levels [146,147]. The *APOE* genotypes are also associated with specific effects

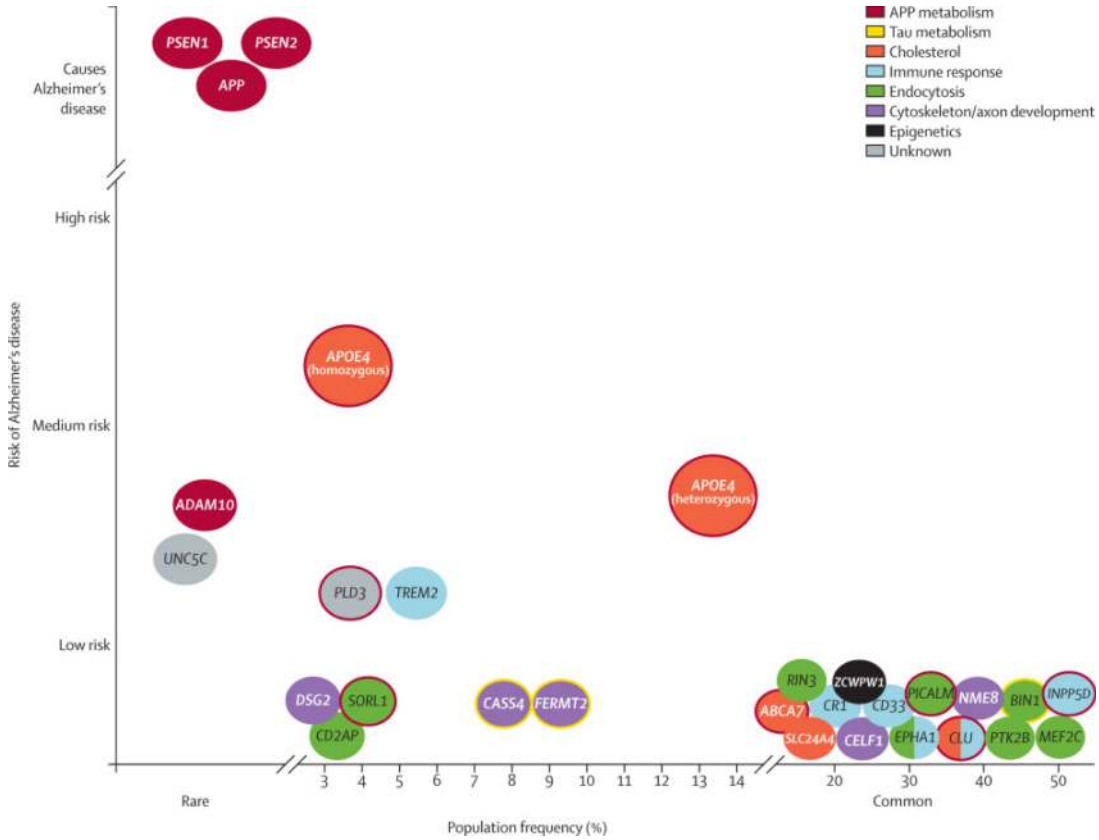

Figure 1.7: *Conceptual overview of the genetic factors involved in AD. Single gene mutations in PSEN1, PSEN2 and APP cause early onset AD, while variants with a low to moderate risk are associated with late onset AD. Colours represent pathways in which genes are implicated. (Reprinted from The Lancet, 388, Scheltens et al 2016, "Alzheimer's disease", 505-517, Copyright 2016, with permission from Elsevier* [141]*).*

on the cognitive domains of episodic memory, executive functioning, perceptual speed and global cognitive ability [136].

ABCA7's *(ATP-binding cassette sub-family A member 7)* known functions include roles in phagocytosis of apoptotic cells and efflux of lipids across the cell membrane into lipoprotein particles, which include *APOE* [137,138]. Knockout of ABCA7 in mice leads to an increased Aβ accumulation as a result of impaired phagocytic Aβ clearance [139,140]. *ABCA7* is associated with neurotic plaque and NFT burden in a mixed case/control analysis of AD autopsied brains [141,142], although not with CSF, Aβ tau or p-tau in MCI patients [117]. In SNP univariate and gene-based analysis, *ABCA7* was associated with a decline in the mini mental state examination (MMSE) in women and 3MS in men, and with a decline in episodic memory in individuals with a final diagnosis of either MCI or LOAD [120].

Table 1.1: *Single Nucleotide Polymorphisms (SNPs) associated with LOAD*

Gene	SNP	Chromosome	Alleles*	MAF†	OR‡	Study
APOE	rs429358 rs7412	19	ε2/ε3/ε4	0.8/0.14	0.54/3.81	[107]
ABCA7	rs3764650	19	T/G	0.11	1.23	[109]
BIN1	rs744373	2	A/G	0.31	1.17	[111]
CASS4	rs7274581	20	T/C	0.11	0.88	[113]
CD2AP	rs9296559	6	T/C	0.27	1.11	[109,110]
CD33	rs34813869	19	A/G	0.3	0.89	[109,110]
CELF1	rs7933019	11	G/C	0.34	1.08	[113]
CLU	rs11136000	8	C/T	0.35	0.88	[108,112]
CR1	rs3818361	1	G/A	0.26	1.17	[112]
DSG2	rs8093731	18	*C/T*	0.01	0.73	[113]
EPHA1	rs11767557	7	T/C	0.2	0.89	[109,110]
FERMT2	rs17125944	14	T/C	0.08	1.14	[113]
HLA-DRB5	rs9271100	6	C/T	0.31	1.11	[113]
INPP5D	rs35349669	2	C/T	0.44	1.08	[113]
MEF2C	rs304132	5	G/A	0.46	0.93	[113]
MS4A4A	rs4938933	11	T/C	0.5	0.88	[110]
MS4A4E	rs670139	11	G/T	0.34	1.08	[109]
MS4A6A	rs610932	11	T/G	0.45	0.9	[109]
NME8	rs2718058	7	A/G	0.36	0.93	[113]
PICALM	rs3851179	11	C/T	0.41	0.88	[108]
PTK2B	rs28834970	8	T/C	0.32	1.1	[113]
SLC24A4-RIN3	rs10498633	14	G/T	0.19	0.91	[113]
SORL1	rs11218343	11	T/C	0.03	0.77	[113]
ZCWPW1	rs1476679	7	T/C	0.32	0.91	[113]

*Major/Minor Allele; †Minor Allele Frequency: HapMap-CEU [143]; ‡OR for minor allele reported by Alzegene or IGAP [113]

Table 1.2: *Characteristics of studies investigating the association between LOAD risk SNPs and cognitive function.*

Study	Sample	Mean Age	Years of Education	Years of Follow up	Cognitive Domains	Genes close to SNPs associated with AD
Barral *et al* 2012 [126]	n = 1,365 European subjects. 337 with LOAD	72.8	14.6	-	EM	*CR1, BIN1, CLU, PICALM, APOE*
Carrasquillo *et al* 2014 [120]	n =2,262 Caucasian subjects. AD free at baseline, 129 incident AD	77	14	3.8	EM	*CLU, PICALM, CR1, ABCA7, BIN1, MS4A6A, EPAH1, CD2AP, CD33, APOE*
Chibnik *et al* 2011 [128]	n =1,666 Caucasian subjects. Dementia free at baseline. 404 incident AD	78.4	16.1	6	EM, EF, GC, PS, VA, VS	*CR1, CLU, PICALM*
Davies *et al* 2015 [122]	n =53,949 European subjects. Dementia free at baseline.	>45	-	-	GC	*APOE, MEF2C, PICALM, EPHA1, ABCA7, ZCWPW1, HLA-DRB5, FERMT2, BIN1, CD33, DSG2, SLC24A4-RIN3, CLU, MS4A6A, CR1, INPP5D, CD2AP, PTK2B, SORL1, CASS*
Davies *et al* 2014 [121]	n = 3,511 Caucasian subjects. Dementia free	68.2	-	-	GC	*APOE, BIN1, CLU, ABCA7, CR1, PICALM, MS4A6A, CD33, CD2AP, APOE*
De Jager *et al* 2012 [116]	n = 749 Caucasian subjects. Dementia free at baseline, 152 incident dementia	75.3	18.2	9	GC	*CLU, CR1, PICALM, BIN1, ABCA7, MS4A, CD2AP, EPHA1, CD33, APOE*
Engelman *et al* 2013 [118]	n = 1,153 Caucasian Subjects with parental history of AD. Dementia free at baseline.	53.6	62% ≥ college	4-6	EM, EF, VA	*APOE, ABCA7, CLU, CR1, BIN1, CD2AP. EPHA1, MS4A4A, PICALM, CD33*
Gui *et al* 2014 [114]	n = 2,408 Chinese Subjects. 224 neurological disorder cases	63.7	26% ≥ college	4	EM	*BIN1, CD2AP, CLU, SORL1, PICALM, MS4A6A, MS4A4E, ABCA7, CD33, APOE*
Hamilton *et al* 2011 [125]	n = 998 Caucasian subjects. Dementia free.	10.9	-	58.68	EM, EF, GC, VS	*BIN1, CLU, CR1, PICALM*
Keenan *et al* 2012 [131]	n =1,709 Caucasian subjects. Dementia free at baseline. 340 incident AD	78.5	16.4	6	EM	*CR1*

Table 1.2 *(Continued)*

Study	Sample	Mean Age	Years of Education	Years of Follow up	Cognitive Domains	Genes close to SNPs associated with AD
Liu *et al* 2014 [132]	n = 719 Caucasian subjects. 162 AD cases	75	15.5	-	EM, GC	*NME8*
Louwersheimer *et al* 2016 [117]	n = 1,730 MCI. 723 incident AD	73.4	-	3.8	EM, GC	*ABCA7, BIN1, CD2AP, CD33, CLU, CR1, EPHA1, MS4A4E, PICALM, PTK2B, SORL1, SLC24A4-RIN3, INPP5D, MEF2C, NME8, ZCWPW1, FERMT2, CASS4*
Mengal-From *et al* 2011 [130]	n = 1,380 Danish subjects	92.5	-	-	GC	*CLU, CR1, PICALM*
Mengal-From *et al* 2013 [129]	n =1,651 Danish subjects n = 689 Danish Twins	92.5 78.8	- -	7 10	GC	*CLU*
Nettiksimmons et al 2016 [123]	n = 3,026 Caucasian males n = 3,267 Caucasian females	73.4 71	56% w/ college 18% w/ college	Up to 10 Up to 10	GC	*ABCA7, BIN1, CD2AP, CD33, CLU, CR1, EPHA1, MS4A6A, PICLAM, HLA-DRB5, PTK2B, SORL1, SLC24A4-RIN3, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERT2, CASS4*
Sweet *et al* 2012 [144]	n = 1,831 Caucasian Subjects. Dementia free at baseline	71.7	39.9% w/ college		AT, GC	*APOE, CLU, PICALM, CR1*
Thambisetty *et al* 2013 [127]	n = 599. 134 African American, 465 Caucasians. Dementia free.	67.5	16.25	6.6	AT, EM, EF, GC, PM, VA, VS	*CLU*
Verhaaren *et al* 2013 [119]	n = 5171 Non-demented Caucasian Subjects.	66.2	12.8% w/ primary only	-	EM, EF, GC, VA	*APOE, CLU, PICALM, BIN1, CR1, ABCA7, MS4A6A, MS4A4E, CD2AP, EPHA1, CD33*
Vivot *et al* 2015 [124]	n = 4,931 French Subjects. Dementia Free	74	36% > 9	Up to 10	EM, EF, GC	*APOE, CR1, BIN1, CLU, PICALM, ABCA7, MS4A6A, CD33, MS4A4E, CD2AP*
Zhange and Pierce 2014 [115]	n = 5,808 European subjects	64	43.8% w/ college	2-13	GC	*APOE, PICALM, CD2AP, CR1, EPHA1, MS4A, CLU, CD33, ABCA7, BIN1*

Cognitive tests are classified into cognitive domains as outlined in Wisdom *et al* 2011 [145]: AT = attention, EM= episodic memory, EF= executive functioning, GC= global cognitive ability, PM= primary memory, PS = perceptual speed, VA= verbal ability, VS = visuospatial functioning

However, associations in other studies are predominantly negative, with no associations observed with episodic memory [114,117-119,124,133], executive function [118,119,124,133], global/general cognitive function [115-117,119,121,122,124] perceptual speed [119,133] and verbal ability [118,124].

BIN1 (Bridging Integrator 1) is involved in regulating clathrin-mediated endocytosis and membrane trafficking [146]. Due to its role in endocytosis, a role in APP processing has been suggested, and it has been associated with neurotic plaque burden [142]. An association between *BIN1* and tau pathology has been established, where *BIN1* knockdown in a Drosophila model suppressed tau-mediated neurotoxicity, while in human neuroblastoma cell lines and the mouse brain BIN1 and tau were observed to colocalize and interact [147]. Accordingly, *BIN1* has also been associated with NFT burden [142]. *BIN1* has been associated with a decline in MMSE in women [123], in a community-based cohort [124], and with general cognitive ability at age 11 [125]. However, negative associations have been observed for episodic memory [114,117,119,120,124-126,133,148], executive function [118,119,124,125], global/general cognitive function [115-117,119,121,122,133], perceptual speed [119,133], verbal ability [118,124] and visuospatial skills [125].

CD2AP (CD2-Associated Protein) is a scaffolding protein that is involved in cytoskeletal reorganization and vesicle trafficking [149], where it is a critical regulator of trafficking to the lysosome, suggesting that it may play a role in Aβ degradation [150]. *CD2AP* is associated with neurotic plaque burden [142]. *CD2AP* is associated with episodic memory in a population-based study of non-demented adults [119]. However, the majority of studies have found negative associations with episodic memory [114,117,118,120,124,133], executive function [118,119,124,133], global/general cognitive function [115-117,119,121-124], perceptual speed [119,133] and verbal ability [118,124].

CD33 (SIGLEC-3) is a immune cell surface receptor that is predominantly expressed in microglia where it promotes immune cell-cell interactions [151]. *CD33* expression and CD33-postive microglia are increased in the brains of AD patients, and are correlated with amyloid plaque burden [142,151,152]. Thus *CD33* likely influences AD by mediating Aβ clearance. Genetic variation in *CD33* is associated with cognitive performance in executive function [119] and decline in

MMSE in women [123]. However, negative associations have been observed for episodic memory [114,117-120,124,133], executive function [118,124,133], global/general cognitive function [115-117,119,121,122,124], perceptual speed [119,133] and verbal ability [118,124].

CLU (Clusterin) is a multifunctional chaperone protein that is involved in several cellular processes including complement regulation, lipid transport, cell-cell interactions, membrane protection and apoptosis [153]. CLU directly interacts with Aβ and influences Aβ aggregation in a concentration-dependent manner such that aggregation is promoted when Aβ levels are 10-fold higher than those of CLU, but prevented when CLU/Aβ ratios are lower [154,155]. Additionally, CLU promotes the clearance of Aβ across the blood brain barrier via LRP-2 and degradation by directing Aβ to microglia for phagocytosis [156]. *CLU* is one of the most extensively studied LOAD risk loci in relation to cognitive performance. It has been associated with a decline in episodic memory in a community-based cohort [120] and in individuals who developed either MCI/LOAD [120,127]. Additionally, *CLU* has been associated with a decline in the Modified Mini-Mental state (3MS) [144] and a measure of global cognition [129,130]. However, negative associations have been observed for attention [127,144], episodic memory [114,117-119,124-126,128,133], executive function [118,119,124,125,127,128,133], global/general cognitive function [115-117,119,121-125,127,128], perceptual speed [124,128,133], primary memory [127], verbal ability [118,124,127,128] and visuospatial skills [125,127,128].

CR1 (Complement receptor type 1) is a component of the complement response system and a crucial mediator in innate immunity. It is predominantly expressed in erythrocytes, where it acts to bind to complement C3b- and C4b-activated particles in the bloodstream and promote their removal by transporting the particles to the liver for degradation [157]. *CR1* expressed in leukocytes promotes phagocytosis of complement activated particles [157]. Circulating $A\beta_{42}$ is bound to complement C3b and is cleared via adherence to CR1 on erythrocytes [158]. *CR1* has been associated with greater amyloid plaque burden [128]. *CR1* is one of the LOAD risk loci most extensively studied in relation to cognitive performance. Genetic variation in *CR1* is associated with more rapid decline in attention [144], episodic memory [128,131], global cognition [116,128], MMSE in women [123], perceptual speed [128,131], verbal ability [124,128,131] and

visuospatial skill [128]. However, negative associations have been observed for episodic memory [114,117-120,124-126,133], executive function [118,119,124,125,128,133], global/general cognitive function [115,117,119,121,122,124,125,130,144], perceptual speed [119,133], verbal ability [118] and visuospatial skills [125].

EPHA1 (EPH Receptor A1) belongs to the ephrin family of tyrosine kinase receptors that bind to membrane-bound ephrin-A ligands on adjacent cells, allowing contact-dependent bidirectional signalling between adjunct cells [159]. *EPAH1* plays a role in synaptic formation and plasticity, axonal guidance and brain development; however, its role in AD is not well understood [160-162]. *EPAH1* is not associated with amyloid or NFT burden [142], although it has been associated with dementia progression [163] and atrophy in the hippocampus and the lateral occipitotemporal and inferior temporal gyri [164]. *EPAH1* has been associated with a more rapid decline in episodic memory in participants who eventually develop MCI/LOAD [120]. No significant associations between *EPAH1* and cognitive performance in episodic memory [114,117-120,133], executive function [118,119,133], global/general cognitive function [115-117,119,121,123], perceptual speed [119,133] or verbal ability [118] have been observed

The *MS4A (Membrane-spanning 4A)* gene cluster contains 18 known genes that have similar polypeptide sequences and topographical structures within cells [165]. SNPs near three genes in the cluster, *MS4A4A, MS4A4E,* and *MS4A6A,* have been associated with LOAD. The function of these genes is not well understood, however, their homologs *MS4A1, 2* and *3* have been implicated in the regulation of calcium homeostasis, immune response, cell activation, growth and development, suggesting they may have a similar role [166]. Genetic variation and increased expression of *MS4A6A* has been associated with neuropathological amyloid and tau burden [142,152]. *MS4A6A* has been associated with more rapid decline in 3MS in men and poor memory performance [133]. Other studies have observed no significant associations between either *MS4A4A, MS4A6A* or *MS4A4E* and episodic memory [114,117-120,124], executive function [118,119,124,133], global/general cognitive function [115-117,119,121,122,124], perceptual speed [119,133] and verbal ability [118,124].

PICALM (Phosphatidylinositol Binding Clathrin Assembly Protein) is involved in clathrin-mediated endocytosis and intracellular trafficking of endocytic

proteins. *PICALM* has been implicated in several processes that influence Aβ pathogenesis including regulating APP internalization and subsequent Aβ generation [167], regulating Aβ blood brain barrier transcytosis and clearance [168] and autophagy-mediated Aβ clearance [169]. *PICALM* is associated with neuropathological amyloid and NFT burden [142]. In addition to *CLU* and *CRI,* the association between *PICALM* and cognitive performance has been extensively studied. *PICALM* has been associated with more rapid decline in episodic memory [128], global cognitive function [115,128], 3MS in men [123], verbal ability [128], and general cognitive function [122,130]. Conversely, other studies have observed non-significant associations for cognitive performance in attention [144], episodic memory [114,117-119,124-126,133,170], executive function [118,119,124,125,128,133], global/general cognitive function [116,117,119,121,124,125,144], perceptual speed [119,128,133], verbal ability [118,124] and visuospatial skills [125,128].

HLA-DRB5/HLA-DRB51 (Major Histocompatibility Complex, Class II, DR Beta 1) are members of the major histocompatibility complex (MHCII), a gene dense region associated with various immune functions [171]. The role of *HLA-DRB5/HLA-DRB51* loci in LOAD is not well characterized, however, GWAS have associated this locus with Parkinson's disease that is characterized by the accumulation of α-synuclein, which induces neurodegeneration [172]. In a PD mouse model, overexpression of α-synuclein induced expression of MHCII signaling and activation of microglia, whereas knocking out MHCII protects against α-synuclein induced neurodegeneration [173]. This suggests that *HLA-DRB5/HLA-DRB51* may play a similar role in LOAD, where the accumulation of Aβ and tau promote microglia activation. Methylation of the *HLA-DRB5* locus has been associated with Aβ and NFT burden [174]. *HLA-DRB5* is associated with greater decline in 3MS and MMSE in men and women, respectively [123], although not with general cognitive function [122], episodic memory, executive function or perceptual speed [133].

PTK2B (Protein Tyrosine Kinase 2 Beta) is a member of the focal adhesion kinase family and is activated by autophosphorylation and phosphorylation by Src-family kinases in response to stimuli such as calcium levels [175,176]. In a recent study, it was observed that the Drosophila PTK2B orthologoue modulated Tau toxicity; that human Tau and PTK2B proteins biochemically interacted in vitro;

and that PTK2B co-localized with hyperphosphorylated and oligomeric Tau in progressive pathological stages in the brains of AD patients and transgenic Tau mice [177]. *PTK2B* was associated with greater decline in 3MS in men [123], though conversely not in MMSE [117,123], episodic memory [117] or with performance in general cognitive function [122], episodic memory, executive function or perceptual speed [133].

SORL1 (Sortilin-Related Receptor, L (DLR Class) A Repeats Containing) is a sorting receptor that directs target proteins to intracellular compartments in neurons [178]. SORL1 directly interacts with APP, redirecting APP to the trans-Golgi network and away from the late endosome pathway, where APP undergoes β- and γ- secretase cleavage to produce Aβ, and directs Aβ peptides to the lysosome for degradation [178,179]. *SORL1* deficient mice have increased Aβ levels [180], and *SORL1* SNPs have been associated with increased CSF tau, ptau, and neuropathological NFT burden [117,142,181]. *SORL1* is associated with greater decline in 3MS in men, though conversely not in MMSE [117,123], episodic memory [114,117] or with performance in general cognitive function [122], episodic memory, executive function or perceptual speed [133].

SLC24A4 (Solute Carrier Family 24 (Sodium/Potassium/Calcium Exchanger), Member 4) is a solute carrier that is abundantly expressed in the brain [182] and has been associated with iris development, hair and skin colour, neuronal development and risk of hypertension [183-186], although its function in AD is not currently known. Methylation of *SLC24A4* has been associated with increased Aβ load [174]. The *SLC24A4* SNP associated with AD is also in the vicinity of the gene *RIN3* (*Ras and Rab Interactor 3),* which interacts with *BIN1*, suggesting that the functional SNP may lie within *RIN3* [187]. *SLC24A4/RIN3* was associated with greater decline in 3MS in men and with general cognitive function [122], although conversely not with a decline in MMSE [117,123], episodic memory [117] or with performance in episodic memory, executive function or perceptual speed [133].

DSG2 (Desmoglein 2) is a desmosomal cadherin and is an essential component of the desmosome, which is involved in cell-cell adhesion [188]. The function of *DSG2* in AD is not currently known, although it should be noted that the *DSG2* SNP was only associated with AD in the stage 1 meta-analysis and was not validated in the replication analysis, suggesting that it may not associate with AD

[113]. *DSG2* was not associated with general cognitive function [122], episodic memory, executive function or perceptual speed [133].

INPP5D (Inositol Polyphosphate-5-Phosphatase, 145 kDa, aka SHIP1) is involved in the immune system, where it regulates cytokine signalling and plays a role in inflammatory responses, and has been implicated in many diseases that are characterized by deregulated immune responses such as cancer, inflammatory diseases, diabetes and atherosclerosis [189-191]. *INPP5D* is functionally linked with two other AD risk loci, *TREM2* and *CD2AP*, and modulates their effect on Aβ degradation, inflammatory responses and phagocytosis [192]. *INPP5D* was not associated with greater decline in 3MS [123], MMSE [117,123] episodic memory [117] or with performance in general cognitive function [122], episodic memory, executive function or perceptual speed [133].

MEF2C (Myocyte Enhancer Factor 2C) is a transcription factor that facilitates neuronal and muscle development, with mutations in *MEF2C* being associated with severe mental retardation [193,194]. *MEF2C* is highly expressed in microglia and *MEF2C* binding sites are enriched near inflammatory genes, suggesting that *MEF2C* may play a role in regulating inflammatory gene expression in response to Aβ and tau [195,196]. *MEF2C* has been associated with neuropathological Aβ and NFT burden [142]. *MEF2C* is associated with greater decline in MMSE in women and with general cognitive function [122], although conversely not with decline in 3MS [123], MMSE [117], episodic memory [117] or performance in episodic memory, executive function or perceptual speed [133].

NME8 (NME/NM23 Family Member 8) is primarily expressed in the testis and respiratory epithelial cells and has been associated with primary ciliary dyskinesia [197], knee osteoarthritis risk, bone mineral density, and susceptibility to oxidative stress in sperm [198-201]. It is not clear how *NME8* influences AD pathogenesis, although it has been suggested it may be a quantitative trait locus regulating expression of other genes directly relevant to AD risk [202]. *NME8* is associated with CSF tau [132]. *NME8* was associated with baseline performance on the CDRSB [132], although conversely not with decline in 3MS [123], MMSE [117,123,132], episodic memory [117], or with performance in general cognitive function [122], episodic memory, executive function or perceptual speed [133].

ZCWPW1 (Zinc Finger, CW Type with PWWP Domain 1) is one of the least studied LOAD risk genes and has been implicated in modulating epigenetic

regulation [203]. However, the LD region surrounding the LOAD associated SNP contains ten other genes, any one of which may be functionally relevant. This uncertaintly was highlighted in a recent finding that the LOAD associated SNP serves as an expression quantitative trait loci (eQTL) for two of these genes: *PILRB* and *GATS* [204]. *PILRB* expression levels are associated with AD, is highly expressed in microglia, and it functionally associates with another AD risk gene, TREM2, suggesting that *PILRB* plays a role in neuroinflammation AD risk [204]. The *ZCWPW1* SNP is associated with neuropathological NFT burden [142]. *ZCWPW1* was not associated with greater decline in 3MS [123], MMSE [117,123] episodic memory [117] or with performance in general cognitive function [122], episodic memory, executive function or perceptual speed [133].

CELF1 (CUGBP, Elav-like Family Member 1) is a member of a protein family that regulates pre-mRAN processing. Its role in AD pathogenesis is not clear, although it has been shown to be a modulator of tau toxicity in Drosophila [205,206]. *CELF1* was associated with better performance in verbal-numerical reasoning [133] and a greater rate of decline in MMSE in women [123], although no significant associations were observed for 3MS [123], episodic memory or perceptual speed [133].

FERMT2 (Fermitin Family Member 2) is a member of the Fermitin family of proteins that regulates cell adhesion, spreading, migration, survival, proliferation and differentiation and the assembly of the extracellular matrix [207]. The role of *FERMT2* in LOAD is not clear, however, an association between the Drosophila ortholog of *FERMT2* and tau toxicity has been described [206]. *FERMT2* was not associated with greater decline in 3MS [123], MMSE [117,123] episodic memory [117] or with performance in general cognitive function [122], episodic memory, executive function or perceptual speed [133].

CASS4 (Cas Scaffolding Protein Family Member 4) is a member of the CAS adaptor protein family, consisting of *CASS4, NEDD9* and *BCAR1,* which act as scaffolds for assembling larger signalling complexes [208]. The function of *CASS4* is not well understood, however *NEDD9* directly interacts another AD risk gene, *PTK2B,* and *CASS4* retains the same sequence motif for this interaction, suggesting it may also interact with *PTK2B* [208]. Based on this interaction several roles in AD pathogenesis have been proposed including hypoxia, vascular changes, inflammation, microtubule stabilization and calcium signaling [209]. SNPs within

CASS4 have been associated with neuropathological amyloid and NFT burden [142] and with longitudinal change in amyloid burden [210]. *CASS4* is not associated with greater decline in 3MS [123], MMSE [117,123] episodic memory [117] or with performance in general cognitive function [122], episodic memory, executive function or perceptual speed [133].

1.3.3 Genetic Risk Scores

Genetic risk scores are based on the cumulative effect of many variants. They can have better predictive ability than individual variants, the effects of which may be too small to be reliably detected in a univariate analysis. GRS composed of genome-wide significant LOAD SNPs identified in the initial LOAD GWAS studies have been associated with baseline general cognition [119], episodic memory [120], visual memory and MMSE [124] and with a decline in episodic memory [120], verbal fluency, visual memory and MMSE [124]. However, these associations largely reflect the effect of APOE as they are not statistically significant when APOE is excluded from the GRS.

Two studies have investigated a GRS composed of the IGAP LOAD SNPs, one of which showed that a GRS, with *APOE* excluded, was associated with a decline in MMSE in participants with MCI [117]. The second study showed that a GRS with APOE included was associated with memory performance at baseline and with a faster rate of decline that accelerated with age. However, after excluding *APOE,* only linear rate of change remained significant [211].

Genome-wide significant IGAP LOAD risk loci do not reflect the full spectrum of genetic susceptibility to LOAD risk loci, explaining only 30% of genetic variance in LOAD [106]. Thus, an alternative approach is to construct a genome-wide polygenic score (GPS), which is calculated with genome wide significant SNPs, plus all nominally associated variants at a given significance level. The first study to use this method did not find an association with cognitive ability or cognitive change [212]. A more recent study using data collected from the UK Biobank (n = 112,151) found that an AD GRS constructed from 20,437 SNPs that were associated with AD at a threshold of $p < 0.05$ in the IGAP study was significantly associated with lower verbal-numerical reasoning, memory and educational attainment [213].

1.4 Environmental and Lifestyle Risk Factors

The greatest risk factors for dementia are age, sex, family history of dementia and genetic risk loci [214]. These risk factors, however, are non-modifiable. In the absence of therapeutical interventions for AD, employing successful intervention and treatment strategies that focus on modifiable environmental and lifestyle factors is currently the only available approach to reducing rates of dementia [215,216]. Targeting these risk factors is likely to promote healthy cognitive aging. Evidence is emerging that supports the association between modifiable risk factors and cognitive aging and the development of dementia.

1.4.1 Health and Medical Factors

A number of health and medical factors are associated with cognitive decline and dementia, including obesity, diabetes, hypertension, hyperlipidaemia (high cholesterol), depression and traumatic brain Injury (TBI).

In a meta-analysis of 21 studies, midlife obesity was associated with a 41% increased risk of dementia, although in late-life the opposite is the case, with obesity being associated with 17% reduction in risk [217]. Evidence is also emerging that obesity impairs normal cognitive function, though more studies are needed [218,219]. Obesity may contribute to cognitive decline and dementia by acting on mediating pathways such as hypertension, hyperlipidaemia, and diabetes [220]. Additionally, adipose tissue secretes a number of hormones, cytokines and growth factors that can cross the blood brain barrier and influence brain health [220].

Diabetes has been associated with a 46 – 56% increased risk of developing AD and a 134 – 156% increased risk of developing vascular dementia [221-223]. Meta-analyses have shown that diabetes results in mild to moderate deficits in all cognitive abilities, with particular effect on episodic memory and cognitive flexibility [224,225]. The biological mechanisms underpinning the association between diabetes, cognitive performance and dementia are likely to be multifactorial in nature, involving vascular changes, hyperglycaemic toxicity, insulin resistance and inflammation [226]. In particular, diabetes is associated with increased risk of cerebrovascular disease [227], accounting for the higher risk of vascular dementia, and hyperinsulinemia as a result of insulin resistance

limiting Aβ degradation as it directly competes against Aβ for degradation by insulin degrading enzyme [228].

Hypertension in midlife is associated with an increased risk of developing VaD but not with AD [229-231]. Increased blood pressure is also correlated with worse performance in global cognition and episodic memory and better performance in attention [232]. Hypertension causes alterations in cerebral artery structure and function that affect cerebral blood flow, resulting in an increased in cerebrovascular injury that accounts for the observed higher risk of VaD [233-235]. Additionally, increased blood pressure has been observed to impair Aβ clearance from the brain and enhance amyloidogenic APP processing, resulting in increased amyloid burden [236-238].

Hyperlipidaemia in midlife is associated with an increased risk of AD but not with VaD, whereas late-life cholesterol levels is not [239]. A non-linear relationship between cholesterol level and cognitive decline has been observed, with higher cholesterol levels in the middle-aged or the young-old and lower cholesterol levels in the old-old associated with a greater rate of cognitive decline [240]. Furthermore, therapeutic intervention using statins to control cholesterol levels in late-life does not prevent cognitive decline or dementia, though further analysis is needed to assess the effects of midlife and long-term statin use [241]. Cholesterol may influence AD pathogenesis by promoting APP processing via the amyloidogenic pathway by increasing the activity of β- and γ-secretases and repressing activity of α-secretases, resulting in excess Aβ accumulation [242,243]. Additionally, cholesterol may influence other non-amyloid factors such as NFT formation, inflammation and neuronal cell growth and survival [242,243].

Major depression in late life is associated with a 98% and 104% increased risk of developing either all-cause dementia or AD, whereas a milder presentation increases risk by 69% and 58% respectively [244]. Higher levels of depressive symptoms are also associated with a more rapid rate of decline in global cognition [245]. Several mechanisms have been proposed that underlie the relationship between depression and cognitive decline and dementia. The vascular depression hypothesis is that cerebrovascular disease predisposes, precipitates or perpetuates some depressive symptoms, implying that vascular disease mediates the effect of depression on dementia [246,247]. Alternatively, individuals with late-life depression have a greater accumulation of Aβ peptides than individuals

with no depressive symptoms suggesting depression may affect Aβ metabolism [247,248]. Other possible mechanisms by which depression affects AD risk include alterations in glucocorticoid steroid levels inducing hippocampal atrophy, inflammatory responses, and deficits of nerve growth factors or neurotrophins [247].

Traumatic Brain Injury is associated with a 40% increased risk of developing AD [249] and moderate to severe TBI is associated with impairments in general intelligence, verbal and visuospatial working memory and verbal short-term memory [250,251]. In animal models TBI is followed by an increase in cerebral Aβ levels, suggesting that TBI may influence AD pathogenesis via Aβ metabolism [252]. The most common pathological feature of TBI is diffuse axonal injury, which causes cytoskeletal disruption that interrupts axonal transport of proteins, and promotes their accumulation in the form of swellings at their disconnected terminals known as axon bulbs [253]. APP, β- and γ-secretases and Aβ are among the proteins that accumulate in axon bulbs, with Aβ been expelled into the extracellular space as injured axons degenerate and lyse [253].

1.4.2 Lifestyle Factors

Lifestyle risk factors that have been associated with dementia and cognitive performance include physical activity, smoking, diet and alcohol consumption.

Physical activity is associated with a 14% reduction in the risk of developing dementia [254] and a 35% reduction in risk of developing cognitive decline [254,255], with both low-moderate and high levels of physical activity having a protective effect. Furthermore, randomized control trials have shown that physical activity interventions improve cognitive function in patients with dementia [256]. Possible mechanisms underlying the positive effect of physical activity on cognitive function have been proposed [257]. Regular exercise stimulates the release of neurotrophins promoting neurogenesis, neuronal survival and improved neurovasculature and protecting against the degenerative brain changes associated with aging and dementia [257]. Physical activity also has positive influences on cardiovascular risk factors such as diabetes, obesity and hypertension, which in turn diminish the risk of dementia [257].

Smoking is strongly associated with the development of dementia, with current smokers showing a 30%, 40% and 38% increased risk of developing all-

cause dementia, AD or VaD, respectively [258]. Quitting smoking may reduce the adverse effects of smoking since former smokers have a similar risk of developing dementia as non-smokers [258-260]. Furthermore, in comparison to people who have never smoked, current and former smokers have poorer cognitive abilities and experience greater rates of cognitive decline [259,261]. The increased risk of dementia and poorer cognitive function due to smoking may be a result of increased cardiovascular disease promoting macro- and micro-vascular cerebral damage [262,263]. Additionally, smoking may promote oxidative stress, with contributes to the development of tau and amyloid pathology [264].

The role of diet in cognitive function and dementia risk has gained attention in recent years with a particular emphasis on the Mediterranean diet, which is characterized as consumption of relatively little read meat and high intake of whole grains, fruits, vegetables, fish, nuts and olive oil. Adherence to the Mediterranean diet reduces risk of developing dementia by 31% [265]. Mediterranean diet has also been associated with less cognitive decline, but results between retrospective studies are inconsistent highlighting the need for intervention studies [266]. Individual nutrients associated with reduced risk of dementia include unsaturated fatty acids, antioxidants, vitamin B and vitamin D [265]. The Mediterranean diet may exert its effects on cognitive health by reducing the risk of cardiovascular comorbidities such as hypertension, dyslipidaemia, and coronary artery disease, in addition to obesity and diabetes [267]. Additionally, the Mediterranean diet may influence dementia pathogenesis via anti-inflammatory and anti-oxidative pathways [267].

Light to moderate alcohol consumption, as distinct from abstinence, has been associated with a 25%-43% reduction in risk of developing all-cause dementia, AD and VaD [268,269]. Associations with cognitive decline were non-significant in early meta-analyses [268,269], although in a recent systematic review, 8 of 18 prospective studies and 9 of 12 cross-sectional studies indicated that moderate alcohol consumption was associated with cognitive outcomes [270]. In contrast, excessive alcohol consumption is associated with brain damage and increased risk of dementia [271,272]. These effects may be mediated partly by decreased cardiovascular risk due to improved lipid profiles and lower platelet aggregation, reduction in oxidative stress associated with the antioxidant properties of flavonoids found in red wine or a direct effect on cognition via the

release of acetylcholine in the hippocampus which enhances learning and memory [270].

1.4.3 Factors Influencing Cognitive Reserve

Cognitive reserve is a theory posited to explain the observed differences in cognitive abilities in individuals with equivalent levels of AD pathology, suggesting that some individuals can compensate for the pathological changes associated with AD better than others [273]. It is thought to reflect more efficient use of pre-existing brain networks (neural reserve) and recruitment of areas of the brain not previously used to compensate for damage (neural compensation) [274]. The cognitive reserve hypothesis explains why certain activities such as education, social engagement, and cognitively stimulating activities can increase an individual's reserve and reduce the risk of dementia and cognitive decline [275].

One of the most consistent associations with dementia and cognitive function is education, with fewer years of formal education associated with an increased risk of dementia, lower cognitive function and higher rate of cognitive decline [270,276]. Education may affect cognitive function by promoting synapse formation and vascularisation in early life. In late-life, individuals with higher levels of education undertake more mentally stimulating activities that may lead to further beneficial changes in brain structure, increasing cognitive reserve [277]. Additionally, greater education is associated with a 'healthier lifestyle' with a lower incidence of cardiovascular disease and greater engagement in healthy behaviours that promote a more favourable trajectory of cognitive decline [278].

The definition and operationalization of cognitively stimulating activities differ between studies and has prevented a meta-analytic summary of the influence cognitively stimulating activities may have on the incidence of dementia. However, systematic reviews of the field suggest that increased participation in cognitively stimulating activities does reduce the risk of dementia [279]. Furthermore, in randomized control trials in demented patients, cognitive stimulation improves cognitive outcomes and in cohort studies of non-demented participants it is associated with improved cognitive function [280,281]. Increased cognitive activity has been associated with reduced β-amyloid disposition and may compensate for the reduced cognitive function associated with lower education [282-284].

As with cognitively stimulating activities, the definition and operationalization of social engagement differs between studies. Lower levels of social participation, frequency of social contact and greater feelings of loneliness are associated with a 41%, 57% and 58% increased risk of developing dementia respectively [285]. No significant associations were observed for social network size or satisfaction with social network [285]. Greater social activity is also associated with better cognitive function, though results with cognitive decline are inconsistent [286,287]. As with engagement in intellectual activities, greater social engagement may increase cognitive reserve, with a socially engaged lifestyle being associated with increased neurogenesis and synaptic density [288]. Other potential mechanisms include: acting as a buffer against stress, which has been associated with an increased risk of AD [288]; promoting the uptake of protective behaviours such as exercise; and providing multiple sources of information about available healthcare and services [289].

1.4.4 Environmental and lifestyle risk scores

Numerous models for predicting dementia that incorporate known risk factors have been developed that allow for the identification of individuals at high risk [290,291]. This information can be used to refine inclusion of participants in clinical trials, allow for targeted treatment and intervention strategies aimed at reducing an individual's personalized risk of dementia, and to inform population health policy. Risk models can be broadly divided into: demographic only models; neuropsychological models, incorporating subjective or objective measures of cognition; health-based models, incorporating self-report or objective measures of health status, with or without genetic and biomarker data; and multifactor models that include multiple types of risk factors [291]. The discriminative accuracy of the currently developed models range from AUC of 0.49-0.91 in development cohorts, with only a minority of test results undergoing external validation, which raises doubts about their generalizability [290,291].

1.5 Methodological Considerations

As highlighted above (see section 1.3), the results of studies investigating the associations between the LOAD risk loci and cognitive performance are characterized by a lack of consensus. This is in contrast to the weight of evidence

associated with environmental, medical and lifestyle risk factors (see section 1.4) in moderating the risk of cognitive decline and dementia, such that management of health medical factors (diabetes, obesity, smoking and hypertension) in conjunction with regular physical activity, a healthy diet and lifelong learning/cognitive training can reduce the risk of cognitive decline and dementia. Different methodological features between studies may account for the lack of consistent findings, and restrict comparisons between studies, between LOAD risk loci and cognitive performance.

1.5.1 Cohort Differences

Differences in cohort design could account for the lack of replication across studies. First, the age of participants in a study and the length of follow-up may affect observed associations. While cognitive decline begins relatively early in adulthood, it has been observed to accelerate at older ages [292,293]. Additionally, the heritability of cognitive performance increases linearly with age, from 20% in infancy to 60% in adulthood [294]. This may be accounted for by the resource-modulation hypothesis, which posits that genetic differences may exert large effects with increasing age due to the loss of anatomical and neurochemical brain resources associated with normal aging [295]. Robust effect sizes may only be observed when investigating cognitive change in older participants.

Second, sex specific effects may affect associations with cognitive performance as a result of sex differences in hormones, immune regulation, inflammatory responses and comorbidities. This is indicated by women having a greater risk of developing AD [296], a faster rate of decline after diagnosis of AD [296], and by differential associations between LOAD risk loci in sex cohorts [123].

Third, population stratification can limit the statistical power to identify an association. This can be due to different racial groups having different linkage disequilibrium blocks, reducing the ability to detect associations between causal SNPs and GWAS tagging SNPs [297]; gene-gene interactions may behave differentially [298]; minor allele frequencies may differ, altering the detectable effect sizes for those SNPs [299]; and comorbidities between different populations may differ, influencing gene-environment interactions [298,299]. Additionally, most studies investigating the association of LAOD risk loci with cognitive decline have been performed in people with European ancestry, as highlighted in Table

1.2, limiting their generalizability to people with ancestry from other parts of the world.

Finally, phenotypic heterogeneity due to the use of different neuropsychological tests between studies may limit replication [122]. While cognitive test results are highly correlated, some tests may lack the sensitivity to identify associations with small effect sizes [300]. MMSE as a cognitive outcome, for example, has strong ceiling effects, limiting its ability to differentiate between medium and high cognitive performers [301].

1.5.2 Sample Size and Statistical Power

Another explanation for the lack of replication across studies is insufficient samples size and thus low statistical power. It has been recommended that to identify a SNP that accounts for 1% of the variance in an cognitive performance with 80% power, a sample size of between 800-1000 is required [302]. However, recent GWAS of cognitive performance have indicated that the effects sizes for individual SNPs associated with cognitive performance are likely to be smaller, suggesting that even larger sample sizes would be required [122,133]. To detect an effect that explains 0.2% of the variance with 80% power, a sample greater than 4000 is required, achieved by only 4 of the studies reviewed in Table 1.2. As LOAD pathology consisting of Aβ and NFT only accounts for ~30% of the variance in cognitive decline, the effect sizes for LOAD risk loci that influence cognitive performance via amyloid and tau pathways are expected to be small [91].

1.5.3 Interactions

As the biological pathways that underlie LOAD are characterised, it is becoming clear that LOAD is a multifactorial disease in which genetic variants are likely to have both additive and interactive effects [303]. Furthermore, the effects of genetic variants may be further moderated by environmental exposure [304]. However, to date, much of the research investigating the association of LOAD genetic risk factors with cognitive performance has focused on the effects of single genes. Interactions are generally only considered for *APOE*. The small effect sizes for other genes limit statistical power to detect interactive effects. The number of interactions between covariates, and thus hypothesis tests, increases exponentially

with the inclusion of additional variables, requiring ever-larger sample sizes [303,304]. Nevertheless, evidence of interactive effects between AD risk variants and environmental variables have been observed, highlighting that further research needs to account for gene-gene and gene-environmental interactions [118,305-308].

1.5.4 Controlling for Dementia

Inclusion of individuals who develop dementia during a study may affect results and bias results in favour of a positive association [122,309]. Table 1.2 shows that of the 20 studies evaluating the associations of LOAD risk loci with cognitive performance, 11 studies retained participants that developed LOAD over the course of the study. Selectively removing participants who develop dementia may not resolve this issue as the preclinical phase of dementia may last decades before clinical diagnosis [93]. Exclusion of participants with biomarker and neuroimaging evidence of preclinical AD in a cohort of cognitively normal individuals greatly attenuated age-related effects on cognitive declines across multiple domains [93]. This suggests that participants who are in the preclinical stages of AD may drive the reports of positive associations of LOAD risk loci with cognitive decline.

1.5.5 Temporal Associations

The GWAS studies that identified the LOAD risk loci were designed to investigate the progression from healthy to a clinical diagnosis of AD. However, as highlighted in Figure 1.1 and Figure 1.5, the development of LOAD spans several cognitive states that are characterized by gradual accumulation of LOAD pathology beginning with amyloidosis, followed by tau accumulation and subsequent structural, functional and cognitive declines [3,54]. Furthermore, cognitive domains associated with fluid intelligence are generally the first to decline with age, followed by measures of crystalized intelligence [310]. This is further reflected in LOAD, with memory impairment being the first cognitive symptom to be reported [311]. Where and when a risk locus is involved in LOAD pathogenesis may influence whether it is associated with processes that predispose, initiate or propagate cognitive decline. This effect will be further influenced by the age of the

cohort and the cognitive tests used, with loci associated with non-memory related domains only being observed in older aged cohorts.

1.5.6 Reverse Causality

An important consideration when investigating the association of lifestyle risk factors with dementia is reverse causation. As the neurodegenerative and cerebrovascular changes that underlie dementia began decades before the onset of clinical symptoms, lifestyle risk factors that are associated the development of dementia in late-life may themselves be a consequence of the same underlying pathological processes and not a casual factor of dementia. As such life-course approaches that study the long term effects of risk factors observed in younger-life on late-life disease processes are required to clearly establish a causative link between an exposure and cognitive impairment or dementia [312].

The issue of reverse causation is observed in studies examining obesity and hypertension. Obesity in late-life has been observed to be associated with a reduced risk of dementia, however, this may be due to weight loss in the prodromal phase of dementia ascribed to diminished self-care as manifested by a poor diet, leading to spurious inverse BMI-dementia association [313,314]. Similarly, a progressive decline in blood pressure during the early stages of dementia may be attributable to neurodegenerative process affecting brain regions that regulate arterial pressure, also resulting in spurious associations between high blood pressure and a reduced risk of dementia [230,315]. Studies examining cognitive, social and physical activities working under the assumption that individuals who are more engaged with their environment are less likely to suffer the adverse effects of cognitive impairment or delay its onset may also be prone to the possibility of reverse causation. Cognitive decline as a result of dementia related pathology may lead to individuals having a reduced interest in or ability to engage in cognitively stimulating activities [316], social interactions [317] and physicaly demanding activities [318,319] and thus potentially overestimating their cognitive benefits. Furthermore, leisure actives may be influenced by prior intelligence and educational attainment, which heavily influences the choice of leisure times activities that are pursued, particularly in regards to mentally stimulating activities [320].

Randomized control trials have also produced conflicting results, with the Dutch Prevention of Dementia by Intensive Vascular Care (PreDiva) trial finding that a multinomial intervention targeting cardiovascular risk factors did not reduce the incidence of all-cause dementia [321]; the Multidomain Alzheimer Preventional Trial (MAPT) did not affect rate of cognitive decline over 3 years after a multidomain intervention consisting of physical activity, cognitive training, nutritional advice and with either omega 3 polyunsaturated fatty acid supplements or placebos; while the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) found that multidomain interventional trial consisting of diet, exercise, cognitive training, and vascular risk monitoring resulted in improved cognitive function over 2 years [216]. As such there is a lack of research from randomized controlled trials confirming the associations observed in observational studies.

1.6 Aims of this Study

Alzheimer's disease is a debilitating neurological disease that is characterized by a progressive deterioration in cognitive function, eventually resulting in a complete loss of independent living that necessitates full-time care, and is ultimately fatal. AD is increasingly understood as a multifactorial neurodegenerative disease, with a long-term, complex, and dynamic etiology. A variety of genetic, health, environmental and lifestyle risk and protective factors can influence whether an individual's genetic predisposition to developing AD is elevated, exacerbated, buffered, or protected. These relevant exposures and experiences begin unfolding well before neuropathology or pronounced cognitive decline can be detected. Thus, an important direction of research is aimed at the early detection of multi-modal risk and protective factors that may exert their influence independently and interactively.

The primary aim of this thesis is to investigate the association of AD genetic, environmental and lifestyle risk factors with normal cognitive decline. I also investigate the effect of selected SNPs that had previously been associated with cognition function.

This thesis uses data collected from the Personality and Total Health (PATH) Through Life Project, a large longitudinal community survey of health and wellbeing in adults [322]. Compared with previous studies, the PATH cohort has

several attributes that allow more robust statistical inference about the effect the selected genetic factors on nonclinical cognitive decline. First, data is collected from 4 assessments over a period of 12 years allowing for the investigation of non-linear cognitive change. Second, a comprehensive cognitive assessment in four different cognitive domains was performed in each participant. Third, the PATH cohort was recruited from a narrow age band, reducing the impact of age differences influencing the results and allowing the study of aging effects distinct from cohort effects.

In this thesis I report results from the following studies:

1. **Interactive Effect of APOE Genotype and Blood Pressure on Cognitive Decline: The PATH Through Life Study.**
   Here, I examine whether *APOE* genotype moderates the effect of late-life hypertension on cognitive decline.
2. **Association of genetic risk factors with cognitive decline: the PATH through life project.**
   Here, I investigate the association between selected genetic risk factors with cognitive decline over eight years in a longitudinally followed community-based cohort of 1689 older adults. First, I investigate whether 12 single nucleotide polymorphisms (SNPs) from the top replicated LOAD associated genes are associated with cognitive decline, either individually or collectively as a genetic risk score (GRS). Second, we investigate whether 16 SNPs, previously associated with either dementia or cognition are also associated with cognitive decline.
3. **Late Onset Alzheimer's disease risk variants in cognitive decline: The PATH Though Life Study.**
   Here, I extend the previous study by reporting the associations of the 24 most significant LOAD risk loci with longitudinal change in cognitive performance, based on four neuropsychological outcomes, over 12 years in 1,626 community-dwelling older adults. I investigate whether these loci are associated, either individually or collectively as genetic risk scores (GRS), with: average differences in cognitive performance; rate of cognitive decline; and acceleration of the rate of decline over time.

4. **Association of *AKAP6* and *MIR2113* with cognitive performance in a population-based sample of older adults.**

   In this study, I extend the research of a previous GWAS study that identified associations of SNPs in the *AKAP6* and *MIR2113* loci with cross-sectional general cognitive function. I investigate whether SNPs with the strongest association at each loci are associated with non-linear cognitive change in episodic memory, working memory, verbal ability and processing speed.

5. **Validating the role of the Australian National University Alzheimer's Disease Risk Index (ANU-ADRI) and a genetic risk score in progression to cognitive impairment in a population-based cohort of older adults followed for 12 years.**

   Here, I examine the association between the Australian National University Alzheimer's Disease Risk Index, an environmental and lifestyle risk index for AD and a LOAD genetic risk score with cognitive impairment, as assessed using a clinical criterion for mild cognitive impairment (MCI) and a psychometric test-based criterion for MCI (MCI-TB) using cox proportional hazard models and multi-state models.

Chapter 2: The Personality and Total Health (PATH) Through Life Project

The PATH project is a longitudinal study of health and wellbeing in community dwelling Australian adults. The original aims of the study of were [322]:

1. to delineate the course of depression, anxiety, substance use and cognitive ability with increasing age across the adult life span;
2. to identify environmental and genetic risk factors influencing individual differences in the courses of these characteristics; and
3. to investigate inter-relationships over time between the three domains of depression and anxiety, substance use, and cognitive ability and dementia.

To examine these aims, the PATH project aims to follow participants for 20 years, spanning the ages from 20-84 using three narrow age ranged cohorts. Participants in PATH were sampled randomly from the electoral rolls of Canberra and the neighbouring town of Queanbeyan into one of three cohorts based on birth year: The '20+' cohort who were born between 1975-79 (aged 20-24 at baseline); the '40+' cohort born between 1956-60 (aged 40-44) and; the '60+' cohort born between 1937-41 (aged 60-64). Participants have been assessed at 4-year intervals, with each cohort interviewed in turn over a 1-year period starting with the 20+. To date four waves of data have been collected for all three cohorts, for a total of 12 years of follow-up (Figure 2.1). The work conducted in this thesis used data from the 60+ cohort.

2.1 Environmental and lifestyle factors

A broad range of fixed and time-varying variable have been collected in the interviews including demographics (eg. Marital Status; Education; Income), Health (eg. BMI; Medical Conditions; Smoking), Stressors (eg Lifetime trauma; Stress; Life events), Physical Measures (Blood Pressure; Eye chart; Reaction Time); Cognitive Measures (See Below), Mental Health (eg. Patient health questionnaire; Goldberg Anxiety and Depression scale), Psychological Scales (eg. Big five personality measure; Mastery; Ruminative style) and General health self-report (Self-report IQCODE; Instrumental activities of daily living) [322].

Figure 2.1: *Sample size of the PATH cohorts across waves. At wave 1 participated refers to participants who joined the study from the random sampling from the electoral role. Retained refers to percentage of participants retained from wave 1. The 60's Cohort is the focus of the work conducted in this thesis.*

The environmental and lifestyle risk factors used in thesis were drawn from the literature (See Chapter 1.4). Descriptive statistics for these environmental and lifestyle variables are presented in Table 2.1 and include: Self-reported education assessed as total number of years spent studying, age, gender, alcohol consumption assessed as number of drinks per week, smoking status for current, past or never smoker. Self-reported medical history of diabetes, epilepsy, stroke or transient ischemic attack (TIA), brain tumours, brain infections and traumatic brain injury with loss of conscious. Hypertension was determined with blood pressure measured twice during interviews while participants were seated. Participants were classified as hypertensive if they met any of the following criteria: i) mean systolic blood pressure ≥140 mm Hg; ii) mean diastolic blood pressure ≥90 mm Hg; iii) taking hypertensive medication at baseline. Obesity was assessed using the Body Mass Index (BMI) as weight/height^2, in kilograms/meters^2. Depression was assessed using the assessed using the Patient Health Questionnaire (PHQ-9) [323] following the coding algorithm provided in the PHQ-9 instruction manual. Social engagement was assessed using self-reported marital status, the Lubben Social Network Scale [324], social support using the Schuster Social Support Scale [325] and level of social activities. Physical activity was assessed using self-reported number of hours performing mild, moderate and vigorous activities. Cognitively stimulating activities was assessed using questions

from the Holland Occupational Themes, which assess an individual's occupational preferences according to six domains of Realistic, Investigative, Artistic, Social, Enterprising, and Clerical [326].

Table 2.1: Descriptive statistics of environmental and lifestyle variables in the PATH 60+ Cohort

Variable	Wave 1	Wave 2	Wave 3	Wave 4
n	2551	2222	1973	1645
Age	62.51 ± 1.51	66.6 ± 1.5	70.6 ± 1.49	75.08 ± 1.49
Male†	1317 (51.63)	1147 (51.62)	1020 (51.7)	854 (51.95)
Education	13.78 ± 2.84	13.89 ± 2.74	-	-
Ethnicity†				
Caucasian	2441 (95.8)	2133 (96.08)	1898 (96.3)	1590 (96.77)
Asian	62 (2.43)	52 (2.34)	44 (2.23)	35 (2.13)
Other	45 (1.77)	35 (1.58)	29 (1.47)	18 (1.1)
Diabetes†	193 (7.58)	218 (10.17)	260 ± 13.2	250 ± 15.39
Alcoholic drinks per week	6.72 ± 8.54	6.81 ± 8.27	6.64 ± 7.6	6.5 ± 7.58
Smoking†				
Current	276 (10.84)	176 (7.99)	108 (5.48)	75 (4.57)
Past	951 (37.34)	854 (38.75)	794 (40.26)	673 (40.99)
Never	1320 (51.83)	1174 (53.27)	1070 (54.26)	894 (54.45)
Epilepsy†	21 (0.82)	14 (0.65)	13 (0.66)	11 (0.68)
Stroke/TIA†	116 (4.55)	139 (6.25)	162 (8.21)	179 (10.88)
Brain Tumours†	-	34 (1.59)	35 (1.77)	-
Brain Infections†	-	55 (2.59)	53 (2.68)	47 (2.85)
Traumatic Brain Injury†	142 (5.58)	148 (6.66%)	132 (6.69%)	113 (6.86%)
Hypertension†	1600 (63.62)	1453 (66.44)	1503 (77.75)	1217 (77.32)
Diastolic blood pressure	83.05 ± 10.72	81.23 ± 10.26	79.46 ± 9.94	74.69 ± 10.12
Systolic blood pressure	139.79 ± 19.52	137.93 ± 19.03	145.62 ± 19.1	140.99 ± 18.46
BMI†				
Underweight	20 (0.87)	20 (0.92)	21 (1.09)	22 (1.37)
Normal	888 (38.52)	862 (39.78)	745 (38.54)	616 (38.43)
Overweight	949 (41.17)	876 (40.42)	782 (40.46)	640 (39.93)
Obese	448 (19.44)	409 (18.87)	385 (19.92)	325 (20.27)
Physical Activity				
Vigorous	294 (13.07)	305 (14.29	281 (14.38)	182 (11.06)
Moderate	744 (33.08)	1067 (49.98)	805 (41.2)	698 (42.43)
None/mild	1211 (53.85)	763 (35.74)	868 (44.42)	765 (46.5)
BPHQ depression score	2.5 ± 3.32	2.48 ± 3.22	2.42 ± 2.97	2.91 ± 3.31
Depression†	89 (3.51)	83 (3.8)	71 (3.6)	67 (4.11)
Marital Status				
Never	68 (2.67)	59 (2.66)	54 (2.74)	47 (2.87)
Widowed	180 (7.06)	198 (8.92)	223 (11.31)	268 (16.35)
Divorced	244 (9.58)	271 (12.21)	241 (12.22)	177 (10.8)
Separated	68 (2.67)	50 (2.25)	32 (1.62)	27 (1.65)
De facto	77 (3.02)	-	-	-
Remarried	-	255 (11.49)	232 (11.76)	186 (11.35)
Married	1911 (75)	1387 (62.48)	1190 (60.34)	934 (56.99)
Social network	-	-	18.26 ± 5.28	18.38 ± 5.06
Social Support				
Friends	-	3.11 ± 1.93	3.2 ± 1.85	2.73 ± 1.81
Partner	-	8.71 ± 4.92	10.05 ± 4.79	8.45 ± 4.54

Family	-	2.49 ± 2.35	2.78 ± 2.22	2.38 ± 2.2
RIASEC Scales				
Realistic	3.95 ± 1.67	3.79 ± 1.65	-	-
Investigative	2.37 ± 1.69	2.27 ± 1.65	-	-
Artistic	2.67 ± 1.71	2.47 ± 1.63	-	-
Social	5.3 ± 1.89	5.04 ± 1.95	-	-
Enterprising	3.27 ± 2.18	2.78 ± 2.04	-	-
Clerical	4.32 ± 2.43	3.95 ± 2.39	-	-

†Categorical variables: n (%); continuous variables mean ± SD

2.3 Neuropsychiatric tests

PATH participants undergo extensive neuropsychiatric testing at each wave assessing a broad range of cognitive domains. Across all four waves participants have been assessed on the following measures: Global cognitive ability assessed using the mini-mental state examination (MMSE), which consists of a series of questions and tests assessing memory, attention and language; Episodic memory using the Immediate and Delayed recall on California Verbal Learning Test, which involves recall a list of 16 nouns [327]; working memory, assessed using the Digit Span Backward from the Wechsler Memory Scale, which presents participants with series of digits increasing in length at the rate of one digit per second and asks them to repeat the Digits Backwards [328]; and vocabulary, assessed with the Spot-the-Word Test, which asks participants to choose the real words from 60 pairs of words and nonsense words [329]. Fine motor control was assessed using the Purdue Pegboard which consists of placing pins into a row of 25 holes moving top to bottom and is repeated with the participants using their dominant, non-dominant and both hands [330]. Simple and choice reaction time (SRT and CRT) tasks were administrated using a hand held box with two depressible buttons, two red stimulus lights and one green 'get ready' light. SRT was measured using four blocks of 20 trials, in which the participant was instructed to press the right hand button (regardless of dominance) in response to the activation of one of the stimulus lights. CRT was measured using two blocks of 20 trials, in which participants were instructed to press the button corresponding to the left or right stimulus light. Mean reaction times were calculated as described previously [331]. The above cognitive measures, except the Purdue pegboard, were used in the work conducted in this thesis. Observed and fitted cognitive trajectories for these measures are displayed in Figure 2.2 and the mean cognitive test scores at each wave for the whole 60+ Cohort and wave 4 completers only are displayed in Table 2.2.

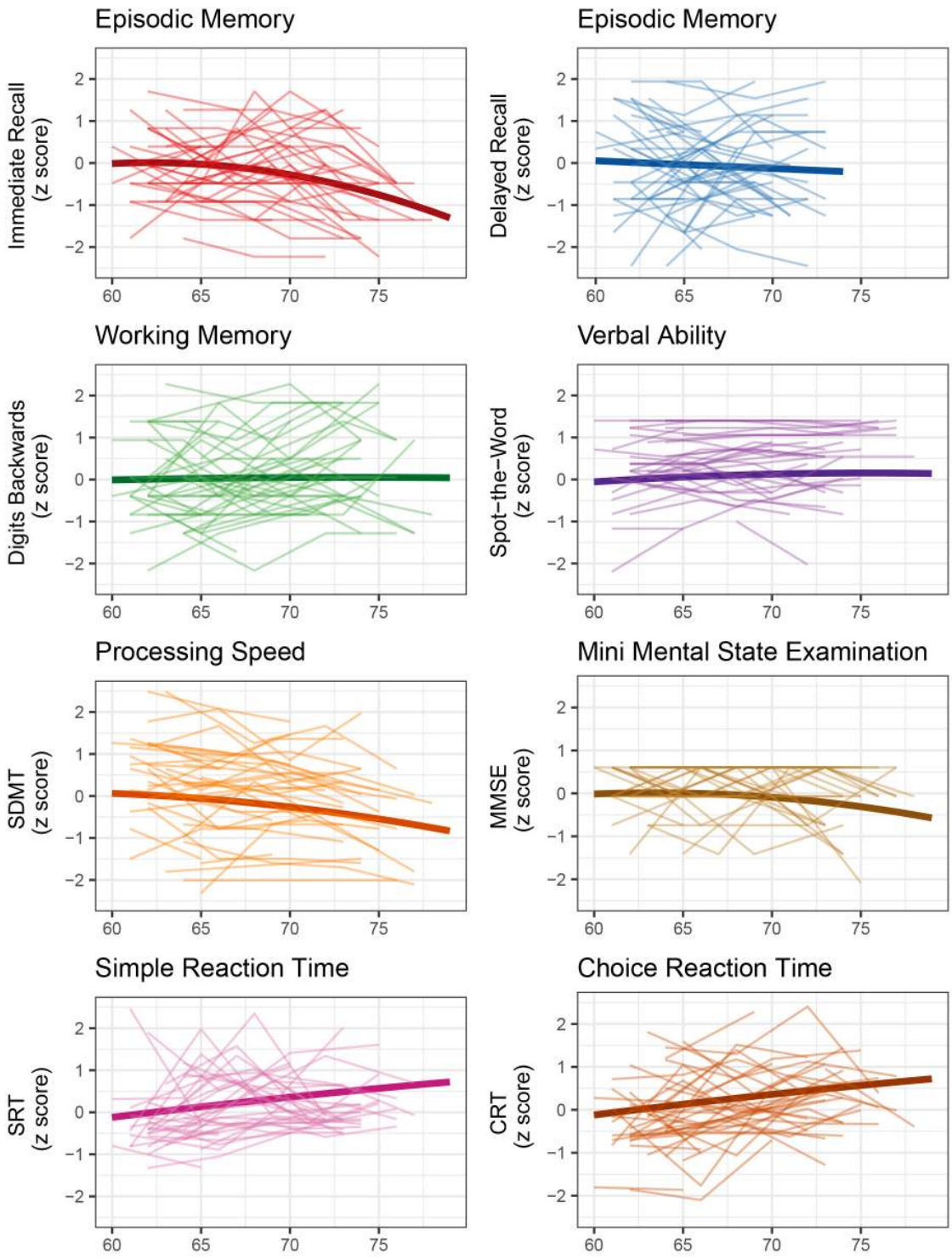

Figure 2.2: *Spaghetti plots displaying cognitive trajectories for 50 randomly select participants in the 60's Cohort and fitted trajectories extracted from linear mixed effects models for the entire 60's cohort (bold trajectory).*

Table 2.2: *Cognitive test scores at each wave for the whole PATH 60's cohort and Wave 4 completers only (mean ± SD)*

Cognitive Variable	Wave 1	Wave 2	Wave 3	Wave 4
Whole Cohort				
CVLT Immediate	7.1 ± 2.29	6.93 ± 2.21	6.62 ± 2.25	5.32 ± 1.92
CVLT Delayed	6.14 ± 2.5	6.09 ± 2.39	5.88 ± 2.32	7.47 ± 3.23
Digits Backwards	4.88 ± 2.25	5.1 ± 2.21	5.03 ± 2.2	5.22 ± 2.25
Spot-the-Word	51.82 ± 5.84	52.96 ± 5.21	53.24 ± 5.04	53.62 ± 5.02
Symbol digits modalities test	49.65 ± 9.8	49.33 ± 9.39	47.61 ± 9.37	45.83 ± 9.76
Simple Reaction Time	251.7 ± 62.57	276.55 ± 77.07	279.41 ± 65.73	277.42 ± 70.67
Choice Reaction Time	317.87 ± 55.42	326.51 ± 54.82	340.29 ± 58.39	343.48 ± 64.45
MMSE	29.1 ± 1.4	29.17 ± 1.26	29.09 ± 1.33	28.78 ± 1.75
Wave 4 Completers				
CVLT Immediate	7.33 ± 2.19	7.05 ± 2.18	6.71 ± 2.22	5.32 ± 1.92
CVLT Delayed	6.35 ± 2.43	6.21 ± 2.35	5.98 ± 2.28	7.47 ± 3.23
Digits Backwards	5.16 ± 2.23	5.29 ± 2.2	5.13 ± 2.21	5.22 ± 2.25
Spot-the-Word	52.63 ± 5.34	53.38 ± 5.04	53.51 ± 4.87	53.62 ± 5.02
Symbol digits modalities test	51.19 ± 8.9	50.32 ± 9.03	48.32 ± 9.05	45.83 ± 9.76
Simple Reaction Time	247.37 ± 54.5	272.69 ± 72.38	276.63 ± 64.22	277.44 ± 70.69
Choice Reaction Time	314.16 ± 45.13	323.38 ± 50.99	337.44 ± 56.14	343.5 ± 64.47
MMSE	29.31 ± 1.14	29.3 ± 1.08	29.17 ± 1.2	28.78 ± 1.75

In addition to the above cognitive measures that have been assessed across all 4 waves, additional cognitive tests that have been assessed at wave 4 include the tests assessing complex attention (the Trail Making Test part A [332]), executive function (Trail Making Test part B, The Stroop Color and Word Test, and the Zoomap Test [332]) visual memory (the Benton Visual Retention Test [332]) and language (the Controlled Oral Word Association Test and the Boston Naming Test [332]). However, as these cognitive tests were not conducted across all 4 waves, these cognitive measures cannot be used to assess cognitive change and as such were not used in the work conducted in this thesis.

2.3 Genetic factors

A number of genetic markers have been genotyped in the 60+ cohort including *APOE* [333], HT1A Serotonin Receptor [334,335] and simple sequence variants [336,337]. As part of the work conducted in this thesis, 80 SNPs were selected for genotyping based on their previously observed associations with dementia, cognition, neuroanatomical differences blood pressure (Table 2.2). Genomic DNA was extracted from cheek swabs (n = 4,597) using Qiagen DNA blood kits or from peripheral blood leukocytes (n = 64) using QIAamp DNA 96

Table 2.3: *SNPs that were genotyped as part of this thesis.*

Gene	SNP	Chromsome	Alleles†	MAF‡	Association
ABCA7	rs3764650	19	T/G	0.11	Alzheimer's disease [109]
ADRB1	rs1801253	10	C/G	0.31	Blood Pressure [338,339]
AGT	rs2004776	1	C/T	0.26	Blood Pressure [338,339]
ARHGAP42	rs633185	11	C/G	0.3	Blood Pressure [340]
ASTN2	rs7852872	9	C/G	0.39	Neuroanatomy [340]
ATP2B1	rs2681472	12	A/G	0.12	Blood Pressure [341]
BAG6	rs805303	6	G/A	0.31	Blood Pressure [340]
BDNF	rs6265	11	C/T	0.2	Cognition [342,343]
BIN1	rs744373	2	A/G	0.31	Alzheimer's disease [110,111]
CD2AP	rs9296559	6	T/C	0.27	Alzheimer's disease [109,110]
CD33	rs34813869	19	A/G	0.29	Alzheimer's disease [109,110]
CETP	rs5882	16	A/G	0.36	Cognition [344]
CHRNA4	rs1044396	20	G/A	0.42	Neuroanatomy [345]
CLU	rs11136000	8	C/T	0.35	Alzheimer's disease [110,112]
COMT	rs4680	22	G/A	0.48	Cognition [346,347]
CR1	rs3818361	1	G/A	0.26	Alzheimer's disease [110,112]
CSK	rs1378942	15	A/C	0.32	Blood Pressure [340]
CTNNBL1	rs6125962	20	T/C	0.6	Cognition [348]
CYP19A1	rs700518	15	C/T	0.42	Neuroanatomy
DPP4	rs6741949	2	G/C	0.43	Neuroanatomy [340]
DRD2	rs6277	11	A/G	0.47	Neuroanatomy [345]
EPHA1-AS1	rs11767557	7	T/C	0.2	Alzheimer's disease [109,110]
F5	rs6703865	1	G/A	0.4	Neuroanatomy [349]
FGF5	rs1458038	4	C/T	0.27	Blood Pressure [340]
FRMD4A	rs17314229	10	C/T	0.09	Alzheimer's disease [350]
FRMD4A	rs2446581				Alzheimer's disease [350]
FRMD4A	rs7081208	10	G/A	0.29	Alzheimer's disease [350]
FTO	rs3751812	16	G/T	0.46	Neuroanatomy [351]
GCFC2	rs2298948	2	T/C	0.33	Neuroanatomy [349]
GNAS-EDN3	rs6015450	20	A/G	0.07	Blood Pressure [340]
GRIN2B	rs10845840	12	C/T	0.46	Neuroanatomy [352]
HFE	rs1799945	6	C/G	0.18	Blood Pressure [340]
Intergenic	rs7294919	12	T/C	0.1	Neuroanatomy [340]
Intergenic	rs11139399	9	T/C	0.41	Neuroanatomy [349]
Intergenic	rs2942354	1	C/A	0.44	Neuroanatomy [349]
Intergenic	rs12007229	X	C/A	0.12	Dementia [353]
LGALS3	rs4644	14	C/A	0.49	Cognition [354]
LHFP	rs9315702	13	C/A	0.43	Neuroanatomy [349]
MECP2	rs2239464	X	G/A	0.22	Neuroanatomy [355]
MMP12	rs12808148	11	T/C	0.2	Dementia [356]
MS4A4A	rs4938933	11	T/C	0.5	Alzheimer's disease [110]
MS4A4E	rs670139	11	G/T	0.34	Alzheimer's disease [109]
MS4A6A	rs610932	11	T/G	0.45	Alzheimer's disease [109]
MSRB3	rs17178006	12	T/G	0.09	Neuroanatomy [340]
MTHFD1L	rs11754661	6	G/A	0.07	Alzheimer's disease [110,357]
MTHFR	rs17367504	1	A/G	0.17	Blood Pressure [340]
NOS3	rs3918226	7	C/T	0.04	Blood Pressure [358]
NPR3	rs1173771	5	G/A	0.49	Blood Pressure [340]
NTSR1	rs4334545	20	C/T	0.29	Neuroanatomy [359]
OPRD1	rs678849	1	T/C	0.47	Neuroanatomy [360]
PAICS	rs11549976	4	A/C	0.08	Dementia [361]
PARP1	rs1136410	1	A/G	0.15	Neuroanatomy [362]
PDE7A	rs10808746	8	G/A	0.48	Cognition [116]
PICALM	rs3851179	11	C/T	0.41	Alzheimer's disease [108,110]
SELP	rs3917836	1	T/C	0.05	Neuroanatomy [349]
SNTG1	rs16914781	8	A/G	0.4	Dementia [361]
SORL1	rs668387	11	C/T	0.48	Alzheimer's disease [363]
SPON1	rs2618516	11	C/T	0.36	Neuroanatomy [364]
SPON1	rs11023139	11	G/A	0.06	Cognition [365]

TNF	rs1800629	6	G/A	0.17	Neuroanatomy [366]
TRIM65	rs3744028	17	T/C	0.2	Neuroanatomy [367]
WDR41	rs163030	5	A/C	0.47	Neuroanatomy [368]
WIF1	rs6581612	12	A/C	0.25	Neuroanatomy [340]
ZNF224	rs3746319	19	G/A	0.19	Dementia [369]
HLA-DRB5	*rs9271100*	6	C/T	0.31	Alzheimer's disease [113]
PTK2B	rs28834970	8	T/C	0.32	Alzheimer's disease [113]
SORL1	rs11218343	11	T/C	0.03	Alzheimer's disease [113]
SLC24A4-RIN3	rs10498633	14	G/T	0.19	Alzheimer's disease [113]
DSG2	rs8093731	18	*C/T*	0.01	Alzheimer's disease [113]
INPP5D	rs35349669	2	C/T	0.44	Alzheimer's disease [113]
MEF2C	rs304132	5	G/A	0.46	Alzheimer's disease [113]
NME8	rs2718058	7	A/G	0.36	Alzheimer's disease [113]
ZCWPW1	rs1476679	7	T/C	0.32	Alzheimer's disease [113]
CELF1	rs7933019	11	G/C	0.34	Alzheimer's disease [113]
FERMT2	rs17125944	14	T/C	0.08	Alzheimer's disease [113]
CASS4	rs7274581	20	T/C	0.11	Alzheimer's disease [113]
MIR2113	rs10457441	6	C/T	0.46	Cognition [122]
AKAP6	rs17522122	14	G/T	0.48	Cognition [122]
TOMM40	rs10119	19	C/T	0.29	Cognition [122]

DNA blood kits. Pre-amplification of the targeted loci was performed using the TaqMan PreAmp Master Mix Kit (Life Technologies). Each reaction included 2.5µl TaqMan PreAmp Master Mix (2x), 1.25µl Pre-amplification Assay Pool, 0.5µl H_20 and 1.2µl genomic DNA. These reactions were incubated in a Biorad thermocycler for 10 min at 95°C, followed by 12 cycles of 95°C for 15 sec and 60°C for 4 min, and then incubated at 99.9°C for 10 minutes. The PreAmplified products were then held at 4°C until they were diluted 1:20 in 1x TE buffer and then stored at -20°C until use. For Format 64 OpenArray Plates, 2.5µl diluted pre-amplified products was mixed with 2.5µl TaqMan OpenArray Master Mix. The resulting samples were dispensed using the OpenArray® AccuFill™ System onto OpenArray plates with each plate containing 48 samples and 64 SNP assays per sample. For Format 32 OpenArray Plates, 2µl diluted pre-amplified products was mixed with 2µl TaqMan OpenArray Master Mix. The resulting samples were dispensed using the OpenArray® AccuFill™ System onto OpenArray plates with each plate containing 96 samples and 16 SNP assays per sample. The QuantStudio™ 12K Flex instrument (Applied Biosystems, Carlsbad, California) was used to perform the real time PCR reactions on the loaded OpenArray plates. The fluorescence emission results were read using the OpenArray® SNP Genotyping Analysis software v1 (Applied Biosystems) and the genotyping analysis was performed using TaqMan® Genotyper v1.3, using the autocalling feature. Participant-specific quality controls included filters for genotype success rate (> 90%), genotype-derived gender concordant with reported gender and sample provenance error

assessed via pairwise comparisons of genotype calls between all samples to identify samples with > 90% similarity. Samples that were flagged in the initial quality control checks were repeated, and those that still failed quality control were excluded. SNP-specific filters included genotype call rate (> 90%) and Hardy-Weinberg equilibrium (p > 0.001) assessed using an exact test. Descriptive statistics for baseline cognitive ability for the SNPs that were used in this thesis to investigate the association of genetic variants with cognitive performance are presented in Table 2.4

2.5 Mild Cognitive Impairment and Dementia

At each wave participants have been screened for MCI and dementia using the following protocol. At waves 1-3, the same predetermined cut-off from a battery of cognitive tests were used for inclusion of participants in a sub-study on mild cognitive disorders and dementia. Participants from the full cohort were selected for clinical assessment if they had any of the following: (i) a Mini Mental State Examination (MMSE) [370] score < 25; (ii) a score below the fifth percentile score on immediate or delayed recall of the first list of the California Verbal Learning Test [327]; or (iii) a score below the fifth percentile on two or more of either the Symbol-Digit Modalities Test [371]; Purdue Pegboard with both hands [372]; or Simple Reaction Time [331]. At wave 4, participants were selected for review if (1) MMSE score <25 or ≤2.5 percentile on one or more cognitive test; or (2) previous diagnosis at waves 1-3; or (3) subjective decline ≥25 on Memory and Cognition Questionnaire (MACQ) or (4) Decline in MMSE score ≥ 3 points.

At waves 1-3, the clinical assessment of MCI and dementia involved a structured clinical assessment for Dementia by one of two physicians [373]. Clinicians used clinical checklists, data from the neuropsychological assessments, neuropsychiatric history, and medical history to formulate consensus diagnoses. Due to the large number of participants screened for review at wave 4, case files consisting of all data derived from the health survey and cognitive testing as well as informant interview for each participant were automatically screened to identify participants meeting criteria for any one of the following diagnoses: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), major neurocognitive disorder (NCD); DSM-IV dementia; DSM-5 mild NCD; MCI; age-associated cognitive decline; age-associated memory impairment; DSM-IV

Table 2.4: *Mean and standard deviation for baseline cognitive tests for SNPs associated with dementia and cognitive ability.*

	Genotype	n	MMSE	Immediate Recall	Delayed Recall	Digits backwards	Spot the word	Symbol Digits Modalities test	Simple Reaction Time	Choice Reaction Time
APOE	e2/e2	19	29.21 ± 1.65	7.26 ± 2.08	6.32 ± 1.86	5.11 ± 2.66	52.79 ± 4.02	48.79 ± 6.77	237.35 ± 35.95	317.3 ± 49.04
	e2/e3	274	29.23 ± 1.2	7.03 ± 2.3	6.04 ± 2.54	5.04 ± 2.39	52.02 ± 5.57	50.06 ± 9.81	248.3 ± 51.11	317.14 ± 47.02
	e2/e4	60	29.37 ± 1.06	7.1 ± 2.32	6.45 ± 2.64	4.82 ± 2.22	52.45 ± 5.1	51.8 ± 7.26	239.95 ± 50.79	315.41 ± 37.54
	e3/e3	1444	29.14 ± 1.43	7.18 ± 2.24	6.22 ± 2.46	4.89 ± 2.22	51.84 ± 5.92	49.82 ± 9.59	250.98 ± 59.12	316.51 ± 52.82
	e3/e4	532	29.17 ± 1.28	7.18 ± 2.26	6.21 ± 2.53	4.93 ± 2.22	52.13 ± 5.63	50.21 ± 9.28	250.66 ± 58.89	316.56 ± 45.24
	e4/e4	49	29.08 ± 1.16	7.12 ± 2.32	6.16 ± 2.62	4.98 ± 2.12	53.75 ± 3.91	50.76 ± 10.43	244.93 ± 38.32	314.15 ± 38.83
ABCA7	G/G	24	28.75 ± 1.62	6.21 ± 1.18	4.88 ± 1.73	5.33 ± 2.33	51.86 ± 5.11	50.87 ± 8.21	256.14 ± 52.93	329 ± 49.04
rs3764650	G/T	396	29.09 ± 1.4	7.06 ± 2.29	5.93 ± 2.59	4.9 ± 2.24	51.85 ± 5.68	49.58 ± 9.41	252.99 ± 56.04	316.93 ± 43.06
	T/T	1940	29.18 ± 1.35	7.19 ± 2.25	6.27 ± 2.47	4.91 ± 2.23	52.01 ± 5.78	50.08 ± 9.52	249.43 ± 57.94	316.26 ± 51.19
BIN1	A/A	1203	29.13 ± 1.38	7.15 ± 2.32	6.22 ± 2.54	4.83 ± 2.26	51.95 ± 5.63	49.66 ± 9.47	250.35 ± 56.99	315.44 ± 50.79
rs744373	A/G	949	29.17 ± 1.36	7.16 ± 2.18	6.18 ± 2.47	5.01 ± 2.23	51.98 ± 5.92	50.31 ± 9.54	250.2 ± 60.28	317.79 ± 48.41
	G/G	206	29.3 ± 1.22	7.09 ± 2.15	6.15 ± 2.24	4.9 ± 2.14	52.17 ± 5.76	50.42 ± 9.36	248.5 ± 47.3	317.44 ± 51.66
BDNF	C/C	1529	29.18 ± 1.37	7.16 ± 2.26	6.21 ± 2.46	4.96 ± 2.24	52.03 ± 5.74	49.91 ± 9.44	249.52 ± 57.05	316.3 ± 50.13
rs6265	C/T	705	29.11 ± 1.34	7.2 ± 2.21	6.26 ± 2.52	4.77 ± 2.18	51.91 ± 5.68	50.17 ± 9.54	253.02 ± 60.75	318.39 ± 50.53
	T/T	74	29.04 ± 1.57	6.41 ± 2.33	5.53 ± 2.57	4.78 ± 2.37	51.64 ± 6.65	49.6 ± 9.5	245.51 ± 46.1	313.78 ± 42.65
CD2AP	C/C	199	29.18 ± 1.24	7.15 ± 2.32	6.17 ± 2.57	4.92 ± 2.17	51.88 ± 5.84	49.65 ± 9.98	251.34 ± 57.67	319.27 ± 51.11
rs9296559	C/T	939	29.18 ± 1.41	7.21 ± 2.27	6.26 ± 2.51	4.93 ± 2.25	52.07 ± 5.74	50.33 ± 9.15	248.59 ± 54.56	314.72 ± 46.09
	T/T	1220	29.15 ± 1.33	7.12 ± 2.23	6.17 ± 2.46	4.88 ± 2.23	51.92 ± 5.76	49.81 ± 9.6	250.82 ± 58.42	317.56 ± 52.45
CD33	A/A	1038	29.15 ± 1.31	7.13 ± 2.24	6.14 ± 2.46	4.92 ± 2.29	51.97 ± 5.67	49.91 ± 9.5	251.14 ± 59.91	317.29 ± 51.67
rs34813869	A/G	1047	29.13 ± 1.47	7.19 ± 2.25	6.22 ± 2.51	4.84 ± 2.18	51.87 ± 5.84	50.12 ± 9.38	248.82 ± 53.88	315.84 ± 48.26
	G/G	267	29.32 ± 1.07	7.18 ± 2.29	6.39 ± 2.51	5.15 ± 2.24	52.45 ± 5.73	49.88 ± 10.09	250.8 ± 61.16	316.55 ± 49.5
CLU	C/C	849	29.09 ± 1.41	7.2 ± 2.35	6.23 ± 2.6	4.83 ± 2.21	51.71 ± 5.76	50.24 ± 9.51	250.52 ± 51.58	316.4 ± 44.44
rs11136000	C/T	1123	29.2 ± 1.35	7.12 ± 2.17	6.17 ± 2.4	4.98 ± 2.27	52.13 ± 5.58	49.87 ± 9.57	249.61 ± 59.86	315.02 ± 48.69
	T/T	376	29.2 ± 1.29	7.15 ± 2.28	6.23 ± 2.53	4.87 ± 2.2	52.21 ± 5.85	49.9 ± 9.35	251.62 ± 63.74	321.15 ± 63.22
COMT	A/A	609	29.14 ± 1.36	7.23 ± 2.25	6.26 ± 2.47	4.87 ± 2.27	51.78 ± 5.98	49.79 ± 9.52	249.72 ± 56.23	317.39 ± 50.53
rs4680	A/G	1161	29.16 ± 1.42	7.19 ± 2.24	6.24 ± 2.5	4.94 ± 2.22	52.09 ± 5.73	50.08 ± 9.47	248.5 ± 55.54	314.75 ± 48.39
	G/G	589	29.19 ± 1.25	7.03 ± 2.27	6.07 ± 2.49	4.9 ± 2.24	51.97 ± 5.57	50.08 ± 9.51	254 ± 62.7	319.14 ± 52.15
CR1	A/A	69	29.39 ± 0.86	7.29 ± 2.38	6.22 ± 2.45	5.45 ± 2.44	53.21 ± 4.84	50.3 ± 11.08	253.45 ± 59	321.71 ± 59.72
rs3818361	A/G	697	29.1 ± 1.49	7.14 ± 2.3	6.13 ± 2.54	4.81 ± 2.22	51.56 ± 6.01	49.65 ± 9.6	252.38 ± 56.28	317.94 ± 48.23
	G/G	1584	29.18 ± 1.32	7.16 ± 2.23	6.23 ± 2.47	4.93 ± 2.23	52.14 ± 5.67	50.13 ± 9.36	249.05 ± 58.18	315.86 ± 50.15

Table 2.4 (*Continued)*

	Genotype	n	MMSE	Immediate Recall	Delayed Recall	Digits backwards	Spot the word	Symbol Digits Modalities test	Simple Reaction Time	Choice Reaction Time
CTNNBL1	C/C	9	29.56 ± 0.53	7.33 ± 0.87	6.11 ± 1.9	4.22 ± 2.05	52.89 ± 5.13	46.89 ± 8.34	262.51 ± 59.35	325.87 ± 59.44
rs6125962	C/T	259	29.15 ± 1.52	7.07 ± 2.17	6.12 ± 2.45	4.88 ± 2.32	51.98 ± 5.56	49.63 ± 9.73	248.76 ± 46.75	312.58 ± 40.9
	T/T	2095	29.16 ± 1.34	7.17 ± 2.26	6.21 ± 2.5	4.91 ± 2.22	51.98 ± 5.79	50.06 ± 9.47	250.27 ± 58.79	317 ± 50.81
LGALS3	A/A	417	29.31 ± 1.1	7.18 ± 2.33	6.21 ± 2.52	5 ± 2.26	52.5 ± 5.36	50.43 ± 8.72	246.9 ± 50.33	314.51 ± 48.73
rs4644	A/C	1095	29.2 ± 1.33	7.19 ± 2.28	6.22 ± 2.54	4.9 ± 2.19	51.9 ± 5.88	50.22 ± 9.91	251.94 ± 61.83	318.31 ± 50.09
	C/C	813	29.02 ± 1.51	7.08 ± 2.16	6.14 ± 2.4	4.89 ± 2.28	51.87 ± 5.75	49.56 ± 9.3	249.15 ± 55.3	315.3 ± 50.49
FRMD4A	A/A	159	29.16 ± 1.26	6.96 ± 2.1	6.02 ± 2.24	5.05 ± 2.26	52.17 ± 5.36	50.41 ± 9.89	252.05 ± 59.18	318.97 ± 43
rs7081208	A/G	884	29.15 ± 1.47	7.18 ± 2.3	6.18 ± 2.54	4.88 ± 2.29	52.15 ± 5.85	49.89 ± 9.69	251.85 ± 63.78	316.54 ± 53.73
	G/G	1303	29.16 ± 1.3	7.16 ± 2.23	6.24 ± 2.47	4.9 ± 2.19	51.83 ± 5.76	50.01 ± 9.3	249.06 ± 52.92	316.54 ± 47.95
FRMD4A	C/C	2052	29.15 ± 1.37	7.14 ± 2.28	6.19 ± 2.5	4.94 ± 2.24	51.91 ± 5.75	50.12 ± 9.58	249.96 ± 58.15	316.24 ± 50.13
rs17314229	C/T	290	29.21 ± 1.36	7.28 ± 2.07	6.27 ± 2.44	4.72 ± 2.2	52.49 ± 5.87	49.56 ± 8.7	250.97 ± 52.28	318.01 ± 47.64
	T/T	12	29.75 ± 0.45	7.83 ± 1.9	6.92 ± 1.93	4.92 ± 2.11	53.17 ± 4.06	47 ± 12.31	281.72 ± 84.24	339.87 ± 60.45
rs12007229	A/A	91	28.68 ± 1.99	6.37 ± 2.02	5.55 ± 2.22	4.62 ± 2.27	51.3 ± 7.17	48.19 ± 9.69	260.65 ± 68.27	320.07 ± 57.5
	A/C	132	29.26 ± 1.45	7.89 ± 2.3	6.95 ± 2.72	4.72 ± 2.36	52.16 ± 5.79	51.05 ± 10.01	254.59 ± 51.74	317.6 ± 39.62
	C/C	2122	29.18 ± 1.31	7.15 ± 2.24	6.19 ± 2.47	4.94 ± 2.22	52.02 ± 5.66	50.04 ± 9.44	249.42 ± 57.53	316.27 ± 50.12
EPHA1	C/C	91	29.38 ± 0.81	7.32 ± 1.97	6.34 ± 2.45	4.91 ± 2.24	53.14 ± 4.36	51.26 ± 10.33	253.27 ± 58.45	324.73 ± 56.41
rs11767557	C/T	755	29.17 ± 1.28	7.04 ± 2.21	6.13 ± 2.44	4.88 ± 2.18	51.95 ± 5.68	49.86 ± 9.11	252.99 ± 61.38	318.81 ± 52.27
	T/T	1513	29.14 ± 1.42	7.2 ± 2.28	6.22 ± 2.51	4.92 ± 2.26	51.93 ± 5.87	49.99 ± 9.62	248.53 ± 55.49	314.97 ± 48.17
MMP12	C/C	50	29.18 ± 0.97	7.3 ± 2.4	6.16 ± 2.85	5.38 ± 2.3	52 ± 6.07	49.48 ± 8.01	263.91 ± 78.23	318.95 ± 43.78
rs12808148	C/T	595	29.16 ± 1.39	7.14 ± 2.15	6.16 ± 2.38	4.83 ± 2.13	51.84 ± 5.8	50.17 ± 9.33	249.32 ± 52.16	316.24 ± 47.63
	T/T	1713	29.16 ± 1.36	7.16 ± 2.28	6.21 ± 2.52	4.92 ± 2.26	52.04 ± 5.74	49.96 ± 9.57	249.94 ± 58.65	316.51 ± 50.8
MS4A4A	C/C	393	29.12 ± 1.48	7.18 ± 2.21	6.19 ± 2.57	4.91 ± 2.33	51.1 ± 6.2	48.95 ± 9.62	255.15 ± 72.41	322.56 ± 62.82
rs4938933	C/T	1140	29.17 ± 1.36	7.18 ± 2.33	6.24 ± 2.52	4.95 ± 2.22	52.36 ± 5.41	50.18 ± 9.46	247.95 ± 52.64	314.89 ± 45.27
	T/T	826	29.17 ± 1.27	7.14 ± 2.15	6.17 ± 2.41	4.84 ± 2.21	51.89 ± 5.94	50.31 ± 9.34	250.46 ± 55.24	315.97 ± 48.69
MS4A4E	G/G	826	29.09 ± 1.46	7.17 ± 2.31	6.2 ± 2.59	4.96 ± 2.31	51.36 ± 6.13	49.34 ± 9.93	252.35 ± 65.78	317.73 ± 54.95
rs670139	G/T	1095	29.21 ± 1.31	7.21 ± 2.24	6.26 ± 2.43	4.94 ± 2.22	52.48 ± 5.49	50.41 ± 9.18	248.9 ± 50.89	315.61 ± 45.8
	T/T	427	29.15 ± 1.31	7.03 ± 2.16	6.07 ± 2.44	4.75 ± 2.13	51.83 ± 5.61	50.12 ± 9.34	249.65 ± 57.02	316.96 ± 50.1
MS4A6A	G/G	784	29.16 ± 1.28	7.07 ± 2.2	6.1 ± 2.43	4.81 ± 2.23	51.97 ± 5.68	50.15 ± 9.33	249.52 ± 53.93	315.96 ± 46.6
rs610932	G/T	1138	29.18 ± 1.37	7.2 ± 2.28	6.25 ± 2.48	4.97 ± 2.2	52.21 ± 5.65	50.13 ± 9.51	249.62 ± 56.64	315.97 ± 50.93
	T/T	432	29.1 ± 1.49	7.2 ± 2.27	6.27 ± 2.61	4.92 ± 2.33	51.34 ± 6.16	49.36 ± 9.75	252.64 ± 65.44	319.24 ± 52.79

Table 2.4 *(Continued)*

	Genotype	n	MMSE	Immediate Recall	Delayed Recall	Digits backwards	Spot the word	Symbol Digits Modalities test	Simple Reaction Time	Choice Reaction Time
MTHFD1L	A/A	11	29.09 ± 1.38	7 ± 2.41	6 ± 2.65	5.18 ± 2.44	51.91 ± 6.77	54.73 ± 15.13	233.83 ± 18.38	290.61 ± 15.49
rs11754661	A/G	344	29.29 ± 1.07	7.17 ± 2.22	6.26 ± 2.52	4.79 ± 2.11	52.07 ± 5.52	50.23 ± 9.01	249.67 ± 54.28	319.6 ± 49.69
	G/G	2006	29.14 ± 1.41	7.16 ± 2.25	6.19 ± 2.48	4.92 ± 2.25	51.96 ± 5.79	49.93 ± 9.53	250.15 ± 58.06	316.14 ± 49.98
MTHFR	A/A	1636	29.14 ± 1.39	7.14 ± 2.3	6.19 ± 2.51	4.88 ± 2.21	52.04 ± 5.7	49.86 ± 9.46	249.38 ± 55.97	316.13 ± 50.05
rs17367504	A/G	652	29.21 ± 1.25	7.21 ± 2.14	6.24 ± 2.47	4.97 ± 2.28	51.93 ± 5.88	50.31 ± 9.68	252.52 ± 61.94	317.42 ± 49.39
	G/G	68	29.06 ± 1.61	7.03 ± 2.28	6.04 ± 2.39	5.06 ± 2.31	50.87 ± 5.69	50.06 ± 8.52	249.64 ± 53.64	320.77 ± 51.97
CETP	A/A	1082	29.18 ± 1.34	7.18 ± 2.22	6.23 ± 2.49	4.95 ± 2.21	51.97 ± 5.78	50.01 ± 9.38	251 ± 59.22	318.47 ± 54.12
rs5882	A/G	1042	29.16 ± 1.4	7.17 ± 2.27	6.21 ± 2.47	4.91 ± 2.25	51.99 ± 5.82	50.19 ± 9.53	249.37 ± 57.47	314.22 ± 44.77
	G/G	240	29.07 ± 1.26	6.97 ± 2.3	6.05 ± 2.54	4.72 ± 2.28	51.95 ± 5.38	49.17 ± 9.8	249.65 ± 50.08	317.83 ± 50.57
PAICS	A/A	2084	29.16 ± 1.35	7.13 ± 2.25	6.17 ± 2.49	4.92 ± 2.26	51.99 ± 5.77	49.98 ± 9.59	250.66 ± 58.52	316.48 ± 49.85
rs11549976	A/C	273	29.14 ± 1.49	7.37 ± 2.22	6.42 ± 2.46	4.8 ± 2.07	51.91 ± 5.66	50.19 ± 8.77	246.26 ± 49.34	317.17 ± 50.42
	C/C	2	29.5 ± 0.71	8 ± 0	6.5 ± 0.71	3 ± 1.41	51 ± 0	51.5 ± 0.71	213.93 ± 5.68	286.78 ± 8.1
PDE7A	A/A	490	29.15 ± 1.28	7.07 ± 2.24	6.15 ± 2.43	4.75 ± 2.13	51.71 ± 5.92	49.41 ± 9.91	251.26 ± 55.32	320.14 ± 55.46
rs10808746	A/G	1117	29.13 ± 1.44	7.15 ± 2.29	6.22 ± 2.5	4.92 ± 2.27	52.09 ± 5.66	50.03 ± 9.5	250.83 ± 61.43	316.75 ± 51.99
	G/G	728	29.21 ± 1.31	7.23 ± 2.2	6.21 ± 2.53	5.01 ± 2.23	52.04 ± 5.78	50.2 ± 9.14	248.36 ± 52.48	314.33 ± 42.42
PICALM	C/C	875	29.21 ± 1.36	7.18 ± 2.32	6.17 ± 2.54	4.96 ± 2.21	52.22 ± 5.68	50.06 ± 9.71	245.97 ± 51.14	313.52 ± 46.98
rs3851179	C/T	1146	29.12 ± 1.37	7.13 ± 2.21	6.23 ± 2.45	4.85 ± 2.26	51.72 ± 5.88	49.93 ± 9.33	252.62 ± 61.45	318.95 ± 53.05
	T/T	334	29.14 ± 1.33	7.18 ± 2.19	6.23 ± 2.49	4.95 ± 2.21	52.25 ± 5.49	50.2 ± 9.53	252.77 ± 59.67	316.28 ± 45.89
SNTG1	A/A	753	29.05 ± 1.33	7.01 ± 2.18	6 ± 2.37	4.87 ± 2.21	52.22 ± 5.44	49.83 ± 9.44	253.5 ± 60.41	318.55 ± 50.9
rs16914781	A/G	1148	29.2 ± 1.36	7.26 ± 2.25	6.31 ± 2.49	4.9 ± 2.23	51.91 ± 5.8	50.13 ± 9.64	247.24 ± 52.9	314.58 ± 46.11
	G/G	457	29.22 ± 1.41	7.16 ± 2.35	6.28 ± 2.63	4.99 ± 2.3	51.82 ± 6.07	50.01 ± 9.21	252.38 ± 63.62	318.6 ± 56.86
SPON1	A/A	5	29.6 ± 0.55	7.8 ± 2.59	6.2 ± 3.9	5.4 ± 1.52	50.8 ± 3.11	52 ± 12.61	251.69 ± 37.38	311.54 ± 41.64
rs11023139	A/G	244	29.13 ± 1.42	7.37 ± 2.28	6.45 ± 2.46	4.75 ± 2.19	51.72 ± 6.71	50.02 ± 9.55	250.42 ± 51.85	315.83 ± 46.96
	G/G	2114	29.16 ± 1.36	7.13 ± 2.24	6.17 ± 2.49	4.92 ± 2.24	52.01 ± 5.64	50 ± 9.48	250.12 ± 58.25	316.64 ± 50.25
ZNF224	A/A	83	29.07 ± 1.49	6.84 ± 1.99	5.67 ± 2.52	4.83 ± 2.28	52.24 ± 6.07	50.63 ± 7	256.36 ± 52.51	322.66 ± 44.69
rs3746319	A/G	659	29.23 ± 1.23	7.23 ± 2.26	6.27 ± 2.45	5.03 ± 2.24	52.28 ± 5.82	50.36 ± 9.43	248.66 ± 52.48	315.42 ± 45.75
	G/G	1621	29.14 ± 1.4	7.14 ± 2.26	6.21 ± 2.5	4.86 ± 2.23	51.85 ± 5.71	49.82 ± 9.62	250.45 ± 59.77	316.73 ± 51.7
MIR2113	C/C	602	29.11 ± 1.5	7.18 ± 2.25	6.23 ± 2.45	4.94 ± 2.26	51.97 ± 5.75	49.7 ± 9.37	249.35 ± 53.8	317.02 ± 53.29
rs10457441	C/T	1149	29.13 ± 1.38	7.1 ± 2.24	6.16 ± 2.52	4.85 ± 2.2	52.02 ± 5.72	50.04 ± 9.59	251.27 ± 58.73	316.74 ± 49.68
	T/T	540	29.28 ± 1.17	7.22 ± 2.27	6.21 ± 2.48	4.99 ± 2.26	51.86 ± 5.95	50.24 ± 9.32	247.53 ± 54.02	315.1 ± 45.41

Table 2.4 *(Continued)*

	Genotype	n	MMSE	Immediate Recall	Delayed Recall	Digits backwards	Spot the word	Symbol Digits Modalities test	Simple Reaction Time	Choice Reaction Time
SLC24A4-RIN3	G/G	1386	29.16 ± 1.33	7.14 ± 2.27	6.15 ± 2.53	4.92 ± 2.25	51.81 ± 5.81	49.97 ± 9.47	248.32 ± 55.32	315.23 ± 48.28
rs10498633	G/T	794	29.16 ± 1.45	7.2 ± 2.23	6.29 ± 2.45	4.89 ± 2.22	52.28 ± 5.75	50.15 ± 9.4	254.31 ± 58.02	320.08 ± 53.61
	T/T	109	29.13 ± 1.27	6.87 ± 2.19	5.94 ± 2.29	4.85 ± 2.15	51.65 ± 5.52	49.28 ± 10.07	238.29 ± 55.44	305.17 ± 32.21
FRMD4A	A/A	32	28.65 ± 1.89	6.97 ± 2.12	6.25 ± 2.29	4.41 ± 2.15	51.71 ± 5.44	48.47 ± 11.09	253.3 ± 60.55	319.99 ± 47.88
rs2446581	A/G	481	29.12 ± 1.39	7.04 ± 2.32	6.05 ± 2.56	4.81 ± 2.19	51.47 ± 5.93	49.5 ± 9.59	255.34 ± 68.62	319.39 ± 60.79
	G/G	1778	29.18 ± 1.35	7.19 ± 2.23	6.23 ± 2.47	4.95 ± 2.24	52.12 ± 5.73	50.16 ± 9.4	248.29 ± 52.41	315.53 ± 46.29
NME8	A/A	943	29.13 ± 1.33	7.14 ± 2.21	6.16 ± 2.48	4.9 ± 2.22	51.77 ± 5.74	50.41 ± 9.15	250.57 ± 55.81	316.2 ± 49.36
rs2718058	A/G	1041	29.19 ± 1.33	7.1 ± 2.25	6.17 ± 2.49	4.84 ± 2.24	52.07 ± 5.63	49.55 ± 9.56	249.79 ± 53.49	318.07 ± 46.75
	G/G	307	29.15 ± 1.59	7.35 ± 2.35	6.37 ± 2.54	5.17 ± 2.2	52.24 ± 6.34	50.28 ± 10.04	248 ± 66.75	311.35 ± 59.37
MEF2C	A/A	402	29.09 ± 1.53	7.14 ± 2.28	6.05 ± 2.67	4.84 ± 2.28	52.14 ± 6.1	49.8 ± 9.48	254.88 ± 59.99	320.54 ± 59.3
rs304132	A/G	1117	29.16 ± 1.37	7.13 ± 2.26	6.18 ± 2.49	4.93 ± 2.24	51.96 ± 5.7	49.67 ± 9.61	248.89 ± 56.1	316.63 ± 46.82
	G/G	774	29.19 ± 1.27	7.18 ± 2.23	6.28 ± 2.4	4.92 ± 2.18	51.91 ± 5.72	50.59 ± 9.22	248.68 ± 54.69	313.99 ± 48.11
INPP5D	C/C	651	29.05 ± 1.5	7.14 ± 2.28	6.13 ± 2.52	4.78 ± 2.24	51.43 ± 5.96	49.58 ± 9.69	252.5 ± 57.56	318.41 ± 49.43
rs35349669	C/T	1077	29.22 ± 1.28	7.14 ± 2.24	6.21 ± 2.47	4.95 ± 2.28	52.15 ± 5.62	50.27 ± 9.39	248.19 ± 52.89	314.62 ± 46.4
	T/T	555	29.16 ± 1.37	7.18 ± 2.24	6.23 ± 2.49	4.98 ± 2.14	52.21 ± 5.83	49.93 ± 9.39	250.28 ± 61.33	317.5 ± 55.4
CELF1	C/C	209	29.2 ± 1.21	7.03 ± 2.24	5.93 ± 2.41	4.86 ± 2.22	51.76 ± 5.64	49 ± 9.83	253.23 ± 60.87	318.98 ± 45.65
rs7933019	C/G	985	29.18 ± 1.33	7.13 ± 2.21	6.22 ± 2.42	4.94 ± 2.22	51.88 ± 5.91	50.21 ± 9.69	249.84 ± 53.47	315.64 ± 46.24
	G/G	1098	29.13 ± 1.43	7.2 ± 2.29	6.22 ± 2.56	4.9 ± 2.24	52.11 ± 5.67	50 ± 9.19	249.16 ± 57.93	316.6 ± 53.25
AKAP6	G/G	619	29.16 ± 1.4	7.3 ± 2.29	6.34 ± 2.57	5.03 ± 2.21	52.14 ± 5.52	50.33 ± 9.34	250.47 ± 56.01	316.78 ± 50.16
rs17522122	G/T	1173	29.2 ± 1.35	7.15 ± 2.23	6.16 ± 2.49	4.9 ± 2.24	52.09 ± 5.83	50.07 ± 9.52	248.69 ± 56	315.31 ± 50.57
	T/T	496	29.07 ± 1.36	6.99 ± 2.24	6.1 ± 2.4	4.78 ± 2.23	51.47 ± 5.97	49.42 ± 9.53	252.26 ± 57.71	318.68 ± 47.07
SORL1	C/C	5	28.4 ± 0.55	6.6 ± 3.36	4.8 ± 2.59	4.2 ± 1.92	48.8 ± 8.32	57 ± 7.52	308.32 ± 91.3	326.15 ± 32.6
rs11218343	C/T	187	29.03 ± 1.45	7.01 ± 2.41	6.22 ± 2.54	4.85 ± 2.17	51.34 ± 6.18	50.83 ± 8.61	248.3 ± 61.72	312.01 ± 47.46
	T/T	2096	29.17 ± 1.36	7.16 ± 2.23	6.19 ± 2.49	4.92 ± 2.24	52.03 ± 5.74	49.91 ± 9.54	249.93 ± 55.76	316.81 ± 49.91
FERMT2	C/C	16	28.81 ± 1.68	7.62 ± 2.96	6.81 ± 2.93	4.94 ± 2.35	51.88 ± 8.2	49.69 ± 8.99	238.22 ± 26.45	316.46 ± 28.73
rs17125944	C/T	378	29.27 ± 1.23	7.26 ± 2.24	6.35 ± 2.53	4.8 ± 2.19	52.07 ± 5.24	51.31 ± 9.39	249.94 ± 59.38	315.56 ± 50.22
	T/T	1899	29.14 ± 1.39	7.12 ± 2.25	6.16 ± 2.48	4.93 ± 2.24	51.96 ± 5.86	49.74 ± 9.46	249.95 ± 55.93	316.59 ± 49.71
HLA-DRB5	C/C	1208	29.11 ± 1.48	7.11 ± 2.27	6.14 ± 2.51	4.81 ± 2.22	51.75 ± 5.96	49.87 ± 9.61	250.2 ± 56.75	316.3 ± 47.99
rs9271100	C/T	933	29.23 ± 1.23	7.19 ± 2.21	6.24 ± 2.43	5.01 ± 2.24	52.24 ± 5.57	50.35 ± 9.22	249.64 ± 56.35	316.47 ± 52.32
	T/T	147	29.18 ± 1.21	7.27 ± 2.39	6.31 ± 2.73	5.08 ± 2.27	52 ± 5.6	48.87 ± 9.86	249.48 ± 54.1	317.4 ± 46.47

Table 2.4 *(Continued)*

	Genotype	n	MMSE	Immediate Recall	Delayed Recall	Digits backwards	Spot the word	Symbol Digits Modalities test	Simple Reaction Time	Choice Reaction Time
DSG2	C/C	2225	29.16 ± 1.35	7.15 ± 2.25	6.19 ± 2.49	4.9 ± 2.24	51.97 ± 5.74	50.02 ± 9.41	249.92 ± 56.56	316.59 ± 49.92
rs8093731	C/T	62	29 ± 1.99	7.05 ± 2.38	6.15 ± 2.65	5.02 ± 2.05	51.62 ± 7.19	49.47 ± 11.35	248.1 ± 50.51	308.91 ± 39.15
	T/T	1	30 ± NA	8 ± NA	7 ± NA	8 ± NA	56 ± NA	58 ± NA	294.56 ± NA	357.9 ± NA
PTK2B	C/C	314	29.24 ± 1.17	7.3 ± 2.2	6.25 ± 2.43	4.89 ± 2.22	52.29 ± 5.44	50.68 ± 9.45	246.4 ± 55.06	313.79 ± 49.4
rs28834970	C/T	1100	29.13 ± 1.4	7.06 ± 2.29	6.16 ± 2.53	4.95 ± 2.27	51.89 ± 6.1	49.59 ± 9.52	251.25 ± 58.42	316.78 ± 51.22
	T/T	874	29.16 ± 1.4	7.2 ± 2.22	6.2 ± 2.47	4.86 ± 2.19	51.93 ± 5.49	50.25 ± 9.39	249.52 ± 54.24	316.96 ± 47.81
ZCWPW1	C/C	213	29.29 ± 1.24	7.16 ± 2.23	6.16 ± 2.56	4.94 ± 2.16	52.3 ± 5.51	50.62 ± 9.2	243.81 ± 55.82	310.8 ± 42.34
rs1476679	C/T	972	29.18 ± 1.31	7.14 ± 2.31	6.18 ± 2.51	4.94 ± 2.24	52.16 ± 5.51	50.25 ± 9.28	249.41 ± 53.01	316.97 ± 49.43
	T/T	1108	29.11 ± 1.43	7.16 ± 2.21	6.21 ± 2.46	4.88 ± 2.23	51.75 ± 6.05	49.67 ± 9.66	251.43 ± 59.18	317.01 ± 51.14
CASS4	A/A	1940	29.17 ± 1.36	7.14 ± 2.24	6.19 ± 2.5	4.92 ± 2.22	51.99 ± 5.83	50.09 ± 9.43	250.54 ± 57.72	316.57 ± 49.98
rs927174	A/C	333	29.11 ± 1.35	7.23 ± 2.35	6.23 ± 2.45	4.85 ± 2.33	51.81 ± 5.5	49.44 ± 9.4	244.25 ± 46.52	314.16 ± 45.88
	C/C	18	29.17 ± 2.09	7 ± 1.88	6.06 ± 2.18	4.61 ± 1.94	53.76 ± 4.72	49.94 ± 13.92	276.53 ± 64.32	341.67 ± 77.23

amnestic disorder not otherwise specified; DSM-IV mild NCD; and DSM-IV other cognitive disorder. Major criteria for meeting most of these diagnoses were operationalised as any of the following: (1) concern of self or informant of significant cognitive decline (MACQ ≥25 or Informant Questionnaire on Cognitive Decline in the Elderly >3.31 or history of dementia diagnosis); (2) substantial impairment on at least one cognitive domain relative to wave 4 normative data (cut-offs less than −2 SD for dementias, less than −1.5 SD for mild cognitive disorders); (3) interference with independence and instrumental activities of daily living (IADL; self-reported IADL impairment or Bayer IADL scale score >3.11 or informant-reported everyday cognitive difficulties); (4) not exclusively during delirium (cognitive changes of >6 months' duration, onset of cognitive changes preceding informant report of onset of delirium like symptoms); and (5) not due to another co-existing disorder (PHQ9 < 9 and no reported history of schizophrenia or other psychosis). Those meeting criteria for one or more diagnoses were screened for case file review by a research neurologist and a diagnoses was made. For complex cases, two physicians formulated a consensus diagnosis. based on the following criteria: (1) comorbid depression, (2) other comorbid psychiatric conditions, (3) stroke and (4) DSM-5 major NCD without memory impairment.

Clinically diagnosed MCI was based on the Petersen criteria at waves 1 and 2 [13], whereas the Winblad criteria [14] were used at wave 3 and 4. Clinically diagnosed dementia was based on the DSM IV criteria [374] at all waves. At wave 4, there were 14 participants who were not interviewed, but were known to have dementia from informant reports and medical records. As expected, the prevalence of MCI and dementia increased over the course of the study with 37 (1.45%), 42 (1.8%), 51 (2.60%) and 144 (8.9%) of participants diagnosed with MCI at waves 1, 2, 3, and 4 respectably. MCI diagnosis were unstable, with 49% of participants between any two waves transitioning from MCI – CN in contrast to 3.07% of participants transitioning from CN – MCI (Table 2.5).

Table 2.5: *Number of transitions between cognitively normal, mild cognitive impairment and dementia during the length of the study*

			To		
From	CN	MCI	Dementia	Death	Censored
CN	5461 (85.57%)	196 (3.07%)	37 (0.58%)	240 (3.76%)	448 (7.02%)
MCI	63 (49.61%)	38 (29.92%)	8 (6.3%)	10 (7.87%)	8 (6.3%)
Dementia	0 (0%)	0 (0%)	6 (75%)	2 (25%)	0 (0%)
Censored	41 (18.39%)	3 (1.35%)	0 (0%)	10 (4.48%)	169 (75.78%)

2.6 Power Analysis

Power curves were calculated to assess the effect size that could be detected at a given power for our sample size using the R package 'simr' [375]. The power calculations are based on Monte Carlo simulations (n = 1000) of linear mixed effects models constructed from the observed interview times of participants in the PATH 60+ cohort for a total of 2551 participants with 8386 observations. The observed variance-covariance matrix in PATH was used to fit the Monte Carlo simulated linear mixed models. Simulated independent variables were constructed for a normal and positivity skewed distributed continuous variable (mean = 0; SD = 1) and a binary categorical independent variable with 50:50, 40:60, 30:70, 20:80, 10:90 and 5:95 split. The effect sizes for the baseline and linear rate of change coefficients were altered in the base model in increments to determine the detectable effect size at 80% power (Table 2.6).

Table 2.6: *Detectable effect size at 80% power that can be observed in the PATH 60's cohort*

	Baseline	**Linear Change**
Continuous Independent Variable		
Normal Distribution	-0.005 (-0.005 - -0.004)	-0.0046 (-0.0051 - -0.004)
Positive Skewed Distribution	-0.044 (-0.052 - -0.036)	-0.0047 (-0.0052 - -0.0042)
Categorical Independent Variable		
50%	-0.087 (-0.093 - -0.08)	-0.0095 (-0.01 - -0.0088)
40%	-0.0891 (-0.0957 - -0.0825)	-0.0094 (-0.01 - -0.0089)
30%	-0.0942 (-0.0996 - -0.0888)	-0.0102 (-0.0107 - -0.0097)
20%	-0.1083 (-0.1122 - -0.1044)	-0.0118 (-0.0122 - -0.0114)
10%	-0.1391 (-0.1437 - -0.1346)	-0.0155 (-0.0158 - -0.0151)
5%	-0.1794 (-0.2592 - -0.0996)	-0.0204 (-0.0249 - -0.016)

Chapter 3: Interactive Effect of *APOE* Genotype and Blood Pressure on Cognitive Decline: The PATH Through Life Study

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Abstract

The *apolipoprotein E* (*APOE*) *ε4 allele and hypertension are two of the most prevalent risk factors for cognitive decline in later life. Here we investigate whether cognitive decline is affected by interaction between these two risk factors. Specifically, we examine whether *APOE***ε4* moderates the association between high blood pressure and cognition in later life. Cognitive function was assessed at three time points over a period of 8 years in 1,474 cognitively normal, community-dwelling adults aged 60-64 years at baseline. Blood pressure and *APOE* genotype were assessed at baseline. Blood pressure was measured categorically as 'Hypertension' and continuously as 'Mean Arterial Pressure' (MAP). Multilevel models were used to investigate main and interactive effects of *APOE* genotype and both hypertension and MAP on the rate of change of episodic memory, working memory, verbal ability, perceptual speed and global cognition. The *APOE*–hypertension interaction was associated with a small but statistically significant increase in the rate of decline of episodic memory, verbal ability and global cognition. However, its inclusion in the model did not increase the amount of outcome variation explained beyond that already explained by the effect of time. In contrast, the *APOE*-MAP interaction had no effect on the rate of decline in any of these domains of cognitive performance. These results provide tentative evidence that *APOE* genotype moderates the association between high blood pressure and cognitive decline in later life.

Keywords: *Apolipoprotein E*; blood pressure; hypertension; cognitive decline; aging; gene-environment interaction

3.1 Introduction

The *apolipoprotein E* (*APOE*) *epsilon 4* (*ε*4*) allele is well-established as the strongest common genetic risk factor for Alzheimer's disease [107,376], with heterozygous and homozygous individuals having approximately 2-3 times and 10-12 times greater risk of developing Alzheimer's disease, respectively [134]. The *APOE* genomic region has also been associated with non-pathological cognitive aging [121], although findings for a role of *APOE***ε4* in cognitive decline is mixed. Two meta-analyses [136,377] concluded that the *APOE***ε4* allele has a relatively small and specific influence on cognitive domains associated with episodic memory, executive functioning, perceptual speed and overall global cognitive ability, but that it does not affect attention, verbal ability, visuospatial skill or primary memory. In contrast, possession of the *APOE epsilon 2* (**ε2*) allele, which has been associated with reduced risk of Alzheimer's Disease [378], may protect against cognitive decline [379,380]. These effects have not been observed in cross-sectional and longitudinal studies of the PATH Cohort used in this study [333,381-383].

The mechanism underlying the association between *APOE* genotype and cognition is not well understood. The predominant theory is that *APOE* alleles bind differentially to amyloid-β (Aβ) peptides resulting in differential regulation of the Aβ aggregation and clearance in the brain, which in turn leads to synaptic dysfunction and neurodegeneration, inducing cognitive decline [135]. However, Aβ-independent pathways have also been proposed, including regulation of brain lipid transport, glucose metabolism, neuro-inflammation and vascular health [135].

High blood pressure has also been linked to dementia and is one of the most important modifiable risk factors for cognitive decline. Hypertension in mid-life has been consistently associated with greater late-life cognitive decline, particularly in executive functioning and attention, and with the development of dementia (reviewed in [384,385]). Evidence for an association between late life (as distinct from mid-life) hypertension and cognition has been mixed. Significant positive [386,387] and negative [388,389] effects, a U-shaped relationship [390], and no effect [391,392] have all been reported. The following mechanistic link between blood pressure and cognition has been suggested: high blood pressure induces atherosclerotic conditions and alters the autoregulation of cerebral blood

flow, which in turn promotes incidents of clinical and subclinical brain damage. These incidents cause brain atrophy and reduce white matter integrity, which affects cognitive functioning [385].

In addition to their direct effects, the interaction between *APOE* genotype and hypertension may also modify the rate of cognitive decline [104]. The *APOE *ε4* allele is associated with an increased risk of hypertension [393], cerebrovascular [394,395] and coronary heart disease [396], and therefore may moderate the association between hypertension and cognitive decline. There is also evidence that the *APOE *ε2* allele is associated with increased risk of cardiovascular disease [397,398], in an age- and sex-dependent manner [399,400].

Longitudinal studies of the effect of this interaction on cognition have produced mixed results (Table 3.1). An initial study found that a late-life hypertension–*APOE***ε4* interaction lowered the risk of cognitive decline [401]. However, several more recent studies have found the opposite effect, both for midlife and late-life hypertension [402-405] and other studies found no such interaction [235,383,406-408]. These divergent results may be due to different participant characteristics (e.g. baseline education, mean age, gender and ethnicity) and methodologies (e.g. sample size, duration of the study, number of follow ups, inclusion of the protective *ε*2* allele, definition of high blood pressure and cognitive measures).

To further elucidate whether the *APOE* genotype moderates the effect of late-life hypertension and cognition, we performed a longitudinal analysis of a community-based sample of older adults.

3.2 Methods

3.2.1 Participants

The sample used in this study is from the Personality and Total Health (PATH) Through Life project, a large community survey of health and wellbeing in adults sampled from the electoral rolls of Canberra and Queanbeyan, Australia (which provide a representative population sample because enrolment to vote is a legal requirement for adult Australian citizens). Written informed consent for participation in the PATH project was obtained from all participants according to the 'National Statement' guidelines of the National Health and Medical Research Council of Australia and following a protocol approved by the Human Research

Table 3.1: *Previous investigations of the effect of APOE-blood pressure interaction on cognitive decline*

Study	Population sample (n)	Age at baseline years/SD	Education, level/mean years/SD	Study time-frame (# time points)	Hypertension	Response Variables	Statistical Models	Effect
Bangen *et al* 2013 [402]	Caucasian (1,436)	54 (9)	40% College degree	N: 8 (2) C: 8 yr previous	>140/90 mmHG or hypertensive medication	Verbal memory Visual memory Attention Executive functioning Visuospatial skills Language	Multivariate linear regression	Reduced language ability
de Frias & Willis 2014 [405]	(563)	51 (12)	15 (2.7)	21 (4)	Physician Diagnosis	(Latent Constructs) Verbal comprehension Episodic memory Numeric facility Inductive reasoning Spatial orientation Perceptual speed Cognitive flexibility	Multilevel modeling	Reduced cognitive flexibility
Kalmijn *et al* 1996 [401]	Caucasian males (353)	74.6 (6)	23% >12 yr	3 yr (2)	Baseline >160/95 mmHG or hypertensive medication	MMSE	Multiple logistic regression	Protective
Kang *et al* 2005 [403]	Caucasian females (4,155)	74 (-)	5.7% Masters or Doctorate degree	N: 2/4 yr (2/3) C: 24 yr previous	Physician Diagnosis	Global cognition Short-term memory Episodic memory Working Memory	Multivariate linear & logistic regression General estimating equations	Reduced working memory

Table 3.1 *(Continued)*

Yasuno *et al* 2012 [404]	Japanese (622)	72 (5.1)	10yr (2.7)	3 yr (2)	Baseline >160/100 mmHG or hypertensive medication	Composite cognitive score Attention Memory Verbal Fluency Abstract reasoning	Repeated Measures ANCOVA	Reduced composite cognitive score
Caselli *et al* 2011 [407]	Caucasian & Latino (808)	60 (13)	15yr (2.7)	11 yr (1 ≤)	>140/90 mmHG	Long-term memory	Mixed models	None
Carmelli *et al* 1998 [406]	Caucasian Male Twins (410)	63 (3)	13yr (3)	N: 10 yr (2) C: 6 yr previous	>140/90 mmHG or hypertensive medication	Perceptual speed Attention Short-term memory	General linear ANCOVA	None
Christensen *et al* 2008 [383]	Caucasian (2,021)	63 (-)	14yr (-)	4 yr (2)	Baseline >140/90 mmHG or hypertensive medication	Short-term memory Episodic memory Perceptual speed Working memory Reaction time MMSE	Bivariate ANOVA	None
Debette *et al* 2011 [235]	Caucasian (1352)	54 (9)	-	N: 8 yr (2) C: 8 yr previous	>140/90 mmHG or hypertensive medication	Verbal memory Visual memory Executive functioning	Multivariate linear & logistic regression	None
Knopman *et al* 2009 [408]	Caucasian & African-American (1,130)	59 (4.3)	-	14 yr (4)	>140/90 mmHG or hypertensive medication	Episodic memory Perceptual speed Verbal fluency	Mixed effects models	None

MMSE: Mini Mental State Exam
N: Neuropsycholgical
C: Cardiovascular

Ethics Committee of The Australian National University. Participants were drawn from three cohorts; those aged 20-24 (20+), 40-44 (40+) and 60-64 (60+) years at baseline, which were assessed at 4-year intervals for a total of 12 years (testing for the 4th wave is currently in progress). Each assessment point is referred to as a wave.

The testing procedures in the PATH study have been previously described in detail [322]. Results presented here are for the first three waves (8 years of follow-up) of the 60+ cohort, conducted in 2001-2002 (n = 2,551), 2005-2006 (n = 2,222) and 2009-2010 (n = 1,973). Individuals were excluded from further analysis if they had only attended one interview (n = 309), were of non-European ancestry (n = 110), had probable dementia (Mini Mental State Examination score < 24; [409] (n = 63) or had a self-reported medical history of epilepsy, stroke, transient ischaemic attack, brain tumours or brain infections (n = 363). There were no group differences between individuals who attended only one wave compared to those who attended more than one assessment, for the variables; *APOE*ε4* genotype, hypertension and MAP (Supplementary Table 1). As missing values can reduce power and introduce bias in the resulting estimate [410], missing values for the continuous variables education, depression and mean systolic and diastolic blood pressure were imputed using expectation-maximization (EM; n = 195). The EM algorithm in SPSS does not impute missing values for categorical variables, so participants with missing values for *APOE* genotype, smoking status, diabetes mellitus or heart trouble were excluded (n = 181). This left a final sample of 1,741 individuals.

3.2.2 Genotyping

Genotyping of the PATH sample for *APOE* variants has been described previously [382]. Briefly, genomic DNA was extracted from cheek swabs using Qiagen DNA Blood kits. TaqMan Assays (Applied Biosystmes Inc., Foster City, CA, USA) were used to genotype two SNPs - rs429358 and rs7412 within *APOE* to identify the six *APOE* genotypes comprising the *ε*2*, *ε*3* and *ε*4* alleles. Genotype frequencies were *ε*2*/*ε*2*: n = 19 (0.8%), *ε*3*/*ε*3*: n = 1444 (60.7%), *ε*4*/*ε*4*: n = 49 (2.1%), *ε*2*/*ε*3*: n = 274 (11.5%), *ε*2*/*ε*4*: n = 60 (2.5%) and ε*3*/ε*4*: n = 532 (22.4%). Allele frequencies did not deviate from Hardy-Weinberg equilibrium [383].

The APOE *ε2 allele has been associated with both protective effects for cognitive decline and increased risk for cardiovascular disease. To avoid potential confounding effects due to these known associations, *APOE *ε2* carriers were excluded from the analysis. Participants were classified as either *APOE*ε4* negative (*ε4-; n= 1,444; 72%, including the *ε3/*ε3* genotype) and *ε4* positive (*ε4+; n= 581; 28%, including *ε4/*ε4* and *ε3/*ε4* genotypes). This left a final sample of 1,474 individuals. A secondary analysis was conducted in which *APOE*ε2* individuals were retained, with participants also classified into *APOE*ε4* negative (*ε4-; n= 1,737; 73% including *ε2/*ε2*, *ε3/*ε3*, *ε2/*ε3*) and *ε4* positive (*ε4+; n= 641; 27%, including *ε4/*ε4*, *ε2/*ε4*, *ε3/*ε4*) groups.

3.2.3 Cardiovascular Risk Factors

Measures of hypertension and MAP were based on assessments taken at baseline. Resting blood pressure was measured twice during the interviews while participants were seated. Participants were asked if they were currently taking medication for high blood pressure. Participants were classified as hypertensive if they met any of the following criteria: i) mean systolic blood pressure ≥140 mm Hg; ii) mean diastolic blood pressure ≥90 mm Hg; iii) taking hypertensive medication at baseline. MAP was calculated as [(2 x diastolic)+systolic] / 3 [411].

3.2.4 Demographic and General Health Variables

Baseline data on gender, smoking status (current or past smoking), education (total years spent studying), history of heart trouble and diabetes mellitus were included in the analysis. Depression symptoms were assessed using the Patient Health Questionnaire (PHQ), which is a short version of the patient questionnaire component of the Primary Care Evaluation of Mental Disorders (PRIME-MD) instrument [323]. We generated measures of depression symptoms from the nine items related to depression [rated on a 4-point scale from “not at all” (1) to “nearly every day” (4)], following the coding algorithm provided in the PHQ instruction manual (available from Patient Health Questionnaire Screeners; http://www.phqscreeners.com/overview.aspx).

3.2.5 Cognitive Assessment

All participants were assessed at baseline and at each subsequent interview for the following six cognitive measures: perceptual speed, measured using the Symbol Digit Modalities Test [371]; episodic memory, assessed using the immediate recall and delayed recall of the first trial of the California Verbal Learning Test (Recall-immediate & Recall-delayed) [327]; working memory, measured using the Digit Span Backward from the Wechsler Memory Scale [328]; and vocabulary, assessed by the Spot-the-Word Test [329]. A global cognition score was computed as the unweighted mean of the standardized scores for the Symbol Digits Modalities Test, Recall-immediate, Digit Span Backwards, Recall-delayed and the Spot-the-Word Test.

3.2.6 Data Preparation and Statistical Analysis

Data were analysed in SPSS Statistics version 21 (IBM SPSS statistics). Group differences in demographic and general health characteristics were examined by unpaired *t* tests and Chi- squared tests. To allow for comparison across all cognitive tasks, the test scores for all cognitive task at all three waves were transformed into *T* scores (M = 50, SD = 10) using the baseline means and standard deviations. Higher scores on all tests indicate better cognitive function. Pearson correlations were computed for the cognitive test scores (Supplementary Table 2).

The variables smoking, diabetes mellitus, heart trouble, hypertension and *APOE* genotype were coded as categorical variables, with non-smokers, non-diabetics, no reported heart trouble, non-hypertensive and *APOE* $\varepsilon 4$- groups treated as the reference group.

Multilevel modelling [412], with maximum likelihood estimation, was used to assess the effect of predictors on change in cognition over time (indicated by age, centred on mean age at baseline). Predictor variables were *APOE* genotype (*$\varepsilon 4$- and *$\varepsilon 4$+) and the blood pressure (BP) variables: Hypertension and MAP. Covariates used in the models were gender, total years of education, depression score, smoking status, diabetes and heart trouble.

In Model 1 (Unconditional Means Model) no predictors were entered. This intercept-only model was used as a baseline index of within- and between-person variation and to estimate the intra-class correlation coefficients

(ICC). In Model 2 (Unconditional Growth Model) time was introduced and used to estimate the average rate of change within the population. In Model 3 covariates were introduced into the Unconditional Growth Model. In Model 4 *APOE* genotype and BP variables were entered as additive terms. Separate models were generated for hypertension and MAP. In Model 5 interaction terms between *APOE* genotype and the BP variables were introduced to determine whether these interactions had effects on the intercept and the rate of change of cognitive test scores.

Due to the disputed effectiveness of assessing single parameter estimates via t-statistics or z-statistics, log-likelihood ratio tests were first used to assess model fit for nested models as compared to their reference model. The parameter estimates for models in which the change in model fit was significant were then assessed to determine significance. Pseudo R-squared statistics were used to quantify the proportion of outcome variation that the models predictors explain [412]. In the final model this approach was used to determine whether inclusion of the interaction terms significantly improved the variance explained.

A power analysis was performed in the R statistical programming language, using the package Longpower [413] for Model 5 of the Global Cognition test score, indicating that this study could detect an effect size of 0.31 SD at 80% power. It should be noted, however, that due to their complexity, power calculations for multilevel models are approximate [414].

3.3 Results

3.3.1 Demographic and General Health Characteristics

There were no group differences in any covariates at baseline between the *APOE*ε4-* and **ε4+* groups (Table 3.2). Means and standard deviations of the cognitive test scores at each wave are presented in Table 3.3.

3.3.2 Multilevel models

Each cognitive measure was first evaluated using unconditional means, unconditional growth and covariate models to provide a baseline for comparing the effects of *APOE* genotype and blood pressure variables (Model 1, 2, and 3; Table 3.4). Significant change in performance across time was observed for all cognitive tests except for Digit Span Backwards test (Model 2; Table 3.4). On average, participants experience significant decline in Recall-immediate, Recall-

Table 3.2: *Demographic and general health characteristics of sample.*

	*APOE*ε4-* (n=1,049)	*APOE*ε4+* (n=425)	Degrees of freedom	t/χ^2	*p*
Age[a]	62.5 ± 1.5	62.4 ± 1.5	1,472	1.33	0.18
Male, n (%)[b]	538 (51.3 %)	220 (51.8 %)	1	0.03	0.87
Years of education[a]	14 ± 2.6	14 ± 2.6	1,472	-0.03	0.98
Depression score[c]	2.1 ± 2.8	2.4 ± 3.1	1,472	-1.51	0.13
Cigarette smoking, n (%)[b]	490 (46.7 %)	202 (47.5 %)	1	0.81	0.78
Diabetes, n (%)[b]	78 (7.4 %)	31 (7.3 %)	1	0.01	0.93
Hypertension, n (%)[b]	656 (62.5 %)	267 (62.8 %)	1	0.01	0.92
Heart Trouble, n (%)[b]	138 (13.2 %)	54 (12.7 %)	1	0.05	0.82
Systolic blood pressure[a]	139.3 ± 18.5	139.4 ± 19.2	1,472	-0.06	0.95
Diastolic blood pressure[a]	82.9 ± 10.6	82.6 ± 10.1	1,472	0.47	0.64
Mean arterial blood pressure[a]	101.7 ± 12.2	101.5 ± 12.1	1,472	0.24	0.81
Cognitive Tests					
Immediate Recall	7.33 ± 2.17	7.47 ± 2.14	794.79	-1.17	0.24
Delayed Recall	6.41 ± 2.43	6.46 ± 2.45	780.04	-0.32	0.74
Symbol Digits Modalities test	51.15 ± 8.75	51.06 ± 8.55	803.47	0.17	0.87
Digit span Backwards	5.04 ± 2.19	5.05 ± 2.17	786.46	-0.03	0.98
Spot-the-Word	52.94 ± 7.01	52.94 ± 6.59	831.01	-0.009	0.99

[a] Unpaired 2-tailed t-test
[b] Pearson's χ^2 2-tailed test
[c] Brief Patient Health Questionnaire

Table 3.3: *Raw cognitive test scores (mean ± standard deviation)*

	Immediate recall	Delayed recall	Symbol digit modalities test	Digit Span Backwards	Spot-the-Word
Wave 1	7.3 ± 2.2	6.4 ± 2.4	51 ± 8.8	5.0 ± 2.2	52 ± 5.3
Wave 2	7.0 ± 2.1	6.2 ± 2.4	50 ± 8.9	5.2 ± 2.2	53 ± 4.9
Wave 3	6.7 ± 2.2	5.9 ± 2.3	48 ± 8.9	5.2 ± 2.16	53 ± 4.9

Higher scores indicate better cognitive function.

delayed, Symbol Digit Modalities Test and Global Cognition. Inclusion of time explained between 70-93% of the variation in the cognitive measures. Conversely, a significant increase in Spot-the-Word test scores was observed over time. Inclusion of the covariates in Model 3 (Table 3.4) improved the model fit for all cognitive test scores and explained additional variation. Parameter estimates for

covariates and random effects for all models can be found in Supplementary Tables 3 & 4.

3.3.3 Blood Pressure and APOE genotype group differences

Statistics for hypertension and MAP models are presented in Table 3.4. Introducing hypertension and *APOE* genotype as additive terms significantly improved model fit for the cognitive variables Recall-immediate and Recall-delayed. Additionally, for these cognitive variables the *APOE*ε4+* group experienced a greater rate of decline in test scores. However, these significant group differences did not explain any additional variance.

Introducing MAP and *APOE* genotype as additive terms significantly improved model fit for the cognitive variables: Recall-immediate Recall-delayed and Global Cognition. Additionally, for these cognitive variables there were significant MAP and *APOE* genotype group differences in both baseline test scores and rate of change. Increased MAP was associated with lower baseline scores for Global Cognition, while the *APOE*ε4+* group experienced a greater rate of decline in test scores for Recall-immediate, Recall-delayed and Global Cognition. These significant group differences did not explain any additional variance.

3.3.4 APOE–blood Pressure Interaction

Statistics for the interaction between *APOE* genotype and hypertension/MAP (Model 5) are presented in Table 3.4 and fitted trajectories are displayed in Figures 3.1 and 3.2. Introducing the interaction term between *APOE* genotype and hypertension significantly improved model fit for the cognitive variables Recall-immediate, Recall-delayed, Spot-the-Word and Global Cognition. Additionally, the interaction term was associated with differences in baseline test scores and rate of change. In individuals who were both hypertensive and *APOE*ε4* carriers, higher baseline test scores were observed for both Recall-immediate and Recall-delayed while a greater rate of decline in test scores was observed for Recall-immediate, Recall-delayed, Spot-the-Word, Global Cognition. The inclusion of the interaction term, however, did not explain any additional variance. Conversely, introducing the interaction term between *APOE* genotype and MAP did not significantly improve model fit for any of the cognitive tests.

Table 3.4: *Fixed effects for hypertension and Mean Arterial Pressure models 1-5*

	Recall-Immediate	Recall-Delayed	Digit Span Backwards	Spot-the-Word	Symbol Digit	Global Cognition
	Estimate (SE)	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*
Model 1 (Unconditional Means)						
Intercept	49 (0.22)***	49 (0.21)***	50 (0.22)***	51 (0.24)***	48 (0.24)***	49 (0.24)***
Model fit, df = 3	30395	29859	29264	26522	28805	28644
ICC	0.52	0.56	0.64	0.86	0.75	0.80
Model 2 (Unconditional Growth)						
Intercept	50 (0.25)***	50 (0.25)***	50 (0.25)***	50 (0.26)***	50 (0.26)***	50 (0.25)***
Time	-0.37 (0.03)***	-0.23 (0.03)***	0.01 (0.03)	0.18 (0.02)***	-0.44 (0.02)***	-0.25 (0.02)***
Change in model fit *df*Δ = 3	135***	68***	5.3	136***	359***	148***
R^2_{yy}	0.73	0.76	0.80	0.93	0.87	0.89
Model 3 (Covariates)						
Intercept	41 (1.19)***	423(1.18)***	36 (1.25)***	25 (1.24)***	38 (1.36)***	31 (1.24)***
Time	-0.37 (0.03)***	-0.23 (0.03)***	0.01 (0.03)	0.18 (0.02)***	-0.44 (0.02)***	-0.26 (0.02)***
Change in model fit *df*Δ = 6	253***	216***	157**	413***	139***	374***
$R^2_{yy}\Delta$	-0.01	0.00	-0.01	0.00	0.00	0.00
Model 4 (*APOE* + Hypertension)						
Initial status						
Intercept	41 (1.24)***	43 (1.23)***	36 (1.30)***	25 (1.29)***	38 (1.42)***	30 (1.29)***
APOE genotype	0.73 (0.52)	0.26 (0.52)	0.16 (0.53)	0.28 (0.50)	-0.02 (0.54)	0.43 (0.50)
Hypertension	-0.22 (0.49)	-0.34 (0.49)	0.24 (0.50)	0.64 (0.48)	0.07 (0.52)	0.08 (0.48)
Rate of Change						
Time	-0.37 (0.06)***	-0.24 (0.05)***	-0.02 (0.05)	0.17 (0.03)***	-0.35 (0.04)***	-0.24 (0.04)***
APOE genotype	-0.20 (0.07)**	-0.20 (0.07)**	0.10 (0.06)	-0.02 (0.04)	-0.07 (0.05)	-0.13 (0.05)*
Hypertension	0.10 (0.07)	0.10 (0.06)	0.01 (0.06)	0.02 (0.04)	-0.11 (0.05)*	0.04 (0.05)
Change in model fit *df*Δ = 4	10*	13*	4.2	3.7	7.9	6.8

	Recall-Immediate	Recall-Delayed	Digit Span Backwards	Spot-the-Word	Symbol Digit	Global Cognition
	Estimate (SE)	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*
$R^2_{yy}\Delta$	0.00	0.00	0.00	0.00	0.00	0.00
Model 5 (*APOE* x Hypertension)						
Initial Status						
Intercept	42 (1.26)***	43 (1.24)***	36 (1.32)***	25 (1.30)***	38 (1.43)***	31 (1.30)***
APOE genotype	-0.61 (0.84)	-1.5 (0.84)	0.53 (0.87)	-0.28 (0.82)	0.45 (0.89)	-0.42 (0.82)
Hypertension	-0.85 (0.58)	-1.1 (0.58)*	0.41 (0.59)	0.37 (0.56)	0.29 (0.61)	-0.32 (0.56)
APOE x HT	2.2 (1.06)*	2.8 (1.07)**	-0.59 (1.10)	0.90 (1.04)	-0.75 (1.12)	1.4 (1.04)
Rate of Change						
Time	-0.43 (0.06)***	-0.31 (0.06)***	-0.03 (0.05)	0.13 (0.04)***	-0.36 (0.04)***	-0.30 (0.04)***
APOE genotype	0.01 (0.12)	0.03 (0.11)	0.12 (0.10)	0.12 (0.06)	-0.03 (0.08)	0.07 (0.08)
Hypertension	0.19 (0.08)*	0.21 (0.07)**	0.01 (0.07)	0.09 (0.04)*	-0.10 (0.06)	0.13 (0.06)*
APOE x HT	-0.34 (0.15)*	-0.36 (0.14)**	-0.03 (0.13)	-0.24 (0.08)**	-0.07 (0.11)	-0.31 (0.11)**
Change in model fit $df\Delta$ = 2	6.3*	8.9*	0.58	8.2*	1.2	8.5*
$R^2_{yy}\Delta$	0.00	0.00	0.00	0.00	0.00	0.00
Model 4 (*APOE* + MAP)						
Initial status						
Intercept	38 (2.35)***	40 (2.35)***	37 (2.43)***	20 (2.33)***	34 (2.53)***	26 (2.33)***
APOE genotype	0.74 (0.52)	0.27 (0.52)	0.16 (0.53)	0.29 (0.50)	-0.01 (0.54)	0.44 (0.50)
MAP	0.04 (0.02)	0.03 (0.02)	-0.00 (0.02)	0.05 (0.02)*	0.04 (0.02)	0.04 (0.02)*
Rate of Change						
Time	-0.13 (0.27)	-0.21 (0.26)	-0.38 (0.24)	0.34 (0.15)*	0.01 (0.20)	-0.16 (0.20)
APOE genotype	-0.20 (0.07)**	-0.20 (0.07)**	0.10 (0.06)	-0.02 (0.04)	-0.07 (0.05)	-0.13 (0.05)*
MAP	-0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-0.00 (0.00)	-0.00 (0.00)	-0.00 (0.00)
Change in model fit $df\Delta$ = 4	11*	13**	6.8	6.2	8.4	11*
$R^2_{yy}\Delta$	0.00	0.00	0.00	0.00	0.00	0.00
Model 5 (*APOE* x MAP)						
Initial Status						
Intercept	39 (2.67)***	43 (2.67)***	35 (2.76)***	21 (2.63)***	33 (2.85)***	27 (2.63)***

	Recall-Immediate	Recall-Delayed	Digit Span Backwards	Spot-the-Word	Symbol Digit	Global Cognition
	Estimate (SE)	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*
APOE genotype	-4.5 (4.36)	-9.7 (4.38)*	4.8 (4.49)	-1.8 (4.25)	3.1 (4.58)	-2.4 (4.26)
MAP	0.02 (0.02)	0.00 (0.02)	0.01 (0.02)	0.04 (0.02)	0.05 (0.02)*	0.03 (0.02)
APOE x MAP	0.05 (0.04)	0.10 (0.04)*	-0.05 (0.04)	0.02 (0.04)	-0.03 (0.04)	-0.03 (0.04)
Rate of Change						
Time	-0.35 (0.32)	-0.50 (0.30)	-0.41 (0.28)	0.16 (0.18)	0.07 (0.23)	-0.36 (0.23)
APOE genotype	0.56 (0.61)	0.80 (0.57)	0.23 (0.53)	0.63 (0.34)	-0.28 (0.44)	0.60 (0.44)
MAP	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-0.00 (0.00)*	0.00 (0.00)
APOE x MAP	-0.01 (0.01)	-0.01 (0.01)	-0.00 (0.00)	-0.01 (0.00)	0.00 (0.00)	-0.01 (0.00)
Change in model fit $df\Delta = 2$	2.0	5.7	1.8	3.7	0.56	2.7
$R^2_{yy}\Delta$	0.00	0.00	0.00	0.00	0.00	0.00

ICC = Intraclass correlation coefficient; R^2_{yy} = Pseudo R^2 statistic for total variance explained
*$p < .05$; **$p < .01$; ***$p < .001$.

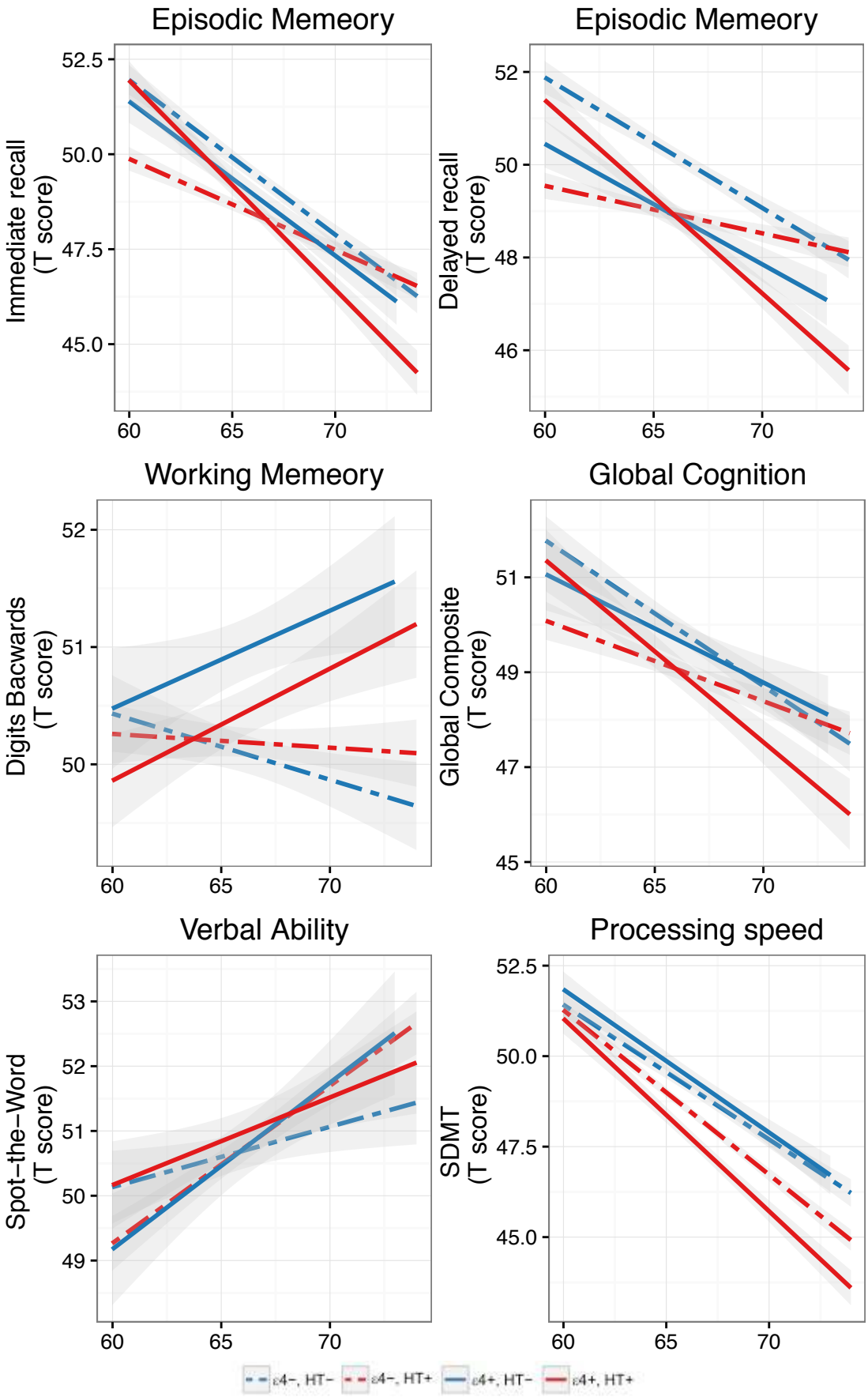

Figure 3.1: *Cognitive trajectories for the APOE and Hypertension interaction.*

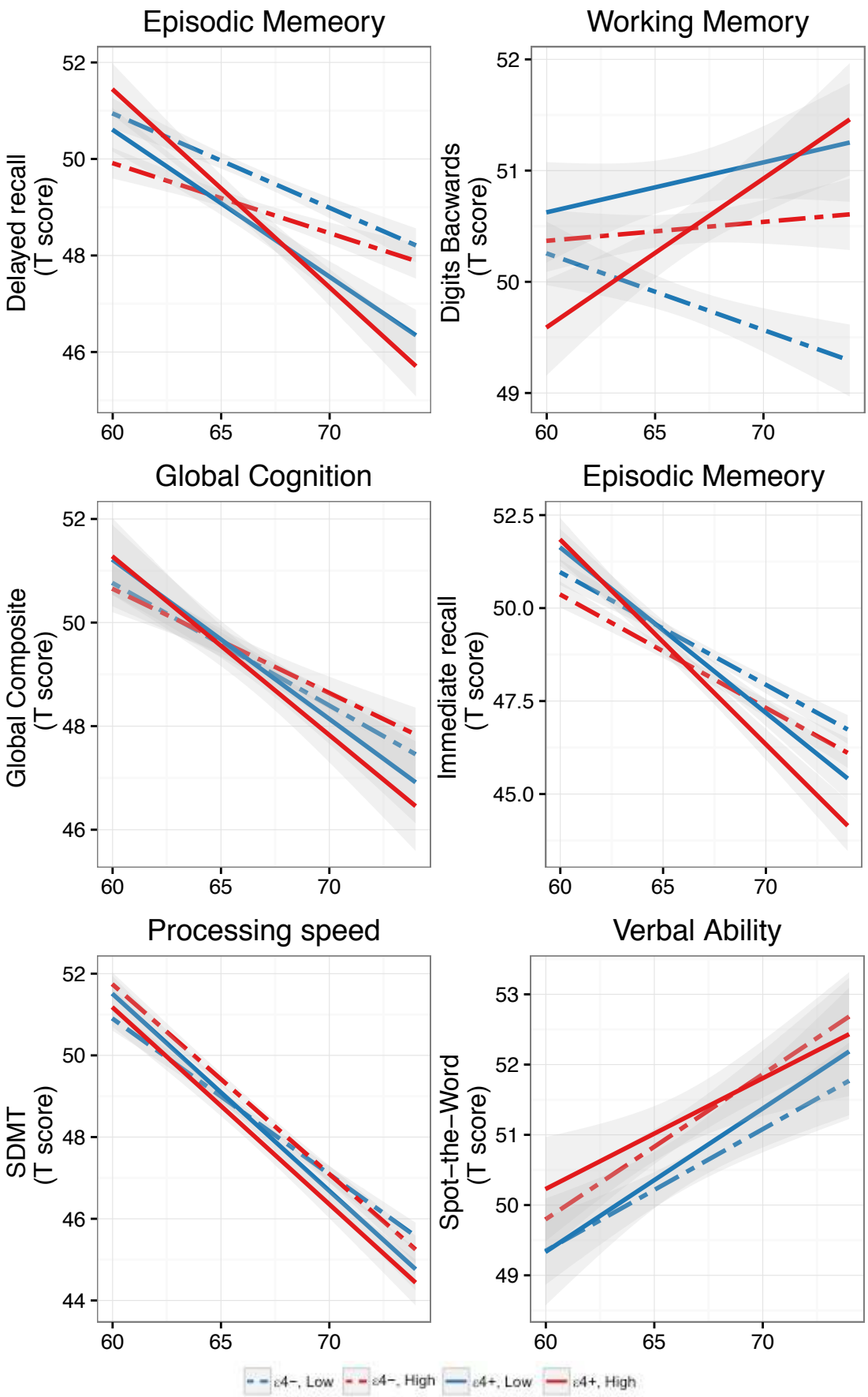

Figure 3.2: *Cognitive trajectories for the APOE and MAP interaction.*

To examine the effect of untreated, in comparison to treated, hypertension on cognitive change we analysed a subsample of 569 individuals with a history of hypertension. 445 (78%) individuals in this group reported taking anti-hypertensive medication at baseline (Med+) and 287 (64%) were classified as hypertensive according to blood pressure readings at baseline (HT+). Of the 124 individuals who reported not taking anti-hypertensive medication (Med-), 87 (70%) were in the HT+ group. The interaction with *APOE* genotype and these groups (Med+/HT+, Med+/HT-, Med-/HT+ and Med-/HT-) were analysed and no significant association with any of the cognitive variables was observed (data not shown). However, given the small size of the groups our study was underpowered to detect an effect of treated vs. untreated hypertension.

3.3.5 Inclusion of APOE*ε2 Carriers

A secondary analysis was performed in which carriers of the AD-protective *APOE***ε2* allele were included. This had no appreciable effect on fixed or random effects for Models 1-3 (Supplementary Table 5), but it changed the effects of introducing *APOE* genotype and the blood pressure variables into the model (Model 4; Supplementary Tables 5 & 6). Specifically, model fit for Symbol Digits Modalities Test becomes significant and for Recall-immediate it was no longer significant in hypertension models, and in MAP models, model fit for Recall-immediate and Global Cognition were no longer significant. There were also small changes in the parameter estimates. No additional variance was explained for any cognitive test.

Inclusion of *APOE***ε2* carriers had a comparatively stronger effect on the *APOE*-hypertension interaction models (Model 5; Supplementary Table 5). A decrease in model fit was observed for Recall-Immediate, Recall-Delayed and Global Cognition. Change in model fit only remained significant for the Spot-the-Word test. The reduction in model fit across all cognitive tests was also accompanied with attenuation in the coefficients for the *APOE*-hypertension interaction term for both baseline scores and rate of change in test scores. No changes in the model fit or the interaction parameter estimates were observed in the *APOE*-MAP interaction models (Model 5; Supplementary Table 6).

3.4 Discussion

We have found evidence that the *APOE*–hypertension interaction has a significant effect on the rate of decline of episodic memory (Recall-immediate & Recall-delayed), vocabulary (Spot-the-Word) and global cognition. However, the effect is small, with the interaction term accounting for a decline in cognitive abilities ranging from -0.024 to -0.038 SD over four years. This is reflected in the *pseudo-R^2* statistics, which indicate that the inclusion of the predictor variables does not increase the amount of total outcome variance explained beyond the effect of time.

In comparison, the *APOE*–MAP interaction did not affect the rate of decline in test scores for any of the cognitive tests. The difference between these results could reflect the confounding effect of hypertensive medication on measurement of MAP, since individuals with controlled hypertension have MAP in the normal range. This interpretation would imply that the effect of hypertension, in interaction with *APOE* genotype has an effect on cognitive decline, even if hypertension is medically controlled.

Consistent with previous findings [379,380], *APOE*ε2* appears to have a protective effect on cognitive decline since its inclusion in the analysis attenuates parameter estimates and the significance of the *APOE*-blood pressure associations, despite the increase in sample size and hence the power to detect an effect. This finding underscores the limitation of investigating effects of *APOE* genotypes based on a simple binary classification of alleles as *ε4+* and *ε4-*.

There are limitations that need to be considered when interpreting our findings. Firstly, this sample is better educated than the general Australian population [322] and the populations sampled in similar, previous studies (Table 3.1). Higher educational attainment is linked with reduced risk of cognitive decline [415] and development of dementia [276], consistent with the cognitive reserve hypothesis [416]. Additionally, the sample is relatively young. Studies that have identified a strong association between the *APOE*ε4* allele and cognitive change have generally been based on a slightly older population then those in the PATH sample [417,418]. The combination of a younger age and a higher potential cognitive reserve could have limited our ability to detect an effect of the *APOE* genotype–BP interaction on cognitive change, which may become apparent in follow-up assessments. Our analysis is also based on measures of hypertension in early old age, whereas studies investigating the interaction between APOE

genotype and mid-life hypertension, which has been consistently associated with cognitive decline in later life [384,385], may observe stronger effects.

This study had a number of strengths that allow for robust statistical inference about how the *APOE*-genotype moderates the effect of hypertension on non-pathological aging. Data were collected from three assessments over a period of 8 years in a representative community-based sample. This included a comprehensive cognitive assessment using a number of different cognitive tests to access different abilities, two measures of blood pressure including a continuous measure of mean arterial pressure (MAP) and a dichotomous hypertension measure. Furthermore, individuals with the protective *APOE**ε*2* allele were excluded.

Our findings suggest an interaction of the biological processes underlying the effects of *APOE* genotype and of hypertension on cognitive aging. Caution is needed in drawing inferences from these findings about dementia. Evidence that *APOE* genotype moderates the effect of hypertension on dementia is inconclusive [419] and the overlap between biological changes associated with normal cognitive aging and dementia is limited [91]. The PATH study is ongoing and the number of incident cases of mild cognitive impairment and dementia among participants is increasing. The work presented here thus provides an excellent basis for investigating this overlap through longitudinal analysis incorporating future waves of the PATH study.

Supplementary Data

Supplementary data is available at: http://dx.doi.org/10.3233/JAD-140630

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Chapter 4: Association of genetic risk factors with cognitive decline: the PATH through life project

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Abstract

We examined the association of 28 single nucleotide polymorphisms (SNPs), previously associated with dementia or cognitive performance, with tests assessing episodic memory, working memory, vocabulary and perceptual speed in 1,689 non-demented older Australians of European ancestry. In addition to testing each variant individually, we assessed the collective association of the 12 risk SNPs for Late-onset Alzheimer's disease (LOAD) using weighted and unweighted genetic risk scores (GRS). Significant associations with cognitive performance were observed for *APOE* ε4 allele, *ABCA7*-rs3764650, *CR1*-rs3818361, *MS4A4E*-rs6109332, *BDNF*-rs6265, *COMT*-rs4680, *CTNNBL*-rs6125962, *FRMD4A*-rs17314229, *FRMD4A*-rs17314229, intergenic SNP chrX-rs12007229, *PDE7A*-rs10808746, *SORL1*-rs668387 and *ZNF224*-rs3746319. Additionally, the weighted GRS was associated with worse performance on episodic memory. The identification of genetic risk factors, that act individually or collectively, may help in screening for people with elevated risk of cognitive decline and for understanding the biological pathways that underlie cognitive decline.

Keywords: Alzheimer's Disease; Cognitive Decline; SNPs; Genetic Risk Scores, Population-Based Study.

4.1 Introduction

Cognitive differences in the elderly consist of differences in stable, life-long cognitive traits and differences in age-associated cognitive change. For both of these there is significant inter-individual variability in the population [420]. Loss of cognitive function due to age-associated cognitive decline is associated with increased difficulties in performing tasks involving memory or rapid information processing and can have a major impact on an individual's quality of life, even in the absence of dementia [5-8,10,421]. Identifying factors that predispose individuals to a faster rate of cognitive decline is an important step for developing intervention and treatment strategies aimed at maintaining cognitive health.

Genetic factors likely contribute to the inter-individual variability observed in cognitive decline, with common genetic variants estimated to account for between 40-50% of the variability associated with general cognitive functioning in later life and 24% of the variability in lifetime cognitive change [102,103]. To date the majority of genetic research on cognitive decline has focused on candidate genes that have been previously associated with age-related disease, traits or mechanisms [104,105], and particularly with genes related to neurotransmitters, neurotrophins, cognitive function and neurodegenerative disease. Two of the most widely studied such genes are *COMT*, which encodes the neurotransmiter catechol-O-methyl transferase, and *BDNF*, which encodes the neurotrophin brain-derived neurotrophic factor. Functional variants in these genes have been primarily associated with decline in executive functioning and memory, respectively, although results are inconsistent [104]. Late-onset Alzheimer's disease (LOAD) susceptibility genes are also good candidates for association with cognitive decline as the pathological features of LOAD progress to varying degrees in individuals without dementia or cognitive impairment and are associated with non-clinical cognitive decline [97,422]. This cross-over effect is exemplified by the *APOE* *ε4 allele, which confers the largest known genetic risk for LOAD, approximately 2-3 times and 10-12 times for heterozygotes and homozygotes respectively [134]. The *APOE* genotype are also associated with specific effects on the cognitive domains of episodic memory, executive functioning, perceptual speed and global cognitive ability [136].

Despite the publication of numerous genetic associations with cognitive decline, the variants identified typically explain a very small fraction of the

phenotypic variability and many remain to be replicated. Furthermore, failure to replicate an initial positive result is common due to differences in participant characteristics (e.g. baseline education, mean age, gender and ethnicity) and methodologies (e.g. sample size, duration of the study, number of follow-ups, population stratification, variation in classification and cognitive measures) [104].

Here we investigate the association between selected genetic risk factors with cognitive decline in a longitudinally followed community-based cohort of 1,689 older adults without dementia who have undergone comprehensive cognitive testing. First, we investigate whether 12 SNPs from the top replicated LOAD associated genes [423] (Table 4.1) are individually, or collectively as a genetic risk score (GRS), associated with cognitive decline. Second, we investigate whether 16 SNPs, previously associated with either dementia or cognition (Table 4.1) are also associated with cognitive decline.

4.2 Methods

4.2.1 Participants

Participants were recruited randomly from the electoral rolls (registration is a legal requirement for Australian Citizens) of Canberra and Queanbeyan into the Personality and Total Health (PATH) Through Life Project. PATH consists of three cohorts 20-24 (20+), 40-44 (40+) and 60-64 (60+) years at baseline, who have participated in a large longitudinal community survey of health and wellbeing in adults, the background and procedures for which have been described in detail elsewhere [322]. Written informed consent was obtained from all participants and approval for the study was obtained from the Human Research Ethics Committee of The Australian National University.

The 60+ cohort is the focus of this study. Individuals were assessed at 4-year intervals for a period of 8 years with interviews conducted in 2001-2002 (n = 2,551), 2005-2006 (n = 2,222) and 2009-2010 (n = 1,973). Individuals were excluded from further analysis based on the following criteria: attendance at only one interview (n = 309), no genomic DNA available for genotyping (n = 185), *APOE* *ε2/*ε4 genotype (n = 60; to avoid the conflation of *ε2 protective and *ε4 risk effect), non-European ancestry (n = 110), probable dementia at any wave (Mini Mental State Examination score < 24 [370]), self-reported medical history of

Table 4.1: *SNPs used in this study*

Gene	Protein	SNP	Chromosome	Alleles†	MAF‡	Odds Ratio§
Top Report Alzheimer's disease risk SNPs						
APOE	Apolipoprotein E	rs429358/rs7412	19	ε2/ε3/ε4	0.8/0.14	0.54/3.81
ABCA7	ATP-binding cassette subfamily A member 7	rs3764650	19	T/G	0.11	1.23
BIN1	Myc box-dependent-interacting protein 1	rs744373	2	A/G	0.31	1.17
CD2AP	CD2-associated protein	rs9296559	6	T/C	0.27	1.11
CD33	Myeloid cell surface antigen CD33	rs34813869	19	A/G	0.29	0.89
CLU	Clusterin	rs11136000	8	C/T	0.35	0.88
CR1	Complement receptor type 1	rs3818361	1	G/A	0.26	1.17
EPHA1	Ephrin type-A receptor 1	rs11767557	7	T/C	0.2	0.89
MS4A4A	Membrane-spanning 4-domains subfamily A member 4A	rs4938933	11	T/C	0.5	0.88
MS4A4E	Membrane-spanning 4-domains subfamily A member 4E	rs670139	11	G/T	0.34	1.08
MS4A6A	Membrane-spanning 4-domains subfamily A member 6A	rs610932	11	T/G	0.45	0.9
PICALM	Phosphatidylinositol-binding clathrin assembly protein	rs3851179	11	C/T	0.41	0.88
Additional AD, dementia and cognition SNPs						
BDNF	Brain-derived neurotrophic factor	rs6265	11	C/T	0.2	
CETP	Cholesteryl ester transfer protein	rs5882	16	A/G	0.36	
COMT	Catechol O-methyltransferase	rs4680	22	G/A	0.48	
CTNNBL1	Beta-catenin-like protein 1	rs6125962	20	T/C	0.6	
FRMD4A	FERM domain-containing protein 4A	rs17314229	10	C/T	0.09	
FRMD4A	FERM domain-containing protein 4A	rs7081208	10	G/A	0.29	
Intergenic	-	rs12007229	X	C/A	0.12	
LGALS3	Galectin-3	rs4644	14	C/A	0.49	
MMP12	Macrophage metalloelastase	rs12808148	11	T/C	0.2	
MTHFD1L	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	rs11754661	6	G/A	0.07	
PAICS	Multifunctional protein ADE2	rs11549976	4	A/C	0.08	
PDE7A	High affinity cAMP-specific 3',5'-cyclic phosphodiesterase 7A	rs10808746	8	G/A	0.48	
SNTG1	Gamma-1-syntrophin	rs16914781	8	A/G	0.4	
SORL1	Sortilin-related receptor	rs668387	11	C/T	0.48	
SPON1	Spondin-1	rs11023139	11	G/A	0.06	
ZNF224	Zinc finger protein 224	rs3746319	19	G/A	0.19	

†Major/Minor Allele; ‡Minor Allele Frequency: HapMap-CEU; §Alzegene reported OR for minor allele

epilepsy, stroke, transient ischaemic attack, brain tumour or brain infection (n = 327). Missing values, which can reduce power and result in biased estimates, were imputed for the covariate 'Education' (total years of education) using random forests via the 'missForest' package available in R [424] (n = 139). This left a final sample of 1,689 individuals. At baseline, the individuals retained in the final sample had on average of 0.69 more years of education and scored 0.74 points higher on the MMSE than those excluded (Table 4.2).

Table 4.2: *Sample Demographics*

Variable	Excluded (n=861)	Included (n=1,689)	Degrees of Freedom	t/χ^2	p
Age†	62.46 ± 1.49	62.54 ± 1.51	1,753	-1.22	0.21
Education†	3.31 ± 3.09	14 ± 2.59	1,488	-5.62	<0.001
Cognitive Tests†					
Immediate Recall	6.61 ± 2.42	7.36 ± 2.18	1578.5	7.57	<0.001
Delayed Recall	5.61 ± 2.55	6.42 ± 2.44	1666.1	7.67	<0.001
Digits Backwards	4.56 ± 2.31	5.05 ± 2.2	1646.4	5.15	<0.001
Spot-the-Word	50.29 ± 6.57	52.57 ± 5.3	1333.7	8.6326	<0.001
SDMT	46.76 ± 11	51.11 ± 8.78	1400.3	9.99	<0.001
MMSE	28.6 ± 2.13	29.35 ± 0.92	1010	-9.77	<0.001
Male n(%)‡	443 (51.4%)	873 (51.7%)	1	0.005	0.94
APOE Genotypes n (%)					
*ε2/*ε2	6 (0.70%)	13 (0.77%)			
*ε3/*ε3	395 (45.82%)	1048 (62%)			
*ε4/*ε4	20 (2.32%)	29 (1.71%)			
*ε2/*ε3	70 (8.12%)	204 (12.07%)			
*ε2/*ε4	60 (6.96%)	0 (0%)			
*ε3/*ε4	137 (15.89%)	395 (23.37%)			

†Unpaired 2-tailed t-test. ‡Pearson's χ^2 2-tailed test.

4.2.2 Cognitive Assessment

All participants were assessed at baseline and at each subsequent interview for the following five cognitive abilities: perceptual speed was assessed using the Symbol Digit Modalities Test, which asks the participant to substitute as many digits for symbols as possible in 90s [371]; episodic memory was assessed using the immediate recall and delayed recall of the first trial of the California Verbal Learning Test, which involves recalling a list of 16 nouns [327]; working memory was assessed using the Digit Span Backward from the Wechsler Memory Scale, which presents participants with series of digits increasing in length at the rate of one digit per second and asks them to repeat the digits backwards [328]; and

vocabulary was assessed with the Spot-the-Word Test, which asks participants to choose the real words from 60 pairs of words and nonsense words [329] (Supplementary Tables 5 & 6).

4.2.3 Genotyping

Sixty-four single nucleotide polymorphisms (SNPs) were selected for genotyping based on previous associations with dementia, cognition, neuroanatomical differences and blood pressure (Table 2.3). Genomic DNA was extracted from cheek swabs (n = 4,597) using Qiagen DNA blood kits or from peripheral blood leukocytes (n = 64) using QIAamp DNA 96 DNA blood kits.

Pre-amplification of the targeted loci was performed using the TaqMan PreAmp Master Mix Kit (Life Technologies). Each reaction included 2.5µl TaqMan PreAmp Master Mix (2x), 1.25µl Pre-amplification Assay Pool, 0.5µl H_20 and 1.2µl genomic DNA. These reactions were incubated in a Biorad thermocycler for 10 min at 95°C, followed by 12 cycles of 95°C for 15 sec and 60°C for 4 min, and then incubated at 99.9°C for 10 minutes. The PreAmplified products were then held at 4°C until they were diluted 1:20 in 1x TE buffer and then stored at -20°C until use. 2.5µl diluted pre-amplified products was mixed with 2.5µl TaqMan OpenArray Master Mix. The resulting samples were dispensed using the OpenArray® AccuFill™ System onto OpenArray plates with each plate containing 48 samples and 64 SNP assays per sample. The QuantStudio™ 12K Flex instrument (Applied Biosystems, Carlsbad, California) was used to perform the real time PCR reactions on the loaded OpenArray plates. The fluorescence emission results were read using the OpenArray® SNP Genotyping Analysis software v1 (Applied Biosystems) and the genotyping analysis was performed using TaqMan® Genotyper v1.3, using the autocalling feature. Participant-specific quality controls included filters for genotype success rate (> 90%), genotype-derived gender concordant with reported gender and sample provenance error assessed via pairwise comparisons of genotype calls between all samples to identify samples with > 90% similarity. Samples that were flagged in the initial quality control checks were repeated, and those that still failed quality control were excluded. SNP-specific filters included genotype call rate (> 90%) and Hardy-Weinberg equilibrium (p > 0.001) assessed using an exact test with the PLINK toolkit [425].

For this study, data for 28 of the 64 genotyped SNPs was extracted based on *a priori* hypotheses (Table 4.1). These SNPs have being previously identified as being associated with dementia or cognition through GWAS or candidate gene studies (Table 2.3) and consist of 12 SNPs that have been highly replicated as being associated with LOAD and an additional 16 SNPs whose associations are ambiguous and are in need of further replication. Genotyping of the PATH sample for *APOE* variants was performed separately and has been described previously [382]. The SNPs were in Hardy-Weinberg equilibrium and genotype frequencies are presented in Supplementary Tables 3 & 4.

4.2.4 Data Preparation and Statistical Analysis

Data were analysed in the R Statistical Computing environment [426]. We created an index for episodic memory using the average scores of the immediate and delayed recall tasks. To allow for comparison across all cognitive tasks, the tests scores for each cognitive task at all three waves were transformed into Z scores (M = 0, SD = 1), using the baseline means and standard deviations. Higher test scores indicate better cognitive function.

Genetic dominance was assumed for previously reported risk alleles [427] for LOAD GWAS SNPs, and for minor alleles (alleles with the lowest frequency in the population) of the 16 additional SNPs. The *APOE* *ε4 and *ε2 alleles were assumed to be dominant to the *ε3 allele. For *APOE* participants were classified as either *APOE* *ε4+ (*ε4/*ε4 + *ε4/*ε3), *ε2+ (*ε2/*ε2 + *ε2/*ε3) or *ε3 (*ε3/*ε3). Because we wanted to assess the independent contributions of *ε4 and *ε2 to cognitive decline, and those with the *ε2/*ε4 genotype were excluded.

Three genetic risk scores [428] were calculated using the LOAD GWAS SNPs: 1) a simple count genetic risk (SC-GRS): the sum of all risk alleles across all loci; 2) an odds-ratio weighted genetic risk score (OR-GRS): the sum of all risk alleles across all loci, weighted by effect size of the risk allele on AD, as reported in the AlzGene Database [427]; 3) an explained variance-weighted genetic risk score (EV-GRS): the sum of all risk alleles across all loci, weighted by minor allele frequency and effect size on AD, as reported in the AlzGene Database. For all genetic risk scores a higher value indicates greater risk. The MAF and OR used to derived the GRS are presented in Table 4.1. Individuals missing any genetic data (n = 69) were excluded from the analysis.

Linear mixed effect models (LMM) with maximum likelihood estimation and subject-specific random slopes and intercepts were used to assess the effect of predictors on change in cognitive test scores over time. Age, centered on mean age at baseline, was used as an indicator of time in the study. The predictor variables included in the analysis were the individual SNPs or the three GRS's: SC-GRS, OR-GRS and EV-GRS. Covariates used in the models included sex, education and for individual SNP models *APOE* genotype. LMM's were estimated using the R package 'lme4' [429] and *F* and *p* values were estimated using Satterthwaite-type approximation to determine the statistical significance of the fixed effects. To evaluate if the random slopes were significantly different from 0 and to determine if there was residual variability in the rate of change that could be explained by predictor variables, LMM's that included random slopes were compared to models that did not include random slopes using parametric bootstrap methods where 1000 simulations of the likelihood ratio test statistic were generated (R package 'pbkrtest', [430]. For each SNP and GRS we compared the model fit of the full model with the covariates-only model to evaluate if there was an overall effect of the SNP or GRS on cognitive decline. Model fit was assessed using a Kenward-Rodger approximation for *F*-tests (R package 'pbkrtest', [430]. Two R^2 statistics were calculated to quantify 1) the proportion of outcome variation explained by the fixed factors (marginal R^2) and 2) the amount of outcome variation explained by the fixed and random factors (conditional R^2; [431,432]; R package 'MuMIn' [433].

Additionally, we performed a secondary analysis in which changes in the rate of cognitive decline by genotype were estimated separately for participants who were classified as cognitive impaired (CI) at wave 3 if they scored <=27 on the MMSE (n = 118) and those classified as cognitively normal (CN, n = 1340). For the secondary analysis, LMM's were performed with the inclusion of the additional terms for a time by cognitive status and separate time by genotype interactions for the CI and CN classifications.

We did not adjust for multiple comparison as strong *a priori* evidence for all our hypothesis based on previous findings for LOAD and cognitive decline was available; a *p*-value < 0.05 was considered statistically significant.

4.3 Results

4.3.1 Population Characteristics of the PATH Cohort

General demographics of the PATH cohort are presented in Table 4.2. Linear Mixed Models 1-3 in Supplementary Table 7-9 show the average rate of change for each cognitive test. Random slopes for all cognitive tests scores were significantly different from 0, indicating that there was sufficient variability in the rate of change between participants thus allowing potential genetic predictors of this change to be tested (bootstrap *p*-value: Episodic Memory = 0.04; Digits backwards = 0.01; Spot-the-Word test = 0.0001; Symbol digits modalities test = 0.01). Significant change in test scores over time was observed for all cognitive tests except Digits Backwards. In model 2, participants experienced an overall decline in test scores for Episodic memory and Symbol digits Modalities Test, and an increase in test scores for Spot-the-Word. 'Time' explained 57-89% of outcome variation for the entire model. The covariates in model 3 improved the model fit for all cognitive tests and explained 7-21% of the outcome variation in the fixed effects, although they did not explain any additional random effect variation for the entire model (Supplementary Table 9).

4.3.2 Main Effects of LOAD GWAS SNPs

There was a significant improvement in model fit for various cognitive tests after the introduction of the *APOE*, *ABCA7*, *CR1* and *MS4A4E* SNPs into their respective models. *APOE* ε4+ was associated with a greater rate of decline in Episodic Memory and the association remained unchanged when *APOE* ε3/ε4 heterozygotes were assessed separately from *APOE* ε4 homozygotes and *APOE* ε2 carriers; *ABCA7*-rs3764650-G was associated with a lower initial status at baseline in Episodic memory test scores; *CR1*-rs3818361-A was associated with a greater rate of decline in Episodic memory and; *MS4A4E*-rs670139-T was associated with a higher baseline Spot-the-Word Test score and a slower decline in Episodic Memory test scores. The group differences resulted in a small increase in the marginal R^2 ranging from 0.001 to 0.002, though there was no increase in the conditional R^2 statistics. Table 4.3, Supplementary Tables 10-21.

The remaining SNPs (*BIN1*, *CD2AP*, *CD33*, *CLU*, *EPHA1*, *MS4A4A*, *MS4A6A* and *PICALM*) were not significantly associated with baseline status or rate of change for any of cognitive tests.

In the secondary analysis assessing the rate of cognitive decline separately for participants who were classified as CI (supplementary Table 44), the *APOE* ε4+ was associated with a faster rate of decline in Episodic memory for CI and CN participants, with a steeper decline observed in CI participants, and a reduced rate of decline in Digits Backwards test scores in CN participants; *ABCA7*-rs3764650-G was associated with a faster rate of decline in Digits backwards tests scores in CI participants and; *EPHA1*-rs11767557-T was associated with a faster rate of decline SDMT tests in CI participants.

4.3.3 *Effect of Genetic Risk Scores*

The equally weighted SC-GRS has an approximately normal distribution in the PATH Cohort (Figure 4.1); mean = 10.5; range = 3-18). The bimodal distribution and long upper tails of the weighted OR- and EV-GRS reflect the strong effect of *APOE* relative to other loci (Figure 4.1); mean = 1.47; range = -0.7-4.5 & mean = 0.92; range = -0.1-2.4 respectively).

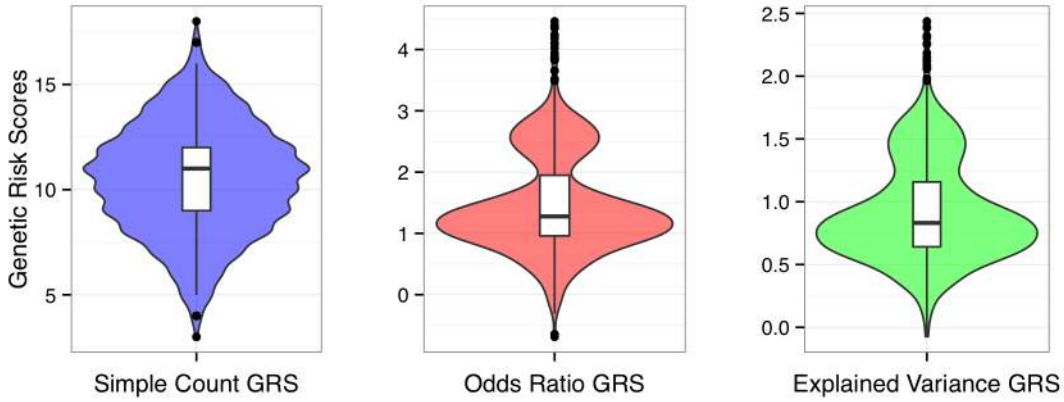

Figure 4.1: *Distributions of the three genetic risk scores: SC-GRS (Mean = 10.5; sd = 2.58), OR-GRS (Mean = 1.47; sd = 0.8) and EV-GRS (Mean = 0.92; sd = 0.4). The variable widths of each violin plot indicate the probability density of the data at each score, with the box plots indicating the first, median and third quartile.*

The SC-GRS was not significantly associated with either initial status at baseline or rate of change for any of the cognitive tests. There was a significant improvement in model fit for Episodic memory for both the OR- and EV-GRS, with

higher OR- and EV-GRS being associated with a greater rate of decline in cognitive performance. These associations resulted in a small increase in the amount of explained variation in the fixed effects, in comparison to that explained by time and the covariates, of 0.001 and 0.002 for the OR-GRS and EV-GRS respectively though there was no increase in the conditional R^2 statistics. (Table 4.3, Supplementary Tables 22-24). The OR- and EV-GRS were not associated with cognitive performance when the *APOE* allele was excluded (Supplementary Tables 25-27).

In the secondary analysis (supplementary Table 44), the OR- and EV-GRS were associated with a faster rate of decline in episodic memory in both CI and CN participants, with a steeper decline observed in CI participants.

4.3.4 Main Effects of SNPs Associated with Dementia or Cognition

Statistics for the models introducing the additional dementia and cognition SNPs are shown in Table 4.4. See Supplementary Tables 28-43 for full models with random and fixed effects.

A significant improvement in model fit was observed for a number of cognitive tests after the introduction of the following SNPs: *BDNF*, *COMT*, *FRMD4A*-rs7081208, Intergenic chrX, *PDE7A* and *ZNF224* into their respective models. In these models significant parameter estimates were observed. *BDNF*-rs6265-T was associated with lower baseline Digits Backwards test scores while *COMT*-rs4680-A was associated with a greater rate of decline in Episodic memory test scores. *FRMD4A*-rs7081208-A was associated with a lower baseline score as well as a slower rate of decline in Digits Backwards test scores and with higher baseline scores, but a greater rate of decline in Spot-the-Word test scores. Intergenic-rs12007229-A was associated with a greater rate of decline in Episodic memory test scores. *PDE7A*-rs10808746-A was associated with a slower rate of decline in Symbol Digits Modalities test scores; *ZNF224*-rs3746319-A was associated with higher baseline Spot-the-Word Test scores.

Statistically significant parameter estimates in the absence of improvement in model fit were also observed, with *CTNNBL1*-rs6125962-C associated with a reduced rate of decline in Episodic memory test scores; *FRMD4A*-rs17314229-T was associated with a greater rate of decline in digits backwards test scores; *PDE7A*-rs10808746-A was associated with lower Digits Backwards test baseline

Table 4.3: *Top LOAD risk SNPs and GRS: Parameter estimates and model fit statistics for SNP/GRS main effects*

		Episodic Memory	**Digits Backwards**	**Spot-the-Word**	**Symbol Digits Modalities Test**
		Estimate (SE)	**Estimate (SE)**	**Estimate (SE)**	**Estimate (SE)**
APOE ε2	Intercept	-0.02 (0.07)	0.01 (0.07)	0.06 (0.06)	-0.04 (0.06)
	Slope	-0.00 (0.01)	0.01 (0.01)	-0.00 (0.00)	0.01 (0.01)
APOE ε4	Intercept	0.03 (0.05)	0.02 (0.05)	0.02 (0.05)	-0.01 (0.05)
	Slope	-0.02 (0.01)**	0.01 (0.01)	-0.00 (0.00)	-0.01 (0.00)
	F -test	2.61*	1.78	0.3	0.8
ABCA7-rs3764650	Intercept	-0.12 (0.06)*	0.02 (0.06)	-0.05 (0.05)	-0.04 (0.06)
	Slope	-0.0003 (0.007)	0.004 (0.007)	-0.0002 (0.004)	0.01 (0.01)
	F-test	2.93	0.46	0.59	0.84
BIN1-rs744373	Intercept	-0.03 (0.04)	0.07 (0.04)	-0.00 (0.04)	0.04 (0.04)
	Slope	-0.006 (0.005)	0.0002 (0.005)	-0.002 (0.003)	-0.001 (0.004)
	F-test	1.71	1.78	0.18	0.49
CD2AP-rs9296559	Intercept	0.02 (0.04)	0.03 (0.05)	-0.02 (0.04)	0.004 (0.04)
	Slope	-0.006 (0.006)	-0.001 (0.005)	-0.003 (0.003)	-0.001 (0.004)
	F-test	0.56	0.27	0.91	0.03
CD33 -rs34813869	Intercept	-0.02 (0.07)	-0.05 (0.07)	-0.07 (0.06)	0.06 (0.07)
	Slope	0.0004 (0.009)	-0.009 (0.008)	0.003 (0.005)	-0.003 (0.006)
	F-test	0.08	1.51	0.66	0.48
CLU-rs11136000	Intercept	0.03 (0.06)	0.07 (0.06)	-0.005 (0.05)	0.05 (0.06)
	Slope	0.0004 (0.007)	-0.001 (0.007)	0.003 (0.004)	0.004 (0.005)
	F-test	0.25	0.75	0.41	0.89
CR1-rs3818361	Intercept	-0.03 (0.05)	0.01 (0.05)	-0.06 (0.04)	-0.05 (0.04)
	Slope	-0.01 (0.01)	-0.01 (0.01)	0.001 (0.003)	-0.0001 (0.004)
	F-test	3.46*	0.49	1.22	0.65
EPHA1-rs11767557	Intercept	0.07 (0.12)	0.08 (0.12)	-0.13 (0.11)	-0.20 (0.11)
	Slope	-0.01 (0.01)	-0.02 (0.01)	0.01 (0.01)	0.01 (0.01)
	F-test	0.37	0.57	0.76	1.54
MS4A4A-rs4938933	Intercept	-0.06 (0.06)	-0.04 (0.06)	0.10 (0.05)	0.06 (0.06)
	Slope	0.01 (0.01)	0.01 (0.01)	0.002 (0.004)	-0.004 (0.005)
	F-test	1.2	1.28	2.9	0.68

Table 4.3 *(Continued)*

		Episodic Memory	**Digits Backwards**	**Spot-the-Word**	**Symbol Digits Modalities Test**
		Estimate (SE)	**Estimate (SE)**	**Estimate (SE)**	**Estimate (SE)**
MS4A4E-rs670139	Intercept	-0.06 (0.04)	-0.04 (0.05)	0.11 (0.04)**	0.08 (0.04)
	Slope	0.01 (0.01)*	0.01 (0.01)	0.0007 (0.003)	-0.0002 (0.004)
	F-test	3.10*	0.73	4.57*	1.71
MS4A6A-rs610932	Intercept	-0.09 (0.06)	-0.03 (0.06)	0.04 (0.05)	0.03 (0.05)
	Slope	0.01 (0.01)	0.01 (0.01)	0.006 (0.004)	-0.002 (0.005)
	F-test	1.64	1.39	2.12	0.17
PICALM-rs3851179	Intercept	0.01 (0.06)	-0.03 (0.06)	-0.02 (0.06)	0.01 (0.06)
	Slope	0.0001 (0.008)	0.006 (0.007)	0.003 (0.004)	0.009 (0.006)
	F-test	0.01	0.35	0.19	1.41
SC-GRS	Intercept	-0.01 (0.009)	-0.001 (0.009)	0.001 (0.008)	0.008 (0.008)
	Slope	-0.001 (0.001)	0.0006 (0.001)	-0.0005 (0.0006)	0.0002 (0.0008)
	F-test	1.95	0.18	0.35	0.53
OR-GRS	Intercept	-0.00001 (0.03)	-0.003 (0.03)	-0.001 (0.02)	0.007 (0.03)
	Slope	-0.009 (0.003)*	0.002 (0.003)	-0.002 (0.002)	-0.004 (0.003)
	F-test	4.20*	0.13	0.58	1.01
EV-GRS	Intercept	-0.007 (0.05)	-0.007 (0.06)	0.004 (0.05)	0.02 (0.05)
	Slope	-0.017 (0.007)*	0.004 (0.007)	-0.004 (0.004)	-0.006 (0.005)
	F-test	4.44*	0.2	0.64	0.75

*p < .05; **p < .01.; adjusted for Sex, APOE and Education

Table 4.4: *Additional SNPs: Parameter estimates and model fit statistics for SNP main effects*

		Episodic Memory	Digits Backwards	Spot-the-Word	Symbol Digits Modalities Test
		Estimate (SE)	**Estimate (SE)**	**Estimate (SE)**	**Estimate (SE)**
BDNF-rs6265	Intercept	0.002 (0.05)	0.1 (0.05)*	0.02 (0.04)	0.05 (0.05)
	Slope	0.008 (0.006)	0.003 (0.006)	0.005 (0.003)	0.001 (0.004)
	F -test	1.31	3.25*	1.75	0.64
CETP -rs5882	Intercept	0.005 (0.043)	0.053 (0.045)	0.007 (0.039)	0.003 (0.042)
	Slope	0.0002 (0.005)	0.01 (0.005)	0.005 (0.003)	0.002 (0.004)
	F -test	0.01	1.77	1.77	0.13
COMT-rs4680	Intercept	0.078 (0.050)	0.032 (0.052)	0.033 (0.045)	0.005 (0.049)
	Slope	0.015 (0.006)*	0.003 (0.006)	0.004 (0.004)	0.009 (0.005)
	F -test	3.09*	0.22	1.57	2.09
CTNNBL1-rs6125962	Intercept	0.054 (0.067)	0.017 (0.07)	0.024 (0.061)	0.077 (0.065)
	Slope	0.018 (0.008)*	0.010 (0.008)	0.002 (0.005)	0.006 (0.006)
	F -test	2.27*	0.89	0.13	0.9
FRMD4A-rs17314229	Intercept	0.022 (0.043)	0.04 (0.045)	0.037 (0.039)	0.006 (0.042)
	Slope	0.007 (0.005)	0.010 (0.005)*	0.005 (0.003)	0.001 (0.004)
	F -test	0.75	1.96*	1.33	0.04
FRMD4A-rs7081208	Intercept	0.001 (0.064)	0.142 (0.067)*	0.135 (0.058)*	0.102 (0.062)
	Slope	0.003 (0.008)	0.022 (0.008)**	0.01 (0.005)*	0.001 (0.006)
	F -test	0.12	4.29*	3.63*	1.39
Intergenic-rs12007229	Intercept	0.031 (0.074)	0.133 (0.079)	0.055 (0.068)	0.016 (0.073)
	Slope	0.023 (0.009)*	0.002 (0.009)	0.004 (0.005)	0.002 (0.007)
	F -test	3.56*	1.64	0.44	0.13
LGALS3-rs4644	Intercept	0.055 (0.045)	0.007 (0.047)	0.031 (0.041)	0.069 (0.044)
	Slope	0.0003 (0.006)	0.007 (0.005)	0.004 (0.003)	0.004 (0.004)
	F -test	0.93	1.08	0.79	1.38
MMP12-rs12808148	Intercept	0.002 (0.048)	0.012 (0.05)	0.007 (0.043)	0.063 (0.047)
	Slope	0.006 (0.006)	0.010 (0.006)	0.001 (0.003)	0.004 (0.004)
	F -test	0.67	1.55	0.03	0.98
MTHFD1L-rs11754661	Intercept	0.025 (0.058)	0.043 (0.061)	0.014 (0.053)	0.033 (0.057)
	Slope	0.001 (0.007)	0.002 (0.007)	0.005 (0.004)	0.005 (0.005)

Table 4.4 *(Continued)*

		Episodic Memory	**Digits Backwards**	**Spot-the-Word**	**Symbol Digits Modalities Test**
		Estimate (SE)	**Estimate (SE)**	**Estimate (SE)**	**Estimate (SE)**
	F -test	0.1	0.44	0.63	0.53
PAICS-rs11549976	Intercept	0.029 (0.066)	0.078 (0.069)	0.070 (0.060)	0.084 (0.064)
	Slope	0.009 (0.008)	0.006 (0.008)	0.004 (0.005)	0.0004 (0.006)
	F -test	0.54	0.66	0.75	0.93
PDE7A-rs10808746	Intercept	0.039 (0.046)	0.103 (0.048)	0.032 (0.042)	0.077 (0.045)
	Slope	0.006 (0.006)	0.010 (0.006)	0.000 (0.003)	0.009 (0.004)*
	F -test	0.69	2.6	0.35	2.76
SNTG1-rs16914781	Intercept	0.086 (0.046)	0.038 (0.048)	0.048 (0.042)	0.008 (0.045)
	Slope	0.008 (0.006)	0.002 (0.006)	0.0002 (0.003)	0.001 (0.004)
	F -test	1.94	0.31	0.74	0.06
SORL1-rs668387	Intercept	0.046 (0.047)	0.006 (0.049)	0.084 (0.043)*	0.010 (0.046)
	Slope	0.007 (0.006)	0.007 (0.006)	0.006 (0.003)	0.005 (0.004)
	F -test	0.85	0.94	2.73	0.92
SPON1-rs11023139	Intercept	0.058 (0.069)	0.093 (0.072)	0.104 (0.063)	0.009 (0.067)
	Slope	0.009 (0.009)	0.007 (0.008)	0.007 (0.005)	0.004 (0.006)
	F -test	0.62	0.88	1.69	0.17
ZNF224-rs3746319	Intercept	0.012 (0.046)	0.071 (0.049)	0.100 (0.042)*	0.047 (0.045)
	Slope	0.002 (0.006)	0.007 (0.006)	0.001 (0.003)	0.005 (0.004)
	F -test	0.06	3.43*	3.04*	1.81

*p < .05; **p < .01.; adjusted for Sex, APOE and Education

scores and a slower rate of decline in Symbol Digits Modalities test scores; *SORL1*-rs668387-T was associated with higher Spot-the-Word Test scores at baseline. These significant associations result in a small increase in explained variation in the fixed effects ranging from 0.0003 to 0.003, though no increase in the conditional R^2.

Results of the secondary analysis assessing the rate of cognitive decline separately for participants who were classified as CI or CN are presented in supplementary Table 45. *BDNF*-rs6265-T, *CETP*-rs5882-G, *MTHFD1L*-rs11754661-A, *CTNNBL1*-rs6125962-C, *FRMD4A*-rs17314229-T, *PAICS*-rs11549976-A and *PED7A*-rs10808746-A were associated with rate of change in participants classified as CI. *COMT*-rs4680-A, *FRMD4A*-rs7081208-A and Intergenic-rs12007229-A were associated with rate of change in participants who were classified as CN.

4.4 Discussion

In this study we investigated the association between common genetic variants that have been previously reported to be associated with LOAD, dementia or cognition with change in episodic memory, working memory, vocabulary and perceptual speed. The top LOAD GWAS SNPs were primarily associated with cognitive performance in episodic memory. This is likely indicative of their role in Alzheimer's disease, as progressive deficits in episodic memory that begin early on in the disease course are one of its defining features [434]. Associations with rate of change in cognitive performance were observed for *APOE*, *CR1*, *MS4A4E*, while *ABCA7* was associated with baseline cognitive performance. The direction of the effect for *APOE*, *CR1*, and *ABCA7* was as expected, however for the *MS4A4E* the AD risk allele was associated with a protective effect on episodic memory, and the same trend was also observed for the SNPs *MS4A6A* and *MS4A4A*, though they were not significant. However, the parameter estimates for the effect of these SNPs on change in cognitive abilities ranged from -0.9% to 0.9% over four years, while the increase in the marginal R^2 statistics after inclusion of the genetic predictors ranged from 0.001 to 0.002, emphasising that the effect of individual SNPs on cognition are extremely small.

Additionally, we constructed three genetic risk scores to investigate the combined effect of the LOAD risk SNPs on cognitive decline. An odds ratio

weighted GRS and a novel combined odds ratio and minor allele frequency weighted GRS were significantly associated with steeper rate of cognitive decline in episodic memory. The EV-GRS takes into account that within the same OR disease risk can vary depending on the risk allele frequencies and has been shown to be a more robust approach for identifying associations in the presence of potential genetic interactions, linkage disequilibrium and false positive predictors [428]. An unweighted GRS was not associated with cognitive performance. This latter score utilised a simple count of the number of risk alleles per individual and does not take into account the varying effect sizes among the LOAD risk SNPs. The lack of significant associations with the SC-GRS, in contrast to the weighted methods, indicates that the significant associations observed for the OR- and EV-GRS can be attributed to the dominant role of the *APOE* *ε4 allele, which was further confirmed when *APOE* was excluded from the GRS.

These results are similar to those of several comparable candidate gene [118,120] and GWAS based [115,121] studies, that reported a lack of robust associations between cognitive decline and non-*APOE* LOAD risk genes. These previous studies have only identified suggestive evidence for *CR1* [116,124], *CLU* [129], *BIN1* [124] and *PICALM* [115]. However, when examining genetic associations with general cognitive function in middle and older age in a large meta-analysis of 31 studies (n = 53,949) no associations were observed with any of these LOAD risk genes, though two other AD related genes, *MEF2C* and *ABCG1*, were associated [122]. Furthermore, when the non *APOE* LOAD GWAS risk loci were assessed collectively as a GRS weighted by their estimated OR's, no associations were observed with cognitive decline [120], though after inclusion of *APOE* the GRS did reach significance [120,124]. An alternative approach using a polygenic risk score of all LOAD associated variants, not just the top associated loci, found no association with cognitive ability in later life or with age-related cognitive change [212]. Investigating interactions between environmental and lifestyle factors and GRS may provide more promising results. A higher GRS composed of *APOE*, *CLU*, *CR1* and *PICALM*, while not independently associated with cognitive decline was shown to exacerbate the deleterious effects of type 2 diabetes on cognitive decline [306].

Alzheimer's related genes may be associated with cognitive decline in subjects who are in the preclinical stages of dementia and who, if followed for long

enough, might eventually develop dementia. We also assessed the effect of the LOAD risk SNPs separately for those who were classified as cognitively impaired according to MMSE at wave 3 and observed faster rates of decline associated with the *ABCA7* risk allele and reduced rates of decline associated with the *EPHA1* risk allele. In previous studies, variants in *ABCA7*, *EPHA1* [120] and *CLU* [127] have been observed to be associated with cognitive decline in subjects who eventually converted to dementia, but not in individuals who remained cognitively normal throughout the study. This also suggests that associations observed between LOAD risk genes and cognitive decline could be due to individuals who are in the preclinical stages of Alzheimer's disease and that the retrospective removal of these individuals could attenuate the observed associations [122,309]. However, removing individuals who are in the preclinical stages of disease in the early analytic stages of a study is likely to be difficult due to the long and asymptomatic nature of the preclinical stages of LOAD.

The above findings, and those of previous studies, suggest that the added predictive value of the top LOAD SNPs for cognitive decline in non-demented individuals may be limited. This is consistent with polygenic models of cognitive decline indicating that there is a large number of variants with modest effects sizes rather than a few variants with large or moderate effect sizes. Additionally, this is consistent with indices of LOAD pathology amyloid and neurofibrillary tangles that only explain ~30% of observed variance in cognitive decline, and cerebrovascular (macro and micro infarcts) and Lewy body disease neuropathologies explaining an additional ~10% of variation [91]. This is consistent with the notion that while LOAD pathology is important in the development of cognitive decline, it occurs in conjunction with other pathological features that are observed in brain ageing. As such, the cognitive deficits observed in brain ageing are unlikely to be due to an isolated pathological feature, but the interaction between multiple neuropathologies [435]. This highlights the need to investigate additional genetic variants in addition to those associated with AD.

For the remaining 16 SNPs investigated, which were previously associated with dementia or cognitive performance, we observed associations with an increased rate of decline in cognitive performance for the minor alleles of *COMT* and Intergenic chrX, while for *CTNNBL1* and *PDE7A* the minor alleles were associated with a reduced rate of cognitive decline. At baseline, *BDNF* and *PDE7A*

minor alleles were associated with worse cognitive performance, while *SORL1* and *ZNF224* minor alleles were associated with better cognitive performance. *FRMD4A*-rs7081208 was associated with a reduced and a greater rate of decline for working memory and vocabulary respectively, though at baseline working memory was associated with worse performance while vocabulary was associated with better performance. Additionally, *FRMD4A*-rs17314229 minor allele was associated with a greater rate of decline in working memory.

In comparison to the top AD related SNPs, the additional AD related genetic variants in *FRMD4A*, *SORL1* and *ZNF224* were associated with cognitive performance in vocabulary and working memory, potentially indicating that they may be involved in the development of atypical AD, in which the development of non-amnestic cognitive deficits occurs early on in the disease process [434]. For the cognition related genetic variants, SNPs in *COMT* and *CTNNBL1* have been associated with differences in regional brain structures and activations that are involved in episodic memory processes, potentially explaining the differential associations of these variants with episodic memory [348,436]. *BDNF* is widely expressed in the prefrontal cortex, which is associated, amongst other functions, with working memory [437]. As with the LOAD risk loci, the additional SNPs were primarily associated with cognitive decline in participants who were classified cognitively impaired. However, as with the LOAD risk loci, the effect sizes for these SNPs were small and inclusion of the SNPs in the model resulted in a negligible increase in the amount of explained variability in cognitive performance.

The presented findings should be interpreted in conjunction with some study limitations. The sample used in this study is somewhat better educated than the population from which it was drawn. Higher education is associated with a reduced risk of cognitive decline and incident dementia. Additionally, the sample is relatively young, which in combination with a higher level of education could limit our ability to detect an effect of the genetic factor with cognitive decline. This is possibly reflected in the limited person specific variation in the rate of decline in the linear mixed models. Second, the subjects in this study are Caucasian and as such our findings need to be replicated in other ethnic groups. Third, despite excluding individuals with probable dementia at each wave, it is still possible that individuals in the preclinical phase of dementia were included in the analysis. Fourth, in concordance with the available data, we have specified time as linear,

however, cognitive decline may accelerate at older ages [293] highlighting the need to investigate nonlinear cognitive trajectories [438]. Finally, although we have a strong *a priori* evidence for all our hypotheses, it should be noted that correcting for multiple testing using Bonferroni correction, all corrected *p*-values would have yielded non-significant results.

Despite these limitations however, this study investigated a large community based cohort followed longitudinally for a period of eight years, with three waves of assessment that included a comprehensive cognitive assessment of different cognitive abilities. These strengths allow for a robust statistical inference about the effect the selected genetic factors have on non-clinical cognitive decline. The narrow age-cohort design also reduced the impact of age-differences influencing results.

To conclude, our findings suggest that the majority of LOAD risk genes are not individually associated with non-clinical cognitive decline in a cohort of older adults who were followed for a period of 8 years. When considered collectively as a genetic risk score, the observed associations are due to the significantly larger weight associated with *APOE* *ε4 allele. The PATH study is ongoing and the number of incident cases of mild cognitive impairment and dementia among participants is increasing. The work presented here thus provides an excellent basis for further investigating the effects of AD risk variants in non-pathological versus pathological decline [309], gene-gene interactions [118,126] and gene-environment interactions [307] in future studies.

Supplementary Data

Supplementary data is available at:
http://dx.doi.org/10.1016/j.neurobiolaging.2016.02.016

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Chapter 5: Late Onset Alzheimer's disease risk variants in cognitive decline: The PATH Through Life Study

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Abstract

Recent genome wide association studies have identified a number of single nucleotide polymorphisms associated with late onset Alzheimer's disease. We examined the associations of 24 LOAD risk loci, individually and collectively as a genetic risk score, with cognitive function. We used data from 1,626 non-demented older Australians of European ancestry who were examined up to four times over 12 years on tests assessing episodic memory, working memory, vocabulary and information processing speed. Linear mixed models were generated to examine associations between genetic factors and cognitive performance. Twelve SNPs were significantly associated with baseline cognitive performance (*ABCA7, MS4A4E, SORL1*), linear rate of change (*APOE, ABCA7, INPP5D, ZCWPW1, CELF1, EPHA1*) or quadratic rate of change (*APOE, CLU, FERMT2*). In addition, a weighted GRS was associated with linear rate of change in episodic memory and information processing speed. Our results suggest that a minority of AD related SNPs may be associated with non-clinical cognitive decline. Further research is required to verify these results and to examine the effect of preclinical AD in genetic association studies of cognitive decline. The identification of LOAD risk loci associated with non-clinical cognitive performance may help in screening for individuals at greater risk of cognitive decline.

Keywords: Alzheimer's disease; Cognitive aging; SNPs; Genetic risk scores; Genetic Epidemiology; Longitudinal Studies

5.1 Introduction

Late onset Alzheimer's disease (LOAD), in which patients show clinical symptoms >65 years of age, is the most common form of dementia and the number of individuals with LOAD is expected to triple by 2050 [25]. LOAD has a long preclinical phase that commences decades before the onset of clinical symptoms, which are characterised by progressive degeneration of brain structure and chemistry resulting in gradual cognitive and functional decline [84]. The neuropathological hallmarks of LOAD are aggregation and accumulation of extracellular Amyloid-β peptides into amyloid plaques and accumulation of intraneuronal hyperphosphorylated and misfolded tau into neurofibrillary tangles. Accumulation of amyloid plaques and neurofibrillary tangles prompt the pathogenesis of AD by promoting alterations in lipid metabolism, neuro-inflammation, endocytosis and synaptic dysfunction and loss that ultimately leads to neuronal cell death [28,29].

LOAD has a large genetic component, with the heritability estimated to be 60-80% [439]. *Apolipoprotein* (*APOE*) *epsilon 4* (*ε4) was the first common genetic variant to be identified [107] and remains the strongest genetic predictor of LOAD. Beyond *APOE,* recent genome-wide association studies (GWAS) and a meta-analysis by the International Genomics of Alzheimer's Project (IGAP) have identified single nucleotide polymorphisms (SNPs) at 23 loci associated with LOAD (*ABCA7*, *BIN1*, *CD2AP*, *CD33*, *CLU*, *CR1*, *EPHA1*, *MS4A4A*, *MS4A4E*, *MS4A6A, PICALM, HLA-DRB5, PTK2B, SORL1, SLC24A4-RIN3, DSG2, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERMT2* and *CASS4*; [108-113]).

The identified LOAD risk loci are clustered in biological pathways that play an important role in disease onset and progression [440] and are involved in the accumulation of the pathological features of LOAD [441]. Furthermore, post-mortem analysis suggests that the neuropathological hallmarks of LOAD progress to varying degrees in individuals without dementia and are associated with cognitive status and nonclinical cognitive decline [90,97]. LOAD risk genes are, therefore, good candidates for investigating potential genetic associations with cognitive performance and decline. How these loci affect normal cognitive function may inform how they influence LOAD onset and progression.

This cross-over effect is exemplified by *APOE*, which is associated with LOAD and has effects on episodic memory, perceptual speed, executive functioning

and global cognitive ability [145,377] mediated predominantly by amyloid-β plaques [92]. Association with cognitive decline of the first 11 LOAD risk loci identified by genome-wide association studies (GWAS) are inconsistent [115,116,118,120,121,124,129,442]. Whether the new risk loci identified by IGAP are associated with cognitive decline has yet to be extensively investigated [117,122,123].

Here, we report associations of the 24 most significant LOAD risk loci with longitudinal change in cognitive performance (based on four neuropsychological outcomes) over 12 years in 1,626 community dwelling older adults. We investigate whether these loci are associated, either individually or collectively, as genetic risk scores (GRS), with: average differences in cognitive performance; rate of cognitive decline; and acceleration of the rate of decline over time.

5.2 Methods

5.2.1 Participants

Participants of this study are community dwelling older adults who were recruited into the Personality and Total Health (PATH) Through Life Project, a longitudinal study of health and wellbeing. Participants in PATH were sampled randomly from the electoral rolls of Canberra and the neighbouring town of Queanbeyan into one of three cohorts based on age at baseline, the 20+ (20-24), 40+ (40-44) and 60+ (60-64) cohorts. Participants are assessed at 4-year intervals, and data from 4 waves of assessment are available. The background and test procedures for PATH have been described in detail elsewhere [322]. Written informed consent for participation in the PATH project was obtained from all participants according to the 'National Statement' guidelines of the National Health and Medical Research Council of Australia and following a protocol approved by the Human Research Ethics Committee of The Australian National University.

In this study, data for the 60+ cohort were used, with interviews conducted in 2001-2002 (n = 2,551), 2005-2006 (n = 2,222), 2009-2010 (n = 1,973), and 2014-2015 (n = 1645), for a total of 12 years of follow-up. Individuals were excluded from analysis based on the following criteria: attendance at only 1 wave (n=309); no available genomic DNA (n = 60); *APOE* ε2/ε4 genotype (n = 60, to avoid conflation of the ε2 protective and ε4 risk affect); non-European ancestry (n = 110); probable dementia at any wave (MMSE < 27 was used due to the high

educational level in PATH [443]; n=269); self-reported medical history of epilepsy, brain tumours or infections, stroke and transient ischemic attacks (n = 450). Missing values in "Education" (total number of years of education, n = 128) were imputed using the 'missForest' package in R [424]. This left a final sample of 1,526 individuals at baseline.

5.2.2 Cognitive Assessment

All participants were assessed at baseline and at each subsequent interview for the following four cognitive abilities: perceptual speed was assessed using the Symbol Digit Modalities Test, which asks the participant to substitute as many digits for symbols as possible in 90 seconds [371]; episodic memory was assessed using the Immediate Recall of the first trial of the California Verbal Learning Test, which involves recalling a list of 16 nouns [327]; working memory was assessed using the Digit Span Backward from the Wechsler Memory Scale, which presents participants with series of digits increasing in length at the rate of one digit per second and asks them to repeat the digits backwards [328]; and vocabulary was assessed with the Spot-the-Word Test, which asks participants to choose the real words from 60 pairs of words and nonsense words [329] Raw cognitive test scores at each wave and Pearson's correlation between test scores are presented in Supplementary Tables 1 & 2.

5.2.3 Genotyping

For this study, we used genotype data for 25 SNPs that have been associated with LOAD (Table 5.1). Genotyping of 11 GWAS LOAD risk SNPs (in the following loci: *ABCA7, BIN1, CD2AP, CD33, CLU, CR1, EPHA1, MS4A4A, MS4A4E, MS4A6A and PICALM*) using TaqMan OpenArray assays has been reported previously [442]. In this study 16 SNPs were selected for genotyping. These included the 12 LOAD GWAS SNPs, which were identified in a meta-analysis of the previous GWAS studies performed by IGAP (in the following loci: *HLA-DRB5, PTK2B, SORL1, SLC24A4-RIN3, DSG2, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERMT2* and *CASS4;* [113]). Three were associated with general cognitive function (MIR2113-rs10457441, AKAP6-rs17522122, TOMM40-rs10119; [122]. One was associated as a haplotype with LOAD (FRMD4A-rs2446581; [350]). We used proxy

SNPs that were in LD with four (*HLA-DRB5/HLA-DRB1*-rs9271192 [r^2 = 1], *MEF2C*-rs190982 [r^2 = 0.89], *CELF1*-rs10838725 [r^2 = 0.99] and *CASS4*-rs7274581 [r^2 = 0.99]) of the SNPs reported by IGAP, as Taqman assays were unavailable [113].

Table 5.1: LOAD risk SNPs used in this study

Gene	SNP	Chromosome	Alleles*	MAF†	OR‡
APOE ε4	rs429358/rs7412	19	*ε2/ε3/ε4*	0.8/0.14	0.54/3.81
ABCA7	rs3764650	19	T/G	0.11	1.23
BIN1	rs744373	2	A/G	0.31	1.17
CD2AP	rs9296559	6	T/C	0.27	1.11
CD33	rs34813869	19	A/G	0.3	0.89
CLU	rs11136000	8	C/T	0.35	0.88
CR1	rs3818361	1	G/A	0.26	1.17
EPHA1	rs11767557	7	T/C	0.2	0.89
MS4A4A	rs4938933	11	T/C	0.5	0.88
MS4A4E	rs670139	11	G/T	0.34	1.08
MS4A6A	rs610932	11	T/G	0.45	0.90
PICALM	rs3851179	11	C/T	0.41	0.88
HLA-DRB5	rs9271100	6	C/T	0.31	1.11
PTK2B	rs28834970	8	T/C	0.32	1.10
SORL1	rs11218343	11	T/C	0.03	0.77
SLC24A4-RIN3	rs10498633	14	G/T	0.19	0.91
DSG2	rs8093731	18	*C/T*	0.01	0.73
INPP5D	rs35349669	2	C/T	0.44	1.08
MEF2C	rs304132	5	G/A	0.46	0.93
NME8	rs2718058	7	A/G	0.36	0.93
ZCWPW1	rs1476679	7	T/C	0.32	0.91
CELF1	rs7933019	11	G/C	0.34	1.08
FERMT2	rs17125944	14	T/C	0.08	1.14
CASS4	rs7274581	20	T/C	0.11	0.88

*Major/Minor Allele; †Minor Allele Frequency: HapMap-CEU; ‡OR for minor allele reported by Alzegene or IGAP [113]

Genomic DNA was extracted from cheek swabs (n = 2,192) using Qiagen DNA kits or from peripheral blood leukocytes (n = 101) using QIAamp 96 DNA blood kits. Pre-amplification of the targeted loci was performed using the TaqMan PreAmp Master Mix Kit (Life Technologies). Each reaction included 2.5µl TaqMan PreAmp Master Mix (2x), 1.25µl Pre-amplification Assay Pool, 0.5µl H_20 and 1.2µl genomic DNA. These reactions were incubated in a Biorad thermocycler for 10 min at 95°C, followed by 12 cycles of 95°C for 15 sec and 60°C for 4 min, and then incubated at 99.9°C for 10 minutes. The PreAmplified products were then held at 4°C until they were diluted 1:20 in 1x TE buffer and then stored at -20°C until use. Post-PreAmplification, samples were genotyped using the TaqMan OpenArray System. 2µl diluted pre-amplified product was mixed with 2µl TaqMan OpenArray Master Mix. The resulting samples were dispensed using the OpenArray®

AccuFill™ System onto Format 32 OpenArray plates with each plate containing 96 samples and 16 SNP assays per sample. The QuantStudio™ 12K Flex instrument (Applied Biosystems, Carlsbad, California) was used to perform the real-time PCR reactions on the loaded OpenArray plates. The fluorescence emission results were read using the OpenArray® SNP Genotyping Analysis software v1 (Applied Biosystems) and the genotyping analysis was performed using TaqMan® Genotyper v1.3, using the autocalling feature. Manual calls were made on selected genotype calls based on the proximity to the nearest cluster and HapMap positive controls.

Participant-specific quality controls included filters for genotype success rate (> 90%) and sample provenance error assessed via pairwise comparisons of genotype calls between all samples to identify samples with > 90% similarity. Analysis of samples that were flagged in the initial quality control checks were repeated. Those samples that still failed quality control were excluded. SNP-specific filters included genotype call rate (> 90%) and Hardy-Weinberg equilibrium ($p > 0.05$) assessed using an exact test.

The two SNPs defining the *APOE* alleles were genotyped separately using TaqMan assays as previously described [382]. All SNPs were in Hardy-Weinberg equilibrium and genotype frequencies are reported in Supplementary Table 3.

5.2.4 Data Preparation and Statistical Analysis

All analyses were performed in the R 3.2.3 Statistical computing environment [426]. Cognitive tests at all 4 waves were transformed into z-scores (Mean = 0, SD = 1) using the means and SD at baseline to facilitate comparisons between cognitive tests. A higher score on all tests indicates better cognitive performance.

Genetic dominance was assumed for the previously reported risk allele for all SNPs, except *SORL1, DSG2* and *CASS4* where a recessive model of inheritance was assumed due to the low frequencies of the non-risk allele. *APOE* alleles were coded as the number of *APOE* *ε4 alleles (0,1,2). Participants with the *APOE* *ε2/ε4 allele were excluded to avoid conflation between the *APOE* *ε2 protective and *APOE* *ε4 risk effects.

Three genetic risk scores were constructed [428]: (1) a simple count genetic risk score (SC-GRS) of the number of risk alleles where SC_GRS = $\sum_{i=1}^{I} G_{ij}$; (2) an odds ratio weighted genetic risk score (OR-GRS) where OR_GRS = $\sum_{i=1}^{I} \log(OR_{ij}) \times G_{ij}$; and (3) an explained variance genetic risk score (EV-GRS) weighted by minor allele frequency and odds ratios where EV_GRS = $\sum_{i=1}^{I} \left(\log(OR_{ij}) \sqrt{2MAF_{ij}(1 - MAF_{ij})}\right) \times G_{ij}$. For the above formulae, risk scores are calculated for the *i*th patient, where $\log(OR_i)$ = the odds ratio for the *j*th SNP; MAF_{ij} = the minor allele frequency for the *j*th SNP; and G_{ij} = the number of risk alleles for *j*th SNP. SNPs were weighted by their previously reported OR for LOAD and by the minor allele frequency (MAF) reported by the International HapMap project for the CEU reference population (Table 5.1). Participants missing genetic data for any SNP were excluded from GRS analysis (n = 121). All three GRS were transformed into z-scores to facilitate comparison between them. A higher score indicates greater genetic risk.

Linear mixed effects models (LMMs) with maximum likelihood estimation and subject-specific random intercepts and slopes were used to evaluate the effect of individual SNPs or GRS on longitudinal cognitive performance. Longitudinal change was modelled as a quadratic growth curve, where age centred on baseline was used as an indicator of time; linear rate of change (age) is estimated from the slope of the line tangential to the curve at the intercept and quadratic rate of change (age^2) is estimated from the acceleration/deceleration in the curve over time. Quadratic growth curves were represented as orthogonal polynomials to avoid collinearity problems and facilitate estimation of the models [444]. Covariates included in the models were sex, total years of education and, for individual SNP models, *APOE* genotype. LMMs were estimated using the R package 'lme4' [445]. Statistical significance of the fixed effects was determined using a Kenward-Roger approximation for *F*-tests, where a full model, containing all fixed effects, is compared to a reduced model that excludes an individual fixed effect (R package 'afex' [446]). Because 24 loci (*APOE* + 23 LOAD GWAS SNPs) and three GRS were tested, $P < 0.0017$ were considered to be study-wide significant after Bonferroni correction. $P < 0.05$ and > 0.0017 were nominally significant. Conditional R^2 (R_c^2), the variance explained by fixed and random effects (i.e. the entire model), and marginal R^2 (R_m^2), the variance explained by the fixed effects

were calculated using the R package 'MuMIn' [431-433] by comparing a full model containing the predictor of interest to a reduced model excluding the predictor.

Power curves were calculated to assess the effect size that could be detected at a given power for our sample size using the R package 'simr' [375]. The power calculations are based on Monte Carlo simulations (n = 1000) of linear mixed effects models for each of the cognitive outcomes considered, where the effect sizes for the baseline, linear and quadratic coefficients were altered in the base model in increments of 0.2 and 0.5 for baseline coefficients and linear/quadratic coefficients respectively. Supplemental Figure 1 shows the results of the power calculations.

5.3 Results

5.3.1 Population Characteristics of the PATH Cohort

Demographic characteristics of the PATH cohort are presented in Table 5.2. LMMs (Supplementary Table 4) showed that all the cognitive tests were associated with significant linear and quadratic rates of change except for the Digits Span Backwards test. Immediate Recall was associated with linear (β = -22.31; SE = 0.71; *P* = <0.0001) and quadratic rate of change (β = -7.68; SE = 0.69; *P* = <0.0001), with Immediate Recall scores declining with age, and with the decline accelerating over time. Digits Span Backwards Test was associated with linear (β = 1.64; SE = 0.71; *P* = .02) but not quadratic (β = -0.31; SE = 0.66; *P* = .64) rate of change, with Digits Span Backwards test scores increasing with age. Spot-the-Word was associated with linear (β = 4.35; SE = 0.36; *P* = <0.0001) and quadratic (β = -1.58; SE = 0.32; *P* = <0.0001) rate of change, with Spot-the-Word scores increasing with age, and with the rate of change decelerating over time. Symbol Digits Modalities Test was associated with linear (β = -14.56; SE = 0.57; *P* = <0.0001) and quadratic (β = -1.88; SE = 0.5; *P* = <0.0001) rate of change, with Symbol Digits Modalities Test scores declining with age, and with the decline accelerating over time.

Linear rate of change explained 53% - 78% of outcome variation for the entire model, with quadratic rate of change explaining an additional 1.2% - 4% of outcome variation. Introducing the covariates into the models explained an additional 3.7% - 19.1% of the variation in the fixed effects (Supplementary Table 5).

Table 5.2: PATH cohort demographics

Variable	Excluded (n = 1,024)	Included (n = 1,526)	t/χ^2	Degrees of Freedom	*P*
Age†	62.51 ± 1.51	62.51 ± 1.5	0.11	2206.27	0.92
Male‡	770 (50.46)	546 (53.32)	1.9	1	0.17
Education†	14.15 ± 2.51	13.21 ± 3.07	8.11	1890.2	<0.001
MMSE†	29.45 ± 0.75	28.57 ± 2.05	13.12	1191.87	<0.001
SC-GRS†	24.67 ± 3.23	24.63 ± 3.18	0.32	1623.27	0.75
OR-GRS†	3.45 ± 0.81	3.45 ± 0.84	0.09	1548.41	0.92
EV-GRS†	1.63 ± 0.41	1.63 ± 0.43	0.03	1554.16	0.97

†unpaired 2-tailed t-test; ‡Pearson's χ^2 2-tailed test

5.3.2 Main Effects of LOAD GWAS SNPs

Associations between single SNPs and cognitive outcomes did not withstand corrections for multiple testing and we report the results that were nominally significant. Introduction of the 24 LOAD GWAS risk loci individually into the LMMs (Table 5.3; for full models including fixed and random effects see Supplementary Tables 6-29) identified 12 loci (*APOE, ABCA7, BIN1, CLU, EPHA1, MS4A4E, SORL1, DSG2, INPP5D, ZCWPW1, CELF1* and *FERMT2*) that were significantly associated with cognitive performance. The remaining 12 loci (*CD2AP, CD33, CR1, MS4A4A, MS4A6A, PICALM, HLA-DRB5, PTK2B, SLC24A4-RIN3, MEF2C, NME8* and *CASS4*) were not significantly associated with cognitive performance.

APOE ε4 allele was associated with a greater rate of decline in Immediate Recall and Symbol Digit Modalities Tests scores. *ABCA7*-rs3764650-G was associated with a lower initial status at baseline in Immediate Recall Test scores and a reduced rate of decline in Symbol Digit Modalities Test scores. *BIN1*-rs744373-G *was* associated with a lower initial status at baseline in Immediate Recall Test scores. *CLU*-rs11136000-C was associated with quadratic rate of change in Digits Span Backwards test scores showing an accelerating positive slope. *EPHA1*-rs11767557-T was associated with a faster rate of decline in Digits Span Backwards test scores. *MS4A4E*-rs670139-T was associated with increased initial status at baseline in Spot-the-word test scores. *SORL1*-rs11218343-T was associated with a lower initial status at baseline in Symbol Digits Modalities Test scores. *DSG2*-rs8093731-C was associated with an improvement in Spot-the-Word test scores. *INPP5D*-rs35349669-T was associated with a reduced rate of decline in Immediate Recall Test scores and a greater rate of decline Symbol Digits Modalities Test scores. *ZCWPW1*-rs1476679-T was associated with an increased rate of growth in Spot-the-word test scores. *CELF1*-rs7933019-C was associated

Table 5.3: *Parameter estimates for the association of LOAD GWAS risk loci with cognitive performance*

SNP	Coefficient	Immediate Recall *Estimate (SE)*	Digits Backwards *Estimate (SE)*	Spot-the-Word *Estimate (SE)*	SDMT *Estimate (SE)*
SC-GRS	Intercept	-0.018 (0.018)	-0.0043 (0.021)	-0.013 (0.018)	0.008 (0.02)
	Age	-0.46 (0.69)	-0.028 (0.71)	0.11 (0.32)	-0.68 (0.56)
	Age^2	0.34 (0.68)	-0.75 (0.65)	-0.18 (0.29)	-0.16 (0.5)
OR-GRS	Intercept	-0.033 (0.018)	-0.013 (0.021)	-0.026 (0.018)	-0.026 (0.02)
	Age	**-1.5 (0.69)***	0.041 (0.71)	-0.18 (0.32)	-1.1 (0.56)
	Age^2	0.62 (0.67)	-0.87 (0.64)	-0.014 (0.29)	-0.35 (0.5)
EV-GRS	Intercept	-0.033 (0.018)	-0.012 (0.021)	-0.023 (0.018)	-0.021 (0.02)
	Age	**-1.5 (0.69)***	0.044 (0.71)	-0.19 (0.32)	**-1.2 (0.56)***
	Age^2	0.58 (0.67)	-0.9 (0.64)	0.016 (0.29)	-0.4 (0.5)
APOE ε4	Intercept	-0.058 (0.036)	-0.014 (0.042)	-0.02 (0.037)	-0.071 (0.041)
	Age	**-3.6 (1.5)***	0.44 (1.5)	-0.13 (0.75)	**-2.7 (1.2)***
	Age^2	0.74 (1.4)	-1.9 (1.4)	-0.032 (0.66)	-1.4 (1)
ABCA7	Intercept	**-0.1 (0.05)***	0.03 (0.05)	-0.05 (0.05)	0.01 (0.05)
	Age	-2.49 (1.84)	-0.55 (1.87)	0.3 (0.93)	**4.0 (1.45)****
	Age^2	-1.91 (1.76)	-2.56 (1.69)	0.83 (0.81)	0.95 (1.28)
BIN1	Intercept	**-0.07 (0.035)***	0.057 (0.04)	-0.016 (0.036)	0.023 (0.039)
	Age	-1.0 (1.4)	-0.8 (1.4)	-0.5 (0.71)	-1.6 (1.1)
	Age^2	1.2 (1.4)	0.48 (1.3)	0.63 (0.63)	-0.64 (1)
CD2AP	Intercept	0.016 (0.035)	0.0064 (0.04)	-0.029 (0.036)	0.00075 (0.04)
	Age	-1.3 (1.4)	-2.5 (1.4)	-1.0 (0.72)	-0.15 (1.1)
	Age^2	1.2 (1.4)	-0.86 (1.3)	0.24 (0.64)	-0.16 (1)
CD33	Intercept	0.0091 (0.055)	-0.066 (0.064)	-0.084 (0.057)	0.023 (0.062)
	Age	1.1 (2.2)	2.2 (2.3)	-0.68 (1.1)	-0.76 (1.8)
	Age2	0.98 (2.2)	3.1 (2.1)	-1.3 (1.0)	0.16 (1.6)
CLU	Intercept	0.046 (0.047)	0.096 (0.055)	0.00095 (0.049)	0.065 (0.054)
	Age	-1.1 (1.9)	0.95 (1.9)	0.14 (0.86)	0.15 (1.5)
	Age^2	0.95 (1.9)	**3.7 (1.8)***	-1.5 (0.79)	0.86 (1.4)
CR1	Intercept	-0.044 (0.037)	0.0085 (0.043)	-0.05 (0.039)	-0.021 (0.042)
	Age	-1.8 (1.5)	-0.2 (1.5)	0.6 (0.77)	-1.6 (1.2)
	Age^2	1.5 (1.5)	-0.66 (1.4)	0.12 (0.69)	0.26 (1.1)

Table 5.3 *(Continued)*

SNP	Coefficient	Immediate Recall *Estimate (SE)*	Digits Backwards *Estimate (SE)*	Spot-the-Word *Estimate (SE)*	SDMT *Estimate (SE)*
EPHA1	Intercept	-0.0059 (0.094)	-0.035 (0.11)	-0.091 (0.097)	-0.15 (0.11)
	Age	-3.8 (3.8)	**-10 (3.8)****	0.34 (2.0)	1.6 (3.1)
	Age^2	2.7 (3.7)	2 (3.5)	-0.16 (1.8)	-1.1 (2.8)
MS4A4A	Intercept	-0.013 (0.047)	-0.024 (0.054)	0.083 (0.048)	0.057 (0.053)
	Age	1 (1.9)	1.8 (1.9)	0.95 (0.95)	0.54 (1.5)
	Age^2	-1.3 (1.8)	-1.9 (1.8)	0.28 (0.85)	0.48 (1.3)
MS4A4E	Intercept	-0.0034 (0.037)	-0.021 (0.042)	**0.1 (0.038)****	0.067 (0.041)
	Age	2.8 (1.5)	2 (1.5)	0.4 (0.75)	0.56 (1.0)
	Age^2	-0.09 (1.4)	-0.53 (1.4)	-0.28 (0.67)	1.2 (1.1)
MS4A6A	Intercept	-0.041 (0.045)	-0.025 (0.052)	0.033 (0.047)	0.012 (0.051)
	Age	1.4 (1.8)	1.7 (1.8)	1.5 (0.92)	0.38 (1.4)
	Age^2	-0.38 (1.8)	-1.9 (1.7)	-0.057 (0.82)	-0.17 (1.3)
PICALM	Intercept	-0.017 (0.05)	-0.0011 (0.057)	0.017 (0.051)	0.031 (0.056)
	Age	0.84 (2.0)	2.3 (2.0)	0.87 (1.0)	-0.31 (1.6)
	Age^2	-0.62 (1.9)	-0.73 (1.9)	-0.15 (0.9)	-1.7 (1.4)
HLA-DRB5	Intercept	-0.016 (0.035)	-0.045 (0.041)	-0.031 (0.036)	0.005 (0.04)
	Age	-0.1 (1.4)	-2.6 (1.4)	-0.78 (0.72)	-0.6 (1.1)
	Age^2	0.43 (1.4)	-0.97 (1.3)	-0.67 (0.64)	-0.16 (1)
PTK2B	Intercept	0.0039 (0.036)	-0.00036 (0.042)	0.02 (0.037)	-0.062 (0.041)
	Age	0.13 (1.5)	-0.48 (1.5)	0.077 (0.75)	0.92 (1.2)
	Age^2	0.38 (1.4)	0.84 (1.4)	-0.17 (0.66)	-1.2 (1.0)
SORL1	Intercept	-0.04 (0.066)	0.018 (0.076)	-0.023 (0.068)	-0.15 (0.074)*
	Age	2.4 (2.6)	0.14 (2.6)	-0.006 (1.3)	-0.9 (2.0)
	Age^2	3.2 (2.5)	-1.8 (2.4)	-0.75 (1.2)	-0.52 (1.8)
SLC24A4-RIN3	Intercept	0.022 (0.081)	0.06 (0.094)	-0.06 (0.084)	-0.074 (0.092)
	Age	-3.9 (3.2)	3.0 (3.3)	-0.97 (1.7)	0.99 (2.6)
	Age^2	0.13 (3.3)	-1.2 (3.2)	-1.7 (1.5)	2.9 (2.4)
DSG2	Intercept	0.023 (0.12)	-0.072 (0.14)	-0.17 (0.12)	-0.14 (0.13)
	Age	1.2 (5.0)	-3.0 (5.1)	**5.9 (2.6)***	3.1 (4.2)
	Age^2	-0.44 (4.9)	-4.2 (4.8)	-2.5 (2.3)	2.4 (3.7)

Table 5.3 *(Continued)*

SNP	Coefficient	Immediate Recall *Estimate (SE)*	Digits Backwards *Estimate (SE)*	Spot-the-Word *Estimate (SE)*	SDMT *Estimate (SE)*
INPP5D	Intercept	-0.032 (0.039)	0.065 (0.045)	0.018 (0.04)	-0.017 (0.044)
	Age	**3.3 (1.6)***	0.63 (1.6)	-1.3 (0.8)	-3.2 (1.3)**
	Age^2	0.71 (1.5)	-2.3 (1.5)	-0.17 (0.7)	-1.2 (1.1)
MEF2C	Intercept	-0.00085 (0.047)	0.022 (0.054)	-0.056 (0.048)	-0.016 (0.052)
	Age	1.1 (1.8)	1.8 (1.9)	-0.34 (0.94)	1.2 (1.5)
	Age^2	-2.5 (1.8)	1.3 (1.7)	1.2 (0.83)	1.5 (1.3)
NME8	Intercept	0.048 (0.051)	-0.0035 (0.059)	0.0091 (0.053)	0.0054 (0.058)
	Age	2.8 (2.1)	3.6 (2.1)	0.26 (1.1)	0.25 (1.7)
	Age^2	-2.9 (2.0)	0.03 (1.9)	-0.13 (0.94)	-2.2 (1.5)
ZCWPW1	Intercept	0.012 (0.059)	-0.0086 (0.068)	0.062 (0.06)	-0.043 (0.066)
	Age	-1.4 (2.3)	0.21 (2.4)	**3.0 (1.2)****	0.41 (1.9)
	Age^2	3.5 (2.2)	3.3 (2.2)	-1.4 (1.0)	0.86 (1.7)
CELF1	Intercept	0.0053 (0.035)	-0.021 (0.041)	-0.0033 (0.036)	-0.0097 (0.04)
	Age	**3.1 (1.4)***	-0.14 (1.4)	1.3 (0.72)	0.28 (1.1)
	Age^2	-0.84 (1.4)	0.71 (1.3)	0.0058 (0.64)	1.4 (1)
FERMT2	Intercept	0.014 (0.047)	-0.1 (0.054)	-0.028 (0.048)	0.077 (0.052)
	Age	-1.9 (1.9)	1.9 (1.9)	0.53 (0.95)	1.6 (1.5)
	Age^2	-0.098 (1.8)	-1.9 (1.8)	-0.3 (0.86)	**2.9 (1.4)***
CASS4	Intercept	-0.00083 (0.049)	0.013 (0.056)	0.018 (0.05)	0.029 (0.055)
	Age	0.82 (1.9)	0.03 (1.9)	0.16 (0.98)	-2.2 (1.5)
	Age^2	0.5 (1.9)	1.1 (1.8)	-0.23 (0.87)	-0.046 (1.4)

***p <0.001, **p <0.01, *p <0.05; Intercept: baseline cognitive function, Age: linear rate of change, Age^2: quadratic rate of change; GRS include *APOE;* negative estimates indicate lower cognitive function and accelerated rates of decline.

with reduced rate of decline in Immediate Recall test scores. *FERMT2*-rs17125944-C was associated with a quadratic rate of change in Symbol Digits Modalities Test scores.

Comparisons in the R^2 statistics between covariate only models and the SNPs showed that there was a negligible increase in marginal R^2 statistics and no increase in conditional R^2 statistics (Supplementary Tables 6-29).

5.3.3 Main Effects of LOAD GRS

We evaluated the association of three genetic risk scores with cognitive performance (Table 5.3; Supplementary Tables 30-32). Mean and SD for the raw GRS at baseline are presented in Table 5.2. The SC-GRS was not associated with cognitive performance. Higher OR- and EV-GRS were associated with a greater rate of decline in Immediate Recall and for the EV-GRS, a greater rate of decline in Symbol Digit Modalities Test scores.

Comparisons in the R^2 statistics between covariates-only models and the GRS models showed that there was a negligible increase in marginal R^2 statistics and no increase in conditional R^2 statistics (Supplementary Tables 30-32). OR- and EV-GRS were not associated with cognitive performance when *APOE* was excluded from the GRS (Supplementary Tables 33-35).

5.4 Discussion

In this study we investigated the association of the 23 most significant LOAD GWAS risk loci with cognitive performance in episodic memory, vocabulary, working memory and processing speed. We identified 11 SNPs as associated with baseline cognitive performance (*ABCA7, BIN1, MS4A4E, SORL1*), linear rate of change (*APOE, ABCA7, EPHA1, DSG2, INPP5D, ZCWPW1, CELF1*) or quadratic rate of change (*CLU, FERMT2*). GRS, weighted by odds ratio and by odds ratio plus minor allele frequency were both associated with a linear rate of change in episodic memory and processing speed. When *APOE* was excluded from these scores neither GRS were significantly associated with cognitive performance indicating that the association was driven by the dominant effect of the *APOE* *ε4 allele. It should be noted, however, that the effect sizes for the observed associations are small, with an increase in marginal R^2 statistics ranging from 0.1-0.2% after

inclusion of the genetic predictors. In comparison, inclusion of the covariate education in the model increases the marginal R^2 statistic around 4.3-19.8%.

Previous studies of associations between the initial GWAS LOAD risk loci and the limited number of studies that have examined the role of the IGAP LOAD risk loci and cognitive performance are characterized by a lack of consistent findings [114-132].

In univariate analysis, SNPs from 7 of the 23 non-*APOE* GWAS loci have been associated with cognitive performance. *ABCA7* with declines in the MMSE score in women [123]; *BIN1* with decline in MMSE score [124]; *CD2AP* with a composite episodic memory [119]; *CD33* with a composite executive function score [119] and decline in MMSE in women [123]; *CLU* with with baseline episodic memory [120], baseline and decline in a composite cognitive score [129,130] and decline in 3MS [144]; *CR1* with declines in verbal fluency [124], global cognition [116,128], episodic memory, perceptual speed, semantic memory [131] and attention [144]; *PICALM* with a composite cognitive score [130] and decline in global cognition [115]; and *NME8* with declines in Clinical Dementia Rating Scale Sum of Boxes Scores [132].

Aggregating SNP variation across genomic regions in a 'gene based' approach, has identified additional AD risk loci as associated with cognitive performance. In a meta-analysis of 31 studies (n = 53,949), *PICALM, MEF2C* and *SLC24A4-RIN3* gene regions were associated with general cognitive function ($p \leq 0.05$). In single sex cohorts, *BIN1*, *CD33*, *CELF1*, *CR1*, *HLA* cluster, and *MEF2C* gene regions were associated with decline in MMSE in an all female cohort and *ABCA7*, *HLA* cluster, *MS4A6E*, *PICALM*, *PTK2B*, *SLC24A4*, and *SORL1* gene regions were associated with decline in 3MS in an all-male cohort.

Genetic risk scores can have greater predictive power than individual variants as they are based on the cumulative effect of many variants that individually may have effects that are too small to be reliably detected in a univariate analysis. GRS composed of LOAD risk SNPs identified in the initial LOAD GWAS have been associated with baseline general cognition [119], episodic memory [120], visual memory and MMSE [124] and with decline in episodic memory [120], verbal fluency, visual memory and MMSE [124]. However, these

associations were no longer statistically significant when *APOE* was excluded from the GRS. GRS that include the IGAP risk loci have been associated with a decline in MMSE in participants with MCI when *APOE* was excluded [117], and with memory performance at baseline and a faster rate of decline that accelerated with age, though only linear rate of change remained significant after *APOE* was excluded [211].

Genome-wide significant IGAP LOAD risk loci only explain only 30.62% of the genetic variance of LOAD [106]. Thus, an alternative approach is to construct a genome-wide polygenic score (GPS) composed of all nominally associated variants at a given significance level. The first study to use this method did not find an association with cognitive ability or cognitive change [212]. A more recent study using data collected from the UK Biobank (n = 112 151) found that an AD GRS constructed from 20,437 SNPs that were associated with AD at a threshold of $p < 0.05$ in the IGAP study was significantly associated with lower verbal-numerical reasoning, memory and educational attainment [213].

Several factors may explain the lack of consistent findings across studies. First, the failure to replicate positive results between studies could result from differences in participant characteristics (e.g., baseline education, mean age, gender, and ethnicity) and methodologies (e.g., sample size, duration of the study, number of follow-ups, non-linear time, population stratification, variation in classification, and cognitive measures) [104]. In particular, studies that did not exclude cognitively impaired individuals from the analysis could bias the observed results in favour of a positive association [122,309].

Selectively removing individuals who develop cognitive impairment during the study from the analysis, as was done in this study, may not resolve the issue because of inadvertent inclusion of participants with preclinical dementia. Inclusion of individuals who are cognitively normal but have biomarker and neuroimaging evidence of preclinical AD greatly exaggerates age-related cognitive decline across multiple cognitive domains [93]. This suggests that AD-related genes may be associated with cognitive decline in participants who are in the preclinical stages of AD. This has been observed in cognitively normal *APOE* *ε4 carriers who had low levels of PET Aβ who remained cognitively stable, in

comparison to an *APOE* *ε4 carriers with high PET Aβ who experienced faster rates of cognitive decline. This suggests that declines in cognitive function observed in *APOE* *ε4 carriers reflects the effect of *APOE* exacerbating Aβ related cognitive decline rather than an independent *APOE* effect [447]. This effect is further indicated by previous studies showing that *ABCA7*, *EPAH1* and *CLU* were associated with cognitive decline in participants classified as cognitively impaired or demented, but not in those who remained cognitively normal [120,127,442].

Second, the rationale for including LOAD risk loci in the analysis is that they may be associated with biological processes, such as neuritic plaque or neurofibrillary tangle burden, that affect both LOAD and general cognitive performance. However, of the 23 loci identified in the IGAP study, only 11 have been associated with neuritic plaque (*ABCA7, BIN1, CASS4, MEF2C, PICALM, MS4A6A, CD33* and *CR1*) or neurofibrillary tangle (*ABCA7, BIN1, CASS4, MEF2C, PICALM, CLU, SORL1* and *ZCWPW1*) burdens in AD case/control autopsies [128,142]. In a longitudinal study, only *BIN1* and *CASS4* were associated with amyloid accumulation [210]. In contrast, in subjects with MCI, none of the LOAD risk loci were associated with levels of Aβ in cerebrospinal fluid (CSF) and only *SORL1* was associated with levels of CSF tau and phosphorylated tau (components of neurofibrillary tangles). Furthermore, neuritic plaques and neurofibrillary tangles only explain 30% of the variation in cognitive decline, with cerebrovascular and Lewy body disease neuropatholgies explaining an additional 10% of variation [91]. This highlights that while LOAD pathology is an important factor in cognitive decline, it occurs in conjunction with other pathological features.

Finally, the pathogenesis of LOAD spans decades, clinically progressing through the preclinical, MCI and dementia stages. As such, where and when a risk locus is involved in the LOAD pathogenesis cascade may influence whether it is associated with processes that predispose, initiate or propagate cognitive decline. Associations have been reported between *CD2AP*, *CLU, MS4A6A* and *INPP5D* and progression from normal cognition to dementia [448]; *CLU*, *CR1*, and *NME8* and progression from MCI to dementia [448-450]; *INPP5D*, *MEFC2, EPHA1, PT2KB,*

FERMT2, CASS4 and rate of progression in AD [163]; and *PICALM* and *MS4A6A* and progression to MCI/Dementia from normal cognition normal [120]

The present findings need to be interpreted with an understanding of their limitations. First, the PATH cohort is better educated then the population it was drawn from. As higher education is associated with a reduced risk of cognitive decline and incident dementia, this may limit our ability to detect an association between genetic factors and cognitive performance. Second, the subjects in this study were of European ancestry, and thus the results presented may not be generalizable to other populations. Finally, there may have been differential attrition from the PATH study of individuals who later became severely impaired and demented, which may have biased results because these individuals would not be excluded from our analysis and are more likely to experience faster rates of cognitive decline [451].

Despite these limitations, this study has a number of strengths. It was performed in a large community-based cohort that has been followed for a period of 12 years with four waves of data assessing four separate cognitive domains. This allows for robust statistical modelling of the association of genetic factors with non-linear declines across a broad spectrum of cognition functions. Additionally, the narrow age range of this cohort reduces the influence of age differences on the results.

In conclusion, our results suggest that a subset of AD-risk loci are associated with non-clinical cognitive decline, although the effect size of each locus is small. Further, when demographic and lifestyle factors are taken into account, neither individual SNPs nor GRS explain a significant proportion of the variance in cognitive decline in our sample. Further investigation of the association of LOAD risk loci with cognitive function needs to account for the inclusion of participants with preclinical AD. The use of neuroimaging and cerebrospinal fluid biomarkers to determine preclinical AD status will allow for a more robust analysis of the role of LOAD risk loci in cognitive aging.

Supplementary Data

Supplementary data for this study is available here:

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Chapter 6: Association of *AKAP6* and *MIR2113* with cognitive performance in a population based sample of older adults

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Abstract

Genetic factors make a substantial contribution to inter-individual variability in cognitive function. A recent meta-analysis of genome-wide association studies identified two loci, *AKAP6* and *MIR2113,* that are associated with general cognitive function. Here, we extend this previous research by investigating the association of *MIR2113* and *AKAP6* with baseline and longitudinal nonlinear change across a broad spectrum of cognitive domains in community-based cohort of older adults without dementia. Two SNPs, *MIR211*-rs10457441 and *AKAP6*-rs17522122 were genotyped in 1,570 non-demented older Australians of European ancestry, who were examined up to 4 times over 12 years. Linear mixed effects models were used to examine the association between *AKAP6* and *MIR2113* with cognitive performance in episodic memory, working memory, vocabulary, perceptual speed and reaction time at baseline and with linear and quadratic rates of change. *AKAP6*-rs17522122*T was associated with worse baseline performance in episodic memory, working memory, vocabulary and perceptual speed, but it was not associated with cognitive change in any domain. *MIR2113*-rs10457441*T was associated with accelerated decline in episodic memory. No other associations with baseline cognitive performance or with linear or quadratic rate or cognitive changes was observed for this SNP. These results confirm the previous finding that *AKAP6* is associated with performance across multiple cognitive domains at baseline but not with cognitive decline, while *MIR2113* primarily affects the rate at which memory declines over time.

6.1 Introduction

Age-associated decline is a general process that affects all cognitive domains, although it is more pronounced in domains associated with fluid cognition (i.e., that involve novel problem solving and speeded information processing) than domains associated with crystalized cognition (i.e., that requires stored knowledge such as vocabulary and general knowledge [4,452].

There is, however, great heterogeneity in the rate of decline between individuels, with some individuals remaining relatively unimpaired across their lifecourse, while others experience a much faster rate of deterioration which can lead to cognitive impairment or dementia [9,10]. Even in the absence of dementia, age-associated cognitive decline may results in increased difficulty performing tasks involving memory and rapid information processing. Decline in cognitive performance is associated with poor decision-making [5], difficulty with instrumental activities of daily living [6,7] and, poor health literacy [8]. Even when no single aspect of daily living is critically impaired, the cumulative effect of small effects in multiple domains can have a major impact on quality of life. Furthermore, a faster rate of decline is associated with dementia [9,10] and mortality [11,12]. Identifying factors that predispose individuals to faster cognitive decline is an important step in developing intervention and treatment strategies for maintaining cognitive health.

Genetic factors contribute to the inter-individual variability observed in cognitive decline, with common genetic variants estimated to account for between 40-50% of the variability associated with general cognitive functioning in later life, and 24% of the variability in lifetime cognitive change [102,103]. A recent meta-analysis of genome-wide association studies (GWAS) performed by the CHARGE consortium (n = 53,949) identified 13 SNPs in three genomic regions, *MIR2113* (n = 11), *AKAP6* (n = 1) and *APOE/TOMM40* (n = 1) associated with general cognitive function [122]. In a follow-up study of the UK Biobank (n = 112,151) *MIR2113* was associated with educational attainment and verbal-numerical reasoning, *AKAP6* was associated with verbal-numerical reasoning, reaction time and memory, while conversely *APOE/TOMM40* was not associated with any measure of cognitive function [133].

The *APOE/TOMM40* region has been consistently associated with Alzheimer's disease [134,453], and in particular for *APOE,* with cognitive performance in episodic memory, executive functioning, perceptual speed, and global cognitive ability [145]. In contrast, the role of *AKAP6* and *MIR2113* in cognitive function is not well understood. The genome-wide significant SNPs identified by Davies *et al* 2015 in the *MIR2113* region are located ~100kb downstream of *MIR2113* and are associated with regulatory elements such open chromatin, histone modifications, DNase hypersensitive sites and position weight matrix sites, suggesting that the associated SNPs associated are in sites of active transcription and may play a regulatory role in transcription [122]. *AKAP6* is highly expressed in various brain regions and cardiac and skeletal muscle where it binds to the regulatory subunit of protein kinase A (PKA) and anchors PKA to the nuclear membrane or sarcoplasmic reticulum. The cAMP-dependent PKA signalling pathway, in turn has been shown to be involved in short and long term memory and working memory [454,455].

Here, we extend this previous research by investigating the association of *MIR2113* and *AKAP6* with baseline and longitudinal nonlinear change in cognitive performance in a community-based cohort of 1,570 older adults without dementia who have undergone cognitive testing in the domains of episodic memory, working memory, verbal ability, processing speed and reaction time.

6.2 Methods

6.2.1 Participants

Participants were from the Personality and Total Health (PATH) Through Life Project, a large community survey of health and wellbeing in adults. Participants were randomly recruited from the electoral rolls of Canberra and the neighbouring town of Queanbeyan into one of three cohorts based on age; the 20+ (20-24 years), the 40+ (40-44 years) and the 60+ (60-64 years), with individuals assessed at 4 year intervals over a period of 12 years. The background and procedures of the PATH cohort have been described in detail elsewhere [322]. Written informed consent was obtained from all participants and approval for the

study was obtained from the Human Research Ethics Committee of the Australian National University.

Data collected from the 60+ cohort was used in this study, with interviews conducted in 2001-2002 (n = 2551), 2005-2006 (n = 2222), 2009-2010 (n = 1973) and 2014-2015 (n = 1645). Individuals were excluded from analysis based on the following criteria: attendance at only 1 wave (n= 309); no genomic DNA available or missing genotype data (n = 264); *APOE* ε2/ε4 carriers (n = 60, to avoid conflation of the ε2 protective and ε4 risk affect); non-European ancestry (n = 107); probable dementia at any wave (MMSE < 24; n = 82); self-reported medical history of epilepsy, brain tumours or infections, stroke and transient ischemic attacks (n = 381). Missing values in "Education" (total number of years of education, n = 129) were imputed using the 'missForest' package in R [424]. These exclusions left a final dataset of 1,570 individuals at baseline.

6.2.2 Cognitive Assessment

All participants were assessed at baseline and at each subsequent interview for the following four cognitive abilities: perceptual speed, assessed using the Symbol Digit Modalities Test, which asks the participant to substitute as many digits for symbols as possible in 90s [371]; episodic memory, assessed using the Immediate Recall of the first trial of the California Verbal Learning Test, which involves recalling a list of 16 nouns [327]; working memory, assessed using the Digit Span Backward from the Wechsler Memory Scale, which presents participants with series of digits increasing in length at the rate of one digit per second and asks them to repeat the Digits Backwards [328]; and vocabulary, assessed with the Spot-the-Word Test, which asks participants to choose the real words from 60 pairs of words and nonsense words [329]. Simple and choice reaction time (SRT and CRT) tasks were administrated using a hand held box with two depressible buttons, two red stimulus lights and one green 'get ready' light. SRT was measured using four blocks of 20 trials, in which the participant was instructed to press the right hand button (regardless of dominance) in response to the activation of one of the stimulus lights. CRT was measured using two blocks of 20 trials, in which participants were instructed to press the button corresponding to the left or right stimulus light. Mean reaction times were calculated as described

previously [331]. Raw cognitive test scores at each wave and Pearson correlations between test scores are presented in Supplementary Tables 1 & 2.

6.2.3 Genotyping

The most strongly associated SNPs identified by the CHARGE consortium, *MIR211*-rs10457441, *AKAP6*-rs17522122 and *TOMM40*-rs10119, were genotyped using TaqMan OpenArray Assays as previously described [456]. For *AKAP6* and *MIR2113* an additive model was used which examines the effect of each minor allele.

TOMM40-rs10119 did not pass quality control in our dataset and as such was excluded from analysis. *TOMM40*-rs10119 is located in the 19q13.32 region, which is a gene-dense region of strong linkage disequilibrium and includes *APOE* and *TOMM40*. Fine mapping of the region indicates that *APOE* variation is driving observed associations with cognitive aging [121]. As such, we included the *APOE* ε2/ε3/ε4 haplotypes in our analysis as covariates. SNP data for rs429358 and rs7412 which define the *APOE* ε2/ε3/ε4 haplotypes, were genotyped separately using TaqMan assays as previously described [382]. *APOE* alleles were coded as the number of *APOE* *ε4 alleles (0,1,2). Participants with the *APOE* *ε2/ε4 allele were excluded to avoid conflation between the *APOE* *ε2 protective and *APOE* *ε4 risk affect.

All SNPs were in Hardy-Weinberg equilibrium ($p < 0.05$). Genotype frequencies are presented in Table 6.1.

6.2.4 Statistical Analysis

Statistical analysis was performed in the R 3.2.3 Computing environment [426]. To facilitate comparisons across cognitive tests, all cognitive test results at all four waves were transformed into z-scores using the means and standard deviations at baseline. Higher scores indicate better cognitive performance.
Demographic characteristics of participants excluded or included from analysis are described using means and standard deviations for continuous variables and frequency and proportions for categorical variables (Table 6.1). Demographic variables were compared using independent sample t-tests and $\chi 2$ tests.

Table 6.1: PATH cohort demographics

Variable	Excluded (n = 981)	Included (n = 1,570)	t/χ^2	Degrees of Freedom	*P*
Age[1]	62.47 ± 1.48	62.54 ± 1.52	1.1	2111.22	0.27
Education[1]	13.35 ± 3.06	14.03 ± 2.56	5.81	1807.7	<0.001
MMSE[1]	28.67 ± 2.04	29.37 ± 0.91	10.07	1206.95	<0.001
Male[2]	513 (52.35)	803 (51.15)	0.4	1	0.53
MIR2113, n (%)					
T/T	188 (26.1)	351 (22.4)			
C/T	343 (47.6)	805 (51.3)			
C/C	189 (26.2)	414 (26.4)			
NA[3]	260 (10.2)	-			
AKAP6, n (%)					
T/T	154 (21.5)	342 (21.8)			
G/T	361 (50.4)	811 (51.7)			
G/G	201 (28.1)	417 (26.6)			
NA[3]	263 (10.3)	-			
APOE ε4 alleles, *n (%)*					
0	613 (76.1)	1183 (75.4)			
1	170 (21.1)	361 (23)			
2	23 (2.9)	26 (1.7)			
NA[3]	173 (6.7)	-			

[1]unpaired 2-tailed t-test; [2]Pearson's χ^2 2-tailed test;
[3]percentage of total sample (excluded + included) with missing genotype data.

Linear Mixed Effects models (LMMs) adjusting for sex and *APOE* genotype, with maximum likelihood estimation, subject specific random slopes and intercepts were used to compare cognitive performance between genotypes. Longitudinal change in cognitive performance was modelled as a quadratic growth curve, where age was centred on baseline, linear rate of change (Time) was the slope at intercept, and quadratic rate of change ($Time^2$) was acceleration of the curve over time. Quadratic growth curves were represented as orthogonal polynomials to avoid colinearity problems and facilitate estimation of the models [444]. LMMs were estimated using the R package 'lme4' [429].

Statistical significance of the fixed effects were determined using a Kenward-Roger approximation for *F*-tests, where a full model, containing all fixed effects, is compared to a reduced model that excludes an individual fixed effect [446]. As this is a follow-up study to replicate the previous findings in the CHARGE consortium and UK biobank studies, a *P*-value < 0.05 was considered statistically significant. To evaluate whether the random slopes were significantly different from 0, and to determine if there was residual variability in the rate of change that could be explained by predictor variables, LMMs that included random slopes

were compared with models that did not include random slopes using parametric bootstrap methods where 1000 simulations of the likelihood ratio test statistic were generated. Conditional R^2 (R^2_c), the variance explained by fixed and random effects (i.e. the entire model), and marginal R^2 (R^2_m), the variance explained by the fixed effects were calculated using the R package 'MuMIn' [431-433]. Power curves were calculated to assess the effect size that could be detected at a given power for our sample size using the R package 'simr' [375]. The power calculations were based on Monte Carlo simulations (n = 1000) of linear mixed effects models for each of the cognitive outcomes considered, where the effect size for *AKAP6* and *MIR2113* baseline, linear and quadratic coefficients were altered in the base model in increments of 0.5 for the linear and quadratic coefficients and 0.02 for baseline coefficients. The power curves are shown in Supplementary Figure 1 and the detectable effect sizes at 80% power is shown in Supplementary Tables 3 & 4.

6.3 Results

6.3.1 Population Characteristics of the PATH cohort

Demographic information on PATH participants is presented in Table 6.1. Individuals retained in the final sample had on average higher MMSE scores and more years of education than those excluded. Unconditional growth LMMs showed that all cognitive tests were associated with linear rate of change and, with the exception for Digits Backwards test, quadratic rate of change (Supplementary Table 5). Random slopes for all cognitive tests scores were significantly different from 0, indicating that there was sufficient variability in the rate of change between participants, thus, allowing potential genetic predictors of this change to be tested (linear random effect boostrap *p* values: Immediate recall = 0.001; Digits Backwards = 0.001; Spot-the-Word = 0.001; SDMT = 0.001; SRT = 0.001; CRT = 0.001. Quadratic random effects bootstrap *p* values: Immediate recall = 0.001; Digits Backwards = 0.002; Spot-the-Word = 0.001; SDMT = 0.001; SRT = 0.001; CRT = 0.001). Supplementary Table 6 shows the results of the covariates only model.

6.3.2 Main Effects of AKAP6 and MIR2113

Parameter estimates for the associations of *AKAP6* and *MIR2113* with cognitive performance are presented in Table 6.2. Figure 6.1 illustrates the differences in cognitive trajectories between *AKAP6* and *MIR2113* genotypes.

AKAP6-rs17522122*T was significantly associated with a lower initial status at baseline in Immediate Recall ($F(1, 1571.13) = 4.09$, $p = 0.04$), Digits Backwards ($F(1, 1572.44) = 5.61$, $p = 0.02$), Spot-the-Word ($F(1, 1581.06) = 10.23$, $p = 0.001$) and Symbol Digits Modalities ($F(1, 1576.76) = 6.11$, $p = 0.01$) test scores. *AKAP6*-rs17522122*T was not significantly associated with baseline SRT, CRT, or linear or quadratic rate of change on any of the cognitive test scores.

MIR2113-rs10457441*T was significantly associated with quadratic rate of change in Immediate Recall test scores showing an accelerating negative slope ($F(1, 1219.93) = 5.80$, $p = 0.02$). *MIR2113,* was not associated with any other measure of cognitive performance.

6.4 Discussion

In this study, we investigated whether SNPs in *MIR2113* and *AKAP6*, which were previously found to be associated with cognitive performance in two large GWAS studies, were also associated with cognitive decline.

AKAP6 was not associated with decline in any of the cognitive abilities tested. However, we did replicate the previously observed association with baseline cognitive performance, with *AKAP6*-rs17522122*T observed to be associated with worse performance in episodic memory, working memory, vocabulary and perceptual speed. In Davies *et al* 2015 & Davies *et al* 2016 *AKAP6*-rs17522122*T was associated with worse general fluid cognitive performance, verbal-numerical reasoning and improved performance in reaction time and memory. The observed differences in the direction of the effect for memory and lack of replication for reaction time between our study and Davies *et al* 2016 could be attributed to methodological differences in the cognitive tests. The cognitive tests utilized by Davies *et al* 2016 were brief bespoke, non-standard tests, with the memory test involving the recall of a single 12 item matrix that contained 6 pairs of matching symbols, with participants instructed to select the matching pairs after observing the matrix for 5 seconds in the fewest number of attempts; and reaction

Table 6.2: *Effect of AKAP6 and MIR2113 on cognitive performance*

	Immediate Recall	**Digits Backwards**	**Spot-the-Word**	**Symbol Digits Modalities Test**	**Simple Reaction Time**	**Choice Reaction Time**
	Estimate (SE)	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*
Initial Status						
Intercept	-0.27 (0.04)*	0.25 (0.05)***	0.37 (0.05)***	-0.03 (0.05)	0.08 (0.05)***	0.06 (0.05)***
Gender	0.37 (0.04)***	-0.14 (0.04)***	-0.11 (0.04)**	0.04 (0.04)	0.33 (0.04)***	0.26 (0.04)***
APOE ε4	-0.05 (0.03)*	-0.07 (0.03)*	-0.10 (0.03)**	-0.07 (0.03)*	0.02 (0.03)	0.04 (0.03)
AKAP6	-0.02 (0.03)	-0.00 (0.03)	-0.01 (0.03)	0.04 (0.03)	-0.01 (0.03)	-0.03 (0.03)
MIR2113	-0.27 (0.04)*	0.25 (0.05)***	0.37 (0.05)***	-0.03 (0.05)	0.08 (0.05)***	0.06 (0.05)***
Linear Rate of Change						
Time	-19.90 (1.70)***	0.62 (1.73)	3.78 (0.90)***	-14.73 (1.38)***	11.08 (2.03)***	12.87 (1.85)***
Gender	-2.35 (1.41)	0.40 (1.43)	0.81 (0.74)	1.11 (1.14)	2.08 (1.67)	5.08 (1.52)***
APOE ε4	1.54 (1.02)	0.85 (1.04)	0.06 (0.54)	0.01 (0.82)	0.70 (1.21)	0.14 (1.10)
AKAP6	-1.50 (1.02)	0.28 (1.03)	-0.15 (0.54)	0.11 (0.83)	-0.64 (1.22)	-0.56 (1.11)
MIR2113	-19.90 (1.70)***	0.62 (1.73)	3.78 (0.90)***	-14.73 (1.38)***	11.08 (2.03)***	12.87 (1.85)***
Quadratic Rate of Change						
$Time^2$	-4.37 (1.65)***	0.78 (1.60)	-1.58 (0.79)	0.24 (1.23)	-5.83 (1.93)**	-2.16 (1.59)
Gender	-3.90 (1.36)**	1.14 (1.32)	0.44 (0.65)	0.65 (1.01)	1.24 (1.59)	0.79 (1.32)
APOE ε4	0.49 (0.99)	-0.28 (0.96)	-0.33 (0.47)	-1.24 (0.73)	-1.85 (1.16)	-0.24 (0.95)
AKAP6	-2.36 (0.98)*	-0.76 (0.95)	-0.01 (0.47)	-0.84 (0.74)	-0.45 (1.16)	-0.21 (0.96)
MIR2113	-4.37 (1.65)***	0.78 (1.60)	-1.58 (0.79)	0.24 (1.23)	-5.83 (1.93)**	-2.16 (1.59)
Log Likelihood	-6671.44	-6558.11	-3777.88	-5351.23	-6991.59	-6228.11
R^2_m	0.14	0.01	0.02	0.05	0.06	0.07
R^2_c	0.58	0.66	0.90	0.80	0.58	0.70
Individuals	1570	1570	1567	1569	1569	1568
Variance						
Intercept	0.39	0.57	0.67	0.62	0.51	0.57
Time	85.56	166.34	56.24	133.42	199.32	259.29
$Time^2$	43.1	63.45	18.83	42.58	132.78	75.41

Table 6.2 *(Continued)*

	Immediate Recall	**Digits Backwards**	**Spot-the-Word**	**Symbol Digits Modalities Test**	**Simple Reaction Time**	**Choice Reaction Time**
	Estimate (SE)	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*	*Estimate (SE)*
Residual	0.40	0.32	0.08	0.18	0.46	0.30
Covariance						
ID x Time	-2.69	-0.82	-0.21	0.62	3.33	6.02
ID x Time^2	-2.9	-0.58	0.72	0.01	-4.7	-1.12
ID x Time x Time^2	17.8	45.07	-31.26	66.41	95.45	107.81

*$p < 0.05$; **$p < 0.01$.; ***$p < 0.001$

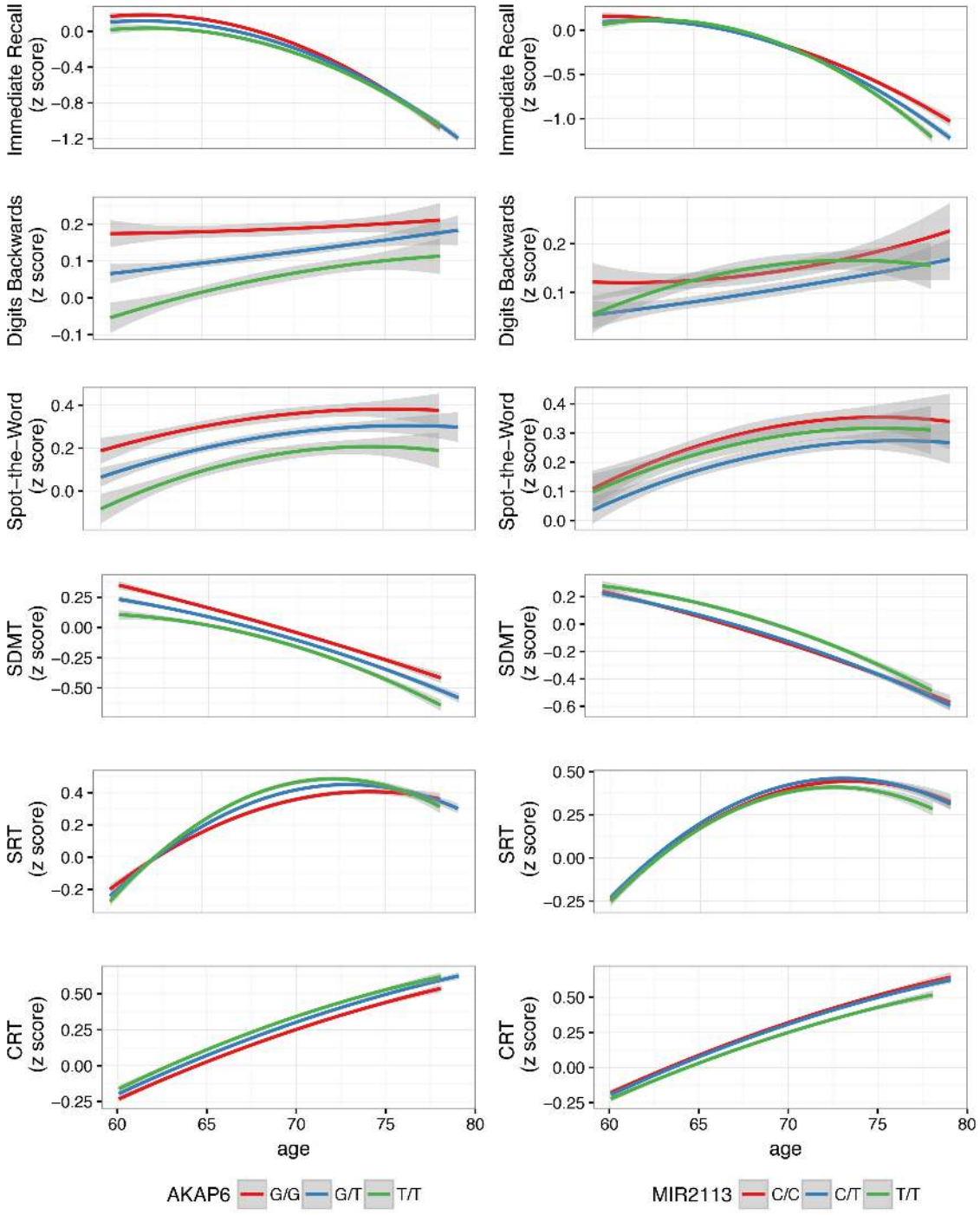

Figure 6.1: *Trajectories of cognitive performance for AKAP6 and MIR2113 genotype*

time assessed using 8 trials of a computerized snap game where participants were directed to push a button if two matching symbols were observed. The memory test has been shown to have low reliability across time [457]. Furthermore, both tests may be susceptible to floor/ceiling effects, where at the upper and lower limit the tests may not be able to distinguish between true differences in participant abilities [457]. Additionally, differences in participants demographics (i.e. age, education and comorbidities) and sample size may result in differential effects sizes, with participants in the UK Biobank being on average 5 years younger and less educated (30.5% vs 76.3% with post school qualifications) than those in PATH. Another possibility is that our study was insufficiently powered, with a dramatically smaller sample size than the UK Biobank.

MIR2113 was associated with an accelerated rate of decline in episodic memory. We did not replicate the previous findings of the association between *MIR2113*-rs10457441*T and worse general fluid cognitive performance. It should be noted, however, that Davies *et al* 2015 used principal component analysis to derive a measure of general cognitive function from cognitive tasks testing at least three different cognitive domains. When investigating individual cognitive domains, Davies *et al* 2016 found that *MIR2113*-rs10457441*T was associated with verbal-numerical reasoning, but not with reaction time and memory. Davies *et al* 2016 suggested that the association with verbal-numerical reasoning may reflect the higher loading of verbal and reasoning abilities have on general cognitive function in comparison to memory and processing speed. As such, our results and those of Davies *et al* 2015; 2016 [122,133] suggest that *MIR2113* may be associated with general cognition rather than specific cognitive abilities.

The present study has a number of strengths that allow for robust statistical modelling of the association of genetic risk factors with cognitive performance. First, this study investigated cognitive change in a randomly selected community dwelling cohort of older adults, and as such the results are likely generalizable to other populations of older adults. Second, the participants were followed for 12 years with four follow-up interviews assessing four separate cognitive domains, allowing for the examination of non-linear declines across a broad spectrum of cognitive abilities. Finally, the narrow age range of the cohort reduces the potential

impact of the confounding effects of age. Despite these strengths, the results from this study should be interpreted with consideration to some study limitations. First, the sample was better educated than the population from which it was drawn. As higher education is associated with a reduced rate of decline this may limit our ability to observe an association with cognitive performance. Second, only the most strongly associated SNP identified by the CHARGE consortium at each locus was genotyped. These SNPs are unlikely to be the causal variants, but are expected to be in linkage disequilibrium with other genetic variation that causes the observed associations with cognitive function. Further, 'gene' based analyses that aggregate the effects of multiple SNPs may further elucidate the role of these loci in cognitive performance.

In conclusion, we have shown that *AKAP6* does not influence the rate of cognitive decline in a population of healthy older adults. However, *AKAP6* is associated with baseline cognitive performance across a broad spectrum of cognitive domains. In contrast, *MIR2113* was associated with an accelerated decline in episodic memory, however, we did not observe an association with baseline cognitive performance. These results extend upon those reported by Davies *et al* [122,133], further elucidating the role these of loci in cognitive performance.

Supplementary Data

Supplementary data is available at: http://dx.doi.org/10.1111/gbb.12368

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Chapter 7: Validating the role of the Australian National University Alzheimer's disease Risk Index (ANU-ADRI) and a Genetic Risk Score in Progression to Cognitive Impairment in a Population-based Cohort of Older Adults Followed for 12 Years

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Abstract

Background: The number of people living with dementia is expected to exceed 130 million by 2050, which will have serious personal, social and economic implications. Employing successful intervention and treatment strategies that focus on disease prevention is currently the only available approach that can have an impact on the projected rates of dementia, with risk assessment being a key component in population-based risk reduction to identifying at risk individuals. Here we evaluate a risk index comprising lifestyle, medical and demographic factors (the Australian National University Alzheimer's Disease Risk Index; ANU-ADRI), and a genetic risk score (GRS) in assessing the risk of progression to Mild Cognitive Impairment (MCI).

Methods: The ANU-ADRI was computed for the baseline assessment of 2,078 participants from the Personality and Total Health (PATH) through life project. GRS were constructed from 25 single nucleotide polymorphisms previously associated with AD. Participants were assessed for clinically diagnosed MCI and Dementia and psychometric test-based MCI (MCI-TB) at 12 years of follow-up. Multi-state models estimated the odds of transitioning from cognitively normal (CN) to MCI, dementia and MCI-TB over 12 years according to baseline ANU-ADRI and GRS.

Results: A higher ANU-ADRI score was associated with increased risk of progressing from CN to both MCI and MCI-TB (HR = 1.07 [1.04-1.11]; 1.07 [1.04-1.09]). The GRS was associated with transitions from CN to Dementia (HR = 4.19 [1.72 – 10.20), but no to MCI or MCI-TB (HR = 1.05 [0.86 - 1.29]; 1.03 [0.87 - 1.21]). Limitations of our study include that the ethnicity in PATH is predominately Caucasian, potentially limiting the generalizability of the results in this study to other ethnicities. Biomarkers of AD were not available to define MCI due to AD. Not all the predictive variables for the ANU-ADRI were available in PATH.

Conclusions: In the general population, the ANU-ADRI comprising lifestyle, medical and demographic factors is associated with the risk of progression from normal cognition to MCI whereas a Genetic Risk Score comprising the main Alzheimer's risk genes was not associated. The ANU-ADRI may be used for population-level risk assessment and screening.

Keywords: Alzheimer's disease; Cognitive aging; MCI (mild cognitive impairment); Cohort studies; Risk factors in epidemiology; Multi-state models

7.1 Background

Accurate risk assessment for cognitive impairment and dementia is increasingly important given the current lack of effective disease modifying treatments for Alzheimer's disease and other dementias. Risk assessment tools may be used in both pharmacological and non-pharmacological trials, clinics, and for population-level screening to guide risk reduction strategies [290,291]. Validated risk assessment tools that can be administered at very low cost provide methods for low-income countries and regions to assess dementia risk and apply prevention strategies. Given current projections of increasing dementia prevalence, there is an urgent need for validated risk assessment tools that have been evaluated on well characterized samples, over long time periods [458]. However, to our knowledge established dementia risk tools [459] have not been evaluated for assessing risk of Mild Cognitive Impairment (MCI) which is a key target group for secondary prevention and pharmaceutical trials. A recently developed risk tool for MCI formulated in the Mayo Clinic Study of Aging found that a basic risk score composed of general demographic (eg age, education, marital status) and clinical features (eg diabetes, hypertension, body mass index) had a C-statistics of 0.60. An augmented version containing additional variables typically collected in clinical and neurological examinations (eg gain speed, anxiety, CDR-Sum of Boxes) had a c-Statistic of 0.70 [460]. Further evaluation of this model in an independent cohort is required.

Recently there has also been an increasing interest in the evaluation of genetic risk scores (GRS) for AD and dementia, which have been associated with the development of AD and incident MCI [119,440,448,461], though they have limited utility in predicting AD beyond that attained with basic demographic variables such as age, gender and education [111,119,120]. The number of studies investigating the association of AD GRS with progression between cognitive states is limited, and the findings mixed. These include reports of a significant association between GRS and progression from CN to either MCI or Late-onset Alzheimer's Disease (LOAD) with a c-statistic of 0.684 (HR = 1.29 [1.19-1.39]) [120]. For the conversion from MCI to LOAD, one study found that participants harbouring six or more AD risk alleles progressed to AD twofold (HR = 1.89 [1.01-3.56]) more rapidly than those with only six alleles [449], while a second study observed that

an AD genetic risk score composed of 19 loci was associated with the conversion to dementia (HR = 1.59 [1.23 – 2.05]), but only when *APOE* was included in the risk score [448]. Conversely, a third study found no association between progression to dementia from MCI using AD GRS composed of 18 loci [450].

Our study has two aims First, to evaluate the association of a non-genetic risk index with the progression from cognitively normal to cognitive impairment. Our measure [462] is a self-report risk index (the Australian National University Alzheimer's Disease Risk Index – ANU-ADRI) that has been externally validated in three cohorts of older adults, in which it was found to be predictive of AD and dementia [463]. The second aim is to compare the ANU-ADRI with a GRS. We examine the association between cognitive impairment and the ANU-ADRI and a LOAD GRS, as assessed using a clinical criterion for MCI or dementia and a psychometric test-based criteria for MCI (MCI-TB) in a community-based cohort of older adults. We first use a Cox proportional hazard model to investigate the association between the ANU-ADRI and a LOAD GRS and incident MCI/dementia, and then extend upon this model using multi-state models to account for back transitions between cognitive states (ie cognitive recovery) and competing risks (ie dementia and death).

7.2 Methods

7.2.1 Participants

Participants were community dwelling adults residing in the city of Canberra or the neighboring town of Queanbeyan, recruited into the Personality and Total Health (PATH) Through Life Project, a longitudinal population-based study of health and wellbeing in adults. Cohorts aged 20-24 (20+), 40-44 (40+) and 60-64 (60+) years at baseline were assessed at four-year intervals for a total of 12 years. The background and procedures for the PATH study have been described elsewhere [322]. Written informed consent was obtained from all participants. This study was approved by the Human Research Ethics Committee of The Australian National University.

This study used data from the 60+ cohort, with interviews conducted in 2001-2002 (n = 2,551), 2005-2006 (n = 2,222), 2009-2010 (n = 1,973), and 2014-

2015 (n = 1645). Individuals were excluded if they were not Caucasian (n = 107), had a self-reported history of stroke, transient ischemic attack, epilepsy, brain tumours or brain infection (n = 381).

7.2.2 ANU-ADRI risk assessment based on demographic, lifestyle and medical risk factors

The development of the ANU-ADRI and the methodology underlying its computation have been described previously [463]. The ANU-ADRI can be computed based on up to 15 predictive variables, 11 of which are available in PATH, including age (self-report), gender (self-report), alcohol consumption (calculated according to NHMRC 2001 guidelines [464] using number of drinks per week. Light to moderate intake, Males: 0.25 – 20.5 per/week; Females: 0.25 – 13.5 per/week), education (self-reported number of years of education), diabetes (self-reported history of diabetes), depression (assessed using the Patient Health Questionnaire (PHQ-9]) [323] following the coding algorithm provided in the PHQ-9 instruction manual with a score of >10 used as the cutoff score), traumatic brain injury (self reported history of TBI with loss of consciousness), smoking (self reported smoking status for current smoker, past smoker or never smoked), social engagement (constructed from 4 domains for marital status, size of social network, quality of social network, level of social activities. A fifth domain for living arrangements was not available in PATH and thus computed pro rata as the average of the above social engagement variables), physical activity (combined self-reported number of hours performing mild, moderate and vigorous activities, weighted by multiples of 1, 2 and 3 respectively [465]), cognitively stimulating activities (assessed as the number of cognitive activities undertaken in the last 6 months for reading, writing, playing games or attending cultural events), and body mass index (BMI equals weight/height^2, in kilograms/meters^2). No data were available for the remaining three predictive variables, cholesterol, fish intake and pesticide exposure. The ANU-ADRI is still predictive of the development of dementia even when a subset of variables is used [463]. Values for predictive variables included in the ANU-ADRI for PATH were selected from baseline measurements or the first occasion on which the variables were measured. A constant of +13 was added to the ANU-ADRI to change range to (from -13–19 to 0–32) to facilitate interpretation.

7.2.3 Genotyping and Genetic Risk Score

The most significant LOAD risk SNPs identified via genome wide association studies from 23 loci [108-113] (*ABCA7, BIN1, CD2AP, CD33, CLU, CR1, EPHA1, MS4A4A, MS4A4E, MS4A6A, PICALM, HLA-DRB5, PTK2B, SORL1, SLC24A4-RIN3, DSG2, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERMT2* and *CASS4)* were genotyped using TaqMan OpenArray assays as previously described [442,456] in addition to the two SNPs defining the APOE alleles which were genotyped using TaqMan assays as previously described [382]. Using these LOAD risk SNPs, an explained variance weighted genetic risk score (EV-GRS) [428] was constructed, which is the sum of all the risk alleles across the individual, weighted by minor allele frequency (MAF) and the Odds Ratio associated with LOAD. The EV-GRS is calculated according to the following formula: EV_GRS = $\sum_{i=1}^{I}\left(\log(OR_{ij})\sqrt{2MAF_{ij}(1-MAF_{ij})}\right) * G_{ij}$ for the *i*th patient, where $\log(OR_i)$ = the odds ratio for the *j*th SNP; MAF_{ij} = the minor allele frequency for the *j*th SNP; and G_{ij} = the number of risk alleles for *j*th SNP. Individuals with missing genetic data were excluded (n = 240). We weighted the LOAD SNPs using the previously reported OR for LOAD and by the MAF for the CEU reference population (Supplementary Table 1). The EV-GRS was transformed into a z-score.

7.2.4 Screening and Clinical Assessment

The screening and clinical assessment methods at waves 1-3 have been described elsewhere [466,467] and are briefly summarised here. At each wave, the same predetermined cut-off from a battery of cognitive tests were used for inclusion of participants in a sub-study on mild cognitive disorders and dementia. Participants from the full cohort were selected for clinical assessment if they had any of the following: (i) a Mini Mental State Examination (MMSE) [370] score < 25; (ii) a score below the fifth percentile score on immediate or delayed recall of the first list of the California Verbal Learning Test [327]; or (iii) a score below the fifth percentile on two or more of either the Symbol-Digit Modalities Test [371]; Purdue Pegboard with both hands [372]; or Simple Reaction Time [331]. At wave 4, participants were selected for review if (1) MMSE score <25 or ≤2.5 percentile on one or more cognitive test; or (2) previous diagnosis at waves 1-3; or (3)

subjective decline ≥25 on Memory and Cognition Questionnaire (MACQ) or (4) Decline in MMSE score ≥ 3 points.

The criteria for the clinical assessment for cognitive impairment at waves 1-3 has been published by our group elsewhere [467]. It involved a Structured Clinical Assessment for Dementia by one of two physicians, a neuropsychological assessment, and the Clinical Dementia Rating Scale [373], which were used to formulate a consensus diagnosis.

Due to the large number of participants screened for review at wave 4, diagnosis was based on neurologist review of interview data as outlined below and in Figure 7.1. For each of the 1644 participants with interview data at Wave 4, assessment data were screened for signs of decline based on the following criteria (screen 1): a previous diagnosis of a cognitive disorder at Waves 1, 2 or 3 OR either evidence of cognitive impairment on the MMSE (≤ 24) or performance on one or more cognitive tests ≤ 6.7th percentile at wave 4 (Immediate recall, Delayed recall, SDMT, F words, A words, Boston Naming Test, Simple RT, Choice RT, Pegboard dominant, Pegboard non-dominant, Pegboard both, Digits Back, Trails B, Stroop Words, Stroop Colour-Word). Additionally, participants had to show evidence of either subjective decline (scores ≥ 25 on the Memory and Cognition Questionnaire (MAC-Q) (32)) or evidence of decline (>3 point decline in MMSE score since wave 3) or evidence of consistent cognitive impairment across time (MMSE ≤ 24 at Waves 3 and 4). All data from the health survey and cognitive testing as well as informant interview were collated into a spreadsheet casefile for each participant. This casefile (Screen 2) automatically screened each participant for meeting criteria for any one of the following diagnoses: DSM 5 Major Neurocognitive Disorder, DSMIV Dementia, DSM 5 Mild Neurocognitive Disorder, Mild Cognitive Impairment, Age Associated Cognitive Decline, Age Associated Memory Impairment, DSMIV Amnestic Disorder not otherwise specified, DSMIV Mild Neurocognitive Disorder, DSMIV Other cognitive disorder. Major criteria for meeting most of the above diagnoses were operationalised as either: 1) Concern of self or informant of significant cognitive decline (MACQ≥25 OR IQCODE>3.31 OR history of dementia diagnosis); 2) Substantial impairment on at least one cognitive domain relative to Wave 4 normative data (cut-offs: < -2SD for dementias, < -1.5SD

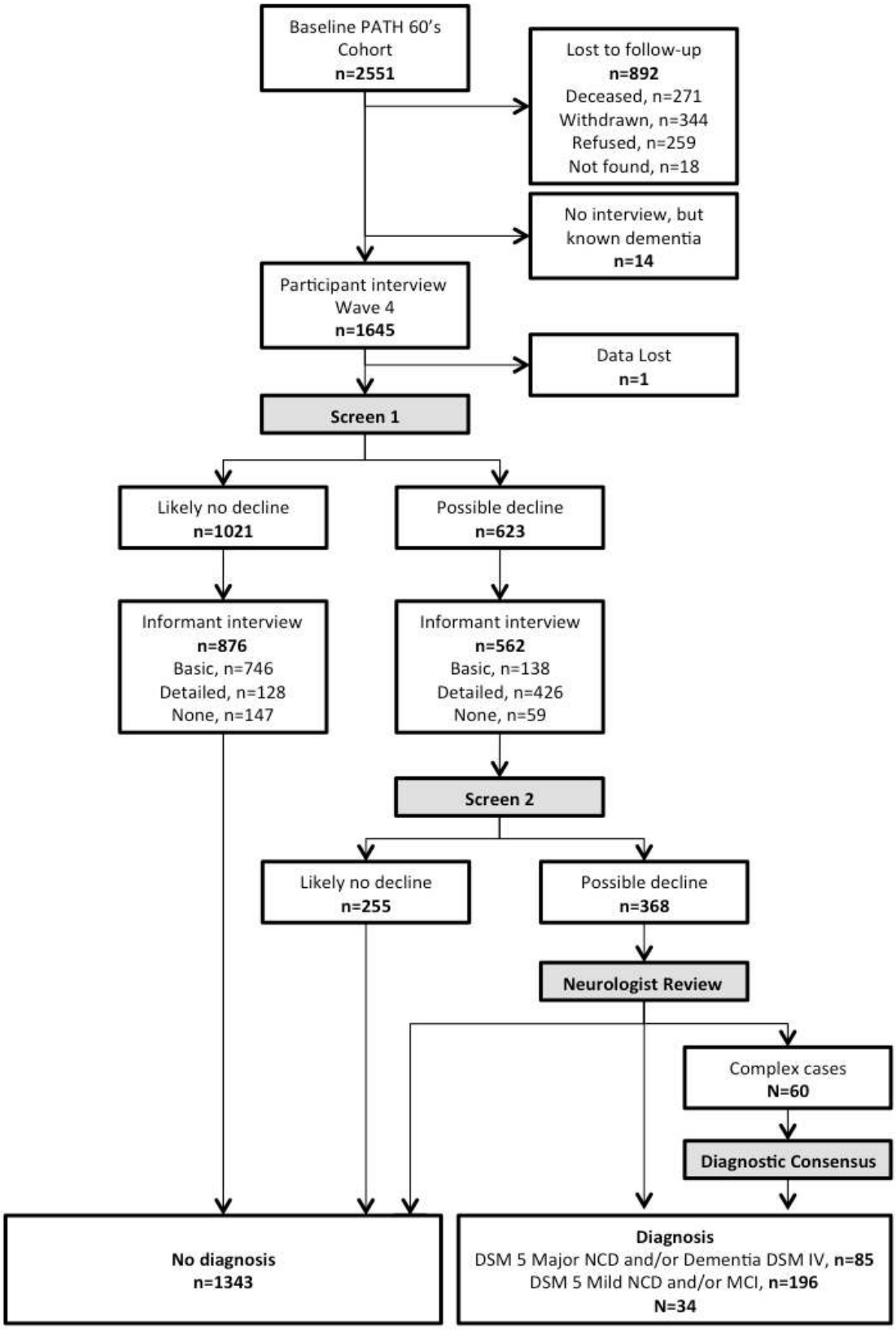

Figure 7.1: *Flowchart depicting the process of screening participants for mild cognitive disorders. DSM-IV Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; DSM-5 Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; MCI Mild cognitive impairment; NCD Neurocognitive disorder; PATH Personality and Total Health Through Life project*

for mild cognitive disorders); 3) Interference with independence and instrumental activities of daily living (Self-reported IADL impairment OR Bayer IADLs>3.11 OR Informant reported everyday cognitive difficulties); 4) Not exclusively during delirium (Cognitive changes since > 6 months, Onset of cognitive changes precede Informant report of onset of delirium-like symptoms); and 5) Not due to another co-existing disorder (PHQ9 < 9 AND No reported history of Schizophrenia or other psychosis). Those meeting criteria for one or more diagnoses (N=368) were screened for case file review by a research neurologist. Diagnoses were made for N= 301 of these cases, of which N=60 complex cases were selected for Diagnostic Consensus based on the following criteria: 1) comorbid depression; 2) other comorbid psychiatric conditions; 3) stroke; and 4) DSM 5 Major Neurocognitive Disorder without memory impairment. Following consensus diagnosis with a clinician specializing in Psychiatry, the final diagnoses included 85 dementia/major NCD, 196 mild cognitive disorders (MCI/mild NCD) and 34 other mild or medical related cognitive disorders.

Clinically diagnosed MCI was based on the Petersen criteria at waves 1 and 2 [13], whereas the Winblad criteria [14] were used at wave 3 and 4. Clinically diagnosed dementia was based on the DSM IV criteria [374] at all waves. At wave 4, there were 14 participants who were not interviewed, but were known to have dementia from informant reports and medical records. Due to the small number of individuals classified with dementia, participants with either MCI or Dementia were grouped into a single MCI/Dementia category.

7.2.5 Test Based MCI

To complement the clinical diagnosis of MCI, a broader psychometric Test-based MCI (MCI-TB) classification was applied to the entire PATH sample [468] at each wave based on education-adjusted cognitive performance (Table 7.1). The PATH sample was first stratified by education (0-12 or 13+ years). Within each of these strata, individuals were classified as MCI-TB if they scored 1.5 standard deviations below the mean on one or more of the psychometric tests used to assess the following cognitive domains: Perceptual speed, measured using the Symbol Digit Modalities Test [371]; episodic memory, assessed using the immediate recall

of the first trial of the California Verbal Learning Test (Recall-immediate) [327]; working memory, measured using the Digit Span Backward from the Wechsler Memory Scale [328]; and vocabulary, assessed by the Spot-the-Word Test [329].

7.2.6 Data analysis

All statistical analyses were performed in R version 3.1.2 [426]. As missing values can reduce power and introduce bias in the resulting estimates, missing values that were not attributable to attrition for the predictive variables utilised in the construction of the ANU-ADRI and the test-based MCI (see above) were imputed using an implementation of the Random Forests algorithm available in the 'missForest' package in R [424,469]. This left 2,078 individuals available for analysis. Supplementary Table 2 shows the proportion of missing variables for each variable.

We first evaluated the risk of progression from normal cognition to MCI/dementia using Cox proportional hazard models with age as the time scale the ANU-ADRI and EV-GRS included as predictor variables in the same model. The outcome of interest in these models was the time to first diagnosis of MCI/dementia, with those subjects who did not develop MCI/dementia at their last assessment right censored. Hazard ratios (HR) and 95% CI were given for the time to MCI/dementia analysis. Concordance index (c-index) for the prediction of conversion from NC to MCI/dementia were calculated. Cox proportional hazard models were estimated using the 'survival' package in R.

To evaluate a more complex model of disease progression, multi-state models (MSMs) were used to examine the association between the ANU-ADRI and EV-GRS and transitions between cognitive states. MSMs allow the modelling of competing risks and back transitions between states (i.e., recovery). Hidden Markov models can be used to estimate misclassification error and the effects of covariates can be allowed to vary by transition [470]. The MSMs utilised in this analysis modelled cognitive deterioration and cognitive recovery by allowing transitions and back transitions between cognitively normal (CN), MCI or MCI-TB states, back transitions from dementia were not allowed, while death was used as a fourth absorbing state (Figure 7.2). Individuals with only a single observation (i.e.,

no recorded transitions) were excluded from the analysis (n = 204). Individuals lost to attrition were considered right censored. The ANU-ADRI and the EV-GRS were included as covariates in the same model. Maximum likelihood estimates of parameters in the MSMs were obtained with the Broyden-Fletcher-Goldfarb-Shanno (BFGS) optimisation method. Normalisation was applied to the likelihood function to improve numerical stability. As the likelihood is maximised using numerical methods, an input of initial values is required to start the search for a maximum. MSMs were fitted using 'msm' [470] in R and multiple models were run using different sets of initial values to ensure the robustness of the parameter estimates. See Supplementary Methods for more detail on the structure of MSMs.

As a sensitivity analysis for the MCI-TB analysis, a more stringent criteria was investigated with MCI-TB based on a score of 1.5 SD below the mean on two or more of the above psychometric tests. Additionally, we performed a complete case analysis to ensure that our imputation method was not biasing the observed results.

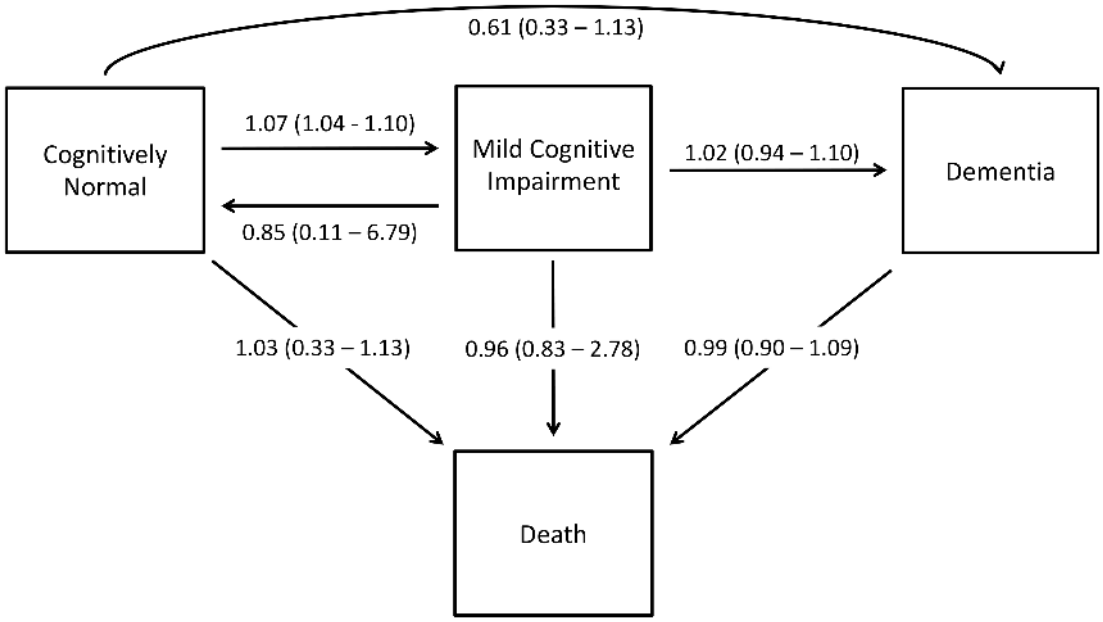

Figure 7.2: *A four state model for possible transitions between cognitive states and death. Hazard ratios (95% confidence intervals) for the effect of the ANU-ADRI on transitions between Cognitively Normal (CN), MCI/Dementia and death are shown. All estimates are from models adjusting for the EV-GRS.*

7.3 Results

7.3.1 Demographics and Other Characteristics of the Sample

Baseline distributions of education, depression, sex, the ANU-ADRI, raw cognitive tests scores and cognitive states at each wave for the PATH cohort are described in Table 7.1. Participants who completed all 4 waves of interviews had a higher level of education in comparison to participants who only completed the wave 1 interview (t = -6.8, df = 331.3, p-value = <0.001). Participants were followed for an average of 9.6 years (after accounting for loss due to attrition) and a total of 13.9 years. Group differences in the sub-indices of the ANU-ADRI between CN and either MCI/Dementia or MCI-TB can be found in Supplementary Table 3. The distribution of the ANU-ADRI and EV-GRS scores is shown in Figure 7.3. As expected, the proportion of individuals classified as MCI/Dementia increased over the course of the study, while the proportion of individuals classified as MCI-TB remained stable (Table 7.1). By wave 4, 36% of the cohort had been lost to follow-up, with 57, 54 and 94 individuals deceased by waves 2, 3 and 4 respectively, and an additional 280, 267 and 329 individuals being lost to follow-up for other reasons (refusal, left catchment area, etc) at waves 2, 3 and 4 respectively.

Table 7.1: Characteristics for the PATH cohort for Waves 1 to 4

	Wave 1 Estimate ± SD	Wave 2 Estimate ± SD	Wave 3 Estimate ± SD	Wave 4 Estimate ± SD
n	2078	1798	1596	1337
Age	63 ± 1.5	67 ± 1.5	71 ± 1.5	75 ± 1.5
Female – n (%)	1009 (49)	-	-	-
Education	14 ± 2.7	-	-	-
Wave 1 completers	12.7 ± (3.0)	-	-	-
Wave 2 completers	13.1 ± (2.7)	-	-	-
Wave 3 completers	13.5 ± (2.7)	-	-	-
Wave 4 completers	14.2 ± (2.6)	-	-	-
Immediate Recall	7.2 ± 2.3	7 ± 2.2	6.7 ± 2.2	5.4 ± 1.9
Digits Backwards	4.9 ± 2.2	5.1 ± 2.2	5.1 ± 2.2	5.3 ± 2.2
Spot-the-Word	52 ± 6	53 ± 5.3	53 ± 5.1	54 ± 5
SDMT	50 ± 9.7	50 ± 9.2	48 ± 9.2	46 ± 9.5
ANU-ADRI	9.4 ± 5.9	-	-	-
EV-GRS	1.6 ± 0.42	-	-	-
Cognitive Status - n (%)				
MCI	23 (1.1)	28 (1.6)	35 (2.2)	103 (7.7)
Dementia	0 (0)	0 (0)	7 (0.44)	37 (2.7)
MCI-TB	384 (18)	373 (21)	347 (22)	261 (19)
Attrition - n (%)				
Death	-	57 (2.7)	54 (3)	94 (5.8)
Dropout	-	280 (13)	167 (9.3)	329 (20.6)

SDMT: Symbol digits modalities test; MCI: Mild cognitive impairment;
MCI-TB: Test-based mild cognitive impairment

Between any two waves, a greater proportion of people transitioned from CN to MCI-TB (10.5%) than from unimpaired to MCI (2.6%), indicating that MCI-TB is a more broad categorization of cognitive impairment. A smaller proportion of individuals transitioned in the opposite direction - from MCI-TB to CN (31.3%) than from either MCI to CN (44%), indicating that MCI-TB is also a more stable category (Table 7.2).

Table 7.2: Number of transitions between CN, MCI, Dementia and MCI-TB during the length of the study.

			To		
From	**CN**	**MCI**	**Dementia**	**Death**	**Censored**
MCI and Dementia					
CN	4459 (86.1%)	137 (2.6%)	32 (0.6%)	189 (3.7%)	359 (6.9%)
MCI	40 (48.2%)	26 (31.3%)	6 (7.2%)	5 (6%)	6 (7.2%)
Dementia	0 (0%)	0 (0%)	5 (71.4%)	2 (28.6%)	0 (0%)
Censored	36 (19.5%)	3 (1.6%)	0 (0%)	8 (4.3%)	138 (74.6%)
MCI-TB					
CN	3403 (80.2%)	446 (10.5%)		144 (3.4%)	249 (5.9%)
MCI	321 (31.3%)	524 (51.2%)		52 (5.1%)	127 (12.4%)
Censored	28 (15.1%)	11 (5.9%)		8 (4.3%)	138 (74.6%)

CN: Cognitively normal; MCI: Mild cognitive impairment; MCI-TB: Test-based mild cognitive impairment

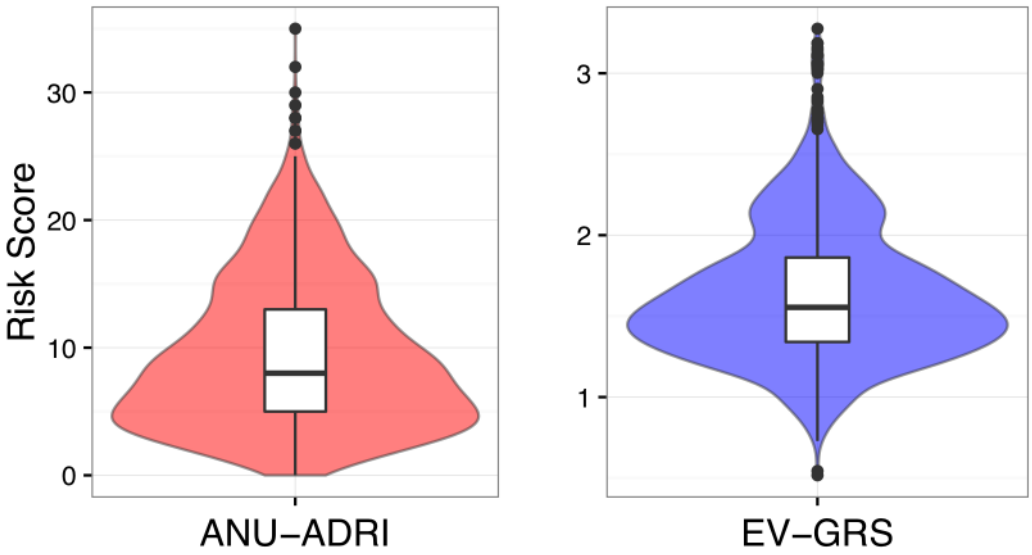

Figure 7.3: *Distribution of the ANU-ADRI and EV-GRS scores within the PATH cohort. The variable width of the violin plot indicates the probability density and the box plot indicates the first, median and third quartile of the ANU-ADRI and EV-GRS scores.*

7.3.2 *Cox proportional hazards models for incident MCI*

A higher ANU-ADRI (indicating greater risk) score was associated with an increased risk of progression to both MCI/Dementia and MCI-TB (Table 7.3). The EV-GRS was not associated with progression from to either MCI/Dementia or MCI-TB. The interaction between the ANU-ADRI and the EV-GRS was non-significant for the MCI/Dementia (HR = 0.99 [0.96-1.01], p = 0.33) and MCI-TB (HR = 0.99 [0.98-1.01], p = 0.11).

In the sensitivity analysis, using a more stringent MCI-TB criterion (scoring 1.5 SD below the mean on two or more test) confirmed that the ANU-ADRI was associated an increased risk of progression from CN – MCI-TB (HR = 1.08 [1.05-1.10], p = < 0.0001). In the complete case analysis, the ANU-ADRI remained significant for both the MCI/Dementia (HR = 1.06 [1.02-1.09], p = 0.001) and MCI-TB (HR = 1.036 [1.01-1.04], p = 0.007) models.

Table 7.3: Associations between the ANU-ADRI and EV-GRS risk scores and cognitive impairment at waves, 1, 2, 3 and 4.

	MCI/Dementia	MCI-TB
ANU-ADRI†, HR (95% CI)	1.06 (1.03-1.09)***	1.04 (1.02-1.50)***
EV-GRS‡, HR (95% CI)	1.14 (0.98-1.33)	1.04 (0.96-1.12)
C-Index (SE)	0.61 (0.03)	0.56 (0.01)
ANU-ADRI	0.60 (0.05)	0.56 (0.02)
EV-GRS	0.53 (0.05)	0.51 (0.02)

*p < .05; **p < .01; ***p < .001; MCI/Dementia: Mild cognitive impairment or Dementia; MCI-TB: Test-based mild cognitive impairment; †per unitary increase in the ANU-ADRI; ‡per SD increase in EV-GRS; all estimates are from models adjusting for the ANU-ADRI and EV-GRS

7.3.3 *Multi-state Models of Transitions*

A higher ANU-ADRI score was associated with an increased risk of transitioning from CN to MCI or MCI-TB (Figure 7.2; Table 7.4). The probability of transitioning from CN to either MCI or MCI-TB after 12 years for individuals scoring 1 SD below the mean on the ANU-ADRI was 10% and for individuals scoring 1 SD above the mean was 20. A higher ANU-ADRI score was not associated with transitions from CN, MCI, dementia or MCI-TB to death; cognitive impairment to dementia; or with cognitive recovery from MCI or MCI-TB to CN. The EV-GRS was associated with an increased risk of transitioning from CN to dementia, with probability of transitioning from CN-dementia for individuals scoring 1 SD above the mean 1.3%. The interaction between the ANU-ADRI and the EV-GRS was not significant for any of the transitions for either the MCI or MCI-TB models.

In the sensitivity analysis, using a more stringent MCI-TB criterion (Supplementary Table 4), confirmed that the ANU-ADRI was associated an increased risk of progression from CN – MCI-TB (HR = 1.12 [1.07-1.17]). For the complete case analysis (Supplementary Table 5), the ANU-ADRI remained statistically significant for both the transition from CN - MCI (1.06 [1.02 - 1.09]) and CN - MCI-TB (HR = 1.05 [1.01 - 1.08]) models.

Table 7.4: Hazard ratios (95% CI) of the ANU-ADRI and EV-GRS scores upon cognitive transition

Transition	MCI/Dementia		MCI-TB	
	ANU-ADRI†	EV-GRS‡	ANU-ADRI†	EV-GRS‡
CN – MCI	1.07 (1.04 - 1.1)*	1.05 (0.86 - 1.29)	1.07 (1.04 - 1.09)*	1.03 (0.87 - 1.21)
CN - Dementia	0.61 (0.33 - 1.13)	4.19 (1.72 - 10.2)*		
CN – Death	1.03 (0.94 - 1.12)	0.70 (0.27 - 1.84)	1.02 (0.98 - 1.06)	0.89 (0.69 - 1.16)
MCI – CN	0.85 (0.11 - 6.79)	0.95 (0 - 181.21)	0.71 (0.50 – 1.00)	0.44 (0.12 - 1.54)
MCI - Dementia	1.02 (0.94 - 1.10)	1.19 (0.76 - 1.85)		
MCI - Death	0.96 (0.83 - 1.11)	0.87 (0.29 - 2.63)	1.05 (0.98 - 1.12)	1.05 (0.65 - 1.71)
Dementia - Death	0.99 (0.90 - 1.09)	0.78 (0.51 - 1.19)		

*p < .05;. CN: Cognitively normal; MCI/Dementia: Mild cognitive impairment or Dementia; MCI-TB: Test-based mild cognitive impairment; †per unitary increase in the ANU-ADRI; ‡per SD increase in EV-GRS; all estimates are from models adjusting for the ANU-ADRI and EV-GRS

7.4 Discussion

To our knowledge, we report the first concurrent evaluation of a non-genetic and genetic risk score in the risk of progression to MCI over a long time period in a population-based cohort. As such this study provides much needed information on the utility of risk assessment tools in assessing the risk of progressing to MCI in the general population. Using cox proportional hazard models, we found that a per unitary increase in the ANU-ADRI at baseline was associated 6% and 4% increased hazard of transitioning from CN - MCI/Dementia and MCI-TB respectively. Additionally, we used multistate models to extend upon the cox proportional hazard models to account for back transitions between cognitive states and the competing risks of death and dementia. We observed that a unitary change in the ANU-ADRI was associated with 7% increased hazard of transitioning from CN to either MCI or MCI-TB. In contrast, the EV-GRS was not

associated with transitions from CN to cognitive impairment, though it was associated with a 4.19 risk of transitioning to dementia from CN.

MSMs are well suited to analysing a more 'realistic' model of cognitive decline in which cognitive deterioration and recovery are modelled simultaneously in addition to misclassification, death and censoring. This is important in the examination of MCI, as pathological cognitive change is often not a linear progression from normal cognition to MCI and finally to dementia, as reversions from MCI back to normal cognition are common, which was also observed in the PATH cohort [22,467]. Individuals with a stable progression to MCI are more likely to progress to dementia than those with an unstable course or no diagnosis of MCI [22]. A higher ANU-ADRI score is associated both with an increased risk of transition to clinically diagnosed MCI and to a psychometric test-based MCI, suggesting that it could be useful for assessing an individual's risk of developing MCI. Additionally, even in individuals who revert to normal cognition, the diagnosis of cognitive impairment may still have prognostic implications as these individuals have a greater likelihood of progressing to dementia or MCI than those who remain cognitively normal [22]. As such individuals that have a higher ANU-ADRI are more likely to revert back to MCI or develop dementia in the future [463]. These results show that the ANU-ADRI may be used to measure risk reduction for clinically significant MCI as well as dementia, and have implications for secondary prevention of dementia. However, while the ANU-ADRI is strongly associated with the progression from CN – MCI, it's predictive ability was limited (c-index of 0.60 for MCI and 0.56 for MCI-TB). This may be due to the relatively young age of the PATH cohort and consequently small number of participants with MCI and the narrow age-range of the sample. We expect that further validation of the ANU-ADRI in slightly older cohort with a higher incidence of MCI, or with a wider age-range, would show that the ANU-ADRI has greater predictive ability.

The ANU-ADRI has several strengths [459]. First, the ANU-ADRI is the only risk assessment tool that has not been developed by identifying risk factors through the analysis of a single cohort and as such the predictive variables are not optimised to a particular study. The ANU-ADRI also does not include any risk factors that require clinical assessments or laboratory tests.

The genetic risk was observed to be associated with the transition from CN to dementia, but not with CN to MCI, or MCI to dementia. This lack of an association may

be a result of the broad categorization of MCI, rather than MCI subtypes, such that it would have included participants with cognitive impairment that was not MCI due to AD [471,472]. This may also explain the reduced risk associated with both MCI and MCI-TB in our sensitivity analysis. Unfortunately, due to the small number of participants with MCI in PATH, further subgroup analysis would likely be underpowered to detect an effect. However, it should be noted that most dementia cases are associated with mixed pathologies rather than singular pathologies, suggesting that an AD GRS would be associated with both amnestic and non-amnestic MCI [473].

Previous studies have investigated the association of AD GRS with MCI. In 3605 participants (360 MCI, 191 dementia) an AD GRS composed of APOE + 19 LOAD GWAS variants was associated with an increased risk of incident MCI and nominally associated with amnestic and non-amnestic [448]. In a second study of 2674 participants (347 MCI, 132 LOAD) a GRS composed of APOE + 9 LOAD GWAS variants, was associated with progression from to normal cognition to MCI/LOAD [120]. Lack of replication in this study could be due to younger and fewer cognitively impaired participants. Furthermore, inclusion of additional AD risk loci that were identified to be nominally significant with AD in GWAS studies may identify a stronger association [461].

Limitations of our study include the relatively high level of education of the PATH cohort [322]; the ethnicity in PATH is predominately Caucasian, potentially limiting the generalizability of the results in this study to other ethnicities, and biomarkers of AD were not available (e.g. CSF, Aβ). Not all the predictive variables for the ANU-ADRI were available in PATH, suggesting the present study may underestimate the sensitivity of this tool in predicting individuals who are at risk of developing cognitive impairment. However, the validation studies also included a subset of the variables contributing to the ANU-ADRI [463].

Study strengths included the large population-based sample with high retention rates and twelve years of follow-up data. The PATH cohort was recruited from a narrow age-band, reducing the impact of age-differences on findings. This is particularly important because age has the largest weighting of risk factors in the ANU-ADRI. Finally, the conservative clinical classifications of MCI/Dementia, based on a thorough clinical assessment and consensus diagnosis by clinicians using published criteria, was complemented by a broader psychometric test-based classification of MCI.

7.5 Conclusions

In conclusion, higher ANU-ADRI scores are associated with increased risk of progressing from cognitively normal to MCI. These results complement previous evidence that the ANU-ADRI is predictive of AD and dementia [463]. In comparison, a genetic risk score comprising the main AD genes was associated with the development of dementia but was not associated with the risk of developing MCI. These results provide further support for using the ANU-ADRI for population-level strategies and individual patient assessment and for informing intervention and treatment strategies aimed at delaying or preventing dementia.

Supplementary Information

Supplementary information is available at http://dx.doi.org/10.1186/s13195-017-0240-3

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Chapter 8. Conclusion

8.1 Summary of Findings in this Thesis

8.1.1 Role of LOAD Risk Loci in Cognitive Decline

The primary aim of this thesis was to elucidate the role of LOAD genetic risk factors in normal cognitive function. Common genetic variants account for 40-50% of the variance in general cognitive function in late life and 24% of the variance in late life cognitive change [102,103]. In recent years, large-scale genome wide association studies (GWAS) have identified over 20 loci associated with Late-onset Alzheimer's disease [108-113]. LOAD risk genes are good candidate genes for associations with cognitive decline as the pathological hallmarks of AD, amyloid-β plaques and neurofibrillary tangles, are observed to occur to varying degrees in individuals without dementia and are associated with nonclinical cognitive decline [82,95-99]. Previous studies of association of the first 11 identified LOAD risk loci with cognitive decline produced mixed results [115,116,118,120,121,124,129,442], and the new risk loci identified by IGAP have yet to be extensively investigated [117,122,123].

In, *'Association of genetic risk factors with cognitive decline: the PATH through life project'* (Chapter 4), I investigated the first 11 LOAD risk loci to be identified by four LOAD genetic consortia for association with change in episodic memory, working memory, verbal ability and perceptual speed over 8 years. I found that *ABCA7* is associated with worse performance in episodic memory at baseline; *APOE *ε4* and *CR1* are associated with increased rate of decline in episodic memory; and *MS4A4E* with reduced rate of decline in episodic memory. Additionally, I observed that a higher weighted genetic risk score is associated with an increased rate of decline in episodic memory, although this is due to the dominant effect of the *APOE *ε4* allele.

In, *'Late Onset Alzheimer's disease risk variants in cognitive decline: The PATH Though Life Study'* (Chapter 5) I extended upon this study too include the 12 additional loci identified in the IGAP meta-analysis, and investigate associations with non-linear cognitive change over 12 years. I found that 11 SNPs are associated with cognitive performance. *ABCA7, MS4A4E* and *SORL1* are associated with baseline cognitive performance in episodic memory, verbal ability and perceptual speed, respectively. For linear rate of change, *APOE* was associated with

episodic memory and perceptual speed*; ABCA7* with perceptual speed; *EPHA1* with working memory; *INPP5D* was associated with episodic memory and perceptual speed; and *ZCWPW1* was associated with verbal ability and; *CELF1* was associated with episodic memory. For quadratic rate of change, *APOE* was associated with a decelerating positive slope in working memory; *CLU* was associated with an accelerating positive slope in working memory; and *FERMT2* was associated with a decelerating negative slope in perceptual speed. Weighted GRS composed of all 25 loci were associated with a greater rate of decline in episodic memory and perceptual speed, although the association was not significant when *APOE* was excluded from the risk score.

For the LOAD risk loci that were significantly associated with cognitive function, the effect sizes and variance they explained was small. This suggests that individual AD-related genetic markers may have limited use in identifying individuals at risk of cognitive decline, especially in relation to environmental and lifestyle risk factors. This finding is supported by previous studies that have found limited utility in including additional LOAD risk loci beyond APOE into predictive models for incidence of LOAD [111,119,120]. The limited improvement in predictive accuracy can be expected, as non-genetic variables such as age, gender and education already have high discriminative accuracy for AD and as such additional variables would need to have large effect sizes to markedly improve model performance [111].

Due to the polygenic nature of AD, it is has been suggested that aggregating the effects of many loci across the genome, rather than restricting analysis to a small number of risk loci, may improve model performance [474]. LAOD genome wide polygenic risk scores composed of all SNPs that were identified by the IGAP meta-analysis to be associated with LOAD below a threshold of $p < 0.05$ and $p < 0.01$ have been associated with cross-sectional and longitudinal cognitive function respectively [213,474]. Nevertheless, a genetic risk score composed of APOE + IGAP risk loci + all genetic variants with a P-value < 0.5 only improved predictive accuracy of LOAD by 3% in comparison to a risk score of just APOE + IGAP risk loci [461].

This highlights that more complex models that investigate other genetic and non-genetic factors need to be investigated, including potential interactions between variables.

8.1.2 Role of SNPs Associated with Cognitive Function in Cognitive Decline

Amyloid and tau pathology only explain 30% of the observed variance in cognitive decline, indicating that while LOAD pathology plays an important role in the development of cognitive decline it operates in conjunction with other pathological features and features of brain aging [91,435]. Additionally, both premorbid levels of cognition and cognitive decline contribute to the development of cognitive deficits in later life, highlighting the need to understand factors that moderate both. As such, a secondary aim of this thesis was to investigate the role of selected SNPs that had been previously linked with cognitive function. In *'Association of genetic risk factors with cognitive decline: the PATH through life project'* (Chapter 4) and '*Association of AKAP6 and MIR2113 with cognitive performance in a population-based sample of older adults'* (Chapter 6) I investigated the role of 9 SNPs (*BDNF, CETP, COMT, CTNNBL1, LGALS3, PDE7A, SPON1, AKAP6*, and *MIR2113*) with cogntive decline.

To date, much of the genetic research in cogntive decline has focused on candidate genes with a biologicaly plausiable role in cogntive performance. Studies of candidate genes have produced inconsistent results often due to insufficent sample sizes leading to false postive and false negative assocations. Two of the most widely studied such genes are COMT, which encodes the neurotransmiter catechol-O-methyl transferase, and BDNF, which encodes the neurotrophin brain-derived neurotrophic factor [104,436,475]. The *COMT* VAL158MET and *BDNF* VAL66MET polymorphisms have been assoicated with excutive function, episodic memory and working memory, though the results are inconsistent between studies (reviewed in [104,436,475]). In this work, the *BDNF* MET allele was associated with worse working memory performance at baseline and the *COMT* MET allele was assoicated with a faster rate of decline in episodic memory.

In contrast to candidate gene approaches, genome wide assocation studies provide a hypothesis free approach that allows for the intetergation of hundreds of thousands SNPs simultaneously to identify robust assocations with a phenoytpe. A GWAS in 1073 Swiss identified *CTNNBL1* as being associated with cross-sectional verbal memory [348], while in this work *CTNNBL1* was associated with a reduced rate of decline episodic memory. A second GWAS in 749 subjects identifed a SNP in *PDE7A* associated with reduced rate of cognitive decline [116]. Our replication of

this study found that *PDE7A* was assoicated with worse perforamnce at baseline in working memory and a reduced rate of decline of perceptual speed.

In two of the largest GWAS's performed to date, by the CHARGE consortium (n = 53,949) and in a follow-up study performed in the UK Biobank (n = 112,151), SNPs within *MIR2113* were associated with general cognitive function, educational attainment, and verbal-numerical reasoning. SNPs within *AKAP6* were associated with general cognitive function, verbal-numerical reasoning, and reaction time [122,133]. In the work presented here, *AKAP6* was not associated with decline in any of the cognitive abilities tested, although we did replicate the previously observed association with baseline cognitive performance, with *AKAP6* associated with worse performance in episodic memory, working memory, vocabulary and perceptual speed. *MIR2113* was associated with an accelerated rate of decline in episodic memory, although we did not replicate the previous findings with baseline cognitive performance.

As with the LOAD risk loci, the effect sizes of these SNPs was small and the amount of variance explained by the SNPs was negligible. These results reflect that cognitive function is a polygenic trait composed of many SNPs of small effect size [122].

8.1.3 Role of Environmental and Lifestyle in Cognitive Decline

LOAD is increasingly understood as a multifactorial neurodegenerative disease, with a long-term, complex, and dynamic etiology. Although genetic variation explains 53% of the variance in AD [106], a variety of biological, health, environmental and lifestyle risk and protective factors can influence whether an individual's genetic predisposition to developing AD is elevated, exacerbated, buffered, or protected. Genetic variants, in contrast to environmental and lifestyle risk factors, are non-modifiable limiting their ability to be used in medical interventions or to modify individual behaviour to reduce risk. It has been estimated that one third of AD cases worldwide may be attributed to modifiable risk factors (see subsection 1.4) and that reducing the prevalence of modifiable risk factors by 10% per decade may reduce the prevalence of AD by 8% by 2050 [476]. As such it is important to further explore role of environmental and lifestyle risk factors in cognitive decline, and their potential interactions with genetic risk factors.

In *'Interactive Effect of APOE Genotype and Blood Pressure on Cognitive Decline: The PATH Through Life Study'* (Chapter 3), I investigated the interaction between *APOE* genotype and hypertension. I found evidence that the *APOE*-hypertension interaction has a significant effect on rate of decline in episodic memory, verbal ability and a composite global cognition measure. In contrast, no significant interaction was observed between *APOE* and Mean Arterial Pressure, possibly due to a confounding effect of hypertension medication, since individuals with controlled hypertension have mean arterial pressure in the normal range.

In *'ANU-ADRI and not Genetic Risk score predicts MCI in a cohort of older adults followed for 12 years'* (Chapter 7) I examined the association of both a weighted AD genetic risk score (GRS) and an environmental and lifestyle risk score with the risk of transitioning to cognitive impairment. The GRS was associated with an increased risk of transitioning to dementia, while the environmental and lifestyle risk score was associated with an increased risk of transitioning to cognitive impairment.

8.1.4 Limitations

The presented findings in this thesis should be interpreted in conjunction with some study limitations. Firstly, the participants in the 60+ cohort have on average a higher level of education (63% with post-school qualifications vs 45%) and a higher socio-economic status (26% of the sample report an income below the national average) then the general Australian Population [322]. As these factors are associated with reduced risk of cognitive decline, cognitive impairment and dementia, this may limit the ability to detect associations between risk factors, and in particular genetic variants, and cognitive performance. Second, the sample is relatively 'young' and only at wave 4 have participants began to experience a higher frequency of transitions between cognitive states. As such, to date the prevalence of MCI and dementia in PATH is low, potentially limiting our ability to detect associations between risk factors and cognitive impairment. Third, the PATH is predominantly of European ancestry, potentially limiting the generalizability of the results in this study to other ethnicities and, due to the limited genetic data available, we are unable to account for population stratification. Fourth, the SNPs genotyped as part of this study that were previously identified by GWAS are the most strongly associated with the trait of

interest. These SNPs are unlikely to be the causal variants, but are expected to be in linkage disequilibrium with other genetic variation that causes the observed associations with cognitive function. Fifth, while the attrition rate has been relatively low between waves, there may have been differential attrition as a result of individuals who later became severely impaired and demented. This may bias results as these individuals would not be excluded from our analysis and are more likely to experience faster rates of cognitive decline [451]. Sixth, despite excluding participants with dementia or cognitive impairment at an each wave in the relevant analysis, it is still possible that individuals in the preclinical phase of dementia were included in the analysis, potentially biasing results [93]. Finally, due to the breadth of the study, PATH is predominately limited to self-reported or lay administrated measures, in contrast to clinical biomarkers and assessments of disease outcomes. In particular biomarkers of AD (e.g. CSF or PET imaging of Tau and Aβ) are not available.

An additional limitation of the work presented in this thesis is the possibility of type 1 errors, particularly for chapters 4 & 5, due to the issue of multiple testing. Due to the large number of genetic variants investigated, in combination with multiple outcomes and the longitudinal analysis, numerous hypothesis tests were performed. This increases the probability of reporting false positives (Type I errors) by falsely rejecting the true null hypothesis. To account for the increased probability of type I errors, significance level adjustment is often employed to account for multiple testing and control for study-wide error rates, and thus lower the probability of type 1 errors. Typically, Bonferroni correction is used to adjust for multiple testing, as was the case in this thesis, whereby the significance level for rejecting the null hypothesis is adjusted such that the significance level one would normally use if only one test was performed (α) is divided by the number of tests performed (n). Bonferroni correction however is often overly conservative and can result in an increased probability of type II errors, in which the null hypothesis is falsely rejected [477]. A key assumption of Bonferroni correction is that all hypothesis tests are independent of each other. However, this assumption is often violated in the context of longitudinal studies with multiple outcomes resulting in Bonferroni correction been overly conservative leading to increased type II errors [477]. This is the case with the

work presented in this thesis as firstly, the cognitive outcomes are highly correlated with each other, and secondly, the baseline, linear and quadratic rate of change coefficients are also correlated. As such, when correcting for multiple testing, a less conservative Bonferroni correction was used in which α was divided by the number of genetic variants tested. Future analysis using novel methods that can account for correlated outcomes, both in the context of Bonferroni correction [478] or false discovery rates [479], or by using of Bayesian modeling is warranted [480].

8.1.5 Strengths

Despite the limitations associated with PATH, the study design has a number of strengths that allows for robust statistical modelling of the association of risk factors with cognitive performance. First, this study investigated cognitive change in a randomly selected community dwelling cohort of older adults, and as such the results are likely generalizable to other populations of older adults. Second, the participants have been followed for 12 years with four follow-up interviews assessing four separate cognitive domains, allowing for the examination of non-linear declines across a broad spectrum of cognitive abilities. Third, while specific clinical biomarkers of disease outcome are not available, the concurrent availability of broad spectrum of variables including demographics, stressors, physical measures, cognitive measures, mental health, psychological scales, general health and genetic variants is a strength. Finally, the PATH cohort was recruited from a narrow age-band, reducing the impact of age-differences on findings allowing for the discrimination between age and cohort effects.

8.1.6 Conclusions

In summary, the research presented in this thesis provides valuable information about the association of common genetic variants with cognitive performance in a large well-characterized cohort. The results suggest that a subset of the AD risk loci are associated with cognitive performance, but effect sizes are small. This suggests that individual AD-related genetic markers may have limited use in identifying individuals at risk of cognitive decline, especially in relation to

environmental and lifestyle risk factors. Nevertheless, these results from the PATH study open up several new lines of inquiry for future research.

8.2 Future Directions

8.2.1 Are AD Genetic Variants Associated with Cognitive Performance in 'Robust' Cognitively Normal Individuals?

Future work investigating the association of AD risk loci with cognitive function needs to account for the inclusion of participants with preclinical dementia. Cognitively normal individuals with abnormal levels of amyloid-β and tau experience a faster rate of cognitive decline compared to individuals with normal measures of amyloid and tau or abnormal measures of only amyloid or tau [481]. Furthermore, inclusion of individuals with neuroimaging and biomarker evidence of preclinical AD greatly exaggerates age-related cognitive decline in 'cognitively normal' populations [93]. As such, examining the association of AD risk loci with cognitive performance separately in 'robust' normal participants (i.e., those with normal measures of amyloid and tau) and preclinical AD participants may further elucidate the role of these loci in normal cognitive aging.

A gold standard approach would use neuroimaging or CSF biomarkers to inform the classification of preclinical AD. In the absence of neuroimaging or CSF biomarkers, other methods for identifying the preclinical stages of AD are available. A greater difference in a discrepancy between fluid and crystallized cognitive ability is associated with greater Aβ disposition and a thinner cortex in AD-vulnerable regions, and may, therefore, be a marker of preclinical AD [482]. Alternatively, using risk scores, such as the ANU-ADRI, to predict future risk of developing AD may be used to segregate the population.

8.2.2 Investigating the Role of AD Loci Involved in the same Biological Pathways or Stage of Pathogenesis

As highlighted in subsection 1.2, the pathogenesis of LOAD spans decades and progresses through preclinical, MCI and dementia stages, with the underlying pathological processes starting with amyloidosis followed by hyperphosphorylated tau accumulation and subsequent structural, functional and cognitive declines [54]. As such, individual LOAD risk loci may be involved at

specific stages in LOAD pathogenesis, and this may influence whether the loci are associated with processes that predispose, initiate or propagate cognitive decline. Therefore, rather than investigating if all known LOAD risk loci are associated with cognitive function, focusing on a subset of LOAD risk loci based on known associations with LOAD neuropathology or transitions between cognitive states may provide more consistent associations. Constructing separate GRSs based on known similarities between risk loci may also prove to be more informative.

In AD case/control autopsies *ABCA7, BIN1, CASS4, MEF2C, PICALM, MS4A6A, CD33* and *CR1* have been associated with neurotic plaque burden and *ABCA7, BIN1, CASS4, MEF2C, PICALM, CLU, SORL1* and *ZCWPW1* with neurofibrillary tangles [128,142]. In relation to transitions between cognitive states, associations have been reported between LOAD risk loci *CD2AP*, *CLU, MS4A6A* and *INPP5D* and progression from normal cognition to dementia [448]; between *CLU*, *CR1*, and *NME8* and progression from MCI to dementia [448-450]; between *INPP5D*, *MEFC2, EPHA1, PT2KB, FERMT2, CASS4* and rate of progression in AD [163]; and between *PICALM* and *MS4A6A* and progression to MCI/Dementia from normal cognition normal [120]. However, these studies are often limited by examining the association between only two cognitive states. The Multi-state Models outlined in Chapter 5 provide a methodology for analysing a more realistic model of cognitive change in which transition between normal cognition, cognitive impairment, dementia and death can be modelled simultaneously.

8.2.3 Gene x Gene Interactions

Known loci for AD only explain 30% of the genetic variance associated with AD, with some of the unexplained variance likely to be accounted for by gene-gene interactions (epistasis) [106,483]. An assumption underlying the construction of LOAD GRS is that the effects of the individual loci exhibit independent, additive and cumulative effects. However, this is likely to be an oversimplification, with combinations of individual genetic variants interacting to affect a phenotype [303]. Initial analyses of epistasis in AD was based on hypothesis-driven approaches and resulted in over 100 claims of epistasis, of which only 27 could be replicated in later comprehensive analyses. The vast majority of these were with *APOE* [484]. The first exhaustive genome-wide association interaction analysis only identified a single gene-gene interaction between *KHDRBS2* and *CRYL1,* which was supported

by meta-analysis of 5 additional replication cohorts [485]. In comparison, a study using *a priori* evidence to select SNP-SNP models based on known gene or protein interactions or participation in common pathways and processes identified significant interactions across 13 data sets between *SIRT1* x *ABCB1*, *PSAP* x *PEBP4*, and *GRIN2B* x *ADRA1A* [486]. Investigation of epistasis between known LOAD risk loci has been limited to the loci identified in the initial GWAS studies, with only an interaction between *CLU-MS4AE* being replicated [305,486-488], while interactions between *APOE-ABCA7* and *APOE-CD33* have been associated with cognitive function [118,489]. Novel methods for evaluating gene-gene interactions such as AGGrEGATOr, which uses a gene-level approach to jointly models all SNPs within a genomic region [490]; aggregated-multifactor dimensionality reduction, a method that exhaustively searches for significant gene-gene interactions and constructs an aggregated epistasis enriched risk score [491]; and machine learning methods that overcome the limitations associated with traditional regression methods [492], will allow for a more robust analysis of epistasis in AD and cognition. Incorporating this information into additive GRS may further improve their predictive power and explain some of the missing variance associated with AD and cognition.

8.2.4 Gene x Environment Interactions

Genetic variation, in total, accounts for 50% of the phenotypic variance in LOAD, highlighting that environmental and lifestyle risk factors also play an important role in the development of LOAD. However, genetic and environmental factors do not act independently of each other but are likely to interact with each other such that environmental exposures may have differential effects that depend on individual genetic risks and vice versa. To date, much of the research on gene-environment interactions in LOAD or LOAD endophenotypes have focused on interactions with *APOE,* with interactions observed between *APOE* and smoking [258], depression [493], vascular risk factors [494], cognitive activity [495], and physical activity [496]. Interactions between *APOE* and depression, education, BMI, diet, and blood pressure have also been associated with cognitive performance [266,497-500]. Interactions between the non-*APOE* risk loci and environmental factors have yet to be extensively investigated, although associations have been observed for risk scores composed of *PICALM-BIN1-CLU* and physical activity;

APOE-CLU-CR1-PICALM and diabetes; and *CLU* and Mediterranean diet [306-308]. As such, further investigation of the interactions between LOAD risk loci and environmental factors are needed, with a particular emphasis on risk factors that share the same underlying biological pathways. The inclusion of this information into risk prediction models would improve predictive accuracy and facilitate prevention strategies.

8.2.5 Mendelian Randomization

The observed associations of environmental and lifestyle risk factors with cognitive impairment or dementia may be subject to reverse causation or confounding and thus may overestimate the cognitive benefits associated with environmental and lifestyle interventions. Due to the difficulties and ethical implications of implementing large scale randomized control trials to evaluate the causal relationships between environmental and lifestyle risk factors and cognitive impairment or dementia, alternative approaches are needed to evaluate causality of observed associations. Mendelian Randomization is one method that uses genetic variants as proxies for environmental exposures to provide an estimate of the causal association between an intermediate exposure and a disease outcome [501]. Mendelian randomization is akin to a 'genetically randomized trial' due to the random allocation of genotypes from parents to offspring and are thus not affected by reverse causation and are independent of confounding factors that may influence disease outcomes [502]. The genetic variants used in Mendelian randomization act as an instrumental variable under the following assumptions: 1) it is associated with exposure; 2) it is independent of measured or unmeasured confounders and; 3) it is associated with the outcome via the causal effect of the exposure [502]. If the assumptions hold for the genetic variant, an association between a genetic variant and a disease outcome would indicate a causal relationship between the exposure and disease outcome. In contrast to standard genetic epidemiological approaches that aim to quantify the magnitude of a genetic variants influence on disease, Mendelian randomization aims to use the observed associations to demonstrate the causal influence of modifiable environmental and lifestyle risk factors on disease outcomes. Using Mendelian randomization to estimate the causal effects of potentially modifiable risk factors on cognitive impairment or dementia would inform the extent to which interventions targeted

at modifiable risk factors can reduce the risk of cognitive impairment and dementia.

8.2.5 Utilizing AD Environmental, Lifestyle and Genetic Risk Factors in Predictive Modelling

One reason for identifying risk factors associated with dementia or its preclinical stages is so that they can be included in risk prediction models. Such predictive models are essential for public health, facilitating the implementation of population-based prevention strategies; for clinical interventions aimed at prevention of dementia using personalized medicine; and for research to identify high-risk individuals for improving inclusion of participants into dementia trials [503]. Additive regression methods that combine genetic and clinical risk scores, such as those constructed in this study, into an easily interpretable report for clinical use are already available [504]. More sophisticated models are required, however, with a particular focus on identifying high-risk individuals who are likely to develop dementia in the near term (3-years) or long-term (10-5 years) and that account for confounding factors that may influence disease onset and potential interactions between variables [291,503]. Furthermore, these models need to leverage the ever-increasing data that is available, both for environmental and lifestyle risk factors due to the increasing availability of electronic health records [505] and genomic data as a consequence of the falling costs of whole genome sequencing [506,507]. Data mining and machine learning methods such as Random Forests [508], Stochastic Gradient Boosting [509] and Support Vector Machines [510] offer an alternative approach to traditional classification methods such as logistic regression. These methods are particularly useful for analysing high dimensional datasets that have a larger number of predictor variables in comparison to observations, and in tree-based methods, implicitly accounting for interactions between variables [511,512]. In addition to constructing highly predictive models, machine learning methods often provide measures of variable importance which can be used for feature selection to identify the key variables in predictive models. The selected features can then be used to develop predictive models targeted at specific subpopulations (i.e. clinical vs. population, near vs. long term) for identifying individuals at risk of developing dementia [511,512].

References

[1] Spearman C. " General Intelligence," objectively determined and measured. The American Journal of Psychology 1904;15:201–92.

[2] Carroll JB. Human cognitive abilities: A survey of factor-analytic studies. Cambridge University Press; 1993.

[3] Hampel H, Lista S. Dementia: The rising global tide of cognitive impairment. Nat Rev Neurol 2016;12:131–2.

[4] Salthouse TA. Decomposing age correlations on neuropsychological and cognitive variables. J Int Neuropsychol Soc 2009;15:650–61.

[5] Boyle PA, Yu L, Wilson RS, Gamble K, Buchman AS, Bennett DA. Poor decision making is a consequence of cognitive decline among older persons without Alzheimer's disease or mild cognitive impairment. PLoS ONE 2012;7:e43647.

[6] Tucker-Drob EM. Neurocognitive functions and everyday functions change together in old age. Neuropsychology 2011;25:368–77.

[7] Yam A, Marsiske M. Cognitive longitudinal predictors of older adults' self-reported IADL function. J Aging Health 2013;25:163S–85S.

[8] Kobayashi LC, Wardle J, Wolf MS, Wagner von C. Aging and Functional Health Literacy: A Systematic Review and Meta-Analysis. J Gerontol B Psychol Sci Soc Sci 2016;71:445–57.

[9] Amieva H, Jacqmin-Gadda H, Orgogozo J-M, Le Carret N, Helmer C, Letenneur L, et al. The 9 year cognitive decline before dementia of the Alzheimer type: a prospective population-based study. Brain 2005;128:1093–101.

[10] Zahodne LB, Manly JJ, MacKay-Brandt A, Stern Y. Cognitive declines precede and predict functional declines in aging and Alzheimer's disease. PLoS ONE 2013;8:e73645.

[11] Batterham PJ, Christensen H, Mackinnon AJ. Fluid intelligence is independently associated with all-cause mortality over 17 years in an elderly community sample: An investigation of potential mechanisms. Intelligence 2009;37:551–60.

[12] Shipley BA, Der G, Taylor MD, Deary IJ. Association between mortality and cognitive change over 7 years in a large representative sample of UK residents. Psychosom Med 2007;69:640–50.

[13] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999;56:303–8.

[14] Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund L-O, et al. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med 2004;256:240–6.

[15] Roberts R, Knopman DS. Classification and epidemiology of MCI. Clin Geriatr Med 2013;29:753–72.

[16] Luck T, Luppa M, Briel S, Riedel-Heller SG. Incidence of mild cognitive impairment: a systematic review. Dement Geriatr Cogn Disord 2010;29:164–75.

[17] Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand 2009;119:252–65.

[18] Ward A, Tardiff S, Dye C, Arrighi HM. Rate of conversion from prodromal

Alzheimer“s disease to Alzheimer”s dementia: a systematic review of the literature. Dement Geriatr Cogn Dis Extra 2013;3:320–32.
[19] Tifratene K, Robert P, Metelkina A, Pradier C, Dartigues J-F. Progression of mild cognitive impairment to dementia due to AD in clinical settings. Neurology 2015;85:331–8.
[20] Petersen RC. Mild Cognitive Impairment. Continuum (Minneap Minn) 2016;22:404–18.
[21] Malek-Ahmadi M. Reversion From Mild Cognitive Impairment to Normal Cognition: A Meta-Analysis. Alzheimer Dis Assoc Disord 2016;Published Ahead of Print:1–7.
[22] Roberts RO, Knopman DS, Mielke MM, Cha RH, Pankratz VS, Christianson TJH, et al. Higher risk of progression to dementia in mild cognitive impairment cases who revert to normal. Neurology 2014;82:317–25.
[23] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer‘s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement 2011;7:270–9.
[24] Prince MJ. World Alzheimer Report 2015: The Global Impact of Dementia: an Analysis of Prevalence, Incidence, Cost and Trends. 2015.
[25] Alzheimer’s Association. 2015 Alzheimer's disease facts and figures. Alzheimers Dement 2015;11:332–84.
[26] Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol n.d.;13:614–29.
[27] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer’s disease: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–9.
[28] Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell 2012;148:1204–22.
[29] Holtzman DM, Morris JC, Goate AM. Alzheimer's disease: the challenge of the second century. Sci Transl Med 2011;3:77sr1.
[30] Sisodia SS. Beta-amyloid precursor protein cleavage by a membrane-bound protease. Proc Natl Acad Sci USA 1992;89:6075–9.
[31] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 1999;286:735–41.
[32] Sisodia SS, St George-Hyslop PH. gamma-Secretase, Notch, Abeta and Alzheimer's disease: where do the presenilins fit in? Nat Rev Neurosci 2002;3:281–90.
[33] Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM. Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid in vivo. Nat Med 2006;12:856–61.
[34] Sagare A, Deane R, Bell RD, Johnson B, Hamm K, Pendu R, et al. Clearance of amyloid-beta by circulating lipoprotein receptors. Nat Med 2007;13:1029–31.
[35] Fuentealba RA, Liu Q, Zhang J, Kanekiyo T, Hu X, Lee J-M, et al. Low-density lipoprotein receptor-related protein 1 (LRP1) mediates neuronal Abeta42 uptake and lysosomal trafficking. PLoS ONE 2010;5:e11884.

[36] Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, et al. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. Nat Med 2000;6:143–50.
[37] Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. J Biol Chem 1998;273:32730–8.
[38] Mueller-Steiner S, Zhou Y, Arai H, Roberson ED, Sun B, Chen J, et al. Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. Neuron 2006;51:703–14.
[39] Carvalho KM, Franca MS, Camarao GC, Ruchon AF. A new brain metalloendopeptidase which degrades the Alzheimer beta-amyloid 1-40 peptide producing soluble fragments without neurotoxic effects. Braz J Med Biol Res 1997;30:1153–6.
[40] Hemming ML, Selkoe DJ. Amyloid beta-protein is degraded by cellular angiotensin-converting enzyme (ACE) and elevated by an ACE inhibitor. J Biol Chem 2005;280:37644–50.
[41] Eckman EA, Watson M, Marlow L, Sambamurti K, Eckman CB. Alzheimer's disease beta-amyloid peptide is increased in mice deficient in endothelin-converting enzyme. J Biol Chem 2003;278:2081–4.
[42] Ledesma MD, Da Silva JS, Crassaerts K, Delacourte A, De Strooper B, Dotti CG. Brain plasmin enhances APP alpha-cleavage and Abeta degradation and is reduced in Alzheimer's disease brains. EMBO Rep 2000;1:530–5.
[43] Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology 2002;58:1791–800.
[44] Wang Y, Mandelkow E. Tau in physiology and pathology. Nat Rev Neurosci 2016;17:5–21.
[45] Iqbal K, Liu F, Gong C-X. Tau and neurodegenerative disease: the story so far. Nat Rev Neurol 2016;12:15–27.
[46] Braak E, Braak H, Mandelkow EM. A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. Acta Neuropathol 1994;87:554–67.
[47] Alonso AD, Beharry C, Corbo CP, Cohen LS. Molecular mechanism of prion-like tau-induced neurodegeneration. Alzheimers Dement 2016;In Press.
[48] Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, et al. Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. J Biol Chem 2013;288:1856–70.
[49] Guo JL, Lee VM-Y. Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. J Biol Chem 2011;286:15317–31.
[50] Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. J Biol Chem 2012;287:3842–9.
[51] Alonso AC, Grundke-Iqbal I, Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. Nat Med 1996;2:783–7.
[52] Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of

Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006;112:389–404.
[53] Musiek ES, Holtzman DM. Three dimensions of the amyloid hypothesis: time, space and 'wingmen'. Nat Neurosci 2015;18:800–6.
[54] Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 2013;12:207–16.
[55] Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, et al. Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. Nature 1992;360:672–4.
[56] Chavez-Gutierrez L, Bammens L, Benilova I, Vandersteen A, Benurwar M, Borgers M, et al. The mechanism of gamma-Secretase dysfunction in familial Alzheimer disease. Embo J 2012;31:2261–74.
[57] Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012;367:795–804.
[58] Reed LA, Grabowski TJ, Schmidt ML, Morris JC, Goate A, Solodkin A, et al. Autosomal dominant dementia with widespread neurofibrillary tangles. Ann Neurol 1997;42:564–72.
[59] Lindquist SG, Holm IE, Schwartz M, Law I, Stokholm J, Batbayli M, et al. Alzheimer disease-like clinical phenotype in a family with FTDP-17 caused by a MAPT R406W mutation. Eur J Neurol 2008;15:377–85.
[60] Hurtado DE, Molina-Porcel L, Iba M, Aboagye AK, Paul SM, Trojanowski JQ, et al. Abeta accelerates the spatiotemporal progression of tau pathology and augments tau amyloidosis in an Alzheimer mouse model. Am J Pathol 2010;177:1977–88.
[61] Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997;41:17–24.
[62] Ingelsson M, Fukumoto H, Newell KL, Growdon JH, Hedley-Whyte ET, Frosch MP, et al. Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. Neurology 2004;62:925–31.
[63] Giannakopoulos P, Herrmann FR, Bussiere T, Bouras C, Kovari E, Perl DP, et al. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. Neurology 2003;60:1495–500.
[64] Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 1992;42:631–9.
[65] Jack CR, Wiste HJ, Vemuri P, Weigand SD, Senjem ML, Zeng G, et al. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. Brain 2010;133:3336–48.
[66] Jack CR, Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, et al. Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer“s disease: implications for sequence of pathological events in Alzheimer”s disease. Brain 2009;132:1355–65.
[67] Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, et al. Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol

2013;12:357–67.
[68] Fennema-Notestine C, Hagler DJ, McEvoy LK, Fleisher AS, Wu EH, Karow DS, et al. Structural MRI biomarkers for preclinical and mild Alzheimer's disease. Hum Brain Mapp 2009;30:3238–53.
[69] Schuff N, Tosun D, Insel PS, Chiang GC, Truran D, Aisen PS, et al. Nonlinear time course of brain volume loss in cognitively normal and impaired elders. Neurobiol Aging 2012;33:845–55.
[70] Petersen RC, Parisi JE, Dickson DW, Johnson KA, Knopman DS, Boeve BF, et al. Neuropathologic features of amnestic mild cognitive impairment. Arch Neurol 2006;63:665–72.
[71] Knopman DS, Parisi JE, Salviati A, Floriach-Robert M, Boeve BF, Ivnik RJ, et al. Neuropathology of cognitively normal elderly. J Neuropathol Exp Neurol 2003;62:1087–95.
[72] Elobeid A, Soininen H, Alafuzoff I. Hyperphosphorylated tau in young and middle-aged subjects. Acta Neuropathol 2012;123:97–104.
[73] Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 1999;45:358–68.
[74] Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin M-L, Yardin C, et al. Tau protein kinases: Involvement in Alzheimer's disease. Ageing Res Rev 2013;12:289–309.
[75] Honjo K, Black SE, Verhoeff NPLG. Alzheimer's disease, cerebrovascular disease, and the beta-amyloid cascade. Can J Neurol Sci 2012;39:712–28.
[76] Guix FX, Wahle T, Vennekens K, Snellinx A, Chavez-Gutierrez L, Ill-Raga G, et al. Modification of gamma-secretase by nitrosative stress links neuronal ageing to sporadic Alzheimer's disease. EMBO Mol Med 2012;4:660–73.
[77] Wahlster L, Arimon M, Nasser-Ghodsi N, Post KL, Serrano-Pozo A, Uemura K, et al. Presenilin-1 adopts pathogenic conformation in normal aging and in sporadic Alzheimer's disease. Acta Neuropathol 2013;125:187–99.
[78] Kukreja L, Kujoth GC, Prolla TA, Van Leuven F, Vassar R. Increased mtDNA mutations with aging promotes amyloid accumulation and brain atrophy in the APP/Ld transgenic mouse model of Alzheimer's disease. Mol Neurodegener 2014;9:16.
[79] Liu Y, Liu X, Hao W, Decker Y, Schomburg R, Fulop L, et al. IKKbeta deficiency in myeloid cells ameliorates Alzheimer's disease-related symptoms and pathology. J Neurosci 2014;34:12982–99.
[80] Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. J Neuropathol Exp Neurol 2011;70:960–9.
[81] Jansen WJ, Ossenkoppele R, Knol DL, Tijms BM, Scheltens P, Verhey FRJ, et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. Jama 2015;313:1924–38.
[82] Scholl M, Lockhart SN, Schonhaut DR, O'Neil JP, Janabi M, Ossenkoppele R, et al. PET Imaging of Tau Deposition in the Aging Human Brain. Neuron 2016;89:971–82.
[83] Johnson KA, Schultz A, Betensky RA, Becker JA, Sepulcre J, Rentz D, et al. Tau positron emission tomographic imaging in aging and early Alzheimer disease. Ann Neurol 2016;79:110–9.
[84] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease:

Recommendations from the National Institute on Aging-Alzheimer“s Association workgroups on diagnostic guidelines for Alzheimer”s disease. Alzheimers Dement 2011;7:280–92.
[85] Chen X, Li M, Wang S, Zhu H, Xiong Y, Liu X. Pittsburgh compound B retention and progression of cognitive status - a meta-analysis. Eur J Neurol 2014;21:1060–7.
[86] Jack CRJ, Therneau TM, Wiste HJ, Weigand SD, Knopman DS, Lowe VJ, et al. Transition rates between amyloid and neurodegeneration biomarker states and to dementia: a population-based, longitudinal cohort study. Lancet Neurol 2016;15:56–64.
[87] Vos SJ, Xiong C, Visser PJ, Jasielec MS, Hassenstab J, Grant EA, et al. Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. Lancet Neurol 2013;12:957–65.
[88] Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement 2016;12:292–323.
[89] Nelson PT, Braak H, Markesbery WR. Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship. J Neuropathol Exp Neurol 2009;68:1–14.
[90] Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol 2012;71:362–81.
[91] Boyle PA, Wilson RS, Yu L, Barr AM, Honer WG, Schneider JA, et al. Much of late life cognitive decline is not due to common neurodegenerative pathologies. Ann Neurol 2013;74:478–89.
[92] Yu L, Boyle PA, Leurgans S, Schneider JA, Bennett DA. Disentangling the effects of age and APOE on neuropathology and late life cognitive decline. Neurobiol Aging 2014;35:819–26.
[93] Hassenstab J, Chasse R, Grabow P, Benzinger TLS, Fagan AM, Xiong C, et al. Certified normal: Alzheimer's disease biomarkers and normative estimates of cognitive functioning. Neurobiol Aging 2016;43:23–33.
[94] Yu L, Boyle PA, Segawa E, Leurgans S, Schneider JA, Wilson RS, et al. Residual decline in cognition after adjustment for common neuropathologic conditions. Neuropsychology 2015;29:335–43.
[95] Hedden T, Oh H, Younger AP, Patel TA. Meta-analysis of amyloid-cognition relations in cognitively normal older adults. Neurology 2013;80:1341–8.
[96] Riley KP, Jicha GA, Davis D, Abner EL, Cooper GE, Stiles N, et al. Prediction of preclinical Alzheimer's disease: longitudinal rates of change in cognition. J Alzheimers Dis 2011;25:707–17.
[97] Boyle PA, Yu L, Wilson RS, Schneider JA, Bennett DA. Relation of neuropathology with cognitive decline among older persons without dementia. Front Aging Neurosci 2013;5:50.
[98] Donohue MC, Sperling RA, Salmon DP, Rentz DM, Raman R, Thomas RG, et al. The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. JAMA Neurol 2014;71:961–70.
[99] Petersen RC, Wiste HJ, Weigand SD, Rocca WA, Roberts RO, Mielke MM, et al. Association of Elevated Amyloid Levels With Cognition and Biomarkers in Cognitively Normal People From the Community. JAMA Neurol 2016;73:85–92.

[100] Wirth M, Oh H, Mormino EC, Markley C, Landau SM, Jagust WJ. The effect of amyloid β on cognitive decline is modulated by neural integrity in cognitively normal elderly. Alzheimers Dement 2013;9:687–698.e1.
[101] Mormino EC, Betensky RA, Hedden T, Schultz AP, Amariglio RE, Rentz DM, et al. Synergistic effect of β-amyloid and neurodegeneration on cognitive decline in clinically normal individuals. JAMA Neurol 2014;71:1379–85.
[102] Davies G, Tenesa A, Payton A, Yang J, Harris SE, Liewald D, et al. Genome-wide association studies establish that human intelligence is highly heritable and polygenic. Mol Psychiatry 2011;16:996–1005.
[103] Deary IJ, Yang J, Davies G, Harris SE, Tenesa A, Liewald D, et al. Genetic contributions to stability and change in intelligence from childhood to old age. Nature 2012;482:212–5.
[104] Payton A. The Impact of Genetic Research on our Understanding of Normal Cognitive Ageing: 1995 to 2009. Neuropsychol Rev 2009;19:451–77.
[105] Harris SE, Deary IJ. The genetics of cognitive ability and cognitive ageing in healthy older people. Trends Cogn Sci 2011;15:388–94.
[106] Ridge PG, Hoyt KB, Boehme K, Mukherjee S, Crane PK, Haines JL, et al. Assessment of the genetic variance of late-onset Alzheimer's disease. Neurobiol Aging 2016;41:200.e13–20.
[107] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–3.
[108] Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009;41:1088–93.
[109] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert J-C, Carrasquillo MM, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 2011;43:429–35.
[110] Naj AC, Jun G, Beecham GW, Wang L-S, Vardarajan BN, Buros J, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 2011;43:436–41.
[111] Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. Jama 2010;303:1832–40.
[112] Lambert J-C, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009;41:1094–9.
[113] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 2013;45:1452–8.
[114] Gui H, Jiang CQ, Cherny SS, Sham PC, Xu L, Liu B, et al. Influence of Alzheimer's disease genes on cognitive decline: the Guangzhou Biobank Cohort Study. Neurobiol Aging 2014;35:2422.e3–8.
[115] Zhang C, Pierce BL. Genetic susceptibility to accelerated cognitive decline in the US Health and Retirement Study. Neurobiol Aging 2014;35:1512.e11–8.
[116] De Jager PL, Shulman JM, Chibnik LB, Keenan BT, Raj T, Wilson RS, et al. A

genome-wide scan for common variants affecting the rate of age-related cognitive decline. Neurobiol Aging 2012;33:1017.e1–1017.e15.
[117] Louwersheimer E, Wolfsgruber S, Espinosa A, Lacour A, Heilmann-Heimbach S, Alegret M, et al. Alzheimer's disease risk variants modulate endophenotypes in mild cognitive impairment. Alzheimers Dement 2016;12:872–81.
[118] Engelman CD, Koscik RL, Jonaitis EM, Okonkwo OC, Hermann BP, La Rue A, et al. Interaction between two cholesterol metabolism genes influences memory: findings from the Wisconsin Registry for Alzheimer's Prevention. J Alzheimers Dis 2013;36:749–57.
[119] Verhaaren BFJ, Vernooij MW, Koudstaal PJ, Uitterlinden AG, van Duijn CM, Hofman A, et al. Alzheimer's Disease Genes and Cognition in the Nondemented General Population. Biol Psychiatry 2013;73:429–34.
[120] Carrasquillo MM, Crook JE, Pedraza O, Thomas CS, Pankratz VS, Allen M, et al. Late-onset Alzheimer“s risk variants in memory decline, incident mild cognitive impairment, and Alzheimer”s disease. Neurobiol Aging 2015;36:60–7.
[121] Davies G, Harris SE, Reynolds CA, Payton A, Knight HM, Liewald DC, et al. A genome-wide association study implicates the APOE locus in nonpathological cognitive ageing. Mol Psychiatry 2014;19:76–87.
[122] Davies G, Armstrong N, Bis JC, Bressler J, Chouraki V, Giddaluru S, et al. Genetic contributions to variation in general cognitive function: a meta-analysis of genome-wide association studies in the CHARGE consortium (N=53 949). Mol Psychiatry 2015;20:183–92.
[123] Nettiksimmons J, Tranah G, Evans DS, Yokoyama JS, Yaffe K. Gene-based aggregate SNP associations between candidate AD genes and cognitive decline. Age 2016;38:41.
[124] Vivot A, Glymour MM, Tzourio C, Amouyel P, Chene G, Dufouil C. Association of Alzheimer’s related genotypes with cognitive decline in multiple domains: results from the Three-City Dijon study. Mol Psychiatry 2015;20:1173–8.
[125] Hamilton G, Harris SE, Davies G, Liewald DC, Tenesa A, Starr JM, et al. Alzheimer's disease genes are associated with measures of cognitive ageing in the lothian birth cohorts of 1921 and 1936. Int J Alzheimers Dis 2011;2011:505984.
[126] Barral S, Bird T, Goate A, Farlow MR, Diaz-Arrastia R, Bennett DA, et al. Genotype patterns at PICALM, CR1, BIN1, CLU, and APOE genes are associated with episodic memory. Neurology 2012;78:1464–71.
[127] Thambisetty M, Beason-Held LL, An Y, Kraut M, Nalls M, Hernandez DG, et al. Alzheimer risk variant CLU and brain function during aging. Biol Psychiatry 2013;73:399–405.
[128] Chibnik LB, Shulman JM, Leurgans SE, Schneider JA, Wilson RS, Tran D, et al. CR1 is associated with amyloid plaque burden and age-related cognitive decline. Ann Neurol 2011;69:560–9.
[129] Mengel-From J, Thinggaard M, Lindahl-Jacobsen R, McGue M, Christensen K, Christiansen L. CLU genetic variants and cognitive decline among elderly and oldest old. PLoS ONE 2013;8:e79105.
[130] Mengel-From J, Christensen K, McGue M, Christiansen L. Genetic variations in the CLU and PICALM genes are associated with cognitive function in the oldest old. Neurobiol Aging 2011;32:554.e7–11.
[131] Keenan BT, Shulman JM, Chibnik LB, Raj T, Tran D, Sabuncu MR, et al. A

coding variant in CR1 interacts with APOE-ε4 to influence cognitive decline. Hum Mol Gen 2012;21:2377–88.
[132] Liu Y, Yu J-T, Wang H-F, Hao X-K, Yang Y-F, Jiang T, et al. Association between NME8 locus polymorphism and cognitive decline, cerebrospinal fluid and neuroimaging biomarkers in Alzheimer's disease. PLoS ONE 2014;9:e114777.
[133] Davies G, Marioni RE, Liewald DC, Hill WD, Hagenaars SP, Harris SE, et al. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N=112 151). Mol Psychiatry 2016;21:758–67.
[134] Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of Age, Sex, and Ethnicity on the Association Between Apolipoprotein E Genotype and Alzheimer Disease: A Meta-analysis. Jama 1997;278:1349–56.
[135] Liu C-C, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 2013;9:106–18.
[136] Wisdom NM, Callahan JL, Hawkins KA. The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. Neurobiol Aging 2011;32:63–74.
[137] Kim WS, Weickert CS, Garner B. Role of ATP-binding cassette transporters in brain lipid transport and neurological disease. J Neurochem 2008;104:1145–66.
[138] Jehle AW, Gardai SJ, Li S, Linsel-Nitschke P, Morimoto K, Janssen WJ, et al. ATP-binding cassette transporter A7 enhances phagocytosis of apoptotic cells and associated ERK signaling in macrophages. J Cell Biol 2006;174:547–56.
[139] Kim WS, Li H, Ruberu K, Chan S, Elliott DA, Low JK, et al. Deletion of Abca7 increases cerebral amyloid-beta accumulation in the J20 mouse model of Alzheimer's disease. J Neurosci 2013;33:4387–94.
[140] Fu Y, Hsiao J-HT, Paxinos G, Halliday GM, Kim WS. ABCA7 Mediates Phagocytic Clearance of Amyloid-β in the Brain. J Alzheimers Dis 2016;Preprint:1–16.
[141] Shulman JM, Chen K, Keenan BT, Chibnik LB, Fleisher A, Thiyyagura P, et al. Genetic susceptibility for Alzheimer disease neuritic plaque pathology. JAMA Neurol 2013;70:1150–7.
[142] Beecham GW, Hamilton K, Naj AC, Martin ER, Huentelman M, Myers AJ, et al. Genome-Wide Association Meta-analysis of Neuropathologic Features of Alzheimer's Disease and Related Dementias. PLoS Genet 2014;10:e1004606.
[143] International HapMap Consortium. The International HapMap Project. Nature 2003;426:789–96.
[144] Sweet RA, Seltman H, Emanuel JE, López OL, Becker JT, Bis JC, et al. Effect of Alzheimer's disease risk genes on trajectories of cognitive function in the Cardiovascular Health Study. Am J Psychiatry 2012;169:954–62.
[145] Wisdom NM, Callahan JL, Hawkins KA. The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. Neurobiol Aging 2011;32:63–74.
[146] Itoh T, De Camilli P. BAR, F-BAR (EFC) and ENTH/ANTH domains in the regulation of membrane-cytosol interfaces and membrane curvature. Biochim Biophys Acta 2006;1761:897–912.
[147] Chapuis J, Hansmannel F, Gistelinck M, Mounier A, Van Cauwenberghe C, Kolen KV, et al. Increased expression of BIN1 mediates Alzheimer genetic

risk by modulating tau pathology. Mol Psychiatry 2013;18:1225–34.
[148] Engelman CD, Baurley JW, Chiu YF, Joubert BR, Lewinger JP, Maenner MJ, et al. Detecting gene-environment interactions in genome-wide association data. Genet Epidemiol, vol. 33 Suppl 1, 2009, pp. S68–73.
[149] Dustin ML, Olszowy MW, Holdorf AD, Li J, Bromley S, Desai N, et al. A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. Cell 1998;94:667–77.
[150] Cormont M, Meton I, Mari M, Monzo P, Keslair F, Gaskin C, et al. CD2AP/CMS regulates endosome morphology and traffic to the degradative pathway through its interaction with Rab4 and c-Cbl. Traffic 2003;4:97–112.
[151] Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, et al. Alzheimer's Disease Risk Gene CD33 Inhibits Microglial Uptake of Amyloid Beta. Neuron 2013;78:631–43.
[152] Karch CM, Jeng AT, Nowotny P, Cady J, Cruchaga C, Goate AM. Expression of Novel Alzheimer's Disease Risk Genes in Control and Alzheimer's Disease Brains. PLoS ONE 2012;7:e50976.
[153] Jones SE, Jomary C. Clusterin. Int J Biochem Cell Biol 2002;34:427–31.
[154] Yerbury JJ, Poon S, Meehan S, Thompson B, Kumita JR, Dobson CM, et al. The extracellular chaperone clusterin influences amyloid formation and toxicity by interacting with prefibrillar structures. Faseb J 2007;21:2312–22.
[155] Wilson MR, Yerbury JJ, Poon S. Potential roles of abundant extracellular chaperones in the control of amyloid formation and toxicity. Mol BioSyst 2008;4:42–52.
[156] Nuutinen T, Suuronen T, Kauppinen A, Salminen A. Clusterin: a forgotten player in Alzheimer's disease. Brain Res Rev 2009;61:89–104.
[157] Liu D, Niu Z-X. The structure, genetic polymorphisms, expression and biological functions of complement receptor type 1 (CR1/CD35). Immunopharmacol Immunotoxicol 2009;31:524–35.
[158] Rogers J, Li R, Mastroeni D, Grover A, Leonard B, Ahern G, et al. Peripheral clearance of amyloid beta peptide by complement C3-dependent adherence to erythrocytes. Neurobiol Aging 2006;27:1733–9.
[159] Yamazaki T, Masuda J, Omori T, Usui R, Akiyama H, Maru Y. EphA1 interacts with integrin-linked kinase and regulates cell morphology and motility. J Cell Sci 2008;122:243–55.
[160] Davy A, Gale NW, Murray EW, Klinghoffer RA, Soriano P, Feuerstein C, et al. Compartmentalized signaling by GPI-anchored ephrin-A5 requires the Fyn tyrosine kinase to regulate cellular adhesion. Genes Dev 1999;13:3125–35.
[161] Martínez A, Otal R, Sieber BA, Ibáñez C, Soriano E. Disruption of ephrin-A/EphA binding alters synaptogenesis and neural connectivity in the hippocampus. Neuroscience 2005;135:451–61.
[162] Lai K-O, Ip NY. Synapse development and plasticity: roles of ephrin/Eph receptor signaling. Curr Opin Neurobiol 2009;19:275–83.
[163] Wang X, López OL, Sweet RA, Becker JT, DeKosky ST, Barmada MM, et al. Genetic determinants of disease progression in Alzheimer's disease. J Alzheimers Dis 2015;43:649–55.
[164] Wang H-F, Tan L, Hao X-K, Jiang T, Tan M-S, Liu Y, et al. Effect of EPHA1 genetic variation on cerebrospinal fluid and neuroimaging biomarkers in healthy, mild cognitive impairment and Alzheimer's disease cohorts. J

Alzheimers Dis 2015;44:115–23.
[165] Liang Y, Buckley TR, Tu L, Langdon SD, Tedder TF. Structural organization of the human MS4A gene cluster on Chromosome 11q12. Immunogenetics 2001;53:357–68.
[166] Eon Kuek L, Leffler M, Mackay GA, Hulett MD. The MS4A family: counting past 1, 2 and 3. Immunol Cell Biol 2016;94:11–23.
[167] Xiao Q, Gil S-C, Yan P, Wang Y, Han S, Gonzales E, et al. Role of phosphatidylinositol clathrin assembly lymphoid-myeloid leukemia (PICALM) in intracellular amyloid precursor protein (APP) processing and amyloid plaque pathogenesis. J Biol Chem 2012;287:21279–89.
[168] Zhao Z, Sagare AP, Ma Q, Halliday MR, Kong P, Kisler K, et al. Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance. Nat Neurosci 2015;18:978–87.
[169] Moreau K, Fleming A, Imarisio S, Lopez Ramirez A, Mercer JL, Jimenez-Sanchez M, et al. PICALM modulates autophagy activity and tau accumulation. Nat Commun 2014;5:4998.
[170] Carrasquillo MM, Zou F, Pankratz VS, Wilcox SL, Ma L, Walker LP, et al. Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. Nat Genet 2009;41:192–8.
[171] Trowsdale J, Knight JC. Major histocompatibility complex genomics and human disease. Annu Rev Genomics Hum Genet 2013;14:301–23.
[172] Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin U-M, Saad M, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet 2011;377:641–9.
[173] Harms AS, Cao S, Rowse AL, Thome AD, Li X, Mangieri LR, et al. MHCII is required for alpha-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. J Neurosci 2013;33:9592–600.
[174] Yu L, Chibnik LB, Srivastava GP, Pochet N, Yang J, Xu J, et al. Association of Brain DNA methylation in SORL1, ABCA7, HLA-DRB5, SLC24A4, and BIN1 with pathological diagnosis of Alzheimer disease. JAMA Neurol 2015;72:15–24.
[175] Lev S, Moreno H, Martinez R, Canoll P, Peles E, Musacchio JM, et al. Protein tyrosine kinase PYK2 involved in Ca(2+)-induced regulation of ion channel and MAP kinase functions. Nature 1995;376:737–45.
[176] Avraham H, Park SY, Schinkmann K, Avraham S. RAFTK/Pyk2-mediated cellular signalling. Cell Signal 2000;12:123–33.
[177] Dourlen P, Fernandez-Gomez FJ, Dupont C, Grenier-Boley B, Bellenguez C, Obriot H, et al. Functional screening of Alzheimer risk loci identifies PTK2B as an in vivo modulator and early marker of Tau pathology. Mol Psychiatry 2016;PrePrint.
[178] Andersen OM, Reiche J, Schmidt V, Gotthardt M, Spoelgen R, Behlke J, et al. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc Natl Acad Sci USA 2005;102:13461–6.
[179] Caglayan S, Takagi-Niidome S, Liao F, Carlo A-S, Schmidt V, Burgert T, et al. Lysosomal sorting of amyloid-beta by the SORLA receptor is impaired by a familial Alzheimer's disease mutation. Sci Transl Med 2014;6:223ra20.
[180] Dodson SE, Andersen OM, Karmali V, Fritz JJ, Cheng D, Peng J, et al. Loss of

LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease. J Neurosci 2008;28:12877–86.
[181] Guo L-H, Westerteicher C, Wang X-H, Kratzer M, Tsolakidou A, Jiang M, et al. SORL1 genetic variants and cerebrospinal fluid biomarkers of Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci 2012;262:529–34.
[182] Li X-F, Kraev AS, Lytton J. Molecular cloning of a fourth member of the potassium-dependent sodium-calcium exchanger gene family, NCKX4. J Biol Chem 2002;277:48410–7.
[183] Adeyemo A, Gerry N, Chen G, Herbert A, Doumatey A, Huang H, et al. A genome-wide association study of hypertension and blood pressure in African Americans. PLoS Genet 2009;5:e1000564.
[184] Han J, Kraft P, Nan H, Guo Q, Chen C, Qureshi A, et al. A genome-wide association study identifies novel alleles associated with hair color and skin pigmentation. PLoS Genet 2008;4:e1000074.
[185] Sulem P, Gudbjartsson DF, Stacey SN, Helgason A, Rafnar T, Magnusson KP, et al. Genetic determinants of hair, eye and skin pigmentation in Europeans. Nature Genetics 2007;39:1443–52.
[186] Larsson M, Duffy DL, Zhu G, Liu JZ, Macgregor S, McRae AF, et al. GWAS findings for human iris patterns: associations with variants in genes that influence normal neuronal pattern development. Am J Hum Genet 2011;89:334–43.
[187] Kajiho H, Saito K, Tsujita K, Kontani K, Araki Y, Kurosu H, et al. RIN3: a novel Rab5 GEF interacting with amphiphysin II involved in the early endocytic pathway. J Cell Sci 2003;116:4159–68.
[188] Chitaev NA, Troyanovsky SM. Direct Ca2+-dependent heterophilic interaction between desmosomal cadherins, desmoglein and desmocollin, contributes to cell-cell adhesion. J Cell Biol 1997;138:193–201.
[189] Viernes DR, Choi LB, Kerr WG, Chisholm JD. Discovery and development of small molecule SHIP phosphatase modulators. Med Res Rev 2014;34:795–824.
[190] Metzner A, Precht C, Fehse B, Fiedler W, Stocking C, Gunther A, et al. Reduced proliferation of CD34(+) cells from patients with acute myeloid leukemia after gene transfer of INPP5D. Gene Ther 2009;16:570–3.
[191] Leung W-H, Tarasenko T, Bolland S. Differential roles for the inositol phosphatase SHIP in the regulation of macrophages and lymphocytes. Immunol Res 2009;43:243–51.
[192] Malik M, Parikh I, Vasquez JB, Smith C, Tai L, Bu G, et al. Genetics ignite focus on microglial inflammation in Alzheimer's disease. Mol Neurodegener 2015;10:1094–12.
[193] Bienvenu T, Diebold B, Chelly J, Isidor B. Refining the phenotype associated with MEF2C point mutations. Neurogenetics 2013;14:71–5.
[194] Le Meur N, Holder-Espinasse M, Jaillard S, Goldenberg A, Joriot S, Amati-Bonneau P, et al. MEF2C haploinsufficiency caused by either microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations. J Med Genet 2010;47:22–9.
[195] Johnson ME, Deliard S, Zhu F, Xia Q, Wells AD, Hankenson KD, et al. A ChIP-seq-defined genome-wide map of MEF2C binding reveals inflammatory pathways associated with its role in bone density

determination. Calcif Tissue Int 2014;94:396–402.
[196] Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 2014;34:11929–47.
[197] Duriez B, Duquesnoy P, Escudier E, Bridoux A-M, Escalier D, Rayet I, et al. A common variant in combination with a nonsense mutation in a member of the thioredoxin family causes primary ciliary dyskinesia. Proc Natl Acad Sci USA 2007;104:3336–41.
[198] Escudier E, Duquesnoy P, Papon JF, Amselem S. Ciliary defects and genetics of primary ciliary dyskinesia. Paediatr Respir Rev 2009;10:51–4.
[199] Smith TB, Baker MA, Connaughton HS, Habenicht U, Aitken RJ. Functional deletion of Txndc2 and Txndc3 increases the susceptibility of spermatozoa to age-related oxidative stress. Free Radic Biol Med 2013;65:872–81.
[200] Shi D, Nakamura T, Nakajima M, Dai J, Qin J, Ni H, et al. Association of single-nucleotide polymorphisms in RHOB and TXNDC3 with knee osteoarthritis susceptibility: two case-control studies in East Asian populations and a meta-analysis. Arthritis Res Ther 2008;10:R54.
[201] Estrada K, Styrkarsdottir U, Evangelou E, Hsu Y-H, Duncan EL, Ntzani EE, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet 2012;44:491–501.
[202] Rosenthal SL, Kamboh MI. Late-Onset Alzheimer's Disease Genes and the Potentially Implicated Pathways. Curr Genet Med Rep 2014;2:85–101.
[203] He F, Umehara T, Saito K, Harada T, Watanabe S, Yabuki T, et al. Structural insight into the zinc finger CW domain as a histone modification reader. Structure 2010;18:1127–39.
[204] Karch CM, Ezerskiy LA, Bertelsen S, Goate AM. Alzheimer's Disease Risk Polymorphisms Regulate Gene Expression in the ZCWPW1 and the CELF1 Loci. PLoS ONE 2016;11:e0148717.
[205] Gallo J-M, Spickett C. The role of CELF proteins in neurological disorders. RNA Biol 2010;7:474–9.
[206] Shulman JM, Imboywa S, Giagtzoglou N, Powers MP, Hu Y, Devenport D, et al. Functional screening in Drosophila identifies Alzheimer's disease susceptibility genes and implicates Tau-mediated mechanisms. Hum Mol Gen 2014;23:870–7.
[207] Rognoni E, Ruppert R, Fassler R. The kindlin family: functions, signaling properties and implications for human disease. J Cell Sci 2016;129:17–27.
[208] Singh MK, Dadke D, Nicolas E, Serebriiskii IG, Apostolou S, Canutescu A, et al. A novel Cas family member, HEPL, regulates FAK and cell spreading. Mol Biol Cell 2008;19:1627–36.
[209] Beck TN, Nicolas E, Kopp MC, Golemis EA. Adaptors for disorders of the brain? The cancer signaling proteins NEDD9, CASS4, and PTK2B in Alzheimer's disease. Oncoscience 2014;1:486–503.
[210] Ramanan VK, Risacher SL, Nho K, Kim S, Shen L, McDonald BC, et al. GWAS of longitudinal amyloid accumulation on 18F-florbetapir PET in Alzheimer's disease implicates microglial activation gene IL1RAP. Brain 2015;138:3076–88.
[211] Marden JR, Mayeda ER, Walter S, Vivot A, Tchetgen Tchetgen EJ, Kawachi I, et al. Using an Alzheimer Disease Polygenic Risk Score to Predict Memory Decline in Black and White Americans Over 14 Years of Follow-

up. Alzheimer Dis Assoc Disord 2016;EPub Ahead of Print:195–202.
[212] Harris SE, Davies G, Luciano M, Payton A, Fox HC, Haggarty P, et al. Polygenic Risk for Alzheimer's Disease is not Associated with Cognitive Ability or Cognitive Aging in Non-Demented Older People. J Alzheimers Dis 2014;39:565–74.
[213] Hagenaars SP, Harris SE, Davies G, Hill WD, Liewald DCM, Ritchie SJ, et al. Shared genetic aetiology between cognitive functions and physical and mental health in UK Biobank (N=112 151) and 24 GWAS consortia. Mol Psychiatry 2016;Epub ahead of print.
[214] Deckers K, van Boxtel MPJ, Schiepers OJG, de Vugt M, Munoz Sanchez JL, Anstey KJ, et al. Target risk factors for dementia prevention: a systematic review and Delphi consensus study on the evidence from observational studies. Int J Geriatr Psychiatry 2015;30:234–46.
[215] Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol 2011;10:819–28.
[216] Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet 2015;385:2255–63.
[217] Pedditizi E, Peters R, Beckett N. The risk of overweight/obesity in mid-life and late life for the development of dementia: a systematic review and meta-analysis of longitudinal studies. Age Ageing 2016;45:14–21.
[218] Sellbom KS, Gunstad J. Cognitive function and decline in obesity. J Alzheimers Dis 2012;30 Suppl 2:S89–95.
[219] Bischof GN, Park DC. Obesity and Aging: Consequences for Cognition, Brain Structure, and Brain Function. Psychosom Med 2015;77:697–709.
[220] Gustafson D. Adiposity indices and dementia. Lancet Neurol 2006;5:713–20.
[221] Chatterjee S, Peters SAE, Woodward M, Mejia Arango S, Batty GD, Beckett N, et al. Type 2 Diabetes as a Risk Factor for Dementia in Women Compared With Men: A Pooled Analysis of 2.3 Million People Comprising More Than 100,000 Cases of Dementia. Diabetes Care 2016;39:300–7.
[222] Cheng G, Huang C, Deng H, Wang H. Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. Intern Med J 2012;42:484–91.
[223] Gudala K, Bansal D, Schifano F, Bhansali A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. J Diabetes Investig 2013;4:640–50.
[224] Sadanand S, Balachandar R, Bharath S. Memory and executive functions in persons with type 2 diabetes: a meta-analysis. Diabetes Metab Res Rev 2016;32:132–42.
[225] Monette MCE, Baird A, Jackson DL. A meta-analysis of cognitive functioning in nondemented adults with type 2 diabetes mellitus. Can J Diabetes 2014;38:401–8.
[226] Ninomiya T. Diabetes mellitus and dementia. Curr Diab Rep 2014;14:487.
[227] Mankovsky BN, Ziegler D. Stroke in patients with diabetes mellitus. Diabetes Metab Res Rev 2004;20:268–87.
[228] Neumann KF, Rojo L, Navarrete LP. Insulin resistance and Alzheimer's disease: molecular links & clinical implications. Curr Alzheimer Res 2008;5:438–47.

[229] Sharp SI, Aarsland D, Day S, Sonnesyn H, Alzheimer's Society Vascular Dementia Systematic Review Group, Ballard C. Hypertension is a potential risk factor for vascular dementia: systematic review. Int J Geriatr Psychiatry 2011;26:661–9.
[230] Power MC, Weuve J, Gagne JJ, McQueen MB, Viswanathan A, Blacker D. The association between blood pressure and incident Alzheimer disease: a systematic review and meta-analysis. Epidemiology 2011;22:646–59.
[231] Guan J-W, Huang C-Q, Li Y-H, Wan C-M, You C, Wang Z-R, et al. No association between hypertension and risk for Alzheimer's disease: a meta-analysis of longitudinal studies. J Alzheimers Dis 2011;27:799–807.
[232] Gifford KA, Badaracco M, Liu D, Tripodis Y, Gentile A, Lu Z, et al. Blood pressure and cognition among older adults: a meta-analysis. Arch Clin Neuropsychol 2013;28:649–64.
[233] Pires PW, Dams Ramos CM, Matin N, Dorrance AM. The effects of hypertension on the cerebral circulation. Am J Physiol Heart Circ Physiol 2013;304:H1598–614.
[234] Muller M, van der Graaf Y, Visseren FL, Mali WPTM, Geerlings MI, SMART Study Group. Hypertension and longitudinal changes in cerebral blood flow: the SMART-MR study. Ann Neurol 2012;71:825–33.
[235] Debette S, Seshadri S, Beiser A, Au R, Himali JJ, Palumbo C, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. Neurology 2011;77:461–8.
[236] Faraco G, Park L, Zhou P, Luo W, Paul SM, Anrather J, et al. Hypertension enhances Abeta-induced neurovascular dysfunction, promotes beta-secretase activity, and leads to amyloidogenic processing of APP. J Cereb Blood Flow Metab 2016;36:241–52.
[237] Carnevale D, Mascio G, D'Andrea I, Fardella V, Bell RD, Branchi I, et al. Hypertension induces brain beta-amyloid accumulation, cognitive impairment, and memory deterioration through activation of receptor for advanced glycation end products in brain vasculature. Hypertension 2012;60:188–97.
[238] Shah NS, Vidal J-S, Masaki K, Petrovitch H, Ross GW, Tilley C, et al. Midlife blood pressure, plasma β amyloid and the risk for Alzheimer's disease: the Honolulu Asia Aging Study. Hypertension 2012;59:780–6.
[239] Anstey KJ, Lipnicki DM, Low L-F. Cholesterol as a risk factor for dementia and cognitive decline: a systematic review of prospective studies with meta-analysis. Am J Geriatr Psychiatry 2008;16:343–54.
[240] Wendell CR, Waldstein SR, Zonderman AB. Nonlinear longitudinal trajectories of cholesterol and neuropsychological function. Neuropsychology 2014;28:106–12.
[241] Power MC, Weuve J, Sharrett AR, Blacker D, Gottesman RF. Statins, cognition, and dementia-systematic review and methodological commentary. Nat Rev Neurol 2015;11:220–9.
[242] Shepardson NE, Shankar GM, Selkoe DJ. Cholesterol level and statin use in Alzheimer disease: I. Review of epidemiological and preclinical studies. Arch Neurol 2011;68:1239–44.
[243] Shepardson NE, Shankar GM, Selkoe DJ. Cholesterol level and statin use in Alzheimer disease: II. Review of human trials and recommendations. Arch Neurol 2011;68:1385–92.
[244] Cherbuin N, Kim S, Anstey KJ. Dementia risk estimates associated with measures of depression: a systematic review and meta-analysis. BMJ

Open 2015;5:e008853.
[245] Wilson RS, Capuano AW, Boyle PA, Hoganson GM, Hizel LP, Shah RC, et al. Clinical-pathologic study of depressive symptoms and cognitive decline in old age. Neurology 2014;83:702–9.
[246] Taylor WD, Aizenstein HJ, Alexopoulos GS. The vascular depression hypothesis: mechanisms linking vascular disease with depression. Mol Psychiatry 2013;18:963–74.
[247] Byers AL, Yaffe K. Depression and risk of developing dementia. Nat Rev Neurol 2011;7:323–31.
[248] Nascimento KKFD, Silva KP, Malloy-Diniz LF, Butters MA, Diniz BS. Plasma and cerebrospinal fluid amyloid-β levels in late-life depression: A systematic review and meta-analysis. J Psychiatr Res 2015;69:35–41.
[249] Perry DC, Sturm VE, Peterson MJ, Pieper CF, Bullock T, Boeve BF, et al. Association of traumatic brain injury with subsequent neurological and psychiatric disease: a meta-analysis. J Neurosurg 2016;124:511–26.
[250] Dunning DL, Westgate B, Adlam A-LR. A Meta-Analysis of Working Memory Impairments in Survivors of Moderate-to-Severe Traumatic Brain Injury. Neuropsychology 2016;Epub ahead of print.
[251] Konigs M, Engenhorst PJ, Oosterlaan J. Intelligence after traumatic brain injury: meta-analysis of outcomes and prognosis. Eur J Neurol 2016;23:21–9.
[252] Bird SM, Sohrabi HR, Sutton TA, Weinborn M, Rainey-Smith SR, Brown B, et al. Cerebral amyloid-beta accumulation and deposition following traumatic brain injury-A narrative review and meta-analysis of animal studies. Neurosci Biobehav Rev 2016;64:215–28.
[253] Johnson VE, Stewart W, Smith DH. Traumatic brain injury and amyloid-beta pathology: a link to Alzheimer's disease? Nat Rev Neurosci 2010;11:361–70.
[254] Blondell SJ, Hammersley-Mather R, Veerman J. Does physical activity prevent cognitive decline and dementia?: A systematic review and meta-analysis of longitudinal studies. BMC Public Health 2014;14:510–2.
[255] Sofi F, Valecchi D, Bacci D, Abbate R, Gensini GF, Casini A, et al. Physical activity and risk of cognitive decline: a meta-analysis of prospective studies. J Intern Med 2010;269:107–17.
[256] Groot C, Hooghiemstra AM, Raijmakers PGHM, van Berckel BNM, Scheltens P, Scherder EJA, et al. The effect of physical activity on cognitive function in patients with dementia: A meta-analysis of randomized control trials. Ageing Res Rev 2016;25:13–23.
[257] Kirk-Sanchez NJ, McGough EL. Physical exercise and cognitive performance in the elderly: current perspectives. Clin Interv Aging 2014;9:51–62.
[258] Zhong G, Wang Y, Zhang Y, Guo JJ, Zhao Y. Smoking is associated with an increased risk of dementia: a meta-analysis of prospective cohort studies with investigation of potential effect modifiers. PLoS ONE 2015;10:e0118333.
[259] Anstey KJ, Sanden von C, Salim A, O'Kearney R. Smoking as a risk factor for dementia and cognitive decline: a meta-analysis of prospective studies. Am J Epidemiol 2007;166:367–78.
[260] Peters R, Poulter R, Warner J, Beckett N, Burch L, Bulpitt C. Smoking, dementia and cognitive decline in the elderly, a systematic review. BMC Geriatr 2008;8:36.

[261] North T-L, Palmer TM, Lewis SJ, Cooper R, Power C, Pattie A, et al. Effect of smoking on physical and cognitive capability in later life: a multicohort study using observational and genetic approaches. BMJ Open 2015;5:e008393.
[262] Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 2004;43:1731–7.
[263] Gons RAR, van Norden AGW, de Laat KF, van Oudheusden LJB, van Uden IWM, Zwiers MP, et al. Cigarette smoking is associated with reduced microstructural integrity of cerebral white matter. Brain 2011;134:2116–24.
[264] Durazzo TC, Mattsson N, Weiner MW. Smoking and increased Alzheimer's disease risk: A review of potential mechanisms. Alzheimers Dement 2014;10:S122–45.
[265] Cao L, Tan L, Wang H-F, Jiang T, Zhu X-C, Lu H, et al. Dietary Patterns and Risk of Dementia: a Systematic Review and Meta-Analysis of Cohort Studies. Mol Neurobiol 2015;Epub ahead of print.
[266] van de Rest O, Berendsen AA, Haveman-Nies A, de Groot LC. Dietary patterns, cognitive decline, and dementia: a systematic review. Adv Nutr 2015;6:154–68.
[267] Frisardi V, Panza F, Seripa D, Imbimbo BP, Vendemiale G, Pilotto A, et al. Nutraceutical properties of Mediterranean diet and cognitive decline: possible underlying mechanisms. J Alzheimers Dis 2010;22:715–40.
[268] Peters R, Peters J, Warner J, Beckett N, Bulpitt C. Alcohol, dementia and cognitive decline in the elderly: a systematic review. Age Ageing 2008;37:505–12.
[269] Anstey KJ, Mack HA, Cherbuin N. Alcohol consumption as a risk factor for dementia and cognitive decline: meta-analysis of prospective studies. Am J Geriatr Psychiatry 2009;17:542–55.
[270] Beydoun MA, Beydoun HA, Gamaldo AA, Teel A, Zonderman AB, Wang Y. Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. BMC Public Health 2014;14:643.
[271] Handing EP, Andel R, Kadlecova P, Gatz M, Pedersen NL. Midlife Alcohol Consumption and Risk of Dementia Over 43 Years of Follow-Up: A Population-Based Study From the Swedish Twin Registry. J Gerontol a Biol Sci Med Sci 2015;70:1248–54.
[272] Langballe EM, Ask H, Holmen J, Stordal E, Saltvedt I, Selbæk G, et al. Alcohol consumption and risk of dementia up to 27 years later in a large, population-based sample: the HUNT study, Norway. Eur J Epidemiol 2015;30:1049–56.
[273] Stern Y. Cognitive reserve in ageing and Alzheimer's disease. Lancet Neurol 2012;11:1006–12.
[274] Stern Y. The concept of cognitive reserve: a catalyst for research. J Clin Exp Neuropsychol 2003;25:589–93.
[275] Scarmeas N, Stern Y. Cognitive reserve and lifestyle. J Clin Exp Neuropsychol 2003;25:625–33.
[276] Meng X, D'Arcy C. Education and dementia in the context of the cognitive reserve hypothesis: a systematic review with meta-analyses and qualitative analyses. PLoS ONE 2012;7:e38268.
[277] Albert MS. How does education affect cognitive function? Ann Epidemiol 1995;5:76–8.

[278] Hendrie HC, Albert MS, Butters MA, Gao S, Knopman DS, Launer LJ, et al. The NIH Cognitive and Emotional Health Project. Report of the Critical Evaluation Study Committee. Alzheimers Dement 2006;2:12–32.
[279] Sajeev G, Weuve J, Jackson JW, VanderWeele TJ, Bennett DA, Grodstein F, et al. Late-life Cognitive Activity and Dementia: A Systematic Review and Bias Analysis. Epidemiology 2016;27:732–42.
[280] Opdebeeck C, Martyr A, Clare L. Cognitive reserve and cognitive function in healthy older people: a meta-analysis. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn 2016;23:40–60.
[281] Huntley JD, Gould RL, Liu K, Smith M, Howard RJ. Do cognitive interventions improve general cognition in dementia? A meta-analysis and meta-regression. BMJ Open 2015;5:e005247–7.
[282] Landau SM, Marks SM, Mormino EC, Rabinovici GD, Oh H, O'Neil JP, et al. Association of lifetime cognitive engagement and low beta-amyloid deposition. Arch Neurol 2012;69:623–9.
[283] Schreiber S, Vogel J, Schwimmer HD, Marks SM, Schreiber F, Jagust W. Impact of lifestyle dimensions on brain pathology and cognition. Neurobiol Aging 2016;40:164–72.
[284] Lachman ME, Agrigoroaei S, Murphy C, Tun PA. Frequent Cognitive Activity Compensates for Education Differences in Episodic Memory. Am J Geriatr Psychiatry 2010;18:4–10.
[285] Kuiper JS, Zuidersma M, Oude Voshaar RC, Zuidema SU, van den Heuvel ER, Stolk RP, et al. Social relationships and risk of dementia: A systematic review and meta-analysis of longitudinal cohort studies. Ageing Res Rev 2015;22:39–57.
[286] Brown CL, Gibbons LE, Kennison RF, Robitaille A, Lindwall M, Mitchell MB, et al. Social activity and cognitive functioning over time: a coordinated analysis of four longitudinal studies. J Aging Res 2012;2012:287438–12.
[287] Seeman TE, Miller-Martinez DM, Stein Merkin S, Lachman ME, Tun PA, Karlamangla AS. Histories of social engagement and adult cognition: midlife in the U.S. study. J Gerontol B Psychol Sci Soc Sci 2011;66 Suppl 1:i141–52.
[288] Fratiglioni L, Paillard-Borg S, Winblad B. An active and socially integrated lifestyle in late life might protect against dementia. Lancet Neurol 2004;3:343–53.
[289] Rizzuto D, Fratiglioni L. Lifestyle factors related to mortality and survival: a mini-review. Gerontology 2014;60:327–35.
[290] Stephan BCM, Kurth T, Matthews FE, Brayne C, Dufouil C. Dementia risk prediction in the population: are screening models accurate? Nat Rev Neurol 2010;6:318–26.
[291] Tang EYH, Harrison SL, Errington L, Gordon MF, Visser PJ, Novak G, et al. Current Developments in Dementia Risk Prediction Modelling: An Updated Systematic Review. PLoS ONE 2015;10:e0136181.
[292] Salthouse TA. When does age-related cognitive decline begin? Neurobiol Aging 2009;30:507–14.
[293] Wilson RS, Hebert LE, Scherr PA, Barnes LL, Mendes de Leon CF, Evans DA. Educational attainment and cognitive decline in old age. Neurology 2009;72:460–5.
[294] Plomin R, Deary IJ. Genetics and intelligence differences: five special findings. Mol Psychiatry 2014;20:98–108.

[295] Papenberg G, Lindenberger U, Backman L. Aging-related magnification of genetic effects on cognitive and brain integrity. Trends Cogn Sci 2015;19:506–14.
[296] Mielke MM, Vemuri P, Rocca WA. Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences. Clin Epidemiol 2014;6:37–48.
[297] Pfaff CL, Parra EJ, Bonilla C, Hiester K, McKeigue PM, Kamboh MI, et al. Population structure in admixed populations: effect of admixture dynamics on the pattern of linkage disequilibrium. Am J Hum Genet 2001;68:198–207.
[298] McClellan J, King M-C. Genetic Heterogeneity in Human Disease. Cell 2010;141:210–7.
[299] Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ, et al. The importance of race and ethnic background in biomedical research and clinical practice. N Engl J Med 2003;348:1170–5.
[300] Deary IJ, Penke L, Johnson W. The neuroscience of human intelligence differences. Nat Rev Neurosci 2010;11:201–11.
[301] Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. J Am Geriatr Soc 1992;40:922–35.
[302] Plomin R. Genetics, genes, genomics and g. Mol Psychiatry 2003;8:1–5.
[303] Wei W-H, Hemani G, Haley CS. Detecting epistasis in human complex traits. Nat Rev Genet 2014;15:722–33.
[304] Hunter DJ. Gene–environment interactions in human diseases. Nat Rev Genet 2005;6:287–98.
[305] Ebbert MTW, Boehme KL, Wadsworth ME, Staley LA, Mukherjee S, Crane PK, et al. Interaction between variants in CLU and MS4A4E modulates Alzheimer's disease risk. Alzheimers Dement 2016;12:121–9.
[306] McFall GP, Wiebe SA, Vergote D, Anstey KJ, Dixon RA. Alzheimer's Genetic Risk Intensifies Neurocognitive Slowing Associated with Diabetes in Non-Demented Older Adults. Alzheimers Dement (Amst) 2015;1:395–402.
[307] Ferencz B, Jonsson Laukka E, Welmer A-K, Kalpouzos G, Angleman S, Keller L, et al. The Benefits of Staying Active in Old Age: Physical Activity Counteracts the Negative Influence of PICALM, BIN1, and CLU Risk Alleles on Episodic Memory Functioning. Psychol Aging 2014;29:440–9.
[308] Martinez-Lapiscina EH, Galbete C, Corella D, Toledo E, Buil-Cosiales P, Salas-Salvado J, et al. Genotype patterns at CLU, CR1, PICALM and APOE, cognition and Mediterranean diet: the PREDIMED-NAVARRA trial. Genes Nutr 2014;9:393–13.
[309] Knight RG, Tsui HSL, Abraham WC, Skeaff CM, McMahon JA, Cutfield NJ. Lack of effect of the apolipoprotein E epsilon4 genotype on cognition during healthy aging. J Clin Exp Neuropsychol 2014;36:742–50.
[310] Park DC, Reuter-Lorenz P. The Adaptive Brain: Aging and Neurocognitive Scaffolding. Annu Rev Psychol 2009;60:173–96.
[311] Barnes J, Dickerson BC, Frost C, Jiskoot LC, Wolk D, van der Flier WM. Alzheimer's disease first symptoms are age dependent: Evidence from the NACC dataset. Alzheimers Dement 2015;11:1349–57.
[312] Kuh D, Ben-Shlomo Y, Lynch J, Hallqvist J, Power C. Life course epidemiology. J Epidemiol Community Health 2003;57:778–83.
[313] Suemoto CK, Gilsanz P, Mayeda ER, Glymour MM. Body mass index and cognitive function: the potential for reverse causation. Int J Obes (Lond) 2015;39:1383–9.

[314] Batty GD, Galobardes B, Starr JM, Jeffreys M, Davey Smith G, Russ TC. Examining if being overweight really confers protection against dementia: Sixty-four year follow-up of participants in the Glasgow University alumni cohort study. Journal of Negative Results in Biomedicine 2016;15:19.
[315] Iadecola C, Yaffe K, Biller J, Bratzke LC, Faraci FM, Gorelick PB, et al. Impact of Hypertension on Cognitive Function: A Scientific Statement From the American Heart Association. Hypertension 2016;68:e67–e94.
[316] Hultsch DF, Hertzog C, Small BJ, Dixon RA. Use it or lose it: engaged lifestyle as a buffer of cognitive decline in aging? Psychol Aging 1999;14:245–63.
[317] Sorman DE, Ronnlund M, Sundstrom A, Adolfsson R, Nilsson L-G. Social relationships and risk of dementia: a population-based study. Int Psychogeriatr 2015;27:1391–9.
[318] Morgan GS, Gallacher J, Bayer A, Fish M, Ebrahim S, Ben-Shlomo Y. Physical activity in middle-age and dementia in later life: findings from a prospective cohort of men in Caerphilly, South Wales and a meta-analysis. J Alzheimers Dis 2012;31.
[319] de Bruijn RFAG, Schrijvers EMC, de Groot KA, Witteman JCM, Hofman A, Franco OH, et al. The association between physical activity and dementia in an elderly population: the Rotterdam Study. Eur J Epidemiol 2013;28:277–83.
[320] Gow AJ, Corley J, Starr JM, Deary IJ. Reverse causation in activity-cognitive ability associations: the Lothian Birth Cohort 1936. Psychol Aging 2012;27:250–5.
[321] van Charante EPM, Richard E, Eurelings LS, van Dalen J-W, Ligthart SA, van Bussel EF, et al. Effectiveness of a 6-year multidomain vascular care intervention to prevent dementia (preDIVA): a cluster-randomised controlled trial. Lancet 2000;388:797–805.
[322] Anstey KJ, Christensen H, Butterworth P, Easteal S, Mackinnon A, Jacomb T, et al. Cohort profile: the PATH through life project. Int J Epidemiol 2012;41:951–60.
[323] Martin A, Rief W, Klaiberg A, Braehler E. Validity of the Brief Patient Health Questionnaire Mood Scale (PHQ-9) in the general population. Gen Hosp Psychiatry 2006;28:71–7.
[324] Lubben J, Blozik E, Gillmann G, Iliffe S, Renteln Kruse von W, Beck JC, et al. Performance of an abbreviated version of the Lubben Social Network Scale among three European community-dwelling older adult populations. Gerontologist 2006;46:503–13.
[325] Schuster TL, Kessler RC, Aseltine RHJ. Supportive interactions, negative interactions, and depressed mood. Am J Community Psychol 1990;18:423–38.
[326] Holland JL. Making vocational choices: A theory of vocational personalities and work environments. Psychological Assessment Resources; 1997.
[327] Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test. San Antonio: Psychological Corporation; 1987.
[328] Wechsler D. A standardized memory scale for clinical use. J Psychol 1945;19:87–95.
[329] Baddeley A, Emslie H, Nimmo-Smith I. The Spot-the-Word test: a robust estimate of verbal intelligence based on lexical decision. Br J Clin Psychol

1993;32:55–65.
[330] Tiffin J, Asher EJ. The Purdue pegboard; norms and studies of reliability and validity. J Appl Psychol 1948;32:234–47.
[331] Anstey KJ, Dear K, Christensen H, Jorm AF. Biomarkers, health, lifestyle, and demographic variables as correlates of reaction time performance in early, middle, and late adulthood. Q J Exp Psychol A 2005;58:5–21.
[332] Strauss E, Sherman EM, Spreen O. A compendium of neuropsychological tests: Administration, norms, and commentary. American Chemical Society; 2006.
[333] Bunce D, Bielak AAM, Anstey KJ, Cherbuin N, Batterham PJ, Easteal S. APOE genotype and cognitive change in young, middle-aged, and older adults living in the community. J Gerontol a Biol Sci Med Sci 2014;69:379–86.
[334] Chipman P, Jorm AF, Tan X-Y, Easteal S. No association between the serotonin-1A receptor gene single nucleotide polymorphism rs6295C/G and symptoms of anxiety or depression, and no interaction between the polymorphism and environmental stressors of childhood anxiety or recent stressful life events on anxiety or depression. Psychiatr Genet 2010;20:8–13.
[335] Chipman P, Jorm AF, Prior M, Sanson A, Smart D, Tan X, et al. No interaction between the serotonin transporter polymorphism (5-HTTLPR) and childhood adversity or recent stressful life events on symptoms of depression: results from two community surveys. Am J Med Genet B Neuropsychiatr Genet 2007;144B:561–5.
[336] Prichard Z, Easteal S. Characterization of simple sequence repeat variants linked to candidate genes for behavioral phenotypes. Human Mutation 2006;27:120–0.
[337] Prichard ZM, Jorm AF, Mackinnon A, Easteal S. Association analysis of 15 polymorphisms within 10 candidate genes for antisocial behavioural traits. Psychiatr Genet 2007;17:299–303.
[338] Johnson AD, Newton-Cheh C, Chasman DI, Ehret GB, Johnson T, Rose L, et al. Association of hypertension drug target genes with blood pressure and hypertension in 86,588 individuals. Hypertension 2011;57:903–10.
[339] Johnson T, Gaunt TR, Newhouse SJ, Padmanabhan S, Tomaszewski M, Kumari M, et al. Blood pressure loci identified with a gene-centric array. Am J Hum Genet 2011;89:688–700.
[340] Bis JC, DeCarli C, Smith AV, van der Lijn F, Crivello F, Fornage M, et al. Common variants at 12q14 and 12q24 are associated with hippocampal volume. Nat Genet 2012;44:545–51.
[341] Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet 2009;41:677–87.
[342] Harris SE, Fox H, Wright AF, Hayward C, Starr JM, Whalley LJ, et al. The brain-derived neurotrophic factor Val66Met polymorphism is associated with age-related change in reasoning skills. Mol Psychiatry 2006;11:505–13.
[343] Mandelman SD, Grigorenko EL. BDNF Val66Met and cognition: all, none, or some? A meta-analysis of the genetic association. Genes Brain Behav 2012;11:127–36.
[344] Izaks GJ, van der Knaap AM, Gansevoort RT, Navis G, Slaets JPJ, Dullaart RPF. Cholesteryl Ester Transfer Protein (CETP) genotype and cognitive

function in persons aged 35 years or older. Neurobiol Aging 2012;33:1851.e7–1851.e16.
[345] Markett S, Reuter M, Montag C, Weber B. The dopamine D2 receptor gene DRD2 and the nicotinic acetylcholine receptor gene CHRNA4 interact on striatal gray matter volume: Evidence from a genetic imaging study. NeuroImage 2013;64:167–72.
[346] Houlihan LM, Harris SE, Luciano M, Gow AJ, Starr JM, Visscher PM, et al. Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. Genes Brain Behav 2009;8:238–47.
[347] Harris SE, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ. The functional COMT polymorphism, Val 158 Met, is associated with logical memory and the personality trait intellect/imagination in a cohort of healthy 79 year olds. Neurosci Lett 2005;385:1–6.
[348] Papassotiropoulos A, Stefanova E, Vogler C, Gschwind L, Ackermann S, Spalek K, et al. A genome-wide survey and functional brain imaging study identify CTNNBL1 as a memory-related gene. Mol Psychiatry 2013;18:255–63.
[349] Melville SA, Buros J, Parrado AR, Vardarajan B, Logue MW, Shen L, et al. Multiple loci influencing hippocampal degeneration identified by genome scan. Ann Neurol 2012;72:65–75.
[350] Lambert JC, Grenier-Boley B, Harold D, Zelenika D, Chouraki V, Kamatani Y, et al. Genome-wide haplotype association study identifies the FRMD4A gene as a risk locus for Alzheimer's disease. Mol Psychiatry 2013;18:461–70.
[351] Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, Leow AD, et al. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proc Natl Acad Sci USA 2010;107:8404–9.
[352] Kohannim O, Hibar DP, Hibar DP, Jahanshad N, JL S, Stein JL, et al. Predicting temporal lobe volume on mri from genotypes using l(1)-l(2) regularized regression. Proc IEEE Int Symp Biomed Imaging 2012:1160–3.
[353] Schrijvers EMC, Schurmann B, Koudstaal PJ, van den Bussche H, van Duijn CM, Hentschel F, et al. Genome-Wide Association Study of Vascular Dementia. Stroke 2012;43:315–9.
[354] Trompet S, Jukema W, Mooijaart SP, Ford I, Stott DJ, Westendorp RGJ, et al. Genetic variation in galectin-3 gene associates with cognitive function at old age. Neurobiol Aging 2012;33:2232.e1–2232.e9.
[355] Joyner AH, J CR, Bloss CS, Bakken TE, Rimol LM, Melle I, et al. A common MECP2 haplotype associates with reduced cortical surface area in humans in two independent populations. Proc Natl Acad Sci USA 2009;106:15483–8.
[356] Kamboh MI, Demirci FY, Wang X, Minster RL, Carrasquillo MM, Pankratz VS, et al. Genome-wide association study of Alzheimer's disease. Transl Psychiatry 2012;2:e117.
[357] Beecham GW, Martin ER, Li Y-J, Slifer MA, Gilbert JR, Haines JL, et al. Genome-wide Association Study Implicates a Chromosome 12 Risk Locus for Late-Onset Alzheimer Disease. Am J Hum Genet 2009;84:35–43.
[358] Salvi E, Kutalik Z, Glorioso N, Benaglio P, Frau F, Kuznetsova T, et al. Genomewide Association Study Using a High-Density Single Nucleotide Polymorphism Array and Case-Control Design Identifies a Novel Essential

Hypertension Susceptibility Locus in the Promoter Region of Endothelial NO Synthase. Hypertension 2012;59:248–55.
[359] Li J, Chen C, Lei X, Wang Y, Chen C, He Q, et al. The NTSR1 gene modulates the association between hippocampal structure and working memory performance. NeuroImage 2013;75:79–86.
[360] Roussotte FF, Jahanshad N, Hibar DP, Sowell ER, Kohannim O, Barysheva M, et al. A commonly carried genetic variant in the delta opioid receptor gene, OPRD1, is associated with smaller regional brain volumes: replication in elderly and young populations. Hum Brain Mapp 2014;35:1226–36.
[361] Velez JI, Chandrasekharappa SC, Henao E, Martinez AF, Harper U, Jones M, et al. Pooling/bootstrap-based GWAS (pbGWAS) identifies new loci modifying the age of onset in PSEN1 p.Glu280Ala Alzheimer's disease. Mol Psychiatry 2013;18:568–75.
[362] Nho K, Corneveaux JJ, Kim S, Lin H, Risacher SL, Shen L, et al. Whole-exome sequencing and imaging genetics identify functional variants for rate of change in hippocampal volume in mild cognitive impairment. Mol Psychiatry 2013;18:781–7.
[363] Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 2007;39:168–77.
[364] Jahanshad N, Rajagopalan P, Hua X, Hibar DP, Nir TM, Toga AW, et al. Genome-wide scan of healthy human connectome discovers SPON1 gene variant influencing dementia severity. Proc Natl Acad Sci USA 2013;110:4768–73.
[365] Sherva R, Tripodis Y, Bennett DA, Chibnik LB, Crane PK, De Jager PL, et al. Genome-wide association study of the rate of cognitive decline in Alzheimer's disease. Alzheimers Dement 2014;10:45–52.
[366] Baune BT, Konrad C, Grotegerd D, Suslow T, Ohrmann P, Bauer J, et al. Tumor necrosis factor gene variation predicts hippocampus volume in healthy individuals. Biol Psychiatry 2012;72:655–62.
[367] Fornage M, Debette S, Bis JC, Schmidt H, Ikram MA, Dufouil C, et al. Genome-wide association studies of cerebral white matter lesion burden. Ann Neurol 2011;69:928–39.
[368] Stein JL, Hibar DP, Madsen SK, Khamis M, McMahon KL, de Zubicaray GI, et al. Discovery and replication of dopamine-related gene effects on caudate volume in young and elderly populations (N=1198) using genome-wide search. Mol Psychiatry 2011;16:927–37.
[369] Shulman JM, Chibnik LB, Aubin C, Schneider JA, Bennett DA, De Jager PL. Intermediate Phenotypes Identify Divergent Pathways to Alzheimer's Disease. PLoS ONE 2010;5:e11244.
[370] Folstein MF, Folstein SE, McHugh PR. “Mini-mental state.” A practical method for grading the cognitive state of patients for the clinician. 1975;12:189–98.
[371] Smith GD, Ebrahim S. “Mendelian randomization”: can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1–22.
[372] Tiffin J. Purdue pegboard examiner manual. Science Research Associates; 1968.
[373] Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 1993;43:2412–4.

[374] Association AP. Diagnostic and statistical manual of mental disorders (DSM). Washington, DC: American Psychiatric Association 1994:143–7.
[375] Green P, MacLeod CJ. SIMR: an R package for power analysis of generalized linear mixed models by simulation. Methods Ecol Evol 2016;7:493–8.
[376] Henderson AS, Easteal S, Jorm AF, Mackinnon AJ, Korten AE, Christensen H, et al. Apolipoprotein E allele epsilon 4, dementia, and cognitive decline in a population sample. Lancet 1995;346:1387–90.
[377] Small BJ, Rosnick CB, Fratiglioni L, Bäckman L. Apolipoprotein E and Cognitive Performance: A Meta-Analysis. Psychol Aging 2004;19:592–600.
[378] Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PCJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 1994;7:180–4.
[379] Wilson RS, Bienias JL, Berry-Kravis E, Evans DA, Bennett DA. The apolipoprotein E epsilon 2 allele and decline in episodic memory. J Neurol Neurosurg Psychiatry 2002;73:672–7.
[380] Berlau DJ, Corrada MM, Head E, Kawas CH. APOE ε2 is associated with intact cognition but increased Alzheimer pathology in the oldest old. Neurology 2009;72:829–34.
[381] Bunce D, Anstey KJ, Burns R, Christensen H, Easteal S. Does possession of apolipoprotein E ε4 benefit cognitive function in healthy young adults? Neuropsychologia 2011;49:1693–7.
[382] Jorm AF, Mather KA, Butterworth P, Anstey KJ, Christensen H, Easteal S. APOE genotype and cognitive functioning in a large age-stratified population sample. Neuropsychology 2007;21:1–8.
[383] Christensen H, Batterham PJ, Mackinnon AJ, Jorm AF, Mack HA, Mather KA, et al. The association of APOE genotype and cognitive decline in interaction with risk factors in a 65-69 year old community sample. BMC Geriatr 2008;8:14.
[384] Novak V, Hajjar I. The relationship between blood pressure and cognitive function. Nat Rev Cardiol 2010;7:686–98.
[385] Gasecki D, Kwarciany M, Nyka W, Narkiewicz K. Hypertension, brain damage and cognitive decline. Curr Hypertens Rep 2013;15:547–58.
[386] Tzourio C, Dufouil C, Ducimetière P, Alpérovitch A. Cognitive decline in individuals with high blood pressure A longitudinal study in the elderly. Neurology 1999;53:1948–52.
[387] Yasar S, Ko JY, Nothelle S, Mielke MM, Carlson MC. Evaluation of the Effect of Systolic Blood Pressure and Pulse Pressure on Cognitive Function: The Women's Health and Aging Study II. PLoS ONE 2011;6:e27976.
[388] Skoog I, Nilsson L, Persson G, Lernfelt B, Landahl S. 15-year longitudinal study of blood pressure and dementia. Lancet 1996;347:1141–5.
[389] Qiu C, Winblad B, Fratiglioni L. Low diastolic pressure and risk of dementia in very old people: a longitudinal study. Dement Geriatr Cogn Disord 2009;28:213–9.
[390] Thorvaldsson V, Skoog I, Hofer SM, Börjesson-Hanson A, Östling S, Sacuiu S, et al. Nonlinear blood pressure effects on cognition in old age: Separating between-person and within-person associations. Psychol Aging 2012;27:375–83.
[391] Herbert LE, Scherr PA, Bennett DA, Bienias JL, Wilson RS, Morris MC, et al. Blood pressure and late-life cognitive function change: a biracial

longitudinal population study. Neurology 2004;62:2021–4.
[392] Solfrizzi V, Panza F, Colacicco AM, D'introno A, Capurso C, Torres F, et al. Vascular risk factors, incidence of MCI, and rates of progression to dementia. Neurology 2004;63:1882–91.
[393] Niu W, Qi Y, Qian Y, Gao P, Zhu D. The relationship between apolipoprotein E epsilon2/epsilon3/epsilon4 polymorphisms and hypertension: a meta-analysis of six studies comprising 1812 cases and 1762 controls. Hypertens Res 2009;32:1060–6.
[394] Sudlow C, González NAM, Kim J, Clark C. Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls. Stroke 2006;37:364–70.
[395] Hamzi K, Tazzite A, Nadifi S. Large-scale meta-analysis of genetic studies in ischemic stroke: Five genes involving 152,797 individuals. Indian J Hum Genet 2011;17:212–7.
[396] Song Y, Stampfer MJ, Liu S. Meta-Analysis: Apolipoprotein E Genotypes and Risk for Coronary Heart Disease. Ann Intern Med 2004;141:137–47.
[397] Khan TA, Shah T, Prieto D, Zhang W, Price J, Fowkes GR, et al. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: systematic review and meta-analysis of 14,015 stroke cases and pooled analysis of primary biomarker data from up to 60,883 individuals. Int J Epidemiol 2013;42:475–92.
[398] Eichner JE, Dunn ST, Perveen G. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 2002;155:487–95.
[399] Lahoz C, Schaefer EJ, Cupples LA, Wilson PW, Levy D, Osgood D, et al. Apolipoprotein E genotype and cardiovascular disease in the Framingham Heart Study. Atherosclerosis 2001;154:529–37.
[400] Kofler BM, Miles EA, Curtis P, Armah CK, Tricon S, Grew J, et al. Apolipoprotein E genotype and the cardiovascular disease risk phenotype: impact of sex and adiposity (the FINGEN study). Atherosclerosis 2012;221:467–70.
[401] Kalmijn S, Feskens EJ, Launer LJ, Kromhout D. Cerebrovascular disease, the apolipoprotein e4 allele, and cognitive decline in a community-based study of elderly men. Stroke 1996;27:2230–5.
[402] Bangen KJ, Beiser A, Delano-Wood L, Nation DA, Lamar M, Libon DJ, et al. APOE genotype modifies the relationship between midlife vascular risk factors and later cognitive decline. J Stroke Cerebrovasc Dis 2013;22:1361–9.
[403] Kang JH, Logroscino G, De Vivo I, Hunter D, Grodstein F. Apolipoprotein E, cardiovascular disease and cognitive function in aging women. Neurobiol Aging 2005;26:475–84.
[404] Yasuno F, Tanimukai S, Sasaki M, Ikejima C, Yamashita F, Kodama C, et al. Effect of plasma lipids, hypertension and APOE genotype on cognitive decline. Neurobiol Aging 2012;33:2633–40.
[405] de Frias CM, Schaie KW, Willis SL. Hypertension moderates the effect of APOE on 21-year cognitive trajectories. Psychol Aging 2014;29:431–9.
[406] Carmelli D, Swan GE, Reed T, Miller B, Wolf PA, Jarvik GP, et al. Midlife cardiovascular risk factors, ApoE, and cognitive decline in elderly male twins. Neurology 1998;50:1580–5.
[407] Caselli RJ, Dueck AC, Locke DEC, Sabbagh MN, Ahern GL, Rapcsak SZ, et al.

Cerebrovascular risk factors and preclinical memory decline in healthy APOE epsilon4 homozygotes. Neurology 2011;76:1078–84.
[408] Knopman DS, Mosley TH, Catellier DJ, Coker LH. Fourteen-year longitudinal study of vascular risk factors, APOE genotype, and cognition: The ARIC MRI Study. Alzheimers Dement 2009;5:207–14.
[409] O'Bryant SE, Humphreys JD, Smith GE, Ivnik RJ, Graff-Radford NR, Petersen RC, et al. Detecting dementia with the mini-mental state examination in highly educated individuals. Arch Neurol 2008;65:963–7.
[410] Roth PL. MISSING DATA: A CONCEPTUAL REVIEW FOR APPLIED PSYCHOLOGISTS. Pers Psychol 1994;47:537–60.
[411] Zheng L, Sun Z, Li J, Zhang R, Zhang X, Liu S, et al. Pulse pressure and mean arterial pressure in relation to ischemic stroke among patients with uncontrolled hypertension in rural areas of China. Stroke 2008;39:1932–7.
[412] Singer JD, Willett JB. Applied longitudinal data analysis: Modeling change and event occurrence. New York: Oxford university press; 2003.
[413] Donohue MC, Gamst AC, Edland SD, Donohue M. Package "longpower." Biometrics 2013;53:937–47.
[414] Scherbaum CA, Ferreter JM. Estimating Statistical Power and Required Sample Sizes for Organizational Research Using Multilevel Modeling. Organ Res Methods 2009;12:347–67.
[415] Richards M, Sacker A. Lifetime antecedents of cognitive reserve. J Clin Exp Neuropsychol 2003;25:614–24.
[416] Barulli D, Stern Y. Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts in cognitive reserve. Trends Cogn Sci 2013;17:502–9.
[417] Hofer SM, Christensen H, Mackinnon AJ, Korten AE, Jorm AF, Henderson AS, et al. Change in cognitive functioning associated with apoE genotype in a community sample of older adults. Psychol Aging 2002;17:194–208.
[418] Wilson RS, Schneider JA, Barnes LL, Beckett LA, Aggarwal NT, Cochran EJ, et al. The apolipoprotein E epsilon 4 allele and decline in different cognitive systems during a 6-year period. Arch Neurol 2002;59:1154–60.
[419] Low L-F, Yap MHW, Brodaty H. Will testing for apolipoprotein E assist in tailoring dementia risk reduction? A review. Neurosci Biobehav Rev 2010;34:408–37.
[420] Wilson RS, Beckett LA, Barnes LL, Schneider JA, Bach J, Evans DA, et al. Individual differences in rates of change in cognitive abilities of older persons. Psychol Aging 2002;17:179–93.
[421] Wilson RS, Boyle PA, Segawa E, Yu L, Begeny CT, Anagnos SE, et al. The influence of cognitive decline on well-being in old age. Psychol Aging 2013;28:304–13.
[422] Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C, et al. Age, neuropathology, and dementia. N Engl J Med 2009;360:2302–9.
[423] Morgan K, Carrasquillo MM. Genetic Variants in Alzheimer's Disease. Springer Science & Business 2013.
[424] Stekhoven DJ, Bühlmann P. MissForest--non-parametric missing value imputation for mixed-type data. Bioinformatics 2012;28:112–8.
[425] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
[426] Ihaka R, Gentleman R. R: a language for data analysis and graphics. J

Comput Graph Stat 1996;5:299–314.
[427] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 2007;39:17–23.
[428] Che R, Motsinger-Reif AA. Evaluation of genetic risk score models in the presence of interaction and linkage disequilibrium. Front Genet 2013;4:138.
[429] Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H, et al. Package "lme4." Convergence 2015;12:1.
[430] Halekoh U, Højsgaard S. A Kenward-Roger Approximation and Parametric Bootstrap Methods for Tests in Linear Mixed Models - The RPackage pbkrtest. J Stat Soft 2014;59:30.
[431] Johnson P. Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. Methods Ecol Evol 2014;5:944–6.
[432] Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol 2013;4:133–42.
[433] Barton K. Package "MuMIn" 2013.
[434] Dubois B, Feldman HH, Jacova C, Cummings JL, DeKosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol 2010;9:1118–27.
[435] Keller JN. Age-related neuropathology, cognitive decline, and Alzheimer's disease. Ageing Res Rev 2006;5:1–13.
[436] Witte AV, Flöel A. Effects of COMT polymorphisms on brain function and behavior in health and disease. Brain Res Bull 2012;88:418–28.
[437] Galloway EM, Woo NH, Lu B. Persistent neural activity in the prefrontal cortex: a mechanism by which BDNF regulates working memory? Prog Brain Res 2008;169:251–66.
[438] Weuve J, Proust-Lima C, Power MC, Gross AL, Hofer SM, Thiébaut R, et al. Guidelines for reporting methodological challenges and evaluating potential bias in dementia research. Alzheimers Dement 2015;11:1098–109.
[439] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, et al. Role of Genes and Environments for Explaining Alzheimer Disease. Archives of General Psychiatry 2006;63:168–74.
[440] Karch CM, Goate AM. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry 2015;77:43–51.
[441] Campion D, Pottier C, Nicolas G, Le Guennec K, Rovelet-Lecrux A. Alzheimer disease: modeling an Aβ-centered biological network. Mol Psychiatry 2016;21:861–71.
[442] Andrews SJ, Das D, Cherbuin N, Anstey KJ, Easteal S. Association of genetic risk factors with cognitive decline: the PATH through life project. Neurobiol Aging 2016;41:150–8.
[443] Spering CC, Hobson V, Lucas JA, Menon CV, Hall JR, O'Bryant SE. Diagnostic accuracy of the MMSE in detecting probable and possible Alzheimer's disease in ethnically diverse highly educated individuals: an analysis of the NACC database. J Gerontol a Biol Sci Med Sci 2012;67:890–6.
[444] Donald H, Robert D G. Longitudinal Data Analysis. vol. 451. Hoboken, NJ, USA: John Wiley & Sons, Inc; 2006.
[445] Bates D, Eigen C, Rcpp LT. Package "lme4" 2014.

[446] Singmann H, Bolker B, Westfall J. Afex: analysis of factorial experiments. 2015.
[447] Lim YY, Laws SM, Villemagne VL, Pietrzak RH, Porter T, Ames D, et al. Aβ-related memory decline in APOE ε4 noncarriers: Implications for Alzheimer disease. Neurology 2016;86:1635–42.
[448] Adams HHH, de Bruijn RFAG, Hofman A, Uitterlinden AG, van Duijn CM, Vernooij MW, et al. Genetic risk of neurodegenerative diseases is associated with mild cognitive impairment and conversion to dementia. Alzheimers Dement 2015;11:1277–85.
[449] Rodríguez-Rodríguez E, Sánchez-Juan P, Vázquez-Higuera JL, Mateo I, Pozueta A, Berciano J, et al. Genetic risk score predicting accelerated progression from mild cognitive impairment to Alzheimer's disease. J Neural Transm 2012;120:1–6.
[450] Lacour A, Espinosa A, Louwersheimer E, Heilmann S, Hernandez I, Wolfsgruber S, et al. Genome-wide significant risk factors for Alzheimer's disease: role in progression to dementia due to Alzheimers disease among subjects with mild cognitive impairment. Mol Psychiatry 2016;Epub ahead of print:1–8.
[451] Wu Q, Tchetgen Tchetgen EJ, Osypuk TL, White K, Mujahid M, Maria Glymour M. Combining direct and proxy assessments to reduce attrition bias in a longitudinal study. Alzheimer Dis Assoc Disord 2013;27:207–12.
[452] Horn JL, Cattell RB. Refinement and test of the theory of fluid and crystallized general intelligences. Journal of Educational Psychology 1966;57:253–70.
[453] Roses AD, Lutz MW, Amrine-Madsen H, Saunders AM, Crenshaw DG, Sundseth SS, et al. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. The Pharmacogenomics Journal 2010;10:375–84.
[454] Taylor JR, Birnbaum S, Ubriani R, Arnsten AF. Activation of cAMP-dependent protein kinase A in prefrontal cortex impairs working memory performance. J Neurosci 1999;19:RC23.
[455] Bernabeu R, Bevilaqua L, Ardenghi P, Bromberg E, Schmitz P, Bianchin M, et al. Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. Proc Natl Acad Sci USA 1997;94:7041–6.
[456] Andrews SJ, Das D, Anstey KJ, Easteal S. Late Onset Alzheimer's Disease Risk Variants in Cognitive Decline: The PATH Through Life Study. J Alzheimers Dis 2017;57:423–36.
[457] Lyall DM, Cullen B, Allerhand M, Smith DJ, Mackay D, Evans J, et al. Cognitive Test Scores in UK Biobank: Data Reduction in 480,416 Participants and Longitudinal Stability in 20,346 Participants. PLoS ONE 2016;11:e0154222.
[458] Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: A systematic review and metaanalysis. Alzheimers Dement 2013;9:63–75.e2.
[459] Anstey KJ, Eramudugolla R, Dixon RA. Contributions of a risk assessment approach to the prevention of Alzheimer's disease and dementia. J Alzheimers Dis 2014;42:S463–73.
[460] Pankratz VS, Roberts RO, Mielke MM, Knopman DS, Jack CR, Geda YE, et al. Predicting the risk of mild cognitive impairment in the Mayo Clinic Study of Aging. Neurology 2015.

[461] Escott-Price V, Sims R, Bannister C, Harold D, Vronskaya M, Majounie E, et al. Common polygenic variation enhances risk prediction for Alzheimer's disease. Brain 2015;138:3673–84.
[462] Anstey KJ, Cherbuin N, Herath PM. Development of a New Method for Assessing Global Risk of Alzheimer's Disease for Use in Population Health Approaches to Prevention. Prev Sci 2013;14:411–21.
[463] Anstey KJ, Cherbuin N, Herath PM, Qiu C, Kuller LH, López OL, et al. A Self-Report Risk Index to Predict Occurrence of Dementia in Three Independent Cohorts of Older Adults: The ANU-ADRI. PLoS ONE 2014;9:e86141.
[464] NHMRC. National Health and Medical Research Council (NHMRC) (2001) Australian Alcohol Guidelines: Health Risks and Benefits. NHMRC; 2001.
[465] Bielak AAM, Cherbuin N, Bunce D, Anstey KJ. Preserved differentiation between physical activity and cognitive performance across young, middle, and older adulthood over 8 years. J Gerontol B Psychol Sci Soc Sci 2014;69:523–32.
[466] Anstey KJ, Cherbuin N, Christensen H, Burns R, Reglade-Meslin C, Salim A, et al. Follow-Up of Mild Cognitive Impairment and Related Disorders over Four Years in Adults in Their Sixties: The PATH Through Life Study. Dement Geriatr Cogn Disord 2008;26:226–33.
[467] Anstey KJ, Cherbuin N, Eramudugolla R, Sargent-Cox K, Easteal S, Kumar R, et al. Characterizing mild cognitive disorders in the young-old over 8 years: prevalence, estimated incidence, stability of diagnosis, and impact on IADLs. Alzheimers Dement 2013;9:640–8.
[468] Dixon RA, DeCarlo CA, MacDonald SWS, Vergote D, Jhamandas J, Westaway D. APOE and COMT polymorphisms are complementary biomarkers of status, stability, and transitions in normal aging and early mild cognitive impairment. Front Aging Neurosci 2014;6:236.
[469] Waljee AK, Mukherjee A, Singal AG, Zhang Y, Warren J, Balis U, et al. Comparison of imputation methods for missing laboratory data in medicine. BMJ Open 2013;3.
[470] Jackson C. Multi-state modelling with R: the msm package. J Stat Soft 2011;38:1–29.
[471] Petersen RC, Negash S. Mild cognitive impairment: an overview. CNS Spectr 2008;13:45–53.
[472] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement, vol. 7, 2011, pp. 270–9.
[473] Kawas CH, Kim RC, Sonnen JA, Bullain SS, Trieu T, Corrada MM. Multiple pathologies are common and related to dementia in the oldest-old: The 90+ Study. Neurology 2015;85:535–42.
[474] Mormino EC, Sperling RA, Holmes AJ, Buckner RL, De Jager PL, Smoller JW, et al. Polygenic risk of Alzheimer disease is associated with early- and late-life processes. Neurology 2016;87:481–8.
[475] Hong C-J, Liou Y-J, Tsai S-J. Effects of BDNF polymorphisms on brain function and behavior in health and disease. Brain Res Bull 2011;86:287–97.
[476] Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-

based data. Lancet Neurol 2014;13:788–94.
[477] Bland JM, Altman DG. Statistics notes: Multiple significance tests: the Bonferroni method. Bmj 1995;310:170–0.
[478] Jensen SM, Pipper CB, Ritz C. Evaluation of multi-outcome longitudinal studies. Statistics in Medicine 2015;34:1993–2003.
[479] Peterson CB, Bogomolov M, Benjamini Y. Many Phenotypes Without Many False Discoveries: Error Controlling Strategies for Multitrait Association Studies. Genetic Epidemiology 2016;40:45–56.
[480] Greenland S, Robins JM. Empirical-Bayes adjustments for multiple comparisons are sometimes useful. Epidemiology 1991;2:244–51.
[481] Soldan A, Pettigrew C, Cai Q, Wang M-C, Moghekar AR, O'Brien RJ, et al. Hypothetical Preclinical Alzheimer Disease Groups and Longitudinal Cognitive Change. JAMA Neurol 2016;73:698–705.
[482] McDonough IM, Bischof GN, Kennedy KM, Rodrigue KM, Farrell ME, Park DC. Discrepancies between fluid and crystallized ability in healthy adults: a behavioral marker of preclinical Alzheimer's disease. Neurobiol Aging 2016;46:68–75.
[483] Ridge PG, Mukherjee S, Crane PK, Kauwe JSK, Alzheimer's Disease Genetics Consortium. Alzheimer's Disease: Analyzing the Missing Heritability. PLoS ONE 2013;8:e79771.
[484] Combarros O, Cortina-Borja M, Smith AD, Lehmann DJ. Epistasis in sporadic Alzheimer's disease. Neurobiol Aging 2009;30:1333–49.
[485] Gusareva ES, Carrasquillo MM, Bellenguez C, Cuyvers E, Colon S, Graff-Radford NR, et al. Genome-wide association interaction analysis for Alzheimer's disease. Neurobiol Aging 2014;35:2436–43.
[486] Hohman TJ, Bush WS, Jiang L, Brown-Gentry KD, Torstenson ES, Dudek SM, et al. Discovery of gene-gene interactions across multiple independent data sets of late onset Alzheimer disease from the Alzheimer Disease Genetics Consortium. Neurobiol Aging 2016;38:141–50.
[487] Belbin O, Carrasquillo MM, Crump M, Culley OJ, Hunter TA, Ma L, et al. Investigation of 15 of the top candidate genes for late-onset Alzheimer's disease. Hum Genet 2010;129:273–82.
[488] Carrasquillo MM, Belbin O, Hunter TA, Ma L, Bisceglio GD, Zou F, et al. Replication of BIN1 association with Alzheimer's disease and evaluation of genetic interactions. J Alzheimers Dis 2011;24:751–8.
[489] Hayden KM, Lutz MW, Kuchibhatla M, Germain C, Plassman BL. Effect of APOE and CD33 on Cognitive Decline. PLoS ONE 2015;10:e0130419.
[490] Emily M. AGGrEGATOr: A Gene-based GEne-Gene interActTiOn test for case-control association studies. Stat Appl Genet Mol Biol 2016;15:151–71.
[491] Dai H, Charnigo RJ, Becker ML, Leeder JS, Motsinger-Reif AA. Risk score modeling of multiple gene to gene interactions using aggregated-multifactor dimensionality reduction. BioData Min 2013;6:1.
[492] Koo CL, Liew MJ, Mohamad MS, Mohamed Salleh AH. A Review for Detecting Gene-Gene Interactions Using Machine Learning Methods in Genetic Epidemiology. Biomed Res Int 2013;2013:1–13.
[493] Karlsson IK, Bennet AM, Ploner A, Andersson TML, Reynolds CA, Gatz M, et al. Apolipoprotein E epsilon4 genotype and the temporal relationship between depression and dementia. Neurobiol Aging 2015;36:1751–6.
[494] Mielke MM, Leoutsakos J-M, Tschanz JT, Green RC, Tripodis Y, Corcoran CD, et al. Interaction between vascular factors and the APOE epsilon4

allele in predicting rate of progression in Alzheimer's disease. J Alzheimers Dis 2011;26:127–34.
[495] Wirth M, Villeneuve S, La Joie R, Marks SM, Jagust WJ. Gene-environment interactions: lifetime cognitive activity, APOE genotype, and beta-amyloid burden. J Neurosci 2014;34:8612–7.
[496] Luck T, Riedel-Heller SG, Luppa M, Wiese B, Kohler M, Jessen F, et al. Apolipoprotein E epsilon 4 genotype and a physically active lifestyle in late life: analysis of gene-environment interaction for the risk of dementia and Alzheimer's disease dementia. Psychol Med 2014;44:1319–29.
[497] Rajan KB, Wilson RS, Skarupski KA, Mendes de Leon CF, Evans DA. Gene-behavior interaction of depressive symptoms and the apolipoprotein E ε4 allele on cognitive decline. Psychosom Med 2014;76:101–8.
[498] Rajan KB, Skarupski KA, Rasmussen HE, Evans DA. Gene-environment interaction of body mass index and apolipoprotein E ε4 allele on cognitive decline. Alzheimer Dis Assoc Disord 2014;28:134–40.
[499] Andrews S, Das D, Anstey KJ, Easteal S. Interactive effect of APOE genotype and blood pressure on cognitive decline: the PATH through life study. J Alzheimers Dis 2015;44:1087–98.
[500] Cook CJ, Fletcher JM. Can education rescue genetic liability for cognitive decline? Soc Sci Med 2015;127:159–70.
[501] Smith GD, Ebrahim S. “Mendelian randomization”: can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1–22.
[502] Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey Smith G. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. Statistics in Medicine 2008;27:1133–63.
[503] Stephan BCM. Models for Predicting Risk of Dementia: Predictive Accuracy and Model Complexity. In: Leist AK, Kulmala J, Nyqvist F, editors. Health and Cognition in Old Age, vol. 10. 1st ed., Cham: Springer International Publishing; 2014, pp. 141–59.
[504] Goldstein BA, Knowles JW, Salfati E, Ioannidis JPA, Assimes TL. Simple, standardized incorporation of genetic risk into non-genetic risk prediction tools for complex traits: coronary heart disease as an example. Front Genet 2014;5:254.
[505] Goldstein BA, Navar AM, Pencina MJ, Ioannidis JP. Opportunities and challenges in developing risk prediction models with electronic health records data: a systematic review. J Am Med Inform Assoc 2016;0:1–11.
[506] Stephens ZD, Lee SY, Faghri F, Campbell RH, Zhai C, Efron MJ, et al. Big Data: Astronomical or Genomical? PLoS Biol 2015;13:e1002195.
[507] Knoppers BM, Zawati MH, Senecal K. Return of genetic testing results in the era of whole-genome sequencing. Nat Rev Genet 2015;16:553–9.
[508] Breiman L. Random Forests. Machine Learning 2001;45:5–32.
[509] Friedman JH. Stochastic gradient boosting. Computational Statistics & Data Analysis 2002;38:367–78.
[510] Cortes C, Vapnik V. Support-vector networks. Machine Learning 1995;20:273–97.
[511] Goldstein BA, Polley EC, Briggs FBS. Random Forests for Genetic Association Studies. Stat Appl Genet Mol Biol 2011;10:1–34.
[512] Falahati F, Westman E, Simmons A. Multivariate data analysis and machine learning in Alzheimer's disease with a focus on structural

magnetic resonance imaging. J Alzheimers Dis 2014;41:685–708.