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Impact of Aquaporin (*AQP1* and *AQP4*) Genetic Variation on the Relationship between Sleep Quality and Alzheimer's disease Pathological Hallmarks

Gavin Noel Mazzucchelli

A Report Submitted in Partial Fulfilment of the Requirements for the Award of Bachelor of Science (Medical Science) Honours

School of Medical and Health Sciences

Edith Cowan University

Submitted (May, 2017)

Supervisors:

Associate Professor Simon Laws

Doctor Stephanie Rainey-Smith

USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

Abstract

Alzheimer's disease (AD) is widely recognised as a growing global health issue with far ranging social and economic implications. The accumulation of Amyloid-β (Aβ) in the brain is a pathological hallmark of AD. A recently discovered lymphatic–like system in the central nervous system (termed the glymphatic system) has been postulated to be both implicit in the clearance of Aβ from the brain, and most effective during sleep—making sleep an important consideration in the investigation of AD. Central nervous system expressed water channel proteins, namely Aquaporin 1 and 4, have been suggested to play a pivotal role in glymphatic function and thus, clearance of Aβ from the brain. However, to-date this has only been investigated in AD rodent models and one human study of aquaporin/Aβ protein co-localisation in post mortem brain tissue.

To partially address this gap in knowledge, the current study sought to investigate whether genetic variations (single nucleotide polymorphisms, SNPs) within the genes encoding aquaporin 1 (*AQP1*) and aquaporin 4 (*AQP4*), were associated with AD risk, brain Aβ burden and self-reported sleep parameters. Further, this study aimed to determine whether genetic variation moderated the relationship between sleep parameters and brain Aβ burden. This study was observational and cross-sectional in design, and utilised Genome-Wide Association Study, Pittsburgh Sleep Quality Index (PSQI), and Aβ positron emission tomography data from the larger Australian Imaging, Biomarkers and Lifestyle (AIBL) study.

Genetic variation in *AQP1* and *AQP4* SNPs was not associated with either an increased AD risk or differences in brain Aβ burden. However, genetic variation in *AQP4*, specifically rs12968026, was associated with altered, self-reported, "overall" sleep quality (PSQI total score). Further, this study reports that several SNPs in *AQP1* and *AQP4* moderate the conditional effect that three PSQI-determined sleep parameters, namely, sleep latency (time taken to fall asleep, in minutes), sleep duration (length of sleep, in hours) and daytime dysfunction (disruption of daytime activities due to sleepiness), had on brain Aβ burden.

Taken together, the results of this study add weight to the argument that the glymphatic system, is a major biological mechanism underpinning Aβ clearance from the brain. The findings also engender a greater understanding of what factors may moderate a sleep-AD phenotype relationship, and suggest that interventions

targeted at improving suboptimal sleep parameters may be most effective at delaying AD onset when tailored to the genetics of the individual.

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I certify that this thesis does not, to the best of my knowledge and belief:

(i) incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education;

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(iii) contain any defamatory material.

Signed…… G. N. Mazzucchelli …..

Date……31/05/2017……

Dedication

This Honours thesis work is dedicated to my grandad, Frank V. Randall, for providing me with a lifetime of love, support and sage advice.

Acknowledgements

It has been a challenging but rewarding year of Honours. I would first and foremost thank my wife Colleen Mazzucchelli for all the encouragement, strength, comfort and support she has given me not only during this year of Honours but for my entire university baccalaureate degree. Without Colleen's constant love and reassurance this thesis would not have happened. A huge thank you to my supervisors, Associate Professor Simon Laws and Doctor Stephanie Rainey-Smith, who have gone above and beyond in providing the highest standard of mentoring and leadership. I hold you both in the highest esteem. Similarly, I thank Dr Brennen Mills, my mentor, who has always been available for me whether it be advice or to just listen to my successes and grievances. I also wish to acknowledge the unconditional help that my co-workers of Edith Cowan University's Collaborative Genomics Department—Simon Laws, Tenielle Porter, Lidija Milicic and Madeline Peretti—have provided through all stages of Honours. On a similar note I express gratitude to the many peers and colleagues from Edith Cowan University who have provided me with assistance along the way which has made my journey of becoming a scientist possible. One of those peers in particular has been Michael Clark. Further, in research nothing is possible without voluntary participants, so I express my sincere gratitude to all of the Australian, Imaging, Biomarkers and Lifestyle (AIBL) study of ageing participants, their families and the AIBL study team. Likewise, I am grateful for the assistance the CRC for Mental Health has provided by provisioning Genome-Wide Association Study data-sets for these analyses.

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1.0 Introduction

The ultimately lethal neurodegenerative condition known as Alzheimer's disease (AD) is the most common form of dementia, and the second leading cause of death in Australia throughout 2013–2015 (Australian Bureau of Statistics, 2016). The 2017 prevalence of AD in Australia is > 413,100 persons, with 55% of those being female (Alzheimer's Australia, 2017), and an Australian Institute of Health and Welfare (AIHW) report disclosed Australia's AD prevalence is expected to increase to 900,000 persons by 2050 (AIHW, 2012). L. Brown, Hansnata, and La (2017), reported the projected estimated incidence (of dementia in Australia) to be > 6.5 million persons from 2016–2056. Furthermore, the 2017 estimated cost of dementia in monetary terms is $14.67 billion (L. Brown et al., 2017). The projected increase in cases poses a dramatic social and economic burden to the Australian healthcare system, and to the family and carers of persons living with AD. AD is, however, a global problem, and Alzheimer's Disease International (ADI) has reported a global prevalence of 46 million persons (with AD) during 2015—with an estimated increase up to 131.5 million persons by 2050 (Prince et al., 2015). Additionally, it is estimated that the global financial cost of AD is expected to rise to a staggering two trillion United States dollars by 2030. Furthermore, the World Health Organization (WHO) in collaboration with ADI have insisted that dementia awareness should be forefront in public health policy and investigated further with scientific inquiry (ADI & WHO, 2012).

AD is complex and multifactorial, with the common symptomology pertaining to: progressive memory loss, apathy, emotional instability; with cognitive deficits in language, visuospatial function, reasoning, judgement and attention (Cacace, Sleegers, & Van Broeckhoven, 2016; Y. Y. Lim et al., 2014; Scheltens et al., 2016). It has been estimated that genetic factors contribute 70% of AD aetiology and by inference the remaining percent is related to environmental and lifestyle determinants (Ballard et al., 2011), including sleep quality and quantity.

A recently postulated paravascular clearance system (or 'glymphatic system') that clears toxins from the brain (Holth, Patel, & Holtzman, 2017) functions best during sleep. The aim of this Honours investigation was to explore if variation within genes encoding water channel proteins (specifically aquaporin 1 and 4) of the brain's glymphatic system were associated with AD pathophysiology and/or whether these

genetic variations modified the relationship between sleep quality and AD pathognomonic features.

2.0 Literature Review

2.1 Alzheimer's disease

The nomenclature, dementia, considered an umbrella term (Alzheimer's Association, 2013), is used to describe many forms of neurodegenerative diseases with associated neuropsychiatric symptoms, including AD; the most common form (McKhann et al., 2011).

Due to a large spectrum of complications (and severity of these) that arise, a broad definition of AD would be that it is a variety of cognitive and behavioural deficits, which are not part of normal senescence, and are characterised by the progressive worsening of symptomology. These neuropsychiatric symptoms include: memory loss, language difficulties, mood changes; deteriorated judgement and initiative (Budson & Kowall, 2011), that interfere with usual social or occupational functioning.

2.2 AD characteristics

Jack et al. (2013) define neurodegeneration as "*a progressive loss of neurons or their processes (axons and dendrites) with a corresponding progressive impairment in neuronal function*" (p. 207). As such, AD exhibits these neurodegenerative characters, plus two major gross morphological changes of the central nervous system (CNS): cortical and hippocampal atrophy. These morphological changes to the brain engender synaptic deficiency and are likely to begin about 20 years before symptomatic presentation (Masters et al., 2015).

Before an individual is diagnosed with fully developed AD there exists a pre-dementia, or prodromal AD stage, which is known as mild cognitive impairment (MCI) (Petersen, 2004). MCI refers to a substantial cognitive decline that fails to hinder one's daily living tasks. MCI is a spectrum disorder that can be broadly dichotomised into amnestic or non-amnestic, with the former usually (but not always) progressing to AD, and the latter proceeding to dementia (mainly frontotemporal dementia (FTD), or dementia with Lewy bodies (DLB)) (Petersen, 2016). Additionally, Gauthier et al. (2006) explain that the global prevalence of MCI might reach up to 19% of people aged > 65 years and the authors assert that the cognitive changes are subtle and these may even revert back into the spectrum of

what is considered normal senescence. It would be most beneficial to identify individuals at risk of AD prior to a diagnosis of MCI because this gives the largest window of opportunity to modify disease course through pharmacological therapy or lifestyle modifications.

2.2.1 Clinical

Individuals with AD exhibit a range of neuropsychiatric symptoms mainly concerning gradual decline of memory together with impairment of judgement and reason (Cacace et al., 2016). Amnestic complaints are common, and AD individuals' recall of past events becomes worse until they forget completely, plus they have impaired learning and recall of new details. Additionally, accompanying these amnestic manifestations, AD affected persons may cease to remember: the meaning of words, how to read and write, what year or season it is, how to get dressed (or to pick suitable attire for the climatic conditions) (McKhann et al., 2011). Further, apraxia along with agnosia (relating to problems recognising previously familiar faces and objects) often present, and as AD progresses and worsens, it is possible for one to become aphasic and completely lose their capacity for language and communication (McKhann et al., 1984).

Behaviourally, AD patients increasingly disengage from social activity, demonstrate a depressed mood; are apathetic, agitated or aggressive; lose their motivation and empathy; plus, display inappropriate deportment (for instance, being overtly sexual in the wrong circumstances) (Nair & Sabbagh, 2014). These behaviours are likely to occur along with other deficits of executive function such as a compromised ability to solve problems, disorientation and confusion (Gauthier et al., 2006; McKhann et al., 2011).

2.2.2 Pathological hallmarks

Concomitant with hippocampal and cortical atrophy are well described pathognomonic hallmarks that are characteristic of AD. These hallmarks include the formation of extracellular neuritic (senile) plaques; neurofibrillary tangles (NFT) (see Figure 1), otherwise known as dystrophic neurites; plus, associated astrocytosis and microgliosis (Serrano-Pozo, Frosch, Masliah, & Hyman, 2011). Microgliosis and

astrocytosis are both downstream events that occur after the formation of NFT and senile plaques. Specifically, microgliosis pertains to the migration and superabundance of microglia; the CNS' resident macrophage-like cells. Whereas, astrocytosis is one of the final responses to brain injury generally responsible for the formation of scar tissue (Boche, Perry, & Nicoll, 2013; Sajja, Hlavac, & VandeVord, 2016; Selkoe & Hardy, 2016).

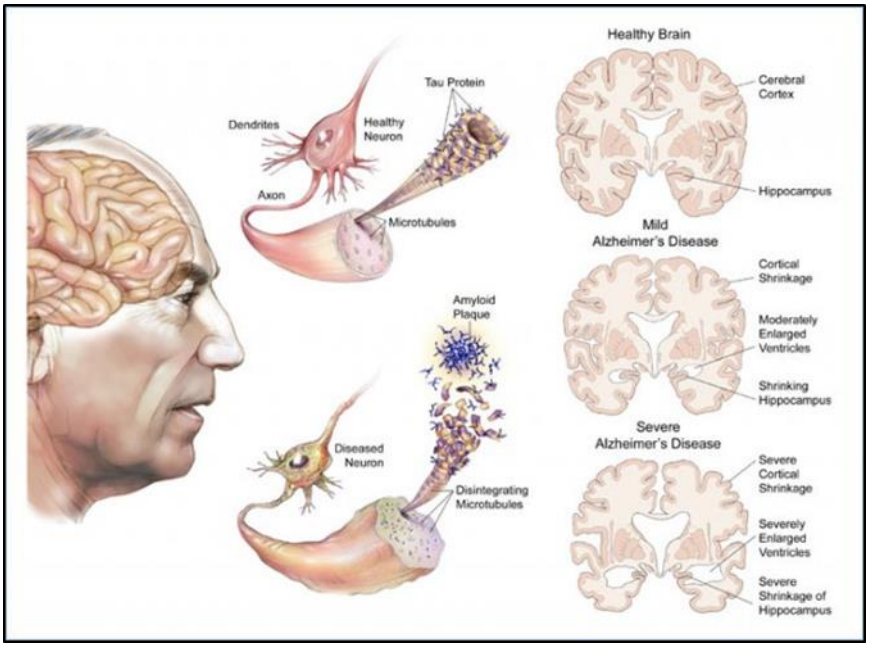

Figure 1: Pathognomonic hallmarks of Alzheimer's disease.

The major pathognomonic features of Alzheimer's disease include: Cortical and hippocampal atrophy; an aggregation of hyperphosphorylated intra-neuronal neurotoxic tau protein; and extracellular build-up of amyloid-beta (Aβ) that aggregates into plaques (senile plaques). Adapted from Delgado-Morales (2014).

The foremost hypothesis pertinent to the pathological process of AD is the 'amyloid hypothesis' (see Figure 2) (Hardy & Higgins, 1992; Hardy & Selkoe, 2002), which posits that the peptide amyloid-beta (Aβ) is deposited in the neural tissue (as insoluble extracellular plaques), preceding other events such as the formation of dystrophic neurites and glial responses (Selkoe & Hardy, 2016). Notably, Aβ is a product expressed from the transmembrane amyloid precursor protein (APP), that has undergone enzymatic (protease processing) cleavage of its N terminus by β-secretase. The C terminus of Aβ is cleaved per the action of γ-secretase, which is a key function of the protein products encoded by the presenilin 1 and 2 (*PSEN1* and *PSEN2*) genes (Goedert & Spillantini, 2006). Furthermore, Aβ has various isoforms ranging from 38–43 amino acids in length, and it is generally considered that Aβ42 is the more neurotoxic species because it easily aggregates into amyloid plaques (Masters et al., 2015). The measurement of soluble Aβ forms is possible from individuals' cerebrospinal fluid (CSF) and blood plasma (Selkoe & Hardy, 2016). Moreover, recent advancement in medical imaging technology has enabled scientific investigators to utilise positron emission tomography (PET) scans with radio-labelled (Carbon-11 or Fluorine-18) tracers, to provide *in vivo* quantitative analyses of non-soluble amyloid deposits in the brain of living individuals (Jack et al., 2013; Villemagne et al., 2013). Previously, such quantitation was only achievable via post mortem and specific histopathologic examination of the autopsied brain (Jack et al., 2010). Furthermore, prospective investigation by Villemagne et al. (2013) indicates that there is a CNS Aβ plaque build-up (and amyloid burden) for about 20 years before an individual's cognitive deficits and diminishing brain volumetrics are observed.

NFT, are formed from the hyperphosphorylated tau protein which is microtubule associated, and expressed from the microtubule-associated protein tau (*MAPT*) gene located on chromosome 17 (Biomedical Research Forum, 2016). These NFT are intracellular and cytotoxic and considered a defining hallmark of AD (Gendreau & Hall, 2013). Nevertheless, it is common to have mixed pathognomonic features of AD with another form of dementia, commonly AD with vascular infarcts, and or Lewy bodies (LB) (Schneider & Yang, 2014).

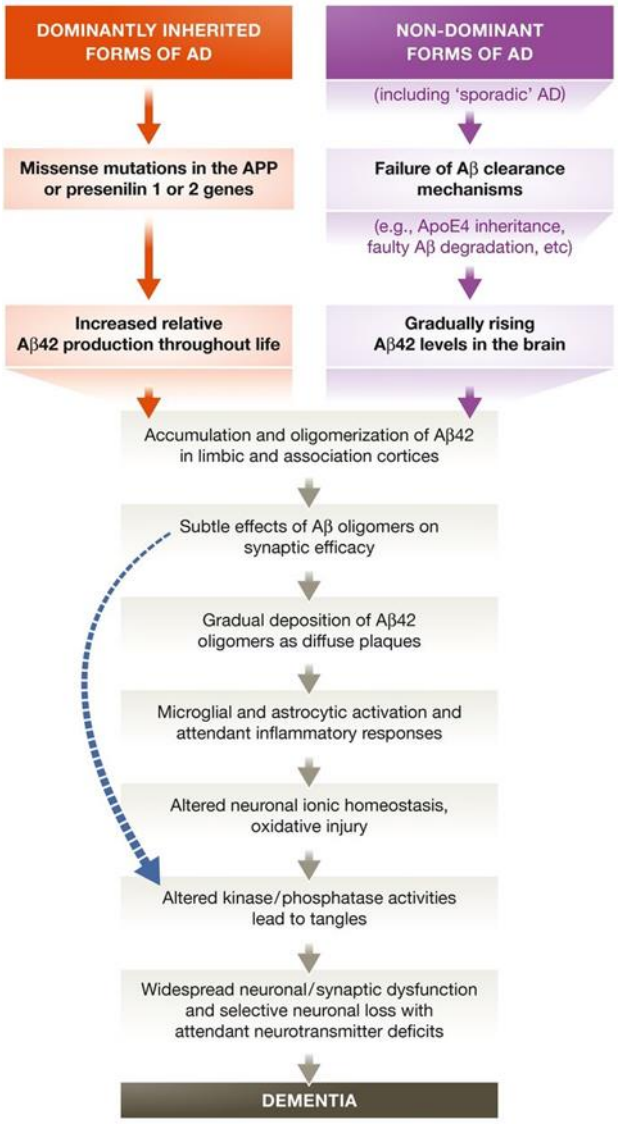

Figure 2: The sequence of major pathogenic events leading to Alzheimer's disease proposed by the amyloid cascade hypothesis.

The curved blue arrow indicates that amyloid-beta (Aβ) oligomers may directly injure the synapses and neurites of brain neurons, in addition to activating microglia and astrocytes. Adapted from Selkoe and Hardy (2016).

2.3 Non-modifiable AD risk factors

A major non-modifiable risk factor for developing AD and other neurodegenerative conditions is advancing age (Riedel, Thompson, & Brinton, 2016). Other non-modifiable risk factors include familial history and sex, with females having a higher prevalence of dementia and AD (Masters et al., 2015). The aforementioned risk of AD that has a genetic basis (70%) will be described in the following section.

2.3.1 Genetic risk of AD

Broadly, AD can be dichotomised into early onset AD (EOAD) and late onset AD (LOAD), where the former presents before the age of 65 years and comprises < 10% of AD cases, whilst the latter occurs beyond 65 years and makes up the remaining AD cases (with a reduced penetrance of its genetic risk). These classifications may be divided further into familial and sporadic forms, with a proportion of the former presenting with the rare (less than 1% of all AD) autosomal dominant AD (ADAD), with a mean onset of 45 years and heritability spanning 92–100% with a highly penetrant Mendelian pattern (see Figure 3) (Cacace et al., 2016; Masters et al., 2015).

There are currently three genes susceptible to mutation (having >280 polymorphisms) which are attributed to the development of ADAD. These include the amyloid precursor protein gene (*APP*, located on chromosome 21), the presenilin 1 (*PSEN1*, located on chromosome 14) and presenilin 2 (*PSEN2*, located on chromosome 1) genes (Cacace et al., 2016; Gaiteri, Mostafavi, Honey, De Jager, & Bennett, 2016; Masters et al., 2015). These mutations mostly cause an overexpression of Aβ, giving rise to faster onset and more pronounced detrimental phenotypes (Selkoe & Hardy, 2016).

It has been well established that variants in the apolipoprotein E gene (*APOE*, located on chromosome 19q13.2) constitute the greatest risk for LOAD: specifically carriage of the ε4 allele (Gaiteri et al., 2016). Furthermore, ε4 allele positive persons' have been shown in a recent study to display faster cognitive decline (especially episodic memory) compared to non-ε4 genotypes (Y. Y. Lim et al., 2016). However, as opposed to mutations in the aforementioned ADAD, the *APOE* ε4 allele is neither essential, nor sufficient, for development of AD, rather the combination of alleles increases or decreases risk. In the case of the ε4 allele, risk increases in a "gene-

dosage" dependent manner (Riedel et al., 2016). Karch and Goate (2015), outline in their review that the combination of data from global genome-wide analysis studies (GWAS) has supplemented scientific understanding of the risk genes attributed to LOAD. These risk genes can be subdivided into broad categories of immune response, endocytosis and cholesterol metabolism. Regarding the latter, GWAS studies have elucidated—aside from the *APOE* genotype—that polymorphisms in the: clusterin gene (*CLU*, located on chromosome 8p21.1), adenosine triphosphate-binding cassette transporter A7 gene (*ABCA7*, located on chromosome 19p13.3) and sortilin related receptor 1 gene (*SORL1*, located on chromosome 11) play a role in AD risk (Karch & Goate, 2015).

The immune response, particularly neuroinflammation along with immune dysfunction, is also implicit in the pathogenesis of AD (Heneka et al., 2015). Correspondingly, variants of the GWAS elucidated risk genes (*CR1, CD33, MS4A, EPHA1* and *TREM2*) also modify risk. Furthermore, disruption of the normal processing of APP via endocytosis dysfunctionality related to variants in genes: *BIN1*, *PICALM* and *CD2AP* (derived from GWAS data) also contribute to LOAD risk (Karch & Goate, 2015). Notwithstanding, this Honours investigation concentrated on the sporadic form of LOAD.

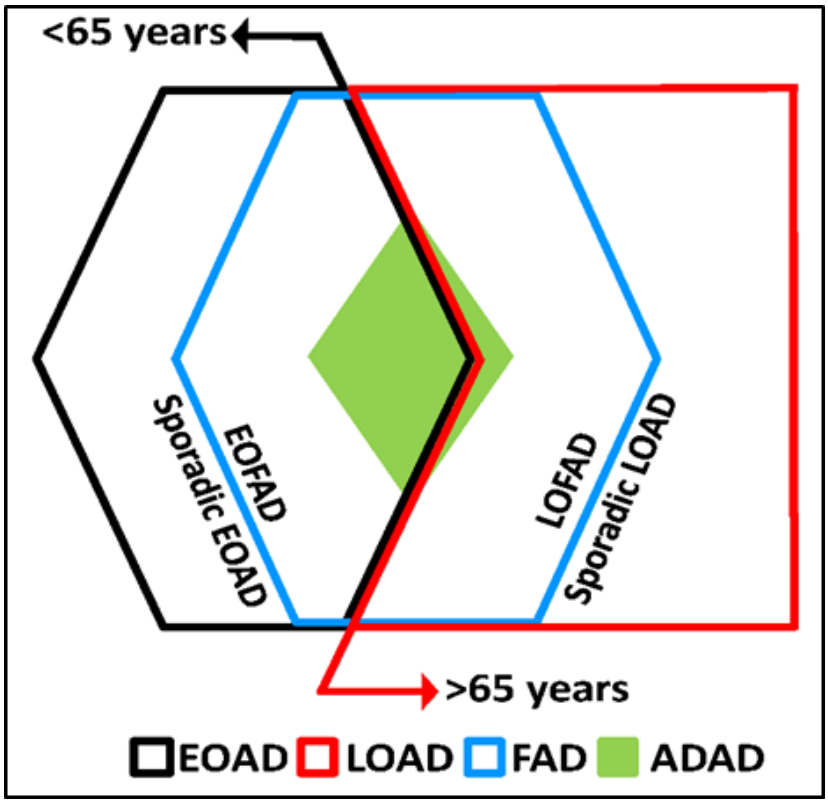

Figure 3: Alzheimer's disease (AD): relationship of the various forms in a diagrammatic representation.

EOAD, Early-onset AD; LOAD, Late-onset AD; FAD, Familial AD; ADAD, Autosomal Dominant AD; EOFAD, Early-onset Familial AD; LOFAD, Late-onset Familial AD. Figure courtesy of Laws, S. M., adapted from Wu et al. (2012).

2.3.2 Lifestyle as a modifiable AD risk factor

Potentially modifiable AD risk factors include lifestyle components such as exercise (or physical activity), diet and sleep (Nair & Sabbagh, 2014). All of these represent good targets for potential preventative AD strategies and are discussed below.

Increasing an individual's level of physical activity by means of exercising has been demonstrated to reduce risk of dementia, and slow cognitive decline. The benefits of exercise positively improve one's mood (that is, depression is reduced), help insulin signalling pathways (impaired glucose tolerance and diabetes are risk factors for dementia), reduce pro-inflammatory mechanisms, and increase neuronal growth, particularly in the hippocampal region, thus promoting better memory retention (Cholerton, Skinner, & Baker, 2014). Exercising also improves cardiovascular and cerebrovascular health which by themselves (in the diseased state) contribute to increased AD and neurodegenerative risk (Hamer & Chida, 2009). Moreover, findings from B. M. Brown et al. (2013) suggest that higher levels of total physical activity are associated with lower PET-determined Aβ burden in the brain of individuals carrying the *APOE* ε4 allele.

A transition to healthier eating in the form of a Mediterranean style diet may also reduce AD risk. This healthy dietary pattern includes consumption of a variety of fresh fruit and vegetables (and a combination of anti-oxidant rich berries), olive oil, minimal meat consumption (excluding fish and seafood, which are increased), and moderate intake of red wine that contains resveratrol and other anti-oxidants. Adherence to such a dietary pattern has been shown to decrease the decline of cognitive function, probably via positive benefits on the cardio-vascular system or via anti-inflammatory properties (Gardener, Rainey-Smith, Barnes, et al., 2015). In contrast, the typical unhealthy and pro-inflammatory western diet, involving excessive consumption of refined sugar, saturated and *trans*-fat that are constituents of most fast-food, is associated with increased cognitive decline. Thus, unhealthy eating behaviours are inflammatory in nature, which may exacerbate current AD symptomology or lead to a premature death through the detrimental co-morbidity of cardiovascular disease (Gardener, Rainey-Smith, & Martins, 2015). These dietary choices are not a stand-alone, lifestyle panacea, but should be combined with the previously mentioned increase of physical activity (if possible) and a healthy sleep routine for maximal benefit (Landry & Liu-Ambrose, 2014). Indeed, sleep represents

a third lifestyle factor of growing interest in AD research: it is the lifestyle factor of focus for this research project, and is discussed in greater depth below.

2.4 Sleep

Inadequate or dysfunctional sleep is a major concomitant morbidity associated with AD, and sleep loss has been demonstrated to impair cognition as well as memory consolidation (Harper, 2011). In fact, sleep is so critically important that flies, cockroaches, rats and humans (elucidated from studying the rare autosomal dominant, fatal familial insomnia) die after prolonged sleep deprivation (Cirelli & Tononi, 2008; Luyster, Strollo, Zee, & Walsh, 2012).

Sleep is a complex activity that invokes many physiological processes in humans and may be thought of as a perceptual disengagement from one's surroundings as one enters a state of quiescence (Carskadon & Dement, 2011). Sleep in humans can be measured quantitatively using a gold standard objective measure, for example polysomnography (requiring an overnight sleep study) or actigraphy (usually a small portable actimetry sensor device worn on the wrist) (Kirsch, 2012; Mellor, 2014). However, the present study utilised a self-reported questionnaire: Pittsburgh Sleep Quality Index (PSQI); Section 4.3.1. The PSQI is a subjective measure of sleep (Kirsch, 2012) that has been validated and assessed as reliable in a cohort of older women (Beaudreau et al., 2012) and older men (Spira et al., 2011).

2.4.1 Normal function of sleep

Normal sleep for one person would not be the same for another due to large inter-individual variability, but generally it is considered to extend for about 6–8 hours (Carskadon & Dement, 2011). Specifically, sleep is a reversible process (people go from wakefulness to sleep and *vice versa*) that oscillates over a 24-hour period, known as the circadian rhythm, that operates from a complex internal pace-maker associated with fluctuating body temperature, the endogenous hormone melatonin (produced by the pineal gland in the brain), and homeostatic sleep pressure (Schmidt, Collette, Cajochen, & Peigneux, 2007). Accordingly, when an individual begins to feel sleepy (that is, homeostatic sleep pressure rises) their core body temperature will decline as they are falling asleep, with a corresponding increase in plasma melatonin levels. Thus, the converse is true when cycling to a period of wakefulness, with

decreasing melatonin levels and an increase in core body temperature (Landry & Liu-Ambrose, 2014; Monk, 2005; Schmidt et al., 2007).

There is not one stage of sleep but several (referred to as sleep architecture) which are neuroanatomically, neurophysiologically, and behaviourally distinct. In humans, sleep is characterised by two prominent stages: rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. NREM is further subdivided into three stages—N1, N2 and N3 (Berry et al., 2015). These NREM divisions are described in relation to the waveforms they produce on an electroencephalogram (EEG) and include: N1, a transition from wakefulness to light sleep (in conjunction with a reduction in brain wave activity); N2, with reductions in heart rate as well as body temperature, relaxation of muscles and EEG readouts featuring K-complexes plus sleep spindles. N3 is also known as slow wave sleep (SWS) or delta sleep and is considered the deepest, most restorative phase of sleep which is characterised by brain waves that display a low frequency and high voltage (Berry et al., 2015; Wolkove, Elkholy, Baltzan, & Palayew, 2007). Furthermore, humans cycle through sleep stages from N1, through N2, into N3 (N1 corresponds with easy arousal to wakefulness, and the opposite for N3) then into REM approximately every 90 minutes. Of interest, dreaming occurs during REM: where the eyes move rapidly in bursts of activity behind an individual's closed lids, motor activity is actively suppressed, and increases in breathing, heart and brain rates are noted (Berry et al., 2015; Carskadon & Dement, 2011).

2.4.2 Sleep and AD

In general, older adults have lower tolerance to changes of their circadian rhythm, and their sleep patterns may change to going to sleep at an earlier time and waking at an earlier time (known as phase shift); which is pronounced more so in AD (Auerbach, 2014). Furthermore, it has been estimated that dysfunctional sleep may be present in up to 45% of people with AD, which is distressing because disturbed sleep has been listed as a possible factor that accelerates the institutionalisation of these people (Peter-Derex, Yammine, Bastuji, & Croisile, 2015). With this in mind, Mander, Winer, Jagust, and Walker (2016) highlight that sleep disturbance (amid others) is one of the first observable symptoms of AD, possibly presenting before a diagnosis of AD or even MCI.

Common sleep dysfunctions that are experienced by persons with AD include: frequent awakenings that occur late at night or early in the morning, referred to as sleep fragmentation; increased time to fall asleep (known as, sleep latency); and poor maintenance of sleep (Wolkove et al., 2007). Of note however, accumulating evidence suggests that rather than simply manifesting as a comorbidity of AD, dysfunctional sleep likely contributes to AD risk and severity. Indeed, a prospective cohort study by Lim, Kowgier, Yu, Buchman and Bennett (2013), suggests that greater sleep fragmentation contributes to the aetiology of AD by increasing the associated risk of developing AD and also accelerating the rate at which cognition declines.

2.4.3 Sleep and AD pathology

As mentioned in the previous section, a bi-directional relationship of sleep and AD phenotypes has been hypothesised (Holth et al., 2017; Ju, Lucey, & Holtzman, 2014). This posits that the AD phenotype might be causal in sleep dysfunction, and alternatively, it is also quite possible that dysfunctional sleeping behaviour could contribute to the AD phenotype (Mander et al., 2016). Moreover, a recent review authored by B. M. Brown, Rainey-Smith, Bucks, Weinborn, and Martins (2016) summarises this bi-directional relationship.

It has been noted in a study of live mice by Xie et al. (2013) that quality sleep enhances Aβ clearance from the brain. Correspondingly, Mander et al. (2015) suggest that human NREM (SWS) is impaired by brain Aβ burden which in turn disrupts memory consolidation. This association between dysfunctional sleep and Aβ burden in the brain is further supported by Branger et al. (2016) in their investigation of healthy adults' sleep behaviour. Further, a recent cross-sectional human study suggests that a greater Aβ burden is associated with increased time to fall asleep (sleep latency period) (B. M. Brown, Rainey-Smith, Villemagne, et al., 2016). Coupled with these findings, Kang et al. (2009) reported an increase in tissue fluid Aβ levels during acute sleep deprivation in two AD mouse models, whilst chronic sleep deprivation was shown to accelerate Aβ deposition in the brain. Furthermore, pharmacologically enhanced sleep (that is, sleep manipulated by the administration of drugs) decreased Aβ plaque deposition in these animals.

Lim et al. (2013) specify that sleep may prove to be a valuable target for AD intervention; possibly slowing the progress of AD pathology, that consequently might improve cognition. As such, many investigators also suggest that further study, particularly optimising sleep as a clinical target, is warranted as it could have positive benefits through the diminished aggregation of Aβ (Branger et al., 2016; B. M. Brown, Rainey-Smith, Villemagne, et al., 2016; Kang et al., 2009). The biological mechanism postulated to underpin Aβ clearance during sleep is a lymphatic–like clearance system, termed the glymphatic system.

2.5 Glymphatic system

Kress and colleagues (2014), describe that the brain has a special type of lymphatic–like clearance system that operates (parallel to the human lymphatic system) through the employment of a network of paravascular clearing mechanisms. This system has been designated as the glymphatic system. The term glymphatic is appropriate as it acknowledges the critical role that the glia or supporting cells of the brain perform, in particular astrocytes (the most common glial cell type) (see Figure 4) (Xiao & Hu, 2014). Moreover, of upmost importance is that the clearance mechanism of the glymphatic system is postulated to function almost entirely during sleep and is mostly suppressed diurnally (Lundgaard et al., 2016).

Astrocytes in human brains are essential to the formation of the blood brain barrier (BBB) which serves to create a tight knit barrier against large molecules (including many drugs), infectious particles (that is, viruses, fungi and bacteria) and inversely operates with the glymphatic system to clear toxins (Ballabh, Braun, & Nedergaard, 2004; Potokar, Jorgačevski, & Zorec, 2016; C. Yang et al., 2016). Furthermore, astrocytes are involved in maintaining brain plasticity and serve to maintain ion, osmotic, neurotransmitter and metabolite homeostasis (see Figure 4) (Sajja et al., 2016; Xiao & Hu, 2014).

Also crucial to the glymphatic system is cerebrospinal fluid (CSF). CSF is a clear fluid that is perfused in the CNS, in particular within the sub-arachnoid space of the spinal cord and the ventricles of the brain (Simon & Iliff, 2016). CSF is produced by the choroid plexus and secreted into the brain's ventricles (see Figure 5) (Damkier, Brown, & Praetorius, 2013). It has recently been hypothesised that CSF is not in circulation, but instead is regularly exchanged with brain extracellular fluid

providing a mechanism for the brain's toxins to exit the CNS (via transport in the CSF) (Orešković & Klarica, 2014). Interestingly, Lee et al. (2015) in an investigation of rodent sleeping posture, suggests sleep posture also influences the effectiveness of the clearance of Aβ from the CNS. In this study, glymphatic clearance was hypothesised to be more efficient in the lateral (lying on your side) position, than the supine (lying on your back) position, and much less effective in the prone position. Whilst the present study did not investigate sleep posture, it may prove prudent to do so in future investigations of glymphatic clearance and sleep.

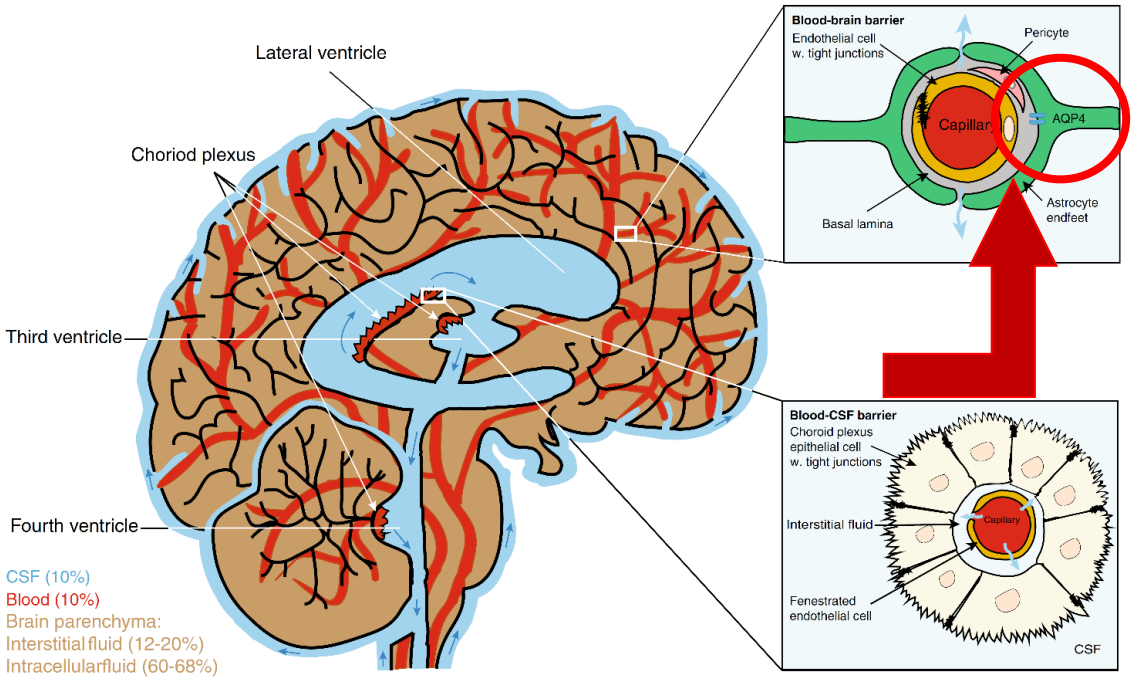

Figure 4: Glymphatic system: Schematic representation of the brain's fluid compartments and barriers showing the location of aquaporin 4 (AQP4) in astrocytic endfeet.

The fluid compartments in the brain consist of intracellular fluid (ICF; 60–68 %), interstitial fluid (ISF) or extracellular fluid (12–20 %), blood (10 %), and cerebrospinal fluid (CSF; 10 %) (Johanson, 2008; Thrane, Thrane, & Nedergaard, 2014). The brain accumulates toxins during wakefulness and during sleep it clears these toxins (including amyloid-beta; Aβ). The 'system' of clearance has been named the glymphatic system. A major protein channel in the glymphatic system is AQP4—illustrated by an arrow and red circle. AQP4 allows for fluid to transfer between astrocytic endfeet: enabling an influx swell and an efflux deflation. Adapted from Jessen, Munk, Lundgaard, and Nedergaard (2015).

2.5.1 Glymphatic system's role in AD

The human brain has a high metabolic rate thereby producing many waste products including Aβ, which, as stated earlier, is prone to accumulation (Jessen et al., 2015): a process which is even more pronounced in the aged (R. Ellis, Croteau, & Hong, 2014). Further, senescent brains (including AD phenotypes) are proposed to demonstrate impairment of glymphatic clearance mechanisms (Kress et al., 2014), thereby exacerbating Aβ accumulation. Animal studies, using *in vivo* imaging, support this notion of an impaired glymphatic clearance (Iliff et al., 2012). Consequently, toxic levels of Aβ are hypothesised to build up thus burdening the brain and exacerbating AD progression (Gallina, Scollato, Conti, Di Lorenzo, & Porfirio, 2015).

However, there remains a paucity of literature examining glymphatic clearance in humans. As such, this Honours research has endeavoured to address an element of this knowledge gap by taking a novel approach investigating a potential mechanism that plausibly impacts glymphatic function that, in turn, may modify the relationship between sleep parameters and AD pathognomonic features. This approach was the investigation of the role of genetic variation in Aquaporins—specifically *Aquaporin 1* (*AQP1*) and *Aquaporin 4* (*AQP4*)—which are discussed in detail in the next section.

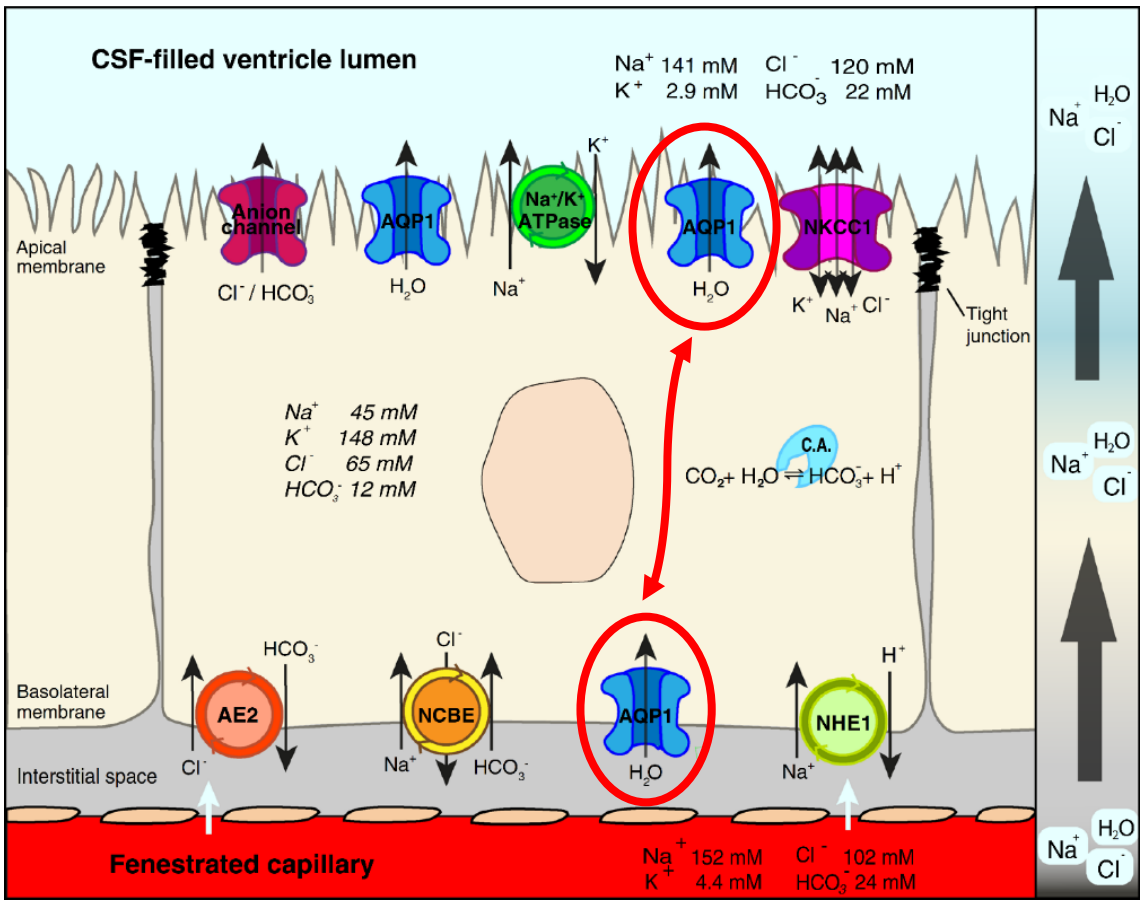

Figure 5: Schematic representation of ion composition and transport across the choroid plexus epithelial cells: With aquaporin 1's (AQP1) location indicated by red circles.

Within the brain, AQP1 (illustrated by red circles with arrow) is expressed primarily in the choroid plexus epithelial cells and is involved in transfer of cerebrospinal fluid (CSF). AQP1 is also an intricate part of the brain's waste clearance mechanism; designated the glymphatic system. Adapted from Jessen et al. (2015).

2.5.2 Aquaporins

Water channel proteins are trans-membrane proteins that serve to transport water in and out of cells, and are also known as aquaporins (AQP) (Nagelhus & Ottersen, 2013; Sorani, Manley, & Giacomini, 2008). Aquaporin water channel proteins are expressed throughout the mammalian body (King, Yasui, & Agre, 2000; Potokar et al., 2016). Of particular relevance to the current study, there are many isoforms of the aquaporin water channels found in the CNS, that is: AQP1, 2, 3, 4, 5, 7, 8, 9, and 11, however AQP4's expression is the more pronounced (Benga & Huber, 2012; Suzuki et al., 2013). Both AQP1 and AQP4 are proposed to play integral roles in the glymphatic system, as illustrated in Figures 4 and 5. Aquaporin 1, also known as channel-like integral membrane protein, 28-kDa (*CHIP28*), was the first water channel protein to be discovered and is encoded by the *AQP1* gene at cytogenetic location 7p14.3 (King et al., 2000; OMIM, 2016). In the CNS, *AQP1's* function is proposed to relate to the production and movement of CSF (Benga & Huber, 2012). Furthermore, an animal study of rats' brains treated with a neurotoxin, demonstrated via immunohistochemical and immunofluorescence techniques that *AQP1* is upregulated. This suggests that *AQP1* involvement in rat brain damage is central to glymphatic clearance (Hoshi et al., 2016). However, Igarashi, Tsujita, Kwee, and Nakada's (2014) magnetic resonance imaging study (MRI) of *AQP1* and *AQP4* knockout mice suggests that *AQP1's* function is secondary to *AQP4*.

Aquaporin 4, also known as human mercurial insensitive water channel (*MIWC*), is encoded by the *AQP4* gene at cytogenetic location 18q11.2 (Lu et al., 1996; B. Yang, Ma, & Verkman, 1995). AQP4 is, as mentioned above, the most common water channel in the CNS (Papadopoulos & Verkman, 2013), is located primarily in the subpial and perivascular endfeet of astrocytic processes and via the glymphatic system is postulated to be involved in Aβ clearance (Xiao & Hu, 2014). Furthermore, a study of autopsied human brains suggests that *Aquaporin* expression is distributed in a manner similar to neuritic plaques (Moftakhar, Lynch, Pomakian, & Vinters, 2010): which by inference suggests that *AQP1* and *AQP4s'* expression in the brain is involved (either by a failure to clear, or some other mechanism) in plaque deposition (Hoshi et al., 2012). Similarly, a recent perivascular AQP4 localization investigation of autopsied human brains (25 cognitively intact individuals < 60 years old, 33 cognitively intact individuals > 60 years, and 21 individuals with AD > 60 years old) conducted by Zeppenfeld et al. (2017), adds weight to the argument of a glymphatic

clearance dysfunction. The authors reported a decreased localization of AQP4 in the perivascular region of AD brains and this was associated, after controlling for age, with increased AD pathognomonic features (that is, NFTs and Aβ burden). By contrast, the cognitively intact (all ages) specimens had no observable loss of AQP4 perivascular localization. Taken together, these findings suggest that the glymphatic system is an important biological mechanism underpinning the clearance of brain Aβ. Further, a decrease in *AQP4* expression—or mislocalization—could be involved with a lack of Aβ clearance.

A human PET imaging study by Suzuki et al. (2015) , demonstrated that aquaporin mediated (AQP4) water influx to the CSF, and interstitial (extracellular space; ECS) flow, is reduced in humans with AD: thereby, negatively affecting the clearance rate of Aβ. These findings are also supported by Conn (2017). Furthermore, Bakker et al. (2016) discuss that the mechanism of Aβ clearance from the human brain is multifactorial and the exact pathways involved are yet to be deduced. However, these authors do report that evidence from animal models is suggestive of a perivascular and paravascular clearing mechanism that involves the bulk flow of interstitial fluid, likely involving AQP4. W. Yang et al.'s (2012) *AQP4* deficient mouse study, also supports the notion that the functionality of this water channel (AQP4) is related to the efficacy of Aβ clearance. Moreover, other mouse model studies have suggested that pericapillary (Virchow-Robin) space water homeostasis is regulated by AQP4 and is a harbinger for the clearing of Aβ by astrocytes and the glymphatic system (Igarashi, Suzuki, Kwee, & Nakada, 2014).

A review by Sorani et al. (2008) highlighted that some genetic variants of *AQP1* and *AQP4* have been elucidated which generally affect the water channels' ability to transfer water by a partial loss of function. Yet, the authors conclude that more investigation into the genetic polymorphisms is warranted. Furthermore, there remains a paucity of human literature relating to the role of Aquaporins in AD, sleep and the glymphatic system. Hence, this Honours research investigated the role of sleep, aquaporin polymorphisms and the AD pathological hallmark of Aβ.

3.0 Theoretical Framework

Aβ accumulates in the brains of individuals who have a propensity towards AD (through genetic and/or lifestyle determinants) (Selkoe & Hardy, 2016). The accumulation of Aβ is thought to begin about 20 years before the onset of AD symptomology and is problematic due to the formation of insoluble aggregates or amyloid plaques which are neurotoxic (Villemagne et al., 2013). Numerous human and animal studies (B. M. Brown, Rainey-Smith, Bucks, et al., 2016; Ju et al., 2014) have suggested that sleep is a potentially useful lifestyle target whereby early intervention could be implemented to induce a lower Aβ burden. Moreover, sleep interventions could potentially prove to be important in improving the quality of life of individuals with AD.

Recently discovered (and relevant to AD research), is a lymphatic–like system in the CNS, termed the glymphatic system, that has been postulated to be implicit in the clearance of Aβ from the brain (Iliff et al., 2012; Jessen et al., 2015). The glymphatic system has been hypothesised to primarily elicit Aβ clearance whilst one is asleep (Xie et al., 2013)—making sleep an important consideration in the investigation of ageing and AD. Furthermore, water channel proteins, namely Aquaporin 1 and 4, have been suggested to be pivotal in the function of glymphatic clearance of Aβ from the human brain.

The motivating research question that provided guidance for the design of the present study was: are genetic variants in *AQP1* and/or *AQP4* associated with AD phenotypes or sleep? To address this research question, I utilised observational data and undertook the investigation within a cross-sectional retrospective study design.

3.1 Hypotheses and aims

The overall aim of the present study was to investigate whether genetic variation (single nucleotide polymorphisms, SNPs) within the genes encoding water channel proteins expressed in the brain, specifically Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*), are associated with AD risk and brain Aβ burden and, further, whether they moderate the relationship between PSQI sleep parameters and brain Aβ burden.

SNPs of interest in *AQP1* and *AQP4* were investigated with regard to consequences of the clearing mechanisms of the glymphatic system—that is, brain Aβ burden. It is

important to realize that the glymphatic system is postulated to only function as an Aβ clearing mechanism during sleep, and glymphatic clearance during dysfunctional sleep is proposed to be sub-optimal and result in a higher Aβ burden. Thus, the study hypotheses are in part formed on the premise that good sleep quality will elicit greater clearance of Aβ (that is, reduced brain Aβ burden) and that the functional implication of genetic variation would manifest through an impact on brain Aβ burden. Therefore, the specific hypotheses of the present study were:

Hypothesis 1 (H1): Genetic variation in *AQP1*/*AQP4* is associated with differences in: i) AD risk, and/or ii) brain Aβ burden.

Hypothesis 2 (H2): Genetic variation in *AQP1*/*AQP4* is associated with differences in PSQI sleep parameters (including sleep quality/quantity).

Hypothesis 3 (H3): Genetic variation in *AQP1*/*AQP4* moderates the relationship between PSQI sleep parameters and brain Aβ burden.

Considering these hypotheses, this Honours investigation was undertaken with the following 3 aims that provided guidance for the research:

Aim 1: To determine whether genetic polymorphisms in *AQP1* and *AQP4* genes are associated with: i) the clinical classification of AD, and/or ii) levels of brain Aβ burden.

Aim 2: To test whether polymorphisms of *AQP1* and *AQP4* are associated with differences in PSQI-determined sleep parameters.

Aim3: Use moderated regression analyses to test whether genetic variants in *AQP1* and *AQP4* moderate the relationship between PSQI sleep parameters ("sleep quality/quantity") and brain Aβ burden.

4.0 Materials and Methods

4.1 Research design

This investigation utilised a cross-sectional study design that incorporated previously collected data from the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study. Briefly, initial participant inclusion/ exclusion criteria (outlined in Section 4.2.1) selected: those samples to be included in the first phase of the study that addressed Aims 1 and 2, and a secondary dataset that was incorporated into the second phase of the study, which addressed Aim 3.

4.2 Participants

This investigation accessed the data of participants already enrolled in the AIBL Study, a prospective longitudinal study of ageing launched in 2006. A paper authored by K. A. Ellis et al. (2009) specifies the AIBL Study's design, including participants' enrolment process, neuropsychological assessments, plus, exclusion and diagnostic criteria. In brief, AIBL participants are males and females over the age of 60 at enrolment who are either cognitively normal healthy controls (HC) or have been classified into an MCI or AD grouping. Approval of the AIBL Study has been granted by each of the ethics committees of each of the member institutions; Austin Health, St Vincent's Health, Hollywood Private Hospital, and Edith Cowan University (ECU), and informed written consent was given by all volunteers. Further ethical considerations are outlined in Appendix 1.

4.2.1 Study specific inclusion criteria

Whilst having access to the complete AIBL cohort, only those individuals with genetic data (outlined in 4.3.2 below) were included in the study. Further, specific inclusion criteria were then applied for each aim of the study.

Within the context of Aim 1 a 'clean' sample of HCs was defined by the inclusion of only those participants with a stable clinical diagnosis of 'cognitively healthy' across the duration of the 72-months of follow-up time-points and, where assessed, were required to have a low brain amyloid-beta (Aβ) burden through positron emission tomography (PET) imaging (outlined in Section 4.3.3 below). Specifically, to be classified as a HC the participant, if scanned, was required to have a ^{11}C-Pittsburgh

compound B PET (PiB-PET) like standardised uptake value ratio (SUVR) of < 1.4 (as calculated using the Before the Centiloid Kernel Transformation (BeCKeT) scale) for all scans. For the classification of AD, the following criteria were applied: the clinical diagnosis of AD or the combination of the clinical diagnosis of amnestic-mild cognitive impairment (aMCI) *and* a high brain Aβ burden (BeCKeT of ≥ 1.4), the combination of which has been shown in the AIBL cohort to be an extremely accurate predictor of AD development within 3-years (Rowe et al., 2013). From these participants, only those with imaging data were included in the second part of Aim 1.

In the context of Aim 2 only participants with genetic and sleep (Section 4.3.2) data were included. Whilst for Aim 3 a further criterion was that imaging data must also be available at the same time point at which the sleep data were aquired.

4.3 Data accessed

4.3.1 Sleep data

A subset of AIBL participants completed, at the 72-month follow-up time point, the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989): a 19-item, self-report measure assessing sleep quality and disturbances over the previous month. The PSQI provides the following factor scores: sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, sleep medication use, and daytime dysfunction, as well as a global score (known as PSQI total). A PSQI total score > 5 indicates poor sleep. In the present study, the relationship between genetic variants in *AQP1* and *AQP4*, brain Aβ burden, and the following sleep parameters was assessed: PSQI total, sleep latency (reported in minutes), sleep duration (reported in hours), and PSQI-derived parameters of sleep disturbance, sleep efficiency, and daytime dysfunction.

4.3.2 Genetic data

Genetic data accessed in this study were derived from a genome-wide single nucleotide polymorphism (SNP) array on 1358 AIBL participants. Briefly, genome wide analysis of 976,713 SNPs (including 273,000 exome variants and an additional 13,000 custom content SNPs) on the Illumina OmniExpressHumanExome+ BeadChip was undertaken by the Australian Genome Research Facility (Melbourne,

Victoria). Genetic markers were subsequently mapped to human genome reference hg19 with only 67 markers being unmappable. Quality control was undertaken at both marker and sample levels. At the marker level 25083 duplicated markers (identical genomic location but different marker identifications), 2925 markers with < 95% call rate and 67 unmappable markers were removed. At the sample level, 9 samples with call rate < 98%, 1 sample with a mismatch gender between reported and calculated based on genotyping data, 32 samples with high heterozygosity rate (defined as more than 3 standard deviations from the cohort mean) were removed. Overall, 948,720 markers and 1316 samples were in the final data set for imputation. Imputation was undertaken using impute2 ver2.3 using 1000 genome reference panel (2015 release).

In addition to the Aquaporin genetic data, the present study also included *APOE* genotype data as a covariate (specifically the presence/absence of the ε4 allele). These data were previously determined through TaqMan® genotyping assays (Life Technologies, USA) for rs7412 (Assay ID: C____904973_10) and rs429358 (Assay ID: C___3084793_20) that were carried out on a QuantStudio 12K Flex real-time PCR system (Applied Biosystems, USA).

4.3.2.1 Aquaporin SNP selection and quality control procedures

The GWAS dataset was analysed within the protocol of assembly 37, annotation release 105 (GRCh37.p13). Initially all SNP genomic regions of interest were extracted from the GWAS dataset for each gene (*AQP1*: GRCh37.p13: Chr.7:30,949,615–30,965,615 base pairs (bp), and *AQP4*: GRCh37.p13: Chr18:24,430–24,450 kilobase pairs (kb), see Appendices 2 and 3), which included a flanking region of 10 kb up- and downstream of the gene. The SNP data from these regions were uploaded separately (by gene) into Golden Helix SNP and Variation Suite (SVS version 8.7.0) and all genetic variants were then subjected to the quality control criteria. Specifically, i) all monomorphic (i.e. those with only one reported allele) SNPs were removed, ii) all SNPs with a successful genotype in < 95% of cases were removed, iii) all SNPs with an observed minor allele frequency of < 5% were removed, and finally iv) all SNPs that departed from Hardy-Weinberg Equilibrium (that is, $p < 0.05$ from that expected) were removed (Hardy–Weinberg equilibrium theorem states that allele and genotype frequencies in a population will

remain constant from generation to generation in the absence of other evolutionary influences).

Subsequently, each SNP dataset underwent Linkage Disequilibrium (LD) pruning as implemented within SVS using the following settings: LD threshold $r^2 > 0.8$, window size 10, and increment 5. LD pruning is a method that inactivates ("prunes") SNPs that are in high LD with other SNPs such that analyses are only undertaken using SNPs that are not in LD with each other. This approach reduced the number of SNPs being analysed but still maintained maximum coverage of genetic variation across the gene of interest. Genetic information for SNPs selected after LD pruning were extracted for all participants selected for inclusion in this study.

4.3.3 Brain imaging data

A subset of participants included in the present study have undergone PET using one of the following radiolabelled Aβ tracers, either; ^{11}C-Pittsburgh Compound B (PiB), ^{18}F-florbetapir or ^{18}F-flutemetamol, as previously described (Clark et al., 2011; Rowe et al., 2010; Vandenberghe et al., 2010). Images were analysed using CapAIBL, a web-based freely availably MR-less methodology to generate PET standardized uptake value ratios (SUVR) for all tracers (Bourgeat et al., 2015). Briefly, SUVs were summed and normalized to either the cerebellar cortex SUV (PiB), whole cerebellum SUV (florbetapir) or pons SUV (flutemetamol), to yield the target-region to reference-region SUVR. To allow for the analysis of these different tracers as a single continuous variable, a linear regression transformation has already been applied to generate PiB-like SUVR units termed the "Before the Centiloid Kernel Transformation" (BeCKeT) scale (Villemagne et al., 2014). These BeCKeT values were utilised in this cross-sectional study.

4.3.4 Demographic data

Participants provided demographic data and medical history via the completion of a questionnaire at their AIBL study assessment closest to their PET scan. At the study visit, participants also undertook a comprehensive neuropsychological assessment, completed lifestyle questionnaires and provided a fasted blood sample. All participants completed the short-form Geriatric Depression Scale (GDS) (Almeida & Almeida, 1999). Specifically for this study, age, sex, calculated body mass index

(BMI), GDS score and information regarding a medical history of cardiovascular disease (CVD), as well as *APOE* genotype data (specifically the presence/absence of the ε4 allele) was extracted from the AIBL Integrated Dataset (IDS version 6.0.0).

4.4 Data analyses

Statistical techniques and inferences were carried out with the aid of Golden Helix (Inc.) SVS (version 8.7.0) (Golden Helix, 2016), for logistic and linear regression analyses in Aim 1 and 2 (Section 4.4.2), and IBM SPSS Statistics, Version 24.0 (Armonk, NY: IBM Corp.) for moderation analyses in Aim 3 (Section 4.4.3). For all Aims, the procedure for regression analyses performed was ordinary least squares (OLS) (Cohen, Cohen, West, & Aiken, 2003; Hayes, 2013).

4.4.1 Genetic Models

In all analyses the *AQP1* and *AQP4* SNPs were grouped using at least two of the below three genetic models:

1. Additive model (Aim 1 and Aim 2): the associations of the minor allele (*M*) are cumulative, that is, where having two minor alleles (*MM*) as opposed to having no minor alleles (*mm*) is two times more likely to affect the outcome in a certain direction as is having just one minor allele (*Mm*) as opposed to no minor alleles (*mm*); where major allele is *m*.
2. Dominant model (all Aims): tests the association of having at the minimum one minor allele *M* (either *Mm or MM*) versus not having any minor alleles (*mm*).
3. Recessive model (all Aims): tests the association of having the minor allele M as both alleles (*MM*) versus at the minimum one major allele *m* (*Mm or mm*).

4.4.2 Risk and linear regression analyses (Aim 1/Aim 2)

Logistic regression analysis was performed with the binary dependent (outcome) variable of clinical classification, as defined in 4.2.1. Covariates included were *APOE* ε4 allele carriage (binary, presence/absence of ε4 allele), sex and age.

Linear regression was performed with the quantitative trait dependent (outcome) variables of brain Aβ burden (in BeCKeTs; Section 4.3.3) or the respective PSQI sleep parameters (Section 4.3.1) for Aim 1 and Aim 2, respectively. *AQP1* and *AQP4* individual SNPs were entered as independent (predictor) variables. Covariates

included were *APOE* ε4 allele carriage (binary, presence/absence of ε4 allele), sex and age—due to these variables being potential confounders. Similarly, covariates for Aim 2 included body mass index (BMI), geriatric depression scale (GDS), a medical history of CVD, sex and age (these potential confounders were also controlled for by B. M. Brown, Rainey-Smith, Villemagne, et al. (2016)).

For both, nominal significance (uncorrected) was reported at $p < 0.05$. However, final levels of significance was ascertained *after* correction for the False Discovery Rate (FDR)—designated as *q-value* with significance threshold set at $q < 0.05$—(Benjamini & Hochberg, 1995; Benjamini & Yekutieli, 2001) as applied in SVS (version 8.7.0).

4.4.3 Moderation analyses (Aim 3)

The moderation analysis was undertaken in SPSS and significance was also met if the p-value was < 0.05. A custom dialog box, called PROCESS (release 2.16.3) (Hayes, 2013), was installed into SPSS and utilised for the moderation analysis. Within Hayes (2013) PROCESS command (in SPSS) moderation model one was selected (see Figure 6), and bootstrapping (5000 bootstrap samples) was additionally chosen.

The equation used for the undertaken moderation (interaction) analysis was: $Y = i_Y + b_1X + b_2W + b_3XW$. Where: Y = outcome, i_Y = Y intercept, b_1 = coefficient of the predictor, X = predictor, b_2 = coefficient of the moderating variable, W = moderator, b_3 = coefficient of the interaction, XW = interaction of the predictor * moderator.

To visualise the moderation of the effect of *X* on *Y* by the moderating variable (*W*) it was necessary to probe the significant interactions with a post hoc analysis. Simple slopes analysis was used, where the mean of *W* and 1 standard deviation above and below that was plotted (Aiken & West, 1993; Hayes & Rockwood, 2016). *AQP1* and *AQP4* SNPs were included as a moderator variable (*W*) in the models established by B. M. Brown, Rainey-Smith, Villemagne, et al. (2016)—to ascertain whether they modify the relationship between sleep parameters and brain Aβ burden.

To facilitate the interpretation of the post hoc probing, the interactions were analysed with respect to the dominant and recessive genetic models only (Section 4.4.1). Brain Aβ burden (in BeCKeTs) was entered as the outcome variable (*Y*) with each of the

six PSQI sleep parameters entered individually as the independent variable (*X*). Finally, SNPs in *AQP1* and *AQP4 (*interaction of *PSQI sleep parameter * AQP1/4 SNP*) was the moderator (*W*) variable. Moderation analyses covaried for age, BMI, medical history of CVD, GDS and *APOE* ε4 allele carriage.

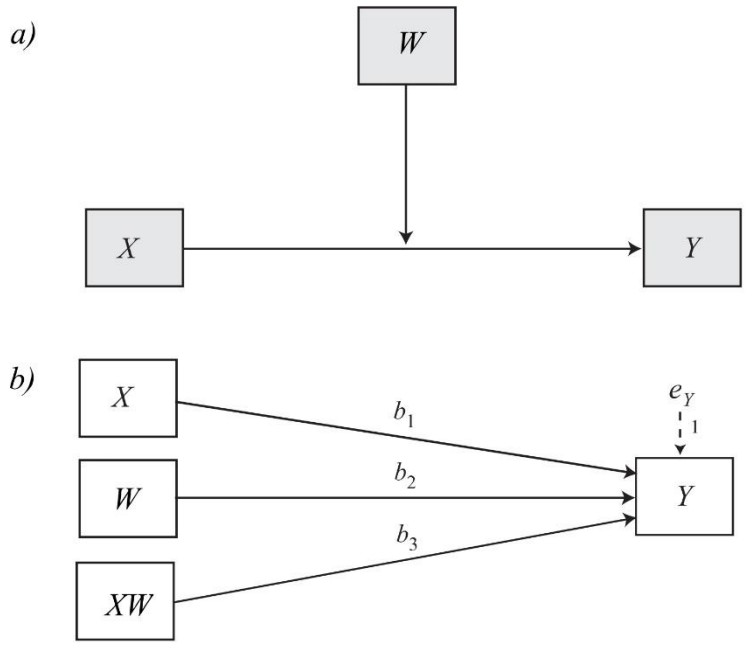

Figure 6: a) Simple conceptual moderation model, b) diagram of the statistical moderation model.

a) X refers to the independent or predictor variable, Y refers to outcome or response variable, and W refers to the moderator variable. Adapted from Hayes (2013, p. 209).

b) X refers to the independent or predictor variable, Y refers to outcome or response variable, W refers to the moderator variable, XW is the interaction (that is, the predictor variable multiplied by the moderator variable); b_1 is the regression coefficient of the predictor (X), b_2 is the regression coefficient of the moderator, b_3 is the regression coefficient of the interaction, and e_{y1} is the residual. Adapted from Hayes (2013, p. 215).

5.0 Results

5.1 SNP selection and quality control

Previously accrued Genome-wide Association Study (GWAS) data from the AIBL study cohort (Section 4.3.2) were leveraged in this study for the selection of single nucleotide polymorphisms (SNPs) within, and 10 kilobase pairs (kb), up- and downstream of the Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) genes (as described in Section 4.3.2.1). This resulted in the initial identification of 525 *AQP1* and 538 *AQP4* SNPs. Quality control (QC) exclusion criteria, outlined in Section 4.3.2.1, were then applied to filter the list of SNPs to take forward for analysis in the subsequent sections of this thesis. These QC measures resulted in a step-wise reduction in SNPs (Figure 7) and retained a final total of 18 and 32 SNPs for *AQP1* and *AQP4*, respectively. A list of these SNPs including minor allele frequency and Hardy-Weinberg equilibrium p-values are presented in *Appendix 4*.

To remove SNP redundancy and therefore reduce total SNP number for analysis, linkage disequilibrium (LD) pruning, as described in Section 4.3.2.1, was undertaken. By eliminating a high degree of correlation (r^2 value of > 0.8), the total SNP numbers were reduced to 8 (from 18) and 13 (from 32) for *AQP1* and *AQP4*, respectively. The minor allele frequencies and Hardy-Weinberg equilibrium p-values for the specific SNPs selected for analysis are presented in Table 1. This reduction in SNP content was made without compromising SNP coverage of the *AQP1* and *AQP4* genes. A visual depiction of the LD prune, both pre- and post-pruning, can be found in Figures 8 and 9 for *AQP1* and *AQP4*, respectively.

5.2 Demographics of the cohort

This cross-sectional project's study cohort demographic information and data (including brain imaging, genetic and sleep data) were extracted from the larger prospective Australian Imaging, Biomarker and Lifestyle (AIBL) study of ageing cohort. As sample sizes within each aim changed due to availability of the data to be analysed, the demographic characteristics are presented *Aim-by-Aim* in Table 2. The study specific inclusion criteria for defining groups to be analysed with specific aims are described in Section 4.2.1.

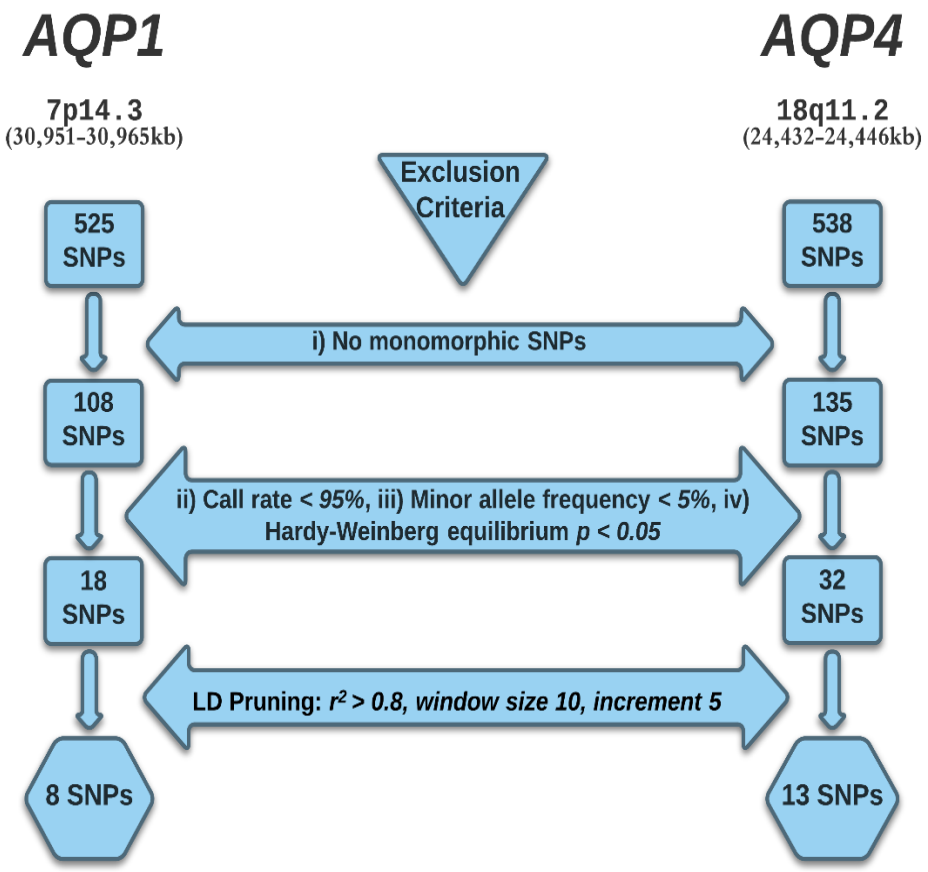

Figure 7: Flow diagram of genetic data quality control and SNP selection for *AQP1* and *AQP4*.

Chromosomal region of *AQP1* (cytogenetic location, 7p14.3) selected from 30,951–30,965 kilobases (kb) and *AQP4* (cytogenetic location, 18q11.2) selected from 24,432–24,446kb. Prior to SNP data quality control there were n = 525 *AQP1* SNPs and n = 538 *AQP4* SNPs. Exclusion criteria applied were: i) removal of monomorphic SNPs (n = 417 *AQP1* and n = 403 *AQP4*), ii) removal of SNPs that had a call rate of < 0.05, iii) removal of those SNPs that had a minor allele frequency (MAF) < 0.05, and iv) removal of those SNPs that deviated from Hardy-Weinberg equilibrium (HWE; p < 0.05 constituted removal). Application of excluding criteria (i-iv) left n = 18 *AQP1* and n = 32 *AQP4* SNPs. Finally, linkage disequilibrium (LD) pruning was undertaken (r^2 cut-off of 0.8, window size 10, increment 5) leaving n = 8 *AQP1* and n = 13 *AQP4* SNPs to take forward for analyses.

Table 1: AQP1 and AQP4 SNPs Obtained Post Linkage Disequilibrium Pruning.

SNP Ref	Major Allele	Call Rate	Minor Allele	MAF	HWE-p
AQP1					
rs2075574	C	1.00	T	0.36	0.64
rs1859838	A	1.00	G	0.17	0.06
rs4419722	T	1.00	G	0.12	0.64
rs1004317	A	1.00	G	0.39	0.79
rs62449133	A	0.97	G	0.22	0.16
rs2299905	A	0.96	T	0.28	0.29
rs28362727	A	0.97	C	0.27	0.47
rs11537660	T	1.00	C	0.07	0.72
AQP4					
rs11661081	C	1.00	A	0.09	0.89
rs9951307	A	1.00	G	0.36	0.65
rs7240333	C	0.99	T	0.11	0.41
rs68006382	A	0.98	G	0.18	0.62
rs71353406	C	0.98	A	0.30	0.45
rs12968026	T	0.97	C	0.12	0.48
rs3875089	T	1.00	C	0.16	0.84
rs162007	G	1.00	A	0.20	0.80
rs162003	C	1.00	T	0.08	0.86
rs151245	T	1.00	G	0.40	0.22
rs151246	G	0.99	T	0.20	0.11
rs2339214	G	0.98	A	0.48	0.63
rs491148	A	1.00	G	0.17	0.11

Final curated list of Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) single nucleotide polymorphisms (SNPs) selected for analysis in subsequent Aims. SNP Ref, the reference single nucleotide polymorphism (SNP) marker (rs). Nucleotides: guanine (G), cytosine (C), adenine (A), thymine (T). Exclusion criteria: Call rate < 95%; MAF, minor allele frequency < 5%; HWE-p, Hardy-Weinberg equilibrium p-value < 0.05.

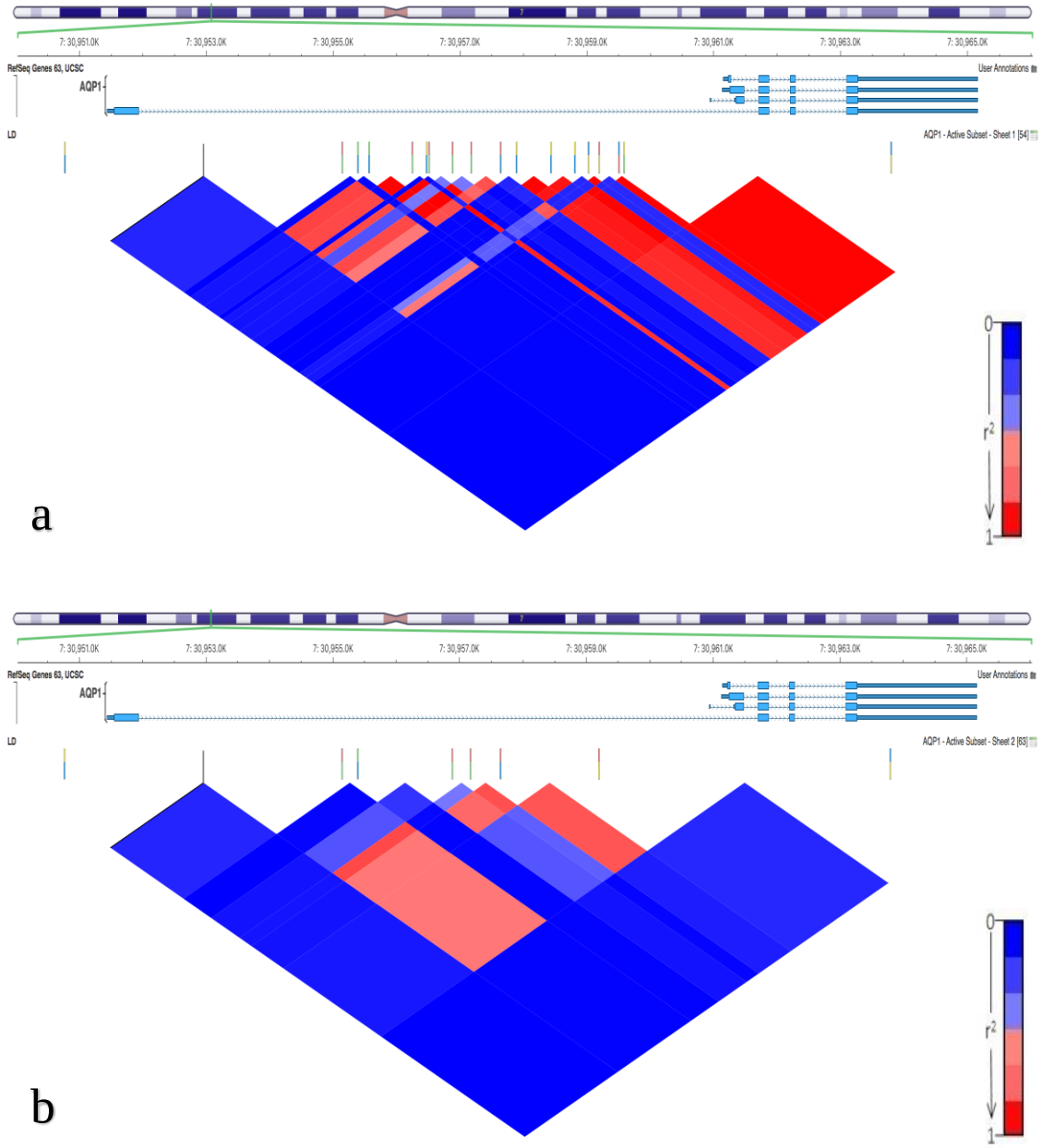

Figure 8: Linkage disequilibrium (LD) structures for *AQP1*

LD structures are presented (a) pre, and (b) post LD pruning of *AQP1* SNPs. LD pruning was undertaken in Golden Helix SVS (v8.7.0) using criteria of: $r^2 > 0.8$, window size 10, increment 5. a) Prior to LD pruning *AQP1* SNPs n = 18, b) after LD pruning *AQP1* SNPs n = 8. Vertical lines above the diamond graph indicate SNPs. Diamond graph: deep red indicates high r^2 value, whilst deep blue indicates a lower r^2 value.

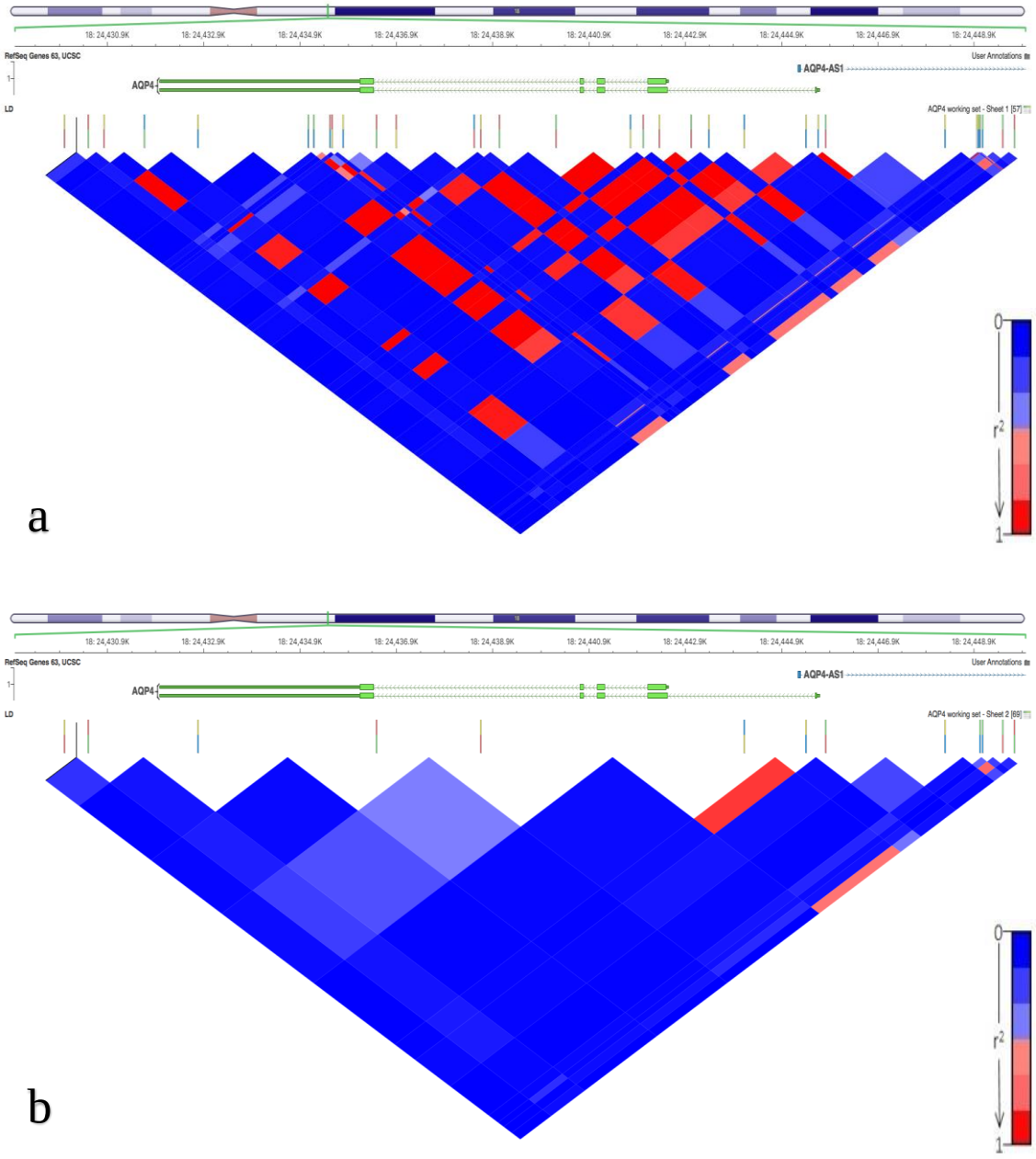

Figure 9: Linkage disequilibrium (LD) structures for *AQP4*

LD structures are presented *(a)* pre, and *(b)* post LD pruning of *AQP4* SNPs. LD pruning was undertaken in Golden Helix SVS (v8.7.0) using criteria of: $r^2 > 0.8$, window size 10, increment 5. a) Prior to LD pruning *AQP4* SNPs n = 32, b) after LD pruning *AQP4* SNPs n = 13. Vertical lines above the diamond graph indicate SNPs. Diamond graph: deep red indicates high r^2 value, whilst deep blue indicates a lower r^2 value.

Table 2: Demographic Characteristics of the Cohort Studied (per Aim).

Cohort Descriptive Statistics	Aim1		Aim 2	Aim 3
	HC	*AD*		
n =	528	402	462	222
Age	69.7 ± 6.5	75.8 ± 7.3	75.0 ± 6.0	75.2 ± 6.1
Sex (% F)	64.4	52.3	58.1	57.2
APOE (% ε4)	19.7	65.4	22.7	23.0
*Aβ: BeCKeT**	1.21 ± 0.09†	2.12 ± 0.5‡		1.38 ± 0.38
BMI			26.5 ± 4.3	26.4 ± 4.2
GDS			1.4 ± 1.7	1.3 ± 1.6
% Good sleepers (n)			50.9 (235)	55.9 (124)
PSQI total			6.2 ± 1.2	5.6 ± 3.2
Sleep latency (minutes)			19.9 ± 19.4	17.0 ± 16.6
Sleep duration (hours)			6.8 ± 1.2	7.0 ± 1.2

All values represented as mean ± standard deviation, unless otherwise indicated. Data are only presented for where data were available for all participants included in the respective study aim analyses. HC, Healthy control; AD, Alzheimer's disease; *APOE*, apolipoprotein E ε4 allele carriage; Aβ, amyloid-beta; BMI, Body Mass Index; GDS, Geriatric Depression Scale; PSQI, Pittsburgh Sleep Quality Index. *^{11}C-Pittsburgh compound B PET (PiB-PET) like standardised uptake value ratio (SUVR) generated using the Before the Centiloid Kernel Transformation (BeCKeT) scale, † n = 376, ‡ n = 288.

Briefly, within the first part of *Aim 1*, associations of *AQP1* and *AQP4* SNPs with AD risk analysis were assessed in healthy controls (HC; n = 528) and AD cases (n = 402). Where HCs were defined as having a stable clinical classification across all time points and a low brain Amyloid-beta (Aβ; PiB-PET-like SUVR < 1.4), whilst the classification of AD required either a clinical diagnosis of AD or the combination of a clinical diagnosis of amnestic-mild cognitive impairment (aMCI) *and* a high brain Aβ burden (SUVR ≥ 1.4). From these, only those individuals with available brain imaging data (HC, n = 376; AD, n = 288) were included in the second part of *Aim 1*, where analyses were performed to ascertain if *AQP1* and *AQP4* SNPs were associated with brain Aβ burden. The impact of *AQP1* and *AQP4* SNPs on Pittsburgh Sleep Quality Index (PSQI) sleep parameters (*Aim 2*) was investigated in those individuals with both PSQI and genetic data (n = 462). Finally, the moderating effects of *AQP1/AQP4* SNPs on the relationship between sleep parameters and brain Aβ burden (*Aim 3*) was investigated in individuals for whom there were available genetic data, PSQI data and imaging data. The PSQI and imaging data were required to be available from the same 72-month follow-up time-point of the AIBL study (n = 222).

5.3 Analysis of association between AQP1/AQP4 SNPs and AD risk

A risk analysis was undertaken to ascertain whether there were differences in genotype and allelic frequencies of *AQP1* and *AQP4* SNPs between the HC (n = 528) and AD (n = 402) groups (Table 3). No significant associations of *AQP1* SNPs with AD risk were observed across the three genetic models used: additive, dominant and recessive (described in Section 4.4). Nominal significance (that is, uncorrected *p-value*) was observed in two *AQP4* SNPs; rs7240333 (additive genetic model $p = 0.04$, and dominant genetic model $p = 0.03$), and rs68006382 (recessive genetic model $p = 0.04$). However, these associations did not remain significant after False Discovery Rate (FDR) correction.

5.4 Association of AQP1 and AQP4 SNPs with brain Aβ burden

To determine if any statistical association between *AQP1* and *AQP4* SNPs and brain Aβ was evident a linear regression analysis was performed. Table 4 shows the

nominal (uncorrected) p-values, across the three genetic models (additive, dominant, and recessive) performed in the absence (base model) or presence (adjusted model) of the covariates of age, sex, *APOE* ε4 allele and clinical classification (HC or AD). No significant associations were detected for *AQP1* SNPs in the adjusted models, though nominal significance was observed in the base model for rs4419722 (p = 0.025) and rs11537660 (p = 0.036). Conversely, no significant associations of *AQP4* variants were observed in the base models, however two *AQP4* variants, rs162007 (additive model, p = 0.044 [β = 0.057]; recessive model, p = 0.047, [β = 0.162]); and rs162003 (dominant model, p = 0.047 [β = 0.086]) presented with nominally significant associations with brain Aβ burden in the adjusted models. Whilst nominally significant associations with brain Aβ burden were observed, they did not survive FDR correction. A complete list of p-values from all models for all SNPs is provided in *Appendix 5*.

Table 3: *AQP1* and *AQP4* Genotype and Allele Frequencies and association with AD risk.

SNP Ref	MAF		MM		Mm		mm		p-value†		
	AD	*HC*	*AD*	*HC*	*AD*	*HC*	*AD*	*HC*	*Add**	*Rec**	*Dom**
AQP1											
rs2075574	0.35	0.34	0.12	0.12	0.45	0.44	0.42	0.44	0.37	0.61	0.16
rs1859838	0.17	0.16	0.04	0.04	0.26	0.25	0.70	0.72	0.94	0.74	0.85
rs4419722	0.12	0.12	0.02	0.01	0.21	0.22	0.77	0.77	0.18	0.10	0.25
rs1004317	0.38	0.39	0.14	0.15	0.47	0.48	0.39	0.37	0.73	0.90	0.48
rs62449133	0.22	0.22	0.06	0.06	0.32	0.32	0.62	0.63	0.88	0.79	0.63
rs2299905	0.27	0.28	0.08	0.08	0.38	0.40	0.55	0.52	0.83	0.65	0.80
rs28362727	0.26	0.27	0.07	0.08	0.39	0.37	0.54	0.55	0.86	0.75	0.60
rs11537660	0.06	0.07	<.01	<.01	0.12	0.13	0.88	0.86	0.26	0.10	0.95
AQP4											
rs11661081	0.09	0.10	0.01	0.01	0.17	0.18	0.82	0.82	0.86	0.70	0.78
rs9951307	0.37	0.34	0.13	0.12	0.48	0.45	0.39	0.43	0.46	0.73	0.21
rs7240333	0.13	0.11	0.01	0.02	0.24	0.18	0.75	0.80	**0.04**	0.42	**0.03**
rs68006382	0.16	0.19	0.02	0.04	0.29	0.31	0.70	0.65	0.08	**0.04**	0.14
rs71353406	0.30	0.30	0.08	0.09	0.43	0.44	0.49	0.48	0.92	0.75	0.72
rs12968026	0.13	0.12	0.02	0.02	0.23	0.20	0.76	0.78	0.69	0.87	0.44
rs3875089	0.17	0.16	0.03	0.03	0.28	0.26	0.69	0.71	0.86	0.90	0.58
rs162007	0.17	0.20	0.03	0.04	0.27	0.32	0.70	0.64	0.16	0.38	0.06
rs162003	0.07	0.09	<.01	0.01	0.13	0.16	0.87	0.82	0.07	0.06	0.07
rs151245	0.41	0.41	0.13	0.17	0.54	0.47	0.32	0.36	0.07	0.13	0.25
rs151246	0.21	0.20	0.05	0.06	0.32	0.29	0.64	0.66	0.63	0.59	0.55
rs2339214	0.48	0.46	0.21	0.22	0.53	0.49	0.26	0.29	0.52	0.92	0.29
rs491148	0.18	0.18	0.03	0.05	0.31	0.26	0.66	0.69	0.18	0.17	0.42

AQP1, Aquaporin 1; *AQP4*, Aquaporin 4; SNP Ref, reference single nucleotide polymorphism marker (rs); HC, Healthy control; AD, Alzheimer's disease; MAF, Minor Allele Frequency; M, Minor allele; m, major allele; MM, homozygote for the minor allele; Mm, heterozygote for the minor allele; mm, homozygote for the major allele. *Genetic models: Add, additive (homozygote for the minor allele (MM) vs heterozygote for the minor allele (Mm) vs homozygote for the major allele (mm)); Rec, recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); Dom, dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)). †Nominally significant ($p < 0.05$; uncorrected) p-values bolded.

Table 4: Association of *AQP1* and *AQP4* genetic variants with brain Aβ burden.

SNP Ref	Additive†		Dominant†		Recessive†	
	Base*	***Adj****	***Base****	***Adj****	***Base****	***Adj****
AQP1						
rs4419722	0.108	0.457	0.242	0.658	**0.025**	0.781
rs11537660	0.586	0.313	0.920	0.528	**0.036**	0.312
AQP4						
rs3875089	0.059	0.504	0.056	0.563	0.488	0.596
rs162007	0.714	**0.044**	0.999	0.100	0.260	**0.047**
rs162003	0.586	0.061	0.751	**0.047**	0.218	0.977

SNP Ref, reference single nucleotide polymorphism marker (rs); *AQP1*, Aquaporin 1; *AQP4*, Aquaporin 4. †Genetic models: Additive (homozygote for the minor allele (MM) vs heterozygote for the minor allele (Mm) vs homozygote for the minor allele (Mm)); Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)). *Statistical models: Base, base statistical model that is, no covariates; Adj, Adjusted statistical model (covaries for: age, sex, Apolipoprotein E status (ε4-carrier/non-carrier) and clinical classification (Healthy control/Alzheimer's disease). Nominally significant ($p < 0.05$; uncorrected) p-values bolded.

5.5 Association of AQP1 and AQP4 SNPs with PSQI sleep parameters

Linear regression analysis was also performed to determine if *AQP1/AQP4* SNPs were associated with PSQI sleep parameters, specifically: PSQI total, sleep latency (minutes), sleep duration (hours), sleep disturbances, sleep efficiency and daytime dysfunction. As per Section 5.4, both a base statistical model (no covariates) and an adjusted statistical model, covarying for age, sex, *APOE ε4* allele and clinical classification (HC or AD), were used. Table 5 summarises those *AQP1* and *AQP4* SNPs for which a nominal significance was observed in one or both statistical models for a respective PSQI sleep parameter. A complete list of p-values derived from these analyses is provided in *Appendix 6.*

For *AQP1* nominally (uncorrected) significant associations were observed for several SNPs across multiple sleep parameters. *AQP1* rs1004317 (additive model, $p = 0.041$; dominant model, $p = 0.025$), rs2299905 (additive model, $p = 0.012$; dominant genetic model, $p = 0.019$) and rs28362727 (additive model, $p = 0.045$) were associated in the base model with PSQI total. However, only rs1004317 (dominant model, $p = 0.047$) and rs2299905 (additive model, $p = 0.015$; dominant genetic model, $p = 0.033$) were significant in the adjusted model, though they did not survive FDR correction. Two of these SNPs, rs2299905 (additive model, $p = 0.045$) and rs28362727 (additive model, $p = 0.037$) in addition to rs1859838 (recessive model, $p = 0.043$) were associated in the base model with sleep duration. However, only rs1859838 (recessive model, $p = 0.031$) was significant after adjusting for covariates, though again failed to survive FDR correction.

Table 5: Association of *AQP1* and *AQP4* SNPs with Pittsburgh Sleep Quality Index Sleep Parameters.

PSQI Sleep Parameter	SNP Ref	Additive†		Dominant†		Recessive†	
		*Base**	*Adj**	*Base**	*Adj**	*Base**	*Adj**
		AQP1					
PSQI total	rs1004317	**0.041**	0.052	**0.025**	**0.047**	0.364	0.289
	rs2299905	**0.012**	**0.015**	**0.019**	**0.033**	0.095	0.067
	rs28362727	**0.045**	0.062	0.088	0.111	0.110	0.135
Sleep duration (hours)	rs1859838	0.065	0.088	0.166	0.238	**0.043**	**0.031**
	rs2299905	**0.045**	0.057	0.093	0.136	0.110	0.081
	rs28362727	**0.037**	0.061	0.094	0.162	0.064	0.064
Sleep disturbances	rs1859838	0.357	0.596	0.841	0.889	**0.015**	**0.037**
		AQP4					
PSQI total	rs71353406	0.130	0.100	**0.042**	**0.045**	0.856	0.871
	rs12968026	0.593	0.836	0.647	0.466	**0.0006‡**	**0.002‡**
	rs3875089	0.494	0.442	0.940	0.931	**0.012**	**0.021**
Sleep disturbances	rs68006382	0.097	0.146	**0.034**	0.077	0.062	0.902
Daytime dysfunction	rs12968026	0.446	0.855	0.981	0.381	**0.005**	**0.032**
	rs3875089	0.556	0.364	0.151	0.096	**0.024**	0.066
	rs162007	0.271	0.116	0.139	**0.044**	0.477	0.476

PSQI, Pittsburgh Sleep Quality Index Sleep Parameters: PSQI total, sleep latency (Latency, in minutes), sleep duration (Duration, in hours), sleep disturbances (Disturbances), Daytime dysfunction. SNP Ref, reference single nucleotide polymorphism marker (rs); *AQP1*, Aquaporin 1; *AQP4*, Aquaporin 4. †Genetic models: Additive (homozygote for the minor allele (MM) vs heterozygote for the minor allele (Mm) vs homozygote for the major allele (mm)); Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)). *Statistical models: Base, base statistical model that is, no covariates; Adj, Adjusted statistical model (covaries for: age, sex, body mass index (BMI), geriatric depression scale (GDS) and a medical history of CVD). ‡Values significant after False Discovery Rate correction ($q < 0.05$). Values that reached nominal significance ($p < 0.05$, uncorrected) are bolded.

AQP1 rs1859838 was also observed to have a nominally significant association with sleep disturbances in both the base (recessive model, $p = 0.015$) and adjusted (recessive model, $p = 0.037$) models. However, this association likewise was not significant after FDR correction.

Several *AQP4* SNPs also demonstrated nominally (uncorrected) significant associations with multiple sleep parameters. Significant associations with PSQI total were observed for *AQP4* SNPs in the base (rs71353406, dominant model, $p = 0.042$; rs12968026, recessive model, $p < 0.001$; rs3875089, recessive model, $p = 0.012$) and adjusted models (rs71353406, dominant model, $p = 0.045$; rs12968026, recessive model, $p = 0.002$; rs3875089, recessive model, $p = 0.021$). The observed associations for rs12968026 (unadjusted, *$\beta = 4.74$ [SE: 1.37];* adjusted, $\beta = 4.15$ *[SE: 1.34])* remained significant after FDR correction for both the base ($q = 0.008$) and adjusted ($q = 0.028$) statistical models. Both rs12968026 (recessive model, $p = 0.005$) and rs3875089 (recessive model, $p = 0.024$) were associated with daytime dysfunction in the base model. However, only rs12968026 (recessive model, $p = 0.032$), in addition to rs162007 (dominant model, $p = 0.044$) were significant after adjusting for covariates, though neither was significant after FDR correction. Finally, a nominally significant association of rs68006382 (dominant model, $p = 0.034$) with sleep disturbances was observed for the base model, which neither survived adjustment for covariates or correction for the FDR.

5.6 Moderating effect of AQP1 and AQP4 SNPs on the relationship between PSQI sleep parameters and brain Aβ burden

Linear regression analysis confirmed the association of sleep latency (minutes) with Aβ burden ($\beta = 0.004$, t (215) = 2.66; *95% CI*, 0.001–0.007; $p = 0.008$) in this subset of the AIBL cohort, independent of *AQP1* and *AQP4* SNPs. No other PSQI sleep parameter was observed to be associated with brain Aβ burden in these analyses, which covaried for age, sex, body mass index (BMI), geriatric depression scale (GDS), a medical history of cardiovascular disease (CVD) and *APOE* ε4 allele status (see *Appendix 7*).

To determine if *AQP1* or *AQP4* SNPs moderated the relationship between PSQI sleep parameters and brain Aβ burden, multivariate linear regression analyses were performed within the moderation model (*SPSS, PROCESS* see Section 4.4.3*)* and

covaried for age, sex, BMI, GDS, a medical history of CVD and *APOE* ε4 allele status. Sixteen of these statistical models revealed significant moderation effects for either *AQP1* or *AQP4* SNPs on the PSQI sleep parameters of sleep latency, sleep duration and daytime dysfunction and are presented in Tables 6–8. Additionally, visual representation of the conditional effects (that is, simple slopes analyses, as described in Section 4.4.2) of those models is depicted in Figures 10–15. A complete list of all *AQP1* and *AQP4* moderation analyses, including those that did not reach significance, are presented in the respective appendices (PSQI Total, Appendix 8; Sleep Latency, Appendix 9; Sleep Duration, Appendix 10; Sleep Disturbances, Appendix 11; Sleep Efficiency, Appendix 12; Daytime Dysfunction, Appendix 13).

The relationship between PSQI-determined sleep latency (in minutes) and brain Aβ burden was observed to be significantly moderated by the largest number of *AQP1* or *AQP4* SNPs (six in total; Table 6). With respect to *AQP1*, the interaction of rs28362727 and sleep latency was significant for both the dominant (R^2*-change* (ΔR^2) = 0.018; $p = 0.034$) and recessive ($\Delta R^2 = 0.035$; $p = 0.003$) genetic models. Visual representation of these conditional effects of *AQP1* rs28362727 for both the dominant and recessive genetic models is presented in Figure 10. A total of five *AQP4* SNPs were also found to significantly moderate the impact of sleep on brain Aβ burden. The interaction of the *AQP4* SNPs rs491148 and sleep latency was statistically significant for both dominant ($\Delta R^2 = 0.017$; $p = 0.036$) and recessive ($\Delta R^2 = 0.020$; $p = 0.022$) genetic models (simple slopes analysis, Figure 11). Whilst rs9951307 ($\Delta R^2 = 0.015$; $p = 0.048$), rs71353406 ($\Delta R^2 = 0.019$; $p = 0.030$), rs3875089 ($\Delta R^2 = 0.019$; $p = 0.028$) and rs151246 (R^2*-change*, 0.039; $p = 0.002$) were significant in the dominant genetic model only (simple slopes analyses, Figure 12).

The interaction of *AQP1* or *AQP4* SNPs with PSQI-determined sleep duration (in hours) was only observed to have a significant moderation effect on brain Aβ burden for three *AQP4* SNPs: rs12968026, rs2339214 and rs491148 (Table 7). For rs12968026 ($\Delta R^2 = 0.019$; $p = 0.034$) and rs491148 ($\Delta R^2 = 0.016$; $p = 0.045$) the association was observed only in the dominant genetic model. Whilst for rs2339214 ($\Delta R^2 = 0.041$; $p = 0.002$), the strongest significant moderation of sleep duration on brain Aβ burden was observed in the recessive model. A visual representation of the conditional effects of those models is presented in Figure 13.

The relationship between PSQI-determined daytime dysfunction and brain Aβ burden was observed to be significantly moderated by several *AQP1* or *AQP4* SNPs (Table 8). The interaction with daytime dysfunction of the *AQP1* SNPs, rs1004317 (ΔR^2 = 0.024; p = 0.015), rs62449133 (ΔR^2 = 0.047; p = 0.001), rs2299905 (ΔR^2 = 0.034; p = 0.002) was observed to be significant in the recessive genetic model. Visual representation of these conditional effects of *AQP1* rs1004317, rs62449133, and rs2299905 for the recessive genetic model is presented in Figure 14. Two *AQP4* SNPs were also found to significantly moderate the relationship of daytime dysfunction with brain Aβ burden (Table 8). The interaction of the *AQP4* SNP rs9951307 (ΔR^2 = 0.021; p = 0.023) and daytime dysfunction was statistically significant for the dominant model. Whereas a statistically significant interaction of rs491148 (ΔR^2 = 0.022; p = 0.0206) and daytime dysfunction was observed in the recessive genetic model. Visual representation, of both conditional effects, is presented in Figure 15.

Finally, Table 9 provides a summary of the 16 significant moderation results from the interaction analyses: Revealing those *AQP1/4* SNPs which had a moderating influence (conditional effect) on the outcome measured—brain Aβ burden.

Table 6: Moderation Analysis for *AQP1 and AQP4* SNPs on Sleep Latency.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs28362727				0.167	<0.001					0.184	<0.001	
Age	0.011	0.004	0.006				0.011	0.004	0.011			
BMI	0.006	0.006	0.326				0.003	0.006	0.661			
CVD risk	-0.004	0.039	0.915				<0.001	0.039	0.995			
GDS	-0.005	0.015	0.716				-0.005	0.015	0.751			
APOE ε4	0.300	0.057	<0.001				0.306	0.057	<0.001			
rs28362727	-0.127	0.069	0.068				-0.298	0.122	0.015			
Latency	-0.001	0.002	0.754				0.002	0.002	0.170			
INT	**0.006**	**0.003**	**0.034**			**0.018**	**0.017**	**0.006**	**0.003**			**0.035**
AQP4												
Model summary: rs9951307				0.186	<0.001					0.166	<0.001	
Age	0.010	0.004	0.019				0.010	0.004	0.013			
BMI	0.003	0.006	0.607				0.004	0.006	0.752			
CVD risk	-0.013	0.039	0.741				-0.012	0.039	0.752			
GDS	-0.007	0.015	0.658				-0.010	0.015	0.485			
APOE ε4	0.312	0.056	<0.001				0.309	0.057	<0.001			
rs9951307	0.015	0.070	0.831				0.025	0.123	0.837			
Latency	0.008	0.002	0.001				0.004	0.002	0.006			
INT	**-0.006**	**0.003**	**0.048**			**0.015**	-0.005	0.006	0.347			0.004
Model summary: rs71353406				0.180	<0.001					0.163	<0.001	
Age	0.010	0.004	0.023				0.010	0.004	0.018			
BMI	0.004	0.006	0.556				0.005	0.006	0.401			
CVD risk	-0.008	0.039	0.833				-0.012	0.040	0.769			
GDS	-0.006	0.015	0.692				-0.009	0.015	0.552			
APOE ε4	0.298	0.058	<0.001				0.307	0.058	<0.001			
rs71353406	-0.063	0.069	0.362				0.050	0.158	0.754			
Latency	0.001	0.002	0.688				0.004	0.002	0.022			
INT	**0.006**	**0.003**	**0.030**			**0.019**	0.003	0.006	0.675			0.001
Model summary: rs3875089				0.184	<0.001					0.165	<0.001	
Age	0.010	0.004	**0.019**				0.011	0.004	0.010			
BMI	0.005	0.006	0.400				0.004	0.006	0.458			
CVD risk	-0.016	0.039	0.683				-0.017	0.040	0.660			
GDS	-0.010	0.015	0.501				-0.012	0.015	0.426			
APOE ε4	0.310	0.057	**<0.001**				0.313	0.058	<0.001			
rs3875089	-0.050	0.074	0.497				-0.005	0.416	0.990			
Latency	0.002	0.002	0.248				0.004	0.002	0.008			
INT	**0.007**	**0.003**	**0.028**			**0.019**	0.010	0.027	0.706			0.001

(continued over)

Table 6 *(cont.)*: Moderation Analysis for *AQP1 and AQP4* SNPs on Sleep Latency.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 (cont.)												
Model summary: rs151246				**0.201**	**<0.001**					0.165	<0.001	
Age	0.009	0.004	**0.023**				0.011	0.004	0.012			
BMI	0.004	0.006	0.525				0.005	0.006	0.448			
CVD risk	-0.015	0.038	0.699				-0.014	0.039	0.731			
GDS	-0.007	0.015	0.654				-0.011	0.015	0.469			
APOE ε4	0.303	0.056	**<0.001**				0.310	0.057	<0.001			
rs151246	0.117	0.070	0.096				0.064	0.175	0.716			
Latency	0.009	0.002	**<0.001**				0.004	0.002	0.006			
INT	**-0.009**	**0.003**	**0.002**			**0.039**	-0.008	0.007	0.294			0.004
Model summary: rs491148				**0.185**	**<0.001**					**0.193**	**<0.001**	
Age	0.010	0.004	**0.016**				0.012	0.004	**0.005**			
BMI	0.005	0.006	0.360				0.005	0.006	0.393			
CVD risk	-0.018	0.039	0.650				-0.017	0.039	0.657			
GDS	-0.011	0.015	0.450				-0.011	0.015	0.459			
APOE ε4	0.316	0.057	**<0.001**				0.320	0.057	**<0.001**			
rs491148	-0.035	0.075	0.639				-0.333	0.271	0.220			
Latency	0.002	0.002	0.639				0.004	0.001	**0.014**			
INT	**0.007**	**0.003**	**0.036**			**0.017**	**0.035**	**0.015**	**0.022**			**0.020**

Model summary statistics for significant Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors; SE, standard error; Sig, p-value; R^2, coefficient of multiple determination; ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Sleep latency * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded).

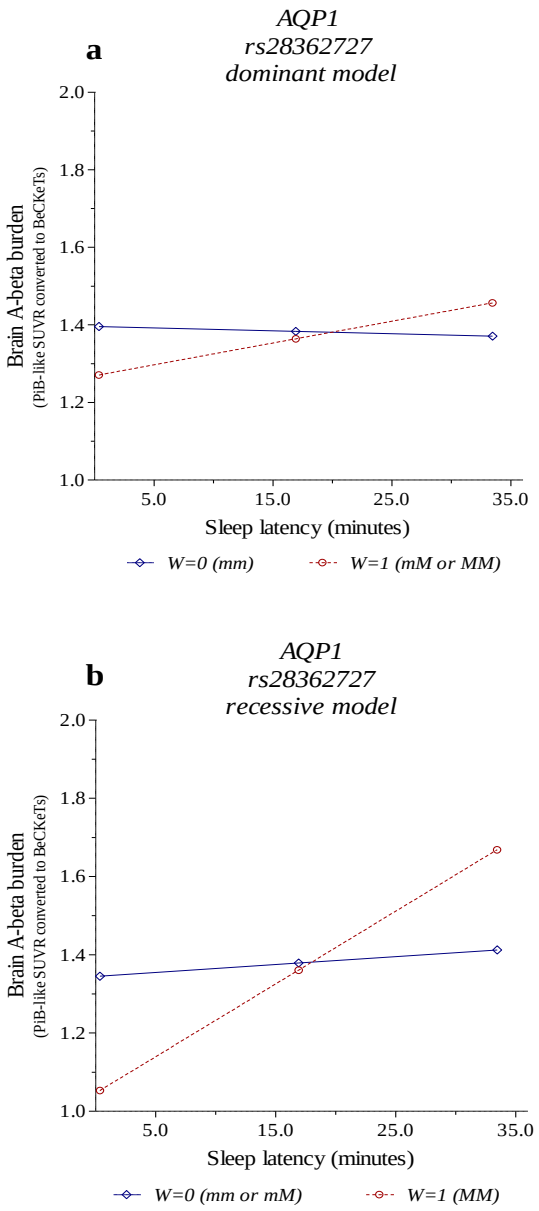

Figure 10: Conditional effects of *AQP1* SNPs on the relationship between sleep latency and brain Aβ burden.

Moderating effects of Aquaporin 1 (*AQP1*) rs28362727, for both the a) dominant and b) recessive genetic models, on the relationship between sleep latency (minutes) and brain Aβ burden. W, moderator variable; M, Minor allele; m, major allele. a) Dominant genetic model: W = 0 (homozygote for the major allele (mm)) compared to W = 1 (heterozygote/homozygote for the minor allele (mM or MM)). b) Recessive genetic model: W = 0 (homozygote/ heterozygote for the major allele (mm or mM)) compared to W = 1 (homozygote for the minor allele (MM)). Brain Aβ burden is presented as ^{11}C-Pittsburgh compound B (PiB) positron emission tomography (PET) like standardised uptake value ratio (SUVR) using the Before the Centiloid Kernel Transformation (BeCKeT) scale.

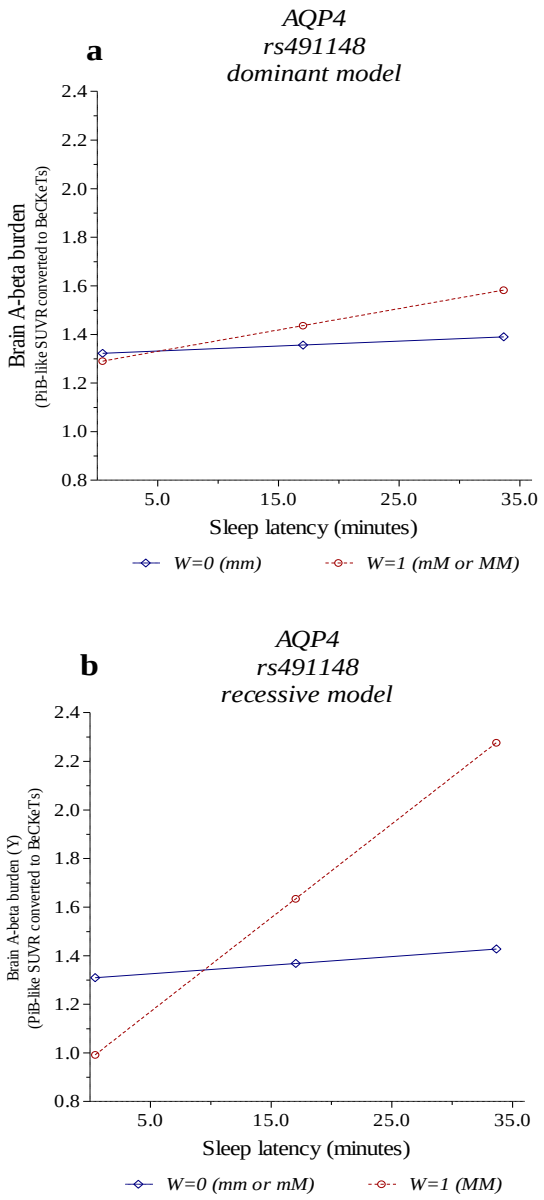

Figure 11: Conditional effects of *AQP4* rs491148 on the relationship between sleep latency and brain Aβ burden

Moderating effects of the Aquaporin 4 (*AQP4*) rs491148, for both the a) dominant and b) recessive genetic models, on the relationship between sleep latency (minutes) and brain Aβ burden. W, moderator variable; M, Minor allele; m, major allele. a) Dominant genetic model: W = 0 (homozygote for the major allele (mm)) compared to W = 1 (heterozygote/homozygote for the minor allele (mM or MM)). b) Recessive genetic model: W = 0 (homozygote/ heterozygote for the major allele (mm or mM)) compared to W = 1 (homozygote for the minor allele (MM)). Brain Aβ burden is presented as ^{11}C-Pittsburgh compound B (PiB) positron emission tomography (PET) like standardised uptake value ratio (SUVR) using the Before the Centiloid Kernel Transformation (BeCKeT) scale.

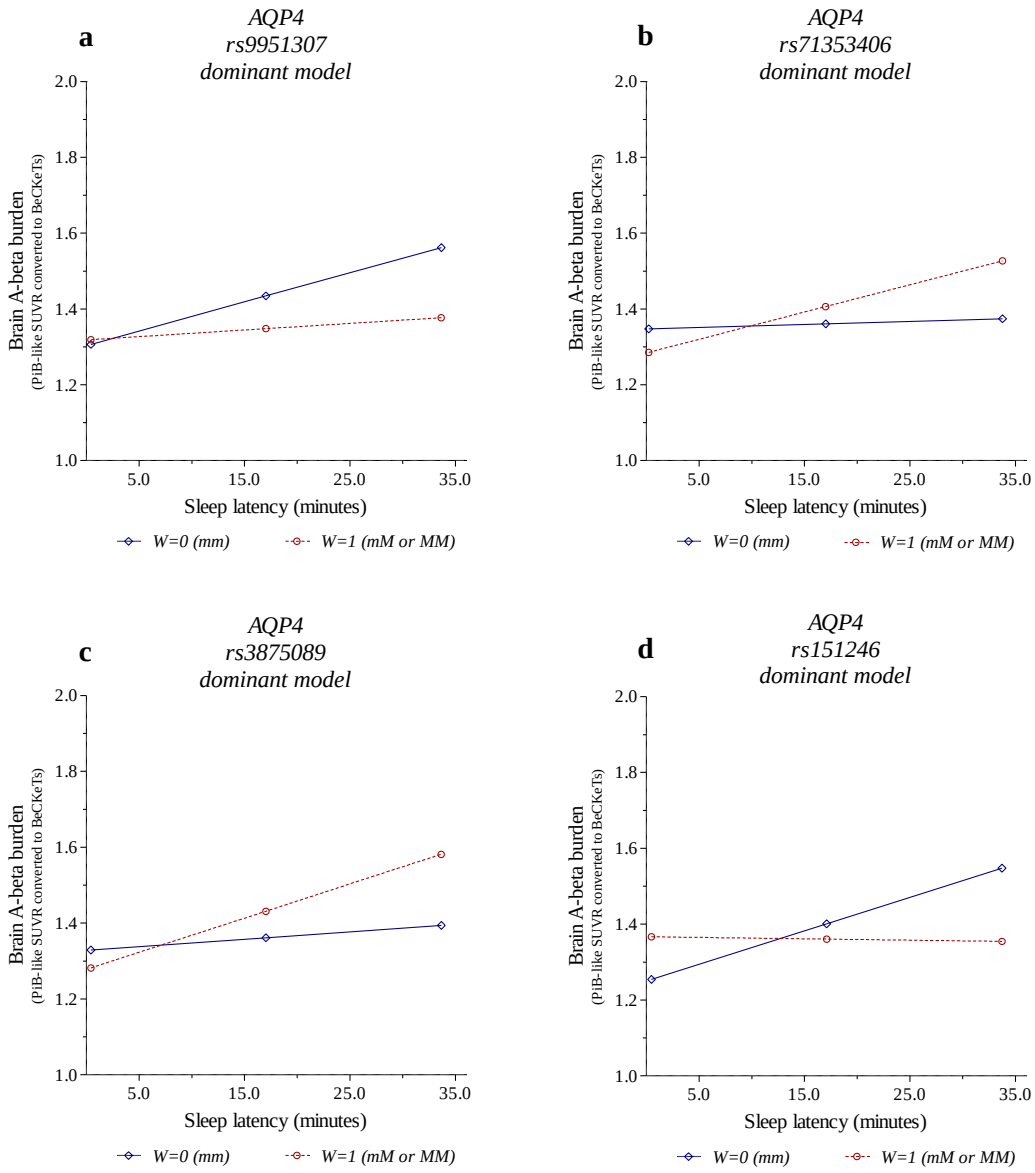

Figure 12: Further conditional effects of *AQP4* SNPs on the relationship between sleep latency and brain Aβ burden.

Moderating effects, in the dominant genetic model, for Aquaporin 4 (*AQP4*) single nucleotide polymorphisms (SNPs) a) rs9951307, b) rs7135406, c) rs3875089 and d) rs151246 on the relationship between sleep latency (minutes) and brain Aβ burden. W, moderator variable; M, Minor allele; m, major allele. Dominant genetic model: W = 0 (homozygote for the major allele (mm)) compared to W = 1 (heterozygote/homozygote for the minor allele (mM or MM)). Brain Aβ burden is presented as ^{11}C-Pittsburgh compound B (PiB) positron emission tomography (PET) like standardised uptake value ratio (SUVR) using the Before the Centiloid Kernel Transformation (BeCKeT) scale.

Table 7: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Duration.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4												
Model summary: rs12968026				0.149	<0.001					0.126	<0.001	
Age	0.012	0.004	0.005				0.011	0.004	0.010			
BMI	0.005	0.006	0.370				0.004	0.006	0.520			
CVD risk	-0.023	0.040	0.565				-0.009	0.040	0.816			
GDS	-0.007	0.015	0.662				-0.003	0.015	0.838			
APOE ε4	0.289	0.058	<0.001				0.283	0.059	<0.001			
rs12968026	0.807	0.352	0.023				0.065	0.715	0.928			
Duration	0.026	0.023	0.251				0.005	0.021	0.817			
INT	**-0.104**	**0.049**	**0.034**			**0.019**	-0.010	0.105	0.923			<0.001
Model summary: rs2339214				0.132	<0.001					0.174	<0.001	
Age	0.010	0.004	0.018				0.011	0.004	0.009			
BMI	0.005	0.006	0.403				0.004	0.006	0.507			
CVD risk	-0.011	0.041	0.796				-0.009	0.040	0.819			
GDS	-0.005	0.016	0.774				-0.008	0.015	0.595			
APOE ε4	0.302	0.059	<0.001				0.307	0.058	<0.001			
rs2339214	0.056	0.324	0.864				-0.993	0.329	0.003			
Duration	0.014	0.038	0.714				-0.031	0.024	0.197			
INT	-0.009	0.045	0.850			<0.001	**0.149**	**0.047**	**0.002**			**0.041**
Model Summary: rs491148				0.156	<0.001					0.146	<0.001	
Age	0.011	0.004	0.007				0.012	0.004	0.005			
BMI	0.005	0.006	0.377				0.004	0.006	0.736			
CVD risk	-0.023	0.040	0.565				-0.016	0.040	0.684			
GDS	-0.012	0.015	0.419				-0.008	0.015	0.574			
APOE ε4	0.317	0.058	<0.001				0.316	0.059	<0.001			
rs491148	0.707	0.320	0.028				-0.135	0.662	0.839			
Duration	0.030	0.024	0.202				0.005	0.021	0.819			
INT	**-0.090**	**0.045**	**0.045**			**0.016**	0.053	0.097	0.584			0.001

Model summary statistics for significant Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs)—no Aquaporin 1 (*AQP1*) SNPs were significant. †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors; SE, standard error; Sig, p-value; R^2, coefficient of multiple determination; ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Sleep duration * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded).

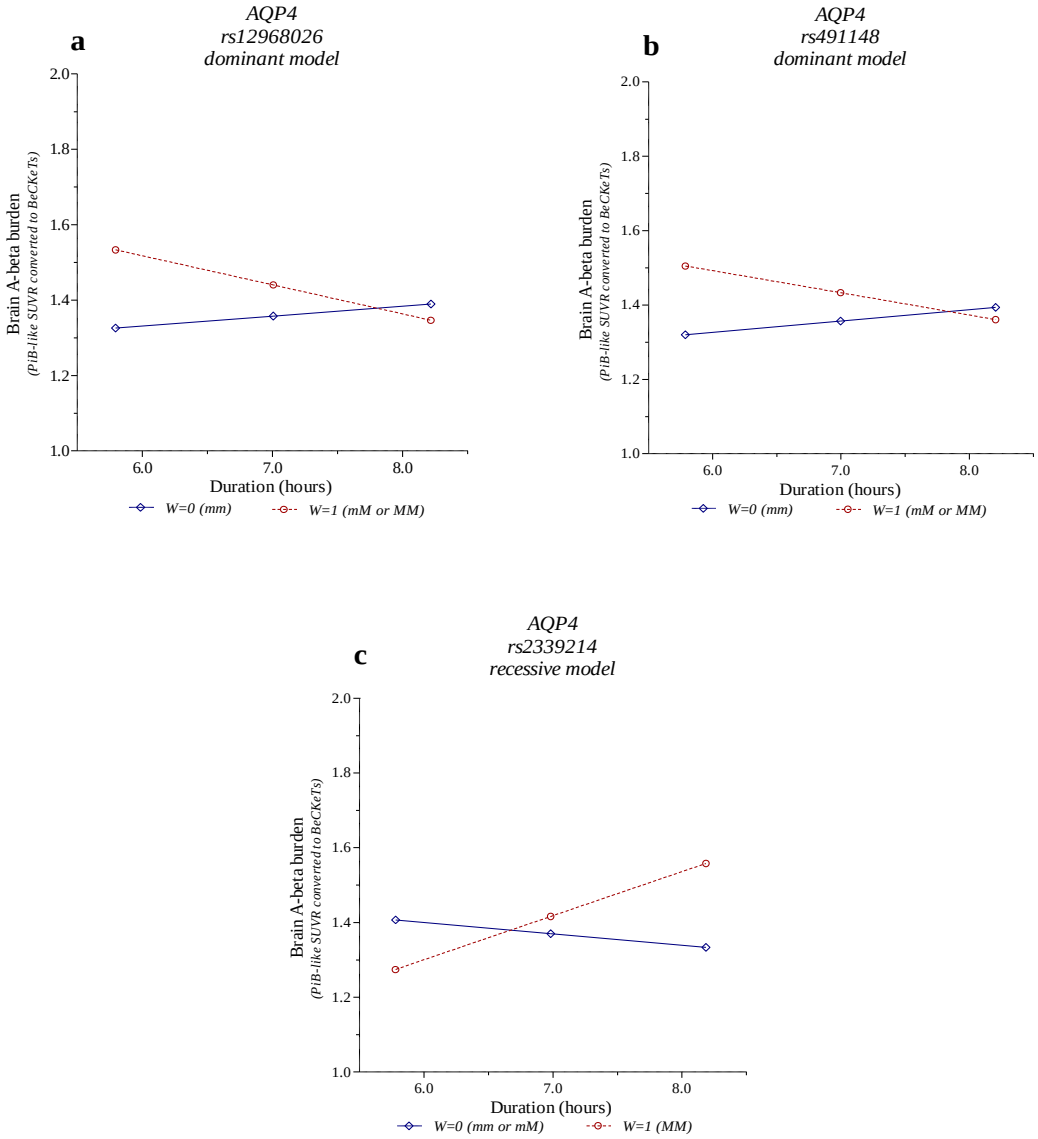

Figure 13: Conditional effects of *AQP4* SNPs on the relationship between sleep duration and brain Aβ burden

Moderating effects of Aquaporin 4 (*AQP4*) single nucleotide polymorphisms (SNPs) a) rs12968026 (dominant model), b) rs491148 (dominant model), and c) rs2339214 (recessive model) on the relationship between sleep duration (hours) and brain Aβ burden. W, moderator variable; M, Minor allele; m, major allele. Dominant genetic model: W = 0 (homozygote for the major allele (mm)) compared to W = 1 (heterozygote/homozygote for the minor allele (mM or MM)). Recessive genetic model: W = 0 (homozygote/heterozygote for the major allele (mm or mM)) compared to W = 1 (homozygote for the minor allele (MM)). Brain Aβ burden is presented as ^{11}C-Pittsburgh compound B (PiB) positron emission tomography (PET) like standardised uptake value ratio (SUVR) using the Before the Centiloid Kernel Transformation (BeCKeT) scale.

Table 8: Moderation Analysis for *AQP1 and AQP4* SNPs on Daytime Dysfunction.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs1004317				0.144	<0.001					0.160	<0.001	
Age	0.010	0.004	0.017				0.009	0.004	0.046			
BMI	0.004	0.006	0.557				0.004	0.006	0.465			
CVD risk	-0.013	0.040	0.739				-0.004	0.040	0.922			
GDS	-0.012	0.016	0.458				-0.013	0.016	0.429			
APOE ε4	0.306	0.058	<0.001				0.307	0.057	<0.001			
rs1004317	-0.029	0.067	0.575				-0.268	0.119	0.025			
Daytime dysfunction	-0.029	0.067	0.668				-0.002	0.043	0.973			
INT	0.090	0.078	0.246			0.005	**0.260**	**0.106**	**0.015**			**0.024**
Model summary: rs62449133				0.141	<0.001					0.191	<0.001	
Age	0.010	0.004	0.018				0.009	0.004	0.037			
BMI	0.003	0.006	0.578				0.002	0.006	0.786			
CVD risk	-0.002	0.040	0.955				0.014	0.039	0.714			
GDS	-0.009	0.017	0.600				-0.007	0.016	0.647			
APOE ε4	0.314	0.059	<0.001				0.345	0.058	<0.001			
rs62449133	-0.006	0.076	0.936				-0.494	0.151	0.001			
Daytime dysfunction	-0.002	0.055	0.965				-0.034	0.042	0.429			
INT	0.022	0.073	0.766			<0.001	**0.411**	**0.119**	**0.001**			**0.047**
Model summary: rs2299905				0.146	<0.001					0.181	<0.001	
Age	0.010	0.004	0.025				0.009	0.004	0.045			
BMI	0.003	0.006	0.578				0.002	0.006	0.786			
CVD risk	-0.003	0.040	0.944				0.012	0.040	0.765			
GDS	-0.009	0.017	0.593				-0.008	0.016	0.607			
APOE ε4	0.318	0.059	<0.001				0.339	0.058	<0.001			
rs2299905	-0.087	0.074	0.245				-0.458	0.149	0.002			
Daytime dysfunction	-0.022	0.058	0.709				-0.027	0.043	0.534			
INT	0.057	0.074	0.440			0.003	**0.336**	**0.115**	**0.002**			**0.034**

(continued over)

Table 8 *(cont.)*: Moderation Analysis for *AQP1 and AQP4* SNPs on Daytime Dysfunction.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4												
Model summary: rs9951307				**0.165**	**<0.001**					0.143	<0.001	
Age	0.010	0.004	**0.013**				0.010	0.004	0.014			
BMI	0.005	0.006	0.405				0.006	0.006	0.340			
CVD risk	-0.012	0.040	0.740				-0.011	0.040	0.788			
GDS	-0.009	0.016	0.593				-0.014	0.017	0.410			
APOE ε4	0.302	0.057	**<0.001**				0.296	0.058	<0.001			
rs9951307	-0.201	0.074	**0.007**				-0.179	0.124	0.152			
Daytime dysfunction	-0.067	0.058	0.245				0.018	0.042	0.667			
INT	**0.165**	**0.072**	**0.023**			**0.021**	0.126	0.111	0.256			0.005
Model summary: rs491148				0.143	<0.001					**0.168**	**<0.001**	
Age	0.010	0.004	0.020				0.010	0.004	**0.015**			
BMI	0.005	0.006	0.394				0.005	0.006	0.384			
CVD risk	-0.012	0.041	0.760				-0.007	0.040	0.866			
GDS	-0.016	0.017	0.351				-0.008	0.016	0.623			
APOE ε4	0.302	0.058	<0.001				0.312	0.058	**<0.001**			
rs491148	0.101	0.082	0.221				0.568	0.197	**0.004**			
Daytime dysfunction	0.046	0.047	0.337				0.043	0.041	0.300			
INT	-0.034	0.082	0.337			0.001	**-0.339**	**0.144**	**0.020**			**0.022**

Model summary statistics for significant Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors; SE, standard error; Sig, p-value; R^2, coefficient of multiple determination,;ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Daytime dysfunction * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded).

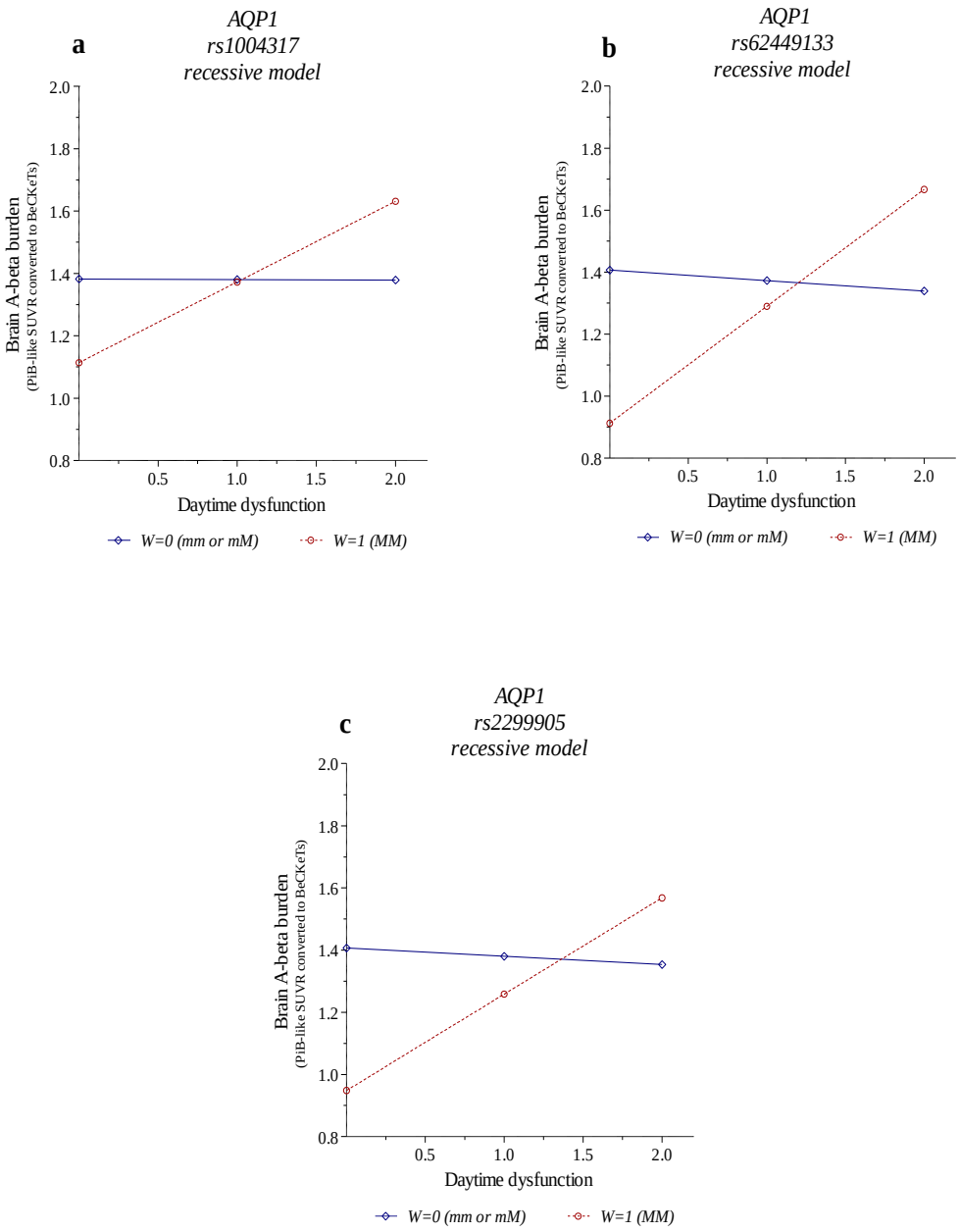

Figure 14: Conditional effects of *AQP1* SNPs on the relationship between daytime dysfunction and brain Aβ burden

Moderating effects, in the recessive genetic model, for Aquaporin 1 (*AQP1*) single nucleotide polymorphisms (SNPs) a) rs1004317, b) rs62449133, and c) rs2299905 on the relationship between daytime dysfunction and brain Aβ burden. W, moderator variable; M, Minor allele; m, major allele. Recessive genetic model: W = 0 (homozygote/heterozygote for the major allele (mm or mM)) compared to W = 1 (homozygote for the minor allele (MM)). Brain Aβ burden is presented as ^{11}C-Pittsburgh compound B (PiB) positron emission tomography (PET) like standardised uptake value ratio (SUVR) using the Before the Centiloid Kernel Transformation (BeCKeT) scale.

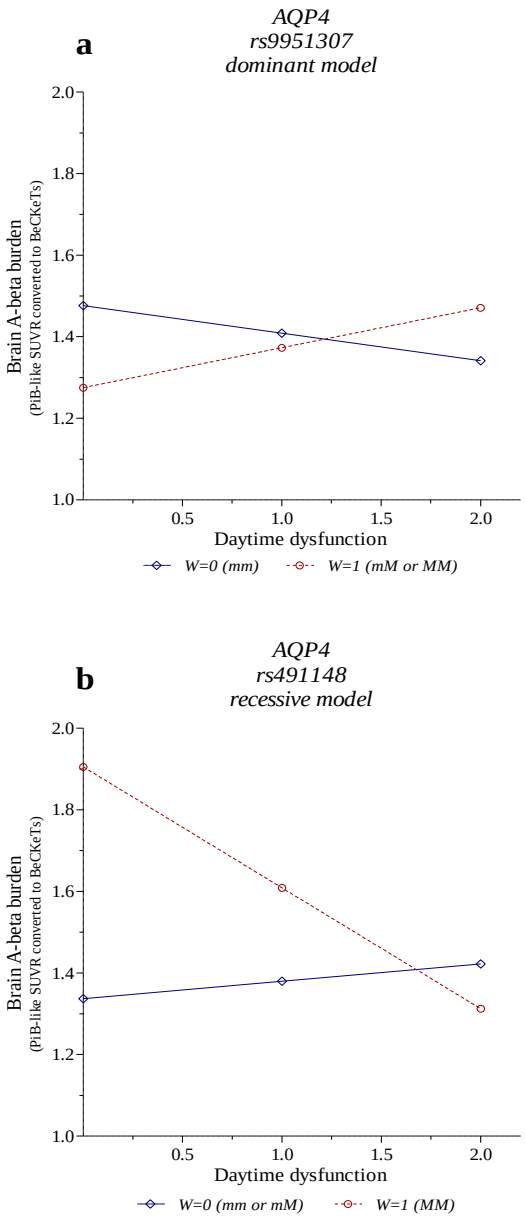

Figure 15: Conditional effects of *AQP4* SNPs on the relationship between daytime dysfunction and brain Aβ burden

Moderating effects of Aquaporin 4 (*AQP4*) single nucleotide polymorphisms (SNPs) a) rs9951307 (dominant model), and b) rs491148 (recessive model) on the relationship between daytime dysfunction and brain Aβ burden. W, moderator variable; M, Minor allele; m, major allele. Dominant genetic model: W = 0 (homozygote for the major allele (mm)), and W = 1 (heterozygote or homozygote for the minor allele (mM or MM)). Recessive genetic model: W = 0 (heterozygote or homozygote for the major allele (mM or mm)), and W = 1 (homozygote for the minor allele (MM)). Brain Aβ burden is presented as ^{11}C-Pittsburgh compound B (PiB): positron emission tomography (PET) tracer, standardised uptake value ratio (SUVR), Before the Centiloid Kernel Transformation (BeCKeT) scale.

Table 9: Summary of Significant Interactions in the Moderation Analyses of *AQP1* and *AQP4* SNPs on PSQI sleep parameters on brain Aβ burden.

PSQI	*SNP Ref*	*Genetic Model*†	β	SE	95% CI (BCa) [LLCI, ULCI]	p-value	f^2
Sleep latency (minutes)							
AQP1							
	rs28362727	*dominant*	0.006	0.003	[0.001, 0.012]	0.034	0.20
	rs28362727	*recessive*	0.017	0.006	[0.006, 0.028]	0.003	0.23
AQP4							
	rs9951307	*dominant*	-0.006	0.003	[-0.012, -0.001]	0.048	0.23
	rs7135406	*dominant*	0.006	0.003	[0.001, 0.012]	0.030	0.22
	rs3875089	*dominant*	0.007	0.003	[0.001, 0.013]	0.028	0.23
	rs151246	*dominant*	-0.009	0.003	[-0.015, -0.004]	0.002	0.25
	rs491148	*dominant*	0.007	0.003	[0.001, 0.013]	0.036	0.23
	rs491148	*recessive*	0.035	0.015	[0.005, 0.065]	0.022	0.24
Sleep duration (hours)							
AQP4							
	rs12968026	*dominant*	-0.104	0.049	[-0.199, -0.008]	0.034	0.18
	rs491148	*dominant*	-0.090	0.045	[-0.178, -0.002]	0.045	0.18
	rs2339214	*recessive*	0.149	0.047	[0.057, 0.240]	0.002	0.21
Daytime dysfunction							
AQP1							
	rs1004317	*recessive*	0.260	0.106	[0.052, 0.468]	0.015	0.19
	rs62449133	*recessive*	0.411	0.119	[0.177, 0.645]	0.001	0.24
	rs2299905	*recessive*	0.336	0.115	[0.109, 0.564]	0.002	0.22
AQP4							
	rs9951307	*dominant*	0.165	0.072	[0.024, 0.307]	0.023	0.20
	rs491148	*recessive*	-0.339	0.144	[-0.623, -0.055]	0.020	0.20

A summary of all Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) single nucleotide polymorphisms (SNPs) with significant ($p < 0.05$) moderation of the relationship between the listed Pittsburgh Sleep Quality Index (PSQI) sleep parameters and brain Aβ burden. SNP Ref, reference SNP marker (rs). †Genetic model: Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)). β, Coefficient of predictors; SE, standard error; 95% CI (BCa), 95% Confidence Interval (bias-corrected and accelerated; based on 5000 bootstrap samples) with lower (LLCI) and upper (ULCI) limits. Cohen's f^2 effect size (Cohen, 1988).

6.0 Discussion

This study aimed to investigate whether genetic variation within the genes encoding water channel proteins expressed in the brain, specifically Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*), were associated with; i) Alzheimer's disease (AD) risk and brain β-amyloid (Aβ) burden, ii) self-reported sleep quality and quantity, and finally, iii) whether these genetic variations moderate the relationship between self-reported sleep parameters and brain Aβ burden. To achieve these aims, an observational and cross-sectional investigation was undertaken using genetic data extracted from a genome-wide association study (GWAS) in participants of the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of ageing. These genetic data were combined with AIBL participant sleep assessment data, determined via the Pittsburgh Sleep Quality Index (PSQI), and brain Aβ burden data, from positron emission tomography.

Overall, the study reports insufficient evidence to support the hypothesis that either *AQP1* or *AQP4* SNPs are associated with either an increased AD risk or differences in brain Aβ burden. However, there was sufficient evidence to suggest that genetic variation in *AQP4*, specifically rs12968026, is associated with altered, self-reported, "overall" sleep quality (PSQI total score). Nonetheless, the major finding from this study was that several *AQP1* and *AQP4* SNPs altered the relationship between PSQI-determined sleep parameters and brain Aβ burden. This finding provides sufficient evidence to support the hypothesis that genetic variation in *AQP1* and *AQP4* moderates the conditional effect that 3 PSQI-determined sleep parameters, namely, sleep latency (time taken to fall asleep, in minutes), sleep duration (length of sleep, in hours) and daytime dysfunction (disruption of daytime activities due to sleepiness), had on brain Aβ burden.

6.1 Genetic variation within AQP1 and AQP4 is not associated with AD risk or brain Aβ burden

Logistic regression risk analysis, based upon the frequency of the minor allele of each genetic variant in the healthy controls and AD cases revealed only a nominal level of significance for two *AQP4* SNPs, rs7240333 and rs68006382 with altered AD risk (section 5.3, Table 3). However, to ensure the reduction of familywise or experimental error when conducting multiple analyses with the same data, it was

prudent to evaluate the findings using a correction for multiple testing. After correction for the False Discovery Rate (FDR), no SNPs across the 3 genetic models retained significance. Therefore, there were no genetic polymorphisms in either *AQP1* or *AQP4* that could be reported to be associated with an increase AD risk, in the AIBL cohort using the clinical grouping criteria outlined in Section 4.2.1. Likewise, results of the linear regression, to see if there was a relationship between *AQP1* and *AQP4* SNPs and brain Aβ burden (Section 5.4, Table 4), suggested only a nominal level of significance for two *AQP4* SNPs (rs162007 and rs162003). However, as with the AD risk analysis, these associations were not significant after FDR correction.

Whilst these results suggest that there is no direct link between *AQP1* and *AQP4* genetic variation and either increased risk for AD or brain Aβ burden, the study of only common variants (minor allele frequency, MAF > 0.05) means that, even when considering the respective levels of gene coverage combined with the respective Linkage Disequilibrium (LD) structures, the possibility cannot be discounted that an untyped rare coding genetic variant may impart some functional impact on either AQP1 or AQP4 that could alter clearance of Aβ in the brain and subsequent AD risk.

6.2 Genetic variation in AQP4, but not AQP1, is associated with overall sleep quality

To ascertain if *AQP1* and *AQP4* SNPs were associated with any of the PSQI-determined sleep parameters in this study, multiple linear regression was carried out. This analysis (Section 5.5, Table 5) indicates that one *AQP4* SNP, rs12968026 recessive genetic model, is significantly associated with self-reported overall sleep quality (PSQI total) after correction for multiple testing and adjusting for potential confounders.

The regression coefficient for this significant finding (unadjusted $\beta = 4.74$ *[SE: 1.37]; adjusted* $\beta = 4.15$ *[SE: 1.34])* suggests a positive linear association or relationship between homozygotes for the minor allele, rs12968026-C, and worse overall sleep quality. A PSQI total score of > 5 indicates poor sleep and the higher the score the worse sleep an individual experiences (Buysse et al., 1989). Thus, this finding suggests that individuals homozygous for the *AQP4 rs12968026-C allele* have worse self-reported overall sleep compared to those with a different genotype.

A National Center for Biotechnology Information (NCBI) genomic reference dbSNP (Sherry et al., 2001) check revealed that rs12968026 is located at Chr18:24,444,150 within the first intron of *AQP4* (NCBI, 2017c). Upon, cross-check with the Ensembl database (Yates et al., 2016) the genetic variant effect predictor (McLaren et al., 2016) confirmed that this SNP is intronic and appears to have no discernible functional implication for the AQP4 protein that would provide a basis for a mechanism by which it impacts overall sleep. An analysis of the LD structure of the *AQP4* gene using the Ensembl database (Yates et al., 2016) with regards to potential linkage with functional *AQP4* SNP variants suggests several variants of interest. Firstly, rs12968026 is in complete LD and thus tags a single coding variant in exon 1, namely, rs35248760 (D' 1.0, r^2 1.0). However, this single coding variant was listed as a synonymous SNP thus creating only a subtle change in the transcribed codon but no change to the amino acid sequence (Hunt, Sauna, Ambudkar, Gottesman, & Kimchi-Sarfaty, 2012). Whilst rs35248760 does not appear to be a SNP that impacts functionality of the protein it encodes, it cannot be ruled out that rs12968026 and rs35248760 may be in linkage with an untyped rare non-synonymous variant in exon 1 (Khoury et al., 2010), that does impact AQP4 functionality. To ascertain whether such variants exist in our study cohort would require targeted resequencing of this region of the *AQP4* gene which is beyond the scope of this Honours project.

Secondly, rs12968026 was observed to tag two further variants, rs72878776 (D' 1.0, r^2 1.0) and rs1058427 (D' 1.0, r^2 1.0), within the 5-prime and 3-prime untranslated regions of the *AQP4* gene, respectively (McLaren et al., 2016). Of the two variants, rs72878776 is possibly of most functional relevance through potentially influencing gene transcription, via modification (creation or deletion) of a transcription factor binding site (Frazer, Murray, Schork, & Topol, 2009). However, analysis of the implications of the base change (T to C) to the DNA sequence using an *in-silico* tool, PROMO (Farré et al., 2003), to interrogate the TRANSFAC database (Wingender, Dietze, Karas, & Knüppel, 1996) suggested that potential transcription factor binding sites were not influenced by this SNP.

6.3 Genetic variation within AQP1 and AQP4 moderates the effect of sleep latency on brain Aβ burden

PSQI-determined sleep latency (time to fall asleep in minutes) was the only sleep parameter in this study to be associated with brain Aβ burden in cognitively healthy controls; confirming results previously reported using the AIBL cohort (B. M. Brown, Rainey-Smith, Villemagne, et al., 2016), among a subset (n = 184) of participants included in the current study (n = 222). One *AQP1* SNP, rs28362727, in both the dominant and recessive models had a significant interaction with self-reported sleep latency and the resultant effect on brain Aβ burden. In both genetic models, carriage of the minor allele (rs28362727-C) in combination with longer time to fall asleep was associated with an elevated PiB-like SUVR (> 1.4). This association was observably stronger in homozygotes, suggesting a potential gene-dosage effect (Figure 10). Ensembl database and NCBI dbSNP check discerned that this *AQP1* variant is intronic, it is however, in medium (D' 0.65, r^2 0.17) to strong (D' 0.99, r^2 0.06) LD with two genetic variants, rs10046506 and rs10046532, respectively, which are located within a regulatory region (open chromatin region) approximately 12kb upstream. It is conceivable that these two variants have a regulatory effect on *AQP1*, which may subsequently affect cerebrospinal fluid formation or movement (Xie et al., 2013). However, a comprehensive study of *AQP1* knockout animal models would be required to confirm such a conclusion.

Five *AQP4* SNPs (rs9951307, rs7135406, rs3875089, rs151246, and rs491148) in the dominant models, had significant interactions with self-reported sleep latency and the resultant effect on brain Aβ burden (Figures 11 and 12). Of these five SNPs, rs491148 also had an observably stronger effect in the recessive model (Figure 12), again suggestive of a gene-dosage effect for the minor allele (rs491148-G). Specifically, carriage of at least one copy of the rs491148-G allele was associated with a PiB-like SUVR approaching 1.6, whilst homozygosity of the G-allele was associated with a PiB-like SUVR approaching 2.3—a level usually associated with a clinical diagnosis of AD. Of note, three of these *AQP4* variants; rs9951307 (D' 0.99, r^2 0.07), rs3875089 (D' 1.00, r^2 0.64) and rs491148 (D' 0.93, r^2 0.46), are in strong LD with the same *AQP4* synonymous coding variant in exon 1 (rs35248760) which was previously linked to self-reported overall sleep quality (Section 6.2). This accumulation of evidence of linkage with exon 1 of *AQP4* suggests sequencing of this exon for rare coding (functional) genetic variants may be warranted.

Previous studies have reported an association of sleep latency with brain Aβ (Branger et al., 2016; B. M. Brown, Rainey-Smith, Villemagne, et al., 2016). The current study supports this association as *AQP4* is ubiquitously expressed in astrocytic end-feet in the brain and is proposed to be implicit in glymphatic clearance of Aβ during sleep (Mander et al., 2016). Accordingly, those *AQP4* SNPs that had a moderating effect on the relationship between sleep latency and Aβ may predispose those individuals to suboptimal sleep parameters due to the Aβ burden within the brain. Alternatively, as a bi-directional relationship between sleep and Aβ has been postulated (B. M. Brown, Rainey-Smith, Bucks, et al., 2016; Ju et al., 2014) it is conceivable that the suboptimal sleep parameters (driven by genetic differences) instead contribute to brain Aβ burden.

6.4 Genetic variation in AQP4, but not AQP1, moderates the effect of sleep duration on brain Aβ burden

This study found three *AQP4* SNPs, rs12968026, rs491148 and rs2339214, interacting with sleep duration to have a moderating effect on levels of Aβ in the brain. Of these three SNPs, rs12968026 was previously associated with overall sleep quality in this study (Section 6.2), whilst rs491148 was shown to interact with sleep latency to moderate brain Aβ burden (Section 6.3). For both rs12968026 and rs491148, the nature of the relationship between sleep duration and brain Aβ was similar, in that carriage of the minor allele of the respective variants was associated with elevated PiB-like SUVR (> 1.4) when self-reported sleep duration was 'short' (approximately 6-hours or less; Figure 13).

The final variant, rs2339214 presents with a potential bi-directional moderation of the relationship between sleep duration and brain Aβ burden. Specifically, homozygosity of the minor allele, rs2339214-A, is suggestive of protecting against the hypothesised negative impact of reduced sleep duration on brain Aβ burden (PiB-like SUVR < 1.3, at 6-hours or less sleep duration) yet is associated with elevated brain Aβ when sleep duration is 'long' (PiB-like SUVR ~ 1.6, > 8-hours sleep duration). To this researcher's knowledge there has been no previous report of a bi-modal relationship between sleep duration and brain Aβ burden. However, there is evidence in the literature that such a bi-modal relationship exists between sleep duration and cognition. Specifically, both short and long sleep duration is purported

to contribute to poorer cognitive function and increased risk of cognitive impairment and AD compared to intermediate sleep duration (Potvin et al., 2012; Schmutte et al., 2007; Xu et al., 2011). It remains to be determined whether this could be a consequence of a bi-modal relationship between sleep duration and Aβ.

The concurrent associations of rs491148 with a moderating effect on the relationship of both sleep latency and duration with brain Aβ provides further evidence to support the role of genetic variation in *AQP4* in brain health. Specifically, the longer one takes to fall asleep (latency) conceivably plays a role in the quantity of sleep (duration), which in turn potentially has an impact on the postulated glymphatic system's clearance of neurotoxins, in this case Aβ—manifesting as a higher brain Aβ burden. Thus, one sleep parameter in isolation may not be detrimental, particularly with respect to clearance mechanisms, but rather sleep dysfunction as a concert of multiple suboptimal sleep parameters negatively impacts brain health (Villa, Ferini-Strambi, & Combi, 2015): This may be particularly apparent when genetic factors, such as those studied herein, are taken into account.

6.5 Genetic variation within AQP1 and AQP4 moderates the relationship of Daytime Dysfunction with brain Aβ burden

Daytime dysfunction, that is, actual disruption of daytime activities due to sleepiness, is highly likely to be significantly impacted by sleep latency and sleep duration, such that poor sleep quality or short duration would likely manifest as poor diurnal functioning (Sprecher et al., 2015). Thus, a relationship between daytime dysfunction and brain Aβ burden is likely not direct but rather a consequence of factors, that is, sleep quality/quantity, that influence it. The association of two *AQP4* SNPs, previously associated with sleep latency (Section 6.3), with a moderating effect on daytime dysfunction's relationship with brain Aβ burden is suggestive of supporting this notion of an indirect relationship between daytime dysfunction and Aβ. However, the implications of this moderation are counterintuitive. Specifically, homozygosity of the rs491148-G allele, in the presence of increased sleep latency, was associated with increased brain Aβ burden, yet this genotype is associated with elevated brain Aβ burden in concert with decreased daytime dysfunction. Similarly, carriage of the minor allele of rs9951307 was suggestive of reducing the impact of

increasing sleep latency on brain Aβ, whereas it is associated with increasing the impact of daytime dysfunction.

Three *AQP1* SNPs (rs1004317, rs62449133 and rs2299905) in the recessive models, demonstrated significant interactions with daytime dysfunction and its relationship with brain Aβ burden (Figure 14). In all cases, homozygosity of the minor allele was associated with an observably higher level of brain Aβ burden, compared to non-homozygotes, when higher levels of daytime dysfunction were reported. Conversely, in the absence of daytime dysfunction, homozygote individuals had observably lower brain Aβ burden. Two of these SNPs, rs1004317 (PSQI total) and rs2299905 (PSQI total and duration) only had nominal significance with sleep parameters, however all three variants were in medium LD (D' 0.52–0.692, r^2 0.16–0.34) with rs10046506 and high LD (D' 0.75–0.99, r^2 0.06–0.11) with rs10046532.rs10046506 and rs10046532 are the same two genetic variants, located within a regulatory region upstream of *AQP1,* discovered to be in LD with *AQP1* rs28362727, which was found to moderate the relationship between sleep latency and brain Aβ burden (Section 6.3).

It is worthy of consideration however, that suboptimal sleep parameters may also be affecting the ability of an individual to perceive any daytime dysfunction, which would suggest that the relationship between daytime dysfunction and brain Aβ should be interpreted with caution regardless of genotype.

6.6 Limitations and Strengths of the study

Whilst the findings of this study are novel and suggest that genetic variation of *AQP1* and *AQP4* can moderate the relationship between sleep parameters and brain Aβ burden, there are some limitations that need to be considered. First, this study was observational and utilised a cross sectional retrospective design; consequently, no conclusions regarding temporal or causal relationships can be drawn (Bonita, Beaglehole, & Kjellström, 2006). Second, a subjective sleep measure was utilised which relies on the accuracy and fidelity of the respondents. Indeed, utilisation of an objective measure of sleep such as actigraphy or polysomnography would circumvent the limitation of self-report. Moreover, use of polysomnography, the 'gold standard' in differentiating sleep from wake, and in identifying sleep stages, would provide detail regarding the association of sleep architecture with brain Aβ.

Notwithstanding, the subjective assessment of sleep using the PSQI was appropriate and justified in this study due to its cost effectiveness and ease of administration to a large cohort (the AIBL cohort from which the current study drew data). Further, the PSQI has demonstrated internal reliability and construct validity (see Mollayeva et al. (2016) for a systematic review and meta-analysis). Third, the brain imaging and PSQI administration were completed on separate days; however, Aβ deposition is a relatively slow process, occurring over many years (Villemagne et al., 2013), and sleep habits are usually chronic, particularly in the age group studied. Finally, this study did not include a measure of glymphatic clearance thus, any inference of potential mechanisms underpinning the association of aquaporin genetic variation with a functional impact on Aβ clearance from the brain is speculative and would require further functional studies to elucidate.

Many aspects of this study however, provide confidence in the findings. A well-characterised cohort was utilised, thereby increasing the internal validity of the results. The sample size was large (over 200) and by means of the central limit theorem the statistical assumptions (for example, assumption of normally distributed data) were not violated (Hayes, 2005). Additionally, the statistically robust measure of bootstrapping was utilised; thereby maintaining precision and helping to keep the results reliable (Wilcox, 2012; Wright, London, & Field, 2011). Furthermore, the calculated effect size for the moderation analyses (Cohen's f-squared) ranged from 0.18–0.25, representing a moderate effect (Aiken & West, 1993; Cohen, 1992) of the interactions of *AQP1* and *AQP4* SNPs with Aβ levels and the sleep parameters of latency, duration, and daytime dysfunction. Plausibly, these data herein could be used as a generalizable measure for other studies that assess similar subjective sleep parameters and their interaction with genetic variants.

6.7 Future implications

This study adds weight to the argument that the brain's paravascular clearance mechanism, the proposed glymphatic system, is the biological mechanism underpinning Aβ clearance from the brain (Iliff et al., 2012). However, further study is warranted, in the context of neurodegenerative disease research, that builds upon the utilisation of *AQP1/4* (for instance, knockout or transgenic) mouse models (for example, Peng et al. (2016) *AQP4* deficient murine study of glymphatic system).

Conceivably, such work may further implicate (or refute) the hypothesized glymphatic clearance mechanism as the biological mechanism underpinning Aβ efflux from the brain, and could provide a platform to investigate the functional implications of aquaporin variants reported in this study. In this respect, the linkage of associated SNPs in this study with genetic regions with potential functional relevance (upstream regulatory region from *AQP1* and exon 1 of *AQP4*) warrants follow-up genetic (for example, sequencing) and functional studies, as described above.

Prospectively, the results of this study engender a greater understanding of what factors may moderate a sleep-AD phenotype relationship, and suggest that establishing interventions targeted at improving sleep parameters may be beneficial for positively modulating cerebral Aβ levels and, thus, potentially delaying AD onset. Indeed, findings from this study could be used to both stratify retrospective analysis of existing datasets, or perhaps more importantly, to derive tailored AD intervention strategies based on the genetics of the individual. For example, a sleep-specific intervention targeted at reducing sleep latency may be most beneficial to individuals who are genetically predisposed to a heightened impact of latency on pathological or clinical outcomes.

Overall, the data from this study have provided proof of concept that genetic variation, at least in genes encoding cerebrally expressed water-channel proteins Aquaporin 1 and Aquaporin 4, likely moderate the relationship between sleep parameters and AD-related imaging, and perhaps, clinical phenotypes. Whether other genetic factors may likewise moderate the relationship between sleep parameters and AD characteristics remains to be determined, however, the current study provides evidence to support future investigation of such interactions.

7.0 Conclusion

In summary, this thesis presents significant evidence that genetic variation within the genes encoding the central nervous system expressed water channel proteins, Aquaporin 1 and 4 (*AQP1* and *AQP4*, respectively) moderate the relationship between self-reported sleep parameters and brain Aβ burden. These findings specifically found that three PSQI-determined sleep parameters: sleep latency (minutes), sleep duration (hours) and daytime dysfunction (disruption of daytime activities due to sleepiness), when interacting with *AQP1/AQP4* genetic variants influenced brain Aβ. However, the study was cross-sectional and observational, so no conclusions can be drawn regarding temporal causal relationships. Nonetheless, the results further support a role for the hypothesised glymphatic system in the clearance of Aβ during sleep. Future studies assessing the functional impact of genetic variation in *AQP1/AQP4* on these processes would greatly assist in furthering our understanding of the contribution of sleep to AD pathogenesis.

This study further highlights that a multitude of factors need to be considered when investigating a complex neurodegenerative condition, such as, Alzheimer's disease. Moreover, the findings of the present study suggest that sleep is intricately influenced by genetic variation: Thereby, providing a rationale for the utilisation of genetics in combination with interventions aimed at improving suboptimal sleep parameters to target those individuals who would benefit the most. Finally, the results reported in this thesis support the notion that further investigation of the interaction of genetic variation with other lifestyle factors may prove advantageous in the quest to develop strategies aimed at preventing or delaying AD onset.

8.0 References

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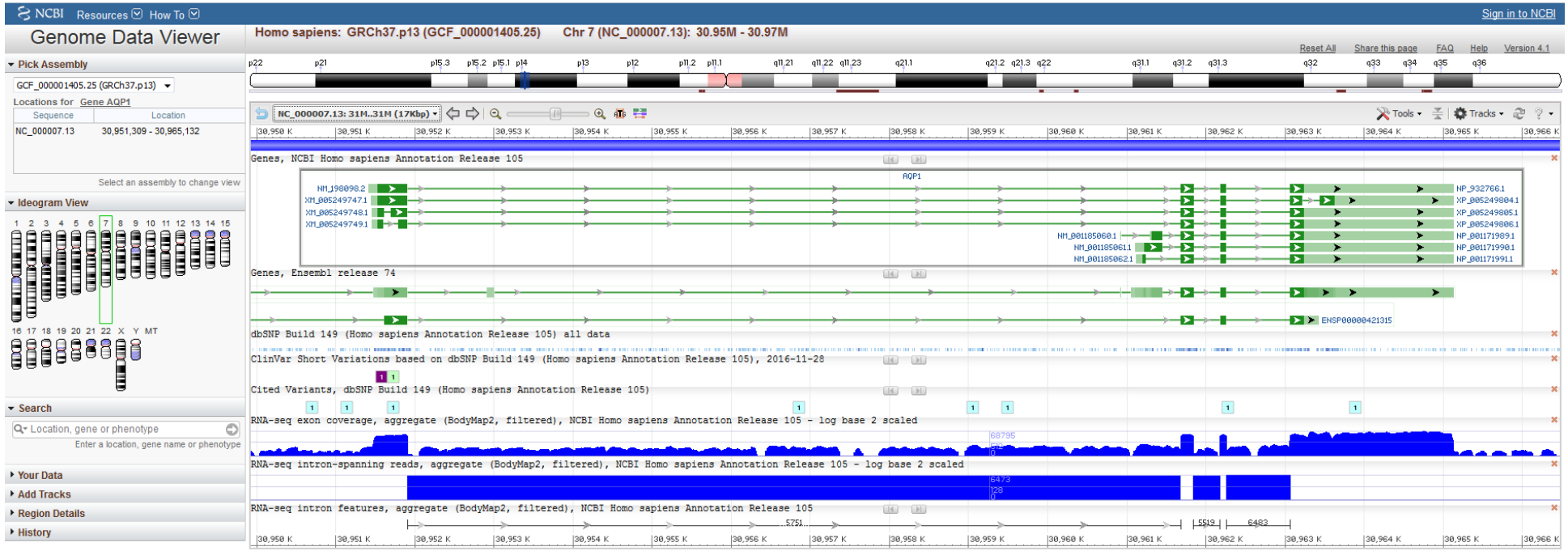
9.0 Appendices

Appendix 1: Ethical considerations.

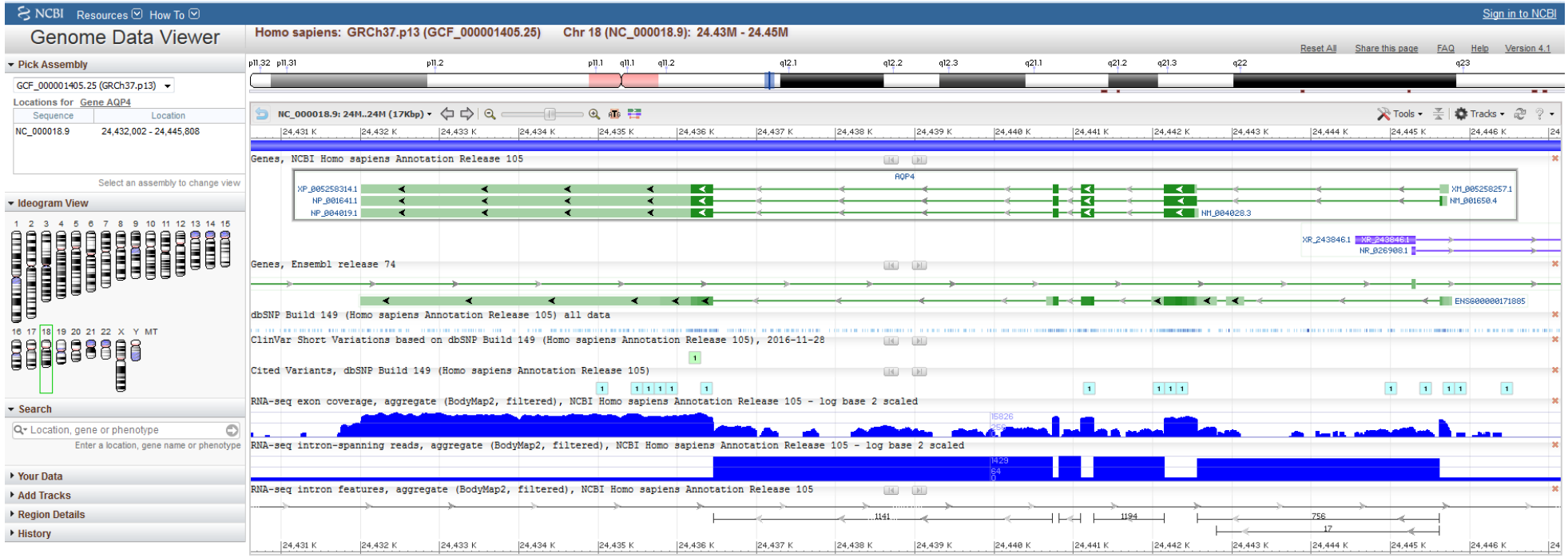
Approval of the AIBL Study has been granted by the respective ethics committees of each of the member institutions: Austin Health, St Vincent's Health (HREC-A 081/07), Hollywood Private Hospital (HPH215), and ECU (ECU-1878-MARTINS); and informed written consent was given by all volunteers. Further, the present study had been granted ethical clearance from ECU Human Research Ethics Committee: ECU-17156-MAZZUCCHELLI. In-line with the National Health and Medical Research Council, Australian Research Council, and Australian Vice-Chancellors' Committee (2015) statement, all secondary data was de-identified and as such ensured participants' anonymity. Also, electronically stored data was secured by password and only accessible by authorised persons.

Appendix 2: Genome data viewer: Aquaporin 1 gene in *Homo sapiens*.



National Center for Biotechnology Information (NCBI) genome data viewer displaying the cytogenetic location (7p14.3) of *AQP1* and the range of base pairs (bp) 30,951,309–30,965,132bp: a region of 13,823bp within the GRCh37.p13 protocol. Adapted from (NCBI, 2017a).

Appendix 3: Genome data viewer: Aquaporin 4 gene in *Homo sapiens*.



NCBI genome data viewer displaying the cytogenetic location (18q11.2) of *AQP4* and the range of base pairs (bp) 24,432,002–24,445,808bp: a region of 13,806bp within the GRCh37.p13 protocol. Adapted from (NCBI, 2017b).

Appendix 4: Complete SNP list for *AQP1* and *AQP4* prior to LD pruning.

SNP Ref	Major Allele	Call Rate	Minor Allele	MAF	HWE-p
AQP1					
rs2075574	C	1.00	T	0.36	0.64
rs1859838	A	1.00	G	0.17	0.06
rs4419722	T	1.00	G	0.12	0.64
rs28362709	G	0.97	T	0.21	0.18
rs10236571	A	0.97	G	0.22	0.17
rs2267719	C	1.00	T	0.06	0.85
rs10276670	A	0.97	G	0.22	0.20
rs1004317	A	1.00	G	0.39	0.79
rs62449133	A	0.97	G	0.22	0.16
rs2299905	A	0.96	T	0.28	0.29
rs2299906	C	1.00	T	0.06	0.40
rs1004318	C	1.00	T	0.06	0.54
rs10255904	C	1.00	T	0.06	0.77
rs17159702	T	1.00	C	0.28	0.16
rs28362727	A	0.97	C	0.27	0.47
rs765840	T	0.99	A	0.06	0.90
rs765839	C	0.99	G	0.06	0.92
rs11537660	T	1.00	C	0.07	0.72
AQP4					
rs11661081	C	1.00	A	0.09	0.89
rs9951307	A	1.00	G	0.36	0.65
rs12455617	C	1.00	A	0.12	0.41
rs16942851	T	0.99	G	0.19	0.98
rs7240333	C	0.99	T	0.11	0.41
rs1058427	G	0.98	T	0.12	0.55
rs14393	G	0.98	T	0.30	0.38
rs1058424	A	0.98	T	0.18	0.59
rs335929	A	1.00	C	0.19	0.98
rs3763043	C	1.00	T	0.31	0.44
rs68006382	A	0.98	G	0.18	0.62
rs335930	A	0.98	C	0.21	0.49
rs11661256	T	0.98	A	0.12	0.49
rs71353406	C	0.98	A	0.30	0.45
rs335931	A	0.98	G	0.19	0.97
rs67207056	G	0.97	A	0.12	0.51
rs55875625	T	0.97	C	0.12	0.49
rs455671	A	0.97	G	0.19	1.00
rs35248760	C	0.97	A	0.12	0.49
rs72878776	G	0.97	A	0.12	0.49
rs63514	C	0.98	T	0.19	0.70
rs12968026	T	0.97	C	0.12	0.48
rs3875089	T	1.00	C	0.16	0.84
rs162008	C	0.99	T	0.19	0.93
rs162007	G	1.00	A	0.20	0.80
rs162003	C	1.00	T	0.08	0.86

Complete SNP list for *AQP1* and *AQP4* prior to LD pruning *(cont.)*.

SNP Ref	Major Allele	Call Rate	Minor Allele	MAF	HWE-p
AQP4 cont.					
rs3834826	-	0.99	C	0.40	0.29
rs11662318	C	0.98	T	0.16	0.67
rs151245	T	1.00	G	0.40	0.22
rs151246	G	0.99	T	0.20	0.11
rs2339214	G	0.98	A	0.48	0.63
rs491148	A	1.00	G	0.17	0.11

Final, post quality control, list of Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) single nucleotide polymorphisms (SNPs) selected for prior to Linkage Disequilibrium (LD) pruning. Nucleotides: guanine (G), cytosine (C), adenine (A), thymine (T). SNP Ref, the reference single nucleotide polymorphism (SNP) marker (rs). Exclusion criteria: Call rate < 95%; MAF, minor allele frequency < 5%; HWE-p, Hardy-Weinberg equilibrium p-value < 0.05.

Appendix 5: Full results from linear regression analysis for the association of *AQP1* and *AQP4* genetic variants with brain Aβ burden.

SNP Ref	Additive†		Dominant†		Recessive†	
	Base*	***Adj****	***Base****	***Adj****	***Base****	***Adj****
AQP1						
rs2075574	0.467	0.551	0.805	0.394	0.272	0.991
rs1859838	0.256	0.561	0.156	0.712	0.868	0.441
rs4419722	0.108	0.457	0.242	0.658	**0.025**	0.781
rs1004317	0.794	0.422	0.741	0.770	0.346	0.255
rs62449133	0.286	0.344	0.262	0.269	0.690	0.884
rs2299905	0.495	0.286	0.327	0.260	0.858	0.652
rs28362727	0.849	0.461	0.516	0.578	0.443	0.477
rs11537660	0.586	0.313	0.920	0.528	**0.036**	0.312
AQP4						
rs11661081	0.117	0.180	0.103	0.142	0.853	0.892
rs9951307	0.113	0.169	0.134	0.095	0.327	0.751
rs7240333	0.392	0.370	0.394	0.464	0.432	0.386
rs68006382	0.502	0.378	0.505	0.251	0.773	0.690
rs71353406	0.934	0.986	0.926	0.644	0.712	0.358
rs12968026	0.146	0.636	0.129	0.637	0.737	0.840
rs3875089	0.059	0.504	0.056	0.563	0.488	0.596
rs162007	0.714	**0.044**	0.999	0.100	0.260	**0.047**
rs162003	0.586	0.061	0.751	**0.047**	0.218	0.977
rs151245	0.828	0.402	0.833	0.656	0.471	0.307
rs151246	0.261	0.086	0.324	0.139	0.408	0.183
rs2339214	0.576	0.988	0.539	0.769	0.768	0.743
rs491148	0.430	0.453	0.235	0.585	0.473	0.393

SNP Ref, reference single nucleotide polymorphism marker (rs); *AQP1*, Aquaporin 1; *AQP4*, Aquaporin 4; MAF, Minor allele frequency; M, Minor allele; m, major allele; MM, homozygote for the minor allele; Mm, heterozygote for the minor allele; mm, homozygote for the major allele. †Genetic models: Additive (homozygote for the minor allele (MM) vs heterozygote for the minor allele (Mm) vs homozygote for the major allele (mm)); Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)). *Statistical models: Base, base statistical model that is no covariates; Adj, Adjusted statistical model (covaries for: age, sex, Apolipoprotein E status (ε4-carrier/non-carrier) and clinical classification (Healthy control/Alzheimer's disease). Nominally significant ($p < 0.05$; uncorrected) p-values bolded.

Appendix 6*:* Full results from linear regression analysis for the association of *AQP1* and *AQP4* genetic variants with PSQI Sleep Parameters.

PSQI Sleep Parameter	SNP Ref	Additive†		Dominant†		Recessive†	
		*Base**	*Adj**	*Base**	*Adj**	*Base**	*Adj**
	AQP1						
PSQI total	rs2075574	0.306	0.628	0.763	0.994	0.102	0.317
	rs1859838	0.162	0.111	0.100	0.087	0.908	0.747
	rs4419722	0.745	0.655	0.680	0.576	0.759	0.701
	rs1004317	**0.041**	0.052	**0.025**	**0.047**	0.364	0.289
	rs62449133	0.058	0.087	0.054	0.104	0.403	0.316
	rs2299905	**0.012**	**0.015**	**0.019**	**0.033**	0.095	0.067
	rs28362727	**0.045**	0.062	0.088	0.111	0.110	0.135
	rs11537660	0.220	0.279	0.218	0.263	0.788	0.938
Sleep Latency (mins)	rs2075574	0.957	0.926	0.567	0.643	0.454	0.613
	rs1859838	0.678	0.794	0.695	0.883	0.818	0.672
	rs4419722	0.553	0.476	0.534	0.434	0.969	0.904
	rs1004317	0.236	0.304	0.284	0.426	0.408	0.372
	rs62449133	0.336	0.447	0.371	0.559	0.547	0.459
	rs2299905	0.072	0.107	0.080	0.148	0.297	0.249
	rs28362727	0.391	0.588	0.513	0.776	0.410	0.451
	rs11537660	0.627	0.771	0.710	0.835	0.472	0.615
Sleep Duration (hours)	rs2075574	0.287	0.365	0.510	0.507	0.237	0.396
	rs1859838	0.065	0.088	0.166	0.238	**0.043**	**0.031**
	rs4419722	0.188	0.175	0.189	0.185	0.716	0.612
	rs1004317	0.550	0.634	0.513	0.634	0.789	0.794
	rs62449133	0.074	0.082	0.142	0.179	0.126	0.091
	rs2299905	**0.045**	0.057	0.093	0.136	0.110	0.081
	rs28362727	**0.037**	0.061	0.094	0.162	0.064	0.064
	rs11537660	0.999	0.890	0.939	0.968	0.688	0.587
Sleep Disturbances	rs2075574	0.260	0.629	0.388	0.716	0.317	0.661
	rs1859838	0.357	0.596	0.841	0.889	**0.015**	**0.037**
	rs4419722	0.232	0.174	0.256	0.187	0.562	0.581
	rs1004317	0.926	0.772	0.899	0.628	0.730	0.926
	rs62449133	0.689	0.817	0.727	0.624	0.070	0.097
	rs2299905	0.616	0.912	0.715	0.994	0.610	0.788
	rs28362727	0.662	0.902	0.890	0.824	0.438	0.489
	rs11537660	0.904	0.764	0.939	0.905	0.291	0.308
Sleep Efficiency	rs2075574	0.799	0.928	0.730	0.688	0.301	0.431
	rs1859838	0.478	0.554	0.309	0.350	0.566	0.476
	rs4419722	0.848	0.616	0.654	0.443	0.264	0.290
	rs1004317	0.488	0.665	0.287	0.499	0.917	0.942
	rs62449133	0.730	0.771	0.916	0.953	0.496	0.521
	rs2299905	0.868	0.777	0.671	0.807	0.254	0.275
	rs28362727	0.729	0.794	0.342	0.398	0.364	0.358
	rs11537660	0.573	0.719	0.535	0.651	0.880	0.692

Appendix 6*:* Full results from linear regression analysis for the association of *AQP1* and *AQP4* genetic variants with PSQI Sleep Parameters *(cont.)*.

PSQI Sleep Parameter	SNP Ref	Additive†		Dominant†		Recessive†	
		*Base**	*Adj**	*Base**	*Adj**	*Base**	*Adj**
	AQP1 (cont.)						
Daytime Dysfunction	rs2075574	0.200	0.826	0.352	0.986	0.223	0.672
	rs1859838	0.597	0.977	0.929	0.810	0.149	0.448
	rs4419722	0.464	0.678	0.441	0.665	0.981	0.974
	rs1004317	0.894	0.824	0.971	0.656	0.838	0.866
	rs62449133	0.948	0.748	0.987	0.661	0.890	0.926
	rs2299905	0.812	0.530	0.683	0.404	0.859	0.974
	rs28362727	0.796	0.535	0.938	0.847	0.643	0.268
	rs11537660	**0**.655	0.968	0.565	0.828	0.576	0.362
	AQP4						
PSQI Total	rs11661081	0.443	0.465	0.412	0.407	0.947	0.770
	rs9951307	0.730	0.794	0.836	0.627	0.297	0.189
	rs7240333	0.539	0.323	0.387	0.205	0.593	0.636
	rs68006382	0.284	0.360	0.251	0.348	0.779	0.722
	rs71353406	0.130	0.100	**0.042**	**0.045**	0.856	0.871
	rs12968026	0.593	0.836	0.647	0.466	**.0006**‡	**0.002**‡
	rs3875089	0.494	0.442	0.940	0.931	**0.012**	**0.021**
	rs162007	0.761	0.545	0.968	0.723	0.374	0.338
	rs162003	0.926	0.802	0.747	0.999	0.307	0.226
	rs151245	0.698	0.697	0.928	0.787	0.396	0.277
	rs151246	0.572	0.636	0.407	0.366	0.715	0.424
	rs2339214	0.775	0.788	0.554	0.416	0.867	0.656
	rs491148	0.550	0.732	0.770	0.973	0.287	0.259
Sleep Latency (minutes)	rs11661081	0.664	0.676	0.562	0.568	0.544	0.526
	rs9951307	0.777	0.756	0.883	0.984	0.721	0.536
	rs7240333	0.787	0.822	0.848	0.713	0.728	0.724
	rs68006382	0.171	0.170	0.189	0.182	0.456	0.470
	rs71353406	0.384	0.604	0.863	0.908	0.084	0.314
	rs12968026	0.283	0.340	0.277	0.372	0.708	0.571
	rs3875089	0.123	0.195	0.098	0.187	0.770	0.648
	rs162007	0.568	0.400	0.656	0.460	0.562	0.545
	rs162003	0.989	0.754	0.951	0.708	0.832	0.857
	rs151245	0.935	0.973	0.843	0.925	0.913	0.852
	rs151246	0.744	0.797	0.756	0.853	0.854	0.776
	rs2339214	0.876	0.630	0.925	0.744	0.868	0.636
	rs491148	0.484	0.583	0.594	0.732	0.457	0.438

Appendix 6*:* Full results from linear regression analysis for the association of *AQP1* and *AQP4* genetic variants with PSQI Sleep Parameters *(cont.)*.

PSQI Sleep Parameter	SNP Ref	Additive†		Dominant†		Recessive†	
		*Base**	*Adj**	*Base**	*Adj**	*Base**	*Adj**
	AQP4 cont.						
Sleep Duration (hours)	rs11661081	0.471	0.523	0.506	0.564	0.648	0.641
	rs9951307	0.722	0.551	0.555	0.695	0.096	0.062
	rs7240333	0.588	0.406	0.410	0.242	0.507	0.469
	rs68006382	0.991	0.750	0.824	0.867	0.546	0.601
	rs71353406	0.730	0.428	0.488	0.315	0.642	0.993
	rs12968026	0.411	0.482	0.163	0.204	0.124	0.126
	rs3875089	0.771	0.658	0.996	0.860	0.341	0.339
	rs162007	0.590	0.689	0.301	0.381	0.205	0.213
	rs162003	0.842	0.979	0.598	0.729	0.149	0.152
	rs151245	0.854	0.891	0.650	0.822	0.798	0.580
	rs151246	0.626	0.751	0.575	0.597	0.967	0.719
	rs2339214	0.769	0.693	0.795	0.726	0.827	0.767
	rs491148	0.510	0.623	0.530	0.662	0.713	0.720
Sleep Disturbances	rs11661081	0.343	0.333	0.405	0.397	0.411	0.400
	rs9951307	0.563	0.418	0.627	0.653	0.660	0.326
	rs7240333	0.939	0.812	0.859	0.996	0.410	0.398
	rs68006382	0.097	0.146	0.034	0.077	0.672	0.902
	rs71353406	0.165	0.181	0.112	0.137	0.733	0.684
	rs12968026	0.886	0.548	0.848	0.576	0.919	0.716
	rs3875089	0.904	0.979	0.968	0.996	0.774	0.920
	rs162007	0.674	0.748	0.382	0.429	0.236	0.209
	rs162003	0.727	0.643	0.849	0.791	0.411	0.304
	rs151245	0.368	0.361	0.159	0.181	0.861	0.958
	rs151246	0.466	0.708	0.442	0.554	0.818	0.726
	rs2339214	0.140	0.093	0.287	0.126	0.171	0.226
	rs491148	0.076	0.188	0.084	0.206	0.382	0.483
Sleep Efficiency	rs11661081	0.511	0.591	0.732	0.830	0.083	0.084
	rs9951307	0.308	0.325	0.272	0.289	0.693	0.708
	rs7240333	0.621	0.511	0.726	0.637	0.521	0.406
	rs68006382	0.275	0.268	0.186	0.207	0.917	0.907
	rs71353406	0.557	0.608	0.414	0.480	0.903	0.923
	rs12968026	0.708	0.519	0.799	0.637	0.593	0.421
	rs3875089	0.660	0.632	0.863	0.886	0.336	0.242
	rs162007	0.704	0.742	0.461	0.492	0.360	0.354
	rs162003	0.562	0.591	0.510	0.499	0.842	0.643
	rs151245	0.832	0.873	0.761	0.830	0.996	0.986
	rs151246	0.783	0.869	0.760	0.790	0.957	0.867
	rs2339214	0.906	0.946	0.827	0.946	0.968	0.845
	rs491148	0.663	0.887	0.510	0.701	0.687	0.571

Appendix 6*:* Full results from linear regression analysis for the association of *AQP1* and *AQP4* genetic variants with PSQI Sleep Parameters *(cont.)*.

PSQI Sleep Parameter	SNP Ref	Additive†		Dominant†		Recessive†	
		*Base**	*Adj**	*Base**	*Adj**	*Base**	*Adj**
	AQP4 cont.						
Daytime Dysfunction	rs11661081	0.971	0.832	0.920	0.996	0.493	0.263
	rs9951307	0.682	0.503	0.931	0.514	0.474	0.715
	rs7240333	0.974	0.545	0.962	0.538	0.974	0.838
	rs68006382	0.726	0.628	0.482	0.711	0.454	0.612
	rs71353406	0.754	0.991	0.635	0.945	0.889	0.878
	rs12968026	0.446	0.855	0.989	0.381	**0.005**	**0.032**
	rs3875089	0.556	0.364	0.151	0.096	**0.024**	0.066
	rs162007	0.271	0.116	0.139	0.044	0.477	0.476
	rs162003	0.831	0.684	0.855	0.679	0.840	0.931
	rs151245	0.531	0.247	0.920	0.442	0.296	0.246
	rs151246	0.521	0.580	0.596	0.575	0.587	0.819
	rs2339214	0.154	0.110	0.113	0.140	0.486	0.259
	rs491148	0.801	0.489	0.843	0.369	0.801	0.816

PSQI, Pittsburgh Sleep Quality Index Sleep Parameters: PSQI total, Sleep Latency (minutes), Sleep Duration (hours), Sleep Disturbances, Sleep Efficiency and Daytime Dysfunction. SNP Ref, reference single nucleotide polymorphism marker (rs); *AQP1*, Aquaporin 1; *AQP4*, Aquaporin 4. †Genetic models: Additive (homozygote for the minor allele (MM) vs heterozygote for the minor allele (Mm) vs homozygote for the major allele (Mm)); Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)). *Statistical models: Base, base statistical model that is no covariates; Adj, Adjusted statistical model (covaries for: age, sex, body mass index (BMI), geriatric depression scale (GDS) and a medical history of CVD). ‡Values significant after False Discovery Rate correction ($q < 0.05$). Values that reached nominal significance ($p < 0.05$, uncorrected) are bolded.

Appendix 7: Linear regression analyses for the association of PSQI Sleep parameters with brain Aβ burden.

PSQI parameter Covariates	***β***	***SE***	***t***	***Sig.***	***R^2***	***F***	***Sig.***
Model summary (PSQI Total)					**0.134**	**5.526**	**<0.001**
PSQI total	0.004	0.008	0.547	0.585			
Age	0.011	0.004	2.513	0.013			
BMI	0.004	0.006	0.730	0.466			
CVD risk	-0.011	0.040	-0.269	0.788			
GDS	-0.010	0.016	-0.617	0.538			
APOE ε4	0.305	0.058	5.265	<0.001			
Model summary (Sleep Latency)					**0.160**	**6.824**	**<0.001**
Sleep latency	**0.004**	**0.001**	**2.656**	**0.008**			
Age	0.010	0.004	2.499	0.013			
BMI	0.004	0.006	0.741	0.459			
CVD risk	-0.012	0.039	-0.314	0.754			
GDS	-0.010	0.015	-0.684	0.495			
APOE ε4	0.306	0.057	5.375	<0.001			
Model summary (Sleep Duration)					**0.133**	**5.483**	**<0.001**
Sleep duration	0.006	0.020	0.276	0.783			
Age	0.011	0.004	2.560	0.011			
BMI	0.004	0.006	0.718	0.473			
CVD risk	-0.011	0.040	-0.279	0.781			
GDS	-0.007	0.015	-0.448	0.654			
APOE ε4	0.302	0.058	5.197	<0.001			
Model summary (Sleep Disturbances)					**0.136**	**5.656**	**<0.001**
Sleep disturbances	-0.047	0.047	-0.988	0.325			
Age	0.010	0.004	2.520	0.012			
BMI	0.004	0.006	0.631	0.528			
CVD risk	-0.004	0.040	-0.091	0.927			
GDS	-0.005	0.015	-0.333	0.740			
APOE ε4	0.304	0.058	5.254	<0.001			
Model summary (Sleep Efficiency)					**0.135**	**5.599**	**<0.001**
Sleep efficiency	-0.027	0.033	-0.822	0.412			
Age	0.011	0.004	2.543	0.012			
BMI	0.004	0.006	0.041	0.534			
CVD risk	-0.007	0.040	-0.177	0.859			
GDS	-0.005	0.015	-0.351	0.726			
APOE ε4	0.303	0.058	5.249	<0.001			

Appendix 7: Linear regression analyses for the association of PSQI Sleep parameters with brain Aβ burden (*cont.).*

PSQI Covariates	***β***	***SE***	***t***	***Sig.***	***R^2***	***F***	***Sig.***
Model summary (Daytime Dysfunction)					**0.111**	**5.584**	**<0.001**
Daytime dysfunction	0.031	0.040	0.776	0.439			
Age	0.010	0.004	2.446	0.015			
BMI	0.004	0.006	0.730	0.466			
CVD risk	-0.014	0.040	-0.345	0.731			
GDS	-0.012	0.016	-0.742	0.459			
APOE ε4	0.304	0.058	5.250	<0.001			

PSQI, Pittsburgh Sleep Quality Index; β, coefficient of predictors; SE, standard error; t, Student's t distribution test statistic; F, Fisher's F ratio/ distribution; Sig, Significance (p-value); R^2, coefficient of multiple determination; BMI, Body Mass Index; GDS, Geriatric Depression Scale; CVD risk, cardiovascular disease risk; *APOE* ε4, Apolipoprotein E ε4 allele carriage (presence/absence).

Appendix 8: Moderation Analysis for *AQP1* and *AQP4* SNPs on PSQI total.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs2075574				0.135	<0.001					0.136	<0.001	
Age	0.010	0.004	0.014				0.011	0.004	0.012			
BMI	0.005	0.006	0.441				0.005	0.006	0.419			
CVD risk	-0.001	0.040	0.783				-0.008	0.040	0.837			
GDS	-0.010	0.016	0.559				-0.010	0.016	0.537			
APOE ε4	0.308	0.059	<0.001				0.307	0.058	<0.001			
rs2075574	-0.014	0.100	0.888				-0.010	0.148	0.944			
PSQI total	0.001	0.012	0.936				0.003	0.009	0.701			
INT	0.006	0.015	0.711			0.001	0.011	0.024	0.652			0.001
Model summary: rs1859838				0.137	<0.001					0.139	<0.001	
Age	0.011	0.004	0.011				0.011	0.004	0.012			
BMI	0.005	0.006	0.426				0.003	0.006	0.609			
CVD risk	-0.012	0.040	0.762				-0.011	0.040	0.785			
GDS	-0.010	0.016	0.551				-0.009	0.016	0.557			
APOE ε4	0.310	0.059	<0.001				0.310	0.058	<0.001			
rs1859838	-0.076	0.105	0.469				-0.144	0.216	0.504			
PSQI total	-0.001	0.010	0.962				0.004	0.008	0.659			
INT	0.014	0.016	0.400			0.003	0.004	0.037	0.916			<0.001
Model summary: rs4419722				0.134	<0.001					0.162	<0.001	
Age	0.011	0.004	0.013				0.011	0.004	0.012			
BMI	0.004	0.006	0.477				0.004	0.006	0.517			
CVD risk	-0.011	0.040	0.788				-0.001	0.039	0.974			
GDS	-0.010	0.016	0.548				-0.016	0.016	0.314			
APOE ε4	0.304	0.058	<0.001				0.289	0.058	<0.001			
rs4419722	0.025	0.126	0.820				0.040	0.524	0.939			
PSQI total	0.006	0.009	0.528				0.005	0.008	0.510			
INT	-0.007	0.020	0.727			0.001	0.126	0.102	0.218			0.006
Model summary: rs1004317				0.139	<0.001					0.137	<0.001	
Age	0.011	0.004	0.011				0.011	0.004	0.013			
BMI	0.004	0.006	0.515				0.004	0.006	0.542			
CVD risk	-0.011	0.040	0.784				-0.010	0.040	0.807			
GDS	-0.010	0.016	0.565				-0.010	0.016	0.538			
APOE ε4	0.307	0.058	<0.001				0.309	0.058	<0.001			
rs1004317	0.011	0.108	0.919				-0.126	0.149	0.398			
PSQI total	0.012	0.014	0.415				0.002	0.009	0.795			
INT	-0.011	0.017	0.529			0.002	0.018	0.024	0.456			0.002
Model summary: rs62449133				0.142	<0.001					0.149	<0.001	
Age	0.010	0.004	0.016				0.010	0.004	0.015			
BMI	0.004	0.006	0.490				0.003	0.006	0.643			
CVD risk	-0.002	0.040	0.957				0.001	0.040	0.999			
GDS	-0.009	0.016	0.560				-0.010	0.016	0.511			
APOE ε4	0.318	0.059	<0.001				0.328	0.060	<0.001			
rs62449133	-0.028	0.100	0.782				-0.226	0.188	0.233			
PSQI total	-0.001	0.011	0.991				0.001	0.008	0.870			
INT	0.007	0.015	0.648			0.001	0.025	0.029	0.388			0.003

Appendix 8: Moderation Analysis for *AQP1* and *AQP4* SNPs on PSQI total *(cont.).*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1 cont.												
Model summary: rs2299905				0.144	<0.001					0.152	<0.001	
Age	0.010	0.004	0.021				0.011	0.004	0.014			
BMI	0.004	0.006	0.544				0.002	0.006	0.735			
CVD risk	-0.004	0.040	0.930				-0.003	0.040	0.946			
GDS	-0.009	0.016	0.561				-0.010	0.016	0.551			
APOE ε4	0.320	0.059	<0.001				0.337	0.060	<0.001			
rs2299905	-0.030	0.101	0.766				-0.290	0.181	0.110			
PSQI total	0.005	0.012	0.712				0.001	0.008	0.983			
INT	-0.002	0.016	0.884			<0.001	0.032	0.028	0.250			0.006
Model summary: rs28362727				0.134	<0.001					0.129	<0.001	
Age	0.011	0.004	0.009				0.011	0.004	0.009			
BMI	0.006	0.006	0.353				0.005	0.006	0.407			
CVD risk	-0.001	0.040	0.998				-0.001	0.040	0.984			
GDS	-0.004	0.016	0.804				-0.004	0.016	0.792			
APOE ε4	0.289	0.058	<0.001				0.288	0.059	<0.001			
rs28362727	-0.110	0.097	0.262				-0.048	0.170	0.778			
PSQI total	-0.007	0.012	0.573				0.002	0.008	0.816			
INT	0.016	0.015	0.277			0.005	0.001	0.032	0.966			<0.001
Model summary: rs11537660				0.147	<0.001					N/A		
Age	0.011	0.004	0.010									
BMI	0.005	0.006	0.438									
CVD risk	-0.015	0.040	0.700									
GDS	-0.010	0.016	0.519									
APOE ε4	0.305	0.058	<0.001									
rs11537660	-0.091	0.143	0.526									
PSQI total	0.006	0.009	0.505									
INT	-0.006	0.022	0.782			<0.001						
AQP4												
Model summary: rs11661081				0.136	<0.001					0.135	<0.001	
Age	0.011	0.004	0.013				0.010	0.004	0.014			
BMI	0.004	0.006	0.496				0.004	0.006	0.469			
CVD risk	-0.009	0.040	0.820				-0.007	0.040	0.866			
GDS	-0.010	0.016	0.526				-0.010	0.016	0.524			
APOE ε4	0.305	0.058	<0.001				0.303	0.058	<0.001			
rs11661081	0.084	0.142	0.554				-0.691	1.820	0.704			
PSQI total	0.007	0.009	0.449				0.005	0.008	0.579			
INT	-0.017	0.023	0.465			0.002	0.082	0.257	0.750			<0.001
Model summary rs9951307				0.151	<0.001					0.142	<0.001	
Age	0.010	0.004	0.015				0.011	0.004	0.011			
BMI	0.005	0.006	0.423				0.006	0.006	0.362			
CVD risk	-0.009	0.039	0.818				-0.007	0.040	0.863			
GDS	-0.008	0.016	0.636				-0.011	0.016	0.490			
APOE ε4	0.306	0.058	<0.001				0.301	0.058	<0.001			
rs9951307	-0.200	0.101	0.049				-0.241	0.177	0.174			
PSQI total	-0.010	0.013	0.480				0.002	0.009	0.780			
INT	0.022	0.016	0.172			0.008	0.025	0.024	0.286			0.005

Appendix 8: Moderation Analysis for *AQP1* and *AQP4* SNPs on PSQI total *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs7240333				0.151	<0.001					0.152	<0.001	
Age	0.010	0.004	0.016				0.011	0.004	0.011			
BMI	0.004	0.006	0.518				0.004	0.006	0.528			
CVD risk	-0.004	0.040	0.915				-0.003	0.040	0.947			
GDS	-0.012	0.016	0.441				-0.014	0.016	0.376			
APOE ε4	0.329	0.059	<0.001				0.320	0.058	<0.001			
rs7240333	0.025	0.125	0.842				0.252	0.351	0.473			
PSQI total	0.005	0.009	0.562				0.007	0.008	0.390			
INT	0.005	0.020	0.823			<0.001	-0.050	0.053	0.348			0.004
Model summary: rs68006382				0.140	<0.001					0.148	<0.001	
Age	0.011	0.004	0.012				0.010	0.004	0.019			
BMI	0.006	0.006	0.340				0.005	0.006	0.409			
CVD risk	-0.005	0.040	0.903				-0.009	0.040	0.823			
GDS	-0.009	0.016	0.574				-0.008	0.016	0.624			
APOE ε4	0.302	0.059	<0.001				0.307	0.059	<0.001			
rs68006382	0.040	0.113	0.725				0.475	0.304	0.119			
PSQI total	0.006	0.009	0.528				0.007	0.008	0.396			
INT	0.001	0.019	0.978			<0.001	-0.082	0.061	0.179			0.008
Model summary: rs71353406				0.136	<0.001					0.150	<0.001	
Age	0.010	0.004	0.017				0.010	0.004	0.023			
BMI	0.005	0.006	0.397				0.005	0.006	0.398			
CVD risk	-0.010	0.040	0.796				-0.014	0.040	0.727			
GDS	-0.009	0.016	0.601				-0.011	0.016	0.512			
APOE ε4	0.303	0.059	<0.001				0.303	0.059	<0.001			
rs71353406	0.026	0.101	0.796				0.450	0.212	0.035			
PSQI total	0.004	0.011	0.682				0.009	0.008	0.294			
INT	0.004	0.016	0.822			<0.001	-0.065	0.036	0.073			0.013
Model summary: rs12968026				0.136	<0.001					0.128	<0.001	
Age	0.011	0.004	0.008				0.011	0.004	0.013			
BMI	0.005	0.006	0.419				0.004	0.006	0.511			
CVD risk	-0.016	0.041	0.699				-0.009	0.040	0.827			
GDS	-0.009	0.016	0.584				-0.007	0.016	0.650			
APOE ε4	0.283	0.058	<0.001				0.284	0.059	<0.001			
rs12968026	-0.003	0.116	0.981				-0.112	0.464	0.810			
PSQI total	0.003	0.009	0.738				0.006	0.008	0.453			
INT	0.013	0.018	0.452			0.002	0.009	0.045	0.846			<0.001
Model summary: rs3875089				0.138	<0.001					0.142	<0.001	
Age	0.011	0.004	0.013				0.011	0.004	0.013			
BMI	0.005	0.006	0.445				0.005	0.006	0.440			
CVD risk	-0.010	0.040	0.812				-0.010	0.040	0.805			
GDS	-0.010	0.016	0.527				-0.009	0.016	0.591			
APOE ε4	0.300	0.058	<0.001				0.309	0.058	<0.001			
rs3875089	0.044	0.104	0.673				0.457	0.317	0.151			
PSQI total	0.004	0.010	0.678				0.005	0.008	0.518			
INT	0.002	0.016	0.893			<0.001	-0.041	0.035	0.235			0.006

Appendix 8: Moderation Analysis for *AQP1* and *AQP4* SNPs on PSQI total *(cont.).*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs162007				0.134	<0.001					0.135	<0.001	
Age	0.010	0.004	0.014				0.010	0.004	0.016			
BMI	0.004	0.006	0.480				0.004	0.006	0.473			
CVD risk	-0.010	0.040	0.807				-0.011	0.040	0.790			
GDS	-0.010	0.016	0.543				-0.011	0.016	0.511			
APOE ε4	0.305	0.058	<0.001				0.306	0.058	<0.001			
rs162007	-0.020	0.105	0.847				-0.264	0.608	0.665			
PSQI total	0.003	0.010	0.737				0.005	0.008	0.574			
INT	0.004	0.017	0.819			<0.001	0.027	0.089	0.762			<0.001
Model summary: rs162003				0.138	<0.001					N/A		
Age	0.010	0.004	0.015									
BMI	0.004	0.006	0.555									
CVD risk	-0.009	0.040	0.821									
GDS	-0.010	0.016	0.544									
APOE ε4	0.303	0.058	<0.001									
rs162003	-0.122	0.137	0.374									
PSQI total	0.002	0.009	0.783									
INT	0.011	0.024	0.650			0.001						
Model summary: rs151245				0.134	<0.001					0.150	<0.001	
Age	0.011	0.004	0.014				0.010	0.004	0.017			
BMI	0.004	0.006	0.468				0.004	0.006	0.504			
CVD risk	-0.011	0.040	0.791				-0.004	0.040	0.916			
GDS	-0.010	0.016	0.541				-0.011	0.016	0.477			
APOE ε4	0.304	0.058	<0.001				0.310	0.058	<0.001			
rs151245	0.008	0.105	0.943				0.263	0.132	0.048			
PSQI total	0.005	0.014	0.713				0.011	0.009	0.212			
INT	-0.001	0.017	0.944			<0.001	-0.035	0.019	0.070			0.013
Model summary: rs151246				0.148	<0.001					0.141	<0.001	
Age	0.011	0.004	0.010				0.011	0.004	0.011			
BMI	0.005	0.006	0.369				0.004	0.006	0.465			
CVD risk	-0.018	0.040	0.648				-0.012	0.040	0.767			
GDS	-0.013	0.016	0.416				-0.010	0.016	0.525			
APOE ε4	0.305	0.058	<0.001				0.308	0.058	<0.001			
rs151246	0.124	0.102	0.224				0.223	0.269	0.408			
PSQI total	0.016	0.010	0.122				0.006	0.008	0.461			
INT	-0.028	0.016	0.079			0.013	-0.050	0.041	0.226			0.006
Model summary: rs2339214				0.140	<0.001					0.194	<0.001	
Age	0.010	0.004	0.028				0.010	0.004	0.019			
BMI	0.005	0.006	0.440				0.005	0.006	0.442			
CVD risk	-0.007	0.040	0.854				-0.007	0.041	0.863			
GDS	-0.008	0.016	0.633				-0.007	0.016	0.666			
APOE ε4	0.303	0.059	<0.001				0.303	0.059	<0.001			
rs2339214	-0.136	0.111	0.221				0.194	0.125	0.123			
PSQI total	-0.012	0.014	0.395				0.009	0.009	0.344			
INT	0.023	0.017	0.170			0.008	-0.027	0.019	0.169			0.008

Appendix 8: Moderation Analysis for *AQP1* and *AQP4* SNPs on PSQI total *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs491148				0.144	<0.001					0.148	<0.001	
Age	0.011	0.004	0.009				0.012	0.004	0.008			
BMI	0.005	0.006	0.387				0.005	0.006	0.448			
CVD risk	-0.020	0.041	0.624				-0.013	0.040	0.746			
GDS	-0.014	0.016	0.396				-0.009	0.016	0.575			
APOE ε4	0.310	0.058	<0.001				0.316	0.058	<0.001			
rs491148	-0.010	0.108	0.926				0.417	0.279	0.137			
PSQI total	0.001	0.010	0.968				0.004	0.008	0.645			
INT	0.015	0.017	0.372			0.003	-0.027	0.033	0.415			0.003

Model summary statistics for all Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors: SE, standard error; Sig, p-value; R^2, coefficient of multiple determination, ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (PSQI total * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded). If a model could not run due to that SNP variable being a constant then not applicable (N/A) is recorded for that model.

Appendix 9: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Latency.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs2075574				0.161	<0.001					0.170	<0.001	
Age	0.010	0.004	0.014				0.011	0.004	0.012			
BMI	0.005	0.006	0.447				0.004	0.006	0.513			
CVD risk	-0.013	0.040	0.744				-0.007	0.039	0.863			
GDS	-0.001	0.015	0.551				-0.010	0.015	0.502			
APOE ε4	-0.009	0.059	<0.001				0.313	0.057	<0.001			
rs2075574	0.035	0.076	0.641				-0.064	0.107	0.552			
Latency	0.005	0.003	0.105				0.003	0.002	0.034			
INT	-0.002	0.004	0.670			0.001	0.007	0.005	0.162			0.008
Model summary: rs1859838				0.160	<0.001					0.163	<0.001	
Age	0.010	0.004	0.014				0.010	0.004	0.013			
BMI	0.004	0.006	0.463				0.003	0.006	0.572			
CVD risk	-0.012	0.040	0.755				-0.013	0.039	0.744			
GDS	-0.010	0.015	0.497				-0.010	0.015	0.506			
APOE ε4	0.307	0.058	<0.001				0.310	0.057	<0.001			
rs1859838	-0.009	0.074	0.904				-0.085	0.172	0.621			
Latency	0.004	0.002	0.067				0.004	0.002	0.011			
INT	0.001	0.003	0.930			<0.001	-0.002	0.012	0.892			<0.001
Model summary: rs4419722				0.161	<0.001					0.188	<0.001	
Age	0.010	0.004	0.013				0.010	0.004	0.012			
BMI	0.004	0.006	0.454				0.004	0.006	0.513			
CVD risk	-0.010	0.039	0.794				-0.003	0.039	0.940			
GDS	-0.010	0.015	0.499				-0.016	0.015	0.286			
APOE ε4	0.306	0.057	<0.001				0.290	0.057	<0.001			
rs4419722	-0.051	0.010	0.603				-0.359	3.274	0.275			
Latency	0.004	0.002	0.020				0.004	0.001	0.007			
INT	0.003	0.005	0.553			0.001	0.258	0.201	0.201			0.006
Model summary: rs1004317				0.163	<0.001					0.163	<0.001	
Age	0.010	0.004	0.013				0.010	0.004	0.016			
BMI	0.004	0.006	0.500				0.005	0.006	0.413			
CVD risk	-0.012	0.040	0.755				-0.015	0.039	0.700			
GDS	-0.010	0.015	0.508				-0.011	0.015	0.477			
APOE ε4	0.308	0.058	<0.001				0.306	0.057	<0.001			
rs1004317	-0.030	0.073	0.685				0.081	0.120	0.504			
Latency	0.004	0.002	0.067				0.004	0.002	0.007			
INT	-0.001	0.003	0.802			<0.001	-0.007	0.008	0.359			0.003
Model summary: rs62449133				0.162	<0.001					0.171	<0.001	
Age	0.010	0.004	0.019				0.010	0.004	0.019			
BMI	0.004	0.006	0.495				0.003	0.006	0.587			
CVD risk	-0.002	0.039	0.965				-0.001	0.039	0.985			
GDS	-0.010	0.015	0.498				-0.010	0.015	0.484			
APOE ε4	0.313	0.059	<0.001				0.322	0.059	<0.001			
rs62449133	0.025	0.071	0.726				0.102	0.149	0.493			
Latency	0.004	0.002	0.070				0.004	0.002	0.018			
INT	-0.001	0.003	0.737			0.001	-0.016	0.011	0.145			0.009

Appendix 9: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Latency *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1 cont.												
Model summary: rs2299905				0.164	<0.001					0.171	<0.001	
Age	0.010	0.004	0.022				0.010	0.004	0.021			
BMI	0.004	0.006	0.551				0.003	0.006	0.648			
CVD risk	-0.004	0.040	0.918				-0.004	0.039	0.915			
GDS	-0.010	0.015	0.511				-0.009	0.015	0.562			
APOE ε4	0.319	0.058	<0.001				0.325	0.058	<0.001			
rs2299905	-0.032	0.381	0.648				0.061	0.148	0.683			
Latency	0.004	0.002	0.098				0.003	0.002	0.023			
INT	-0.001	0.003	0.845			<0.001	-0.013	0.010	0.196			0.007
Model summary: rs28362727				0.167	<0.001					0.184	<0.001	
Age	0.011	0.004	0.006				0.011	0.004	0.011			
BMI	0.006	0.006	0.326				0.003	0.006	0.661			
CVD risk	-0.004	0.039	0.915				<0.001	0.039	0.995			
GDS	-0.005	0.015	0.716				-0.005	0.015	0.751			
APOE ε4	0.300	0.057	<0.001				0.306	0.057	<0.001			
rs28362727	-0.127	0.069	0.068				-0.298	0.122	0.015			
Latency	-0.001	0.002	0.754				0.002	0.002	0.170			
INT	**0.006**	**0.003**	**0.034**			**0.018**	**0.017**	**0.006**	**0.003**			**0.035**
Model summary: rs11537660				0.178	<0.001					N/A		
Age	0.010	0.004	0.014									
BMI	0.004	0.006	0.454									
CVD risk	-0.016	0.039	0.683									
GDS	-0.010	0.015	0.485									
APOE ε4	0.308	0.057	<0.001									
rs11537660	-0.240	0.122	0.050									
Latency	0.003	0.002	0.023									
INT	0.008	0.006	0.237			0.005						
AQP4												
Model summary: rs11661081				0.169	<0.001					0.162	<0.001	
Age	0.010	0.004	0.019				0.010	0.004	0.015			
BMI	0.004	0.006	0.502				0.004	0.006	0.467			
CVD risk	-0.011	0.039	0.785				-0.008	0.040	0.839			
GDS	-0.010	0.015	0.504				-0.011	0.015	0.466			
APOE ε4	0.307	0.057	<0.001				0.304	0.057	<0.001			
rs11661081	0.111	0.099	0.262				-0.144	0.397	0.717			
Latency	0.005	0.002	0.003				0.004	0.002	0.008			
INT	-0.007	0.005	0.132			0.009	-0.001	0.008	0.927			<0.001
Model summary: rs9951307				0.186	<0.001					0.166	<0.001	
Age	0.010	0.004	0.019				0.010	0.004	0.013			
BMI	0.003	0.006	0.607				0.004	0.006	0.752			
CVD risk	-0.013	0.039	0.741				-0.012	0.039	0.752			
GDS	-0.007	0.015	0.658				-0.010	0.015	0.485			
APOE ε4	0.312	0.056	<0.001				0.309	0.057	<0.001			
rs9951307	0.015	0.070	0.831				0.025	0.123	0.837			
Latency	0.008	0.002	0.001				0.004	0.002	0.006			
INT	**-0.006**	**0.003**	**0.048**			**0.015**	-0.005	0.006	0.347			0.004

Appendix 9: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Latency *(cont.).*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs7240333				0.184	<0.001					0.182	<0.001	
Age	0.010	0.004	0.015				0.011	0.004	0.010			
BMI	0.004	0.006	0.554				0.004	0.006	0.518			
CVD risk	-0.008	0.039	0.845				-0.004	0.039	0.921			
GDS	-0.011	0.015	0.473				-0.013	0.015	0.378			
APOE ε4	0.328	0.058	<0.001				0.319	0.057	<0.001			
rs7240333	-0.027	0.081	0.736				0.251	0.297	0.398			
Latency	0.003	0.002	0.111				0.004	0.001	0.005			
INT	0.004	0.003	0.218			0.006	-0.016	0.013	0.225			0.006
Model summary: rs68006382				0.174	<0.001					0.178	<0.001	
Age	0.011	0.004	0.012				0.010	0.004	0.017			
BMI	0.005	0.006	0.406				0.005	0.006	0.460			
CVD risk	-0.001	0.040	0.980				-0.008	0.040	0.847			
GDS	-0.008	0.015	0.582				-0.009	0.015	0.536			
APOE ε4	0.301	0.058	<0.001				0.309	0.058	<0.001			
rs68006382	-0.036	0.083	0.667				-0.113	0.203	0.578			
Latency	0.003	0.002	0.062				0.004	0.002	0.019			
INT	0.004	0.004	0.231			0.006	0.013	0.008	0.108			0.011
Model summary: rs71353406				0.180	<0.001					0.163	<0.001	
Age	0.010	0.004	0.023				0.010	0.004	0.018			
BMI	0.004	0.006	0.556				0.005	0.006	0.401			
CVD risk	-0.008	0.039	0.833				-0.012	0.040	0.769			
GDS	-0.006	0.015	0.692				-0.009	0.015	0.552			
APOE ε4	0.298	0.058	<0.001				0.307	0.058	<0.001			
rs71353406	-0.063	0.069	0.362				0.050	0.158	0.754			
Latency	0.001	0.002	0.688				0.004	0.002	0.022			
INT	**0.006**	**0.003**	**0.030**			**0.019**	0.003	0.006	0.675			0.001
Model summary: rs12968026				0.176	<0.001					0.157	<0.001	
Age	0.010	0.004	0.011				0.011	0.004	0.012			
BMI	0.005	0.006	0.414				0.004	0.006	0.515			
CVD risk	-0.016	0.039	0.683				-0.009	0.039	0.816			
GDS	-0.006	0.015	0.687				-0.007	0.015	0.647			
APOE ε4	0.287	0.057	<0.001				0.287	0.058	<0.001			
rs12968026	-0.041	0.083	0.620				-0.145	0.432	0.738			
Latency	0.002	0.002	0.145				0.004	0.001	0.007			
INT	0.006	0.003	0.056			0.015	0.012	0.029	0.687			0.001
Model summary: rs3875089				0.184	<0.001					0.165	<0.001	
Age	0.010	0.004	**0.019**				0.011	0.004	0.010			
BMI	0.005	0.006	0.400				0.004	0.006	0.458			
CVD risk	-0.016	0.039	0.683				-0.017	0.040	0.660			
GDS	-0.010	0.015	0.501				-0.012	0.015	0.426			
APOE ε4	0.310	0.057	**<0.001**				0.313	0.058	<0.001			
rs3875089	-0.050	0.074	0.497				-0.005	0.416	0.990			
Latency	0.002	0.002	0.248				0.004	0.002	0.008			
INT	**0.007**	**0.003**	**0.028**			**0.019**	0.010	0.027	0.706			0.001

Appendix 9: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Latency *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs162007				0.163	<0.001					0.161	<0.001	
Age	0.010	0.004	0.015				0.010	0.004	0.015			
BMI	0.004	0.006	0.481				0.004	0.006	0.464			
CVD risk	-0.012	0.039	0.753				-0.013	0.039	0.745			
GDS	-0.009	0.015	0.557				-0.010	0.015	0.498			
APOE ε4	0.307	0.057	<0.001				0.306	0.057	<0.001			
rs162007	0.041	0.071	0.564				-0.071	0.263	0.786			
Latency	0.005	0.002	0.010				0.004	0.002	0.010			
INT	-0.003	0.003	0.364			0.003	0.001	0.018	0.963			<0.001
Model summary: rs162003				0.168	<0.001					N/A		
Age	0.009	0.004	0.024									
BMI	0.003	0.006	0.560									
CVD risk	-0.011	0.039	0.784									
GDS	-0.009	0.015	0.558									
APOE ε4	0.302	0.057	<0.001									
rs162003	-0.007	0.092	0.941									
Latency	0.005	0.092	0.941									
INT	-0.004	0.004	0.286			0.005						
Model summary: rs151245				0.165	<0.001					0.163	<0.001	
Age	0.010	0.004	0.013				0.011	0.004	0.011			
BMI	0.004	0.006	0.498				0.004	0.006	0.504			
CVD risk	-0.012	0.039	0.763				-0.012	0.039	0.754			
GDS	-0.009	0.015	0.543				-0.012	0.015	0.439			
APOE ε4	0.300	0.057	<0.001				0.308	0.057	<0.001			
rs151245	0.065	0.079	0.414				0.069	0.107	0.524			
Latency	0.007	0.003	0.032				0.004	0.002	0.011			
INT	-0.004	0.004	0.275			0.005	-0.001	0.005	0.928			<0.001
Model summary: rs151246				**0.201**	**<0.001**					0.165	<0.001	
Age	0.009	0.004	**0.023**				0.011	0.004	0.012			
BMI	0.004	0.006	0.525				0.005	0.006	0.448			
CVD risk	-0.015	0.038	0.699				-0.014	0.039	0.731			
GDS	-0.007	0.015	0.654				-0.011	0.015	0.469			
APOE ε4	0.303	0.056	**<0.001**				0.310	0.057	<0.001			
rs151246	0.117	0.070	0.096				0.064	0.175	0.716			
Latency	0.009	0.002	**<0.001**				0.004	0.002	0.006			
INT	**-0.009**	**0.003**	**0.002**			**0.039**	-0.008	0.007	0.294			0.004
Model summary: rs2339214				0.164	<0.001					0.165	<0.001	
Age	0.010	0.004	0.024				0.010	0.004	0.023			
BMI	0.005	0.006	0.435				0.005	0.006	0.423			
CVD risk	-0.012	0.040	0.768				-0.005	0.040	0.896			
GDS	-0.007	0.015	0.638				-0.007	0.015	0.642			
APOE ε4	0.306	0.058	<0.001				0.301	0.058	<0.001			
rs2339214	-0.059	0.078	0.451				-0.029	0.092	0.758			
Latency	0.002	0.003	0.506				0.003	0.002	0.039			
INT	0.003	0.003	0.342			0.004	0.004	0.004	0.368			0.003

Appendix 9: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Latency *(cont.)*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs491148				**0.185**	**<0.001**					**0.193**	**<0.001**	
Age	0.010	0.004	**0.016**				0.012	0.004	**0.005**			
BMI	0.005	0.006	0.360				0.005	0.006	0.393			
CVD risk	-0.018	0.039	0.650				-0.017	0.039	0.657			
GDS	-0.011	0.015	0.450				-0.011	0.015	0.459			
APOE ε4	0.316	0.057	**<0.001**				0.320	0.057	**<0.001**			
rs491148	-0.035	0.075	0.639				-0.333	0.271	0.220			
Latency	0.002	0.002	0.639				0.004	0.001	**0.014**			
INT	**0.007**	**0.003**	**0.036**			**0.017**	**0.035**	**0.015**	**0.022**			**0.020**

Model summary statistics for all Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors: SE, standard error; Sig, p-value; R^2, coefficient of multiple determination, ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Sleep latency * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded). If a model could not run due to that SNP variable being a constant then not applicable (N/A) is recorded for that model.

Appendix 10: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Duration.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs2075574				0.138	<0.001					0.135	<0.001	
Age	0.010	0.004	0.013				0.011	0.004	0.011			
BMI	0.005	0.006	0.402				0.005	0.006	0.442			
CVD risk	-0.008	0.040	0.849				-0.009	0.040	0.831			
GDS	-0.006	0.015	0.668				-0.007	0.015	0.654			
APOE ε4	0.300	0.059	<0.001				0.303	0.058	<0.001			
rs2075574	0.286	0.295	0.330				0.186	0.431	0.667			
Duration	0.028	0.032	0.385				0.007	0.022	0.749			
INT	-0.039	0.042	0.356			0.003	-0.019	0.058	0.742			<0.001
Model summary: rs1859838				0.134	<0.001					0.139	<0.001	
Age	0.011	0.004	0.011				0.011	0.004	0.012			
BMI	0.005	0.006	0.428				0.003	0.006	0.609			
CVD risk	-0.011	0.040	0.782				-0.013	0.040	0.739			
GDS	-0.007	0.015	0.647				-0.005	0.015	0.728			
APOE ε4	0.302	0.059	<0.001				0.308	0.058	<0.001			
rs1859838	0.165	0.307	0.591				-0.479	0.673	0.478			
Duration	0.014	0.026	0.581				0.004	0.021	0.835			
INT	-0.024	0.043	0.583			0.001	0.047	0.090	0.601			0.001
Model summary: rs4419722				0.133	<0.001					0.160	<0.001	
Age	0.011	0.004	0.011				0.011	0.004	0.010			
BMI	0.004	0.006	0.472				0.004	0.006	0.548			
CVD risk	-0.011	0.040	0.775				0.001	0.039	0.995			
GDS	-0.007	0.015	-0.037				-0.013	0.015	0.387			
APOE ε4	0.302	0.059	<0.001				0.290	0.058	<0.001			
rs4419722	-0.037	0.334	0.913				-1.977	2.010	0.327			
Duration	0.005	0.023	0.840				-0.008	0.021	0.706			
INT	0.003	0.047	0.947			<0.001	0.266	0.206	0.197			0.007
Model summary: rs1004317				0.139	<0.001					0.134	<0.001	
Age	0.011	0.004	0.011				0.011	0.004	0.011			
BMI	0.004	0.006	0.547				0.004	0.006	0.520			
CVD risk	-0.011	0.040	0.791				-0.010	0.040	0.799			
GDS	-0.007	0.015	0.641				-0.007	0.015	0.646			
APOE ε4	0.307	0.058	<0.001				0.302	0.059	<0.001			
rs1004317	-0.276	0.316	0.383				0.086	0.453	0.850			
Duration	-0.016	0.039	0.686				0.008	0.022	0.720			
INT	0.033	0.045	0.472			0.002	-0.017	0.063	0.791			<0.001
Model summary: rs62449133				0.141	<0.001					0.144	<0.001	
Age	0.010	0.004	0.015				0.011	0.004	0.012			
BMI	0.004	0.006	0.486				0.003	0.006	0.615			
CVD risk	-0.002	0.040	0.954				-0.002	0.040	0.966			
GDS	-0.007	0.015	0.638				-0.008	0.015	0.620			
APOE ε4	0.313	0.060	<0.001				0.319	0.060	<0.001			
rs62449133	0.046	0.301	0.878				0.024	0.581	0.967			
Duration	0.010	0.027	0.711				0.010	0.021	0.651			
INT	-0.005	0.042	0.902			<0.001	-0.016	0.081	0.845			<0.001

Appendix 10: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Duration *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1 cont.												
Model summary: rs2299905				0.145	<0.001					0.148	<0.001	
Age	0.010	0.004	0.019				0.011	0.004	0.013			
BMI	0.004	0.006	0.541				0.002	0.006	0.696			
CVD risk	-0.005	0.040	0.901				-0.004	0.040	0.920			
GDS	-0.007	0.015	0.650				-0.007	0.015	0.647			
APOE ε4	0.320	0.059	<0.001				0.325	0.059	<0.001			
rs2299905	-0.163	0.293	0.578				0.111	0.550	0.840			
Duration	0.001	0.031	0.982				0.011	0.021	0.611			
INT	0.017	0.042	0.682			0.001	-0.032	0.077	0.679			0.001
Model summary: rs28362727				0.134	<0.001					0.129	<0.001	
Age	0.011	0.004	0.007				0.012	0.004	0.007			
BMI	0.006	0.006	0.326				0.005	0.006	0.407			
CVD risk	-0.003	0.040	0.950				-0.001	0.040	0.975			
GDS	-0.004	0.015	0.784				-0.003	0.015	0.843			
APOE ε4	0.277	0.058	<0.001				0.284	0.059	<0.001			
rs28362727	0.421	0.286	0.142				0.106	0.525	0.841			
Duration	0.046	0.030	0.127				0.015	0.022	0.494			
INT	-0.063	0.040	0.121			0.010	-0.021	0.070	0.760			<0.001
Model summary: rs11537660				0.061	<0.001					N/A		
Age	0.011	0.004	0.009									
BMI	0.004	0.006	0.521									
CVD risk	-0.014	0.040	0.732									
GDS	-0.007	0.015	0.628									
APOE ε4	0.301	0.058	<0.001									
rs11537660	0.061	0.418	0.884									
Duration	0.006	0.022	0.771									
INT	-0.027	0.061	0.655			0.001						
AQP4												
Model summary: rs11661081				0.140	<0.001					0.134	<0.001	
Age	0.011	0.004	0.010				0.011	0.004	0.012			
BMI	0.004	0.006	0.500				0.004	0.006	0.477			
CVD risk	-0.009	0.040	0.823				-0.007	0.041	0.856			
GDS	-0.010	0.015	0.537				-0.007	0.015	0.640			
APOE ε4	0.304	0.058	<0.001				0.301	0.059	<0.001			
rs11661081	-0.526	0.405	0.196				-0.688	1.825	0.707			
Duration	-0.006	0.022	0.801				0.005	0.020	0.806			
INT	0.074	0.057	0.199			0.007	0.083	0.258	0.748			<0.001
Model summary rs9951307				0.143	<0.001					0.139	<0.001	
Age	0.011	0.004	0.009				0.011	0.004	0.009			
BMI	0.004	0.006	0.484				0.005	0.006	0.402			
CVD risk	-0.011	0.040	0.782				-0.010	0.040	0.797			
GDS	-0.005	0.015	0.725				-0.008	0.015	0.588			
APOE ε4	0.303	0.058	<0.001				0.303	0.058	<0.001			
rs9951307	-0.174	0.299	0.561				0.301	0.416	0.470			
Duration	-0.003	0.034	0.920				0.011	0.022	0.622			
INT	0.014	0.042	0.744			<0.001	-0.055	0.061	0.372			0.003

Appendix 10: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Duration *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs7240333				0.151	<0.001					0.147	<0.001	
Age	0.010	0.004	0.019				0.011	0.004	0.009			
BMI	0.004	0.006	0.505				0.004	0.006	0.548			
CVD risk	-0.002	0.040	0.951				-0.003	0.040	0.942			
GDS	-0.008	0.015	0.620				-0.009	0.015	0.545			
APOE ε4	0.322	0.059	<0.001				0.321	0.059	<0.001			
rs7240333	0.321	0.372	0.390				-0.462	1.035	0.656			
Duration	0.010	0.023	0.665				0.002	0.021	0.938			
INT	-0.039	0.052	0.458			0.002	0.065	0.148	0.659			0.001
Model summary: rs68006382				0.138	<0.001					0.142	<0.001	
Age	0.011	0.004	0.010				0.011	0.004	0.014			
BMI	0.006	0.006	0.350				0.006	0.006	0.368			
CVD risk	-0.005	0.040	0.894				-0.007	0.040	0.871			
GDS	-0.006	0.016	0.723				-0.007	0.015	0.670			
APOE ε4	0.302	0.059	<0.001				0.307	0.059	<0.001			
rs68006382	-0.021	0.348	0.951				-0.621	0.776	0.425			
Duration	0.002	0.024	0.923				-0.001	0.021	0.958			
INT	0.009	0.049	0.859			<0.001	0.099	0.103	0.340			0.004
Model summary: rs71353406				0.135	<0.001					0.144	<0.001	
Age	0.190	0.004	0.017				0.010	0.004	0.016			
BMI	0.005	0.006	0.426				0.005	0.006	0.397			
CVD risk	-0.009	0.041	0.823				-0.012	0.040	0.776			
GDS	-0.004	0.016	0.793				-0.007	0.015	0.656			
APOE ε4	0.297	0.060	<0.001				0.307	0.059	<0.001			
rs71353406	0.190	0.305	0.535				-0.679	0.578	0.241			
Duration	0.013	0.028	0.634				-0.005	0.021	0.830			
INT	-0.021	0.043	0.623			0.001	0.112	0.080	0.162			0.008
Model summary: rs12968026				0.149	<0.001					0.126	<0.001	
Age	0.012	0.004	0.005				0.011	0.004	0.010			
BMI	0.005	0.006	0.370				0.004	0.006	0.520			
CVD risk	-0.023	0.040	0.565				-0.009	0.040	0.816			
GDS	-0.007	0.015	0.662				-0.003	0.015	0.838			
APOE ε4	0.289	0.058	<0.001				0.283	0.059	<0.001			
rs12968026	0.807	0.352	0.023				0.065	0.715	0.928			
Duration	0.026	0.023	0.251				0.005	0.021	0.817			
INT	**-0.104**	**0.049**	**0.034**			**0.019**	-0.010	0.105	0.923			<0.001
Model summary: rs3875089				0.143	<0.001					0.138	<0.001	
Age	0.011	0.004	0.008				0.011	0.004	0.008			
BMI	0.004	0.006	0.464				0.004	0.006	0.477			
CVD risk	-0.016	0.400	0.694				-0.014	0.040	0.737			
GDS	-0.008	0.015	0.588				-0.008	0.015	0.603			
APOE ε4	0.297	0.058	<0.001				0.309	0.059	<0.001			
rs3875089	0.448	0.311	0.151				-0.301	0.677	0.657			
Duration	0.023	0.025	0.343				0.003	0.021	0.876			
INT	-0.056	0.044	0.200			0.007	0.064	0.098	0.515			0.002

Appendix 10: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Duration *(cont.).*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs162007				0.133	<0.001					0.134	<0.001	
Age	0.011	0.004	0.012				0.011	0.004	0.014			
BMI	0.004	0.006	0.470				0.004	0.006	0.482			
CVD risk	-0.012	0.040	0.767				-0.012	0.040	0.767			
GDS	-0.007	0.015	0.652				-0.007	0.015	0.668			
APOE ε4	0.302	0.059	<0.001				0.302	0.059	<0.001			
rs162007	-0.051	0.309	0.870				-0.217	1.742	0.901			
Duration	0.003	0.025	0.900				0.005	0.020	0.820			
INT	0.007	0.043	0.872			<0.001	0.022	0.275	0.936			<0.001
Model summary: rs162003				0.144	<0.001					N/A		
Age	0.011	0.004	0.011									
BMI	0.004	0.006	0.554									
CVD risk	-0.007	0.040	0.866									
GDS	-0.007	0.015	0.620									
APOE ε4	0.301	0.058	<0.001									
rs162003	0.662	0.571	0.248									
Duration	0.015	0.021	0.466									
INT	-0.100	0.077	0.194			0.007						
Model summary: rs151245				0.142	<0.001					0.137	<0.001	
Age	0.011	0.004	0.008				0.011	0.004	0.011			
BMI	0.004	0.006	0.505				0.004	0.006	0.518			
CVD risk	-0.012	0.040	0.772				-0.010	0.040	0.808			
GDS	-0.010	0.015	0.529				-0.008	0.015	0.599			
APOE ε4	0.309	0.058	<0.001				0.308	0.057	<0.001			
rs151245	0.437	0.298	0.144				-0.159	0.366	0.665			
Duration	0.043	0.033	0.184				-0.001	0.023	0.995			
INT	-0.062	0.042	0.140			0.009	0.031	0.052	0.547			0.002
Model summary: rs151246				0.135	<0.001					0.134	<0.001	
Age	0.011	0.004	0.012				0.011	0.004	0.010			
BMI	0.005	0.006	0.449				0.004	0.006	0.461			
CVD risk	-0.013	0.040	0.749				-0.012	0.040	0.766			
GDS	-0.007	0.015	0.645				-0.006	0.015	0.677			
APOE ε4	0.302	0.059	<0.001				0.303	0.059	<0.001			
rs151246	-0.208	0.308	0.500				0.050	0.626	0.937			
Duration	-0.003	0.025	0.905				0.008	0.021	0.710			
INT	0.026	0.044	0.559			0.001	-0.016	0.082	0.843			<0.001
Model summary: rs2339214				0.132	<0.001					0.174	<0.001	
Age	0.010	0.004	0.018				0.011	0.004	0.009			
BMI	0.005	0.006	0.403				0.004	0.006	0.507			
CVD risk	-0.011	0.041	0.796				-0.009	0.040	0.819			
GDS	-0.005	0.016	0.774				-0.008	0.015	0.595			
APOE ε4	0.302	0.059	<0.001				0.307	0.058	<0.001			
rs2339214	0.056	0.324	0.864				-0.993	0.329	0.003			
Duration	0.014	0.038	0.714				-0.031	0.024	0.197			
INT	-0.009	0.045	0.850			<0.001	**0.149**	**0.047**	**0.002**			**0.041**

Appendix 10: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Duration *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model Summary: rs491148				0.156	<0.001					0.146	<0.001	
Age	0.011	0.004	0.007				0.012	0.004	0.005			
BMI	0.005	0.006	0.377				0.004	0.006	0.736			
CVD risk	-0.023	0.040	0.565				-0.016	0.040	0.684			
GDS	-0.012	0.015	0.419				-0.008	0.015	0.574			
APOE ε4	0.317	0.058	<0.001				0.316	0.059	<0.001			
rs491148	0.707	0.320	0.028				-0.135	0.662	0.839			
Duration	0.030	0.024	0.202				0.005	0.021	0.819			
INT	**-0.090**	**0.045**	**0.045**			**0.016**	0.053	0.097	0.584			0.001

Model summary statistics for all Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors: SE, standard error; Sig, p-value; R^2, coefficient of multiple determination, ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Sleep duration * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded). If a model could not run due to that SNP variable being a constant then not applicable (N/A) is recorded for that model.

Appendix 11: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Disturbances.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs2075574				0.139	<0.001					0.141	<0.001	
Age	0.010	0.004	0.012				0.010	0.004	0.015			
BMI	0.004	0.006	0.509				0.004	0.006	0.517			
CVD risk	-0.004	0.041	0.920				-0.002	0.040	0.958			
GDS	-0.004	0.015	0.801				-0.004	0.015	0.818			
APOE ε4	0.305	0.058	<0.001				0.300	0.058	<0.001			
rs2075574	0.122	0.140	0.385				0.234	0.228	0.305			
Disturbances	-0.009	0.066	0.896				-0.036	0.050	0.471			
INT	-0.075	0.093	0.417			0.003	-0.120	0.144	0.407			0.003
Model summary: rs1859838				0.137	<0.001					0.141	<0.001	
Age	0.011	0.004	0.013				0.011	0.004	0.012			
BMI	0.003	0.006	0.571				0.003	0.006	0.660			
CVD risk	-0.001	0.041	0.973				-0.005	0.040	0.905			
GDS	-0.005	0.015	0.746				-0.005	0.015	0.738			
APOE ε4	0.303	0.058	<0.001				0.308	0.058	<0.001			
rs1859838	0.053	0.159	0.737				-0.124	0.396	0.756			
Disturbances	-0.037	0.054	0.495				-0.042	0.040	0.905			
INT	-0.038	0.107	0.720			0.001	0.003	0.232	0.989			<0.001
Model summary: rs4419722				0.137	<0.001					0.165	<0.001	
Age	0.011	0.004	0.012				0.011	0.004	0.011			
BMI	0.004	0.006	0.545				0.003	0.006	0.593			
CVD risk	-0.004	0.040	0.916				0.007	0.040	0.865			
GDS	-0.005	0.015	0.748				-0.011	0.015	0.484			
APOE ε4	0.302	0.058	<0.001				0.287	0.058	<0.001			
rs4419722	-0.037	0.170	0.830				-0.434	0.804	0.590			
Disturbances	-0.050	0.054	0.355				-0.054	0.047	0.250			
INT	0.017	0.112	0.882			<0.001	0.692	0.512	0.177			0.007
Model summary: rs1004317				0.139	<0.001					0.137	<0.001	
Age	0.011	0.004	0.011				0.011	0.004	0.012			
BMI	0.003	0.006	0.564				0.003	0.006	0.576			
CVD risk	-0.005	0.040	0.898				-0.005	0.041	0.895			
GDS	-0.005	0.015	0.739				-0.005	0.015	0.742			
APOE ε4	0.308	0.058	<0.001				0.306	0.058	<0.001			
rs1004317	-0.062	0.147	0.675				-0.093	0.229	0.686			
Disturbances	-0.051	0.084	0.543				-0.051	0.052	0.326			
INT	0.012	0.101	0.902			<0.001	0.045	0.136	0.740			<0.001
Model summary: rs62449133				0.143	<0.001					0.149	<0.001	
Age	0.010	0.004	0.018				0.010	0.004	0.016			
BMI	0.004	0.006	0.534				0.003	0.006	0.016			
CVD risk	0.004	0.040	0.922				0.006	0.040	0.880			
GDS	-0.006	0.015	0.703				-0.005	0.015	0.742			
APOE ε4	0.315	0.059	<0.001				0.317	0.059	<0.001			
rs62449133	-0.015	0.142	0.916				0.212	0.326	0.517			
Disturbances	-0.050	0.066	0.455				-0.022	0.049	0.648			
INT	0.022	0.094	0.815			<0.001	-0.184	0.195	0.349			0.004

Appendix 11: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Disturbances *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1 cont.												
Model summary: rs2299905				0.146	<0.001					0.149	<0.001	
Age	0.010	0.004	0.021				0.010	0.004	0.017			
BMI	0.003	0.006	0.544				0.002	0.006	0.715			
CVD risk	0.006	0.040	0.988				0.001	0.040	0.974			
GDS	-0.006	0.015	0.701				-0.005	0.015	0.757			
APOE ε4	0.321	0.059	<0.001				0.324	0.060	<0.001			
rs2299905	-0.087	0.141	0.536				0.022	0.308	0.943			
Disturbances	-0.050	0.072	0.489				-0.024	0.049	0.635			
INT	0.034	0.095	0.719			0.001	-0.082	0.185	0.659			0.001
Model summary: rs28362727				0.131	<0.001					0.135	<0.001	
Age	0.011	0.004	0.010				0.011	0.004	0.013			
BMI	0.005	0.006	0.406				0.004	0.006	0.488			
CVD risk	0.004	0.040	0.915				0.002	0.040	0.957			
GDS	-0.002	0.015	0.921				-0.001	0.015	0.968			
APOE ε4	0.286	0.059	<0.001				0.284	0.058	<0.001			
rs28362727	-0.025	0.140	0.858				0.197	0.264	0.457			
Disturbances	-0.041	0.062	0.506				-0.025	0.049	0.604			
INT	0.005	0.093	0.954			<0.001	-0.169	0.174	0.334			0.004
Model summary: rs11537660				0.149	<0.001					N/A		
Age	0.011	0.004	0.010									
BMI	0.004	0.006	0.525									
CVD risk	-0.006	0.040	0.875									
GDS	-0.005	0.015	0.740									
APOE ε4	0.304	0.058	<0.001									
rs11537660	-0.168	0.207	0.417									
Disturbances	-0.050	0.051	0.327									
INT	0.032	0.139	0.819			<0.001						
AQP4												
Model summary: rs11661081				0.137	<0.001					0.138	<0.001	
Age	0.011	0.004	0.013				0.010	0.004	0.014			
BMI	0.004	0.006	0.553				0.004	0.006	0.533			
CVD risk	-0.004	0.040	0.931				0.001	0.041	0.987			
GDS	-0.005	0.015	0.766				-0.005	0.015	0.732			
APOE ε4	0.304	0.058	<0.001				0.302	0.058	<0.001			
rs11661081	-0.046	0.178	0.796				-0.459	0.819	0.576			
Disturbances	-0.052	0.054	0.332				-0.049	0.048	0.305			
INT	0.027	0.116	0.814			<0.001	0.233	0.515	0.652			0.001
Model summary rs9951307				0.154	<0.001					0.140	<0.001	
Age	0.011	0.004	0.012				0.011	0.004	0.013			
BMI	0.003	0.006	0.584				0.005	0.006	0.446			
CVD risk	-0.004	0.040	0.931				-0.004	0.040	0.925			
GDS	-0.003	0.015	0.826				-0.005	0.015	0.765			
APOE ε4	0.311	0.058	<0.001				0.302	0.058	<0.001			
rs9951307	-0.276	0.143	0.055				-0.215	0.253	0.395			
Disturbances	-0.140	0.081	0.085				-0.053	0.050	0.287			
INT	0.147	0.098	0.133			0.009	0.102	0.157	0.517			0.002

Appendix 11: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Disturbances *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs7240333				0.151	<0.001					0.151	<0.001	
Age	0.010	0.004	0.014				0.011	0.004	0.010			
BMI	0.003	0.006	0.580				0.003	0.006	0.590			
CVD risk	0.001	0.040	0.972				0.002	0.040	0.957			
GDS	-0.007	0.015	0.634				-0.008	0.015	<0.001			
APOE ε4	0.326	0.059	<0.001				0.318	0.058	<0.001			
rs7240333	0.056	0.175	0.749				0.458	0.628	0.467			
Disturbances	-0.036	0.053	0.500				-0.033	0.048	0.491			
INT	-0.006	0.117	0.959			<0.001	-0.355	0.444	0.425			0.003
Model summary: rs68006382				0.145	<0.001					0.142	<0.001	
Age	0.011	0.004	0.012				0.011	0.004	0.014			
BMI	0.005	0.006	0.414				0.005	0.006	0.399			
CVD risk	0.003	0.041	0.938				-0.002	0.041	0.970			
GDS	-0.004	0.016	0.800				-0.006	0.016	0.712			
APOE ε4	0.304	0.059	<0.001				0.307	0.059	<0.001			
rs68006382	0.200	0.166	0.232				-0.150	0.452	0.740			
Disturbances	-0.014	0.055	0.798				-0.047	0.050	0.347			
INT	-0.127	0.119	0.289			0.005	0.190	0.319	0.552			0.002
Model summary: rs71353406				0.139	<0.001					0.139	<0.001	
Age	0.011	0.004	0.013				0.010	0.004	0.016			
BMI	0.004	0.006	0.476				0.005	0.006	0.427			
CVD risk	-0.006	0.041	0.892				-0.006	0.041	0.892			
GDS	-0.004	0.016	0.775				-0.006	0.012	0.718			
APOE ε4	0.303	0.059	<0.001				0.305	0.059	<0.001			
rs71353406	0.146	0.141	0.302				0.234	0.325	0.472			
Disturbances	-0.001	0.065	0.988				-0.034	0.050	0.489			
INT	-0.079	0.096	0.410			0.003	-0.090	0.229	0.489			0.001
Model summary: rs12968026				0.366	<0.001					0.129	<0.001	
Age	0.011	0.004	0.008				0.110	0.004	0.010			
BMI	0.004	0.006	0.497				0.003	0.006	0.582			
CVD risk	-0.006	0.041	0.878				-0.004	0.041	0.918			
GDS	-0.003	0.015	0.838				-0.003	0.015	0.870			
APOE ε4	0.280	0.059	<0.001				0.285	0.059	<0.001			
rs12968026	-0.012	0.178	0.946				-0.245	0.553	0.658			
Disturbances	-0.041	0.052	0.361				-0.038	0.048	0.425			
INT	0.057	0.116	0.621			0.001	0.154	0.333	0.644			0.001
Model summary: rs3875089				0.141	<0.001					0.141	<0.001	
Age	0.010	0.004	0.014				0.011	0.004	0.011			
BMI	0.004	0.006	0.538				0.004	0.006	0.519			
CVD risk	-0.001	0.040	0.988				-0.006	0.041	0.879			
GDS	-0.004	0.015	0.771				-0.006	0.015	0.704			
APOE ε4	0.303	0.058	<0.001				0.310	0.058	<0.001			
rs3875089	0.124	0.149	0.407				0.362	0.462	0.435			
Disturbances	-0.032	0.057	0.584				-0.044	0.048	0.356			
INT	-0.049	0.099	0.622			0.001	-0.149	0.284	0.602			0.001

Appendix 11: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Disturbances *(cont.).*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs162007				0.134	<0.001					0.135	<0.001	
Age	0.011	0.004	0.013				0.010	0.004	0.015			
BMI	0.004	0.006	0.528				0.004	0.006	0.539			
CVD risk	-0.004	0.040	0.926				-0.003	0.040	0.938			
GDS	-0.005	0.015	0.739				-0.006	0.015	0.695			
APOE ε4	0.304	0.058	<0.001				0.304	0.058	<0.001			
rs162007	0.015	0.144	0.919				-0.302	0.523	0.564			
Disturbances	-0.043	0.058	0.467				-0.500	0.048	0.295			
INT	-0.012	0.097	0.905			<0.001	0.177	0.413	0.668			0.001
Model summary: rs162003				0.138	<0.001					N/A		
Age	0.010	0.004	0.019									
BMI	0.003	0.006	0.659									
CVD risk	-0.001	0.040	0.978									
GDS	-0.005	0.015	0.720									
APOE ε4	0.299	0.058	<0.001									
rs162003	-0.189	0.220	0.391									
Disturbances	-0.058	0.050	0.245									
INT	0.083	0.155	0.593			0.001						
Model summary: rs151245				0.134	<0.001					0.150	<0.001	
Age	0.010	0.004	0.014				0.010	0.004	0.016			
BMI	0.004	0.006	0.543				0.003	0.006	0.570			
CVD risk	-0.004	0.041	0.931				0.003	0.041	0.950			
GDS	-0.006	0.015	0.701				-0.008	0.015	0.599			
APOE ε4	0.304	0.058	<0.001				0.301	0.058	<0.001			
rs151245	0.085	0.153	0.580				0.261	0.206	0.206			
Disturbances	-0.006	0.094	0.951				-0.028	0.051	0.586			
INT	-0.056	0.108	0.602			0.001	-0.144	0.138	0.586			0.004
Model summary: rs151246				0.148	<0.001					0.141	<0.001	
Age	0.011	0.004	0.011				0.011	0.004	0.012			
BMI	0.005	0.006	0.407				0.004	0.006	0.509			
CVD risk	-0.008	0.040	0.844				-0.005	0.041	0.898			
GDS	-0.009	0.015	0.547				-0.004	0.015	0.779			
APOE ε4	0.300	0.058	<0.001				0.306	0.059	<0.001			
rs151246	0.171	0.146	0.243				-0.124	0.392	0.753			
Disturbances	0.010	0.061	0.872				-0.048	0.048	0.318			
INT	-0.143	0.098	0.144			0.009	0.039	0.256	0.880			<0.001
Model summary: rs2339214				0.140	<0.001					0.136	<0.001	
Age	0.010	0.004	0.020				0.010	0.004	0.021			
BMI	0.005	0.006	0.472				0.004	0.006	0.472			
CVD risk	0.001	0.041	0.992				0.001	0.041	0.984			
GDS	-0.005	0.016	0.749				-0.003	0.016	0.843			
APOE ε4	0.301	0.059	<0.001				0.300	0.059	<0.001			
rs2339214	-0.124	0.167	0.459				0.064	0.173	0.711			
Disturbances	-0.101	0.091	0.270				-0.038	0.053	0.470			
INT	0.076	0.107	0.478			0.478	-0.020	0.123	0.869			<0.001

Appendix 11: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Disturbances *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs491148				0.145	<0.001					0.151	<0.001	
Age	0.011	0.004	0.012				0.012	0.004	0.004			
BMI	0.004	0.006	0.473				0.004	0.006	0.506			
CVD risk	-0.004	0.041	0.916				-0.013	0.040	0.744			
GDS	-0.007	0.015	0.626				-0.008	0.015	0.590			
APOE ε4	0.305	0.058	<0.001				0.319	0.058	0.590			
rs491148	0.070	0.162	0.666				-0.050	0.401	0.901			
Disturbances	-0.056	0.056	0.317				-0.055	0.048	0.248			
INT	0.006	0.104	0.955			<0.001	0.179	0.249	0.473			0.002

Model summary statistics for all Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors: SE, standard error; Sig, p-value; R^2, coefficient of multiple determination, ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Sleep disturbances * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded). If a model could not run due to that SNP variable being a constant then not applicable (N/A) is recorded for that model.

Appendix 12: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Efficiency.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs2075574				0.136	<0.001					0.137	<0.001	
Age	0.010	0.004	0.014				0.011	0.004	0.012			
BMI	0.004	0.006	0.523				0.004	0.006	0.497			
CVD risk	-0.008	0.040	0.853				-0.005	0.040	0.894			
GDS	-0.005	0.015	0.742				-0.006	0.015	0.722			
APOE ε4	0.305	0.058	<0.001				0.305	0.058	<0.001			
rs2075574	0.032	0.062	0.603				0.034	0.087	0.699			
Efficiency	-0.012	0.052	0.813				-0.029	0.035	0.409			
INT	-0.024	0.067	0.720			0.001	0.023	0.096	0.811			<0.001
Model summary: rs1859838				0.137	<0.001					0.139	<0.001	
Age	0.010	0.004	0.020				0.011	0.004	0.012			
BMI	0.003	0.006	0.640				0.003	0.006	0.674			
CVD risk	-0.001	0.040	0.979				-0.010	0.040	0.811			
GDS	-0.005	0.015	0.755				-0.004	0.015	0.794			
APOE ε4	0.293	0.059	<0.001				0.308	0.058	<0.001			
rs1859838	0.050	0.067	0.453				-0.100	0.133	0.452			
Efficiency	-0.003	0.037	0.936				-0.025	0.034	0.460			
INT	-0.102	0.076	0.185			0.007	-0.072	0.145	0.623			0.001
Model summary: rs4419722				0.139	<0.001					N/A		
Age	0.010	0.004	0.015									
BMI	0.004	0.006	0.555									
CVD risk	-0.008	0.040	0.835									
GDS	-0.005	0.015	0.761									
APOE ε4	0.301	0.058	<0.001									
rs4419722	0.031	0.074	0.675									
Efficiency	-0.009	0.037	0.801									
INT	-0.074	0.076	0.335			0.004						
Model summary: rs1004317				0.152	<0.001					0.137	<0.001	
Age	0.010	0.004	0.015				0.010	0.004	0.014			
BMI	0.003	0.006	0.665				0.004	0.006	0.554			
CVD risk	-0.005	0.040	0.910				-0.009	0.040	0.821			
GDS	-0.001	0.015	0.968				-0.004	0.015	0.785			
APOE ε4	0.297	0.058	<0.001				0.303	0.058	<0.001			
rs1004317	0.019	0.065	0.769				-0.009	0.089	0.921			
Efficiency	0.042	0.051	0.414				-0.021	0.035	0.543			
INT	-0.120	0.067	0.073			0.013	-0.054	0.102	0.593			0.001
Model summary: rs62449133				0.146	<0.001					0.145	<0.001	
Age	0.010	0.004	0.025				0.011	0.004	0.014			
BMI	0.004	0.006	0.550				0.003	0.006	0.651			
CVD risk	0.004	0.040	0.922				-0.001	0.040	0.997			
GDS	-0.005	0.015	0.760				-0.005	0.015	0.723			
APOE ε4	0.307	0.059	<0.001				0.321	0.059	<0.001			
rs62449133	0.050	0.062	0.422				-0.066	0.118	0.578			
Efficiency	0.008	0.040	0.838				-0.017	0.034	0.626			
INT	-0.077	0.069	0.266			0.005	-0.051	0.135	0.705			0.001

Appendix 12: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Efficiency *(cont.).*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1 cont.												
Model summary: rs2299905				0.149	<0.001					0.148	<0.001	
Age	0.010	0.004	0.023				0.011	0.004	0.014			
BMI	0.003	0.006	0.582				0.002	0.006	0.735			
CVD risk	0.001	0.040	0.995				-0.002	0.040	0.963			
GDS	-0.004	0.015	0.802				-0.005	0.015	0.739			
APOE ε4	0.312	0.059	<0.001				0.327	0.059	<0.001			
rs2299905	-0.011	0.062	0.861				-0.119	0.114	0.300			
Efficiency	0.006	0.044	0.886				-0.020	0.034	0.571			
INT	-0.065	0.067	0.333			0.004	0.007	0.120	0.954			<0.001
Model summary: rs28362727				0.137	<0.001					0.142	<0.001	0.137
Age	0.011	0.004	0.011				0.011	0.004	0.009			
BMI	0.005	0.006	0.448				0.004	0.006	0.473			
CVD risk	0.005	0.040	0.895				0.006	0.040	0.891			
GDS	0.001	0.015	0.956				0.002	0.015	0.918			
APOE ε4	0.283	0.058	<0.001				0.284	0.058	<0.001			
rs28362727	-0.007	0.061	0.909				0.008	0.101	0.940			
Efficiency	-0.033	0.042	0.436				-0.033	0.034	0.336			
INT	-0.032	0.067	0.633			0.001	-0.134	0.114	0.235			0.006
Model summary: rs11537660				0.149	<0.001					N/A		
Age	0.011	0.004	0.009									
BMI	0.004	0.006	0.522									
CVD risk	-0.010	0.040	0.807									
GDS	-0.005	0.015	0.748									
APOE ε4	0.302	0.058	<0.001									
rs11537660	-0.112	0.083	0.180									
Efficiency	-0.025	0.035	0.476									
INT	-0.027	0.093	0.772			<0.001						
AQP4												
Model summary: rs11661081				0.135	<0.001					N/A		
Age	0.011	0.004	0.012									
BMI	0.004	0.006	0.530									
CVD risk	-0.008	0.040	0.842									
GDS	-0.005	0.015	0.735									
APOE ε4	0.304	0.058	<0.001									
rs11661081	-0.019	0.077	0.807									
Efficiency	-0.031	0.037	0.409									
INT	0.018	0.078	0.818			<0.001						
Model summary rs9951307				0.151	<0.001					0.142	<0.001	
Age	0.011	0.004	0.012				0.011	0.004	0.011			
BMI	0.005	0.006	0.451				0.004	0.006	0.486			
CVD risk	-0.010	0.040	0.798				-0.007	0.040	0.861			
GDS	-0.005	0.015	0.750				-0.005	0.015	0.746			
APOE ε4	0.310	0.058	<0.001				0.303	0.058	<0.001			
rs9951307	-0.132	0.061	0.033				-0.064	0.100	0.522			
Efficiency	-0.097	0.057	0.087				-0.026	0.035	0.464			
INT	0.112	0.069	0.107			0.010	0.002	0.094	0.984			<0.001

Appendix 12: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Efficiency *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs7240333				0.151	<0.001					0.148	<0.001	
Age	0.011	0.004	0.013				0.011	0.004	0.012			
BMI	0.003	0.006	0.568				0.003	0.006	0.570			
CVD risk	-0.001	0.040	0.995				-0.001	0.040	0.985			
GDS	-0.007	0.015	0.632				-0.007	0.015	0.655			
APOE ε4	0.325	0.059	<0.001				0.319	0.059	<0.001			
rs7240333	0.064	0.075	0.392				0.017	0.263	0.949			
Efficiency	-0.012	0.037	0.742				-0.017	0.033	0.603			
INT	-0.030	0.083	0.719			0.001	-0.101	0.450	0.823			<0.001
Model summary: rs68006382				0.140	<0.001					0.146	<0.001	
Age	0.011	0.004	0.012				0.010	0.004	0.017			
BMI	0.005	0.006	0.400				0.005	0.006	0.460			
CVD risk	-0.004	0.042	0.931				-0.007	0.041	0.860			
GDS	-0.004	0.016	0.777				-0.004	0.016	0.789			
APOE ε4	0.303	0.059	<0.001				0.306	0.059	<0.001			
rs68006382	0.051	0.070	0.469				0.232	0.186	0.215			
Efficiency	-0.018	0.040	0.661				-0.018	0.034	0.587			
INT	-0.023	0.079	0.776			<0.001	-0.386	0.319	0.228			0.006
Model summary: rs71353406				0.139	<0.001					0.144	<0.001	
Age	0.011	0.004	0.014				0.010	0.004	0.023			
BMI	0.005	0.006	0.432				0.004	0.006	0.471			
CVD risk	-0.010	0.041	0.806				-0.011	0.041	0.796			
GDS	-0.004	0.016	0.823				-0.003	0.016	0.873			
APOE ε4	0.306	0.059	<0.001				0.301	0.059	<0.001			
rs71353406	0.071	0.062	0.256				0.209	0.134	0.121			
Efficiency	-0.001	0.047	0.980				-0.017	0.034	0.612			
INT	-0.052	0.067	0.442			0.003	-0.289	0.229	0.208			0.007
Model summary: rs12968026				0.136	<0.001					0.128	<0.001	
Age	0.011	0.004	0.008				0.011	0.004	0.011			
BMI	0.004	0.006	0.487				0.003	0.006	0.600			
CVD risk	-0.007	0.040	0.866				-0.006	0.040	0.888			
GDS	-0.004	0.015	0.808				-0.002	0.015	0.915			
APOE ε4	0.281	0.058	<0.001				0.283	0.059	<0.001			
rs12968026	0.043	0.075	0.568				-0.030	0.207	0.717			
Efficiency	-0.032	0.036	0.384				-0.026	0.034	0.439			
INT	0.047	0.084	0.577			0.001	0.038	0.204	0.852			<0.001
Model summary: rs3875089				0.139	<0.001					0.139	<0.001	
Age	0.011	0.004	0.012				0.011	0.004	0.009			
BMI	0.004	0.006	0.516				0.004	0.006	0.510			
CVD risk	-0.005	0.040	0.894				-0.011	0.040	0.786			
GDS	-0.005	0.015	0.730				-0.007	0.015	0.667			
APOE ε4	0.300	0.058	<0.001				0.310	0.058	<0.001			
rs3875089	0.053	0.067	0.425				0.176	0.179	0.326			
Efficiency	-0.025	0.038	0.508				-0.024	0.033	0.326			
INT	-0.002	0.074	0.977			<0.001	-0.090	0.191	0.638			0.001

Appendix 12: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Efficiency *(cont.).*

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs162007				0.139	<0.001					0.148	<0.001	
Age	0.011	0.004	0.012				0.010	0.004	0.016			
BMI	0.003	0.006	0.568				0.004	0.006	0.480			
CVD risk	-0.008	0.040	0.848				-0.005	0.040	0.893			
GDS	-0.004	0.015	0.811				-0.008	0.015	0.620			
APOE ε4	0.301	0.058	<0.001				0.305	0.058	<0.001			
rs162007	-0.036	0.061	0.555				-0.508	0.308	0.100			
Efficiency	-0.049	0.041	0.229				-0.033	0.033	0.323			
INT	0.063	0.069	0.361			0.003	0.374	0.219	0.088			0.012
Model summary: rs162003				0.142	<0.001					N/A		
Age	0.010	0.004	0.015									
BMI	0.003	0.006	0.660									
CVD risk	-0.010	0.040	0.808									
GDS	-0.005	0.015	0.751									
APOE ε4	0.301	0.058	<0.001									
rs162003	-0.111	0.084	0.186									
Efficiency	-0.039	0.034	0.257									
INT	0.087	0.122	0.478			0.002						
Model summary: rs151245				0.135	<0.001					0.144	<0.001	
Age	0.010	0.004	0.014				0.011	0.004	0.009			
BMI	0.004	0.006	0.538				0.003	0.006	0.646			
CVD risk	-0.007	0.040	0.856				-0.005	0.040	0.896			
GDS	-0.006	0.015	0.714				-0.007	0.015	0.662			
APOE ε4	0.304	0.058	<0.001				0.305	0.058	<0.001			
rs151245	-0.001	0.063	0.997				0.123	0.087	0.159			
Efficiency	-0.035	0.059	0.554				-0.012	0.035	0.738			
INT	0.011	0.071	0.874			<0.001	-0.109	0.092	0.237			0.006
Model summary: rs151246				0.138	<0.001					0.143	<0.001	
Age	0.011	0.004	0.010				0.011	0.004	0.009			
BMI	0.004	0.006	0.507				0.003	0.006	0.615			
CVD risk	-0.008	0.040	0.839				-0.007	0.040	0.868			
GDS	-0.006	0.015	0.677				-0.006	0.015	0.689			
APOE ε4	0.297	0.059	<0.001				0.305	0.058	<0.001			
rs151246	0.002	0.064	0.971				0.066	0.161	0.680			
Efficiency	-0.004	0.044	0.927				-0.018	0.034	0.599			
INT	-0.050	0.066	0.453			0.002	-0.197	0.153	0.197			0.007
Model summary: rs2339214				0.137	<0.001					0.139	<0.001	
Age	0.010	0.004	0.018				0.009	0.004	0.031			
BMI	0.004	0.006	0.501				0.004	0.006	0.478			
CVD risk	-0.004	0.041	0.925				-0.002	0.041	0.964			
GDS	-0.003	0.016	0.851				-0.003	0.016	0.852			
APOE ε4	0.303	0.059	<0.001				0.304	0.059	<0.001			
rs2339214	-0.024	0.070	0.735				0.068	0.072	0.348			
Efficiency	-0.062	0.067	0.355				-0.024	0.039	0.546			
INT	0.034	0.077	0.654			0.001	-0.049	0.080	0.539			0.002

Appendix 12: Moderation Analysis for *AQP1* and *AQP4* SNPs on Sleep Efficiency *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs491148				0.146	<0.001					0.148	<0.001	
Age	0.011	0.004	0.012				0.012	0.004	0.005			
BMI	0.005	0.006	0.454				0.004	0.006	0.515			
CVD risk	-0.009	0.040	0.815				-0.014	0.040	0.729			
GDS	-0.011	0.016	0.483				-0.007	0.015	0.626			
APOE ε4	0.305	0.058	<0.001				0.317	0.058	<0.001			
rs491148	0.030	0.068	0.654				0.231	0.174	0.186			
Efficiency	-0.046	0.038	0.224				-0.027	0.033	0.416			
INT	0.074	0.073	0.313			0.004	-0.016	0.183	0.932			<0.001

Model summary statistics for all Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors: SE, standard error; Sig, p-value; R^2, coefficient of multiple determination, ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Sleep efficiency * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded). If a model could not run due to that SNP variable being a constant then not applicable (N/A) is recorded for that model.

Appendix 13: Moderation Analysis for *AQP1* and *AQP4* SNPs on Daytime Dysfunction.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1												
Model summary: rs2075574				0.136	<0.001					0.137	<0.001	
Age	0.010	0.004	0.015				0.010	0.004	0.016			
BMI	0.005	0.006	0.439				0.005	0.006	0.427			
CVD risk	-0.015	0.040	0.715				-0.013	0.040	0.751			
GDS	-0.013	0.017	0.454				-0.011	0.017	0.499			
APOE ε4	0.307	0.058	<0.001				0.304	0.058	<0.001			
rs2075574	0.036	0.074	0.631				0.016	0.139	0.890			
Dysfunction	0.045	0.057	0.437				0.025	0.044	0.572			
INT	-0.025	0.073	0.732			0.001	0.031	0.107	0.769			<0.001
Model summary: rs1859838				0.148	<0.001					0.152	<0.001	
Age	0.010	0.004	0.018				0.010	0.004	0.016			
BMI	0.004	0.006	0.475				0.003	0.006	0.618			
CVD risk	-0.010	0.040	0.803				-0.012	0.040	0.761			
GDS	-0.011	0.016	0.493				-0.012	0.016	0.461			
APOE ε4	0.304	0.058	<0.001				0.313	0.058	<0.001			
rs1859838	-0.110	0.080	0.170				-0.352	0.170	0.040			
Dysfunction	0.010	0.004	0.018				0.010	0.004	0.016			
INT	0.004	0.006	0.475				0.003	0.006	0.618			
Model summary: rs4419722				0.137	<0.001					0.161	<0.001	
Age	0.010	0.004	0.018				0.010	0.004	0.013			
BMI	0.005	0.006	0.443				0.004	0.006	0.522			
CVD risk	-0.014	0.040	0.725				-0.004	0.040	0.925			
GDS	-0.012	0.017	0.453				-0.016	0.016	0.323			
APOE ε4	0.299	0.058	<0.001				0.288	0.058	<0.001			
rs4419722	-0.070	0.095	0.464				-0.369	0.804	0.647			
Dysfunction	0.020	0.044	0.646				0.022	0.040	0.580			
INT	0.068	0.095	0.472			0.002	0.637	0.512	0.215			0.006
Model summary: rs1004317				0.144	<0.001					0.160	<0.001	
Age	0.010	0.004	0.017				0.009	0.004	0.046			
BMI	0.004	0.006	0.557				0.004	0.006	0.465			
CVD risk	-0.013	0.040	0.739				-0.004	0.040	0.922			
GDS	-0.012	0.016	0.458				-0.013	0.016	0.429			
APOE ε4	0.306	0.058	<0.001				0.307	0.057	<0.001			
rs1004317	-0.029	0.067	0.575				-0.268	0.119	0.025			
Dysfunction	-0.029	0.067	0.668				-0.002	0.043	0.973			
INT	0.090	0.078	0.246			0.005	**0.260**	**0.106**	**0.015**			**0.024**
Model summary: rs62449133				0.141	<0.001					0.191	<0.001	
Age	0.010	0.004	0.018				0.009	0.004	0.037			
BMI	0.003	0.006	0.578				0.002	0.006	0.786			
CVD risk	-0.002	0.040	0.955				0.014	0.039	0.714			
GDS	-0.009	0.017	0.600				-0.007	0.016	0.647			
APOE ε4	0.314	0.059	<0.001				0.345	0.058	<0.001			
rs62449133	-0.006	0.076	0.936				-0.494	0.151	0.001			
Dysfunction	-0.002	0.055	0.965				-0.034	0.042	0.429			
INT	0.022	0.073	0.766			<0.001	**0.411**	**0.119**	**0.001**			**0.047**

Appendix 13: Moderation Analysis for *AQP1* and *AQP4* SNPs on Daytime Dysfunction *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP1 cont.												
Model summary: rs2299905				0.146	<0.001					0.181	<0.001	
Age	0.010	0.004	0.025				0.009	0.004	0.045			
BMI	0.003	0.006	0.578				0.002	0.006	0.786			
CVD risk	-0.003	0.040	0.944				0.012	0.040	0.765			
GDS	-0.009	0.017	0.593				-0.008	0.016	0.607			
APOE ε4	0.318	0.059	<0.001				0.339	0.058	<0.001			
rs2299905	-0.087	0.074	0.245				-0.458	0.149	0.002			
Dysfunction	-0.022	0.058	0.709				-0.027	0.043	0.534			
INT	0.057	0.074	0.440			0.003	**0.336**	**0.115**	**0.002**			**0.034**
Model summary: rs28362727				0.136	<0.001					0.131	<0.001	
Age	0.010	0.004	0.015				0.011	0.004	0.010			
BMI	0.005	0.006	0.393				0.005	0.006	0.413			
CVD risk	-0.001	0.040	0.996				-0.004	0.040	0.916			
GDS	-0.006	0.017	0.738				-0.007	0.017	0.690			
APOE ε4	0.286	0.058	<0.001				0.289	0.058	<0.001			
rs28362727	-0.084	0.072	0.246				-0.092	0.133	0.863			
Dysfunction	-0.024	0.056	0.668				0.019	0.043	0.665			
INT	0.087	0.072	0.668			0.006	0.043	0.103	0.675			0.001
Model summary: rs11537660				0.145	<0.001					N/A		
Age	0.010	0.004	0.017									
BMI	0.004	0.006	0.485									
CVD risk	-0.015	0.040	0.699									
GDS	-0.013	0.016	0.446									
APOE ε4	0.302	0.058	<0.001									
rs11537660	-0.175	0.112	0.119									
Dysfunction	0.026	0.042	0.541									
INT	0.064	0.114	0.575			0.001						
AQP4												
Model summary: rs11661081				0.135	<0.001					N/A		
Age	0.010	0.004	0.016									
BMI	0.004	0.006	0.472									
CVD risk	-0.015	0.040	0.714									
GDS	-0.012	0.017	0.470									
APOE ε4	0.303	0.058	<0.001									
rs11661081	-0.034	0.105	0.745									
Dysfunction	0.028	0.043	0.745									
INT	0.029	0.108	0.788			<0.001						
Model summary: rs9951307				**0.165**	**<0.001**					0.143	<0.001	
Age	0.010	0.004	**0.013**				0.010	0.004	0.014			
BMI	0.005	0.006	0.405				0.006	0.006	0.340			
CVD risk	-0.012	0.040	0.740				-0.011	0.040	0.788			
GDS	-0.009	0.016	0.593				-0.014	0.017	0.410			
APOE ε4	0.302	0.057	**<0.001**				0.296	0.058	<0.001			
rs9951307	-0.201	0.074	**0.007**				-0.179	0.124	0.152			
Dysfunction	-0.067	0.058	0.245				0.018	0.042	0.667			
INT	**0.165**	**0.072**	**0.023**			**0.021**	0.126	0.111	0.256			0.005

Appendix 13: Moderation Analysis for *AQP1* and *AQP4* SNPs on Daytime Dysfunction *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs7240333				0.165	<0.001					0.150	<0.001	
Age	0.012	0.004	0.007				0.010	0.004	0.016			
BMI	0.004	0.006	0.539				0.004	0.006	0.511			
CVD risk	-0.003	0.040	0.949				-0.005	0.040	0.909			
GDS	-0.015	0.016	0.348				-0.010	0.017	0.557			
APOE ε4	0.333	0.058	<0.001				0.315	0.059	<0.001			
rs7240333	0.174	0.088	0.049				-0.205	0.334	0.540			
Dysfunction	0.059	0.044	0.180				0.017	0.041	0.683			
INT	-0.172	0.091	0.059			0.014	0.191	0.265	0.472			0.002
Model summary: rs68006382				0.139	<0.001					0.151	<0.001	
Age	0.011	0.004	0.014				0.010	0.004	0.023			
BMI	0.006	0.006	0.355				0.005	0.006	0.442			
CVD risk	-0.006	0.041	0.883				-0.012	0.040	0.765			
GDS	-0.010	0.017	0.554				-0.009	0.017	0.601			
APOE ε4	0.302	0.059	<0.001				0.304	0.059	<0.001			
rs68006382	0.025	0.083	0.766				0.356	0.214	0.097			
Dysfunction	0.022	0.048	0.656				0.041	0.042	0.324			
INT	0.016	0.081	0.842			<0.001	-0.254	0.154	0.101			0.011
Model summary: rs71353406				0.138	<0.001					0.148	<0.001	
Age	0.011	0.004	0.015				0.010	0.004	0.023			
BMI	0.005	0.006	0.403				0.005	0.006	0.431			
CVD risk	-0.017	0.041	0.682				-0.017	0.040	0.668			
GDS	-0.012	0.017	0.498				-0.012	0.017	0.483			
APOE ε4	0.302	0.059	<0.001				0.308	0.059	<0.001			
rs71353406	0.078	0.074	0.295				0.299	0.159	0.061			
Dysfunction	0.059	0.055	0.292				0.051	0.043	0.233			
INT	-0.049	0.075	0.518			0.002	-0.205	0.128	0.110			0.011
Model summary: rs12968026				0.137	<0.001					0.131	<0.001	
Age	0.011	0.004	0.012				0.010	0.004	0.018			
BMI	0.005	0.006	0.444				0.004	0.006	0.504			
CVD risk	-0.015	0.040	0.704				-0.013	0.040	0.757			
GDS	-0.012	0.016	0.479				-0.010	0.017	0.540			
APOE ε4	0.281	0.058	0.281				0.281	0.059	<0.001			
rs12968026	0.051	0.089	0.571				-0.027	0.363	0.940			
Dysfunction	0.039	0.046	0.398				0.046	0.041	0.269			
INT	0.030	0.087	0.726			0.001	-0.005	0.208	0.981			<0.001
Model summary: rs3875089				0.140	<0.001					0.144	<0.001	
Age	0.011	0.004	0.016				0.010	0.004	0.019			
BMI	0.004	0.006	0.456				0.005	0.006	0.437			
CVD risk	-0.013	0.040	0.745				-0.011	0.040	0.779			
GDS	-0.013	0.017	0.438				-0.011	0.017	0.527			
APOE ε4	0.300	0.058	<0.001				0.307	0.058	<0.001			
rs3875089	0.043	0.079	0.583				0.369	0.245	0.133			
Dysfunction	0.028	0.049	0.560				0.037	0.041	0.377			
INT	0.021	0.792	0.792			<0.001	-0.207	0.163	0.206			0.007

Appendix 13: Moderation Analysis for *AQP1* and *AQP4* SNPs on Daytime Dysfunction *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs162007				0.136	<0.001					0.139	<0.001	
Age	0.011	0.004	0.015				0.010	0.004	0.017			
BMI	0.005	0.006	0.456				0.005	0.006	0.422			
CVD risk	-0.013	0.040	0.740				-0.014	0.040	0.728			
GDS	-0.013	0.017	0.449				-0.011	0.017	0.493			
APOE ε4	0.305	0.058	<0.001				0.308	0.058	<0.001			
rs162007	0.024	0.079	0.764				0.058	0.213	0.786			
Dysfunction	0.042	0.047	0.379				0.033	0.041	0.419			
INT	-0.034	0.079	0.672			0.001	-0.321	0.335	0.338			0.004
Model summary: rs162003				0.140	<0.001					N/A		
Age	0.010	0.004	0.020									
BMI	0.004	0.006	0.566									
CVD risk	-0.011	0.040	0.784									
GDS	-0.013	0.017	0.431									
APOE ε4	0.299	0.058	<0.001									
rs162003	-0.019	0.117	0.872									
Dysfunction	0.037	0.042	0.378									
INT	-0.078	0.133	0.559			0.001						
Model summary: rs151245				0.135	<0.001					0.152	<0.001	
Age	0.010	0.004	0.016				0.009	0.004	0.029			
BMI	0.004	0.006	0.472				0.005	0.006	0.404			
CVD risk	-0.015	0.040	0.714				-0.003	0.040	0.933			
GDS	-0.012	0.017	0.459				-0.011	0.017	0.498			
APOE ε4	0.305	0.058	<0.001				0.298	0.058	<0.001			
rs151245	-0.005	0.078	0.949				0.213	0.106	0.046			
Dysfunction	0.023	0.062	0.708				0.058	0.043	0.176			
INT	0.014	0.075	0.857			<0.001	-0.197	0.104	0.059			0.014
Model summary: rs151246				0.137	<0.001					0.138	<0.001	
Age	0.011	0.004	0.014				0.011	0.004	0.013			
BMI	0.005	0.006	0.455				0.005	0.006	0.447			
CVD risk	-0.013	0.040	0.744				-0.017	0.040	0.680			
GDS	-0.013	0.017	0.441				-0.012	0.017	0.470			
APOE ε4	0.303	0.058	<0.001				0.305	0.058	<0.001			
rs151246	-0.001	0.076	0.993				-0.257	0.288	0.372			
Dysfunction	0.043	0.048	0.370				0.030	0.041	0.478			
INT	-0.038	0.078	0.627			0.001	0.185	0.260	0.477			0.477
Model summary: rs2339214				0.141	<0.001					0.138	<0.001	
Age	0.009	0.004	0.040				0.010	0.004	0.024			
BMI	0.006	0.006	0.367				0.005	0.006	0.423			
CVD risk	-0.010	0.041	0.815				-0.011	0.041	0.789			
GDS	-0.010	0.017	0.567				-0.009	0.017	0.583			
APOE ε4	0.296	0.059	<0.001				0.303	0.059	<0.001			
rs2339214	-0.091	0.088	0.301				0.088	0.094	0.350			
Dysfunction	-0.054	0.081	0.506				0.046	0.046	0.317			
INT	0.114	0.089	0.203			0.007	-0.063	0.087	0.468			0.002

Appendix 13: Moderation Analysis for *AQP1* and *AQP4* SNPs on Daytime Dysfunction *(cont.)*.

	Dominant†						Recessive†					
	β	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**	**β**	**SE**	**Sig.**	**R^2**	**Sig.**	**ΔR^2**
AQP4 cont.												
Model summary: rs491148				0.143	<0.001					**0.168**	**<0.001**	
Age	0.010	0.004	0.020				0.010	0.004	**0.015**			
BMI	0.005	0.006	0.394				0.005	0.006	0.384			
CVD risk	-0.012	0.041	0.760				-0.007	0.040	0.866			
GDS	-0.016	0.017	0.351				-0.008	0.016	0.623			
APOE ε4	0.302	0.058	<0.001				0.312	0.058	**<0.001**			
rs491148	0.101	0.082	0.221				0.568	0.197	**0.004**			
Dysfunction	0.046	0.047	0.337				0.043	0.041	0.300			
INT	-0.034	0.082	0.337			0.001	**-0.339**	**0.144**	**0.020**			**0.022**

Model summary statistics for all Aquaporin 1 (*AQP1*) and Aquaporin 4 (*AQP4*) reference single nucleotide polymorphism (SNP) markers (rs). †Genetic models: Dominant (heterozygote/homozygote for the minor allele (Mm or MM) vs homozygote for the major allele (mm)), Recessive (homozygote for the minor allele (MM) vs heterozygote/homozygote for the major allele (Mm/mm)); β, Coefficient of predictors: SE, standard error; Sig, p-value; R^2, coefficient of multiple determination, ΔR^2, multiple correlation coefficient (R) squared change; BMI, Body Mass Index; CVD risk, cardiovascular disease risk; GDS, Geriatric Depression Scale; *APOE*, Apolipoprotein E ε4 allele carriage (presence/absence); INT, Interaction (Daytime dysfunction * model summary SNP). Models where the interaction term (INT) resulted in a statistically significant R^2-change ($p < 0.05$) are indicated (bolded). If a model could not run due to that SNP variable being a constant then not applicable (N/A) is recorded for that model.