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# Identification of genetic loci associated with pathophysiological changes in Alzheimer's disease progression using neuroimaging genetics

Graduate School of Chosun University

Department of Biomedical Sciences

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# Identification of genetic loci associated with pathophysiological changes in Alzheimer's disease progression using neuroimaging genetics

## 알츠하이머병의 뇌손상과 베타아밀로이드 축적에 대한

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*This dissertation is submitted to the Graduate School of Chosun University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Science*

**May 2020**

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# ABSTRACT

## Identification of genetic loci associated with pathophysiological changes in Alzheimer's disease progression using neuroimaging genetics

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Alzheimer's disease (AD) is a complex neurodegenerative disorder and considered as one of the main forms of dementia. AD is clinically characterised by progressive deficits in memory, behavioural problems and cognitive impairments. The extracellular deposition of β-amyloid peptides and the intracellular tau hyperphosphorylation are the pathological hallmarks of AD. In particular, the apolipoprotein E (*APOE*) □4 is the most potent genetic risk factor of AD and is associated with both β-amyloid deposition and brain atrophy. Apart from the *APOE* gene, 21 common genetic variants are found to be associated with AD. The focus was on the identification of comprehensive novel genetic variants associated with brain β-amyloid deposition, atrophy and AD progression. In addition, the role of

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ethnicity in AD risk at different progressive stages were evaluated. The brain cortical thickness from structural Magnetic Resonance Images (MRI), cerebral β-amyloid standard uptake value ratio (SUVR) from $^{18}$F-Florbetaben ($^{18}$F-FBB) positron emission tomography (PET) images and cognitive measures were applied for genome-wide association study (GWAS) and polygenic analysis. The results of MRI GWA study showed that the *PRDM4* gene was associated with brain temporal lobe atrophy, AD risk and cerebral β-amyloid deposition. The results suggested that rs117303558 and rs73398399 SNPs in *PRDM4* gene are associated with AD pathology by promoting Aβ deposition in the brain. The $^{18}$F-FBB amyloid PET GWAS results showed that there was an association of two novel risk loci with cerebral β-amyloid deposition. Those two novel loci are located at rs12975891 in *CPAMD8* gene and rs113454949 in *ARL14* gene. A missense variant, rs3745339 was also identified in the *CPAMD8* gene. In addition, *CPAMD8*, *ARL14*, *SMC4*, *KPNA4*, *TRIM59* and *IFT80* genes were identified to play an important role in β-amyloid pathology in the brain. Next, in a polygenic risk score (PGRS) analysis, it was identified that there was a synergistic effect among AD biomarkers involved in β-amyloid, hippocampal volume and cognitive domain, which drives AD risk in both early and late stages of AD dementia. Additionally, it was also identified that SNPs based on their effect size direction in the association analysis could influence the correlation of AD biomarker with PGRSs of AD. In conclusion, several novel genetic risk loci were identified for the association with AD mediated pathophysiological

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changes in the brain. Moreover, East Asian specific SNPs were identified to promote AD mediated pathology. Taken together, findings from this study could provide novel insights in understanding this complex neurodegenerative disease.

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# PART 1

## *General Introduction*

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# I. GENERAL INTRODUCTION

## I-1. Overview of Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease, often characterized by a cluster of symptoms including cognitive impairments, memory loss and behavioural problems. AD is commonly thought to be caused by intracellular tau hyperphosphorylation and neuronal dysfunction due to extracellular β-amyloid deposition in the brain. AD is also a common form of dementia that predominantly affects the elderly population (Hong-Qi, Zhi-Kun, & Sheng-Di, 2012). An estimate showed that around 5.8 million Americans aged 65 and above are affected by Alzheimer's disease in 2020, and among them, 85% are aged 75 and older (Association, 2020). In 2016, the prevalence of dementia was found to be 43.8 million (95% uncertainty interval 37.8-51.0) people worldwide, and that has been increased from 20.2 million (95% uncertainty interval 17.4-23.5) in 1990 (Nichols, 2019). Although drug therapies like N-methyl-D aspartate receptor antagonist, cholinesterase inhibitors and some neuroprotective agents are available, an effective treatment for Alzheimer's disease currently remains out of reach (Hong-Qi et al., 2012).

The term "Alzheimer's disease" was coined after a German physician, Alois Alzheimer, who worked with Professor Emil Kraepelin, the father of modern psychiatry. In 1906, Alzheimer performed an autopsy on a brain from his patient named Auguste D, a 51-year-old woman, who was admitted to the Frankfurt State Asylum with conditions like auditory hallucinations, aphasia, severe progressive impairments in cognition function, delusions, paranoia, impaired social functioning and erratic behaviour. In the autopsy, he found some abnormal changes in her brain, which includes the formation of neurofibrillary tangles, argyrophilic plaques, and atherosclerotic changes. Then, on November 3, 1906, Alzheimer reported his

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findings at a conference held at Tübingen in Germany. Later, in 1910, Emil Kraepelin named the disease as Alzheimer's disease to differentiate it from dementia (Gunasekaran & Ohn, 2015).

**I-2. Alzheimer's disease associated changes in the brain**

After the onset of Alzheimer's disease, the primary brain regions that are affected are hippocampus, entorhinal cortex and cerebral cortex. Initially, the brain atrophy associated with Alzheimer's disease starts from entorhinal cortex that connects to hippocampus, and later, the atrophy progression affects hippocampus, which is responsible for memory formation. Then, the atrophies further progress to the cerebral cortex. During the disease progression, neuronal loss triggers brain shrinkage and the cerebrospinal fluid (CSF) fills the space caused by it. As the disease reaches its severity, there will be increase in the size of lateral and third ventricles, and this stage eventually end up in long-term memory loss, weight loss and even failure in recognizing their own family members (Jackson-Siegal, 2005). Alzheimer's disease exists as early onset form, the familial and the late onset form, the sporadic. Familial Alzheimer's disease (FAD) is a rare Mendelian dominant inheritance that affects patients less than fifty or sixty years of age and is caused due to mutations in *APP*, Presenilin 1 (*PSEN1*) and Presenilin 2 (*PSEN2*) genes. Whereas, several genetic and environmental factors collectively instigate the late onset Alzheimer's disease (LOAD) and it is commonly found among older population. Although there are several genes associated with LOAD, a gene called apolipoprotein E gene (*APOE*) found on chromosome 19 is attributed to high risk for LOAD (Barber, 2012; Whalley, 2018). Mutations in *APP* genes cause abnormal cleavage of β-secretase which in turn increases the production of fibrillogenic Aβ42 over Aβ40 (Jürgen Götz, Andreas Schild, Fred Hoerndli, & Luis Pennanen, 2004). Moreover, mutations in *PSEN1* and *PSEN2* genes are also associated with Aβ42 deposition (Hutton & Hardy, 1997).Whereas, mutations in *APOE* gene is associated

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with novel monofibrils deposition, which precipitates into dense structures (R. J. Guerreiro, Gustafson, & Hardy, 2012).

**I-3. Pathological changes associated with Alzheimer's disease**

The β-amyloid (Aβ) plaques are the resultant of a large amyloid precursor protein (*APP*), which is an integral membrane glycoprotein. It is usually expressed in the brain and central nervous system, and it undergoes proteolytic α and β pathways. The cleavage of *APP* through α pathway yields non-amyloidogentic product by α-secretase and γ-secretase. But, the β pathway cleavage of *APP* by β-secretase generates β-amyloid plaques and the soluble peptide *APP*β (sAPPβ). This sAPPβ is released into extracellular space by β-secretase, leaving behind the 99-amino-acid C-terminal sequence (C99) within the cellular membrane. Then, the γ-secretase converts C99 into 38-43 amino acids and produces β-amyloid and *APP* intracellular C-terminal domain (AICD). However, the cleavage by γ-secretase generally produces a soluble Aβ1-40, whereas in Alzheimer's disease, the γ-secretase produces a toxic insoluble Aβ1-42 (Dong, Duan, Hu, & Zhao, 2012). Mutations in the enzymes that cleave *APP* causes elevated deposition of β-amyloid, specifically Aβ1-42, which leads to early-onset familial Alzheimer's disease (EOFAD). The common late-onset Alzheimer's disease (LOAD) is thought to be caused by microglial cells, low level expression of β-amyloid degrading protease, reduced level of low density lipoprotein receptor- related protein 1 (*LRP1*) to clear β-amyloid from the brain and reduction in the vascular and perivascular drainage in the brain (Mokhtar, Bakhuraysah, Cram, & Petratos, 2013).

Neurofibrillary tangles are comprised of highly phosphorylated tau protein, which is associated with microtubules (Brion, 1998). The main proteinaceous component of neurofibrillary tangles is either straight filament (SF) or paired helical filament (PHF) and sometimes, it was also reported that both SF and PHF can be

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found as hybrid filaments in the AD patients. Abnormal phosphorylation of tau involves altered conformation, which re-localizes from axonal region to somatodendritic region (Crowther, 1991; J. Götz, A. Schild, F. Hoerndli, & L. Pennanen, 2004). In addition, phosphorylation causes tau to dissociate from microtubules. The accumulation of highly phosphorylated tau proteins in the neurons is also associated with the imbalance between functions of protein kinases and phosphatases acting on tau protein during the early onset of Alzheimer's disease (Brion, 1998).

Although Alzheimer's disease causes several million deaths each year, the potential method of diagnosis is only possible after the histological tests on brain tissues during autopsy (Reitz, 2012; Rosén, Hansson, Blennow, & Zetterberg, 2013). However, unambiguous diagnosis of Alzheimer's disease needs both clinical and histopathological post-mortem results. Moreover, the early diagnosis of Alzheimer's disease still remains challenging due to the common symptoms that are similar to other neurodegenerative diseases (Humpel, 2011). Since the current method of diagnosis is limited in accuracy, the bio-marker based diagnosis of Alzheimer's disease is emerging as a promising method (Fagan & Perrin, 2012). There are three biomarkers to diagnose AD in CSF that are inter- nationally validated: β-amyloid (1-42), phosphorylated-tau and total tau. Nevertheless, the combination of all three of these biomarkers can only provide significant sensitivity to the results.

### I-4. Brain imaging in Alzheimer’s disease

Neuroimaging has played a vital role in studying Alzheimer’s disease over past few decades. Magnetic resonance imaging (MRI) and computed tomography (CT) were initially used in diagnosing dementia. In recent years, different imaging modalities including structural MRI, functional MRI and positron emission tomography (PET) imaging with amyloid tracers. These images further helped in

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detecting pathophysiological changes in the brains of AD patients. However, not just single imaging modality can be helpful in all the scenarios since each imaging modality has its own advantages and disadvantages. Recently, many studies tried to combine these imaging biomarker to detect pathophysiological changes in the brain (Johnson, Fox, Sperling, & Klunk, 2012).

**I-5. Genome-wide association studies in Alzheimer's disease**

In recent years, genome-wide association studies (GWASs) have drawn more attention in identifying novel genetic risk factors for many disease. Given that the role of multiple genes in AD, GWAS is being widely adopted in the field of AD researches. Several GWASs on AD even successfully identified several novel risk loci susceptible to AD. As discussed in the above sections, a cluster of genes have identified to be associated with familial AD and additional genes are being reported through several studies. Due to complex genetic pathways associated with late-onset or sporadic AD, researchers suggested that there could several genetic loci that are yet to be identified, even though several AD susceptible loci are already reported.

Brain atrophy with excessive neurodegeneration is an inevitable sign of Alzheimer's disease (AD). Earlier studies on neuroimaging have reported a consistent reduction in the brain hippocampal volume of the clinically diagnosed AD patients. In addition to the hippocampal atrophy, several other anatomically defined region of interests (ROI) are associated with the progressive cortical atrophy in AD. Recent advancements on the high-throughput genotyping and brain imaging technique facilitates to study the effect of genetic variation on the brain structure. In this study, a genome-wide association study on brain structural and pathological measures were performed to identify high-risk genetic loci associated with AD. In addition, this study evaluated the effects of polygenic risk scores for AD on brain structural, pathological and cognitive domain biomarkers.

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# PART 2

***PRDM4 is associated with the Alzheimer's disease and contributes β-amyloid deposition in the brain***

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## SUMMARY

Brain atrophy with excessive neurodegeneration is an inevitable sign of Alzheimer's disease (AD). Earlier studies on neuroimaging have reported a consistent reduction in the brain hippocampal volume of the clinically diagnosed AD patients. In addition to the hippocampal atrophy, several other anatomically defined region of interests (ROI) are associated with the progressive cortical atrophy in AD. Recent advancements on the high-throughput genotyping and brain imaging technique facilitate to study the effect of genetic variation on the brain structure. In this study, brain ROI based linear regression method was performed to identify high-risk genetic loci associated with AD. The structural Magnetic Resonance Images (MRI) of 1.5 T and 3 T field strength and genetic dataset were investigated in the genome-wide association study (GWAS). The linear regression was performed on each AD-specific ROI cortical thickness including 5,519,663 single nucleotide polymorphisms (SNPs) and 2,277 participants (197 AD, 988 amnestic mild cognitive impairment (aMCI), mild cognitive impairment (MCI) and 1,092 cognitively normal). GWAS results are considered significant when $p < 10^{-6}$. In concordance with the previous studies, SNPs in the *APOE*, *APOC*, *TOMM40* and *PVRL2* genes in the chromosome 19 showed high significance in the AD-specific ROIs. Apart from the genes in chromosome 19, a functional gene-enrichment analysis on GWAS-derived genes showed that *PRDM4* gene was associated with temporal lobe atrophy, AD risk and β-amyloid deposition in the brain. The regional SNP-based association analysis and haplotype association analysis on *PRDM4* locus identified that rs117303558 and rs73398399 SNPs were associated with AD risk and Aβ deposition. This suggests that rs117303558 and rs73398399 SNPs in *PRDM4* were associated with AD pathology by promoting Aβ deposition in the brain. Moreover, these two SNPs were associated with memory domain in MCI and AD patients. In addition, a previous study reported that *PRDM4* is associated with the lower psychological

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endurance threshold and likely prone to abnormal behaviour. Interestingly, rs117303558 and rs73398399 SNPs are East Asian specific. However, this study focuses on the temporal lobe, which is associated with lateral stages of Alzheimer's disease. Although the association of *PRDM4* gene for AD risk and Aβ deposition were moderate, *PRDM4* might play an important role in the AD related gene pathways during lateral stages.

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## II-1. INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, affecting individuals aged 65 and above (R. Guerreiro & Bras, 2015; Wang, Gu, Masters, & Wang, 2017). It is a progressive neurodegenerative disease, characterized pathologically by the abnormal deposition of amyloid-β (Aβ) proteins in the form of senile plaques and hyperphosphorylated tau proteins in the form of neurofibrillary tangles in the brain (Karantzoulis & Galvin, 2011). These abnormal protein deposition causes disruption of neurotransmitters and receptors, alterations in the synaptic densities, neuronal loss and eventually resulting in brain atrophy (Vlassenko, Benzinger, & Morris, 2012). Assessment of this brain atrophy using structural magnetic resonance imaging (MRI) has been reported as a valid biomarker in detecting AD mediated neurodegeneration (Pini et al., 2016). Previous studies reported that brain atrophy measured from structural MRI in the anatomical regions of medial temporal lobe, paralimbic system, temporal and parietal lobes are associated with AD mediated neurodegeneration (Vlassenko et al., 2012).

Recently, large-scale genome-wide association studies have reported more than 20 genetic loci associated with late onset Alzheimer's disease (LOAD) (Jansen et al., 2019; Kunkle et al., 2019). Past studies reported that some of the AD risk variants were also observed in the GWASs involving MRI measures that included mixed groups of cognitively normal, mild cognitively impaired and AD patients (Chauhan et al., 2015). In addition, the genetic studies that employ AD-related endophenotypes may provide opportunities in finding additional AD related risk variants that were not identified in previous studies.

Among the AD biomarkers, hippocampal atrophy in the brain is considered as one of the strongly established biomarker that is involved in the early stages of dementia and aging (Pini et al., 2016). This AD related brain atrophy starts in

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hippocampus and later extends to temporal, occipital, parietal and frontal cortices (Migliaccio et al., 2015). In the early stages of AD, it was also found that the accumulation of neurofibrillary tangles in the brain initiates in the hippocampus, entorhinal cortex and progress to the temporal, parietal and frontal cortices. Similarly, Aβ deposition in the brain involves parietal, temporal and frontal regions (Karantzoulis & Galvin, 2011).

Earlier GWASs with brain MRI measures involving hippocampus and entorhinal cortex have successfully identified AD susceptible genes including *APOE, LHF, GCFC2, F5/SELP, TOMM40, EPHA4, TP63, NXPH1* and *ZNF292* (Melville et al., 2012; Li Shen et al., 2010). However, several past studies were focused on identifying AD susceptible SNPs and genes that are associated with hippocampus and entorhinal cortex atrophy, but MRI GWASs involving other AD specific brain region including temporal, parietal and frontal regions are limited. In this study, GWAS with temporal lobe cortical thickness measures was performed and novel SNPs that are associated with temporal lobe atrophy in the Korean population were identified. In addition, this study analysed whether the temporal lobe atrophy associated SNPs and genes promote Aβ deposition and AD risk. This study also investigated the association of temporal lobe specific SNPs with cognitive decline.

## Ⅱ-2. METHODS

### Ⅱ-2-1. Study participants

The study participants included 2,693 Korean elderly (196 AD patients, 419 other dementias, 987 mild cognitive impairment and 1,091 cognitively normal older adults) selected from the Gwangju Alzheimer's & Related Dementias (GARD) Study in Korea, a database established by the National Research Center for Dementia (NRCD) at Chosun University in Gwangju, South Korea. According to NINCDS-

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ADRDA criteria, the clinical diagnosis was performed for probable AD (G. McKhann et al., 1984). The cognitively normal older adults had no sign of any neurological diseases or impairments on cognitive function or daily life activities. From the large cohort of NRCD database, a subset of samples (2,693 participants) with MR images were included in this study (Table 1). In addition, 296 subjects underwent β-amyloid PET imaging scans (72 amyloid positive and 224 amyloid negative) (Table 1). All participants or authorized guardians for cognitively impaired patients gave written informed consent that was approved by the institutional review board of Chosun University Hospital, South Korea.

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**Table 1.** Demographic characteristics of participants with brain structural and β-amyloid imaging data.

| Temporal lobe measures | Groups | N | Age (y, mean ± SD) | Female (n, %) | Years of education (y, mean ± SD) |
|---|---|---|---|---|---|
| Cortical thickness | Control | 1091 | 72.45 ± 5.25 | 673 (61.6%) | 9.57 ± 4.87 |
| | MCI | 987 | 72.89 ± 5.45 | 591 (59.9%) | 8.53 ± 5.05 |
| | Other dementias | 419 | 73.74 ± 5.58 | 265 (63.25%) | 8.84 ± 4.76 |
| | AD | 196 | 73.84 ± 5.95 | 109 (55.6%) | 8.05 ± 5.14 |
| β-amyloid | Positive | 72 | 73.44 ± 7.86 | 40 (55.56%) | 9.00 ± 4.58 |
| | Negative | 224 | 70.26 ± 8.0 | 142 (63.39%) | 9.85 ± 4.98 |

Abbreviations: N, number of samples; SD, standard deviation; y, years; MCI, mild cognitive impairment; AD, Alzheimer's disease.

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### Ⅱ-2-2. Cognitive assessment and clinical measures

All participants were assessed for the cognitive ability in multiple domain at the NRCD using Seoul Neuropsychological Screening Battery (SNSB). This battery measures five cognitive domains that include memory, language, attention, visuospatial and frontal/executive functions (Ahn et al., 2010). Tests in SNSB include Korean Mini-Mental State Exam (K-MMSE), Clinical Dementia Rating (CDR) Sum of boxes, Digit Span test backward, Digit Span test forward, Digit Span test Forward/Backward, Short form of Korean version of the Boston Naming Test (S-K-BNT), Praxis Ideomotor, Rey Complex Figure Test (RCFT) Copy score, Seoul Verbal Learning Test (SVLT) Immediate Recall, SVLT Delayed Recall, SVLT Recognition, RCFT Immediate Recall, Delayed Recall, Recognition, Contrasting Program, Go-No Go test, Controlled Oral Word Association Test (COWAT) Animal, COWAT Supermarket, COWAT Phenomic, StroopWordreading Time, StroopColorreading Time, Korean-Trail Making Test-Elderly's version A Time (K-TMT-E-A) and Korean-Trail Making Test-Elderly's version B Time (K-TMT-E-B).

### Ⅱ-2-3. SNP genotyping and imputation

Genomic DNA derived from peripheral blood leukocytes for each individuals were genotyped using an Affymetrix Genome-wide genotyping array (Affymetrix® Axiom KORV1.0). This Affymetrix chip was designed and optimized for Korean population by the Center for Genome Science, Korea National Institute of Health, South Korea (Jee, Lee, Jung, & Jee, 2016). Genotyping was further performed at DNALink, Seoul, South Korea. In the genotyping data, individuals with call-rate < 95%, gender inconsistencies and extremely high or low heterozygosity rate (±3 SD from the mean) were excluded. SNPs with call-rate < 95%, minor allele frequency (MAF) < 1% and Hardy-Weinberg equilibrium (HWE) test $p$-value < $10^{-5}$ were excluded using PLINK software (https://www.cog-genomics.org/plink2)

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version 1.09. In addition, After the quality control, the genotype data was imputed using pre-phased reference haplotypes of the East Asian population from the Haplotype Reference Consortium (HRC) panel version 1.1 (McCarthy et al., 2016). Moreover, the low quality imputed SNPs were removed with info score < 0.5. After the quality control, 6,706,236 SNPs were included in the dataset. Since this study included a pooled subset of samples from the NRCD database, no samples were lost during quality control.

### Ⅱ-2-4. Magnetic resonance (MR) imaging

MR brain imaging data were acquired for 2,693 individuals including 196 AD patients, 419 other dementias, 987 mild cognitive impairment and 1,091 cognitively normal older adults at the Chosun University Hospital (affiliated to NRCD). The whole brain contiguous 0.9 mm axial MPRAGE images were acquired using a 1.5 T MR scanner (Magnetom Avanto, Siemens) with the following protocol: TE = 3.43 ms; TR = 1800 ms; TI = 1100 ms; FoV = 224×224; 15 flip angle; number of slices = 176; matrix = 256×256. In addition, the whole brain contiguous 0.8 mm sagittal MPRAGE images were acquired using a 3 T MR scanner (Skyra, Siemens) with the following parameters: TE = 2.143 ms; TR = 2300 ms; TI = 900 ms; FoV = 256×256; 9 flip angle; number of slices = 178; matrix = 320×320.

### Ⅱ-2-5. Positron emission tomography (PET) imaging

A total of 352 participants from NRCD was subjected to PET scanning session at the Chosun University Hospital. The PET images were acquired 90 minutes after intravenous administration of approximately 300 MBq $^{18}$F-Florbetaben ($^{18}$F-FBB) using a Discovery ST PET-CT scanner (General Electric Medical Systems, Milwaukee, WI, USA). Non-contrast-enhanced CT scan were included for attenuation correction with following parameters: 120 Kvp, 8 slice, 10-130 mAs,

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helical and 3.79 mm slice thickness. After attenuation correction involving 2 iterations and 21 subsets, the reconstruction of PET and CT scan data were performed with ordered subset expectation maximization.

Another cohort of independent PET samples were enrolled at the Inha University Hospital. 68 participants subjected to three-dimensional $^{11}$C-Pittsburgh Compound B (PiB) PET scanning session. A 3.0 T Biograph mMR (PET-MR) scanner (Siemens, Washington, DC, USA) was used to acquire $^{11}$C-PiB PET images. 40 minutes after intravenous administration of 555 MBq $^{11}$C-PiB (450-610 MBq), the scanning session was performed. The images were corrected for ultrashort echo time-based attenuation, decay reduction, and uniformity. Then, the images were reconstructed with 256 × 256 image matrix using 6 iterations and 21 subsets. Additionally, 121 participants enrolled at the Inha University hospital undertook $^{18}$F-Flutemetamol ($^{18}$F-FMM) PET scanning session. All the participants were scanned 90 minutes after intravenous administration of 185 MBq $^{18}$F-Flutemetamol ($^{18}$F-FMM), followed by 10 mL saline flush. Images were acquired using Biography MCT PET/CT scanner (Siemens), Discovery 600 PET/CT scanner (GE), Discovery 690 PET/CT scanner (GE), Discovery STE PET/CT scanner or Gemini TF PET/CT scanner (Philips). Low-dose CT scan was used for attenuation correction before scans. Images were reconstructed with OSEM algorithm by involving 4 iterations and 16 subsets.

### Ⅱ-2-6. MRI data processing

MR T1-weighted images from the subjects were processed with FreeSurfer software version 5.3.0 (https://surfer.nmr.mgh.harvard.edu/fswiki) using automated processing stream to reconstruct 3-dimensional cortical surface model (Dale, Fischl, & Sereno, 1999; B. Fischl & Dale, 2000; Bruce Fischl, Sereno, & Dale, 1999). In detail, the FreeSurfer automated reconstruction protocol includes Talairach

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transformation, intensity normalization, non-brain tissue removal, separation of hemispheres, exclusion of cerebellum and brain stem, tessellation of the gray-matter (GM) and white-matter (WM) boundaries, topology correction, GM and WM subcortical segmentation, atlas registration and surface deformation for the optimization of GM/WM and gray/cerebrospinal fluid boundaries (Gorbach et al., 2017).

### Ⅱ-2-7. PET image data processing

The PET images were processed with SPM12 toolbox implemented in MATLAB (R2018a, Mathworks, Natick, MA, USA). Initially, F-18 PET images were co-registered to the respective T1-weighted MR images of the same subject, which are taken at the same time or within 6 months of interval time. Then, the transformation parameters were estimated for the spatial normalization of the respective T1-weighted images to the standard MNI template. Furthermore, these parameters were used to spatially normalize the PET images to the standard MNI template. After that, the spatially normalized PET images were smoothened with an 8-mm full-width at half maximum Gaussian kernel (Park, Han, Mook-Jung, et al., 2019). The segmentations from respective structural MR images with FreeSurfer software version 6.0 were applied to extract standard value uptake ratios from 68 predefined region of interests (ROIs) (Palmqvist et al., 2017). SUVRs from frontal, lateral parietal, anterior and posterior cingulate and lateral temporal regions were used to calculate global cortical retention mean SUVRs with reference to whole cerebellum (Maass et al., 2017). $^{18}$F-FMM and $^{11}$C-PiB PET images were co-registered with the respective T1-weighted MR images and the detailed pre-processing procedures are described elsewhere (Hwang et al., 2019).

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### Ⅱ-2-8. Statistical analyses

#### Ⅱ-2-8-1. Association analyses with temporal lobe cortical thickness

The linear regression on brain temporal lobe cortical thickness involving 6,706,236 SNPs and 2,693 samples was performed using PLINK. Moreover, the logistic regression involving 6,706,236 SNPs and clinically diagnosed 544 samples (196 AD cases and 348 cognitively normal controls) was also performed using PLINK. The principle component (PC) analysis for the cohort that accounts for the population substructure was calculated using smartpca program with EIGENSOFT (Patterson, Price, & Reich, 2006). Tracy-Widom statistics identified that 3 PCs were significantly contributing to the genetic variations in the cohort. The linear regression was performed with age, sex, 3 PCs and MRI field strength as covariates, while the logistic regression was performed with age, sex and 3 PCs as covariates. Then, the summary statistics from the linear regression and logistic regression were used in the gene-based association analysis performed using the GATES (Gene-based Association Test using Extended Simes procedure) method implemented in the KGG software version 4.0. In this extended Simes procedure, the model integrates functional information and the association evidence to combine *p*-value of each SNPs within a gene to get an overall association *p*-value for that entire gene (Mo et al., 2015).

#### Ⅱ-2-8-2 Association analyses on the genetic locus of interest

To evaluate the association of the genetic locus of interest with AD risk, a logistic regression was performed with 1,091 cognitively normal controls and 196 AD cases from the temporal lobe cortical thickness cohort of 2,693 subjects (Table 1). Additionally, Aβ risk was evaluated with logistic regression involving cohort of 224 Aβ negative and 72 Aβ positive subjects using PLINK software (Table 1). Subjects from this 296 Aβ cohort are independent of 2,693 MR imaging cohort. PCs were

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estimated using smartpca program with EIGENSOFT and the Tracy-Widom statistics identified 3 PCs were significantly contributing to the genetic variations in both Aβ and AD case-control cohort. The logistic regressions were performed involving Aβ status and AD case-control groups with age, sex and 3 PCs as covariates. The summary statistics from logistic regressions were used in the gene-based association analysis using the GATES method implemented in the KGG software.

**Ⅱ-2-8-3. Regional SNP based association analysis**

The regional association analysis on temporal cortical atrophy was performed with SNPs in the defined region from 2 kb upstream to 2 kb downstream of interested gene were included. The regional analysis was performed using linear regression involving 2,693 samples adjusted with age, sex, 3 PCs and field strength as covariates. Regional association analysis were plotted with LocusZoom (Willer, Abecasis, et al., 2010) software. After that, a subset of 244 subjects (Table 2) in the 2,693 MR imaging cohort, who underwent $^{18}$F-FBB β-amyloid PET scanning were assessed for the association of SNPs in the interested gene region with amyloid deposition in temporal lobe. In the regional analysis of $^{18}$F-FBB β-amyloid PET SUVR, the linear regression was performed including 244 individuals with age, sex and 3 PCs as covariates. The SNP-based regional analysis from both cortical thickness and β-amyloid SUVR were compared with the regional analysis involving clinically diagnosed 196 AD cases and 1,091 cognitively normal controls. The logistic regression was performed with the clinically diagnosed groups adjusting for age, sex and 3 PCs as covariates.

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**Table 2.** Demographic characteristics of participants with temporal lobe Aβ deposition.

| Demography | Temporal Aβ SUVR |
|---|---|
| Number of subjects | 244 |
| Age (y, mean ± SD) | 72.57 ± 5.57 |
| Female (n, %) | 131 (53.69%) |
| Years of education (y, mean ± SD) | 9.94 ± 4.88 |
| MMSE (mean ± SD) | 25.87 ± 3.97 |

Abbreviations: SD, standard deviation; y, years; MMSE, Mini-Mental State Examination.

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### Ⅱ-2-8-4. Neuroimaging analysis

SNPs that were most associated with temporal lobe atrophy in the interested gene region were analysed for the influence on cortical thickness measures. In this analysis, 1,287 samples (196 AD cases and 1,091 cognitively normal controls) were included to assess whether the atrophy in temporal lobe are evidential in AD patients. This study analysed the genotype effect under dominant model. The effects of brain atrophy associated SNP genotypes were assessed using general linear model (GLM) with the SurfStat toolbox (http://www.math.mcgill.ca/keith/surfstat/) in MATLAB (R2012a, The Mathworks, Natick, MA, USA) adjusting for age, sex, education and field strength (Fan et al., 2010). A random field theory (RFT)-based correction was applied for the multiple point-wise cortical thickness comparisons. After corrections at the peak level, $p = 0.05$ was considered as the significant threshold (Taylor & Adler, 2003). Furthermore, the cortical thickness difference in temporal lobe among the subgroups of most associated SNP genotypes were evaluated using analysis of covariance (ANCOVA) in R (version 3.3.1) program. The ANCOVA was performed with SNP genotypes as a fixed factor while age, sex and field strength as covariates. In addition, the differences in β-amyloid burden among the individuals with most associated SNP genotypes were assessed by ANCOVA, while adjusted for age and sex as covariates.

### Ⅱ-2-8-5 Haplotype based association analysis

The pairwise linkage disequilibrium $D'$ and $r^2$ values were calculated for the SNPs of interest in the gene locus using Haploview software version 4.2 (http://www.broad.mit.edu/mpg/haploview/) (Z. Wu et al., 2017). The haplotypes and its frequencies were estimated using “haplo.stats” package implemented in R program. Haplotypes were constructed from the alleles of interested SNPs. Then, the haplotype association analysis was performed using logistic regression with age, sex

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and 3 PCs as covariates. The odds ratios for AD risk from the haplotype analysis were calculated using “haplo.gm” function available in the “haplo.stats” package (Lescai et al., 2011). Moreover, the survival analysis was performed with haplotypes using cox-proportional regression model and hazard ratios were calculated with AD as event, age as time and adjusted for sex and 3PCs as covariates. This cox proportional regression model was performed using “survival” package available in R program (Tregouet & Tiret, 2004).

#### Ⅱ-2-8-6 Association analysis on cognitive measures

Furthermore, partial correlations of cortical thickness with cognitive measures were analysed. Temporal lobe cortical thickness were assessed for the partial correlations with 5 cognitive domains involving 23 neuropsychological tests. The partial correlations were performed with the “ppcor” package using Pearson’s method in R program while including age, sex, field strength and phenotypes as covariates (Jiang et al., 2016; Ottino-González et al., 2019). Participants with missing scores for any neuropsychological tests were excluded for that analysis. Partial correlation plots were plotted with “ggplot” package in R program. All the SNPs included in the haplotype analysis were assessed for the association with cognitive domains. This association analysis with neuropsychological tests were performed using linear regression in PLINK program adjusted for age, sex and field strength as covariates.

## Ⅱ-3. RESULTS

### Ⅱ-3-1. Identification of novel gene associated with brain cortical atrophy in temporal lobe and AD risk

The linear regression on brain temporal lobe cortical thickness with SNP based GWAS showed rs145565989 ($p$=2.25 × $10^{-15}$) and gene based GWAS showed

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*TXNRD1* ($p$=1.28×10$^{-13}$) gene were most associated with temporal lobe atrophy (Figure 1A, 1B, 2A, 2B, Table 3 and 4). Most associated locus with temporal lobe was further selected from the defined region starting from 5 Mb upstream to 5 Mb downstream of the most associated *TXNRD1* gene obtained from the gene-based association study. From this locus, 18 protein coding genes including *TXNRD1* gene were found passing threshold ($p$<0.05) (Table 5 and Figure 3A). This study also hypothesized that the locus, which is associated with the temporal lobe atrophy, may also be associated with the AD risk and Aβ deposition. To evaluate this hypothesis, this study performed SNP based GWAS and gene-based association analysis with logistic regression involving cognitively normal and AD case groups (Figure 1C and D). Subsequently, a logistic regression was performed in the region of 5Mb upstream and 5Mb downstream to *TXNRD1* locus involving Aβ positive and negative groups in the same locus. Then, this study compared 18 protein coding genes from the *TXNRD1* locus for AD and Aβ risk. Then, the overlapping genes were selected in the *TXNRD1* locus, which resulted in *PRDM4* gene (Figure 3B).

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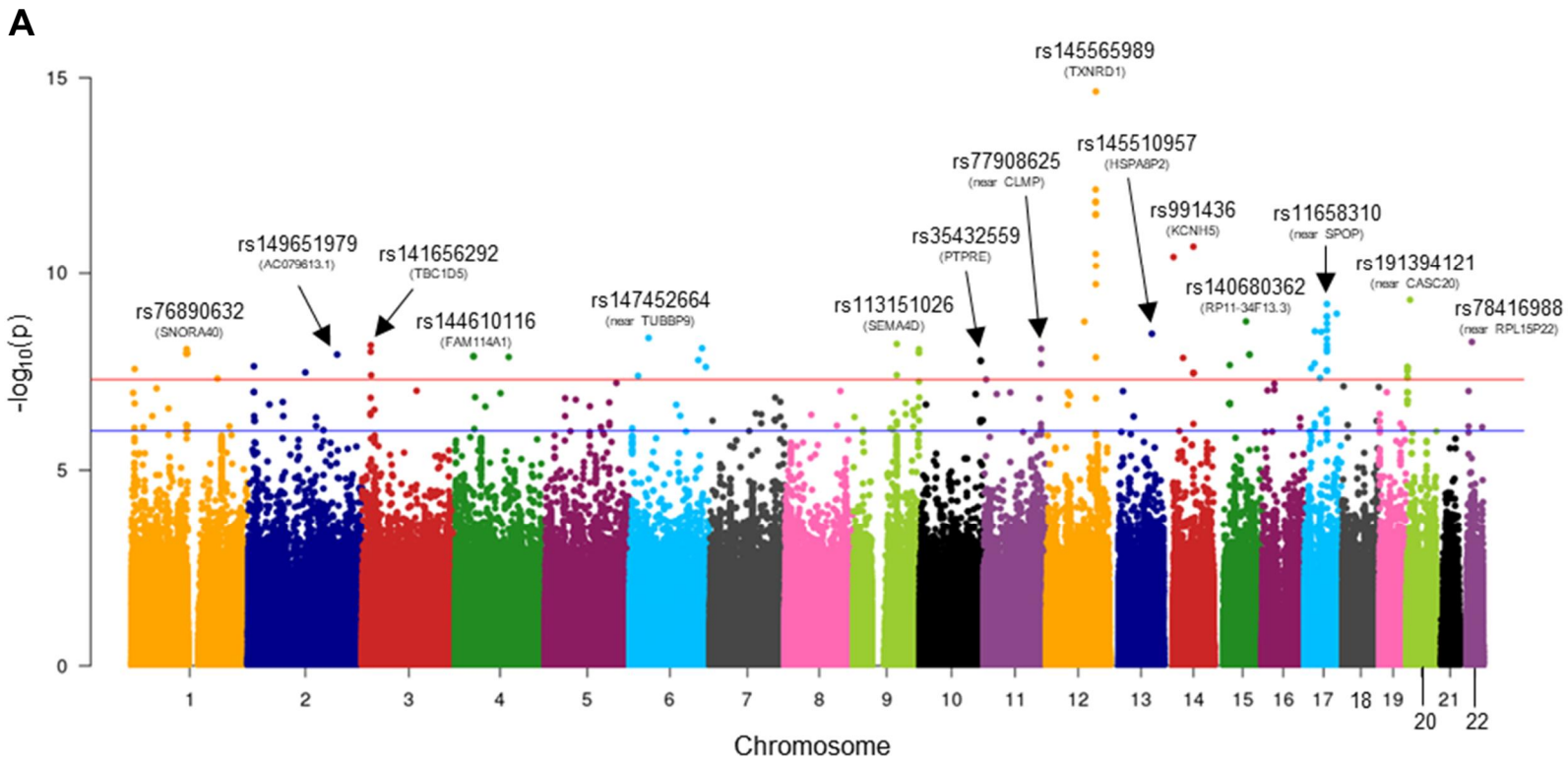


**Figure 1.** Continued

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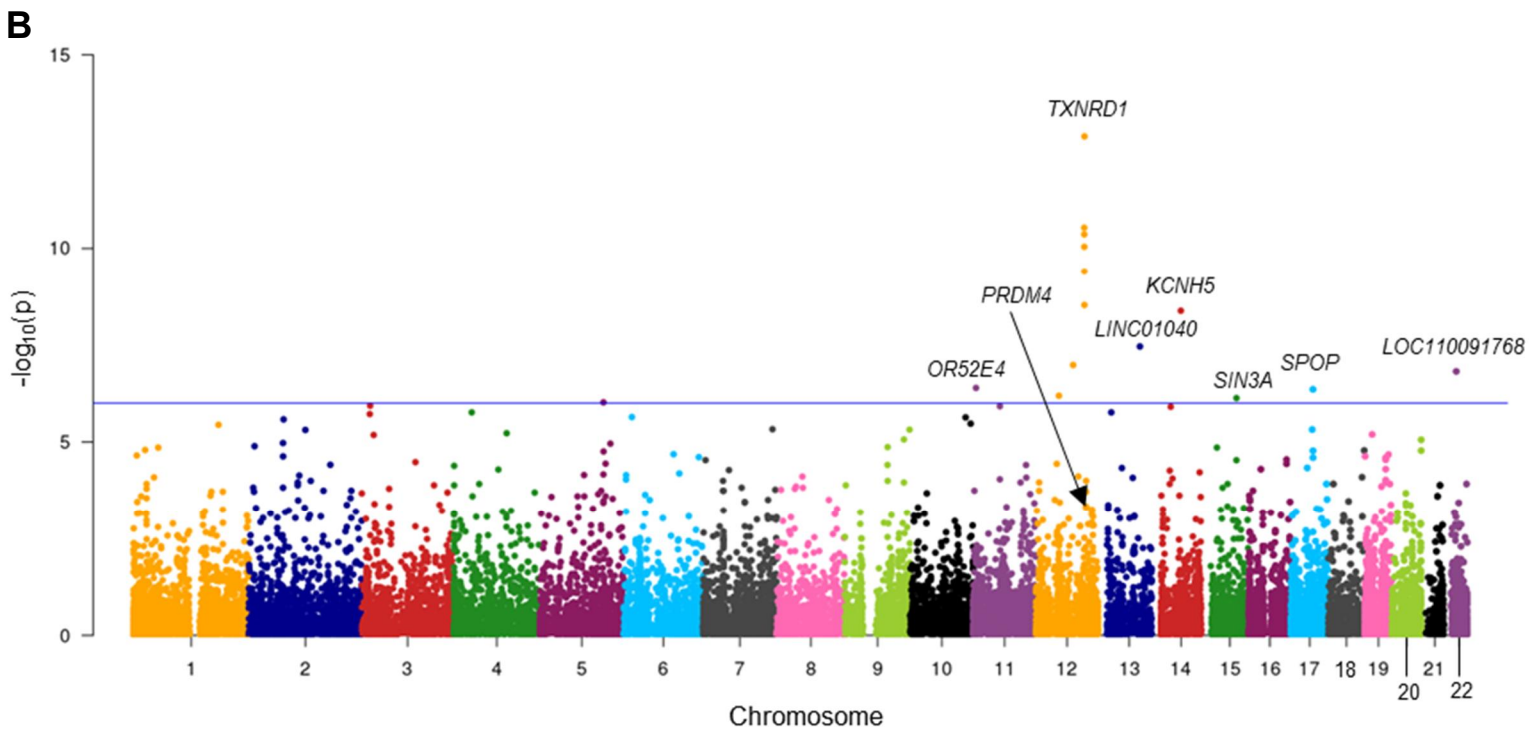


**Figure 1. Genetic variants and genes associated with temporal lobe atrophy and AD risk.** Manhattan plot shows the linear regression *p*-value for each genetic variant (A) and gene (B) in the genome (*p*-value is displayed as $-\log_{10}$) with brain cortical thickness of temporal lobe adjusted for 3 PCs, age, sex and field strength.

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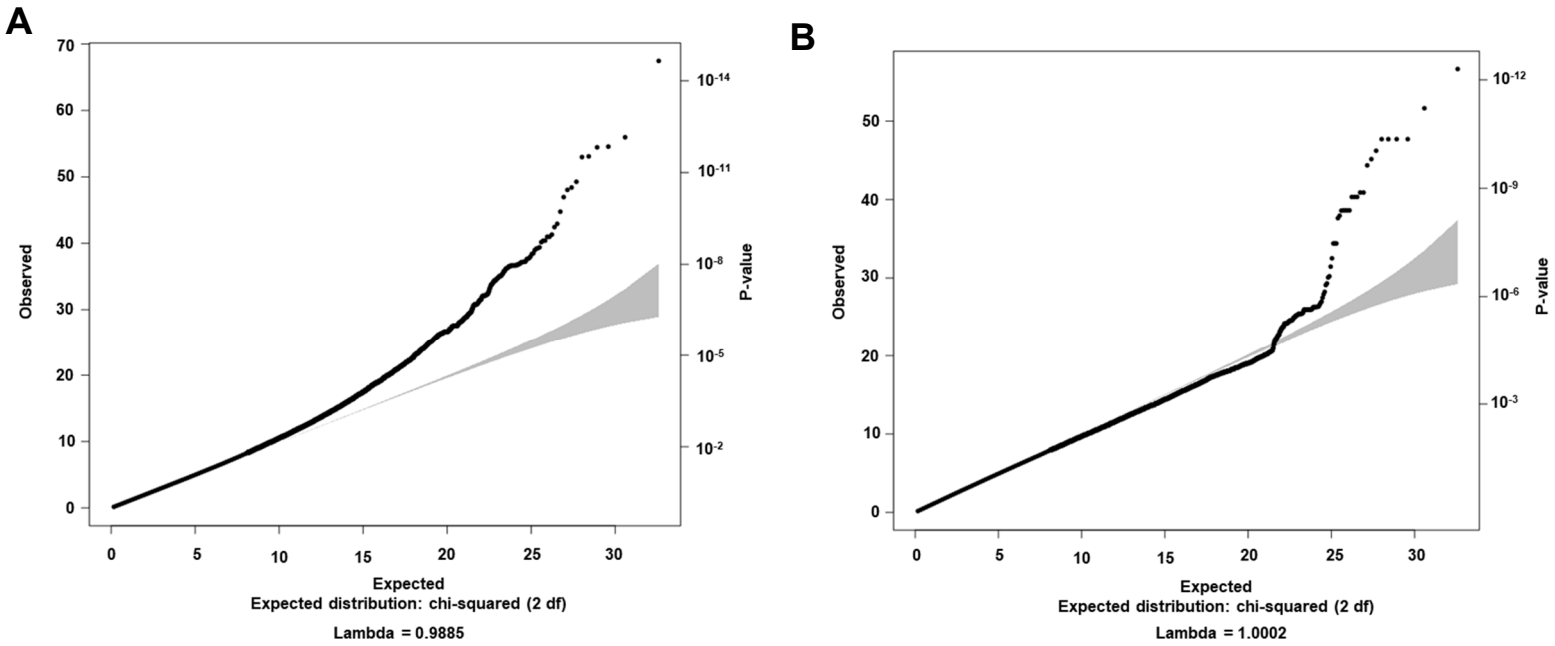


**Figure 2. SNP and gene based GWAS of temporal lobe atrophy and AD risk.** (A) Quantile-Quantile (QQ) plots of the SNP GWAS of brain cortical thickness of temporal lobe adjusted for 3 PCs, age, sex and field strength. (B) QQ plots of the SNP based GWAS from logistic regression with 1,091 controls and 196 cases adjusted for 3 PCs, age and sex.

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**Table 3.** SNPs associated with brain temporal lobe atrophy.

| Chromosome # | SNP | Gene | *p*-value[a] | BP | SNP type | m/M | MAF |
|---|---|---|---|---|---|---|---|
| 12 | rs145565989 | *TXNRD1* | **2.25E-15** | 104633324 | Intron variant | G/A | 0.01671 |
| 12 | rs113876254 | *RP11-341G23.4* | **7.03E-13** | 104162729 | Upstream gene variant | T/C | 0.02135 |
| 12 | rs149213014 | *RP11-642P15.1* | **1.42E-12** | 104249410 | Intron variant | T/C | 0.02135 |
| 12 | rs139674131 | *RP11-642P15.1* | **1.50E-12** | 104246771 | Intron variant | T/C | 0.02172 |
| 12 | rs182011489 | *RPL18AP3* | **2.97E-12** | 104654106 | Upstream gene variant | T/A | 0.01931 |
| 12 | rs75992048 | *STAB2* | **3.13E-12** | 104156403 | Sequence feature | G/A | 0.02172 |
| 14 | rs991436 | *KCNH5* | **2.01E-11** | 63425070 | Sequence feature | A/C | 0.01058 |
| 12 | rs2293622 | *HSP90B1* | **3.10E-11** | 104347442 | Downstream gene variant | T/C | 0.01690 |
| 14 | rs137889652 | *OR6S1-Y_RNA* | **3.69E-11** | 21135052 | Intergenic region | T/C | 0.01708 |
| 12 | rs188354221 | *TXNRD1-CHST11* | **6.37E-11** | 104773107 | Intergenic region | G/C | 0.02562 |
| 12 | rs201386542 | *NFYB* | **1.90E-10** | 104513982 | Sequence feature | A/G | 0.03119 |
| 20 | rs191394121 | *CASC20-RP5-859D4.3* | **4.76E-10** | 6564056 | Intergenic region | C/T | 0.01522 |
| 17 | rs11658310 | *SPOP-SLC35B1* | **6.08E-10** | 47764402 | Intergenic region | G/A | 0.01003 |
| 17 | rs149819935 | *RP11-1058G23.1-RP11-238F2.1* | **1.07E-09** | 68653917 | Intergenic region | G/A | 0.01355 |
| 17 | rs12450870 | *SPOP* | **1.26E-09** | 47754199 | Intron variant | C/T | 0.01133 |
| 17 | rs8067844 | *SPOP* | **1.26E-09** | 47755741 | Upstream gene variant | T/G | 0.01133 |

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**Table 3.** Continued.

| Chromosome # | SNP | Gene | *p*-value[a] | BP | SNP type | m/M | MAF |
|---|---|---|---|---|---|---|---|
| 15 | rs140680362 | *RP11-34F13.3-RP11-282M16.1* | **1.68E-09** | 68210751 | Intergenic region | A/G | 0.01652 |
| 12 | rs146037207 | *PPP1R12A* | **1.70E-09** | 80232214 | Sequence feature | A/G | 0.01467 |
| 17 | rs35388001 | *FAM117A* | **1.89E-09** | 47857324 | Intron variant | T/C | 0.01318 |
| 17 | rs17712721 | *SPOP* | **2.79E-09** | 47742277 | Intron variant | C/T | 0.01170 |
| 17 | rs192376622 | *RP11-846F4.10-RP11-285M22.3* | **2.97E-09** | 22145170 | Intergenic region | C/T | 0.01207 |
| 17 | rs2049037 | *ACACA* | **3.09E-09** | 35682921 | Downstream gene variant | G/C | 0.01374 |
| 13 | rs145510957 | *LINC01040* | **3.45E-09** | 90136219 | Intron variant | T/C | 0.01300 |
| 6 | rs147452664 | *TUBBP9-RP11-552E20.1* | **4.40E-09** | 39999794 | Intergenic region | T/C | 0.02228 |
| 17 | rs10514970 | *SPOP* | **4.60E-09** | 47688526 | Sequence feature | T/C | 0.01188 |
| 22 | rs78416988 | *RP1-40G4P.1* | **5.57E-09** | 27280468 | Downstream gene variant | G/C | 0.01634 |
| 9 | rs113151026 | *SEMA4D-GADD45G* | **6.27E-09** | 92132416 | Intergenic region | G/T | 0.02191 |
| 17 | rs146438004 | *SPOP* | **6.57E-09** | 47733393 | Intron variant | T/C | 0.01188 |
| 3 | rs141656292 | *TBC1D5* | **6.75E-09** | 18104238 | Intron variant | A/G | 0.03862 |
| 6 | rs12210741 | *RNA5SP225-RP11-331O9.1* | **8.04E-09** | 153884270 | Intergenic region | T/G | 0.01225 |

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**Table 3.** Continued.

| Chromosome # | SNP | Gene | *p*-value[a] | BP | SNP type | m/M | MAF |
|---|---|---|---|---|---|---|---|
| 11 | rs77908625 | *CTD-2216M2.1-U8* | **8.31E-09** | 123121736 | Intergenic region | A/G | 0.01504 |
| 17 | rs3815473 | *SPOP* | **8.33E-09** | 47700311 | Upstream gene variant | G/T | 0.01170 |
| 1 | rs76890632 | *AL365331.2-SNORA40* | **8.48E-09** | 118201142 | Intergenic region | A/G | 0.03899 |
| 9 | rs35116034 | *NOTCH1* | **8.54E-09** | 139421837 | Sequence feature | T/C | 0.01522 |
| 1 | rs17037506 | *AL365331.2-SNORA40* | **9.23E-09** | 118191177 | Intergenic region | T/C | 0.03899 |
| 17 | rs4794060 | *SPOP-SLC35B1* | **9.78E-09** | 47767025 | Intergenic region | A/G | 0.01411 |
| 3 | rs148500897 | *AC104451.2* | **9.88E-09** | 17752334 | Upstream gene variant | T/C | 0.01968 |
| 9 | rs11574863 | *NOTCH1* | **1.05E-08** | 139421548 | Sequence feature | A/G | 0.01522 |
| 9 | rs35863377 | *NOTCH1* | **1.05E-08** | 139421684 | Sequence feature | T/C | 0.01522 |
| 1 | rs12071172 | *AL365331.2-SNORA40* | **1.09E-08** | 118192073 | Intergenic region | A/G | 0.03918 |
| 1 | rs55718481 | *AL365331.2-SNORA40* | **1.09E-08** | 118192342 | Intergenic region | T/G | 0.03918 |
| 1 | rs74114358 | *AL365331.2-SNORA40* | **1.09E-08** | 118191927 | Intergenic region | T/C | 0.03918 |
| 1 | rs74630332 | *AL365331.2-SNORA40* | **1.09E-08** | 118192845 | Intergenic region | A/G | 0.03918 |
| 1 | rs75335364 | *AL365331.2-SNORA40* | **1.09E-08** | 118191893 | Intergenic region | T/C | 0.03918 |
| 1 | rs75679456 | *AL365331.2-SNORA40* | **1.09E-08** | 118192160 | Intergenic region | A/G | 0.03918 |
| 1 | rs75984342 | *AL365331.2-SNORA40* | **1.09E-08** | 118191814 | Intergenic region | T/A | 0.03918 |
| 2 | rs149651979 | *AC079613.1* | **1.16E-08** | 189495399 | Intron variant | A/C | 0.01114 |

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**Table 3.** Continued.

| Chromosome # | SNP | Gene | *p*-value[a] | BP | SNP type | m/M | MAF |
|---|---|---|---|---|---|---|---|
| 15 | rs189349408 | *SIN3A* | **1.16E-08** | 75696140 | Sequence feature | A/G | 0.01374 |
| 4 | rs144610116 | *FAM114A1* | **1.27E-08** | 38942848 | Downstream gene variant | C/T | 0.01207 |
| 4 | rs2306923 | *FAM114A1* | **1.31E-08** | 38930893 | Missense variant | A/G | 0.01263 |
| 4 | rs117188623 | *RP11-650J17.1* | **1.34E-08** | 113915959 | Intron variant | C/T | 0.01987 |
| 12 | rs139395315 | *RP11-818F20.4-TXNRD1* | **1.37E-08** | 104603836 | Intergenic region | A/G | 0.03323 |
| 14 | rs143179167 | *RP11-129M6.1-RP11-629F19.1* | **1.41E-08** | 41497372 | Intergenic region | G/A | 0.01133 |
| 6 | rs141636652 | *EPM2A* | **1.61E-08** | 145926399 | Intron variant | C/T | 0.01021 |
| 10 | rs35432559 | *PTPRE* | **1.67E-08** | 129838748 | Intron variant | G/C | 0.01485 |
| 10 | rs6482651 | *PTPRE* | **1.67E-08** | 129838742 | Intron variant | G/A | 0.01485 |
| 17 | rs79985182 | *AC144838.2* | **1.95E-08** | 21545562 | Upstream gene variant | A/C | 0.01188 |
| 11 | rs74749324 | *RP11-335F8.2-RPL34P23* | **2.00E-08** | 122897223 | Intergenic region | A/T | 0.01764 |
| 15 | rs4780111 | *RYR3* | **2.15E-08** | 33654013 | Sequence feature | T/C | 0.01578 |
| 2 | rs75063391 | *AC096559.1* | **2.30E-08** | 12183600 | Intron variant | G/A | 0.01578 |
| 6 | rs149172185 | *PARK2* | **2.40E-08** | 161995431 | Sequence feature | C/A | 0.03361 |
| 20 | 20:1000072 | *RSPO4-PSMF1* | **2.43E-08** | 1000072 | Intergenic region | A/G | 0.01170 |
| 20 | 20:1000152 | *RSPO4-PSMF1* | **2.43E-08** | 1000152 | Intergenic region | C/T | 0.01170 |

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**Table 3.** Continued.

| Chromosome # | SNP | Gene | *p*-value[a] | BP | SNP type | m/M | MAF |
|---|---|---|---|---|---|---|---|
| 20 | 20:1000179 | *RSPO4-PSMF1* | **2.43E-08** | 1000179 | Intergenic region | A/C | 0.01170 |
| 17 | 17:14281791 | *AC022816.2* | **2.59E-08** | 14281791 | Intron variant | C/T | 0.05681 |
| 1 | rs146646674 | *CAMTA1* | **2.70E-08** | 7308145 | Sequence feature | T/C | 0.01207 |
| 20 | 20:999784 | *RSPO4-PSMF1* | **2.85E-08** | 999784 | Intergenic region | C/T | 0.01207 |
| 17 | rs11079876 | *FAM117A* | **2.95E-08** | 47855616 | Intron variant | A/C | 0.01652 |
| 17 | rs4793620 | *FAM117A* | **2.95E-08** | 47858389 | Intron variant | T/C | 0.01652 |
| 20 | 20:1000303 | *RSPO4-PSMF1* | **3.06E-08** | 1000303 | Intergenic region | T/C | 0.01188 |
| 2 | rs3768693 | *GLI2* | **3.30E-08** | 121744716 | Downstream gene variant | A/G | 0.01615 |
| 14 | rs60749058 | *KCNH5* | **3.42E-08** | 63459469 | Sequence feature | A/T | 0.01021 |
| 14 | rs74058739 | *KCNH5* | **3.42E-08** | 63454264 | Sequence feature | T/C | 0.01021 |
| 14 | rs74058741 | *KCNH5* | **3.42E-08** | 63455627 | Sequence feature | T/C | 0.01021 |
| 14 | rs74058747 | *KCNH5* | **3.42E-08** | 63460464 | Sequence feature | T/C | 0.01021 |
| 9 | rs35340801 | *SEMA4D-GADD45G* | **3.85E-08** | 92131699 | Intergenic region | A/T | 0.02674 |
| 3 | rs145481419 | *AC144521.1* | **3.89E-08** | 18802458 | Intron variant | G/A | 0.03045 |
| 6 | rs117088663 | *NUP153* | **4.04E-08** | 17659759 | Intron variant | G/A | 0.01374 |
| 20 | 20:999572 | *RSPO4-PSMF1* | **4.34E-08** | 999572 | Intergenic region | G/A | 0.01244 |
| 20 | 20:1000386 | *RSPO4-PSMF1* | **4.52E-08** | 1000386 | Intergenic region | T/C | 0.01151 |

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**Table 3.** Continued.

| Chromosome # | SNP | Gene | *p*-value[a] | BP | SNP type | m/M | MAF |
|---|---|---|---|---|---|---|---|
| 17 | rs191020402 | *RP11-642M2.1-CCT6B* | **4.58E-08** | 33196632 | Intergenic region | G/C | 0.01133 |
| 1 | rs76101694 | *RGL1* | **4.71E-08** | 183878209 | Intron variant | C/T | 0.03156 |
| 11 | rs3816467 | *OR52E4* | **4.98E-08** | 5902276 | Upstream gene variant | G/A | 0.01021 |

Abbreviations: SNP, single nucleotide polymorphism; BP, base pair position; MAF, minor allele frequency.

SNPs with genome-wide significant association to cerebral β-amyloid deposition were shown.

[a]Adjusted for age, sex, field strength, diagnosis, PC1, PC2 and PC3.

Significant *p*-values are indicated in bold letters.

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**Table 4.** Top 20 most associated genes with brain temporal lobe atrophy.

| Gene | Chromosome # | Start position | Temporal thickness[a] *p*-value[b] | No. of SNPs |
|---|---|---|---|---|
| *TXNRD1* | 12 | 104680459 | **1.28E-13** | 401 |
| *STAB2* | 12 | 103981068 | **2.92E-11** | 522 |
| *NT5DC3* | 12 | 104166080 | **4.32E-11** | 340 |
| *TTC41P* | 12 | 104237526 | **9.07E-11** | 393 |
| *C12orf73* | 12 | 104343980 | **4.00E-10** | 66 |
| *NFYB* | 12 | 104510857 | **3.03E-09** | 55 |
| *KCNH5* | 14 | 63173290 | **4.22E-09** | 940 |
| *LINC01040* | 13 | 90129860 | **3.50E-08** | 84 |
| *PPP1R12A* | 12 | 80167342 | **1.05E-07** | 243 |
| *LOC110091768* | 22 | 27256173 | **1.53E-07** | 90 |
| *OR52E4* | 11 | 5905522 | **4.07E-07** | 50 |
| *SPOP* | 17 | 47676245 | **4.43E-07** | 95 |
| *TUBA1A* | 12 | 49578577 | **6.46E-07** | 17 |
| *SIN3A* | 15 | 75661719 | **7.44E-07** | 132 |
| *HNRNPA0* | 5 | 137087072 | **9.55E-07** | 6 |
| *LOC339862* | 3 | 18004063 | **1.17E-06** | 551 |
| *SERPING1* | 11 | 57365704 | **1.20E-06** | 52 |
| *LINC02315* | 14 | 41423915 | **1.24E-06** | 897 |
| *FAM114A1* | 4 | 38869353 | **1.73E-06** | 331 |
| *URAD* | 13 | 28552242 | **1.73E-06** | 55 |

[a]Temporal lobe includes inferior temporal, middle temporal and superior temporal cortex.

[b]Adjusted for age, sex, field strength, diagnosis, PC1, PC2 and PC3.

Significant *p*-values are indicated in bold letters.

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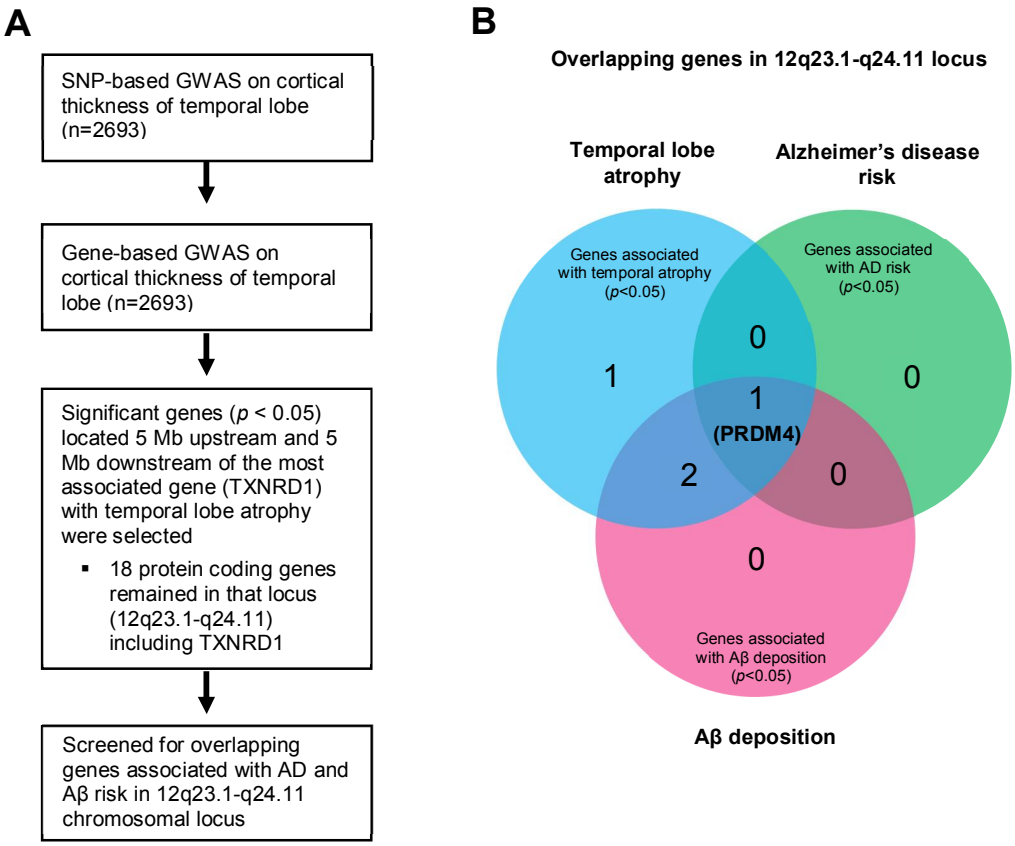


**Figure 3. *PRDM4* gene associated with temporal lobe atrophy, AD risk and Aβ deposition.** (A) Flow chart showing the screening steps to identify a most associated genetic loci with brain temporal lobe atrophy. (B) Venn diagram explains identification of candidate gene associated with brain temporal lobe atrophy, AD risk and Aβ deposition.

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**Table 5.** *PRDM4* gene associated with brain temporal lobe atrophy, AD risk and β-amyloid deposition.

| Gene | Chromosome # | Start position | *p*-value[b] (Temporal lobe[a]) | *p*-value[c] (Control vs. AD case) | *p*-value[c] (Aβ Positive vs. Negative) | No. of SNPs |
|---|---|---|---|---|---|---|
| *MYBPC1* | 12 | 101988708 | **0.05** | 0.45 | 0.19 | 334 |
| *GNPTAB* | 12 | 102139274 | **0.02** | 0.90 | 0.65 | 207 |
| *DRAM1* | 12 | 102271104 | **0.02** | 0.99 | 0.33 | 258 |
| *PARPBP* | 12 | 102513948 | **0.05** | 0.38 | 0.69 | 129 |
| *ASCL1* | 12 | 103351451 | **0.01** | 0.46 | 0.16 | 14 |
| *C12orf42* | 12 | 103695718 | **0.006** | 0.97 | 0.74 | 623 |
| *STAB2* | 12 | 103981068 | **2.92E-11** | 0.38 | 0.72 | 522 |
| *NT5DC3* | 12 | 104166080 | **4.32E-11** | 0.50 | 0.51 | 340 |
| *TTC41P* | 12 | 104237526 | **9.07E-11** | 0.62 | 0.98 | 393 |
| *C12orf73* | 12 | 104343980 | **4E-10** | 0.29 | 0.25 | 66 |
| *GLT8D2* | 12 | 104382760 | **0.003** | 0.43 | 0.07 | 165 |
| *NFYB* | 12 | 104510857 | **3.03E-09** | 0.62 | 0.99 | 55 |
| *TXNRD1* | 12 | 104680459 | **1.28E-13** | 0.40 | 0.99 | 401 |
| *POLR3B* | 12 | 106751859 | **0.02** | 0.40 | 0.26 | 270 |
| *BTBD11* | 12 | 107974633 | **0.005** | 0.69 | 0.43 | 877 |
| *PWP1* | 12 | 108079818 | **1.90E-04** | 0.08 | **0.001** | 84 |
| *PRDM4* | 12 | 108126642 | **4.90E-04** | **0.02** | **0.001** | 101 |

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**Table 5.** Continued.

| Gene | Chromosome # | Start position | *p*-value[b] (Temporal lobe[a]) | *p*-value[c] (Control vs. AD case) | *p*-value[c] (Aβ Positive vs. Negative) | No. of SNPs |
|---|---|---|---|---|---|---|
| *ASCL4* | 12 | 108168161 | **9.96E-05** | 0.50 | **0.02** | 36 |

[a]Temporal lobe includes inferior temporal, middle temporal and superior temporal cortex.

[b]Adjusted for age, sex, field strength, diagnosis, PC1, PC2 and PC3.

[c]Adjusted for age, sex, PC1, PC2 and PC3.

Significant *p*-values are indicated in bold letters.

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**Table 6.** SNPs in *PRDM4* region associated with cortical atrophy and Aβ deposition in temporal lobe.

| Chromosome # | BP | SNP | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[b] | OR | *p*-value[c] |
| 12 | 108122424 | rs10861772 | 0.44 | -0.001 | 0.98 | 0.002 | 0.85 | 0.88 | 0.23 |
| 12 | 108123866 | rs1558824 | 0.30 | 0.012 | **0.03** | -0.001 | 0.94 | 0.88 | 0.31 |
| 12 | 108123969 | rs67647504 | 0.10 | -0.032 | **1.93E-04** | -0.024 | 0.36 | 1.03 | 0.88 |
| 12 | 108124013 | rs17234071 | 0.10 | -0.032 | **1.93E-04** | -0.024 | 0.36 | 1.03 | 0.88 |
| 12 | 108124178 | rs1558823 | 0.44 | -0.001 | 0.99 | 0.002 | 0.85 | 0.89 | 0.30 |
| 12 | 108124384 | rs10778572 | 0.29 | 0.012 | **0.03** | -0.001 | 0.94 | 0.87 | 0.24 |
| 12 | 108124749 | rs113073640 | 0.04 | 0.021 | 0.12 | -0.049 | 0.10 | 0.63 | 0.18 |
| 12 | 108125731 | rs35756650 | 0.11 | -0.034 | **2.79E-05** | -0.027 | 0.27 | 1.03 | 0.86 |
| 12 | 108126209 | rs7137854 | 0.11 | -0.034 | **3.12E-05** | -0.027 | 0.27 | 1.03 | 0.88 |
| 12 | 108126443 | rs3741788 | 0.11 | -0.034 | **3.12E-05** | -0.027 | 0.27 | 1.03 | 0.37 |
| 12 | 108126854 | rs1045749 | 0.10 | -0.032 | **2.15E-04** | -0.024 | 0.36 | 1.02 | 0.91 |
| 12 | 108126866 | rs117637335 | 0.05 | 0.017 | 0.13 | -0.007 | 0.79 | 0.38 | **9.46E-03** |
| 12 | 108127273 | rs1045741 | 0.44 | -0.001 | 0.99 | 0.002 | 0.85 | 0.89 | 0.30 |
| 12 | 108127778 | rs1045739 | 0.11 | -0.033 | **3.98E-05** | -0.027 | 0.27 | 1.00 | 0.99 |
| 12 | 108127965 | rs17306603 | 0.11 | -0.033 | **3.98E-05** | -0.027 | 0.27 | 1.00 | 0.99 |
| 12 | 108128762 | rs10083011 | 0.30 | 0.012 | **0.03** | 0.005 | 0.72 | 0.90 | 0.38 |
| 12 | 108129716 | rs7978575 | 0.30 | 0.012 | **0.03** | 0.002 | 0.90 | 0.89 | 0.36 |

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**Table 6.** Continued.

| Chromosome # | BP | SNP | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[b] | OR | *p*-value[c] |
| 12 | 108129964 | rs11113456 | 0.11 | -0.034 | **2.36E-05** | -0.027 | 0.27 | 1.02 | 0.93 |
| 12 | 108130032 | rs11113457 | 0.11 | -0.034 | **2.36E-05** | -0.027 | 0.27 | 1.02 | 0.93 |
| 12 | 108130041 | rs7968311 | 0.30 | 0.012 | **0.03** | 0.002 | 0.87 | 0.90 | 0.37 |
| 12 | 108130187 | rs139760615 | 0.01 | -0.050 | **0.05** | -0.07 | 0.23 | 0.21 | 0.13 |
| 12 | 108130312 | rs34265083 | 0.11 | -0.030 | **2.36E-05** | -0.027 | 0.27 | 1.02 | 0.93 |
| 12 | 108131052 | rs10778574 | 0.44 | 0.001 | 0.98 | 0.002 | 0.85 | 0.89 | 0.28 |
| 12 | 108131123 | rs7957541 | 0.03 | 0.022 | 0.11 | 0.034 | 0.29 | 0.76 | 0.43 |
| 12 | 108132001 | rs11113460 | 0.11 | -0.033 | **3.47E-05** | -0.027 | 0.27 | 1.02 | 0.93 |
| 12 | 108132420 | rs140062738 | 0.02 | 0.004 | 0.83 | 0.046 | 0.34 | 1.40 | 0.34 |
| 12 | 108132431 | rs12227593 | 0.11 | -0.034 | **4.03E-05** | -0.027 | 0.27 | 1.02 | 0.93 |
| 12 | 108132584 | rs150297450 | 0.02 | 0.008 | 0.68 | NA | NA | 1.31 | 0.51 |
| 12 | 108133688 | rs4258462 | 0.09 | -0.031 | **3.81E-04** | -0.024 | 0.36 | 0.99 | 0.95 |
| 12 | 108133769 | rs58981531 | 0.05 | 0.005 | 0.69 | 0.025 | 0.37 | 0.86 | 0.56 |
| 12 | 108134032 | rs73189568 | 0.05 | 0.005 | 0.69 | 0.025 | 0.37 | 0.86 | 0.56 |
| 12 | 108135911 | rs2302736 | 0.44 | -0.001 | 0.96 | 0.002 | 0.85 | 0.89 | 0.31 |
| 12 | 108136172 | rs2302734 | 0.11 | -0.034 | **3.55E-05** | -0.024 | 0.27 | 1.02 | 0.91 |
| 12 | 108137424 | rs61938569 | 0.04 | 0.018 | 0.15 | -0.051 | 0.08 | 0.69 | 0.23 |

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**Table 6.** Continued.

| Chromosome # | BP | SNP | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[b] | OR | *p*-value[c] |
| 12 | 108137525 | rs2160591 | 0.44 | -0.001 | 0.99 | 0.002 | 0.85 | 0.89 | 0.29 |
| 12 | 108137534 | rs1861678 | 0.11 | -0.034 | **3.55E-05** | -0.027 | 0.27 | 1.02 | 0.91 |
| 12 | 108137631 | rs11113461 | 0.11 | -0.034 | **3.55E-05** | -0.027 | 0.27 | 1.02 | 0.91 |
| 12 | 108137839 | rs35531735 | 0.11 | -0.034 | **3.55E-05** | -0.027 | 0.27 | 1.02 | 0.91 |
| 12 | 108138027 | rs117893122 | 0.08 | 0.005 | 0.59 | 0.034 | 0.11 | 1.28 | 0.19 |
| 12 | 108138565 | rs117307558 | 0.05 | -0.007 | 0.54 | 0.050 | **0.03** | 1.95 | **9.27E-04** |
| 12 | 108138698 | rs12829738 | 0.09 | -0.031 | **3.83E-04** | -0.024 | 0.36 | 1.03 | 0.90 |
| 12 | 108138706 | rs12829746 | 0.09 | -0.031 | **3.83E-04** | -0.024 | 0.36 | 1.03 | 0.90 |
| 12 | 108139363 | rs10861773 | 0.34 | -0.005 | 0.34 | -0.022 | 0.10 | 1.07 | 0.54 |
| 12 | 108139591 | rs12579462 | 0.44 | -0.001 | 0.99 | 0.002 | 0.85 | 0.89 | 0.29 |
| 12 | 108139879 | rs78639015 | 0.02 | 0.015 | 0.43 | -0.018 | 0.75 | 0.52 | 0.16 |
| 12 | 108140032 | rs3803120 | 0.11 | -0.034 | **4.40E-05** | -0.027 | 0.27 | 1.03 | 0.89 |
| 12 | 108140621 | rs11113463 | 0.44 | -0.001 | 0.99 | 0.002 | 0.85 | 0.89 | 0.29 |
| 12 | 108141452 | rs117007749 | 0.01 | 0.011 | 0.65 | 0.036 | 0.40 | 0.96 | 0.94 |
| 12 | 108141488 | rs73189570 | 0.05 | 0.006 | 0.59 | 0.025 | 0.37 | 0.88 | 0.64 |
| 12 | 108141516 | rs17041588 | 0.05 | -0.004 | 0.70 | 0.047 | 0.11 | 0.94 | 0.82 |
| 12 | 108141752 | rs117978304 | 0.02 | -0.008 | 0.71 | 0.009 | 0.87 | 1.29 | 0.54 |

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**Table 6.** Continued.

| Chromosome # | BP | SNP | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[b] | OR | *p*-value[c] |
| 12 | 108142033 | rs55929921 | 0.11 | -0.033 | **5.64E-05** | -0.027 | 0.27 | 1.04 | 0.82 |
| 12 | 108142496 | rs35373113 | 0.11 | -0.033 | **7.92E-05** | -0.027 | 0.27 | 1.04 | 0.82 |
| 12 | 108142557 | rs10861774 | 0.30 | 0.013 | 0.022 | 0.006 | 0.66 | 0.88 | 0.29 |
| 12 | 108142805 | rs17306876 | 0.11 | -0.033 | **5.21E-05** | -0.027 | 0.27 | 1.07 | 0.68 |
| 12 | 108143284 | rs117732799 | 0.04 | -0.014 | 0.3 | 0.006 | 0.88 | 1.11 | 0.71 |
| 12 | 108143445 | rs56332415 | 0.44 | -0.001 | 0.99 | 0.002 | 0.85 | 0.89 | 0.29 |
| 12 | 108144091 | rs10861775 | 0.21 | 0.013 | 0.037 | -0.014 | 0.35 | 0.72 | **0.02** |
| 12 | 108144101 | rs11113464 | 0.11 | -0.034 | **3.83E-05** | -0.027 | 0.27 | 1.04 | 0.83 |
| 12 | 108144208 | rs73189574 | 0.05 | 0.005 | 0.67 | 0.017 | 0.56 | 0.80 | 0.43 |
| 12 | 108144263 | rs117535962 | 0.08 | 0.003 | 0.68 | 0.039 | 0.08 | 1.38 | 0.07 |
| 12 | 108144710 | rs1468879 | 0.29 | 0.012 | 0.03 | 0.006 | 0.69 | 0.89 | 0.33 |
| 12 | 108144740 | rs1468880 | 0.09 | -0.033 | **1.51E-04** | -0.024 | 0.36 | 1.03 | 0.87 |
| 12 | 108145022 | rs1468881 | 0.11 | -0.034 | **3.69E-05** | -0.027 | 0.27 | 1.06 | 0.76 |
| 12 | 108146071 | rs7302814 | 0.29 | 0.012 | 0.028 | 0.005 | 0.72 | 0.88 | 0.30 |
| 12 | 108146281 | rs7303327 | 0.09 | -0.034 | **1.04E-04** | -0.024 | 0.37 | 1.05 | 0.79 |
| 12 | 108146472 | rs11836200 | 0.14 | -0.021 | **4.54E-03** | -0.009 | 0.64 | 0.98 | 0.90 |
| 12 | 108146905 | rs60513804 | 0.44 | -0.001 | 0.97 | 0.003 | 0.80 | 0.88 | 0.24 |

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**Table 6.** Continued.

| Chromosome # | BP | SNP | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[b] | OR | *p*-value[c] |
| 12 | 108147441 | rs35726106 | 0.11 | -0.035 | **1.75E-05** | -0.027 | 0.27 | 1.02 | 0.92 |
| 12 | 108147982 | rs4964617 | 0.09 | -0.035 | **7.78E-05** | -0.025 | 0.35 | 1.03 | 0.88 |
| 12 | 108148528 | rs2374722 | 0.44 | -0.001 | 0.94 | 0.003 | 0.80 | 0.88 | 0.24 |
| 12 | 108148917 | rs7137753 | 0.09 | -0.037 | **3.01E-05** | -0.025 | 0.35 | 1.04 | 0.85 |
| 12 | 108149611 | rs10861776 | 0.44 | -0.001 | 0.99 | 0.003 | 0.80 | 0.89 | 0.30 |
| 12 | 108149649 | rs139624723 | 0.03 | 0.012 | 0.43 | 0.018 | 0.63 | 0.68 | 0.38 |
| 12 | 108150758 | rs1814335 | 0.09 | -0.038 | **2.71E-05** | -0.025 | 0.35 | 1.05 | 0.79 |
| 12 | 108150920 | rs1861677 | 0.44 | -0.001 | 0.98 | 0.003 | 0.80 | 0.89 | 0.30 |
| 12 | 108151801 | rs12367937 | 0.44 | -0.001 | 0.99 | 0.003 | 0.80 | 0.91 | 0.39 |
| 12 | 108151811 | rs12368330 | 0.44 | -0.001 | 0.93 | 0.003 | 0.80 | 0.90 | 0.33 |
| 12 | 108152633 | rs3851639 | 0.29 | 0.012 | 0.03 | 0.005 | 0.74 | 0.94 | 0.58 |
| 12 | 108153200 | rs150315190 | 0.01 | 0.013 | 0.54 | 0.022 | 0.67 | 1.19 | 0.71 |
| 12 | 108153571 | rs3741791 | 0.05 | 0.005 | 0.68 | 0.017 | 0.56 | 0.82 | 0.47 |
| 12 | 108154199 | rs2302733 | 0.10 | -0.039 | **3.88E-06** | -0.028 | 0.27 | 1.08 | 0.67 |
| 12 | 108154632 | rs76964322 | 0.10 | -0.039 | **4.47E-06** | -0.028 | 0.27 | 1.08 | 0.67 |
| 12 | 108154937 | rs11113466 | 0.10 | -0.039 | **5.38E-06** | -0.028 | 0.27 | 1.08 | 0.65 |
| 12 | 108155005 | rs181617446 | 0.04 | 0.006 | 0.64 | -0.033 | 0.29 | 0.58 | 0.09 |

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**Table 6.** Continued.

| Chromosome # | BP | SNP | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[b] | OR | *p*-value[c] |
| 12 | 108155108 | rs4964618 | 0.10 | -0.040 | **2.26E-06** | -0.028 | 0.27 | 1.08 | 0.65 |
| 12 | 108155128 | rs4964223 | 0.10 | -0.040 | **2.94E-06** | -0.028 | 0.27 | 1.08 | 0.65 |
| 12 | 108155353 | rs73189581 | 0.03 | 0.021 | 0.14 | 0.023 | 0.48 | 0.78 | 0.45 |
| 12 | 108155632 | rs73398399 | 0.08 | 0.004 | 0.68 | 0.050 | 0.02 | 1.31 | 0.14 |
| 12 | 108156456 | rs4015237 | 0.10 | -0.040 | **2.41E-06** | -0.028 | 0.27 | 1.08 | 0.65 |
| 12 | 108156907 | rs4964224 | 0.10 | -0.040 | **2.41E-06** | -0.028 | 0.27 | 1.08 | 0.70 |
| 12 | 108157564 | rs34911908 | 0.08 | -0.040 | **2.78E-05** | -0.039 | 0.16 | 1.08 | 0.70 |
| 12 | 108157700 | rs1819872 | 0.08 | -0.040 | **2.78E-05** | -0.039 | 0.16 | 1.25 | 0.57 |
| 12 | 108158091 | rs2216265 | 0.01 | -0.030 | 0.15 | 0.015 | 0.79 | 0.83 | 0.70 |
| 12 | 108158278 | rs4964620 | 0.24 | 0.014 | **0.02** | -0.017 | 0.24 | 0.74 | **0.03** |
| 12 | 108158360 | rs73398401 | 0.07 | 0.003 | 0.74 | 0.050 | **0.03** | 1.29 | 0.18 |
| 12 | 108158392 | rs77651314 | 0.01 | 0.005 | 0.84 | 0.050 | 0.26 | 0.76 | 0.60 |
| 12 | 108158985 | rs10861777 | 0.20 | 0.012 | **0.05** | -0.026 | 0.09 | 0.77 | 0.07 |
| 12 | 108159186 | rs10861778 | 0.44 | -0.002 | 0.70 | -0.002 | 0.87 | 0.90 | 0.32 |
| 12 | 108159191 | rs35864914 | 0.09 | -0.040 | **2.65E-06** | -0.037 | 0.15 | 1.03 | 0.87 |

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**Table 6.** Continued.

| Chromosome # | BP | SNP | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[b] | OR | *p*-value[c] |
| 12 | 108159306 | rs34156340 | 0.10 | -0.040 | **2.19E-06** | -0.028 | 0.27 | 1.09 | 0.63 |
| 12 | 108159932 | rs61938578 | 0.44 | -0.002 | 0.67 | -0.003 | 0.84 | 0.89 | 0.29 |

Abbreviations: SNP, single nucleotide polymorphism; Ref, reference; Alt, alternate; OR, odds ratio; AD, Alzheimer's disease.

[a]Temporal lobe includes inferior temporal, middle temporal and superior temporal cortex.

[b]Adjusted for age, sex, field strength, diagnosis, PC1, PC2 and PC3.

[c]Adjusted for age, sex, PC1, PC2 and PC3.

Significant *p*-values are indicated in bold letters.

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### Ⅱ-3-2. *PRDM4* rs34156340 associated with brain atrophy in the temporal lobe

Regional association analysis involving SNPs in the *PRDM4* gene region (from 2 kb upstream to 2 kb downstream) showed rs34156340 ($p$=2.19×10$^{-06}$) was strongly associated with the temporal lobe atrophy (Figure 4A and Table 6). To investigate the effects of rs34156340 on temporal lobe atrophy and AD risk, this study evaluated the association of this SNP with brain MRI measures in the sample that includes 196 AD cases and 1,096 cognitively normal controls using dominant model. The results showed that both rs34156340 (C-allele) were associated with cortical thinning in temporal lobe ($p$<0.05) (Figure 4B).

### Ⅱ-3-3. *PRDM4* rs117307558 associated with β-amyloid deposition in the brain temporal lobe

To assess whether the SNPs in *PRDM4* region associated with β-amyloid deposition in the brain, regional SNP-based association analysis were performed with β-amyloid SUVR values from temporal lobe. Regional association analysis involving SNPs showed that rs117307558 ($p$<0.05) was associated with β-amyloid deposition in temporal lobe (Table 6). Furthermore, genotype effect on β-amyloid deposition in the anatomical regions of temporal lobe was evaluated using dominant genotype model. These results showed that greater β-amyloid deposition was observed among rs117307558-T individuals compared to rs117303558-GG individuals in temporal lobe (F=8.11, $p$=0.0048) (Figure 4D and Table 7). Similarly, rs73398399 SNP, which was in LD with rs117303558, was also tested for Aβ risk and the results showed that rs73398399-T individuals had greater Aβ deposition than rs73398399-CC individuals in the temporal lobe (F=6.87, $p$=0.009) (Table 7).

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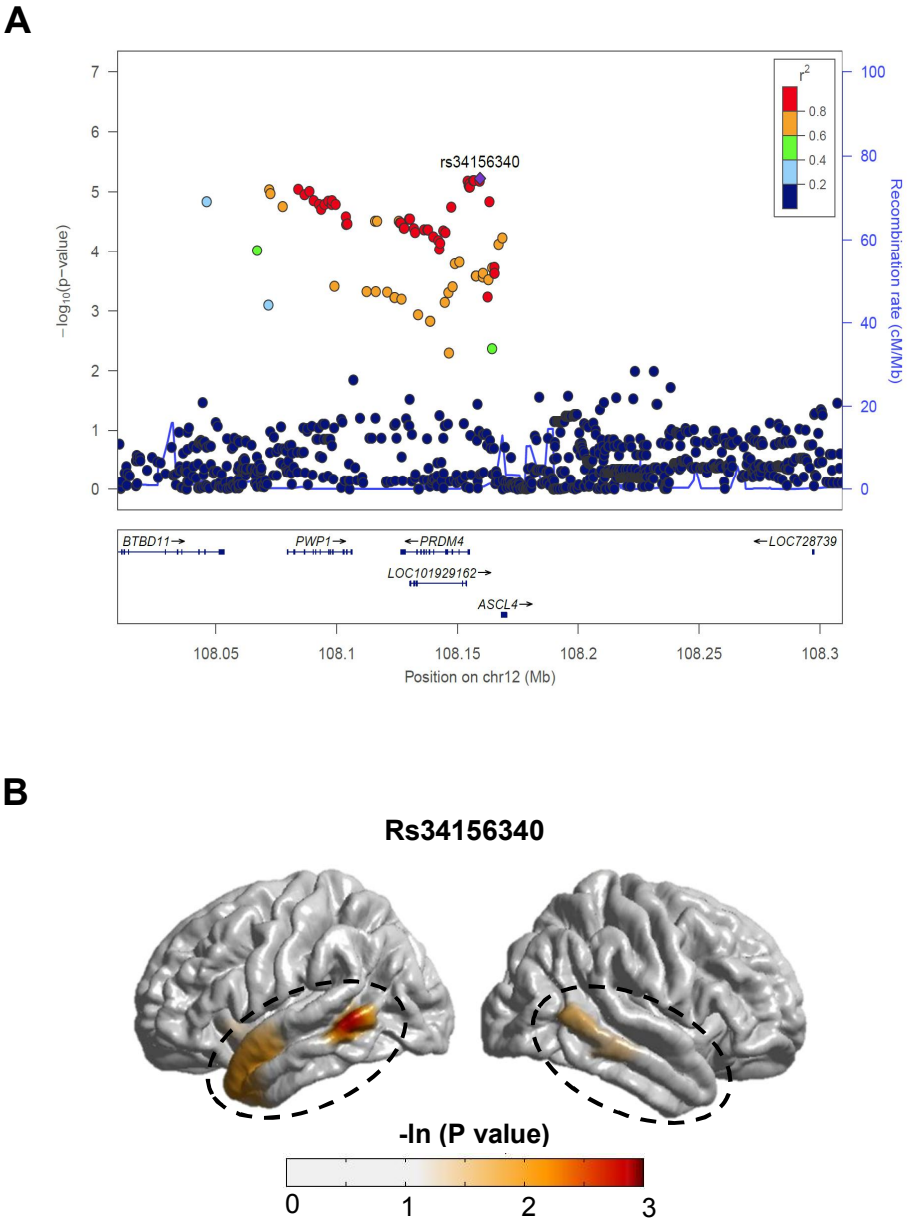


**Figure 4.** Continued

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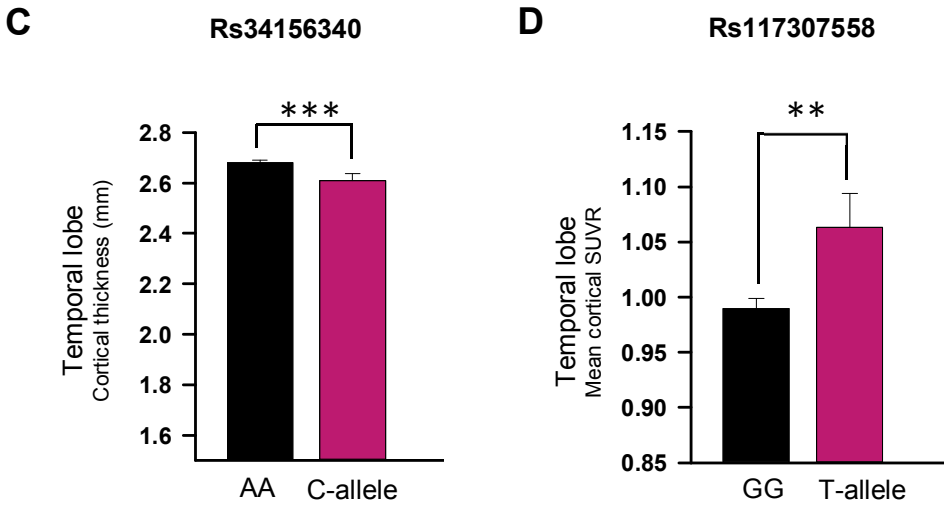


**Figure 4. SNPs in *PRDM4* gene associated with brain cortical atrophy and β-amyloid deposition in the temporal lobe.** (A) Regional association plot of *PRDM4* showing rs34156340 is associated with brain temporal lobe atrophy. The purple dot shows the most associated SNP in the *PRDM4* gene region. The colours for all other SNPs indicate the LD $r^2$ value with most associated SNP ($r^2 \geq 0.8$ in red, $r^2$ between 0.6 and 0.8 in orange, $r^2$ between 0.4 and 0.6 in green, $r^2$ between 0.2 and 0.4 in light blue and $r^2 < 0.2$ in dark blue) . The x-axis indicates base-pair position and the y-axis is $-\log_{10}$ *p*-value. (B) Cortical thinning maps associated with rs34156340. A general linear model was applied to identify point-wise cortical thickness differences using rs34156340 genotypes (AA vs. C- allele) as a predictor with age, sex and field strength as covariates. Statistically, greater thinning in C-allele compared to AA individuals is shown for temporal lobe and fusiform gyrus (indicated in dotted black and blue line respectively). (C) The average cortical thickness in the temporal lobe were compared between rs34156340 AA and C-allele individuals. (D) The β-amyloid deposition (mean cortical SUVR) in the temporal lobe were compared between rs117307558 GG and T-allele individuals.

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**Table 7.** Effect of rs117307558 and rs73398399 genotypes with β-amyloid deposition in temporal lobe.

| Region of interest | Temporal lobe SUVR | | | |
|---|---|---|---|---|
| | rs117307558 | | rs73398399 | |
| | GG vs. GT+TT (n=244) | | CC vs. TC+TT (n=244) | |
| | ***F*-value** | ***p*-value[b]** | ***F*-value** | ***p*-value[b]** |
| Temporal lobe | 8.11 | **0.0048** | 6.87 | **0.009** |

[a]Temporal lobe includes inferior temporal, middle temporal and superior temporal cortex.

[b]Adjusted for age, sex, PC1, PC2 and PC3.

Significant *p*-values are indicated in bold letters.

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### Ⅱ-3-4. Haplotype association analysis on AD risk with SNPs of interest in *PRDM4* gene

Even though rs34156340 SNP in *PRDM4* gene showed association with cortical atrophy, rs117307558 showed association with β-amyloid deposition. In addition, there are many other SNPs which are located in the functional regions of the *PRDM4* gene having strong LD with these 2 SNPs (rs34156340 and rs117307558). To analyse the haplotype effect in the *PRDM4* gene regulation, this study selected one SNP in each functional regions of the *PRDM4* gene. The selected SNPs in each functional region were also in strong LD with any of these 2 SNPs. The selected SNPs of interest were rs3741788 (distal regulatory), rs17306603 (3' UTR), rs117307558 (intron), rs2302733 (intron), rs11113466 (5' UTR), rs73398399 (promotor) and rs34156340 (promotor). Association analysis involving temporal lobe cortical thickness and β-amyloid SUVR measures were re-analysed with these SNPs of interest using linear regression and compared with association analysis that included 196 AD cases and 1,096 cognitively normal controls using logistic regression (Table 8). In the LD analysis, this study found that rs3741788, rs17306603, rs2302733, rs11113466, and rs34156340 are in strong LD ($D' > 0.8$ and $r^2 > 0.80$) with each other and strongly associated with the temporal lobe cortical atrophy. In another group, rs117307558 and rs73398399 are in moderate LD ($D' > 0.8$ and $r^2 = 0.51$) with each other and associated with temporal lobe β-amyloid deposition. Anyhow, these two groups do not have LD $r^2$ ($r^2 = 0$) but has strong LD $D'$ ($D' > 0.8$) with low likelihood odds (LOD < 2) (Figure 5A and 5B). To determine whether these two groups of SNPs increase AD risk as haplotypes, initially, this study analysed the haplotype effect with the cortical atrophy associated SNPs. Haplotypes estimated with rs3741788, rs17306603, rs2302733, rs11113466, and rs34156340 were analysed for the AD risk association. Haplotype CAACA with highest frequency in both controls (88%) and AD cases (88%) was considered as

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reference. The results showed that the haplotypes constructed with cortical atrophy associated SNPs were not associated with AD risk while considering $P<0.05$ as significance (Figure 6A and Table 9). Then, this study constructed haplotypes including both cortical atrophy associated SNPs and β-amyloid deposition associated SNPs to evaluate the combined effect. In this analysis, haplotypes were constructed with rs3741788, rs17306603, rs117307558, rs2302733, rs11113466, rs73398399 and rs34156340. This study considered CAGACCA haplotype with highest frequency in both controls (80%) and AD cases (77%) as reference. The association analysis showed that CATACTA haplotype is associated with AD risk (OR=1.85, $p$=0.0036) and the cox proportional regression results showed this haplotype is associated with early age at onset for AD (HR=1.69, $p$=0.020) (Figure 6B and Table 10). Interestingly, this haplotype includes two risk alleles (rs117307558-T and rs73398399-T) from β-amyloid deposition associated SNPs and 5 non risk alleles from (rs3741788-C, rs17306603-A, rs2302733-A, rs11113466-C and rs34156340-A) cortical atrophy associated SNPs. Moreover, results of CATACTA haplotype suggests that β-amyloid deposition associated SNP group and cortical atrophy associated SNP group are not working in tandem, which was also evidential in LD analysis. Nevertheless, β-amyloid deposition associated SNPs confers AD risk but not the cortical atrophy associated SNPs.

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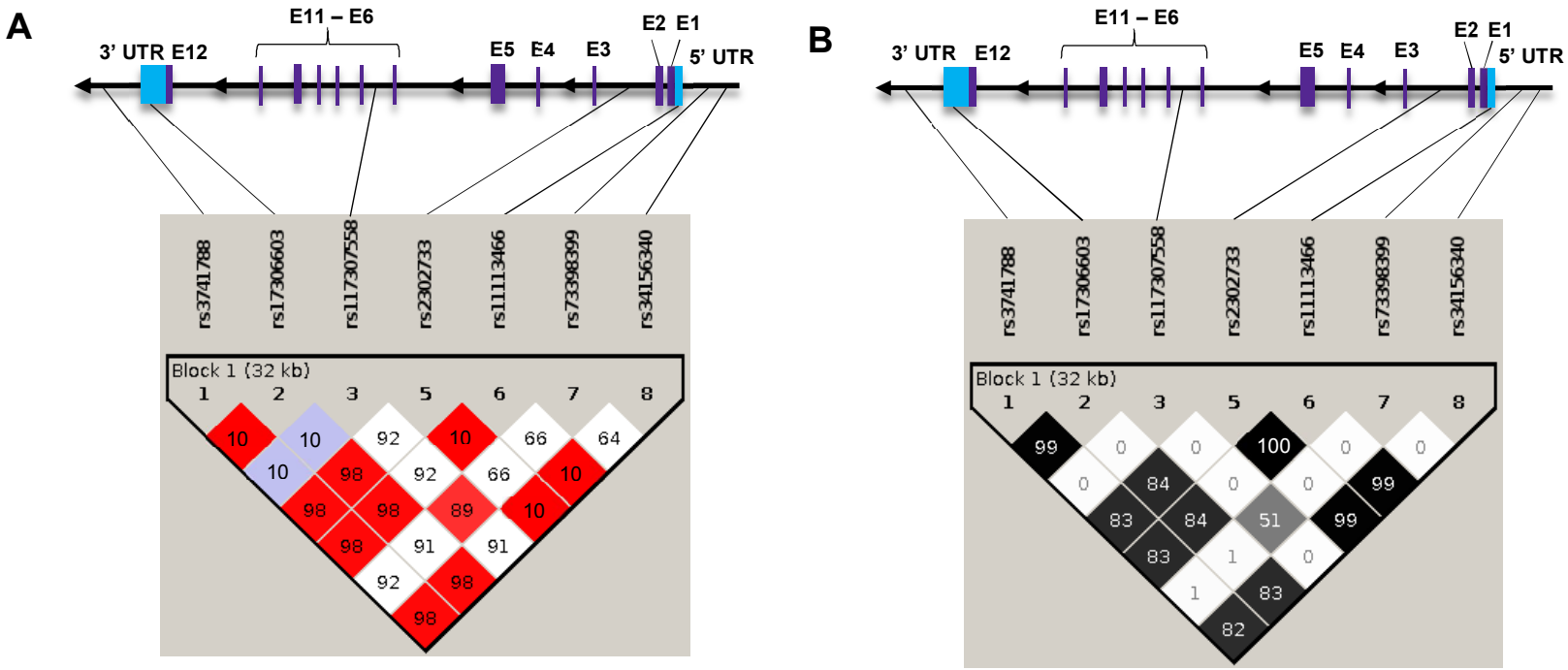


**Figure 5. Linkage disequilibrium (LD) among SNPs in the *PRDM4* gene region.** LD block constructed from 7 SNPs in the *PRDM4* region. (A) SNPs with strong LD *D'* (*D'* ≤ 1 and log-odds (LOD) > 2) are shown in red, whereas white indicates high LD *D'* (*D'* ≤ 1) but low LOD (LOD < 2), and light blue indicates LD *D'* = 1 but low LOD (LOD < 2). (B) Black square indicates LD $r^2$ = 1, which explains perfect LD between two SNPs.

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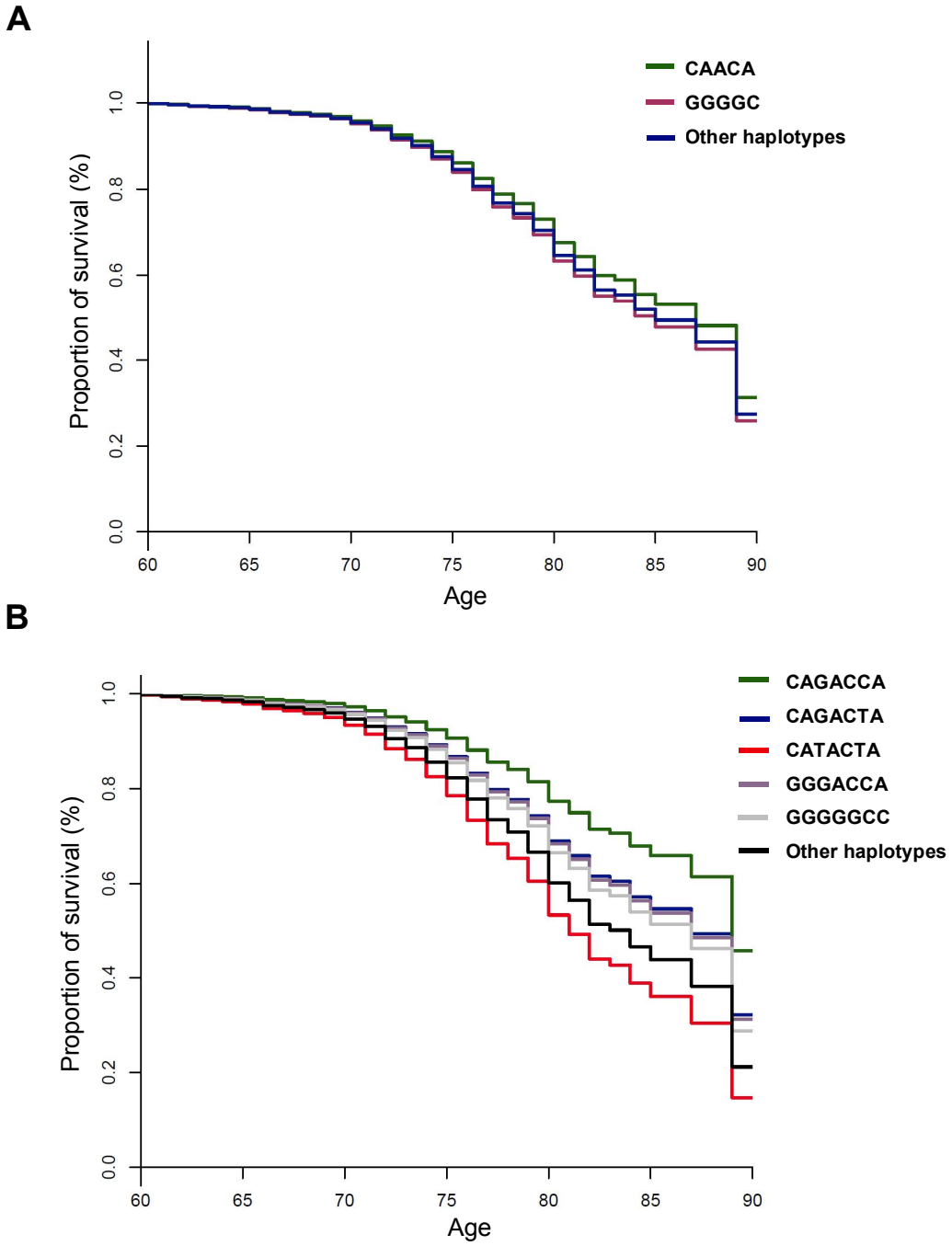


**Figure 6. Haplotype associated with β-amyloid deposition increases risk for AD onset.** SNPs associated with cortical atrophy and β-amyloid deposition were

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analysed for haplotype effect. (A) Effect of the haplotype constructed from 5 cortical atrophy (temporal lobe and fusiform gyrus) associated SNPs including rs3741788, rs17306603, rs2302733, rs11113466 and rs34156340 were analysed with cox-proportional regression model adjusted for sex and 3PCs. Haplotype with highest frequency in both controls and AD cases was used as reference. Curves are shown for individuals with the following combination of haplotypes: green line = rs3741788-C + rs17306603-A + rs2302733-A + rs11113466-C + rs34156340-A, maroon line = rs3741788-G + rs17306603-G + rs2302733-G + rs11113466-G + rs34156340-C and blue line = other haplotypes with very low frequencies. (B) Effect of the haplotype constructed from 5 cortical atrophy associated SNPs (rs3741788, rs17306603, rs2302733, rs11113466 and rs34156340) and 2 β-amyloid burden associated SNPs (rs117307558 and rs73398399) were analysed with cox-proportional regression model adjusted for sex and 3PCs. Haplotype with highest frequency in both controls and AD cases was used as reference. Curves are shown for individuals with the following combination of haplotypes: green line = rs3741788-C + rs17306603-A + rs117307558-G + rs2302733-A + rs11113466-C + rs73398399-C + rs34156340-A, blue line = rs3741788-C + rs17306603-A + rs117307558-G + rs2302733-A + rs11113466-C + rs73398399-T + rs34156340-A, red line = rs3741788-C + rs17306603-A + rs117307558-T + rs2302733-A + rs11113466-C + rs73398399-T + rs34156340-A, purple line = rs3741788-G + rs17306603-G + rs117307558-G + rs2302733-A + rs11113466-C + rs73398399-C + rs34156340-A, grey line = rs3741788-G + rs17306603-G + rs117307558-G + rs2302733-G + rs11113466-G + rs73398399-C + rs34156340-C and black line = other haplotypes with very low frequencies.

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**Table 8.** *PRDM4* region SNPs of interest associated with cortical atrophy and β-amyloid deposition in temporal lobe.

| Chromosome # | SNP | Ref/Alt Allele | MAF | Cortical thickness | | β-amyloid | | Control vs. AD case | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Beta | *p*-value[b] | Beta | *p*-value[c] | Odds ratio (95% CI) | *p*-value[c] |
| 12 | rs3741788 | C/G | 0.11 | -0.034 | **3.12E-05** | -0.027 | 0.27 | 1.03 (0.57-1.23) | 0.88 |
| 12 | rs17306603 | A/G | 0.11 | -0.033 | **3.98E-05** | -0.027 | 0.27 | 1.00 (0.55-1.21) | 0.99 |
| 12 | rs117307558 | G/T | 0.05 | -0.007 | 0.54 | 0.050 | **0.03** | 1.95 (1.36-3.78) | **9.27E-04** |
| 12 | rs2302733 | A/G | 0.10 | -0.039 | **3.88E-06** | -0.028 | 0.27 | 1.08 (0.62-1.39) | 0.67 |
| 12 | rs11113466 | C/G | 0.10 | -0.039 | **5.38E-06** | -0.028 | 0.27 | 1.08 (0.62-1.39) | 0.65 |
| 12 | rs73398399 | C/T | 0.08 | 0.004 | 0.68 | 0.050 | **0.02** | 1.31 (0.91-2.15) | 0.14 |
| 12 | rs34156340 | A/C | 0.10 | -0.040 | **2.19E-06** | -0.028 | 0.27 | 1.09 (0.66-1.10) | 0.63 |

Abbreviations: SNP, single nucleotide polymorphism; Ref, reference; Alt, alternate; MAF, minor allele frequency; OR, odds ratio; AD, Alzheimer's disease; CI, confidence interval.

[a]Temporal lobe includes inferior temporal, middle temporal and superior temporal cortex.

[b]Adjusted for age, sex, field strength, diagnosis, PC1, PC2 and PC3. [c]Adjusted for age, sex, PC1, PC2 and PC3.

Significant *p*-values are indicated in bold letters.

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**Table 9.** *PRDM4* region haplotype associated with cortical atrophy does not increase AD risk.

| Haplotype[a] | Haplotype frequency | | | Odds ratio[c] (95% CI) | *p*-value | Hazard ratio[d] (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|
| | Pooled[b] (n=1288) | Control (n=1092) | Case (n=196) | | | | |
| CAACA | 0.8828 | 0.8832 | 0.8807 | Ref | NA | Ref | NA |
| GGGGC | 0.0977 | 0.0970 | 0.1015 | 1.10 (0.73-1.50) | 0.78 | 1.06 (0.74-1.51) | 0.75 |
| GGACA | 0.0136 | 0.0142 | 0.0102 | 0.90 (0.39-2.10) | 0.90 | 1.01 (0.45-2.29) | 0.98 |
| CAGGC[e] | 0.0032 | NA | 0.0051 | NA | NA | NA | NA |
| GAACA[e] | 0.0004 | NA | 0.0025 | NA | NA | NA | NA |
| GGGGA[e] | 0.0012 | 0.0014 | NA | NA | NA | NA | NA |

Abbreviations: CI, confidence interval; Ref, reference haplotype; NA, not applicable.

[a]Haplotype = rs3741788 + rs17306603 + rs2302733 + rs11113466 + rs34156340.

[b]Pooled haplotype frequency includes frequency from control and case groups. [c]Adjusted for age, sex, PC1, PC2 and PC3. [d]Adjusted for sex, PC1, PC2 and PC3. [e]Haplotypes with pooled frequency less than 1% were combined as a single haplotype in the logistic regression and cox proportional regression model.

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**Table 10.** *PRDM4* region SNPs associated with β-amyloid deposition increases AD risk.

| Haplotype[a] | Haplotype frequency | | | Odds ratio[c] (95% CI) | *p*-value | Hazard ratio[d] (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|
| | Pooled[b] (n=1289) | Control (n=1092) | Case (n=196) | | | | |
| CAGACCA | 0.8000 | 0.8000 | 0.77359 | Ref | Ref | Ref | Ref |
| CAGACTA | 0.0340 | 0.0370 | 0.02041 | 0.61 (0.28-1.30) | 0.19 | 0.69 (0.32-1.48) | 0.34 |
| GGGGGCC | 0.0960 | 0.0960 | 0.0940 | 1.05 (0.72-1.50) | 0.87 | 1.03 (0.69-1.53) | 0.90 |
| CATACTA | 0.0490 | 0.0043 | 0.0778 | **1.85 (1.22-2.80)** | **3.60E-03** | **1.69 (1.09-2.63)** | **0.02** |
| GGGACCA | 0.0130 | 0.0140 | 0.0102 | 1.47 (0.72-3.00) | 0.5 | 1.10 (0.41-2.99) | 0.85 |
| CATACCA[e] | 0.0027 | 0.00160 | 0.00888 | NA | NA | NA | NA |
| GGGGGTC[e] | 0.0013 | 0.00065 | 0.00522 | NA | NA | NA | NA |
| CAGGGCC[e] | 0.0028 | 0.00230 | 0.00513 | NA | NA | NA | NA |
| GGTGGCC[e] | 0.0005 | 0.00029 | 0.00153 | NA | NA | NA | NA |

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**Table 10.** Continued.

| Haplotype[a] | Haplotype frequency | | | Odds ratio[c] (95% CI) | *p*-value | Hazard ratio[d] (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|
| | Pooled[b] (n=1289) | Control (n=1092) | Case (n=196) | | | | |
| GAGACCA[e] | 0.0003 | NA | 0.00254 | NA | NA | NA | NA |
| GGGGGCA[e] | 0.0001 | 0.0014 | NA | NA | NA | NA | NA |
| GGTGGTC[e] | 1.2e-07 | 4.2e-08 | 0.00065 | NA | NA | NA | NA |

Abbreviations: OR, odds ratio; HR, hazard ratio; CI, confidence interval; Ref, reference haplotype; NA, not applicable.

[a]Haplotype = rs3741788 + rs17306603 + rs117307558 + rs2302733 + rs11113466 + rs73398399 + rs34156340.

[b]Pooled haplotype frequency includes frequency from control and case groups.

[c]Adjusted for age, sex, PC1, PC2 and PC3. [d]Adjusted for sex, PC1, PC2 and PC3.

[e]Haplotypes with pooled frequency less than 1% were combined as a single haplotype in the logistic regression and cox proportional regression models.

Significant *p*-values are indicated in bold letters.

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### Ⅱ-3-5. Association of *PRDM4* gene SNPs with neuropsychological tests

Correlation analyses on cortical thickness of temporal lobe with cognitive measures showed that all cognitive domains including attention, language and related functions, visuospatial functions, memory and functional/executive functions were strongly correlated with temporal lobe (Table 11). In addition, strong association of temporal lobe was found with MMSE and CDR sum of boxes. Sequentially, correlation analyses on temporal Aβ deposition with cognitive measures were performed, and the results showed that except attention and functional/executive functions, all other domains were strongly correlated with Aβ deposition. Therefore, this study included all the cognitive domains to evaluate the association of SNPs of interest with neuropsychological tests. The association analysis was performed with control, mild cognitively impaired (MCI) and AD case groups separately and as well as with total group including other dementia groups. In controls, the result suggested that rs73398399 was associated with ($p<0.05$) SVLT recognition. While in MCI group, rs73398399 was associated ($p<0.05$) with SVLT delayed recall and K-TMT-E-A. However, in AD group, rs117307558 was associated ($p<0.05$) with Rey CFT copy score. In total group, rs73398399 was associated ($p<0.05$) with COWAT supermarket (Table 12).

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**Table 11.** Partial correlation of neuropsychological tests with temporal lobe cortical thickness and temporal lobe β-amyloid deposition.

| Tests | Temporal lobe thickness[a] | | | | Temporal lobe SUVR[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean (SD) | *r*-value | *p*-value | N | Mean (SD) | *r*-value | *p*-value |
| K-MMSE | 2672 | 25.87 (3.79) | **0.22** | **6.71E-30** | 243 | 25.80 (4.25) | **-0.26** | **4.48E-05** |
| CDR Sum of boxes | 1747 | 0.58 (1.14) | **-0.13** | **3.36E-08** | 215 | 0.67 (1.10) | **0.14** | **0.04** |
| **Attention** | | | | | | | | |
| Digit Span test backward | 2655 | 3.23 (1.16) | **0.05** | **9.37E-03** | 242 | 3.34 (1.05) | -0.08 | 0.22 |
| Digit Span test forward | 2662 | 5.52 (1.50) | **0.04** | **0.04** | 242 | 5.49 (1.50) | -0.01 | 0.92 |
| Digit Span test Forward/Backward | 2655 | 2.30 (1.40) | 0.00 | 0.98 | 242 | 2.15 (1.24) | 0.06 | 0.37 |
| **Language & related functions** | | | | | | | | |
| S-K-BNT | 2331 | 11.23 (2.71) | **0.18** | **4.77E-18** | 236 | 44.52 (8.38) | **-0.23** | **4.57E-04** |
| Praxis Ideomotor | 2614 | 4.89 (0.44) | **0.11** | **2.64E-08** | 234 | 4.85 (0.46) | -0.03 | 0.63 |

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**Table 11.** Continued.

| Tests | Temporal lobe thickness[a] | | | | Temporal lobe SUVR[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean (SD) | *r*-value | *p*-value | N | Mean (SD) | *r*-value | *p*-value |
| **Visuospatial functions** | | | | | | | | |
| Rey CFT Copy score | 2653 | 30.51 (10.63) | **0.09** | **1.82E-06** | 243 | 31.21 (6.52) | **-0.17** | **9.13E-03** |
| **Memory** | | | | | | | | |
| SVLT Immediate Recall | 2668 | 17.15 (5.26) | **0.14** | **3.02E-13** | 243 | 17.17 (5.13) | **-0.27** | **2.32E-05** |
| SVLT Delayed Recall | 2662 | 4.93 (2.69) | **0.13** | **2.08E-11** | 243 | 4.55 (2.75) | **-0.21** | **1.17E-03** |
| SVLT Recognition | 2662 | 19.62 (2.88) | **0.13** | **1.32E-11** | 243 | 19.25 (2.76) | **-0.27** | **2.32E-05** |
| RCFT Immediate Recall | 2622 | 11.92 (7.32) | **0.12** | **3.45E-09** | 240 | 12.39 (7.56) | **-0.2** | **2.39E-03** |
| RCFT Delayed Recall | 2611 | 11.93 (7.01) | **0.13** | **1.30E-10** | 239 | 4.55 (2.75) | **-0.21** | **1.04E-03** |
| RCFT Recognition | 2629 | 18.90 (2.44) | **0.15** | **6.86E-15** | 242 | 19.25 (2.75) | **-0.18** | **6.15E-03** |

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**Table 11.** Continued.

| Tests | Temporal lobe thickness[a] | | | | Temporal lobe SUVR[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean (SD) | *r*-value | *p*-value | N | Mean (SD) | *r*-value | *p*-value |
| **Frontal/Executive Functions** | | | | | | | | |
| Go-No Go | 2598 | 18.09 (3.77) | **0.11** | **4.36E-08** | 235 | 18.60 (2.96) | 0.03 | 0.63 |
| COWAT Animal | 2659 | 13.30 (4.60) | **0.12** | **4.69E-10** | 242 | 13.21 (4.39) | -0.11 | 0.08 |
| COWAT Supermarket | 2625 | 14.41 (5.45) | **0.15** | **4.24E-14** | 236 | 14.71 (5.60) | -0.09 | 0.19 |
| COWAT Phonemic | 2522 | 20.59 (11.22) | **0.07** | **3.77E-04** | 234 | 22.20 (11.38) | -0.04 | 0.54 |
| StroopWordreading Time | 2546 | 84.46 (23.01) | **-0.09** | **1.15E-05** | 239 | 107.57 (13.59) | **-0.14** | **0.03** |
| StroopColorreading Time | 2556 | 73.42 (26.35) | -0.02 | 0.29 | 237 | 73.63 (26.72) | 0.1 | 0.12 |
| K-TMT-E-A | 1910 | 35.64 (22.35) | **-0.09** | **5.12E-05** | 233 | 34.49 (18.44) | 0.08 | 0.23 |

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**Table 11.** Continued.

| Tests | Temporal lobe thickness[a] | | | | Temporal lobe SUVR[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean (SD) | *r*-value | *p*-value | N | Mean (SD) | *r*-value | *p*-value |
| | Frontal/Executive Functions | | | | | | | |
| K-TMT-E-B | 1609 | 68.95 (46.47) | **-0.06** | **0.02** | 198 | 68.76 (45.27) | 0.10 | 0.18 |

Abbreviations: SD, standard deviation; N, number of samples; K-MMSE, Korean Mini-Mental State Exam; CDR, Clinical Dementia Rating Sum of boxes; S-K-BNT, Short form of Korean version of the Boston Naming Test; Rey CFT, Rey Complex Figure Test Copy score; SVLT, Seoul Verbal Learning Test; RCFT, Rey Complex Figure Test Copy score; COWAT, Controlled Oral Word Association Test; K-TMT-E-A, Korean-Trail Making Test-Elderly's version A Time; K-TMT-E-B, Korean-Trail Making Test-Elderly's version B Time.

[a]Adjusted for Age, Sex, Field Strength, Phenotype.

Significant *p*-values are indicated in bold letters.

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**Table 12.** Association of *PRDM4* SNPs of interest with neuropsychological tests.

| Tests | CN | | | | MCI | | | | AD | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **rs117307558[b]** | | **rs73398399[b]** | | **rs117307558[b]** | | **rs73398399[b]** | | **rs117307558[b]** | | **rs73398399[b]** | |
| | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** |
| K-MMSE | 0.03 | 0.56 | 0.01 | 0.8 | 0.04 | 0.22 | 0.03 | 0.31 | 0.07 | 0.08 | 0.04 | 0.25 |
| CDR Sum of boxes | -0.52 | 0.27 | -0.50 | 0.17 | -0.10 | 0.46 | -0.01 | 0.94 | -0.22 | 0.14 | -0.07 | 0.55 |
| Digit Span test backward | -0.03 | 0.78 | 0.02 | 0.77 | 0.16 | 0.06 | 0.03 | 0.66 | 0.36 | 0.07 | 0.32 | 0.07 |
| Digit Span test forward | -0.08 | 0.31 | -0.02 | 0.69 | 0.10 | 0.07 | 0.08 | 0.17 | 0.15 | 0.30 | 0.15 | 0.25 |
| Digit Span test Forward/ Backward | -0.07 | 0.4 | -0.05 | 0.44 | 0.03 | 0.63 | 0.06 | 0.33 | -0.11 | 0.50 | -0.08 | 0.59 |
| S-K-BNT | -0.04 | 0.5 | -0.01 | 0.81 | -0.02 | 0.71 | 0.02 | 0.63 | -0.04 | 0.48 | 0.04 | 0.47 |
| Praxis Ideomotor | -0.34 | 0.21 | -0.15 | 0.54 | 0.03 | 0.88 | 0.04 | 0.83 | 0.23 | 0.47 | 0.02 | 0.95 |
| Rey CFT Copy score | 0.00 | 0.97 | -0.01 | 0.66 | 0.01 | 0.34 | 0.01 | 0.51 | **0.06** | **0.02** | 0.02 | 0.24 |

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**Table 12.** Continued.

| Tests | CN | | | | MCI | | | | AD | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | rs117307558[b] | | rs73398399[b] | | rs117307558[b] | | rs73398399[b] | | rs117307558[b] | | rs73398399[b] | |
| | Beta | *p*-value | Beta | *p*-value | Beta | *p*-value | Beta | *p*-value | Beta | *p*-value | Beta | *p*-value |
| SVLT Immediate Recall | -0.03 | 0.22 | -0.02 | 0.37 | -0.01 | 0.61 | -0.02 | 0.25 | 0.04 | 0.31 | 0.03 | 0.45 |
| SVLT Delayed Recall | -0.02 | 0.70 | 0.00 | 1.00 | -0.04 | 0.25 | **-0.07** | **0.03** | 0.04 | 0.59 | 0.03 | 0.64 |
| SVLT Recognition | 0.04 | 0.41 | **0.09** | **0.04** | 0.02 | 0.62 | 0.02 | 0.45 | 0.06 | 0.40 | 0.02 | 0.78 |
| RCFT Immediate Recall | -0.00 | 0.97 | -0.01 | 0.49 | 0.00 | 1.00 | 0.01 | 0.66 | -0.05 | 0.88 | 0.02 | 0.61 |
| RCFT Delayed Recall | -0.02 | 0.70 | -0.01 | 0.62 | -0.00 | 0.78 | -0.01 | 0.93 | 0.03 | 0.92 | 0.02 | 0.52 |
| RCFT Recognition | -0.05 | 0.40 | 0.03 | 0.44 | -0.03 | 0.45 | -0.02 | 0.53 | 0.13 | 0.08 | 0.07 | 0.26 |
| Contrasting Program | 0.33 | 0.14 | 0.04 | 0.59 | 0.01 | 0.85 | 0.01 | 0.98 | 0.05 | 0.41 | 0.01 | 0.76 |

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**Table 12.** Continued.

| Tests | CN | | | | MCI | | | | AD | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | rs117307558[b] | | rs73398399[b] | | rs117307558[b] | | rs73398399[b] | | rs117307558[b] | | rs73398399[b] | |
| | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** |
| Go-No Go | 0.08 | 0.10 | 0.03 | 0.37 | 0.02 | 0.42 | 0.04 | 0.13 | 0.04 | 0.30 | 0.05 | 0.19 |
| COWAT Animal | -0.01 | 0.71 | 0.01 | 0.95 | 0.01 | 0.79 | 0.01 | 0.59 | 0.01 | 0.85 | 0.02 | 0.65 |
| COWAT Supermarket | 0.00 | 1.00 | 0.02 | 0.35 | 0.02 | 0.41 | 0.02 | 0.17 | 0.07 | 0.08 | 0.06 | 0.11 |
| COWAT Phonemic | -0.01 | 0.23 | -0.04 | 0.64 | 0.01 | 0.15 | 0.08 | 0.31 | 0.02 | 0.29 | 0.02 | 0.24 |
| Stroop Wordreading Time | 0.00 | 0.20 | -0.01 | 0.22 | 0.00 | 1.00 | -0.00 | 0.99 | -0.04 | 0.59 | -0.05 | 0.55 |
| Stroop Colorreading Time | -0.01 | 0.67 | -0.02 | 0.20 | 0.01 | 0.71 | -0.01 | 0.29 | 0.07 | 0.53 | 0.09 | 0.50 |
| K-TMT-E-A | -0.001 | 0.90 | 0.00 | 0.70 | -0.01 | 0.13 | **-0.01** | **0.03** | 0.00 | 0.96 | -0.00 | 0.82 |

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**Table 12.** Continued.

| Tests | CN | | | | MCI | | | | AD | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | rs117307558[b] | | rs73398399[b] | | rs117307558[b] | | rs73398399[b] | | rs117307558[b] | | rs73398399[b] | |
| | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** | **Beta** | ***p*-value** |
| K-TMT-E-B | 0.002 | 0.55 | 0.00 | 0.79 | 0.00 | 0.96 | 0.00 | 0.85 | 0.01 | 0.23 | 0.00 | 0.74 |

Abbreviations: K-MMSE, Korean Mini-Mental State Exam; CDR, Clinical Dementia Rating Sum of boxes; S-K-BNT, Short form of Korean version of the Boston Naming Test; Rey CFT, Rey Complex Figure Test Copy score; SVLT, Seoul Verbal Learning Test; RCFT, Rey Complex Figure Test Copy score; COWAT, Controlled Oral Word Association Test; K-TMT-E-A, Korean-Trail Making Test-Elderly's version A Time; K-TMT-E-B, Korean-Trail Making Test-Elderly's version B Time.

[a]Adjusted for Age, Sex, 3PCs and Phenotype.

Significant *p*-values are indicated in bold letters.

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## Ⅱ-4. DISCUSSION

In this study, the association of genome-wide 6,706,236 SNPs on the temporal lobe (including inferior, middle and superior temporal gyrus) thickness from MRI data of 2,693 Korean subjects was investigated. This study identified that thioredoxin reductase1 (*TXNRD1*) genetic locus was most associated with temporal lobe atrophy. In that locus, rs145565989 (P=2.25 × 10-15) SNP was top most associated from the SNP-based GWAS and the *TXNRD1* (P=1.28×10-13) gene was top most associated from the gene-based GWAS. Earlier studies have demonstrated that thioredoxin reductase plays a protective role against cytotoxicity induced by the generation of reactive oxygen species (ROS). It was also reported that thioredoxin reductase is associated with increased oxidative stress mediated neurodegeneration in Alzheimer's disease patients. Moreover, thioredoxin reductase activity was also found to be increased in all AD related brain regions (Lovell, Xie, Gabbita, & Markesbery, 2000). In concordance with the previous study, the results confirm the association of *TXNRD1* gene with the brain temporal lobe region. In addition, *TXNRD1* is also associated with aging (Dato, De Rango, Crocco, Passarino, & Rose, 2015), genetic generalized epilepsy (Kudin et al., 2017) and cerebellar hypoplasia (Soerensen et al., 2008).

This study hypothesized that the *TXNRD1* gene and its neighbouring genes might also be associated with AD risk in the Korean cohort as well. Therefore, this study compared 18 protein coding genes located 5 Mb upstream and 5Mb downstream to *TXNRD1* with 1196 genes that showed nominal significance level ($P<0.05$) in the gene-based GWAS of case-control subjects (Figure 3). This resulted in a single gene, *PRDM4* overlapping in both temporal lobe atrophy and AD risk. Furthermore, the results showed that rs34156340 ($p=2.19\times10^{-06}$) in the *PRDM4* gene region was most significantly associated with temporal lobe atrophy (Figure 4A). In

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addition, whole-brain cortical thickness analysis under dominant model using case-control subjects showed rs34156340-C allele individuals was associated with greater temporal lobe atrophy than rs34156340-AA allele individuals (Figure 4B and C). However, the rs34156340 SNP exhibited strong association with temporal lobe atrophy but not with AD risk.

Since temporal lobe region is involved in both cortical atrophy (Migliaccio et al., 2015) and Aβ deposition (Karantzoulis & Galvin, 2011) in AD patients, this study analysed the association of all *PRDM4* gene region SNPs with temporal lobe Aβ deposition. The results showed that rs117307558 SNP in the *PRDM4* gene was most associated ($p<0.05$) with β-amyloid deposition in temporal lobe (Table 8). However, the LD map of *PRDM4* gene region including top cortical atrophy associated SNPs and Aβ deposition associated SNPs showed that rs34156340 and rs117307558 were not in LD (Figure 5A and B). Nevertheless, this study found that rs117307558 SNP was in moderate LD (r2=0.5) with rs73398399 SNP in the same region. Moreover, the haplotype analysis with AD case-control groups involving top cortical atrophy associated SNPs and Aβ deposition associated SNPs revealed that CATACTA haplotype was associated with AD risk. This CATACTA haplotype included two risk alleles (rs117307558-T and rs73398399-T) of Aβ deposition associated SNPs and five non-risk alleles (rs3741788-C, rs17306603-A, rs2302733-A, rs11113466-C and rs34156340-A) of cortical atrophy associated SNPs. Although previous studies reported that the brain atrophy in the temporal cortex is part of AD pathology (Migliaccio et al., 2015), the haplotype analysis results showed that rs117307558 and rs73398399 SNPs in the *PRDM4* gene were associated with the AD risk and Aβ deposition but not associated with brain atrophy. However, a previous study reported a group of AD patients showed no brain atrophy (Ferreira et al., 2017) and similar to that previous study, the results showed that rs117307558 and rs73398399 SNPs were not associated with temporal lobe atrophy but associated

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with AD risk and Aβ deposition. It should be noted that our cohort included 196 AD subjects and the MAF for rs117307558 and rs73398399 were 0.05 and 0.07 respectively, which were East Asian specific. Future MRI GWASs involving larger AD samples is needed to confirm the role of the rs117307558 and rs73398399 SNPs in the Aβ mediated AD pathology. In the replication analysis, by involving independent subjects, the results showed that rs73398399 (OR=4.41; $p$=0.0034) SNP was strongly associated with Aβ deposition, while rs117307558 (OR=1.22; $p$=0.67) SNP showed increased Aβ risk but no statistical significance was observed. Among AD patients, the results suggests that *PRDM4* gene promotes Aβ deposition-mediated AD risk. Whereas in non-AD individuals, *PRDM4* gene might be associated with ageing-mediated temporal lobe atrophy.

Previous MRI studies showed that temporal lobe cortex is correlated with memory, verbal abilities and executive functioning (Hartberg et al., 2010). In concordance with the results of previous study, this study found that cortical thickness of temporal lobe was strongly correlated with attention, language and related functions, visuospatial functions, memory and functional/executive functions. In addition, the correlation analysis of temporal lobe Aβ SUVR with cognitive measure suggested that language and related functions, visuospatial functions and memory domains were strongly correlated. Another study found that the abnormal expression of *PRDM4* is associated with lower psychological endurance threshold and the individuals are prone to commit abnormal behaviour certainly and thus, suggesting the association of *PRDM4* with psychological behaviour (Xu, Jing, Zhang, & Liu, 2019). From the results, this study found that rs73398399 SNP in the *PRDM4* gene was associated ($P$<0.05) with memory domain and functional/executive functions among MCI individuals. Whereas, rs117307558 was associated ($P$<0.05) with memory domain among AD patients. Since rs117307558 and rs73398399 are in moderate LD, it is likely that both SNPs might

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promote memory decline and functional/executive functions in AD patients (Table 11). However, it requires careful interpretation of the neuropsychological test analysis due to smaller AD sample size. Overall, the results suggest that *PRMD4* gene promote Aβ deposition in the AD patients and likely causes decline in memory and frontal/executive functions.

Earlier studies reported that *APOE* genetic locus could explain for 6% heritability for sporadic AD dementia and the additional 21 common genetic variants that were identified in the large AD GWAS meta-analyses could explain 2% of heritability, whereas the remaining 2 million common genetic variants could explain 25% of heritability. However, the effect sizes for those additional 2 million variants are found to be small (OR<1.22), and this suggests that the Aβ associated SNPs, rs117307558 and rs73398399 might be associated with AD risk while exhibiting moderate effect size.

In addition to *PRDM4* gene, this study also identified 14 genes having genome-wide significance in the temporal lobe cortical thickness GWAS, but none of them showed significant association with neither AD risk nor Aβ deposition. This suggests that these 14 genes might be associated with aging mediated atrophy but not AD related atrophy. Moreover, this study primarily focused on the identification of temporal lobe related genes that are involved in the AD pathology, and it should be noted that the temporal lobe is associated with the lateral stage AD pathology. This might also be a reason that *PRDM4* gene showed moderate association for AD risk. Future temporal lobe analysis involving larger sample size might help us to understand the genes associated with lateral stage AD pathology.

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# PART 3

***Genome-wide association study on amyloid PET identifies novel risk loci that regulates cerebral β-amyloid deposition in the East Asians***

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## SUMMARY

Beta-amyloid (β-amyloid) deposition is a pathological hallmark of Alzheimer's disease (AD) and genetic factors contribute to both AD risk and β-amyloid deposition. Previous genome-wide association studies (GWAS) involving β-amyloid burden are based on autopsy results, were only semi-quantitative, and only Caucasians were studied. To identify ethnic-specific novel genetic loci associated to cerebral β-amyloid deposition, a GWAS using brain cerebral β-amyloid levels as the trait, measured with florbetaben ($^{18}$F) positron emission tomography (PET) imaging was performed. The sample was 712 Korean subjects, and included normal controls, and subjects with MCI and AD. This study identified two novel risk loci located at rs12975891 on chromosome 19 ($P = 4.42 \times 10^{-09}$, closest gene *CPAMD8*), and at rs113454949 on chromosome 3 ($P = 1.08 \times 10^{-08}$, in *ARL14*). This study also observed a strong signal at rs429358 on chromosome 19 ($P = 3.22 \times 10^{-26}$) which is in *APOE*, a known AD risk gene. Both rs12975891 at *CPAMD8* and rs113454949 at *ARL14* were associated with severe patterns of β-amyloid deposition in brain. In addition, the *ARL14* locus exhibited strong linkage disequilibrium (LD) block extending to multiple genes including *KPNA4*, *TRIM59*, *SMC4* and *IFT80*, and thus along with *ARL14*, are candidate AD genes. These genes also showed genome-wide significant association to β-amyloid deposition in the gene-based GWAS and the gene network analysis confirmed that these genes including *CPAMD8* were together related to the *TOMM40* and *APOE* genes. This study also identified a missense variant, rs3745339 ($P = 1.26 \times 10^{-08}$) in the *CPAMD8* gene. In this study, two novel genetic loci (*CPAMD8* and *ARL14*) were identified that includes *CPAMD8*, *ARL14*, *SMC4*, *KPNA4*, *TRIM59* and *IFT80* genes. These genes are likely playing an important role in β-amyloid pathology in the brain. The findings on the Korean specific novel genetic loci associated with β-

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amyloid deposition can improve the understanding in ethnic-specific genetic pathways related to β-amyloid deposition mediated AD pathology.

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## Ⅲ-1. INTRODUCTION

Alzheimer's disease (AD) is pathologically characterised by the accumulation of extracellular amyloid-β (Aβ) proteins comprised of neuritic plaques and the intracellular hyperphosphorylated tau proteins composed of neurofibrillary tangles in the brain (Hardy & Selkoe, 2002; He et al., 2018; Rosenberg, Lambracht-Washington, Yu, & Xia, 2016). The deposition of Aβ plaques is considered as a primary event in the pathological cascade of AD that occurs before the onset of neurodegeneration and neuronal dysfunction (He et al., 2018; Jack et al., 2013). Neuropathological and genetic evidences have also supported the Aβ hypothesis in the AD pathology. Mutations in the Amyloid Precursor Protein (*APP*), Presenilin1 (*PSEN1*) and Presenilin2 (*PSEN2*) genes that are associated with early onset autosomal dominant AD is the strongest genetic evidence supporting Aβ hypothesis (Rosenberg et al., 2016).

Among overall AD cases, the sporadic or late onset AD (LOAD) accounts for 99% and the genetic factors are strongly contributing for the disease risk. However, the sporadic AD is genetically more complex and less clear. The strongest genetic risk factor in AD is apolipoprotein E (*APOE*) ε4 allele (Musiek & Holtzman, 2015). Given that AD is genetically complex form of disease, it is very important to search for the additional genetic risk factors contributing to the disease mechanism (Van Cauwenberghe, Van Broeckhoven, & Sleegers, 2015). Recently, 21 common genetic variants associated with late onset AD have been reported in a large genome-wide association study (GWAS) meta-analyses (J. C. Lambert et al., 2013). Another study suggested that there could be numerous additional AD susceptible genetic loci, which could not be identified in the previous AD GWAS (Mormino, Sperling, Holmes, Buckner, De Jager, Smoller, & Sabuncu, 2016). The use of quantitative AD

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related endophenotypes in GWAS have provided novel insights into genes and pathways that are associated with disease mechanisms (Li Shen et al., 2014).

Recent advancements in the amyloid radiotracers in combination with positron emission tomography (PET) imaging facilitates non-invasive detection of Aβ plaques in the brain and the radiotracers with enhanced half-life provides capability to collect β-amyloid PET images for large number of samples (Li Shen et al., 2014). The in vivo detection of Aβ burden in the brain using amyloid PET has strong correlation with Aβ measures from the cerebrospinal fluid (CSF) and brain autopsy samples (Clark et al., 2012; Palmqvist et al., 2015). The first study of amyloid PET GWAS identified a novel gene, *BCHE* that showed genome-wide significant association to amyloid burden using measures from $^{18}$F-Florbetapir PET images and in addition, this study confirmed the vital role of *APOE* gene in the amyloid pathology (Li Shen et al., 2014). Subsequent genome-wide studies on amyloid PET using either $^{11}$C-labelled Pittsburgh Compound-B (PiB) or $^{18}$F-Florbetapir radiotracer identified few novel genetic loci associated with amyloid burden mediated AD pathology, but later studies could not identify genetic loci with genome-wide significant association to amyloid burden (Roostaei, Nazeri, Felsky, De Jager, Schneider, Pollock, Bennett, Voineskos, et al., 2017; Yan et al., 2018). Moreover, all the previous amyloid PET genome-wide studies were limited to Caucasian specific populations. In our previous study, the ethnic variability in the risk of *APOE* ε4 for AD was explained and it was observed stronger in East Asians than Caucasians (Choi et al., 2019). Due to the possibility of ethnic-specific AD susceptible genetic loci that was demonstrated in multiple studies (Luxi Shen & Jia, 2016), it is also important to identify ethnic-specific amyloid associated genetic loci related to AD pathology.

In this study, GWAS with β-amyloid measures as quantitative phenotype using the $^{18}$F-Florbetaben PET imaging data was performed from the samples

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enrolled in the Gwangju Alzheimer's & Related Dementias (GARD) cohort. Till date, this is the first GWAS study on cerebral β-amyloid deposition in the Koreans. This study hypothesized that this GWAS study would identify novel ethnic-specific genetic loci associated with cerebral β-amyloid deposition mediated AD pathology in the Korean samples.

## Ⅲ-2. METHODS

### Ⅲ-2-1. Study participants

The study cohort utilized data for 712 participants aged 55-90 years from the GARD cohort collected by National Research Center for Dementia (NRCD) at Chosun University in Gwangju, South Korea. The sample comprised of 135 AD patients, 270 mild cognitive impairments (MCI), 252 cognitively normal adults and 55 subjects with other dementias (Vascular dementia, frontotemporal dementia and Lewy body dementia) (Table 13). Probable AD dementia was clinically diagnosed according to criteria of the National Institute of Neurological and Communicative Disorders Association (NINCDS-ADRDA) (G. M. McKhann et al., 2011). The cognitively normal individuals had no sign of neurological disease or impairments in the cognitive abilities or activities of daily life. All the samples included in this study had amyloid positron emission tomography (PET) imaging. The institutional review board of Chosun University Hospital, South Korea approved the study protocol and all the participants or their legal guardians of cognitively impaired individuals gave informed consent before participation in this study.

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**Table 13.** Demographic and clinical characteristics of participants with β-amyloid imaging data.

| Groups | N | Age (y, mean ± SD) | Female (n, %) | *APOE* ε4 carriers (n, %) | Years of education (y, mean ± SD) | MMSE (mean ± SD) |
|---|---|---|---|---|---|---|
| Control | 252 | 72.14 ± 5.40 | 134 (53.17%) | 67 (26.59%) | 10.59 ± 4.69 | 26.81 ± 4.11 |
| MCI | 270 | 72.70 ± 6.15 | 139 (51.48%) | 81 (30.0%) | 9.45 ± 4.99 | 24.31 ± 5.49 |
| Other dementias | 55 | 74.59 ± 6.53 | 32 (58.18%) | 7 (12.72%) | 8.85 ± 4.85 | 23.05 ± 8.37 |
| AD | 135 | 71.28 ± 7.80 | 71 (52.59%) | 67 (49.63%) | 7.23 ± 5.40 | 17.66 ± 7.89 |
| Positive | 251 | 72.00 ± 6.74 | 132 (52.59%) | 133 (52.99%) | 8.63 ± 5.10 | 21.84 ± 7.74 |
| Negative | 461 | 72.59 ± 6.08 | 244 (52.93%) | 89 (19.31%) | 9.80 ± 5.04 | 24.92 ± 5.79 |

Abbreviations: N, number of samples; SD, standard deviation; y, years; MMSE, Mini-Mental State Examination; MCI, mild cognitive impairment; AD, Alzheimer's disease.

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### Ⅲ-2-2. Florbetaben Positron Emission Tomography (PET) imaging

All the study participants underwent PET scanning session at the Chosun University Hospital, Korea. Participants were administered 300 MBq $^{18}$F-Florbetaben ($^{18}$F-FBB) β-amyloid tracer intravenously and scanned after 90 minutes using a Discovery ST PET-CT scanner (General Electric Medical Systems, Milwaukee, WI, USA). Attenuation correction of PET image was performed with non-contrast-enhanced CT scans with technical parameters of 10-130 mAs, 8 slice, 120 Kvp, helical and 3.79 mm slice thickness. After applying ordered subset expectation maximization (OSEM), PET and CT images were reconstructed followed by the attenuation correction with iterative reconstruction algorithm.

### Ⅲ-2-3. PET image data processing

$^{18}$F-FBB PET images were pre-processed with SPM12 toolbox implemented in MATLAB (R2018a, Mathworks, Natick, MA, USA). All the $^{18}$F-FBB PET images were co-registered with the respective T1-weighted MR images from the same subject that are acquired on the same day or within 6 months of time interval. Then, the transformation parameters for the spatial normalization of T1-weighted MR images to the standard MNI template were estimated. Subsequently, the estimated parameters were applied to spatially normalize the PET images to the standard MNI template. Furthermore, these normalized PET images were smoothened with an 8-mm full-width at half maximum Gaussian kernel (Park, Han, Yi, et al., 2019). The respective MR images were segmented using FreeSurfer software version 6.0) and applied to the smoothened PET images to extract values (SUVRs) from 68 predefined brain anatomical region of interests (ROIs) (Palmqvist et al., 2017). The global cortical retention mean SUVRs were calculated from anterior and posterior cingulate, lateral temporal, frontal and lateral parietal regions with reference to the whole cerebellum (Maass et al., 2017).

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### Ⅲ-2-4. PET image data for replication analyses

An independent Korean amyloid PET samples recruited at the Inha University (INU) hospital were used as replication Aβ cohort. 124 participants enrolled at the Inha University hospital underwent $^{18}$F-Fliutemetamol ($^{18}$F-FMM) PET scanning session 90 minutes after administering 185 MBq of $^{18}$F-FMM intravenously. PET scan images were acquired using Discovery 690 PET/CT scanner (GE), Discovery 600 PET/CT scanner (GE), Biography MCT PET/CT scanner (Siemens), Discovery STE PET/CT scanner or Gemini TF PET/CT scanner (Philips). For attenuation correction, low-dose CT scan was used. Then, OSEM algorithm was used to reconstruct images by 4 iterations and 16 subsets. In addition, 68 underwent 3-dimensional $^{11}$C-Pittsburgh Compound B ($^{11}$C-PiB) PET image scanning. A 3.0 T Biograph mMR (PET-MR) scanner (Siemens) was used to acquire $^{11}$C-PiB PET images 40 minutes after administering 555 MBq $^{11}$C-PiB (450-610 MBq) intravenously. Then, the images were corrected for decay reduction, uniformity and ultrashort echo time-based attenuation. Images were reconstructed using 6 iterations and 21 subsets with 256 x 256 image matrix.

### Ⅲ-2-5. PET image data processing for replication analysis

$^{18}$F-FMM and $^{11}$C-PiB PET images were co-registered with the respective T1- weighted MR images from the same participant. Additional details on pre-processing procedures were described elsewhere. The $^{18}$F-FMM PET scan individuals with SUVR value ≥ 0.634 or $^{11}$C-PiB PET scan individuals with SUVR value > 1.21 were considered as Aβ positive (Hwang et al., 2019). $^{18}$F-Florbetapir ($^{18}$F-FBP) amyloid PET images for ADNI participants were downloaded from ADNI database (http://adni.loni.usc.edu). According to ADNI guidelines, individuals with SUVR value > 1.11 were classified as Aβ positive (Jagust et al., 2010).

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### Ⅲ-2-6. SNP genotyping and imputation

Genomic DNA for all the participants were isolated from the peripheral blood leukocytes and genotyped using Affymetrix Axiom KORV1.0 and Affymetrix Axiom KORV1.1 Genome-wide genotyping array (Affymetrix® Axiom KORV1.0 and Axiom KORV1.1, Santa Clara, CA, USA). These genotyping chips were optimized and designed for Korean population by the Center for Genome Science, Korea National Institute of Health, South Korea (Jee et al., 2016). All the samples were genotyped at DNALink, Seoul, South Korea. After genotyping, the samples with individual call rate <95%, extremely low or high heterozygosity rate (±3 SD from the mean), individual pairs with significant relatedness (identity by descent PI_HAT >0.4) and gender inconsistencies between recorded sex and genotyped X-chromosome SNPs were excluded. SNPs with minor allele frequency (MAF) <1%, Hardy-Weinberg equilibrium (HWE) test *p*-value $<10^{-5}$ and genotype call rate <95% were removed using PLINK software, version 1.09 (https://www.cog-genomics.org/plink/1.9/). After the quality control of genotype data, the imputation was performed using pre-phased reference haplotypes of the East Asian population available from the Haplotype Reference Consortium (HRC) panel version 1.1 (McCarthy et al., 2016). The low quality imputed SNPs with info score <0.5 and MAF <1% were excluded after imputation. After quality control, 712 samples and 6,103,636 SNPs were included in the dataset. The GARD study cohort has the collection of large quality controlled genotype dataset for 5,345 samples and this study employed a pooled subset of 712 samples that had PET images.

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### Ⅲ-2-7. Cognitive assessment and clinical measures

All the participants were screened for the cognitive ability in multiple domains using Seoul Neuropsychological Screening Battery (SNSB) by the NRCD at Chosun University in Gwangju, Korea. This battery assesses 5 cognitive domains that include attention, language, visuospatial, memory and frontal/executive functions (Ahn et al., 2010). The neuropsychological tests in the SNSB consisted of Clinical Dementia Rating (CDR) Sum of boxes, Korean Mini-Mental State Exam (K-MMSE), Digit Span test Forward/Backward, Digit Span test forward, Digit Span test backward, Praxis Ideomotor, Short form of Korean version of the Boston Naming Test (S-K-BNT), Copy score, Rey Complex Figure Test (RCFT), Seoul Verbal Learning Test (SVLT) Immediate Recall, SVLT Delayed Recall, SVLT Recognition, RCFT Recognition, Contrasting Program, Go-No Go test, Controlled Oral Word Association Test (COWAT) Phenomic, COWAT Supermarket, COWAT Animal, Korean-Trail Making Test-Elderly's version A Time (K-TMT-E-A), Korean-Trail Making Test-Elderly's version B Time (K-TMT-E-B), StroopColorreading Time and StroopWordreading Time.

### Ⅲ-2-8. H3K27ac ChIP-seq data of entorhinal cortex

Due to the presence of huge LD block in the *ARL14* genetic locus, the candidate risk gene for β-amyloid burden in that region was unclear. To identify active enhancers and promoters in that region, this study evaluated the lysine H3K27 acetylation (H3K27ac) chromatin immunoprecipitation combined with highly-parallel sequencing (ChIP-seq) data of brain entorhinal cortex tissue from 24 AD samples with neuropathology and 23 neuropathology-free control samples that were downloaded from Gene Expression Omnibus (GEO) (accession number GSE102538) database (https://www.ncbi.nlm.nih.gov/geo/). The brain samples included in that dataset was obtained from MRC London Neurodegenerative Disease

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Brain Bank. H3K27ac tracks and association statistics of AD cases and controls were downloaded from UCSC Genome Browser. The downloaded H3K27ac tracks were annotated with gene symbols using bedmap tool. More description about the samples and association tests were available elsewhere (S. J. Marzi et al., 2018). H3K27ac narrow peaks annotated regional plot was plotted using LocusZoom program and the H3K27ac tracks were plotted using UCSC Genome Browser. H3K27ac *p*-values were plotted using R program.

### Ⅲ-2-9. Statistical analysis

#### Ⅲ-2-9-1. Genome-wide association analyses

The linear regression on β-amyloid SUVR involving 6,103,636 SNPs and 712 samples was performed using PLINK to analyse the association of SNPs with brain β-amyloid deposition. The principal components (PCs) that account for the population substructure in the cohort were calculated using smartpca program with EIGENSOFT (Patterson et al., 2006). Tracy-Widom statistics identified that 4 PCs were significantly associated with β-amyloid levels. Then, the linear regression was performed involving additive genetic model with age, sex, education and 4 PCs as covariates. Conditional analyses were performed with age, sex education, 4PCs and *APOE* □4 status or AD status as covariates. In the GWAS results, $p<5\times10^{-8}$ was considered as conservative threshold to account for multiple comparisons (Pe'er, Yelensky, Altshuler, & Daly, 2008). Manhattan and Q-Q plots of GWAS results were plotted using “qqman” and “snpStats” packages in R program, version 3.6.1 (https://www.r-project.org/) (Solé, Guinó, Valls, Iniesta, & Moreno, 2006; Turner, 2014). In addition, the regional association plots of significant loci were plotted using LocusZoom standalone version of program (Pruim et al., 2010). The proportion of variance accounted by additive effects of all SNPs in the significant loci were

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estimated using GCTA (version 1.92.3) (https://cnsgenomics.com/software/gcta) software (Brand et al., 2018).

Gene-based association analysis was performed to identify candidate genes associated with β-amyloid deposition using MAGMA software (de Leeuw, Mooij, Heskes, & Posthuma, 2015). All SNPs were annotated to the respective genes using NCBI 37.3 build positions with no extra boundary regions around the genes, which resulted in 18,011 genes. Then, the gene-level association analysis was performed with "multi = all" command that computes principle components regression model, SNP-wise Top 1 (snp-wise = top) model and SNP-wise Mean (snp-wise = mean) model. The aggregate *p*-values from all these three models were calculated. The gene-based association analysis was controlled for age, sex, education and 4PCs. For the gene-based association analyses, the genome-wide significance was determined based on the Bonferroni method ($\alpha = 0.05/18{,}011$; $p < 2.78 \times 10^{-6}$).

Moreover, the functional gene pathway analyses were performed with Reactome, GeneOntology and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases downloaded from the MSigDB (version 7.0) database (Conrad et al.).

**Ⅲ-2-9-2. Gene network-based functional analyses**

Gene network analysis was performed using web-based GeneMANIA algorithm to evaluate the relationships among the candidate gene list using publicly available multiple biological datasets of protein-protein, genetic, protein-DNA interactions, co-localization, co-expression and shared protein domains (Bonham et al., 2018).

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### Ⅲ-2-9-3. Whole-brain β-amyloid deposition analyses

This study assessed the association of candidate risk SNPs with the β-amyloid depositions in the whole-brain using MATLAB's "fitglm" function for the general linear model with age, sex and education as covariates (Huijbers et al., 2019; Mathotaarachchi et al., 2016; Yue, Lindquist, & Loh, 2012). In addition, the model was corrected for multiple voxel-wise comparisons with the false discovery rate (FDR $q$-value<0.05) (Yao et al., 2019). The 3-D visualisation of brain map was plotted using Brain Net Viewer (Xia, Wang, & He, 2013) and the cerebral β-amyloid SUVR pattern was plotted using MRICRO software (version 1.40). This study also evaluated the effects of candidate SNP genotypes with overall mean SUVRs of β-amyloid by analysis of covariance (ANCOVA) using R program.

### Ⅲ-2-9-4. Replication analysis

SNPs in the most significantly associated genetic loci with Aβ deposition were further analysed using an independent replication Aβ cohort from Korean population. The replication Aβ cohort included 124 $^{18}$F-FMM and 68 $^{11}$C-PiB PET images (Table 14). The logistic regression was performed with amyloid status (2=Aβ positive and 1=Aβ negative) by adjusting for age, sex, education, amyloid probe (coded 2 for $^{11}$C-PiB and 1 for $^{18}$F-FMM) and 2PCs as covariates. Then, the sex stratified logistic regression was performed with amyloid status by adjusting for age, education, amyloid probe and 2PCs as covariates. Results from the Aβ study cohort and Aβ replication cohort were combined by meta-analysis using "metaphor" package in R software (Viechtbauer, 2010). Heterogeneity between the cohorts was evaluated using $I^2$ statistics and Cochran's Q test, and considered significant if $P_{Heterogeneity}$ ($P_{Het}$) <0.05 and $I^2$ >50%. A fixed effect inverse variance method to combine the estimated effects across the cohorts and a random effect method was used if there was significant heterogeneity. Moreover, this study evaluated the SNPs

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of interest in a European Ancestry Aβ cohort (ADNI) (Table 15). The linear regression with SUVR values and adjusted for age, sex, education and 6PCs as covariates was performed. Additionally, the logistic regression with amyloid status by adjusting for age, sex, education and 6PCs as covariates was performed. Sex stratified analysis were repeated with both SUVR values and amyloid status with age, education and 6PCs as covariates.

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**Table 14.** Demographic characteristics of participants for the Korean replication Aβ cohort from Inha University (INU).

| Amyloid probe | Groups | N | Age (y, mean ± SD) | Female (n, %) | Years of education (y, mean ± SD) |
|---|---|---|---|---|---|
| $^{18}$F-Flutemetamol | Positive | 50 | 73.78 ± 8.08 | 25 (50.00%) | 8.84 ± 4.51 |
| | Negative | 74 | 68.20 ± 8.95 | 41 (55.41%) | 10.61 ± 4.84 |
| $^{11}$C-Pittsburgh Compound B | Positive | 16 | 70.88 ± 7.50 | 11 (68.75%) | 8.47 ± 4.54 |
| | Negative | 52 | 68.35 ± 7.04 | 30 (57.69%) | 8.88 ± 5.06 |

Abbreviations: N, number of samples; SD, standard deviation; y, years.

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**Table 15.** Demographic characteristics of participants for the European Ancestry Aβ cohort from Alzheimer's Disease Neuroimaging Initiative (ADNI).

| Amyloid probe | Groups | N | Age (y, mean ± SD) | Female (n, %) | Years of education (y, mean ± SD) |
|---|---|---|---|---|---|
| $^{18}$F-Florbetapir | Positive | 331 | 73.84 ± 6.77 | 151 (45.62%) | 15.77 ± 2.86 |
| | Negative | 313 | 72.58 ± 7.15 | 135 (43.13%) | 16.52 ± 2.60 |

Abbreviations: N, number of samples; SD, standard deviation; y, years.

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Top Aβ deposition associations were also examined for AD status association within GARD and using common variant results from meta-analysis of non-Hispanic white GWAS datasets imputed to the Haplotype Reference Consortium (HRC) r1.1 reference panel (McCarthy et al., 2016) in the Alzheimer's disease Genetics Consortium (ADGC). For ADGC data, single-variant case-control association testing was performed within dataset using logistic regression under an additive genetic model on allelic dosages from imputation, with covariate adjustment only for principal components for population substructure (Chang et al., 2015; Price et al., 2006). Individual family datasets were analysed using GWAF/R (citation) with generalized estimating equations (GEE) implemented for common variants (MAF ≥ 0.01) (Chen & Yang, 2010). Within-dataset results were meta-analysed using an inverse-variance-weighted fixed-effects approach with genomic control in METAL (Willer, Li, & Abecasis, 2010). Additionally, the summary statistics of late onset AD association analysis downloaded from the International Genomics of Alzheimer's Project (IGAP) (J.-C. Lambert et al., 2013) were used to evaluate the AD risk in *CPAMD8* and *ARL14* loci SNPs for European Ancestry population. This study used the stage-1 meta-analysis results from the IGAP for the evaluation.

## Ⅲ-3. RESULTS

### Ⅲ-3-1. Novel genome-wide SNPs associated with β-amyloid deposition

In this study, 712 participants from the Korean population were analysed for the genome-wide SNP association with β-amyloid deposition in the brain. All the participants were clinically diagnosed as cognitively normal controls, MCI, other dementias and AD dementia at the time of β-amyloid PET scan.

The GWAS showed no evidence of spurious inflation in the association test statistics ($\lambda$ = 1.0) (Figure 7B). Moreover, the GWAS results revealed three independent genome-wide significant loci ($p < 5\times 10^{-8}$) associated with the cerebral

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β-amyloid deposition (Figure 7A, Table 16 and 17). The standard Alzheimer's disease associated peak was observed on chromosome 19 from rs429358 ($p = 3.22 \times 10^{-26}$) (Figure 7C) that codes for apolipoprotein (*APOE*) ε4 allele as anticipated. Additional SNPs around the same locus that showed genome-wide significant association to β-amyloid deposition were found in apolipoprotein C1 (*APOC1*), translocase of outer mitochondrial membrane 40 (*TOMM40*), apolipoprotein C1 pseudogene 1 (*APOC1P1*), and poliovirus receptor related 2 (*PVRL2*) genes. The two novel loci were located on chromosome 19 with index SNP, rs12975891 ($p = 4.42 \times 10^{-9}$) (Figure 7D) that codes for C3 and PZP like Alpha-2-Macroglobulin Domain containing 8 (*CPAMD8*) gene and chromosome 3 with index SNP, rs113454949 ($p = 1.09 \times 10^{-8}$) (Figure 7E) that codes for ADP-ribosylation factor-like 14 (*ARL14*) gene. Interestingly, a missense variant, rs3745339 in the *CPAMD8* gene showed genome-wide significance ($p = 1.26 \times 10^{-8}$). The locus on chromosome 3 showed additional SNPs with genome-wide significance in karyopherin subunit alpha 4 (*KPNA4*), intraflagellar transport 80 (*IFT80*), structural maintenance of chromosomes 4 (*SMC4*) and tripartite motif containing 59 (*TRIM59*) genes, and these SNPs were in strong linkage disequilibrium (LD) with rs113454949 (Figure 7E). When *APOE* ε4 allele status were added as covariate, the association strength at the *CPAMD8* and *ARL14* loci were not reduced (Figure 8A and B).

Furthermore, the variance explained by SNPs in the loci that showed genome-wide significant association to β-amyloid deposition were assesed. *APOE* rs429358 locus including SNPs in 400kb window explained for 8.9% (Standard error (SE) = 0.032, $p = 3.19 \times 10^{-9}$) of the variance, whereas the *CPAMD8* rs12975891 locus with 400kb window accounted for 3.2% (SE = 0.02, $p = 0.005$) of the variance. Due to huge LD block in *ARL14* rs113454949 locus, 1Mb window size was included and this locus explained for 2.9% (SE = 0.019, $p = 0.003$) of the variance. After the inclusion of *APOE* ε4 allele status as covariate, *CPAMD8* locus explained for 2.7%

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(SE = 0.019, $p$ = 0.01) of variance, and similarly *ARL14* locus accounted 2.7% (SE = 0.019, $p$ = 0.004) of variance. *APOE* rs429358 locus showed no variance as expected after ε4 allele status adjustment.

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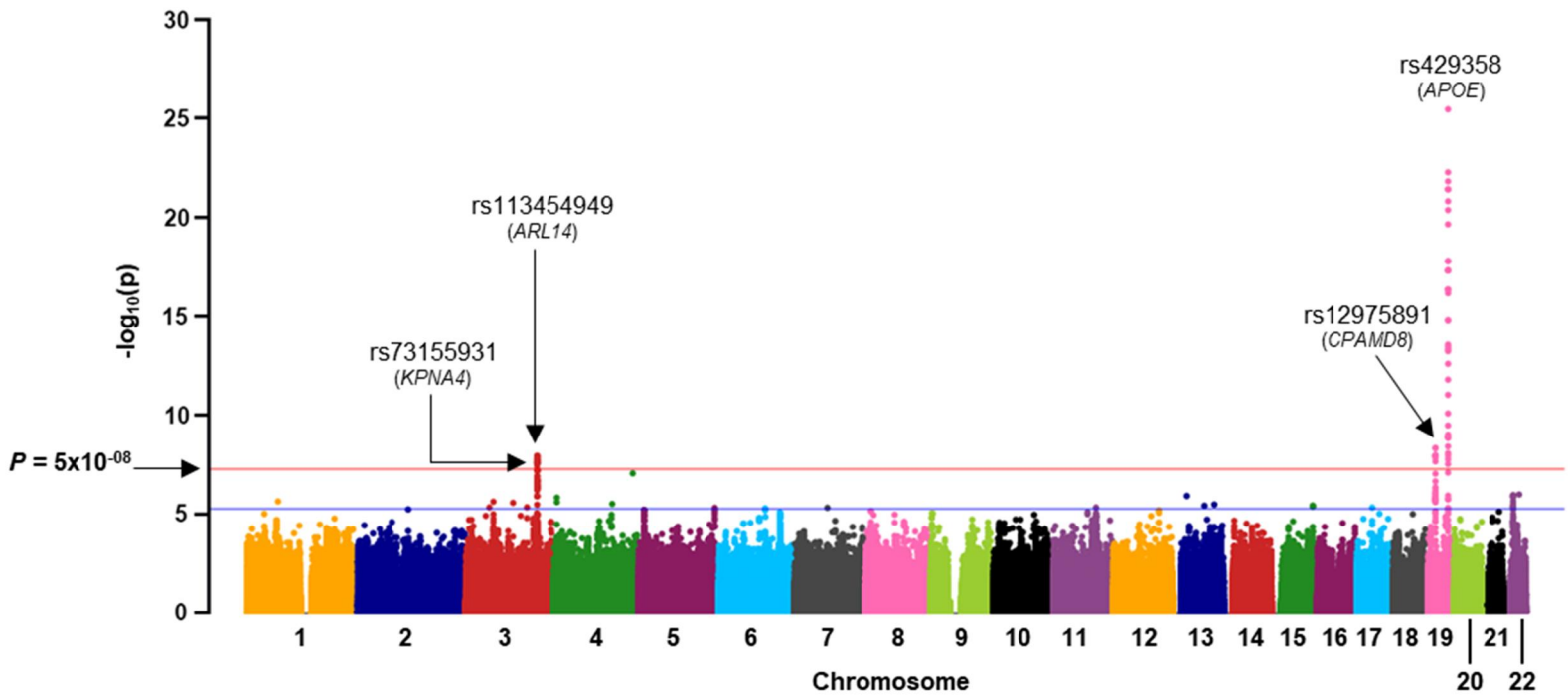


**Figure 7.** Continued

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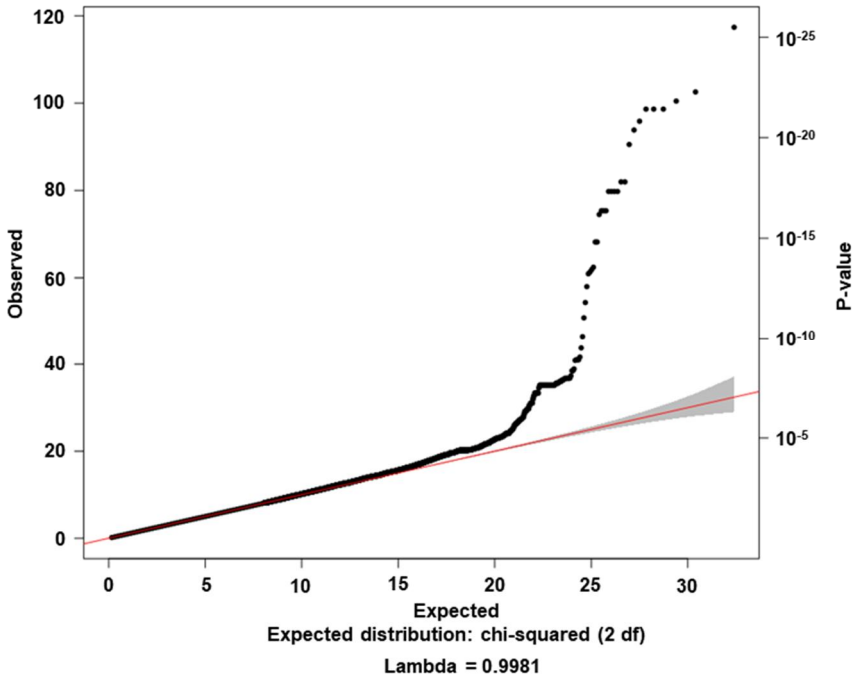
**B**




**C**

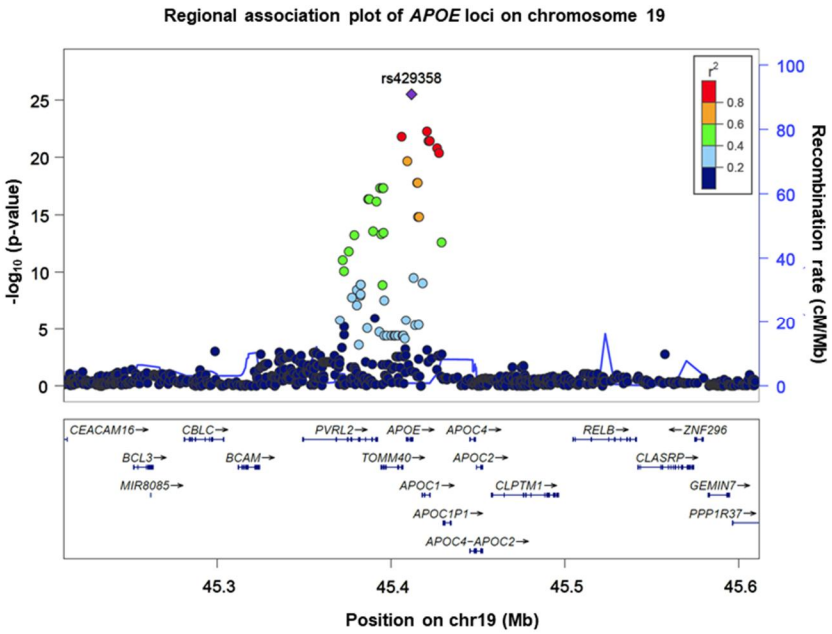



**Figure 7.** Continued

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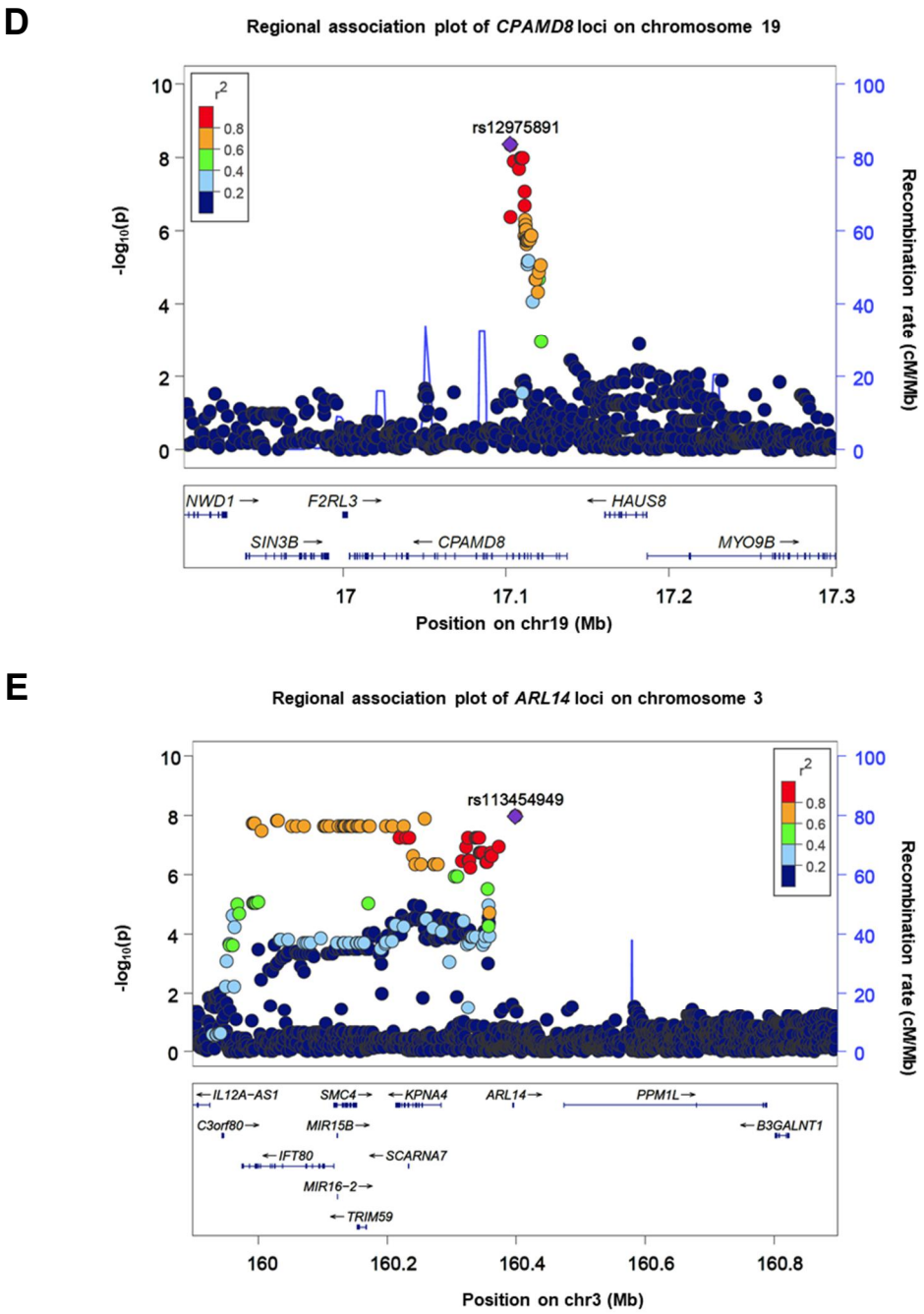


**Figure 7. Genetic variants associated with cerebral β-amyloid deposition.** (A) Manhattan plot shows the linear regression *p*-value for each single nucleotide

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polymorphism (SNP) in the genome (*p*-values displayed as $-\log_{10}$) with cerebral β-amyloid deposition under an additive genetic model adjusted for 4 PCs, age, sex and education as covariates. Genome-wide significance is displayed in red line for $p = 5 \times 10^{-8}$ threshold and suggestive significance displayed in blue line for $p = 5 \times 10^{-6}$ threshold. Three loci with genome-wide significance were identified on chromosome 19 at *APOE* and *CPAMD8* gene regions and on the chromosome 3 at *ARL14* gene region. Annotations are given for the top SNP with genome-wide significant associations. (B) Quantile-Quantile (QQ) plot shows observed data in $-\log_{10}$ *p*-values from the SNP-based GWAS of cerebral β-amyloid deposition adjusted for 4 PCs, age, sex and education as covariates (n=712). The observed data is indicated in black dots, expected data in red line and 95% confidence level zone in grey shade. The QQ plot showed no evidence of spurious genomic inflation ($\lambda = 1.0$). (C) Magnified regional association plots show regions around rs429358 at *APOE* locus (D) rs12975891 at the *CPAMD8* locus and (E) rs113454949 at the *ARL14* locus. The purple dot shows the most associated SNP in the region. The colours for all other SNPs indicate the LD $r^2$ value with most associated SNP ($r^2 \geq 0.8$ in red, $r^2$ between 0.6 and 0.8 in orange, $r^2$ between 0.4 and 0.6 in green, $r^2$ between 0.2 and 0.4 in light blue and $r^2 < 0.2$ in dark blue). The x-axis indicates base-pair position annotated to NCBI 37.3 build, the y-axis shows GWAS $-\log_{10}$ *p*-value and the blue peaks demonstrate recombination rates calculated from the 1000 genome project.

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**Table 16.** Top genetic variants at three loci with genome-wide significant association to cerebral β-amyloid deposition.

| SNP | Chromosome # | Gene | Base pair | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs429358 | 19 | *APOE* | 45411941 | Missense variant | C/T | 0.124 | **3.22E-26** | 0.102 | **6.93E-21** |
| rs12975891 | 19 | *CPAMD8* | 17102491 | Intron variant | A/C | 0.094 | **4.42E-09** | 0.077 | **1.34E-07** |
| rs113454949 | 3 | *ARL14* | 160398210 | Downstream gene variant | G/T | 0.121 | **1.09E-08** | 0.086 | **9.32E-06** |

Abbreviations: SNP, single nucleotide polymorphism; m, minor allele; M, major allele; N, number of samples.

[a]Adjusted for age, sex, education and 4PCs.

[b]Conditional analysis=Adjusted for age, sex, education, AD status and 4PCs.

Significant *p*-values are indicated in bold letters.

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**Table 17.** SNPs that were genome-wide significant association to cerebral β-amyloid deposition.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs429358 | 19 | *APOE* | 45411941 | Missense variant | C/T | 0.125 | **3.22E-26** | 0.102 | **6.93E-21** |
| rs12721046 | 19 | *APOC1* | 45421254 | Sequence feature | A/G | 0.115 | **5.30E-23** | 0.092 | **1.15E-17** |
| rs10119 | 19 | *TOMM40* | 45406673 | 3 prime UTR variant | A/G | 0.112 | **1.51E-22** | 0.092 | **1.03E-17** |
| rs12721051 | 19 | *APOC1* | 45422160 | Sequence feature | G/C | 0.112 | **3.79E-22** | 0.090 | **3.7E-17** |
| rs4420638 | 19 | *APOC1* | 45422946 | Downstream gene variant | G/A | 0.112 | **3.79E-22** | 0.090 | **3.7E-17** |
| rs56131196 | 19 | *APOC1* | 45422846 | Downstream gene variant | A/G | 0.112 | **3.79E-22** | 0.090 | **3.7E-17** |
| rs111789331 | 19 | *APOC1P1* | 45427125 | Upstream gene variant | A/T | 0.113 | **1.52E-21** | 0.092 | **9.72E-17** |
| rs66626994 | 19 | *APOC1P1* | 45428234 | Upstream gene variant | A/G | 0.113 | **4.16E-21** | 0.091 | **2.28E-16** |
| rs769449 | 19 | *APOE* | 45410002 | Sequence feature | A/G | 0.126 | **2.18E-20** | 0.098 | **1.24E-14** |
| rs10414043 | 19 | *APOC1* | 45415713 | Upstream gene variant | A/G | 0.116 | **1.58E-18** | 0.089 | **4.23E-13** |
| rs7256200 | 19 | *APOC1* | 45415935 | Upstream gene variant | T/G | 0.116 | **1.58E-18** | 0.089 | **4.23E-13** |
| rs11556505 | 19 | *TOMM40* | 45396144 | Synonymous variant | T/C | 0.109 | **4.73E-18** | 0.085 | **2.25E-13** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs2075650 | 19 | *CTB-129P6.4* | 45395619 | Upstream gene variant | G/A | 0.109 | **4.73E-18** | 0.085 | **2.25E-13** |
| rs34404554 | 19 | *CTB-129P6.4* | 45395909 | Upstream gene variant | G/C | 0.109 | **4.73E-18** | 0.085 | **2.25E-13** |
| rs71352238 | 19 | *CTB-129P6.4* | 45394336 | Upstream gene variant | C/T | 0.109 | **4.73E-18** | 0.085 | **2.25E-13** |
| rs12972156 | 19 | *PVRL2* | 45387459 | Sequence feature | G/C | 0.107 | **4.32E-17** | 0.082 | **2.64E-12** |
| rs12972970 | 19 | *PVRL2* | 45387596 | Sequence feature | A/G | 0.107 | **4.32E-17** | 0.082 | **2.64E-12** |
| rs34342646 | 19 | *PVRL2* | 45388130 | Sequence feature | A/G | 0.107 | **4.32E-17** | 0.082 | **2.64E-12** |
| rs6857 | 19 | *PVRL2* | 45392254 | 3 prime UTR variant | T/C | 0.105 | **6.60E-17** | 0.080 | **6.27E-12** |
| rs438811 | 19 | *APOC1* | 45416741 | Upstream gene variant | T/C | 0.084 | **1.55E-15** | 0.068 | **1.92E-12** |
| rs483082 | 19 | *APOC1* | 45416178 | Upstream gene variant | T/G | 0.084 | **1.55E-15** | 0.068 | **1.92E-12** |
| rs283815 | 19 | *PVRL2* | 45390333 | Sequence feature | G/A | 0.077 | **2.78E-14** | 0.063 | **1.26E-11** |
| rs157582 | 19 | *CTB-129P6.4* | 45396219 | Upstream gene variant | T/C | 0.077 | **3.68E-14** | 0.062 | **1.27E-11** |
| rs184017 | 19 | *CTB-129P6.4* | 45394969 | Upstream gene variant | G/T | 0.076 | **4.84E-14** | 0.063 | **1.06E-11** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs412776 | 19 | *PVRL2* | 45379516 | Sequence feature | A/G | 0.085 | **5.88E-14** | 0.070 | **1.40E-11** |
| rs60049679 | 19 | *APOC1P1* | 45429708 | Upstream gene variant | C/G | 0.091 | **2.56E-13** | 0.076 | **1.77E-11** |
| rs519113 | 19 | *PVRL2* | 45376284 | Sequence feature | G/C | 0.077 | **1.58E-12** | 0.064 | **9.46E-11** |
| rs404935 | 19 | *PVRL2* | 45372794 | Sequence feature | A/G | 0.075 | **9.18E-12** | 0.063 | **3.23E-10** |
| rs395908 | 19 | *PVRL2* | 45373565 | Sequence feature | A/G | 0.072 | **7.94E-11** | 0.060 | **2.98E-09** |
| rs75627662 | 19 | *APOC1* | 45413576 | Upstream gene variant | T/C | 0.072 | **3.19E-10** | 0.054 | **2.06E-07** |
| rs5117 | 19 | *APOC1* | 45418790 | Sequence feature | C/T | 0.070 | **9.19E-10** | 0.051 | **9.70E-07** |
| rs34224078 | 19 | *PVRL2* | 45383115 | Sequence feature | G/A | 0.077 | **1.25E-09** | 0.058 | **6.22E-07** |
| rs35879138 | 19 | *PVRL2* | 45383139 | Sequence feature | A/T | 0.077 | **1.25E-09** | 0.058 | **6.22E-07** |
| rs71352237 | 19 | *PVRL2* | 45383079 | Sequence feature | C/T | 0.077 | **1.25E-09** | 0.058 | **6.22E-07** |
| rs157581 | 19 | *TOMM40* | 45395714 | Synonymous variant | C/T | 0.058 | **1.35E-09** | 0.049 | **1.30E-08** |
| rs3865427 | 19 | *PVRL2* | 45380961 | Sequence feature | A/C | 0.075 | **3.59E-09** | 0.056 | **1.40E-06** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs12975891 | 19 | *CPAMD8* | 17102491 | Intron variant | A/C | 0.094 | **4.42E-09** | 0.077 | **1.34E-07** |
| rs7256009 | 19 | *CPAMD8* | 17102939 | Intron variant | A/C | 0.094 | **4.42E-09** | 0.077 | **1.34E-07** |
| 19:45383061 | 19 | *PVRL2* | 45383061 | Sequence feature | G/T | 0.057 | **7.88E-09** | 0.045 | **5.00E-07** |
| rs11666342 | 19 | *CPAMD8* | 17109548 | Downstream gene variant | G/A | 0.089 | **1.04E-08** | 0.070 | **6.52E-07** |
| rs11670464 | 19 | *CPAMD8* | 17110128 | Downstream gene variant | T/C | 0.089 | **1.04E-08** | 0.070 | **6.52E-07** |
| rs7251841 | 19 | *CPAMD8* | 17110572 | Downstream gene variant | T/C | 0.089 | **1.04E-08** | 0.070 | **6.52E-07** |
| rs8105013 | 19 | *CPAMD8* | 17109350 | Downstream gene variant | C/T | 0.089 | **1.04E-08** | 0.070 | **6.52E-07** |
| 19:45382966 | 19 | *PVRL2* | 45382966 | Sequence feature | C/T | 0.056 | **1.07E-08** | 0.044 | **8.97E-07** |
| rs113454949 | 3 | *ARL14* | 160398210 | Downstream gene variant | G/T | 0.121 | **1.09E-08** | 0.086 | **9.32E-06** |
| rs7648271 | 3 | *ARL14-SNORA72* | 160401724 | Intergenic region | G/T | 0.121 | **1.09E-08** | 0.086 | **9.32E-06** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| 19:45382966 | 19 | *PVRL2* | 45382966 | Sequence feature | C/T | 0.056 | **1.07E-08** | 0.044 | **8.97E-07** |
| rs113454949 | 3 | *ARL14* | 160398210 | Downstream gene variant | G/T | 0.121 | **1.09E-08** | 0.086 | **9.32E-06** |
| rs7648271 | 3 | *ARL14-SNORA72* | 160401724 | Intergenic region | G/T | 0.121 | **1.09E-08** | 0.086 | **9.32E-06** |
| rs3745339 | 19 | *CPAMD8* | 17108053 | Missense variant | T/C | 0.090 | **1.26E-08** | 0.072 | **5.25E-07** |
| rs8107156 | 19 | *CPAMD8* | 17105057 | Intron variant | C/T | 0.090 | **1.26E-08** | 0.072 | **5.25E-07** |
| rs73155931 | 3 | *KPNA4* | 160259437 | Sequence feature | A/G | 0.107 | **1.29E-08** | 0.079 | **3.91E-06** |
| rs112036095 | 3 | *RP11-432B6.3* | 160031445 | Intron variant | C/T | 0.114 | **1.51E-08** | 0.082 | **7.89E-06** |
| rs73154549 | 3 | *IFT80* | 160030330 | Upstream gene variant | C/T | 0.114 | **1.51E-08** | 0.082 | **7.89E-06** |
| rs73154552 | 3 | *RP11-432B6.3* | 160031986 | Intron variant | A/G | 0.114 | **1.51E-08** | 0.082 | **7.89E-06** |
| rs34278513 | 19 | *PVRL2* | 45378144 | Sequence feature | T/C | 0.072 | **1.63E-08** | 0.054 | **3.40E-06** |
| rs112239616 | 3 | *RP11-432B6.3* | 159992556 | Intron variant | A/G | 0.117 | **1.84E-08** | 0.082 | **1.77E-05** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs3737214 | 3 | *IFT80* | 159995356 | Upstream gene variant | G/A | 0.117 | **1.84E-08** | 0.082 | **1.77E-05** |
| rs55930449 | 3 | *RP11-432B6.3* | 159994587 | Intron variant | C/T | 0.117 | **1.84E-08** | 0.082 | **1.77E-05** |
| rs56030166 | 3 | *RP11-432B6.3* | 159994763 | Intron variant | C/T | 0.117 | **1.84E-08** | 0.082 | **1.77E-05** |
| rs12982311 | 19 | *CPAMD8* | 17108283 | Downstream gene variant | G/A | 0.088 | **2.05E-08** | 0.070 | **8.52E-07** |
| rs35281166 | 3 | *KPNA4* | 160227220 | Sequence feature | C/T | 0.110 | **2.29E-08** | 0.081 | **6.23E-06** |
| rs111800736 | 3 | *RP11-432B6.3* | 160107100 | Intron variant | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs112186271 | 3 | *IFT80* | 160044691 | Sequence feature | A/T | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs113773657 | 3 | *IFT80* | 160105241 | Upstream gene variant | C/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs1141023 | 3 | *RP11-432B6.3* | 160156951 | Synonymous variant | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs17236389 | 3 | *IFT80* | 160060573 | Sequence feature | G/T | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs17236452 | 3 | *SMC4* | 160145296 | Sequence feature | G/A | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs17826161 | 3 | *IFT80* | 160111960 | 5 prime UTR variant | T/A | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs1979160 | 3 | *SMC4* | 160147686 | Sequence feature | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs2029361 | 3 | *KPNA4* | 160209895 | Downstream gene variant | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs41272955 | 3 | *SMC4* | 160142424 | Sequence feature | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs41272957 | 3 | *SMC4* | 160142554 | Sequence feature | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs4254702 | 3 | *RP11-227J5.3* | 160174138 | Downstream gene variant | C/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs4679883 | 3 | *SMC4* | 160125603 | Upstream gene variant | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs4679887 | 3 | *TRIM59* | 160208546 | Upstream gene variant | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs4680577 | 3 | *IFT80* | 160053532 | Sequence feature | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs4680582 | 3 | *SMC4* | 160135286 | Sequence feature | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs4680590 | 3 | *TRIM59* | 160199834 | Intron variant | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs55853078 | 3 | *RP11-432B6.3* | 160159899 | Intron variant | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs56051094 | 3 | *SMC4* | 160133344 | Sequence feature | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs56322040 | 3 | *SMC4* | 160131055 | Sequence feature | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs6776582 | 3 | *IFT80* | 160072020 | Sequence feature | G/T | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs73154567 | 3 | *IFT80* | 160103123 | Upstream gene variant | C/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs73154591 | 3 | *SMC4* | 160136726 | Sequence feature | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs73154596 | 3 | *SMC4* | 160144863 | Sequence feature | T/C | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs73154600 | 3 | *TRIM59* | 160154627 | 3 prime UTR variant | A/G | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs73154602 | 3 | *TRIM59* | 160154971 | 3 prime UTR variant | C/T | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs73155903 | 3 | *TRIM59* | 160155021 | 3 prime UTR variant | C/T | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |
| rs7622031 | 3 | *RP11-432B6.3* | 160172180 | Upstream gene variant | A/T | 0.112 | **2.35E-08** | 0.081 | **9.43E-06** |

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**Table 17.** Continued.

| SNP | Chromosome # | Gene | BP | SNP type | m/M | Beta[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Beta | *p*-value |
| rs59007384 | 19 | *CTB-129P6.4* | 45396665 | Upstream gene variant | T/G | 0.062 | **2.82E-08** | 0.047 | **4.19E-06** |
| rs2152424 | 3 | *RP11-432B6.3* | 160006351 | Intron variant | C/T | 0.113 | **3.31E-08** | 0.080 | **2.09E-05** |

Abbreviations: SNP, single nucleotide polymorphism; BP, base pair position; MAF, minor allele frequency; N, number of samples.

[a]Adjusted for age, sex, education and 4PCs.

[b]Conditional analysis=Adjusted for age, sex, education, AD status and 4PCs.

Significant *p*-values are indicated in bold letters.

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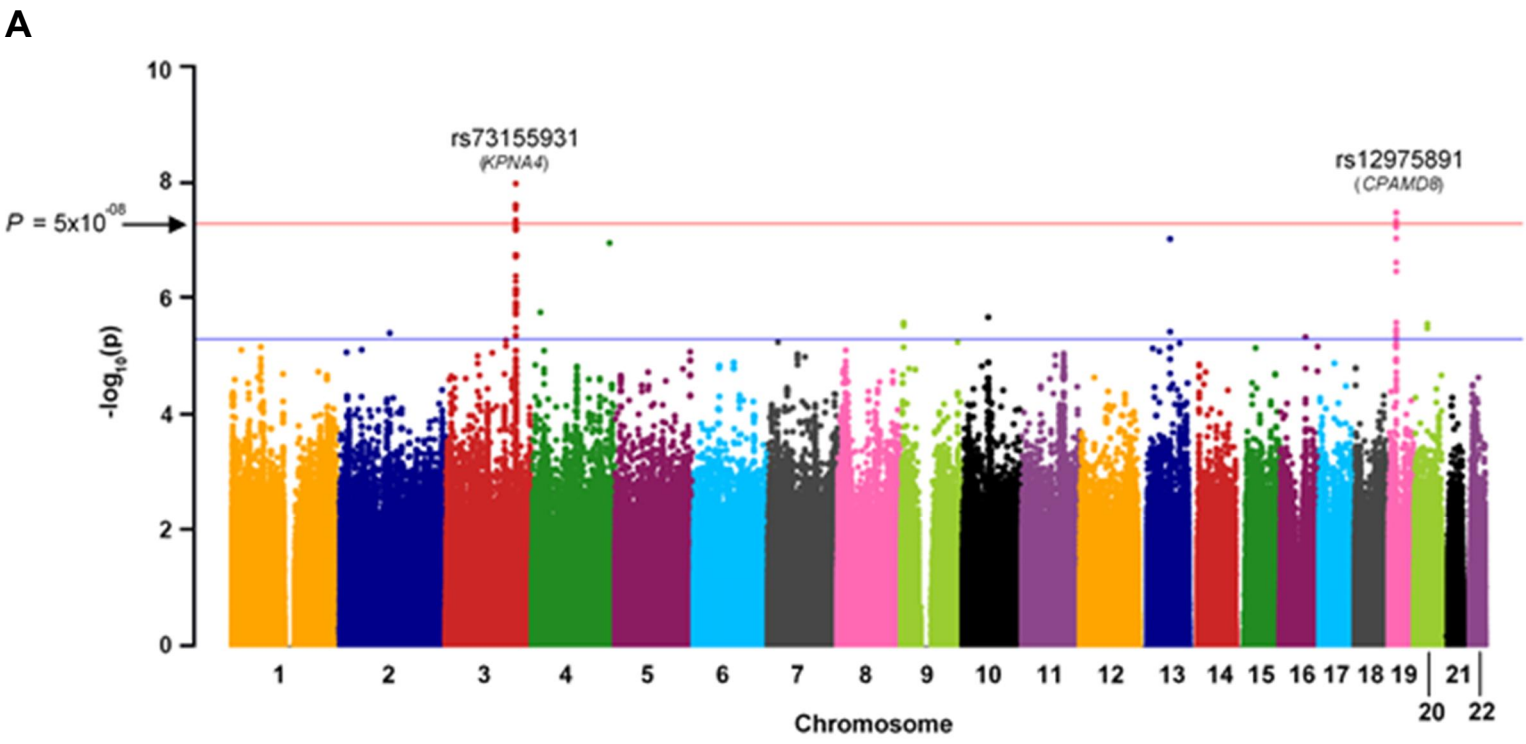


**Figure 8.** Continued

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B

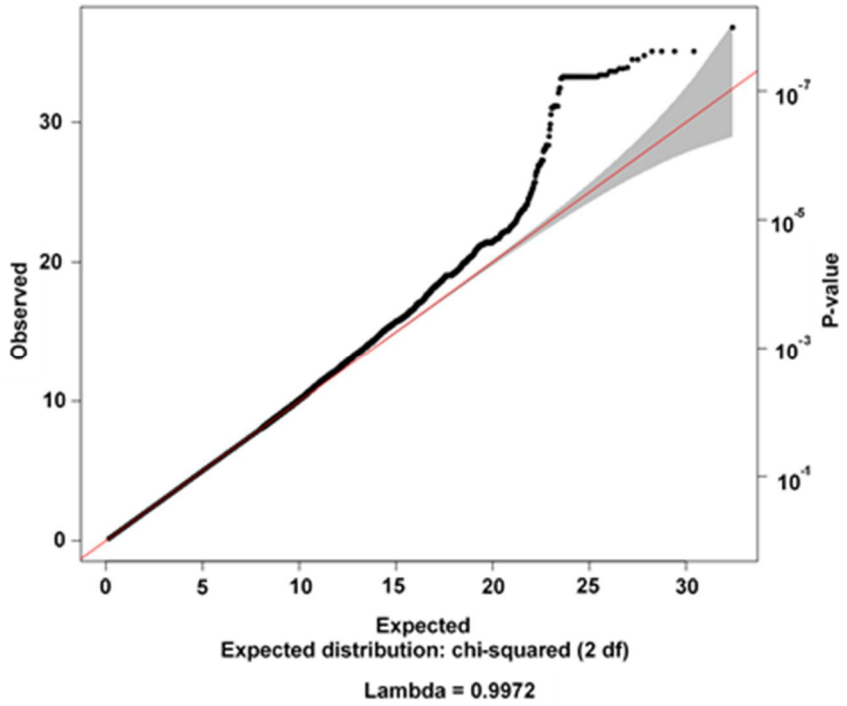


**Figure 8. Genetic variants associated with cerebral β-amyloid deposition after correcting for *APOE* □4 status.** (A) Manhattan plot shows the linear regression *p* value for each single nucleotide polymorphism (SNP) in the genome (*p*-values displayed as $-\log_{10}$) with cerebral β-amyloid deposition under an additive genetic model adjusted for 4 PCs, age, sex, education and *APOE* □4 allele as covariates (n=712). Genome-wide significance is displayed in red line for $p = 5 \times 10^{-8}$ threshold and suggestive significance displayed in blue line for $p = 5 \times 10^{-6}$ threshold. *CPAMD8* and *KPNA4* (lies proximal to *ARL14*) genetic loci on the chromosome 19 and 3 showed genome-wide significance. (B) Quantile-Quantile (QQ) plot shows observed data in $-\log_{10}$ *p*-values from the SNP-based GWAS of cerebral β-amyloid deposition adjusted for 4 PCs, age, sex, education and *APOE* □4 allele as covariates. The observed data is indicated in black dots, expected data in red line and 95%

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confidence level zone in grey shade. The QQ plot showed no evidence of spurious genomic inflation ($\lambda = 1.0$).

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### Ⅲ-3-2. Genes associated with β-amyloid deposition

In the gene-based analysis, the results showed three independent peaks (Figure 9A) with genome-wide significance with similarity to the SNP-based GWAS results and observed no inflation in the association statistic ($\lambda = 0.96$) (Figure 9B). In the *APOE* locus, *APOC1* ($p = 7.38 \times 10^{-21}$) emerged as the most significantly associated gene to β-amyloid deposition and the *APOE* ($p = 6.08 \times 10^{-19}$) gene, which is located near *APOC1* also exhibited genome-wide significant association. In addition to *APOE* locus in chromosome 19, *CPAMD8* ($p = 4.33 \times 10^{-8}$) gene showed genome-wide significant association that was observed in the SNP-based GWAS. Another novel locus on chromosome 3 revealed *TRIM59* ($p = 5.33 \times 10^{-8}$), *SMC4* ($p = 1.02 \times 10^{-7}$), *KPNA4* ($p = 1.41 \times 10^{-7}$) and *IFT80* ($p = 1.85 \times 10^{-7}$) genes with genome-wide significance (Table 18 and 19). This shows that there was a strong LD in the *ARL14* rs113454949 locus as reflected in the SNP-based GWAS.

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**Table 18**. Top 10 most associated genes with cerebral Aβ deposition.

| Gene | Chromosome # | Start | Stop | No. of SNPs | N | ZSTAT[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ZSTAT | *p*-value |
| *APOC*1 | 19 | 45417577 | 45422606 | 6 | 712 | 9.29 | **7.38E-21** | 7.85 | **2.13E-15** |
| *APOE* | 19 | 45409039 | 45412650 | 5 | 712 | 8.81 | **6.08E-19** | 8.42 | **1.94E-17** |
| *PVRL2* | 19 | 45349393 | 45392485 | 132 | 712 | 8.57 | **5.17E-18** | 6.96 | **1.70E-12** |
| *TOMM40* | 19 | 45394477 | 45406946 | 37 | 712 | 8.56 | **5.83E-18** | 7.87 | **1.84E-15** |
| *CPAMD8* | 19 | 17003762 | 17137625 | 336 | 712 | 5.35 | **4.33E-08** | 4.23 | **1.19E-05** |
| *TRIM59* | 3 | 160153291 | 160167626 | 25 | 712 | 5.32 | **5.33E-08** | 3.89 | **4.97E-05** |
| *SMC4* | 3 | 160117078 | 160152755 | 64 | 712 | 5.20 | **1.02E-07** | 3.87 | **5.44E-05** |
| *KPNA4* | 3 | 160212783 | 160283376 | 110 | 712 | 5.14 | **1.41E-07** | 3.99 | **3.29E-05** |
| *IFT80* | 3 | 159974774 | 160118028 | 176 | 712 | 5.08 | **1.85E-07** | 3.54 | **1.97E-04** |
| *LYZL6* | 17 | 34261522 | 34270714 | 30 | 712 | 4.15 | **1.63E-05** | 2.26 | **0.01** |

Abbreviations: Start, start base pair position; Stop, end base pair position; SNP, single nucleotide polymorphism; NSNPs, number of single nucleotide polymorphsims; N, number of samples.

[a]Adjusted for age, sex, education and 4PCs.

[b]Conditional analysis=Adjusted for age, sex, education, AD status and 4PCs. Significant *p*-values are indicated in bold letters.

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**Table 19**. Association of top 10 genes with cerebral β-amyloid deposition after LD pruning.

| Gene | Chromosome # | Start | Stop | No. of SNPs | N | ZSTAT[a] | *p*-value[a] | [b]Conditional analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ZSTAT | *p*-value |
| *APOC1* | 19 | 45417577 | 45422606 | NA | 712 | NA | NA | NA | NA |
| *APOE* | 19 | 45409039 | 45412650 | NA | 712 | NA | NA | NA | NA |
| *PVRL2* | 19 | 45349393 | 45392485 | 14 | 712 | **4.77** | **9.19E-07** | **3.48** | **2.52E-04** |
| *TOMM40* | 19 | 45394477 | 45406946 | 1 | 712 | 0.88 | 0.19 | 0.54 | 0.29 |
| *CPAMD8* | 19 | 17003762 | 17137625 | 46 | 712 | **2.33** | **9.86E-03** | 0.95 | 0.17 |
| *TRIM59* | 3 | 160153291 | 160167626 | 2 | 712 | -1.70 | 0.96 | -0.37 | 0.64 |
| *SMC4* | 3 | 160117078 | 160152755 | 10 | 712 | **1.91** | **0.03** | 1.55 | 0.06 |
| *KPNA4* | 3 | 160212783 | 160283376 | 19 | 712 | **2.58** | **5.28E-03** | **2.50** | **6.27E-03** |
| *IFT80* | 3 | 159974774 | 160118028 | 18 | 712 | **2.97** | **1.48E-03** | **2.59** | **4.76E-03** |
| *LYZL6* | 17 | 34261522 | 34270714 | 1 | 712 | 0.44 | 0.33 | 0.70 | 0.24 |

Abbreviations: Start, start base pair position; Stop, end base pair position; SNP, single nucleotide polymorphism; NSNPs, number of single nucleotide polymorphsims; N, number of samples.

[a]Adjusted for age, sex, education and 4PCs.

[b]Conditional analysis=Adjusted for age, sex, education, AD status and 4PCs. Significant *p*-values are indicated in bold letters.

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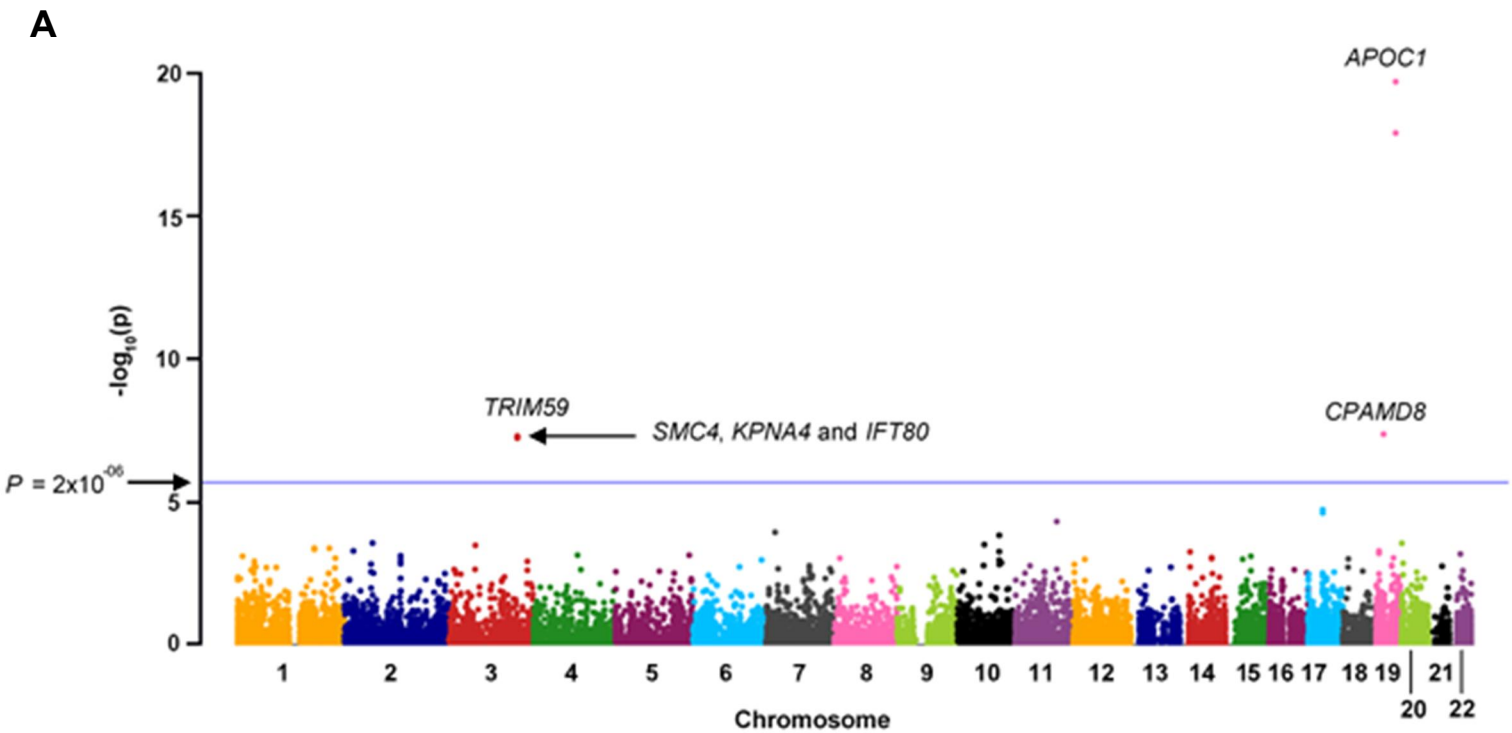


**Figure 9.** Continued

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**B**

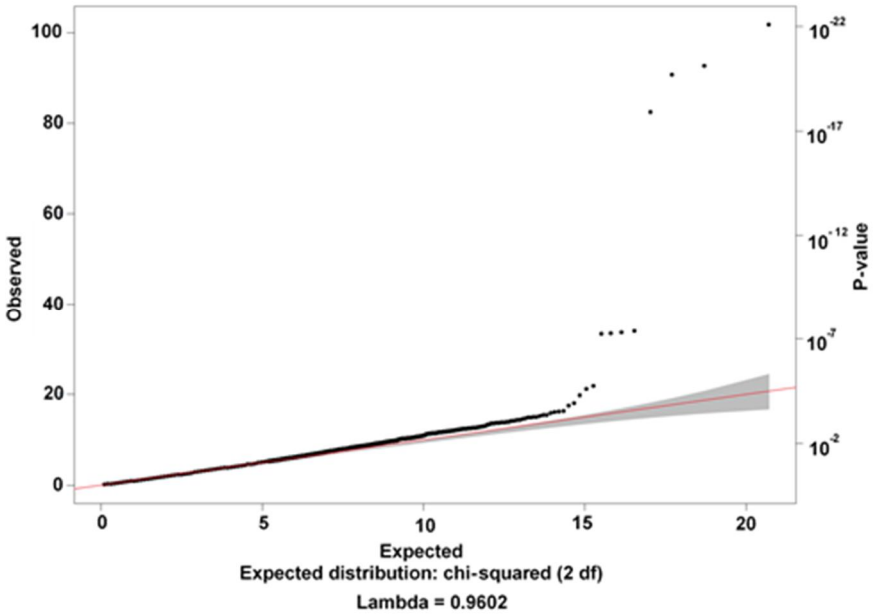


**Figure 9. Genes associated with cerebral β-amyloid deposition.** (A) Manhattan plot shows the linear regression *p*-value for each genes in the genome (*p*-values displayed as $-\log_{10}$) with cerebral β-amyloid deposition adjusted for 4 PCs, age, sex and education as covariates (n=712). Genome-wide significance is displayed in blue line for $p = 2 \times 10^{-6}$ threshold. *CPAMD8*, *TRIM59*, *SMC4*, *KPNA4* and *IFT80* genes on the chromosome 19 and 3 showed genome-wide significance. (B) Quantile-Quantile (QQ) plot shows observed data in $-\log_{10}$ *p*-values from the gene-based GWAS of cerebral β-amyloid deposition adjusted for 4 PCs, age, sex and education as covariates. The observed data is indicated in black dots, expected data in red line and 95% confidence level zone in grey shade. The QQ plot showed no evidence of spurious genomic inflation ($\lambda = 0.96$).

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### Ⅲ-3-3. Gene networks involved in β-amyloid deposition

To evaluate the relationship among the candidate genes, this study included a list of 34 genes ($p < 0.001$) for the gene network analysis that were associated with β-amyloid deposition. The candidate genes showed relationship with known AD risk genes through co-expression and co-localization (Figure 10). *CPAMD8* gene was related to *APCO1* and *APOE* genes by co-expression with *AGBL2*, *SCEC31B* and *NFIA* genes. Moreover, the *IFT80*, *SMC4* and *KPNA4* genes were connected to *TOMM40* through co-expression with *SMC3*, *ACTR2*, *TOMM70* and *CDK1* genes. *SMC4* was functionally related to *APOE* and *APOA2* in protein *heterodimerization activity*.

### Ⅲ-3-4. Gene pathways associated with β-amyloid deposition

All the genes from gene-based GWAS were included in the gene pathway analyses with Reactome, KEGG and GO pathway libraries to identify the gene functional pathways associated with β-amyloid deposition. In Reactome pathway analysis, the *transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer* ($p = 4.60 \times 10^{-5}$) (Figure 11A and Table 20) pathway was the most significantly associated pathway with cerebral β-amyloid deposition, which also showed significance after Bonferroni correction ($p \leq 0.05$). However, the KEGG and GO pathway analyses showed no significant pathways after Bonferroni correction ($p \leq 0.05$). The *KEGG TGF beta signalling pathway* ($p = 0.002$) was the most significant pathway in KEGG pathway analysis (Figure 11B and Table 21), whereas the GO pathway analysis showed *GO feeding behaviour* ($p = 8.30 \times 10^{-5}$) as the top significant pathway (Figure 11C and Supplementary Table 22).

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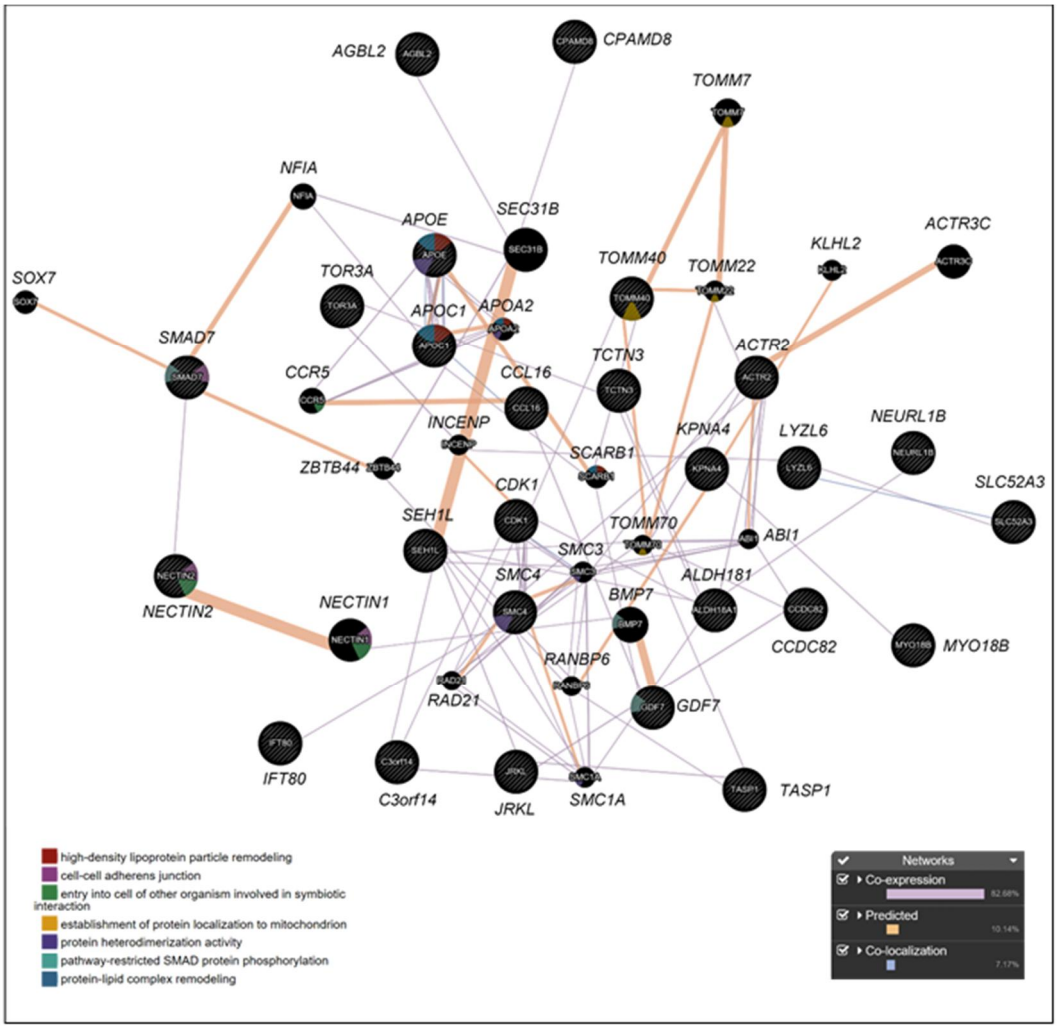


**Figure 10. Gene network for candidate genes associated with cerebral Aβ deposition.** Gene network was plotted with 34 genes ($p < 0.001$) that were most associated with cerebral Aβ deposition from the gene-based GWAS. The six candidate genes were found anchored to the known AD specific genes (*APOE*, *APOC1* and *TOMM40*). Shared functional relations between the genes were shown in different shades of colours within the circles.

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**Table 20**. Reactome pathways associated with cerebral Aβ deposition.

| Reactome pathway | Number of genes | Beta | Beta standard | Standard error | *p*-value |
|---|---|---|---|---|---|
| Transcriptional activity of *SMAD2/SMAD3*:*SMAD4* Heterotrimer | 30 | 0.56 | 0.02 | 0.14 | **4.61E-05** |
| Loss of function of *TGFBR1* in cancer | 52 | 0.37 | 0.02 | 0.11 | **4.03E-04** |
| *SMAD2/SMAD3*:*SMAD4* Heterotrimer regulates transcription | 19 | 0.59 | 0.02 | 0.18 | **5.91E-04** |
| G0 and early G1 | 20 | 0.48 | 0.02 | 0.16 | **1.89E-03** |
| Metabolism of polyamines | 10 | 0.65 | 0.02 | 0.25 | **3.87E-03** |
| Ribosomal scanning and start codon recognition | 27 | 0.38 | 0.02 | 0.15 | **4.45E-03** |
| Downregulation of *SMAD2/3*:*SMAD4* transcriptional activity | 17 | 0.50 | 0.02 | 0.19 | **5.50E-03** |
| *CDC20*:Phospho-APC/C mediated degradation of cyclin A | 44 | 0.27 | 0.01 | 0.10 | **5.50E-03** |
| Signalling by BMP | 21 | 0.45 | 0.02 | 0.18 | **6.01E-03** |
| Incretin synthesis, secretion, and inactivation | 16 | 0.55 | 0.02 | 0.22 | **6.00E-03** |

Top 10 most associated Reactome gene pathways with cerebral Aβ deposition were shown.

Significant *p*-values are indicated in bold letters.

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**Table 21**. KEGG pathways associated with cerebral Aβ deposition.

| KEGG pathway | Number of genes | Beta | Beta standard | Standard error | *p*-value |
|---|---|---|---|---|---|
| KEGG *TGF*-beta signalling pathway | 84 | 0.26 | 0.02 | 0.09 | **2.01E-03** |
| KEGG O-glycan biosynthesis | 28 | 0.38 | 0.02 | 0.18 | **0.02** |
| KEGG Endocytosis | 176 | 0.13 | 0.01 | 0.06 | **0.02** |
| KEGG Pentose phosphate pathway | 22 | 0.31 | 0.01 | 0.17 | **0.03** |
| KEGG Cell cycle | 119 | 0.13 | 0.01 | 0.07 | **0.04** |
| KEGG *P53* signalling pathway | 66 | 0.15 | 0.01 | 0.10 | 0.07 |
| KEGG Ether lipid metabolism | 33 | 0.21 | 0.01 | 0.14 | 0.07 |
| KEGG Proteasome | 43 | 0.15 | 0.01 | 0.11 | 0.08 |
| KEGG Taurine and hypota urine metabolism | 10 | 0.34 | 0.01 | 0.24 | 0.08 |
| KEGG Oocyte meiosis | 106 | 0.11 | 0.01 | 0.08 | 0.08 |

Top 10 most associated KEGG gene pathways with cerebral Aβ deposition were shown.

Significant *p*-values are indicated in bold letters.

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**Table 22**. GO pathways associated with cerebral Aβ deposition.

| GO pathway | Number of genes | Beta | Beta standard | Standard error | *p*-value |
|---|---|---|---|---|---|
| GO Feeding behaviour | 93 | 0.35 | 0.03 | 0.09 | **8.30E-05** |
| GO Eating behaviour | 32 | 0.54 | 0.02 | 0.15 | **1.16E-04** |
| GO Regulation of feeding behaviour | 26 | 0.64 | 0.02 | 0.18 | **1.26E-04** |
| GO Protein targeting to lysosome | 19 | 0.62 | 0.01 | 0.17 | **1.32E-04** |
| GO Tubulin complex assembly | 6 | 1.08 | 0.02 | 0.30 | **1.56E-04** |
| GO Histone kinase activity | 17 | 0.66 | 0.02 | 0.18 | **1.57E-04** |
| GO N-terminal protein amino acid acetylation | 15 | 0.68 | 0.02 | 0.19 | **2.29E-04** |
| GO Histone methyl-transferase activity H4-K20 specific | 5 | 1.16 | 0.02 | 0.33 | **2.72E-04** |
| GO Regulation of protein oligomerization | 42 | 0.43 | 0.02 | 0.12 | **2.83E-04** |
| GO Regulation of protein localization to nucleus | 108 | 0.25 | 0.02 | 0.07 | **3.20E-04** |

Top 10 most associated GO gene pathways with cerebral Aβ deposition were shown.

Significant *p*-values are indicated in bold letters.

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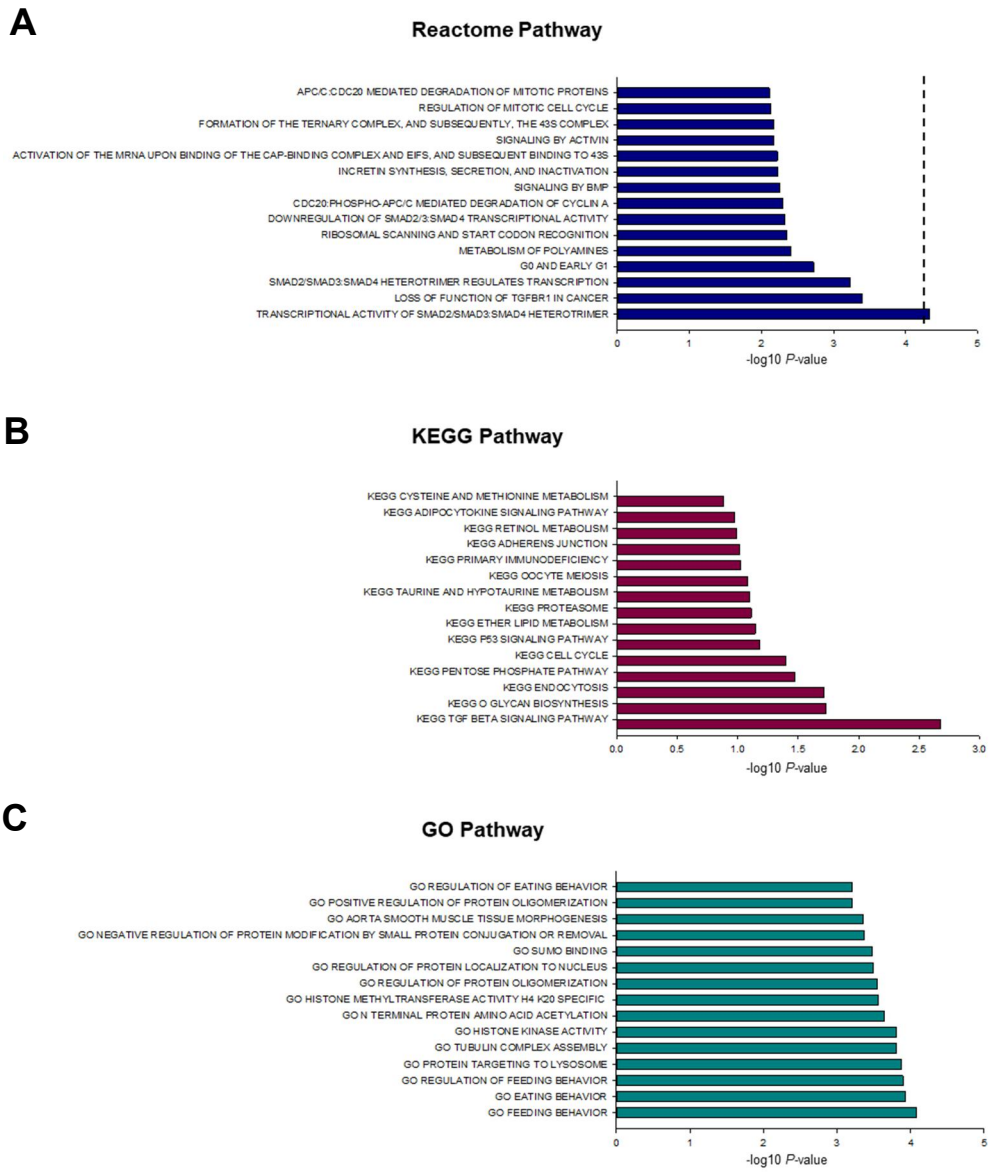


**Figure 11. Gene pathways associated with cerebral β-amyloid deposition.** Gene pathway analysis from (A) Reactome (B) KEGG and (C) GO pathway libraries showed the significantly associated gene pathways with cerebral β-amyloid deposition. The dotted line indicates the gene pathway that survived Bonferroni correction ($p \leq 0.05$).

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### Ⅲ-3-5. Association of *CPAMD8* rs12975891 and *ARL14* rs113454949 risk alleles in the whole brain β-amyloid deposition

To confirm the effects of rs12975891 SNP in *CPAMD8* locus and rs113454949 SNP in *ARL14* locus on the brain β-amyloid burden, the association of dominant model SNP genotypes with the β-amyloid load in the whole brain were assesed. *CPAMD8* rs12975891-A allele individuals exhibited high levels of β-amyloid load than the rs12975891-C/C allele individuals specifically in the brain anatomical regions of entorhinal, frontal, precuneus, temporal and insula (Figure 12A-D). Similarly, *ARL14* rs113454949-G allele individuals showed greater β-amyloid load than the rs113454949-T/T individuals in the anatomical regions of entorhinal, frontal, cuneus, precuneus, temporal, triangularis, opercularis, precentral and postcentral (Figure 12A-E). However, rs113454949-G allele individuals exhibited more severe β-amyloid load in the right hemisphere of frontal, cuneus and precuneus regions than the left hemisphere. Whereas, the triangularis, opercularis, precentral and postcentral regions showed greater β-amyloid deposition in the left hemisphere than the right hemisphere.

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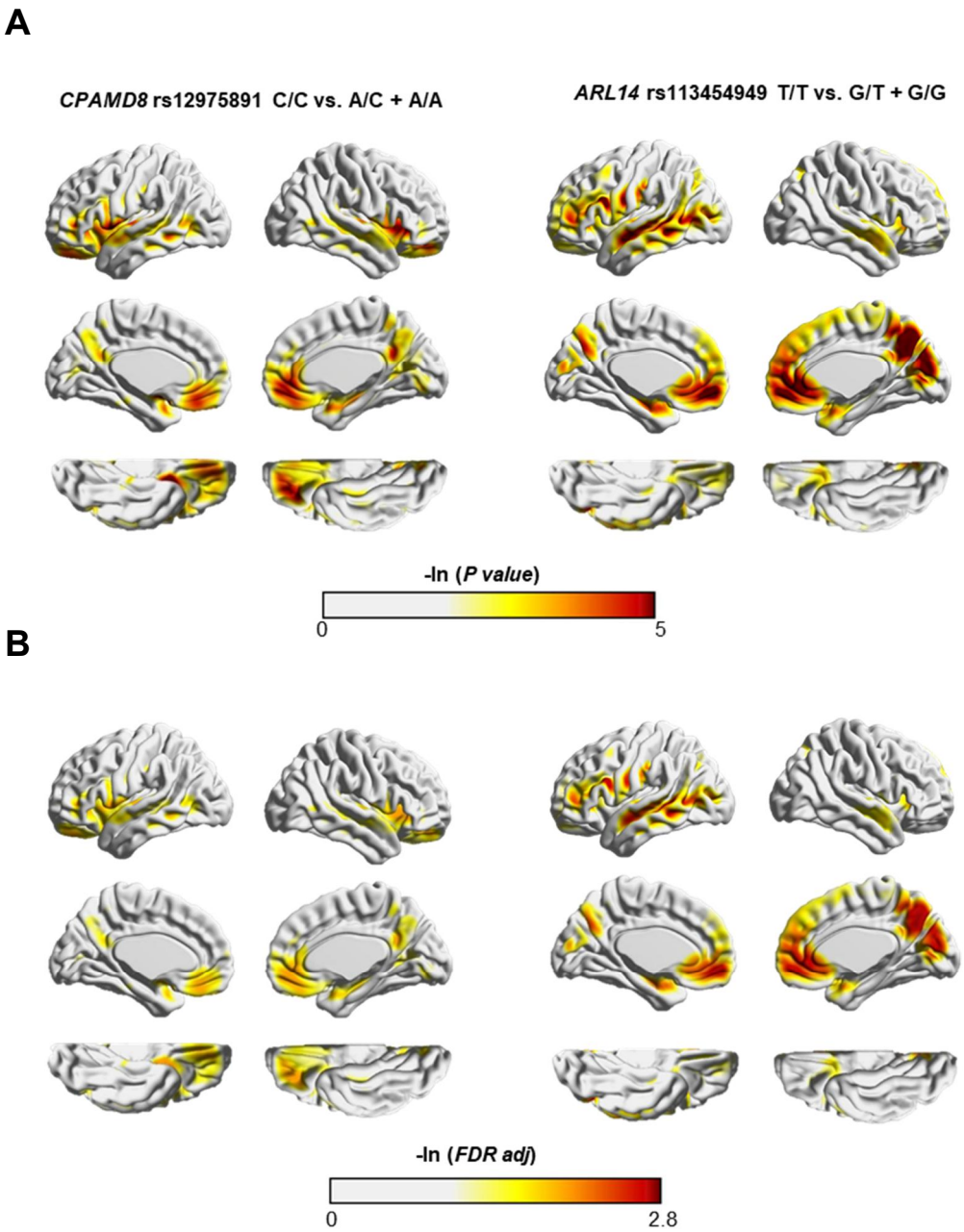


**Figure 12.** Continued.

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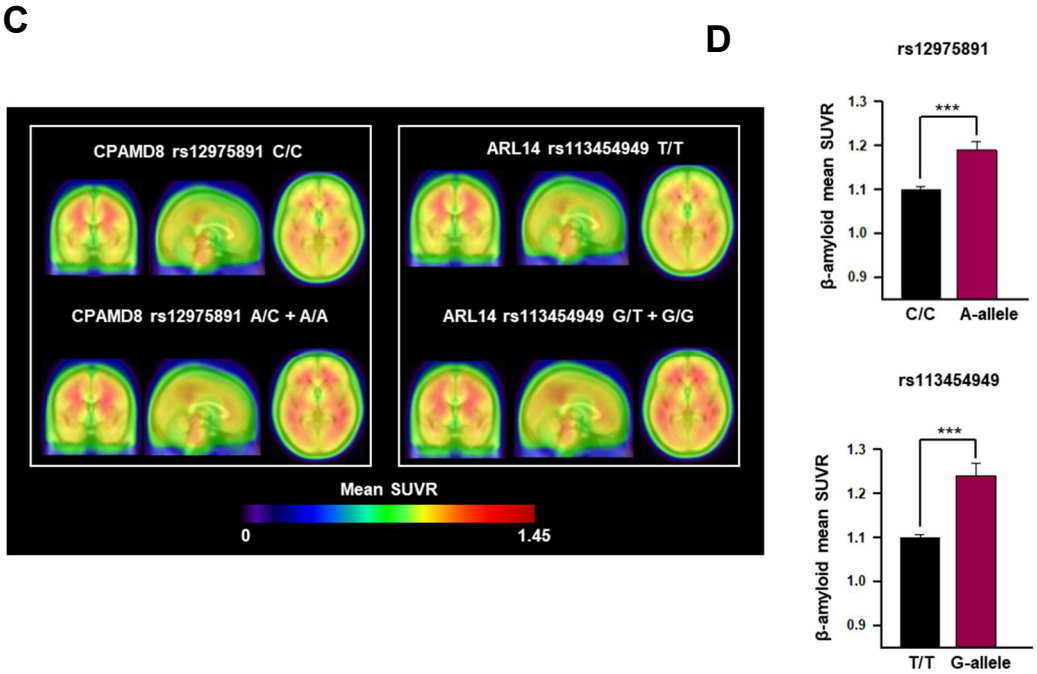


**Figure 12. *CPAMD8* rs12975891-A allele and *ARL14* rs113454949-G allele individuals exhibit severe patterns of cerebral β-amyloid deposition.** (A) Brain map illustrates the spatial pattern of voxel-wise cerebral β-amyloid deposition. A general linear model was performed to observe the differences in voxel-wise β-amyloid burden using *CPAMD8* rs12975891 (rs12975891-C/C (n=591) vs. rs12975891-A/C + A/A (n=121)) and *ARL14* rs113454949 (rs113454949-T/T (n=646) vs. rs113454949-G/T + G/G (n=66)) genotypes as predictor with age, sex and education as covariates. The voxel-wise $-\log_{10}$ *p*-values are superimposed on the standard ICBM152 brain template. (B) The voxel-wise false discovery rate (FDR) corrected $-\log_{10}$ *Q*-values are superimposed on the standard ICBM152 brain. (C) The voxel-wise mean standard value uptake ratios (SUVRs) were compared between *CPAMD8* rs1297589-C/C and rs1297589-A/C + A/A individuals in left panel, and the voxel-wise SUVRs were compared between *ARL14* rs113454949-T/T

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and rs113454949-G/T + G/G individuals in the right panel. The plot displays cerebral β-amyloid SUVR topology overlaid on the standard avg152T1 brain template and the colour bar below indicates the mean β-amyloid SUVR levels. (D) Bar plot indicates the comparison of average β-amyloid burden between *CPAMD8* rs1297589-C/C and rs1297589-A/C + A/A individuals. (E) Differences in the average β-amyloid load between *ARL14* rs113454949-T/T and rs113454949-G/T + G/G individuals are shown in bar plot. The error bars are indicated above the bar plots (*$p$<0.05,**$p$<0.01,***$p$<0.001).

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### Ⅲ-3-6. Gender influences the association of *CPAMD8* rs12975891 and *ARL14* rs113454949 with β-amyloid deposition

To evaluate the role of genders in the association of *CPAMD8* rs12975891 and *ARL14* rs113454949 with β-amyloid burden, sex-stratified SNP-based regional association test was performed. SNPs in the *CPAMD8* (400 kb window of rs12975891) and *ARL14* (1 Mb window of rs113454949) loci were included in the association test with age, education and 4PCs as covariates. Sex-stratified analysis revealed that *CPAMD8* rs12975891 was highly associated with the β-amyloid deposition in the female group ($p = 2.49 \times 10^{-9}$) than male group ($p = 0.07$) (Figure 13A and B), whereas *ARL14* rs113454949 exhibited strong association with β-amyloid deposition in the male-specific group ($p = 4.02 \times 10^{-6}$) than the female-specific group ($p = 0.003$) (Figure 13C and D). Anyhow, in the *ARL14* locus, rs35281166 in *KPNA4* gene emerged as the top significant ($p = 2.02 \times 10^{-8}$) variant in the male-specific group, which was also in strong LD ($r^2 = 0.78$) with rs113454949 in *ARL14* gene. To confirm the results from the sex-stratified analyses, the mean SUVR score of β-amyloid between the SNP genotypes split by male and female groups were compared. Even though there was a significant difference between rs12975891-C/C and rs12975891-A allele individuals in the β-amyloid levels, the strength of association with β-amyloid burden was different among male and female groups. Rs12975891-A allele individuals (F = 31.21, $p = 4.52 \times 10^{-8}$) in female group showed stronger association with β-amyloid burden than the rs12975891-A allele individuals (F = 6.37, $p = 0.012$) in male groups compared to rs12975891-C/C individuals (Figure 13E). In contrast, rs113454949-G allele individuals (F = 28.82, $p = 1.50 \times 10^{-7}$) in male group exhibited greater association with β-amyloid burden than the rs113454949-G allele individuals (F = 11.06, $p = 0.001$) in female group in comparison with rs113454949-T/T individuals (Figure 13F).

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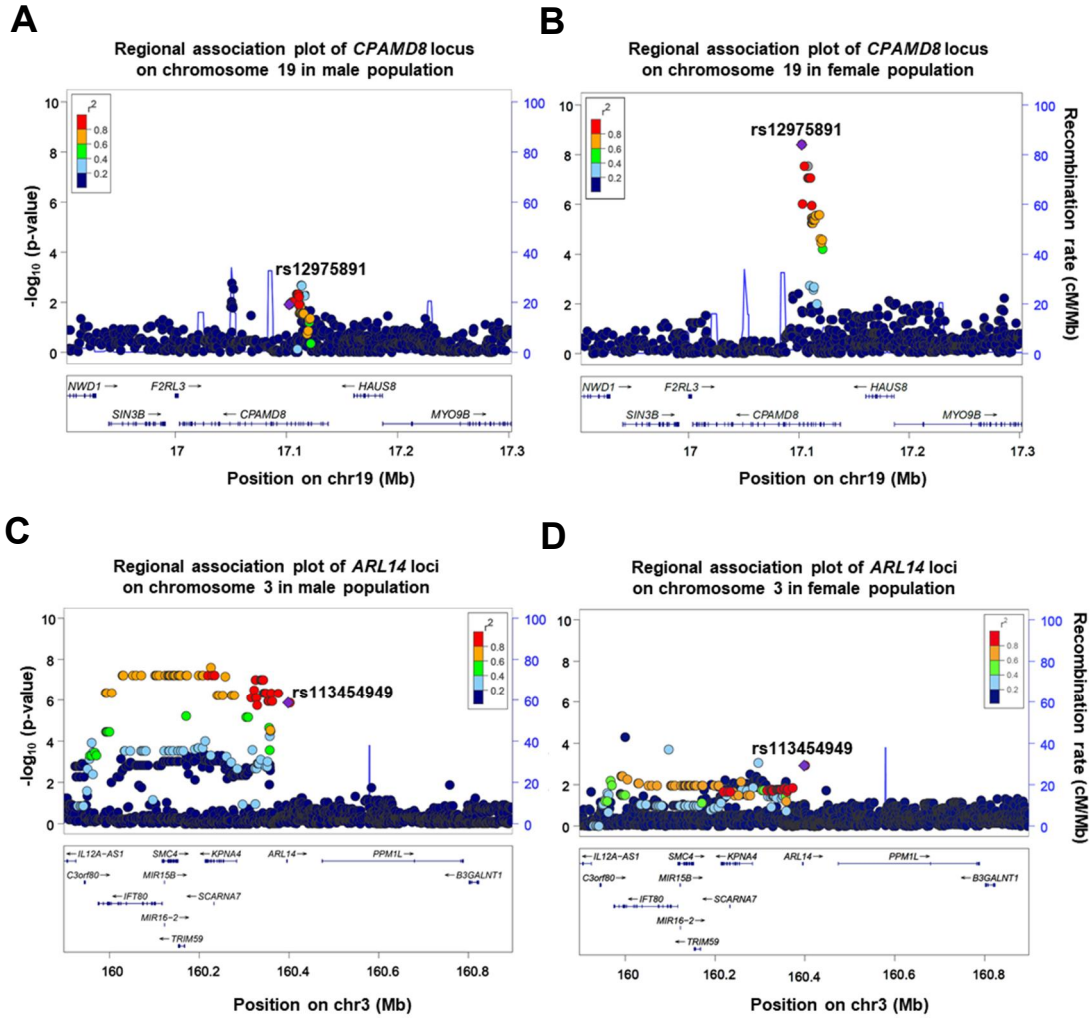


**Figure 13.** Continued.

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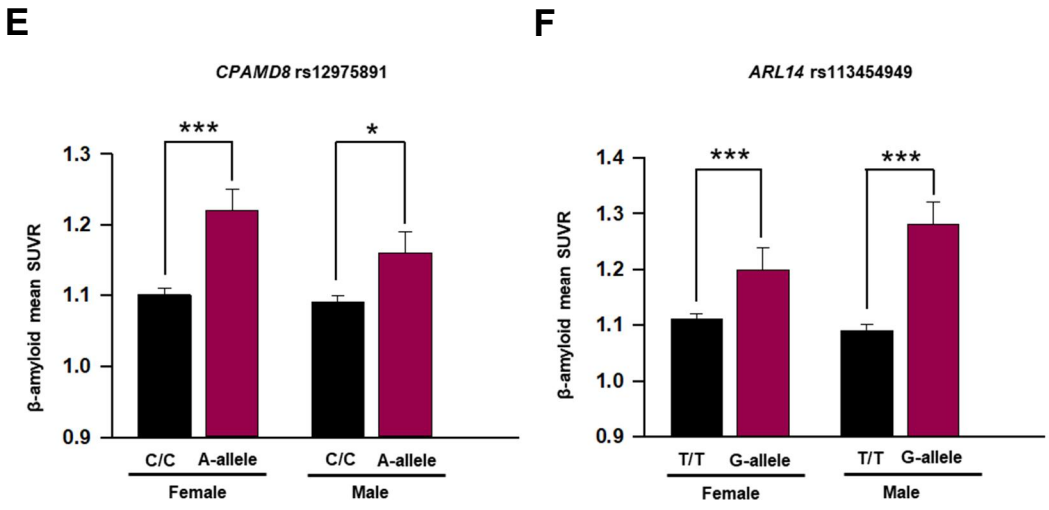


**Figure 13. Gender influences the association of *CPAMD8* rs12975891 and *ARL14* rs113454949 with cerebral β-amyloid deposition.** The linear regression *p*-value with cerebral Aβ deposition for each single nucleotide polymorphism (SNP) in the *ARL14* (*p*-values displayed as $-\log_{10}$) genetic loci adjusted for age, education and four PCs for population substructure as covariates were plotted. Regional plots of *ARL14* rs113454949 in male (n=337) **(a)** and female (n=375) **(b)** specific population. **(c)** Bar plot shows the comparison of average Aβ burden between *CPAMD8* rs1297589-C/C (n=279 in males; n=312 in females) and rs1297589-A/C + A/A (n=58 in males; n=63 in females) individuals stratified by male and female specific groups (*$p<0.05$,**$p<0.01$,***$p<0.001$). **(d)** The average Aβ burden compared between *ARL14* rs113454949-T/T (n=309 in males; n=337 in females) and rs113454949-G/T + G/G (n=28 in males; n=38 in females) individuals stratified by male and female specific groups (*$p<0.05$,**$p<0.01$,***$p<0.001$).

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### Ⅲ-3-7. *CPAMD8* rs12975891 and *ARL14* rs113454949 mediates β-amyloid deposition in *APOE* ε4 independent manner

To determine whether the *APOE* ε4 allele status mediate the β-amyloid deposition in the *CPAMD8* and *ARL14* loci, this study performed *APOE* ε4 stratified SNP-based association analysis. SNPs in the 400 kb window of rs12975891 and the SNPs in the 1 Mb window of rs113454949 were included in the association analysis with age, sex education and 4PCs as covariates. The results showed that the association of *CPAMD8* rs12975891 with β-amyloid was stronger among *APOE* ε4 non-carriers ($p = 1.07 \times 10^{-5}$) compared to *APOE* ε4 carriers ($p = 0.004$) (Figure 14A and B). Likewise, *ARL14* rs113454949 exhibited stronger association with β-amyloid in *APOE* ε4 non-carriers ($P = 9.44 \times 10^{-7}$) than *APOE* ε4 carriers ($P = 0.04$) (Figure 14C and D). This study further evaluated the results with the mean SUVR score of β-amyloid levels between the SNP genotypes stratified by *APOE* ε4 allele status. This study observed that mean SUVR scores in both rs12975891 and rs113454949 genotypes among *APOE* ε4 carriers were greater than the mean SUVR score of these genotypes among *APOE* ε4 non-carriers. However, the strength of differences for the β-amyloid levels between rs12975891-C/C and rs12975891-A allele were greater among *APOE* ε4 non-carriers (F = 14.01, $p = 2.04 \times 10^{-4}$) than *APOE* ε4 carriers (F = 10.24, $p = 0.002$) (Figure 14E). Similar pattern of strong differences in the β-amyloid levels between rs113454949-T/T and rs113454949-G allele were observed among *APOE* ε4 non-carriers (F = 26.99, $p = 3.03 \times 10^{-7}$) compared to *APOE* ε4 carriers (F = 6.62, $p = 0.01$) (Figure 14F).

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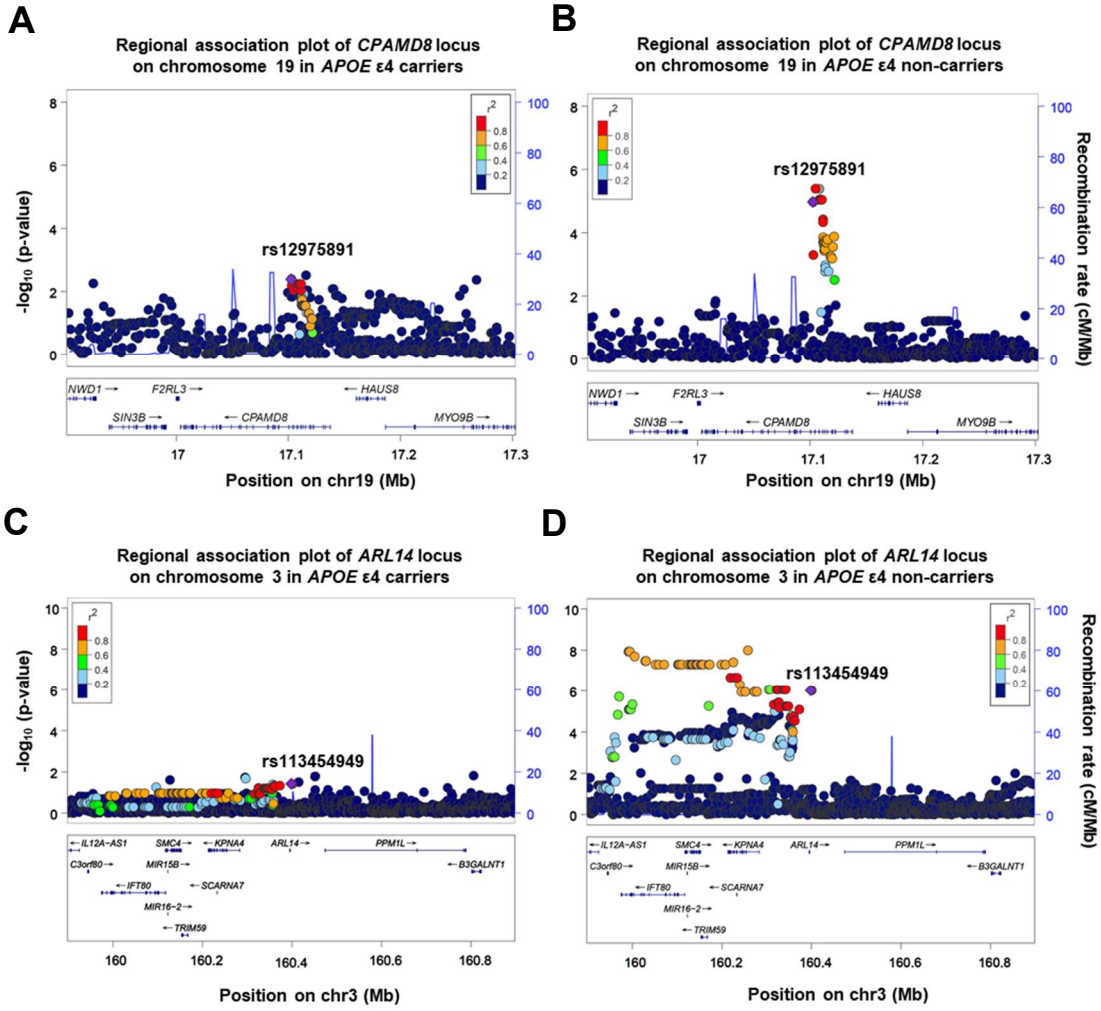


**Figure 14.** Continued.

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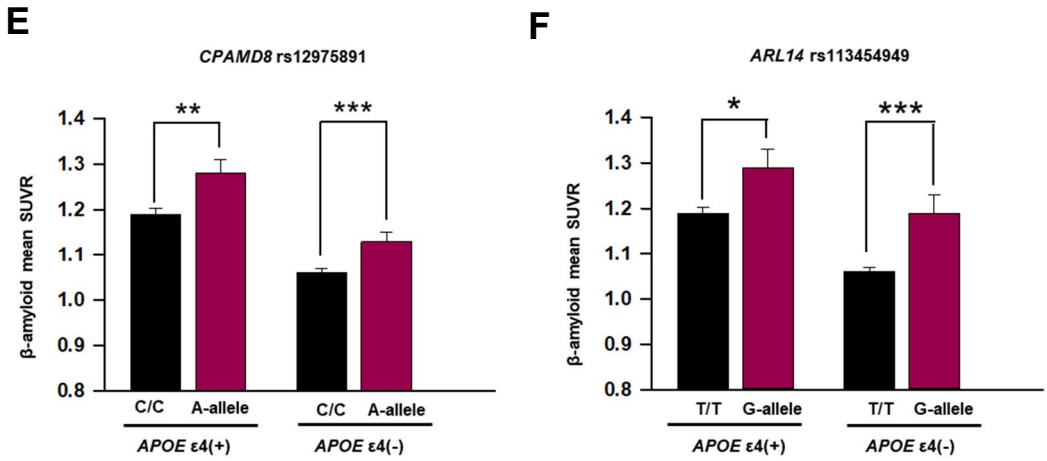


**Figure 14. *CPAMD8* rs12975891 and *ARL14* rs113454949 mediates cerebral β-amyloid deposition in *APOE* ε4 independent manner.** The linear regression *p*-value with cerebral β-amyloid deposition for each single nucleotide polymorphism (SNP) in the *CPAMD8* and *ARL14* (*p*-values is displayed as $-\log_{10}$) genetic loci adjusted for age, sex, education and 4PCs as covariates were plotted. Regional plots of *CPAMD8* rs12975891 in *APOE* ε4 (n=222) (A) and *APOE* non-ε4 (n=490) (B) carriers for association with β-amyloid deposition. Regional plots of *ARL14* rs113454949 in *APOE* ε4 (n=222) (C) and *APOE* non-ε4 (n=490) (D) carriers. (E) Bar plot shows the comparison of average β-amyloid burden between *CPAMD8* rs1297589-C/C (n=173 for *APOE* ε4 carriers; n=418 for *APOE* non-ε4 carriers) and rs1297589-A/C + A/A (n=49 for *APOE* ε4 carriers; n=72 for *APOE* non-ε4 carriers) individuals stratified by *APOE* ε4 allele status (*$p<0.05$,**$p<0.01$,***$p<0.001$). (F) The average β-amyloid burden compared between *ARL14* rs113454949-T/T (n=193 for *APOE* ε4 carriers; n=453 *APOE* for non-ε4 carriers) and rs113454949-G/T + G/G (n=29 for *APOE* ε4 carriers; n=37 for *APOE* non-ε4 carriers) individuals stratified by *APOE* ε4 allele status (*$p<0.05$,**$p<0.01$,***$p<0.001$).

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### Ⅲ-3-8. AD-associated variation of H3K27ac in the *CPAMD8* and *ARL14* loci

The SNP-based GWAS and gene-based GWAS showed strong association of *CPAMD8* gene with β-amyloid deposition, but the candidate risk gene in the *ARL14* genetic locus remain unclear due to the presence of huge LD block. Entorhinal cortex tissue specific AD associated variation in H3K27ac and DNA modifications were analysed to identify the active enhancers and promoters in the *APOE*, *CPAMD8* and *ARL14* genetic loci. The top five most associated H3K27ac peaks with AD in these three genetic loci were shown in Table 23. In the *APOE* locus, the most significant AD associated hyper-acetylated peak ($p = 2.13 \times 10^{-4}$; log fold change = 0.48; FDR corrected $p$-value < 0.05) was located in the *TOMM40* gene, which is also located close to *APOE* gene. Similarly, in the *CPAMD8* genetic locus, a strong AD associated hyper-acetylated peak ($p = 8.44 \times 10^{-4}$; log fold change = 0.42; FDR corrected $p$-value < 0.05) was located in the *CPAMD8* gene. This H3K27ac peak of *CPAMD8* gene was within the top five most AD associated peaks in that locus. Moreover, in the *ARL14* genetic locus, among the top five most AD associated H3K27ac peaks, one of the strong AD associated hypo-acetylated peak ($p = 4.57 \times 10^{-4}$; log fold change = -0.50; FDR corrected $p$-value < 0.05) was located in the *IFT80* gene and this gene exhibited genome-wide significance ($p < 2.78 \times 10^{-6}$) in the gene-based GWAS (Figure 15). The other top AD associated H3K27ac peaks in the *ARL14* genetic locus were located in the *C3orf80*, *PPM1L* and *AK097161* genes, but these genes showed no statistical significance ($p < 0.05$) in the gene-based GWAS.

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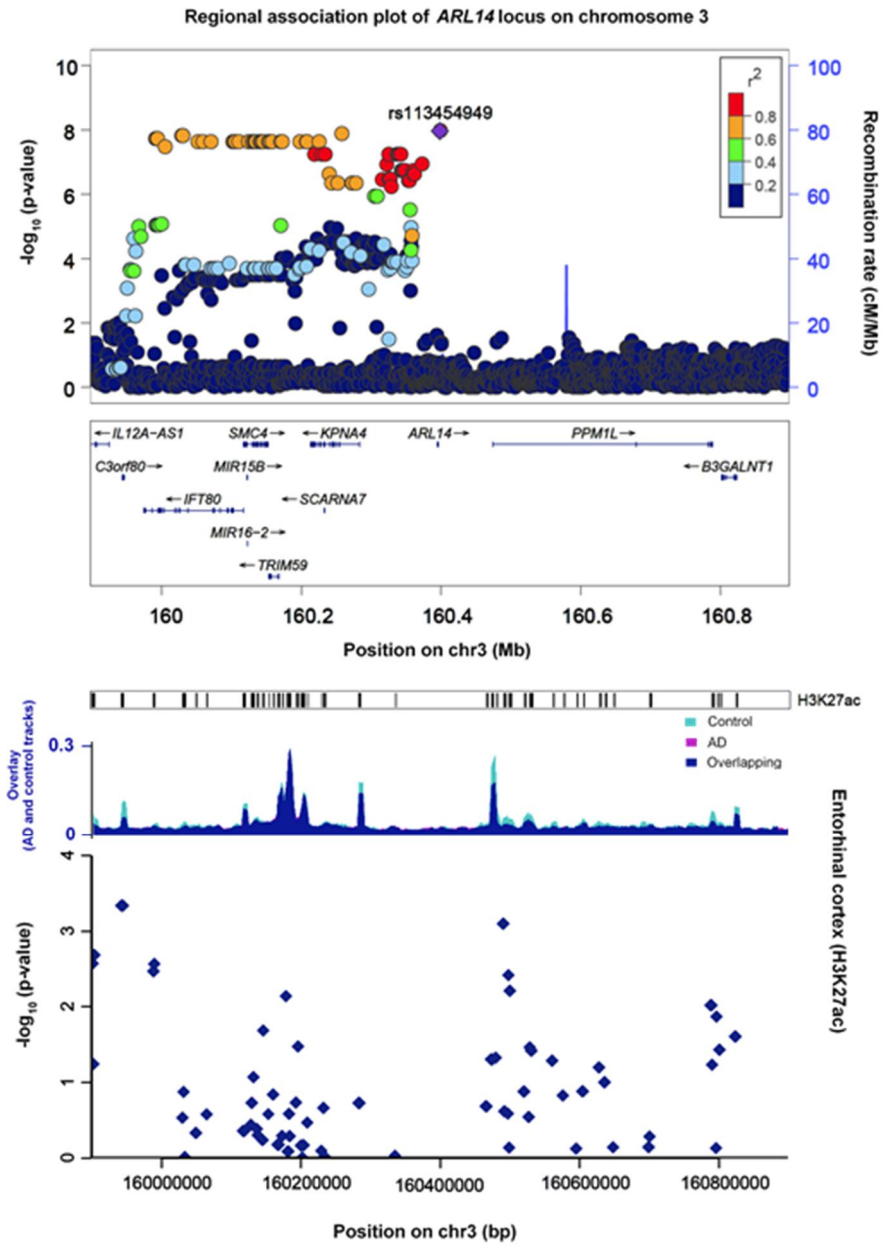


**Figure 15. Genome-wide significant association of *ARL14* genetic locus with cerebral Aβ deposition.** Magnified regional association plot around rs113454949 at the *ARL14* locus. The linear regression *p* value with cerebral Aβ deposition for each single nucleotide polymorphism (SNP) in the *ARL14* (*p*-values displayed as $-\log_{10}$)

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locus are shown. The purple dot in the top regional plot shows the most associated SNP in the region. The colours for all other SNPs indicate the LD $r^2$ value with most associated SNP ($r^2 \geq 0.8$ in red, $r^2$ between 0.6 and 0.8 in orange, $r^2$ between 0.4 and 0.6 in green, $r^2$ between 0.2 and 0.4 in light blue and $r^2 < 0.2$ in dark blue). The x-axis indicates base-pair position annotated to NCBI 37.3 build, the y-axis shows GWAS $-\log_{10}$ *p*-value and the blue peaks demonstrate recombination rates calculated from the 1000 genome project. Black bars below the regional plot indicate the narrow peaks of brain entorhinal cortex H3K27ac downloaded from GEO (accession number GSE102538 submitted by Marzi *et al*.,). H3K27ac tracks from AD (magneta), control samples (turquoise) and the overlay (AD and control) shows the acetylation differences. Below the track peaks, *p*-values of differentially acetylated peaks are displayed as $-\log_{10}$ in the dark blue coloured diamond dots.

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**Table 23.** Association of top cerebral Aβ deposition risk genetic loci with the H3K27ac peaks in the brain entorhinal cortex of AD patients.

| Loci | Chromosome # | Start-end (BP) | *p*-value | *Q*-value | Log FC | Annotated genes |
|---|---|---|---|---|---|---|
| *APOE* | 19 | 45394441-45395396 | **2.13E-04** | **0.01** | 0.48 | *TOMM40* |
| | 19 | 45222271-45222766 | **1.78E-03** | **0.05** | 0.67 | *BCL3* |
| | 19 | 45221395-45222215 | **2.80E-03** | **0.05** | 0.65 | *BCL3* |
| | 19 | 45606465-45607958 | **3.29E-03** | **0.05** | -0.31 | *PPP1R37* |
| | 19 | 45226845-45227788 | **4.40E-03** | **0.05** | 0.48 | *BCL3* |
| *CPAMD8* | 19 | 16989663-16991138 | **1.47E-04** | **0.01** | 0.67 | *SIN3B* |
| | 19 | 17006786-17007721 | **8.44E-04** | **0.02** | 0.42 | *CPAMD8* |
| | 19 | 17259966-17260983 | **2.68E-03** | **0.04** | 0.44 | *MYO9B* |
| | 19 | 17186553-17187959 | **8.33E-03** | 0.08 | 0.28 | *MYO9B* |
| | 19 | 16999631-17000196 | **0.02** | 0.13 | 0.52 | *F2RL3* |

**Table 23.** Continued.

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| Loci | Chromosome # | Start-end (BP) | *P*-value | *Q*-value | Log FC | Annotated genes |
|---|---|---|---|---|---|---|
| *ARL14* | 3 | 159942017-159944844 | **4.57E-04** | **0.03** | -0.50 | *C3orf80* |
| | 3 | 160489537-160490587 | **7.95E-04** | **0.03** | -0.64 | *PPM1L* |
| | 3 | 159902680-159904014 | **2.05E-03** | **0.04** | -0.69 | *AK097161* |
| | 3 | 159901026-159901516 | **2.65E-03** | **0.04** | -0.72 | *AK097161* |
| | 3 | 159989243-159989765 | **2.70E-03** | **0.04** | -0.81 | *IFT80* |

Abbreviations: BP, base pair position; *Q*-value, false-discovery rate adjusted *p*-value, Log FC, log fold change.

Top-5 most associated brain entorhinal cortex H3K27ac narrow peaks were shown in each genetic locus. H3K27ac data were downloaded from GEO database (accession number GSE102538 submitted by Marzi et al.,).

Significant *p*-values are indicated in bold letters.

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### Ⅲ-3-9. Replication analysis

The Aβ risk of most associated SNPs in the *CPAMD8* and *ARL14* genetic loci with an independent Aβ cohort from Korean population were examined. This study included the top significant SNPs from each gene in those two genetic loci (rs12975891, rs113454949, rs73155931, rs4680590 and rs3737214) and a missense variant (rs3745339) in *CPAMD8*. Analysis on the replication Aβ cohort showed that no significant ($p$<0.05) SNPs in that two loci. However, the study cohort showed that SNPs in the *CPAMD8* were associated with female-specific Aβ deposition, whereas *ARL14* locus SNPs were associated with male specific Aβ deposition (Figure 16). Therefore, this study hypothesized similar gender influence in the replication Aβ cohort as well. Similar to the study cohort results, the sex-stratified analysis with the replication Aβ cohort showed that rs12975891 (OR (odds ratio) = 2.53, $p$ = 0.04) and rs3745339 (OR = 2.34, $p$ = 0.04) in *CPAMD8* were associated with female-specific Aβ deposition (Table 24). While, *ARL14* loci SNPs showed no sex-specific significant ($p$<0.05) association with Aβ deposition. However, rs4680590 (OR = 1.85, $p$ = 0.51) and rs3737214 (OR = 1.85, $p$ = 0.51) showed same direction of allelic effect as in the study cohort (rs4680590: OR = 6.11, $p$ = 4.62E-05; rs3737214: OR = 5.82, $p$ = 7.93E-05) among males (Table 24). Therefore, this study combined the effect sizes from study cohort and the replication Aβ cohort using meta-analysis. The combined effect sizes revealed that rs12975891 (OR = 2.83, $p$-meta = 1.02E-05) and rs3745339 (OR = 2.68, $p$-meta = 2.05E-05) were associated with female-specific Aβ deposition, whereas rs113454949 (OR = 3.47, $p$-meta = 1.72E-03), rs4680590 (OR = 4.89, $p$-meta = 7.43E-05) and rs3737214 (OR = 4.70, $p$-meta = 1.20E-04) were associated with male-specific Aβ deposition (Table 25).

To examine ethnic specific effect of *CPAMD8* and *ARL14* loci, this study analysed the Aβ risk of these SNPs in the European Ancestry population. The results

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showed that rs12975891 (OR = 0.53, $p$ = 0.02) and rs3745339 (OR = 0.53, $p$ = 0.01) were associated with protective role against Aβ deposition in females, but in males, rs12975891 (OR = 1.42, $p$ = 0.08) and rs3745339 (OR = 1.48, $p$ = 0.11) showed allelic Aβ risk effect direction with no statistical significance. Moreover, in *ARL14* loci, rs3737214 (β = 0.04, $p$ = 0.03) of *IFT80* gene exhibited male-specific Aβ deposition, which was in concordance with the Korean Aβ cohort results (Table 26).

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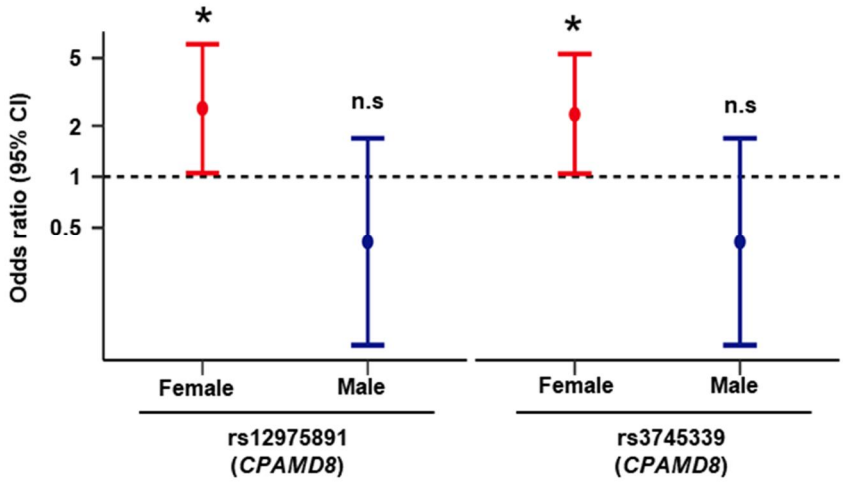


**Figure 16. *CPAMD8* mediates female-specific Aβ deposition.**

Korean Aβ replication cohort confirms female-specific *CPAMD8* mediated Aβ deposition. Sex stratified logistic regression was performed involving top cerebral Aβ associated SNPs under additive genetic model to analyse Aβ risk with age, education, amyloid probe and two PCs for population substructure as covariates. The *p*-values were labelled above the odds ratio error bar (**p*<0.05; n.s, not significant).

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**Table 24**. *CPAMD8* SNPs were associated with Aβ deposition in the Korean replication Aβ cohort (INU).

| Chromosome # | SNP | Base pair position | Gene | Groups | No. of samples | Odds ratio[a] (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|
| 19 | rs12975891 | 17102491 | *CPAMD8* | Female | 107 | **2.53 (1.05-6.04)** | **0.04** |
| | | | | Male | 85 | 0.41 (0.10-1.68) | 0.22 |
| | | | | All | 192 | 1.33 (0.67-2.60) | 0.41 |
| 19 | rs3745339 | 17108053 | *CPAMD8* | Female | 107 | **2.34 (1.04-5.29)** | **0.04** |
| | | | | Male | 85 | 0.41 (0.10-1.68) | 0.22 |
| | | | | All | 192 | 1.32 (0.70-2.50) | 0.39 |
| 3 | rs113454949 | 160398210 | *ARL14* | Female | 107 | 0.62 (0.12-3.31) | 0.58 |
| | | | | Male | 85 | 0.88 (0.16-4.77) | 0.88 |
| | | | | All | 192 | 0.78 (0.25-2.47) | 0.67 |
| 3 | rs73155931 | 160259437 | *KPNA4* | Female | 107 | 0.81 (0.15-4.42) | 0.80 |
| | | | | Male | 85 | 1.03 (0.21-5.01) | 0.97 |
| | | | | All | 192 | 1.01 (0.36-2.89) | 0.98 |

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**Table 24**. Continued.

| Chromosome # | SNP | Base pair position | Gene | Groups | No. of samples | Odds ratio[a] (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|
| 3 | rs4680590 | 160199834 | *TRIM59* | Female | 107 | 0.93 (0.21-4.03) | 0.92 |
| | | | | Male | 85 | 1.85 (0.30-11.47) | 0.51 |
| | | | | All | 192 | 1.18 (0.36-3.91) | 0.78 |
| 3 | rs3737214 | 159995356 | *IFT80* | Female | 107 | 0.96 (0.17-5.50) | 0.96 |
| | | | | Male | 85 | 1.85 (0.30-11.47) | 0.51 |
| | | | | All | 192 | 1.34 (0.40-4.55) | 0.64 |

Abbreviations: SNP, single nucleotide polymorphism; No. of samples, number of samples; CI, confidence interval.

[a]Female and male groups were adjusted for age, probe, education and two PCs capturing population substructure. All group including female and male was adjusted for age, sex, probe, education and two PCs capturing population substructure.

Significant *p*-values are indicated in bold letters.

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**Table 25**. *CPAMD8* and *ARL14* loci SNPs were associated with Aβ deposition in the meta-analysis involving Korean study cohort (GARD) and the replication cohort (INU).

| Chromosome # | SNP | Gene | Groups | GARD | | INU | | Meta-analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | OR[a] (95% CI) | *p*-value | OR[b] (95% CI) | *p*-value | OR (95% CI) | *p*-value |
| 19 | rs12975891 | *CPAMD8* | Female | **2.96 (1.72-5.12)** | **9.48E-05** | **2.53 (1.05-6.04)** | **0.04** | **2.83 (1.78-4.50)** | **1.02E-05** |
| | | | Male | **2.77 (1.54-5.06)** | **7.07E-04** | 0.41 (0.10-1.68) | 0.22 | 1.19 (0.19-7.65) | 0.85 |
| | | | All | **2.77 (1.86-4.10)** | **4.04E-07** | 1.33 (0.67-2.60) | 0.41 | **2.30 (1.63-3.22)** | **1.68E-06** |
| 19 | rs3745339 | *CPAMD8* | Female | **2.85 (1.65-4.92)** | **1.72E-04** | **2.34 (1.04-5.29)** | **0.04** | **2.68 (1.70-4.22)** | **2.05E-05** |
| | | | Male | **2.84 (1.59-5.06)** | **3.97E-04** | 0.41 (0.10-1.68) | 0.22 | 1.21 (0.18-7.96) | 0.84 |
| | | | All | **2.75 (1.86-4.06)** | **3.90E-07** | 1.32 (0.70-2.50) | 0.39 | 1.99 (0.98-4.06) | 0.06 |

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**Table 25**. Continued.

| Chromosome # | SNP | Gene | Groups | GARD | | INU | | Meta-analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | OR[a] (95% CI) | *p*-value | OR[b] (95% CI) | *p*-value | OR (95% CI) | *p*-value |
| 3 | rs113454949 | *ARL14* | Female | **2.06 (1.03-4.12)** | **0.04** | 0.62 (0.12-3.31) | 0.58 | 1.73 (0.91-3.27) | 0.09 |
| | | | Male | **5.01 (2.09-12.01)** | **3.08E-04** | 0.88 (0.16-4.77) | 0.88 | **3.47 (1.59-7.54)** | **1.72E-03** |
| | | | All | **2.92 (1.74-4.88)** | **4.57E-05** | 0.78 (0.25-2.47) | 0.67 | **1.68 (0.47-6.00)** | **4.30E-01** |
| 3 | rs73155931 | *KPNA4* | Female | **2.01 (1.10-3.69)** | **0.02** | 0.81 (0.15-4.42) | 0.8 | **1.80 (1.03-3.15)** | **0.04** |
| | | | Male | **8.07 (3.32-19.63)** | **4.20E-06** | 1.03 (0.21-5.01) | 0.97 | 3.21 (0.43-23.91) | 0.25 |
| | | | All | **3.30 (2.07-5.26)** | **5.78E-07** | 1.01 (0.36-2.89) | 0.98 | 2.01 (0.64-6.29) | 0.23 |

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**Table 25**. Continued.

| Chromosome # | SNP | Gene | Groups | GARD | | INU | | Meta-analysis | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | OR[a] (95% CI) | *p*-value | OR[b] (95% CI) | *p*-value | OR (95% CI) | *p*-value |
| 3 | rs4680590 | *TRIM59* | Female | **1.96 (1.03-3.74)** | **0.04** | 0.93 (0.21-4.03) | 0.92 | 1.76 (0.96-3.21) | 0.07 |
| | | | Male | **6.11 (2.56-14.59)** | **4.62E-05** | 1.85 (0.30-11.47) | 0.51 | **4.89 (2.23-10.74)** | **7.43E-05** |
| | | | All | **3.01 (1.85-4.92)** | **1.03E-05** | 1.18 (0.36-3.91) | 0.78 | **2.63 (1.67-4.14)** | **2.88E-05** |
| 3 | rs3737214 | *IFT80* | Female | **2.13 (1.08-4.21)** | **0.03** | 0.96 (0.17-5.50) | 0.96 | **1.92 (1.01-3.62)** | **0.04** |
| | | | Male | **5.82 (2.43-13.94)** | **7.93E-05** | 1.85 (0.30-11.47) | 0.51 | **4.70 (2.13-10.33)** | **1.20E-04** |
| | | | All | **3.17 (1.90-5.28)** | **9.26E-06** | 1.34 (0.40-4.55) | 0.64 | **2.79 (1.74-4.47)** | **1.92E-05** |

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval. [a]Female and male groups were adjusted for age, education and two PCs capturing population substructure. [b]Female and male groups were adjusted for age, probe, education and two PCs capturing population substructure. All group including female and male was adjusted for age, sex, probe, education and two PCs capturing population substructure. Significant *p*-values are indicated in bold letters.

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**Table 26**. *CPAMD8* SNPs were associated with Aβ risk in the European Ancestry Aβ cohort (ADNI).

| Chromosome # | SNP | BP | Gene | Groups | N | Positive vs. Negative | | SUVR | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OR[a] (95% CI) | *p*-value | Beta[a] | *p*-value |
| 19 | rs12975891 | 17102491 | *CPAMD8* | Female | 286 | **0.53 (0.32-0.89)** | **0.02** | -0.04 | 0.13 |
| | | | | Male | 358 | 1.42 (0.92-2.20) | 0.08 | 0.02 | 0.32 |
| | | | | All | 644 | 0.93 (0.68-1.27) | 0.64 | -0.01 | 0.79 |
| 19 | rs3745339 | 17108053 | *CPAMD8* | Female | 286 | **0.53 (0.32-0.87)** | **0.01** | -0.05 | 0.10 |
| | | | | Male | 358 | 1.48 (0.96-2.27) | 0.11 | 0.02 | 0.32 |
| | | | | All | 644 | 0.94 (0.69-1.28) | 0.68 | -0.01 | 0.72 |
| 3 | rs113454949 | 160398210 | *ARL14* | Female | 286 | 0.89 (0.44-1.81) | 0.75 | -0.02 | 0.70 |
| | | | | Male | 358 | 0.72 (0.40-1.28) | 0.26 | -0.02 | 0.59 |
| | | | | All | 644 | 0.78 (0.50-1.22) | 0.28 | -0.02 | 0.46 |
| 3 | rs73155931 | 160259437 | *KPNA4* | Female | 286 | 0.87 (0.59-1.28) | 0.47 | -0.02 | 0.29 |
| | | | | Male | 358 | 1.11 (0.77-1.61) | 0.57 | 0.02 | 0.26 |
| | | | | All | 644 | 0.99 (0.76-1.29) | 0.94 | 0.00 | 0.96 |

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**Table 26**. Continued.

| Chromosome # | SNP | BP | Gene | Groups | N | Positive vs. Negative | | SUVR | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OR[a] (95% CI) | *p*-value | Beta[a] | *p*-value |
| 3 | rs4680590 | 160199834 | *TRIM59* | Female | 286 | 0.83 (0.55-1.27) | 0.39 | -0.03 | 0.26 |
| | | | | Male | 358 | 1.27 (0.87-1.87) | 0.22 | 0.03 | 0.11 |
| | | | | All | 644 | 1.05 (0.79-1.39) | 0.74 | 0.01 | 0.71 |
| 3 | rs3737214 | 159995356 | *IFT80* | Female | 286 | 0.82 (0.55-1.22) | 0.32 | -0.04 | 0.11 |
| | | | | Male | 358 | 1.35 (0.93-1.95) | 0.11 | **0.04** | **0.03** |
| | | | | All | 644 | 1.07 (0.82-1.40) | 0.62 | 0.01 | 0.63 |

Abbreviations: BP, base pair position; SNP, single nucleotide polymorphism; N, number of samples; OR, odds ratio; CI, confidence interval.

[a]Female and male groups were adjusted for age, education and six PCs capturing population substructure. All group including female and male was adjusted for age, sex, education and six PCs capturing population substructure.

Significant *p*-values are indicated in bold letters.

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Then, the number of AD associated SNPs in the *CPAMD8* and *ARL14* genetic with IGAP GWAS results were examined. In the *CPAMD8* gene region, 10 SNPs showed nominal significance ($p<0.05$) with AD risk. In the *ARL14* genetic loci, 33 SNPs in *KPNA4*, 3 SNPs in *SMC4*, 6 SNPs in *IFT80* and 1 SNP in *TRIM59* showed nominal significance ($p<0.05$) with AD risk (Table 27 and Figure 17). *ARL14* gene showed no SNPs with significant association, however 7 SNPs showed nominal significance ($p<0.05$) when 2 kilo-base pair (kbp) flank regions were added around the gene (Table 27 and 28).

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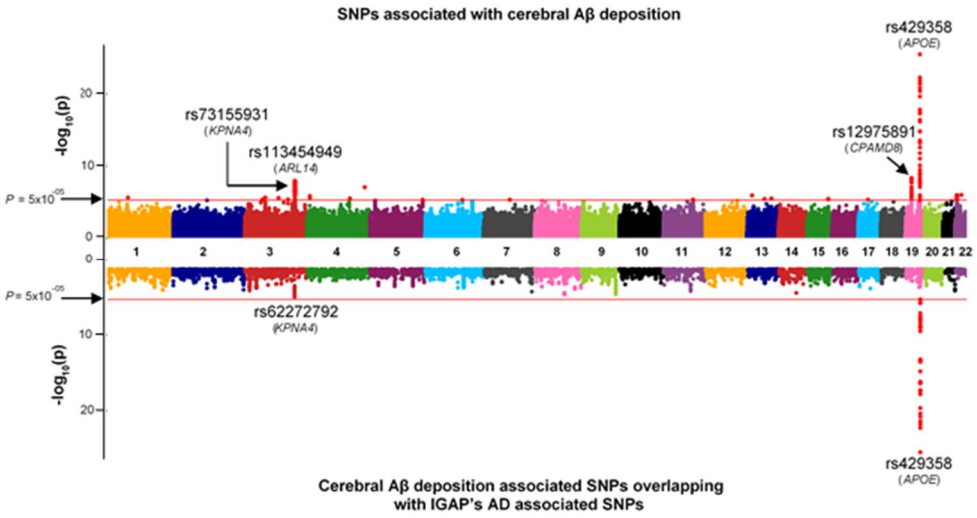


**Figure 17. Hudson plot illustrates SNP association with cerebral Aβ deposition (Top) and the overlapping AD associated SNPs in the IGAP study (Bottom).** The top Manhattan plot shows genome-wide *p*-value (displayed as $-\log_{10}$) of each single nucleotide polymorphism (SNP) from linear regression with cerebral Aβ deposition tested under additive genetic model adjusted for four population substructure PCs, age, sex, education. The bottom Manhattan plot shows the significantly associated ($p<0.05$) SNPs in the cerebral Aβ association study that overlaps with the AD associated ($p<0.05$) SNPs from the IGAP study. Genome-wide suggestive significance considered as $p = 5\times10^{-5}$ and indicated in red line. SNPs less than the suggestive significance were highlighted in red.

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**Table 27**. Top cerebral Aβ deposition associated genetic loci in IGAP case-control study.

| Chromosome # | Gene | Start BP | End BP | No. of SNPs in the region | No. of SNPs with *p*<0.05 | Top SNP in the gene | Minor/ Major | OR | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| 19 | *CPAMD8* | 17003762 | 17137625 | 349 | 10 | *CPAMD8*/rs3745333 | A/C | 0.93 | **1.70E-03** |
| 3 | *ARL14* | 160394948 | 160396236 | 1 | 0 | *ARL14*/rs75884210 | A/G | 1.07 | 0.14 |
| 3 | *KPNA4* | 160212783 | 160283376 | 114 | 33 | *KPNA4*/rs116810064 | A/C | 1.21 | **2.8E-03** |
| 3 | *SMC4* | 160117078 | 160152755 | 61 | 3 | *SMC4*/rs73154594 | G/A | 0.87 | **0.03** |
| 3 | *IFT80* | 159974774 | 160118028 | 182 | 6 | *IFT80*/rs6764829 | C/T | 0.84 | **0.01** |
| 3 | *TRIM59* | 160153291 | 160167626 | 26 | 1 | *TRIM59*/rs114692699 | C/T | 1.11 | **0.04** |

Abbreviations: BP, base pair position; SNP, single nucleotide polymorphism; OR, odds ratio.

Significant *p*-values are indicated in bold letters.

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**Table 28**. Top cerebral Aβ deposition genetic loci in IGAP case-control study with 2kbp flank regions around the genes.

| Chromosome # | Gene | Start BP | End BP | No. of SNPs in the region | No. of SNPs with *p*<0.05 | Top SNP in the gene | Minor/ Major | OR | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| 19 | *CPAMD8* | 17001762 | 17139625 | 355 | 10 | *CPAMD8*/rs3745333 | A/C | 0.93 | **1.67E-03** |
| 3 | *ARL14* | 160392948 | 160398236 | 19 | 7 | *ARL14*/rs7610939 | A/T | 1.04 | **0.02** |
| 3 | *KPNA4* | 160210783 | 160285376 | 122 | 36 | *KPNA4*/rs116810064 | A/C | 1.21 | **2.76E-03** |
| 3 | *SMC4* | 160115078 | 160154755 | 73 | 4 | *SMC4*/rs73154594 | G/A | 0.87 | **0.03** |
| 3 | *IFT80* | 159972774 | 160120028 | 188 | 7 | *IFT80*/rs6764829 | C/T | 0.84 | **0.01** |
| 3 | *TRIM59* | 160151291 | 160169626 | 35 | 2 | *TRIM59*/rs114692699 | C/T | 1.11 | **0.04** |

Abbreviations: BP, base pair position; SNP, single nucleotide polymorphism; OR, odds ratio.

Significant *p*-values are indicated in bold letters.

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## Ⅲ-4. DISCUSSION

In this study, $^{18}$F-FBB PET image data of 712 samples collected from Korean population were used to identify the novel genetic loci associated with AD β-amyloid pathology by GWAS approach. This is the first study, to our knowledge for the largest Korean specific $^{18}$F-FBB PET GWAS. From the results, this study identified three independent genetic loci with genome-wide significant association to cerebral β-amyloid deposition, in which two were novel: rs12975891 ($p = 4.42 \times 10^{-9}$) and rs113454949 ($p = 1.09 \times 10^{-8}$). These two novel loci accounted 6.1% of the variance, whereas the *APOE* explained 8.9% of variance on the β-amyloid deposition.

Similar to the previous β-amyloid PET GWAS (Li Shen et al., 2014; Yan et al., 2018), *APOE* locus exhibited the most significant association with β-amyloid deposition in this study. Moreover, this confirms the association of *APOE* with β-amyloid deposition in Koreans, though the earlier studies of β-amyloid PET GWAS were mainly on the European Ancestry population. In addition, *APOE* ε4 allele is one of the strongest AD risk factors and the several previous studies demonstrated the role of *APOE* ε4 on β-amyloid peptide deposition in the form of senile plaques on the brain promoting synaptic dysfunction, neurodegeneration and cognitive impairment (Hashimoto et al., 2012; Lesné et al., 2006; Shankar et al., 2008; H.-Y. Wu et al., 2010). In our previous study, the effect of *APOE* ε4 homozygotes on amyloid burden was observed to be stronger among East Asians (odds ratio (OR) = 54.75) than in European Ancestry (OR = 18.98) (Choi et al., 2019), and in this study, the results confirm the role of *APOE* gene in the β-amyloid mediated risks for AD among East Asians.

The association of *CPAMD8* (C3 and PZP like, Alpha-2-Macroglobulin Domain Containing 8) locus to the β-amyloid deposition is the first to be reported through this study.

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An intron variant, rs12975891 located in the *CPAMD8* gene was initially identified showing genome-wide significant association to cerebral β-amyloid burden from the SNP-based GWAS. Then, this *CPAMD8* gene as a candidate gene with genome-wide significant *p*-value ($p = 4.33 \times 10^{-8}$) from the gene-based GWAS was confirmed. A recent family-based study proposed that this *CPAMD8* is associated with the familial late onset AD (fLOAD). It was also reported that *CPAMD8* interacts with previously known AD related genes directly (genetic interaction) or indirectly (co-localization or shared functions). Therefore, this study hypothesized that the *CPAMD8* might interact with AD related genes in the β-amyloid pathology as well. From the gene network analysis with the candidate gene list ($p < 1 \times 10^{-3}$), this study identified that *CPAMD8* interacts with *APOE*, *APOC1* and *APOA2* (AD related genes) through co-expression with SEC31 homolog B (*SEC31B*) and Nuclear Factor I A (*NFIA*) genes. Additionally, *CPAMD8* is also associated with multiple sclerosis (de-la-Torre et al., 2019) and Anterior Segment Dysgenesis (Cheong et al., 2016) and found to be expressed in the brain and eye tissues (de-la-Torre et al., 2019).

Interestingly, a missense variant, rs3745339 of *CPAMD8* gene with Methonine303Isoleucine (M303I) mutation showed genome-wide significant *p*-value ($p = 1.26 \times 10^{-8}$) and it was found to be in strong LD ($r^2 = 0.96$) with top significant SNP, rs12975891 in that locus. However, the missense variant was genotyped but not imputed. Subsequently, the effects of this M303I missense mutation on the *CPAMD8* protein structure was evaluated. Also, *CPAMD8* was found to be inactivated by the missense mutation and thereby increases the β-amyloid mediated pathology in the brain.

In the another novel locus, the most significant SNP, rs113454949 in *ARL14* forms huge LD block with the group of significant SNPs extending to *KPNA4*, *TRIM59*, *SMC4* and *IFT80* genes. This is the first study to report this novel genetic

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locus, which was associated with β-amyloid deposition. The *ARL14* gene is involved in the GTP binding and signalling pathways that includes transforming growth factor beta (TGFβ) (Paul et al., 2011). Nevertheless, the gene pathway analysis involving KEGG pathways showed that the *KEGG TGF Beta signalling* pathway ($p = 0.002$) was most associated with β-amyloid deposition, but the pathway showed no significance after Bonferroni correction ($p \leq 0.05$). Previous studies reported that the impairment in the TGFβ-Smad signalling facilitates cytotoxic activation of microglia and induces microglia-mediated neurodegeneration. This mechanism was also observed in the AD pathogenesis and other neurodegenerative diseases (von Bernhardi, Cornejo, Parada, & Eugenín, 2015). In consistent with previous studies, from the gene pathway analysis involving Reactome pathways, this study identified *Transcriptional activity of SMAD2/SMAD3:SMAD4 Heterotrimer* pathway ($p = 4.60 \times 10^{-5}$) was most associated with β-amyloid deposition, which also survived Bonferroni correction ($p \leq 0.05$). Together, it suggests that the *ARL14* might be involved in TGFβ signalling mechanism that plays a role in the β-amyloid mediated AD pathogenesis.

*KPNA4* was another risk gene located at the close proximity to *ARL14*. *KPNA4*, Karyopherin Subunit Alpha 4 is associated with frontotemporal dementia and amyotrophic lateral sclerosis (Solomon et al., 2018). The gene network analyses showed that the *KPNA4* was related to *APOC1* and *APOE* through co-expression with Inner Centromere protein (*INCENP*) and Torsin family 3 member A (*TOR3A*) genes. This *TOR3A* gene is associated with hypo-acetylation ($p = 7.03 \times 10^{-8}$) in the brain entorhinal cortex of AD patients (S. Marzi et al., 2018).

*TRIM59*, Tripartite Motif containing 59, on hypermethylation, it causes aberrations in cell cycle control and DNA repair mechanisms in the familial early onset AD (fEOAD) (Wezyk et al., 2018). In the gene pathway analysis using GO pathways, this study identified that the *G0 and Early G1* pathway ($p = 0.0019$) was

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associated with β-amyloid deposition, but this pathway did not survive Bonferroni correction ($p \leq 0.05$). Anyhow, the pathological evidences in the form of regulatory proteins and cell cycle markers identified that the cell cycle re-entry in AD precedes the β-amyloid deposition and neurofibrillary tangles (Bonda et al., 2010). Intriguingly, the results suggest that there might be a strong association of *TIRM59* with abnormalities in the cell cycle, which eventually leading to β-amyloid burden in the AD. However, further validation is needed to identify the underlying functional mechanisms.

*SMC4*, structural maintenance of chromosomes protein 4 was identified to promote inflammatory innate immune response (Qinlan et al., 2018). A previous study reported that a variant, rs6441306 ($p = 1.22 \times 10^{-6}$) in 5' region of *IFT80* (Intraflagellar Transport 80) is associated with the CSF *APOE* protein levels (Cruchaga et al., 2012). Additional studies showed that the *IFT80* is associated with retinal degeneration (Moran et al., 2018) and osteogenesis (Yang & Wang, 2012). This study observed that the *IFT80* and *SMC4* were related to each other in the gene network analysis and further, these two genes along with Cyclin Dependent Kinase 1 (*CDK1*) are related to Translocase of Outer Mitochondrial membrane 40 (*TOMM40*) genes through co-expression. *TOMM40* gene is located in the *APOE* locus and past studies reported its strong association with Alzheimer's disease (Chiba-Falek, Gottschalk, & Lutz, 2018). In addition, from the pathway analysis using GO pathways, this study identified that the *GO Feeding Behaviour* pathway was most associated with β-amyloid deposition but no significance was observed after Bonferornni correction ($p \leq 0.05$). However, prior studies reported that there are changes in the eating behaviours among AD patients (Ikeda, Brown, Holland, Fukuhara, & Hodges, 2002; Kai et al., 2015) and this suggest that there might be an association between β-amyloid deposition and feeding or eating behaviours.

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The huge LD block in the *ARL14* locus provided less clarity in identifying the candidate risk gene associated to β-amyloid deposition in that region. However, in the gene-based GWAS, *KPNA4*, *TRIM59*, *SMC4* and *IFT80* genes showed genome-wide significant ($p < 2.78 \times 10^{-6}$) association to β-amyloid deposition except *ARL14* gene ($p = 0.68$). The AD associated variation in the H3K27ac of entorhinal cortex tissue (S. Marzi et al., 2018) from GEO database were evaluated to identify active enhancers and promoters in the *ARL14* genetic locus. The results suggested that one of the most AD-associated hypo-acetylated peak ($p = 4.57 \times 10^{-4}$) in *ARL14* locus was located in the *IFT80* gene (Figure15 and Table 23). Nonetheless, further experiments are needed to conclude this finding.

Both *CPAMD8* and *ARL14* genetic loci showed genome-wide significance after correcting for *APOE* □4 allele status. This study hypothesized that variants in the *CPAMD8* and *ARL14* genetic loci might be associated with β-amyloid deposition by *APOE* □4 independent manner. The *APOE* □4 allele stratified association analyses on these loci showed that the variants in both *CPAMD8* and *ARL14* regions were strongly associated with β-amyloid deposition among *APOE* non-□4 carriers than *APOE* □4 carriers. This suggests that these genetic loci could be associated with the risk of β-amyloid deposition in an *APOE* □4 independent manner. The influence of gender in *CPAMD8* and *ARL14* genetic loci was evaluated. The study results revealed that the variants in *CPAMD8* locus were strongly associated with β-amyloid deposition in the female specific group than in male-specific group. In contrast, the variants in *ARL14* locus were strongly associated with β-amyloid deposition in the male specific group. Anyhow, further validation with bigger sample size is needed to confirm the influences of gender and *APOE* □4 allele in these two genetic loci.

The replication analysis involving independent Aβ replication cohort from Korean population revealed that rs12975891 and rs3745339 in *CPAMD8* were associated with the female-specific Aβ deposition (Figure 16 and Table 24). This

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result further confirms the Aβ study cohort results, suggesting that *CPAMD8* mediates female-specific Aβ deposition in Korean population. In addition, the meta-analysis involving Aβ study cohort and Aβ replication cohort showed similar sex-specific results (Table 25).

To evaluate whether *CPAMD8* and *ARL14* loci SNPs are ethnic specific, this study analysed the association of Aβ risk with European Ancestry Aβ cohort. The results suggested that *CPAMD8* might play a protective role against female-specific Aβ deposition in European Ancestry, though *CPAMD8* was associated with female-specific Aβ deposition in Koreans. In contrast, male group showed allelic Aβ risk effect direction in European Ancestry. Anyhow, this disparity in sex-specific *CPAMD8* mediated Aβ deposition in different ethnicities need further validation. In addition, in *ARL14* loci, the results revealed that *IFT80* SNP, rs3737214 promotes male-specific Aβ deposition in both Korean and European Ancestry populations.

Moreover, this study found that top genome-wide Aβ risk SNPs in the study cohort showed no association with AD risk in the ADGC GWAS results. Nevertheless, in the *ARL14* genetic locus, this study found multiple SNPs in *KPNA4* gene showed association with Aβ deposition in the study cohort GWAS and as well as with AD risk in the IGAP GWAS (Figure 17). This suggests that *KPNA4* gene might be associated with Aβ deposition mediated AD pathology in both East Asian and European Ancestry populations

Like previous amyloid PET GWAS, this study also has limitation in the sample size. By involving larger sample size, genetic loci that showed suggestive genome-wide significance might achieve genome-wide significance level. However, the continuous collection of amyloid PET samples by the GARD would help performing large scale Korean specific amyloid PET GWAS in future. In spite of limitations, this study is the first amyloid PET GWAS in the East Asian population,

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specifically Koreans. The novel findings on the β-amyloid pathology in Koreans would be helpful in future follow-up studies. Moreover, the detailed study involving analytical methods with supporting replication results revealed the functional genes involved in the β-amyloid mediated AD pathogenesis, which are not identified in the previous East Asian specific AD GWASs.

In conclusion, this study identifies two novel risk loci in *CPAMD8* and *ARL14* gene regions associated with β-amyloid pathology in the brain using large Korean amyloid PET GWAS. The findings supported with the functional experiments and homology modelling of protein, it could be confirmed that the genes in these two novel genetic loci may play a critical role in the β-amyloid induced AD pathology in the Korean population.

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# PART 4

***Multimodal brain imaging and cognitive measures reveal multistage specific multi-polygenic risk for Alzheimer's disease***

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## SUMMARY

The pathogenesis of Alzheimer's disease (AD) is thought to be an early event, during which the β-amyloid deposition in the brain is under progress despite the absence of clinical symptoms. A recent study suggests that anti-amyloid therapy in the early stages of AD may slow cognitive decline among cognitively normal (CN) individuals, who are at the risk of developing AD dementia. Therefore, it is critical to find the risk factors before the onset of clinical symptoms for AD dementia. In this study, the aim is to evaluate the polygenic risk of AD biomarkers that are associated with AD risk in both preclinical or early stages and late stages of AD dementia. Moreover, identifying ethnic specific genetic risk factors are also important. In this study, the association between AD polygenic risk scores (PRSs) and AD markers that includes β-amyloid deposition, hippocampus volume atrophy and cognitive decline in East Asian population were analyzed. The β-amyloid positron emission tomography (PET) images and T1-weighted structural images were used to extract the PET β-amyloid standard uptake value ratio (SUVR) and hippocampus volume. Memory domain was included to analyze the cognitive decline in East Asian population. In this study, CN, individuals with mild cognitive impairments (MCI) and AD dementia patients were included. The AD markers including β-amyloid ($r^2 = 0.116$; $p = 2.76 \times 10^{-20}$), hippocampus volume ($r^2 = 0.054$; $p = 1.55 \times 10^{-39}$) and memory domain ($r^2 = 0.066$; $p = 3.31 \times 10^{-42}$; $r^2 = 0.089$; $p = 1.70 \times 10^{-54}$) showed significant association with AD PRSs. Even in the early stage of dementia, AD biomarker including β-amyloid ($r^2 = 0.104$; $p = 1.14 \times 10^{-14}$), hippocampus volume ($r^2 = 0.013$; $p = 8.52 \times 10^{-10}$) and memory domain ($r^2 = 0.020$; $p = 1.45 \times 10^{-12}$; $r^2 = 0.030$; $p = 1.95 \times 10^{-17}$) with PRSs of AD. Furthermore, the analysis of mean best-fit PGRSs of AD revealed that SNPs based on their effect size direction from the association analysis were better in discriminating the PGRSs of AD. In conclusion, this study identified that there is a synergistic effect among AD

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biomarkers in driving AD risk in both early and late stages of AD dementia. This study also identified that SNPs based on their effect size direction could influence the correlation of AD biomarker with PGRSs of AD.

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## IV-1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease, in which the asymptomatic stage is found to last longer, then followed by a symptomatic pre-dementia stage, and finally leads to a dementia stage (Parnetti, Chipi, Salvadori, D'Andrea, & Eusebi, 2019). With the advancements in AD pathological markers, it has been made possible to identify the disease at the asymptomatic or pre-clinical stage before the onset of clinical symptoms. This asymptomatic or preclinical phase of AD has become more focus for the research since it provides higher probability of therapeutic success when the disease is intervened at early stage (Dubois et al., 2016). It has been found that AD is caused by 70% genetic and 30% environmental factors, and this makes it a multifactorial disease.

AD is categorised into sporadic, which commonly occurs and another is familial, which causes due to familial history. In familial AD, amyloid precursor protein (*APP*), Presenilin1 (*PSEN1*) and Presenilin2 (*PSEN2*) genes are found to be the causative genetic factors. Whereas in sporadic AD, apolipoprotein E (*APOE*) is found to be most potent genetic risk factor (Jolanta, Michal, Anna, Mateusz, & Wojciech, 2016). Apart from *APOE* gene, 21 common genetic variants associated with late onset AD or sporadic AD have been reported in a large genome-wide association study (GWAS) meta-analyses (J. C. Lambert et al., 2013). However, the effect sizes of those AD associated variants are small (odds ratio < 1.22), and this suggests that there could be numerous additional AD susceptible genetic loci, which could not be identified in the previous AD GWAS (Mormino, Sperling, Holmes, Buckner, De Jager, Smoller, & Sabuncu, 2016). Moreover, it has been identified that genetic variants associated with sporadic AD accounts for over 60% of heritability estimates, which appears to have a huge influence in the sporadic AD. Anyhow, the previous studies have identified 6% of heritability accounting for *APOE* locus, while

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2% heritability accounts for GWAS-confirmed genetic loci that are reported in the previous studies. Nevertheless, around 2 million genetic variants accounts for additional 25% of heritability estimates (Mormino, Sperling, Holmes, Buckner, De Jager, Smoller, Sabuncu, et al., 2016).

The most important aim of biomedical research is to identify potential biomarkers that can be adopted in the early stage diagnosis of AD and therapeutic interventions. In the past decade, several advancements in the development of biomarker has led to the development of AD biomarkers using neuroimaging methods. These neuroimaging approaches provide opportunity in understanding AD mediated pathophysiological changes in the brain such as brain volume loss and cortical thinning through magnetic resonance imaging (MRI). Similarly, amyloid and tau positron emission tomography (PET) imaging can track pathological aggregation of β-amyloid (Aβ) and tau proteins in the brain (Márquez & Yassa, 2019). The Aβ deposition in the brain is associated with early stage pathological cascade of AD that occurs well before the brain atrophy and cognitive decline (Roostaei, Nazeri, Felsky, De Jager, Schneider, Pollock, Bennett, & Voineskos, 2017). In general, the symptoms of AD begins with slight memory difficulties and progress towards cognitive impairments and several other cognitive related functions (Mantzavinos & Alexiou, 2017).

Given the association of Aβ accumulation in the early stages of AD and the association of brain atrophy and cognitive decline in the lateral stages of AD, these biomarkers can be adopted as composite biomarker in studying early stage and lateral stages mechanisms of AD. Even previous studies have adopted these biomarkers in the genome-wide studies and identified genetic variants associated with these AD biomarkers. In this study, a measure of polygenic risk scores using large AD case-control GWASs was used to identify whether the polygenic risk scores (PGRS) of these biomarkers are associated with early and late stage of AD. In addition, this

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study will identify comprehensive polygenic risk variants that are associated with both early stage and late stage biomarkers. Although previous studies have reported association of PGRSs from some of these biomarkers with AD in European Ancestry population, this study combines multiple early and late stage AD biomarkers to identify East Asian specific polygenic variants that are associated with both early and late stages of AD.

## Ⅳ-2. METHODS

### Ⅳ-2-1. Study participants

This study utilized data from participants aged 55-90 years from the GARD cohort collected by National Research Center for Dementia (NRCD) at Chosun University in Gwangju, South Korea. The training dataset comprised of 1,369 AD patients, 1,573 cognitively normal adults (Table 29). The biomarker cohorts that were used to calculate PGRSs includes 672 subjects from cerebral Aβ cohort, 2,176 subjects from MRI cohort and 2,120 subjects from cognitive test cohort (Table 30). Probable AD dementia was clinically diagnosed according to criteria of the National Institute of Neurological and Communicative Disorders Association (NINCDS-ADRDA) (G. M. McKhann et al., 2011). The cognitively normal individuals had no sign of neurological disease or impairments in the cognitive abilities or activities of daily life. The institutional review board of Chosun University Hospital, South Korea approved the study protocol and all the participants or their legal guardians of cognitively impaired individuals gave informed consent before participation in this study.

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**Table 29.** Demography characteristic of subjects in training data.

| Demography | Cognitively normal | AD cases |
|---|---|---|
| Number | 1573 | 1369 |
| Age (y, mean ± SD) | 70.80 ± 9.47 | 72.61 ± 8.55 |
| Female (n, %) | 920 (58.49%) | 846 (61.80%) |
| Years of education (y, mean ± SD) | 9.75 ± 4.74 | 8.81 ± 5.46 |
| MMSE (mean ± SD) | 27.04 ± 2.52 | 20.78 ± 5.96 |

Abbreviations: n, number; SD, standard deviation; y, years; MMSE, Mini-Mental State Examination; AD , Alzheimer's disease.

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**Table 30.** Demography characteristic of subjects in biomarker cohorts (PET Aβ, MRI imaging and cognitive test cohorts).

| Demography | Cortical β-amyloid | | | Total hippocampus | | | SVLT Immediate recall | | | SVLT Delayed recall | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CN | MCI | AD | CN | MCI | AD | CN | MCI | AD | CN | MCI | AD |
| n | 267 | 270 | 135 | 1213 | 705 | 258 | 1202 | 685 | 233 | 1201 | 685 | 230 |
| Age (y, mean±SD) | 72.25 ± 5.39 | 72.70 ± 6.15 | 71.28 ± 7.80 | 72.83 ± 5.44 | 73.27 ± 5.47 | 73.06 ± 7.09 | 72.86 ± 5.45 | 73.32 ± 5.37 | 73.13 ± 7.07 | 72.83 ± 5.44 | 73.32 ± 5.37 | 73.14 ± 7.11 |
| Female (n, %) | 142 (53.18%) | 139 (51.48%) | 71 (52.59%) | 743 (61.25%) | 399 (56.60%) | 154 (59.69%) | 736 (61.23%) | 387 (56.49%) | 141 (60.52%) | 736 (61.2%) | 387 (56.50%) | 139 (60.4%) |
| MMSE (mean±SD) | 27.21 ± 2.33 | 25.15 ± 3.16 | 19.23 ± 6.12 | 26.72 ± 4.19 | 24.51 ± 6.61 | 16.79 ± 9.60 | 27.05 ± 2.43 | 25.49 ± 3.35 | 19.24 ± 5.96 | 26.73 ± 4.19 | 25.48 ± 3.35 | 19.37 ± 5.86 |

Abbreviations: SD, standard deviation; y, years; MMSE, Mini-Mental State Examination; CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; SVLT, Seoul Verbal Learning Test.

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### Ⅳ-2-2. Florbetaben Positron Emission Tomography (PET) imaging

All the study participants underwent PET scanning session at the Chosun University Hospital, Korea. Participants were administered 300 MBq $^{18}$F-Florbetaben ($^{18}$F-FBB) β-amyloid tracer intravenously and scanned after 90 minutes using a Discovery ST PET-CT scanner (General Electric Medical Systems, Milwaukee, WI, USA). Attenuation correction of PET image was performed with non-contrast-enhanced CT scans with technical parameters of 10-130 mAs, 8 slice, 120 Kvp, helical and 3.79 mm slice thickness. After applying ordered subset expectation maximization (OSEM), PET and CT images were reconstructed followed by the attenuation correction with iterative reconstruction algorithm.

### Ⅳ-2-3. PET image data processing

$^{18}$F-FBB PET images were pre-processed with SPM12 toolbox implemented in MATLAB (R2018a, Mathworks, Natick, MA, USA). All the $^{18}$F-FBB PET images were co-registered with the respective T1-weighted MR images from the same subject that are acquired on the same day or within 6 months of time interval. Then, the transformation parameters for the spatial normalization of T1-weighted MR images to the standard MNI template were estimated. Subsequently, the estimated parameters were applied to spatially normalize the PET images to the standard MNI template. Furthermore, these normalized PET images were smoothened with an 8-mm full-width at half maximum Gaussian kernel (Park, Han, Yi, et al., 2019). The respective MR images were segmented using FreeSurfer software version 6.0 and applied to the smoothened PET images to extract values (SUVRs) from 68 predefined brain anatomical region of interests (ROIs) (Palmqvist et al., 2017). The global cortical retention mean SUVRs were calculated from anterior and posterior cingulate, lateral temporal, frontal and lateral parietal regions with reference to the whole cerebellum (Maass et al., 2017).

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### Ⅳ-2-4. MRI data processing

MR T1-weighted images from the subjects were processed with FreeSurfer software version 5.3.0 (https://surfer.nmr.mgh.harvard.edu/fswiki) using automated processing stream to reconstruct 3-dimensional cortical surface model (Dale et al., 1999; B. Fischl & Dale, 2000; Bruce Fischl et al., 1999). In detail, the FreeSurfer automated reconstruction protocol includes Talairach transformation, intensity normalization, non-brain tissue removal, separation of hemispheres, exclusion of cerebellum and brain stem, tessellation of the gray-matter (GM) and white-matter (WM) boundaries, topology correction, GM and WM subcortical segmentation, atlas registration and surface deformation for the optimization of GM/WM and gray/cerebrospinal fluid boundaries (Gorbach et al., 2017).

### Ⅳ-2-5. Cognitive assessment and clinical measures

All the participants were screened for the cognitive ability in multiple domains using Seoul Neuropsychological Screening Battery (SNSB) by the NRCD at Chosun University in Gwangju, Korea. This battery assesses 5 cognitive domains that include attention, language, visuospatial, memory and frontal/executive functions (Ahn et al., 2010). The neuropsychological tests in the SNSB consisted of Clinical Dementia Rating (CDR) Sum of boxes, Korean Mini-Mental State Exam (K-MMSE), Digit Span test Forward/Backward, Digit Span test forward, Digit Span test backward, Praxis Ideomotor, Short form of Korean version of the Boston Naming Test (S-K-BNT), Copy score, Rey Complex Figure Test (RCFT), Seoul Verbal Learning Test (SVLT) Immediate Recall, SVLT Delayed Recall, SVLT Recognition, RCFT Recognition, Contrasting Program, Go-No Go test, Controlled Oral Word Association Test (COWAT) Phenomic, COWAT Supermarket, COWAT Animal, Korean-Trail Making Test-Elderly's version A Time (K-TMT-E-A), Korean-Trail

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Making Test-Elderly's version B Time (K-TMT-E-B), StroopColorreading Time and StroopWordreading Time.

### Ⅳ-2-6. Statistical analysis

Linear regression on cerebral Aβ, hippocampal volume, memory domain that included SVLT Immediate recall and SVLT Delayed recall tests involving 6,513,520 SNPs were performed using PLINK. The principal components (PCs) accounting for the population substructure in the dataset were calculated using smartpca program with EIGENSOFT (Patterson et al., 2006). Tracy-Widom statistics identified significantly associated PCs with all endophenotypes (cerebral Aβ, hippocampal volume and memory domain). Linear regression on cerebral Aβ was performed with age, sex, education and 4 PCs as covariates. Linear regression on hippocampal volume was performed with age, sex, education, intracranial volume, field strength and 5 PCs as covariates. Linear regression on memory domain was performed with age, sex, education and 5 PCs as covariates. Logistic regression was performed with AD case-control group while adjusted for age, sex and 3 PCs as covariates.

### Ⅳ-2-7. Estimation of Polygenic Risk Scores (PGRS)

PGRSs were estimated using profile option implemented in PLINK software. PGRSs were calculated with the training data from AD case-control cohort involving 1,369 cases and 1,573 controls. The data were linkage disequilibrium (LD) clumped using clump function (--clump) implemented in the PLINK by removing SNPs located within 500 kilobase and $r^2 > 0.25$ with the significantly associated SNP. Then, this study iterated over a range of *p*-values (GWAS –level or polygenic threshold $p < 5 \times 10^{-8}$, $p < 1 \times 10^{-7}$, $p < 1 \times 10^{-6}$, $p < 1 \times 10^{-5}$, $p < 1 \times 10^{-4}$, $p < 1 \times 10^{-3}$, $p < 0.01$, $p < 0.05$, $p < 0.1$, $p < 0.1$, $p < 0.3$, $p < 0.5$) to determine an ambient *p*-value threshold. Analyses were performed with and without *APOE* ε4

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status adjustment. PGRSs were calculated for early stage by including cognitively normal (CN) and mild cognitively impaired individuals (MCI). Then the PGRSs for late stage were calculated by involving CN, MCI and AD.

## Ⅳ-3. RESULTS

### Ⅳ-3-1. Correlation of AD biomarkers with PGRSs for AD

In this study, large AD case-control group association analysis from Korean population was used as training data for estimating PGRSs with AD biomarkers including hippocampal volume, cortical Aβ and memory domain. All the biomarker cohorts had sub-groups of cognitively normal, MCI and AD. The results showed that PGRSs for AD were strongly correlated with hippocampal volume, cortical Aβ deposition and memory domain (Table 31). Moreover, PGRSs for AD calculated with all threshold are also strongly associated with hippocampal volume, cortical Aβ deposition and memory domain (Table 31). The best fit *p*-value thresholds ($P_T$) for Aβ deposition was found at $P_T < 1 \times 10^{-5}$ ($r^2 = 0.116$; $p = 2.76 \times 10^{-20}$). For hippocampal volume, best fit *p*-value threshold was found at $P_T < 0.01$ ($r^2 = 0.054$; $p = 1.55 \times 10^{-39}$) and for memory domain, involving SVLT immediate recall test, the threshold was at $P_T < 0.01$ ($r^2 = 0.066$; $p = 3.31 \times 10^{-42}$) and SVLT delayed recall test showed that the threshold was at $P_T < 0.01$ ($r^2 = 0.089$; $p = 1.70 \times 10^{-54}$). This study hypothesized that SNPs based on the effective size direction can improve the effectiveness of PGRSs for AD. Then, the SNP groups were split based on their effect size direction and analysed PGRSs for AD using AD biomarker cohort. Furthermore, the results showed that cortical Aβ deposition was strongly correlated with PGRSs for AD when SNPs with positive effect size group was involved. Whereas, other biomarkers including hippocampal volume and memory domain showed strong correlation with PGRSs for AD when SNPs with negative effect size group was involved (Figure 18).

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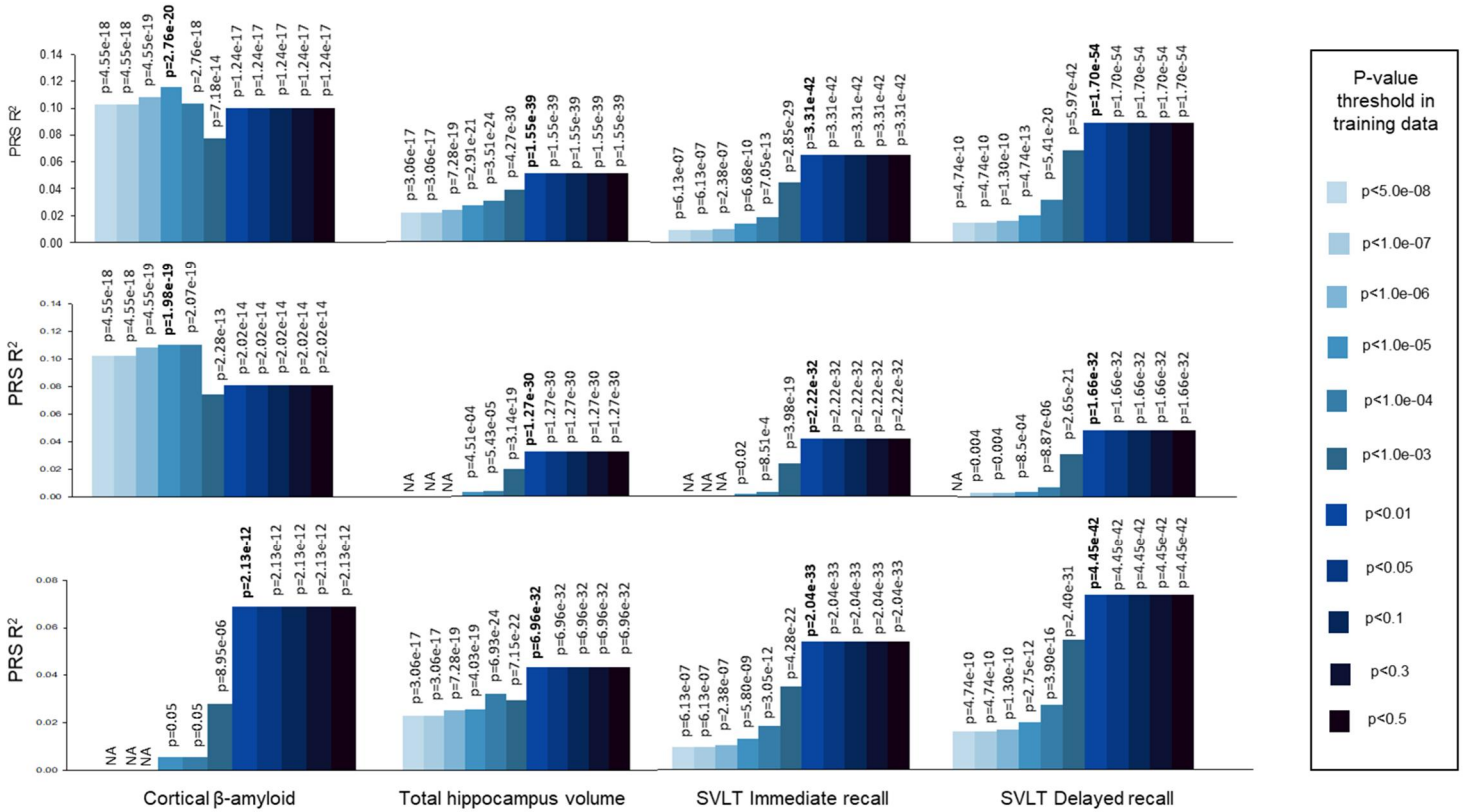


**Figure 18. Effects of AD polygenic risk scores (PGRSs) on cortical β-amyloid, hippocampal volume and memory domain.** The bar plot shows strong correlation of PGRSs for AD on cortical β-amyloid, hippocampal volume and memory domain.

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Horizontal axis denotes AD biomarkers showing correlation (indicated as bar) and association (*p*-value) with AD PGRSs. Top bar plots show PGRSs for AD with all SNPs included, the middle bar plot show PGRSs for AD with SNPs having positive effect size and the bottom bar plot show PGRSs for AD with SNPs having negative effect size. The best-fit thresholds are shown in bold.

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**Table 31.** Effects of AD polygenic risk scores (PGRSs) on AD biomarker.

| Training $P_T$ value | Cortical β-amyloid $R^2$ (*p*-value) | | | Total hippocampus $R^2$ (*p*-value) | | | SVLT Immediate Recall $R^2$ (*p*-value) | | | SVLT Delayed Recall $R^2$ (*p*-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size |
| $P_T < 5\times10^{-8}$ | 0.103 (4.55e-18) | 0.103 (4.55e-18) | NA | 0.023 (3.06e-17) | NA | 0.023 (3.06e-17) | 0.009 (6.13e-07) | NA | 0.009 (6.13e-07) | 0.015 (4.74e-10) | NA | 0.015 (4.74e-10) |
| $P_T < 1\times10^{-7}$ | 0.103 (4.55e-18) | 0.103 (4.55e-18) | NA | 0.023 (3.06e-17) | NA | 0.023 (3.06e-17) | 0.009 (6.13e-07) | NA | 0.009 (6.13e-07) | 0.015 (4.74e-10) | 0.003 (0.004) | 0.015 (4.74e-10) |
| $P_T < 1\times10^{-6}$ | 0.108 (4.55e-19) | 0.108 (4.55e-19) | NA | 0.025 (7.28e-19) | NA | 0.025 (7.28e-19) | 0.010 (2.38e-07) | NA | 0.010 (2.38e-07) | 0.016 (1.30e-10) | 0.003 (0.004) | 0.016 (1.30e-10) |
| $P_T < 1\times10^{-5}$ | **0.116 (2.76e-20)** | **0.111 (1.98e-19)** | 0.005 (0.05) | 0.029 (2.91e-21) | 0.004 (4.51e-04) | 0.026 (4.03e-19) | 0.014 (6.68e-10) | 0.002 (0.02) | 0.012 (5.80e-09) | 0.020 (4.74e-13) | 0.004 (8.5e-04) | 0.019 (2.75e-12) |
| $P_T < 1\times10^{-4}$ | 0.104 (2.76e-18) | 0.110 (2.07e-19) | 0.006 (0.05) | 0.033 (3.51e-24) | 0.005 (5.43e-05) | 0.032 (6.93e-24) | 0.019 (7.05e-13) | 0.040 (8.51e-4) | 0.018 (3.05e-12) | 0.032 (5.41e-20) | 0.007 (8.87e-06) | 0.025 (3.90e-16) |

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**Table 31.** Continued.

| Training $P_T$ value | Cortical β-amyloid $R^2$ (*p*-value) | | | Total hippocampus $R^2$ (*p*-value) | | | SVLT Immediate Recall $R^2$ (*p*-value) | | | SVLT Delayed Recall $R^2$ (*p*-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size |
| $P_T < 1\times10^{-3}$ | 0.078 (7.18e-14) | 0.075 (2.28e-13) | 0.028 (8.95e-06) | 0.041 (4.27e-30) | 0.026 (3.14e-19) | 0.029 (7.15e-22) | 0.045 (2.85e-29) | 0.029 (3.98e-19) | 0.034 (4.28e-22) | 0.069 (5.97e-42) | 0.033 (2.65e-21) | 0.051 (2.40e-31) |
| $P_T < 0.01$ | 0.100 (1.24e-17) | 0.081 (2.02e-14) | **0.069 (2.13e-12)** | **0.054 (1.55e-39)** | **0.042 (1.27e-30)** | **0.044 (6.96e-32)** | **0.066 (3.31e-42)** | **0.050 (2.22e-32)** | **0.052 (2.04e-33)** | **0.089 (1.70e-54)** | **0.050 (1.66e-32)** | **0.069 (4.45e-42)** |
| $P_T < 0.05$ | 0.100 (1.24e-17) | 0.081 (2.02e-14) | 0.069 (2.13e-12) | 0.054 (1.55e-39) | 0.042 (1.27e-30) | 0.044 (6.96e-32) | 0.066 (3.31e-42) | 0.050 (2.22e-32) | 0.052 (2.04e-33) | 0.089 (1.70e-54) | 0.050 (1.66e-32) | 0.069 (4.45e-42) |
| $P_T < 0.1$ | 0.100 (1.24e-17) | 0.081 (2.02e-14) | 0.069 (2.13e-12) | 0.054 (1.55e-39) | 0.042 (1.27e-30) | 0.044 (6.96e-32) | 0.066 (3.31e-42) | 0.050 (2.22e-32) | 0.052 (2.04e-33) | 0.089 (1.70e-54) | 0.050 (1.66e-32) | 0.069 (4.45e-42) |
| $P_T < 0.3$ | 0.100 (1.24e-17) | 0.081 (2.02e-14) | 0.069 (2.13e-12) | 0.054 (1.55e-39) | 0.042 (1.27e-30) | 0.044 (6.96e-32) | 0.066 (3.31e-42) | 0.050 (2.22e-32) | 0.052 (2.04e-33) | 0.089 (1.70e-54) | 0.050 (1.66e-32) | 0.069 (4.45e-42) |
| $P_T < 0.5$ | 0.100 (1.24e-17) | 0.081 (2.02e-14) | 0.069 (2.13e-12) | 0.054 (1.55e-39) | 0.042 (1.27e-30) | 0.044 (6.96e-32) | 0.066 (3.31e-42) | 0.050 (2.22e-32) | 0.052 (2.04e-33) | 0.089 (1.70e-54) | 0.050 (1.66e-32) | 0.069 (4.45e-42) |

Abbreviation: $P_T$, *p*-value threshold; SNP, single nucleotide polymorphism; SVLT, Seoul Verbal Learning Test.

Best-fit thresholds are indicated in bold.

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### Ⅳ-3-2. Effects of AD biomarker PGRSs in early stage of AD dementia

To evaluate the changes attributed to cortical Aβ deposition, hippocampus and memory domain in early stage of AD dementia, this study excluded the AD dementia group from all the biomarker cohorts by including cognitively normal and MCI groups. Then, PGRS analysis by using AD case-control cohort as training dataset was performed. The analysis was performed with iteration of threshold that was described in the methods. The results showed that even in the absence AD dementia group, PGRSs for AD were strongly correlated with hippocampal volume, cortical Aβ deposition and memory domain (Table 32). In addition, best fit *p*-value thresholds ($P_T$) for Aβ deposition was found at $P_T < 1 \times 10^{-5}$ ($r^2 = 0.104$; $p = 1.14 \times 10^{-14}$). For hippocampal volume, best fit *p*-value threshold was found at $P_T < 0.01$ ($r^2 = 0.013$; $p = 8.52 \times 10^{-10}$) and for memory domain, involving SVLT immediate recall test, the threshold was at $P_T < 0.01$ ($r^2 = 0.020$; $p = 1.45 \times 10^{-12}$) and SVLT delayed recall test showed that the threshold was at $P_T < 0.01$ ($r^2 = 0.030$; $p = 1.95 \times 10^{-17}$) (Table 32). This study also hypothesized that grouping of SNPs based on their effect size direction from the association analysis can improve the PGRSs correlation for AD even in the early stage of AD dementia. As expected, the results showed that the cortical Aβ deposition was strongly correlated with PGRSs for AD when SNPs with positive effect size group was involved. Whereas, other biomarkers including hippocampal volume and memory domain showed strong correlation with PGRSs for AD when SNPs with negative effect size group was involved (Figure 19).

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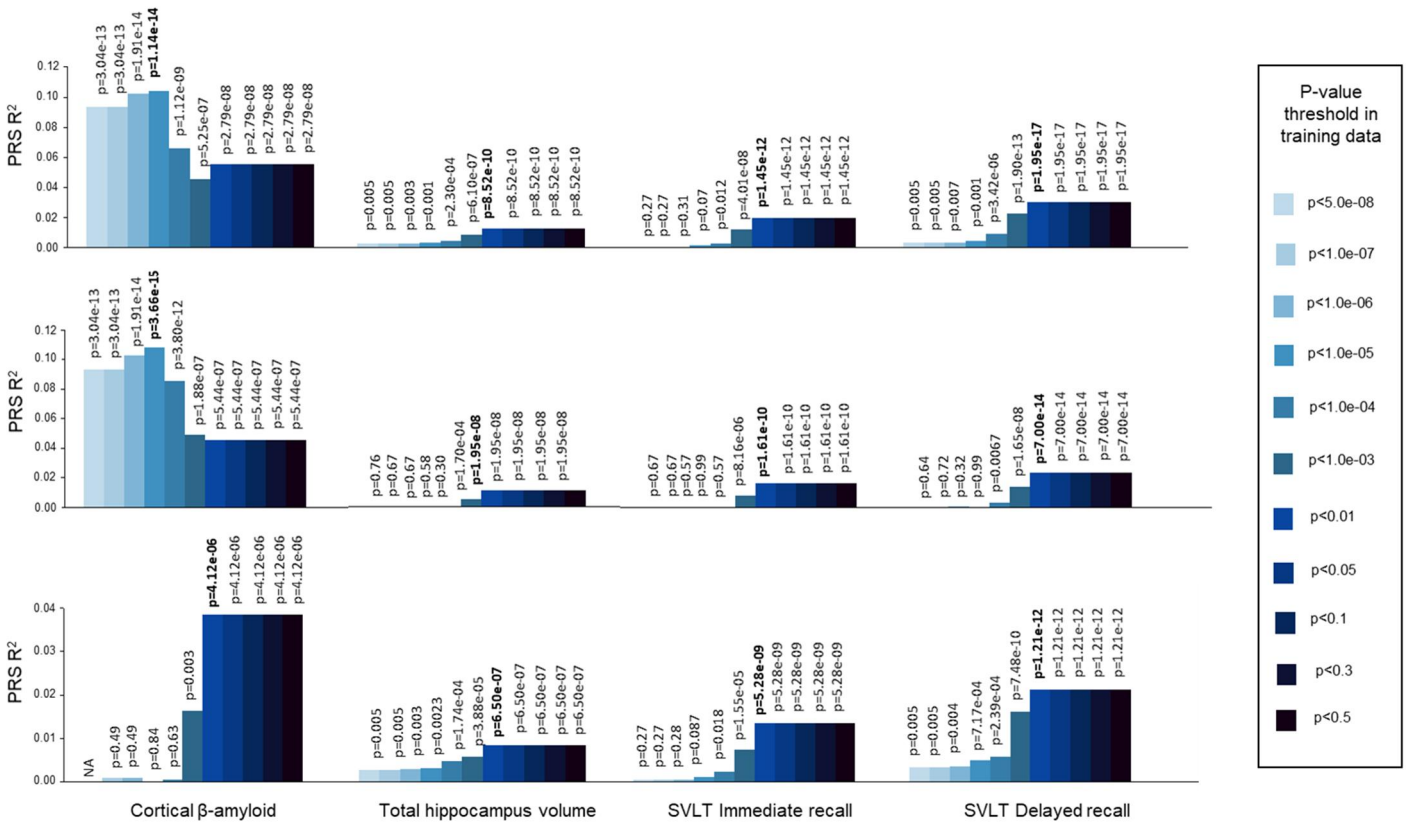


**Figure 19. Effects of AD polygenic risk scores (PGRSs) in early stage of AD.** The bar plot shows strong correlation of PGRSs for AD on cortical β-amyloid, hippocampal volume and memory domain. Horizontal axis denotes AD biomarkers showing

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correlation (indicated as bar) and association (*p*-value) with AD PGRSs. Top bar plots show PGRSs for AD with all SNPs included, the middle bar plot show PGRSs for AD with SNPs having positive effect size and the bottom bar plot show PGRSs for AD with SNPs having negative effect size. The best-fit thresholds are shown in bold.

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**Table 32.** Effects of AD polygenic risk scores (PGRSs) on AD biomarker in the early stage of AD.

| Training $P_T$ value | Cortical β-amyloid $R^2$ (*p*-value) | | | Total hippocampus $R^2$ (*p*-value) | | | SVLT Immediate Recall $R^2$ (*p*-value) | | | SVLT Delayed Recall $R^2$ (*p*-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size |
| $P_T < 5\times10^{-8}$ | 0.094 (3.04e-13) | 0.094 (3.04e-13) | NA | 0.003 (0.005) | 3.06e-05 (0.76) | 0.003 (0.005) | 0.001 (0.27) | 7.26e-05 (0.67) | 0.001 (0.27) | 0.003 (0.005) | 9.40e-05 (0.64) | 0.003 (0.005) |
| $P_T < 1\times10^{-7}$ | 0.094 (3.04e-13) | 0.094 (3.04e-13) | 8.90e-04 (0.49) | 0.003 (0.005) | 6.38e-05 (0.67) | 0.003 (0.005) | 0.001 (0.27) | 7.26e-05 (0.67) | 0.001 (0.27) | 0.003 (0.005) | 5.54e-05 (0.72) | 0.003 (0.005) |
| $P_T < 1\times10^{-6}$ | 0.103 (1.91e-14) | 0.103 (1.91e-14) | 8.90e-04 (0.49) | 0.003 (0.003) | 6.38e-05 (0.67) | 0.030 (0.003) | 4.00e-04 (0.31) | 1.30e-04 (0.57) | 0.001 (0.28) | 0.003 (0.007) | 4.30e-04 (0.32) | 0.004 (0.004) |
| $P_T < 1\times10^{-5}$ | **0**.104 **(1.14e-14)** | **0.108 (3.66e-15)** | 7.93e-05 (0.84) | 0.004 (0.001) | 1.02e-04 (0.58) | 0.032 (0.002) | 0.001 (0.07) | 4.42e-08 (0.99) | 0.001 (0.09) | 0.004 (0.001) | 1.28e-07 (0.99) | 0.005 (7.17e-04) |
| $P_T < 1\times10^{-4}$ | 0.066 (1.12e-09) | 0.085 (3.80e-12) | 4.20e-04 (0.63) | 0.005 (2.30e-04) | 3.62e-04 (0.30) | 0.005 (1.74e-04) | 0.003 (0.012) | 1.40e-04 (0.57) | 0.022 (0.02) | 0.009 (3.42e-06) | 0.003 (0.007) | 0.006 (2.39e-04) |
| $P_T < 1\times10^{-3}$ | 0.045 (5.25e-07) | 0.049 (1.88e-07) | 0.016 (0.003) | 0.008 (6.10e-07) | 0.005 (1.70e-04) | 0.058 (3.88e-05) | 0.012 (4.01e-08) | 0.008 (8.16e-06) | 0.007 (1.55e-05) | 0.023 (1.90e-13) | 0.014 (1.65e-08) | 0.016 (7.48e-10) |
| **$P_T < 0.01$** | 0.055 (2.79e-08) | 0.045 (5.44e-07) | **0.038 (4.12e-06)** | **0.013 (8.52e-10)** | **0.011 (1.95e-08)** | **0.008 (6.50e-07)** | **0.020 (1.45e-12)** | **0.016 (1.61e-10)** | **0.014 (5.28e-09)** | **0.030 (1.95e-17)** | **0.024 (7.00e-14)** | **0.021 (1.21e-12)** |

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**Table 32.** Continued.

| Training $P_T$ value | Cortical β-amyloid $R^2$ (*p*-value) | | | Total hippocampus $R^2$ (*p*-value) | | | SVLT Immediate Recall $R^2$ (*p*-value) | | | SVLT Delayed Recall $R^2$ (*p*-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size |
| **$P_T$ < 0.05** | 0.055 (2.79e-08) | 0.045 (5.44e-07) | 0.038 (4.12e-06) | 0.013 (8.52e-10) | 0.011 (1.95e-08) | 0.008 (6.50e-07) | 0.020 (1.45e-12) | 0.016 (1.61e-10) | 0.014 (5.28e-09) | 0.030 (1.95e-17) | 0.024 (7.00e-14) | 0.021 (1.21e-12) |
| **$P_T$ < 0.1** | 0.055 (2.79e-08) | 0.045 (5.44e-07) | 0.038 (4.12e-06) | 0.013 (8.52e-10) | 0.011 (1.95e-08) | 0.008 (6.50e-07) | 0.020 (1.45e-12) | 0.016 (1.61e-10) | 0.014 (5.28e-09) | 0.030 (1.95e-17) | 0.024 (7.00e-14) | 0.021 (1.21e-12) |
| **$P_T$ < 0.3** | 0.055 (2.79e-08) | 0.045 (5.44e-07) | 0.038 (4.12e-06) | 0.013 (8.52e-10) | 0.011 (1.95e-08) | 0.008 (6.50e-07) | 0.020 (1.45e-12) | 0.016 (1.61e-10) | 0.014 (5.28e-09) | 0.030 (1.95e-17) | 0.024 (7.00e-14) | 0.021 (1.21e-12) |
| **$P_T$ < 0.5** | 0.055 (2.79e-08) | 0.045 (5.44e-07) | 0.038 (4.12e-06) | 0.013 (8.52e-10) | 0.011 (1.95e-08) | 0.008 (6.50e-07) | 0.020 (1.45e-12) | 0.016 (1.61e-10) | 0.014 (5.28e-09) | 0.030 (1.95e-17) | 0.024 (7.00e-14) | 0.021 (1.21e-12) |

Abbreviation: $P_T$, *p*-value threshold; SNP, single nucleotide polymorphism; SVLT, Seoul Verbal Learning Test.

Best-fit thresholds are indicated in bold.

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After that, this study evaluated the PGRSs estimated from the best fit p-value thresholds by calculating the mean polygenic scores for each AD biomarker. Initially, this study calculated the mean polygenic score for AD with CN, MCI and AD dementia group, and then compared the mean polygenic scores calculated with CN and MCI groups. The results showed that that PGRSs for AD were associated with cortical β-amyloid in both early and late stage of AD dementia. The results also showed that the PGRSs increases with increased Aβ deposition and the PGRSs decreases with decreased hippocampal volume and cognitive performance (Figure 20A). When the SNPs were grouped based on the effect size direction, the results showed that PGRSs for AD increases when cortical Aβ deposition increases. This trend was observed when this study included SNPs that had positive effect size from association analysis (Figure 20B). Subsequently, when SNPs that had negative effect size were included, the PGRSs for AD increased with the decrease in hippocampal volume and cognitive decline. The trend is also in contrast when SNPs having positive effect size for hippocampal volume and cognitive domain were included. Similarly, PGRSs for AD decreases with decreased cortical amyloid deposition when SNPs with negative effect size (Figure 20C) were included. All AD biomarkers showed increased polygenic risk scores for AD in both early and late stages of AD (Table 33).

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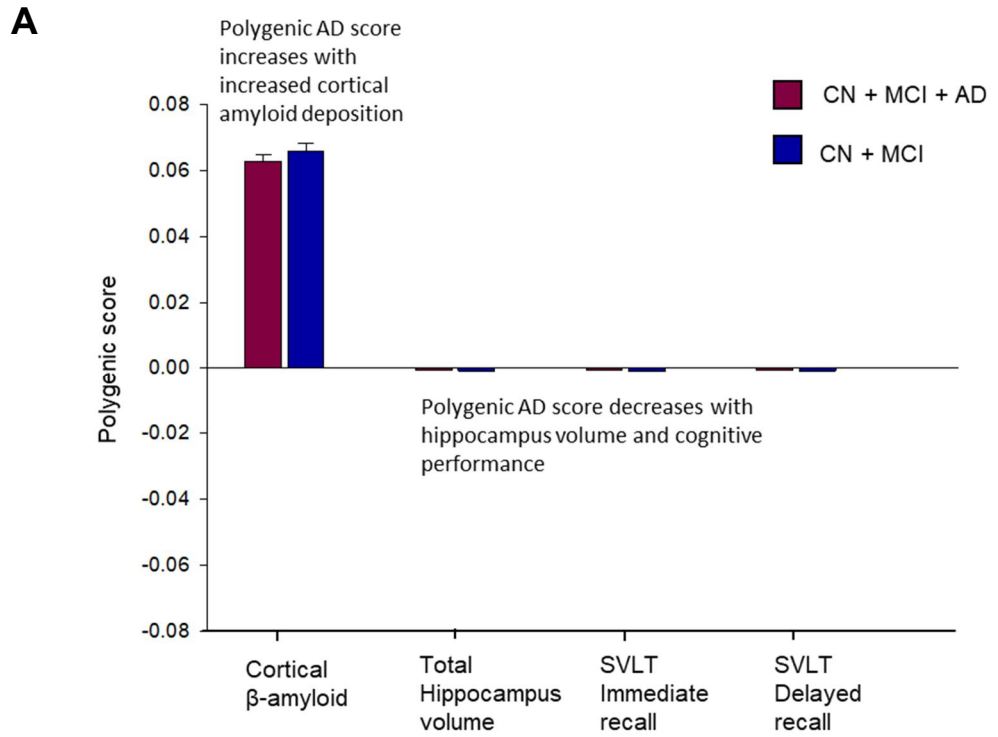


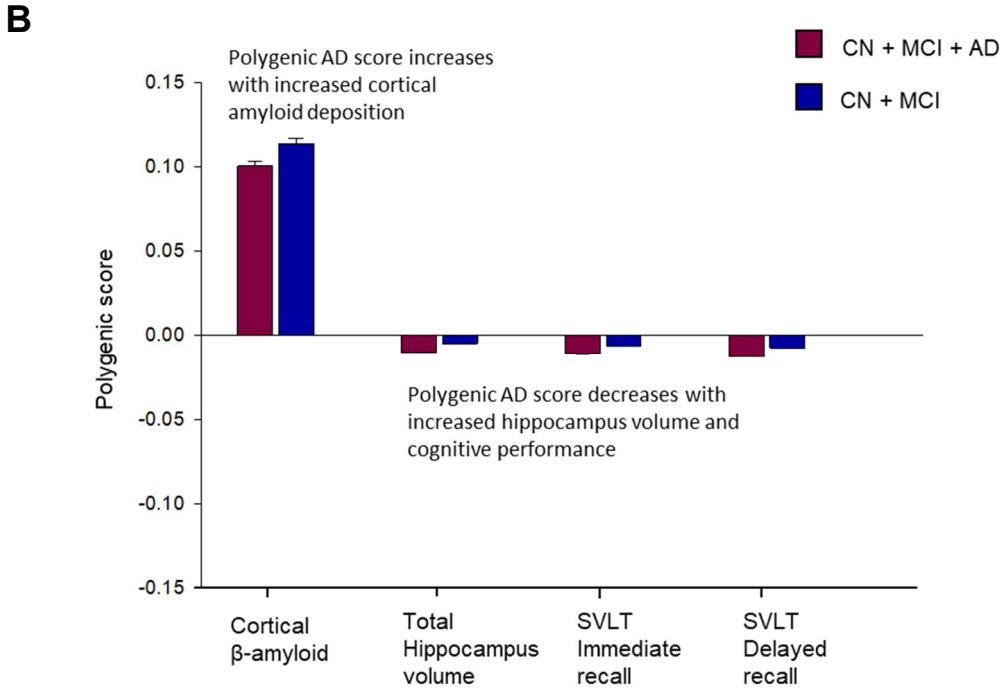


**Figure 20.** Continued.

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**C**

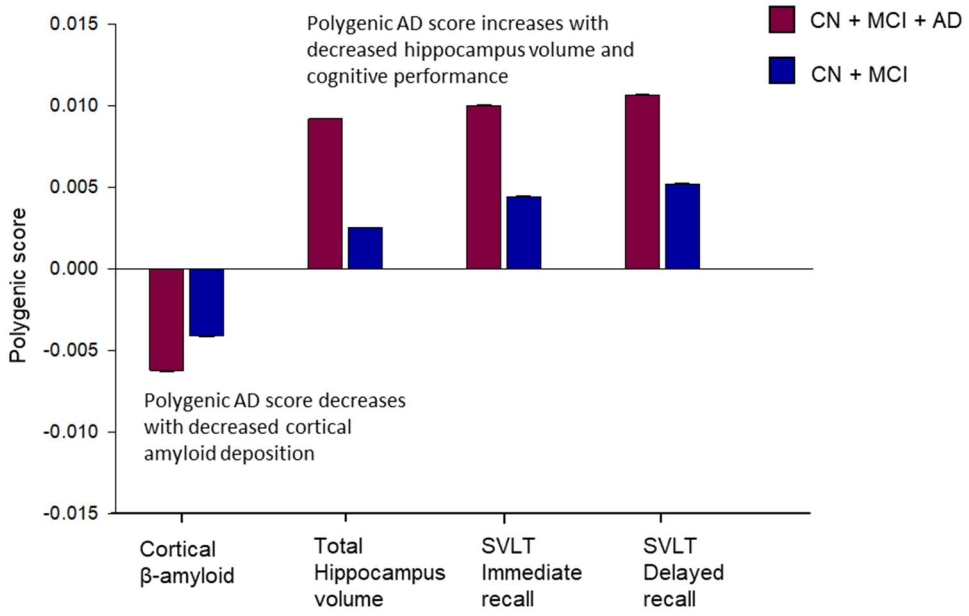


**Figure 20. Elevated polygenic risk scores (PGRSs) of Alzheimer's disease (AD) are visible in both early and late stages of AD dementia. (A)** Bar plot shows mean of best-fit polygenic risk score calculated with and without AD dementia group in the AD biomarker cohort. Mean of best-fit polygenic risk score calculated with SNPs having positive effect size **(B)** and negative effect size **(C)** are also showed with and without AD dementia group.

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**Table 33.** Mean best-fit polygenic score by diagnostic status calculated with SNPs discriminated by positive and negative effect sizes and overall SNPs.

| Phenotypes | CN + MCI + AD group mean polygenic score (S.E) | | | CN + MCI group mean polygenic score (S.E) | | |
|---|---|---|---|---|---|---|
| | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size | Overall SNPs | SNPs with positive effect size | SNPs with negative effect size |
| Cortical β-aamyloid | **0.063** **(0.002)** | **0.100** **(0.003)** | -0.006 (7.62e-05) | **0.066** **(0.002)** | **0.114** **(0.0032)** | -0.004 (8.39e-05) |
| Total Hippocampus volume | -0.001 (4.36e-05) | -0.010 (4.44e-05) | **0.009** **(4.57e-05)** | -0.001 (4.29e-05) | -0.005 (4.487e-05) | **0.003** **(4.48e-05)** |
| SVLT Immediate recall | -0.001 (4.36e-05) | -0.011 (4.45e-05) | **0.010** **(4.55e-05)** | -0.001 (4.30e-05) | -0.006 (4.44e-05) | **0.004** **(4.54e-05)** |
| SVLT Delayed recall | -0.001 (4.36e-05) | -0.013 (4.45e-05) | **0.011** **(4.55e-05)** | -0.001 (4.30e-05) | -0.007 (4.45e-05) | **0.005** **(4.54e-05)** |

Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; S.E, standard error, SNP, single nucleotide polymorphism. Significant *p*-values are indicated in bold letters.

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## IV-4. DISCUSSION

In this cross-sectional study, both structural brain imaging pathological brain imaging and cognitive domain were included. Then, the association strength of PGRSs for AD dementia with all these AD biomarkers were evaluated. From the results, this study found that the association strength of PGRSs for AD dementia varies with AD biomarkers. As expected, this study found strong correlation for PGRSs of AD with cortical β-amyloid deposition than other AD biomarker. In addition, other biomarkers like hippocampal volume and cognitive domain showed stronger association of PGRSs for AD. Then, this study hypothesized that the strength of PGRSs for AD may vary based on the SNP's effect size direction obtained from the association analysis. The results also suggested that cortical amyloid deposition show stronger correlation for AD PGRSs with SNPs having positive effect size. Similarly, the hippocampal volume and cognitive domain show stronger correlation for AD PGRSs with SNPs having negative effect size. The results are also in concordance with the previous studies suggesting that hippocampal volume, cortical Aβ and cognitive domain are strongly associated with PGRSs for AD (Ge, Sabuncu, Smoller, Sperling, & Mormino, 2018) (Foley et al., 2017).

Moreover, this study also hypothesized that PGRSs of AD likely associated even in the early stages of AD dementia. To evaluate the hypothesis, this study excluded AD dementia group in all the AD biomarker cohorts and retained cognitively normal and mild cognitively impaired individuals. The results suggested that all the AD biomarker group showed stronger correlation for PGRSs of AD. Furthermore, SNPs stratified by effect size directions showed that cortical amyloid deposition is strongly correlated with PGRSs of AD with SNPs having positive effect size direction, while hippocampal volume and cognitive domain strongly correlated with PGRSs of AD with negative effect size direction. This suggests that AD

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biomarkers may show varied strength of association for AD PGRSs based on the effect size direction and this pattern is visible even in the early stage of AD dementia.

This study also evaluated the mean best-fit PGRSs of AD with AD biomarkers in both late stage and early stage AD dementia. The results suggested that in overall AD biomarker, cortical Aβ measure showed stronger association for PGRSs of AD with and without AD dementia group suggesting that cortical Aβ measure could be a strong AD biomarker in cross-sectional cohorts which lack longitudinal subjects. However, other biomarkers like hippocampal volume and cognitive domain showed no clear pattern when all the SNPs were included in the analysis. When SNPs were stratified based on their effect size direction for biomarker like hippocampal volume and cognitive domain, the PGRSs for AD exhibited clear pattern of discrimination between increased and decreased PGRSs. For instance, SNPs with negative effect size direction showed increased PGRSs for AD with decreased hippocampal volume and cognitive domain. Furthermore, this pattern was clear in both early and late stages of AD dementia suggesting that AD biomarker like hippocampal volume and cognitive domain can efficiently adopted in the calculating early and late stage PGRSs of AD when the SNPs are stratified based on their effect size direction.

Furthermore, the analysis of mean best-fit PGRSs of AD revealed that SNPs based on their effect size direction from the association analysis were better in discriminating the PGRSs of AD. This also suggests that there is a synergistic effect among AD biomarkers in both early and late stages of AD dementia, which were reflected in the analysis of mean best-fit PGRSs of AD (Figure 20A-C). This study has limitation in the sample size and there were around 20% overlapping AD dementia subjects in the AD case-control cohort and AD biomarker cohorts. However, this study confirmed the association of AD biomarker like cortical Aβ deposition, hippocampal volume and cognitive domain with PGRSs of AD in an East

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Asian population. In addition, this study identified that SNPs based on their effect size direction could influence the correlation of AD biomarker with PGRSs of AD.

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# PART 5

***Overall conclusion***

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## V-1. OVERALL CONCLUSION

In this study, by involving large scale Magnetic Resonance imaging (MRI), cerebral β-amyloid standard uptake value ratios (SUVR) measured from florbetaben ($^{18}$F) positron emission tomography (PET) images, cognitive measures and genetic datasets were investigated in the genome-wide association study (GWAS). From MRI GWAS, PR/SET domain 4 (*PRDM4*) gene was identified for the association with temporal lobe atrophy, Alzheimer's disease (AD) risk and β-amyloid deposition in the brain. The regional SNP-based association analysis and haplotype association analysis on *PRDM4* locus identified that rs117303558 and rs73398399 SNPs were associated with AD risk and Aβ deposition, which were also specific to East Asian population. In addition, earlier studies the role of *PRDM4* in neutrophin signaling is another interesting factor to look into further (Kawalia et al., 2017). Moreover, the regulation of Aβ deposition by *PRDM4* in the brain could be possibly another gene target which need to be studied further their detailed functional role in AD pathology.

In addition, from a GWAS using brain cerebral β-amyloid levels as the trait, this study identified two novel risk loci located at rs12975891 on chromosome 19 (closest gene *CPAMD8*), and at rs113454949 on chromosome 3 (*ARL14*). This study also identified a missense variant, rs3745339 in the *CPAMD8* gene. In these two novel genetic loci (*CPAMD8* and *ARL14*) several genes including *CPAMD8*, *ARL14*, *SMC4*, *KPNA4*, *TRIM59* and *IFT80* genes, which are likely playing an important role in β-amyloid pathology in the brain. This is also a first study in an East Asian population to identify β-amyloid associated genes using amyloid PET imaging. Identification of ethnic specific risk genes are always in the interest of scientific communities to develop a personalized medicine for a particular population, and in such a way this study provides additional opportunity in the field of therapeutics, since existing approved and in trail AD drugs are not targeting the risk genes efficiently (Kwok, Lin, & Schooling, 2018). Additionally, from a polygenic risk

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score (PGRS) analysis, this study identified AD biomarkers that exhibit synergistic effect in promoting AD progression during early and late stages of AD. This study also reported an efficient method in the PGRS based AD prediction models.

In future, expansion of MRI GWAS with very large cohort have been planned to identify more ethnic specific risk variants and genes that contributes for brain atrophy and AD risk. With amyloid PET cohort, second stage GWAS meta-analysis with additional subjects have been planned. Furthermore, the study identifying ethnic specific amyloid associated risk variants and genes are also planned. Additionally, the expansion of PGRSs analysis in identifying comprehensive ethnic specific polygenic risk variants associated with early and late stage AD biomarkers are also planned.

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# VI. 초록

## 알츠하이머병의 뇌손상과 베타아밀로이드 축적에 대한 영상 유전학 연구

구나세카란 타밀어 이니얀
지도교수: 이 건 호
의과학과
조선대학교 대학원

알츠하이머병은 복합적인 신경퇴행성 질환으로 치매의 주요 원인 중 하나이다. 알츠하이머병은 뇌에서 베타 아밀로이드 단백질 침착과 신경섬유다발 엉킴과 더불어 특정 뉴런과 시냅스 손실, 신경실 등에 의해 인지능력이 점진적으로 감소되는 특징이 있다. *APOE* e4는 알츠하이머병의 가장 강력한 유전적 위험인자로 뇌의 베타 아밀로이드 침착과 뇌 위축과 관련이 있으며, *APOE* 유전자와는 별도로 21개의 유전변이가 알츠하이머병과 연관되어 있다는 보고가 있다. 본 연구는 베타 아밀로이드 침착, 뇌 위축 및 알츠하이머병 진행과 관련된 새로운 유전변이를 발굴하고, 알츠하이머병 진행에서 인종적 차이를 규명하는 연구이다.

본 연구에서는 구조적 자기공명영상에서 측정된 대뇌 피질 두께,

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$^{18}$F-Florbetaben($^{18}$F-FBB) 양전자방출 단층촬영영상에서 측정된 베타 아밀로이드 표준섭취계수율, 인지측정값과 유전체 데이터셋을 사용하여 전장유전체 연관성분석을 수행하였다. 첫번째로 자기공명영상 기반 전장유전체 연관성분석 결과, *PRDM4* 유전자가 대뇌 측두엽의 위축, 알츠하이머병 및 베타 아밀로이드 침착과 연관이 있었음을 확인하였다. 단일염기다형성 기반 연관성 분석 및 haplotype 연관성 분석에서 *PRDM4*의 rs117303558과 rs73398399는 알츠하이머병과 베타 아밀로이드 침착과 연관이 있었다. 따라서, *PRDM4*의 rs117303558과 rs73398399는 베타 아밀로이드 침착을 촉진하여 병리학적으로 알츠하이머병과 연관되었음을 시사한다. $^{18}$F-FBB 양전자방출 단층촬영영상에서 측정한 뇌의 베타 아밀로이드 침착정도를 전장유전체 연관성 분석한 결과, 19번 염색체(가장 가까운 유전자 *CPAMD8*)의 rs1297591과 3번 염색체(*ARL14*)의 rs11345449에 위치한 2개의 새로운 유전변이를 추출하였다. 또한 *CPAMD8* 유전자에서 missense variant(rs374533)를 검출하였다. 요약하면 뇌의 베타 아밀로이드 병리학에서 중요한 역할을 하는 *CPAMD8*, *ARL14*, *SMC4*, *KPNA4*, *TRIM59*, *IFT80* 유전자를 포함하는 2개의 새로운 유전자(*CPAMD8*, *ARL14*)를 추출하였다. 다음으로 다중유전변이 위험점수분석에서 알츠하이머병 초기 및 후기 단계에서 알츠하이머병을 유발하는 베타 아밀로이드, 해마 부피 및 인지영역 등의 알츠하이머병 바이오마커 간 시너지 효과를 확인하였다. 또한 연

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관성 분석에서 단일염기다형성의 효과 크기 방향이 다중유전변이 위험점수와 알츠하이머병 바이오마커 간 상관관계에 영향을 줄 수 있다는 것을 확인하였다.

본 연구에서는 대규모 분석을 통해 알츠하이머병 환자들의 뇌 병리학적 변화와 연관된 새로운 유전변이를 추출하였다. 더욱이 본 연구는 동아시안 특이 단일염기다형성을 보여주었고 뇌 위축과 대뇌 베타 아밀로이드 침착 및 알츠하어머병과 관련된 유전자를 보여주었으며, 복잡한 신경퇴행성 질환인 치매 원인 및 기전 이해에 중요한 결과이다.

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