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Family-based association analysis of NAV2 gene with the risk and age at onset of Alzheimer's disease

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Abstract

The neuron navigator 2 (NAV2) gene is highly expressed in brain and involved in the nervous system development and may play a role in Alzheimer's disease (AD). We aimed to investigate the associations of 317 single-nucleotide polymorphisms (SNPs) in the NAV2 gene with risk and age at onset (AAO) of AD using a family-based sample (1266 AD cases and 1279 healthy relatives). Association with the risk of AD was assessed using family-based association test -generalized estimating equations (FBAT- GEE) statistics while the association with AAO as a quantitative trait was evaluated using the FBAT-Wilcoxon statistic. Single marker analysis showed that 20 SNPs were significantly associated with AD (top SNP rs7112354 with $p = 8.46\times10^{-4}$) and 11 SNPs were associated with AAO (top SNP rs1354269 with $p = 2.87\times10^{-3}$). Interestingly, two SNPs rs17614100 and rs12364788 were associated with both the risk ($p = 1.7\times10^{-2}$ and 2.71×10^{-2}; respectively) and AAO ($p = 1.85\times10^{-3}$ and 6.06×10^{-3}; respectively). Haplotype analyses further supported the results of single marker analyses. The present study is the first study providing evidence of several genetic variants within the NAV2 gene influencing the risk and AAO of AD.

Keywords

Alzheimer's disease; age at onset; family-based design; NAV2; polymorphisms; haplotype

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Conflict of interest

The authors declare that they have no conflict of interest.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease and the prevalence dramatically increases when people getting older than 65 years (Hebert et al., 2003). It has been reported that AD is one of the top 10 leading causes of death in the United States (US) (Xu et al., 2010). In 2013, approximately 5 million people in the US aged 65 years or older were living with AD; while this number is projected to rise to 13.8 million, a nearly three-fold increase, by 2050 (Hebert et al., 2013). World Health Organization estimated that 0.379% of people worldwide had dementia in 2005 and the prevalence would increase to 0.441% in 2015 and to 0.556% in 2030 (WHO, 2006). Family and twin studies have shown that the heritability of the risk of AD ranges from 49% to 79% (Gatz et al., 2006). In addition to the disease risk, age-at-onset (AAO) of AD is also genetically influenced with an estimated heritability of about 42% (Daw et al., 2000; Li et al., 2002).

The neuron navigator 2 (NAV2) (also known as HELAD1; RAINB1; POMFIL2; UNC53H2; STEERIN2) gene is located at 11p15.1 (Coy et al., 2002; Maes et al., 2002) and contains 50 distinct gt-ag introns and 38 exons38 exons (Merrill et al., 2002)38 exons38 exons. 38 exonsThe NAV2 gene was most highly expressed in brain and also in kidney, live, thyroid, mammary gland, and spinal cord (Coy et al., 2002; Maes et al., 2002; Merrill et al., 2002) and may play a role in cellular growth, migration, and nervous system development (Coy et al., 2002;, Ishiguro et al., 2002; Merrill et al., 2002; Peeters et al., 2004; Clagett-Dame et al., 2006; McNeill et al., 2010; Marzinke et al., 2013). Recently, NAV2 was found to be associated with episodic memory scores in AD (Yan et al., 2015).

However, no study has focused on the association of NAV2 gene with the risk or AAO of AD to date. Based on previous reports on the functions of NAV2 in nervous system development and its role in episodic memory scores in AD, we hypothesized that NAV2 gene polymorphisms might play a role in AD. We investigated the associations of 317 single-nucleotide polymorphisms (SNPs) in the NAV2 gene with the risk and AAO of AD using a family-based sample.

2. Materials and Method

2.1. Subjects

A family-based sample was available from the National Institute on Aging - Late Onset Alzheimer's Disease (NIA-LOAD) Family Study: Genome-Wide Association Study for Susceptibility Loci – Study Accession: phs000168.v1.p1. The purpose of the study was to (1) identify and recruit families with two or more siblings with the late-onset form of AD and a cohort of unrelated, non-demented controls similar in age and ethnic background, and (2) make the samples, the clinical and genotyping data, and preliminary analyses available to qualified investigators world-wide. Genotyping by the Center for Inherited Disease Research (CIDR) was performed using the Illumina Infinium II assay protocol. The details about these subjects were described in previous studies (Lee et al., 2008). Totally, 1266 AD cases and 1279 non-AD individuals (including 1070 with AAO values) were from 1386 pedigree (589 nuclear families) (Table 1). There are 317 single-nucleotide polymorphisms (SNPs) within the NAV2 gene available in this sample.

2.2. Detection of mRNA expression of NAV2 in human brains

We examined the mRNA expression of NAV2 using Affymetrix Human ST 1.0 exon arrays in ten human brain regions in 134 UK Europeans without neurodegenerative disorders (Trabzuni et al., 2011). These ten brain regions included cerebellar cortex (CRTX), frontal cortex (FCTX), temporal cortex (TCTX), occipital cortex (OCTX), putamen (PUTM), thalamus (THAL), hippocampus (HIPP), substantia nigra (SNIG), and intralobular white matter and medulla (WHMT). The expression levels with raw intensity > 36, i.e., normalized log2(raw intensity) > 5.17, were taken as "expressed". The association between APOE and AD is most robust and the role of APOE in the development of AD has been well-recognized. Based on this fact, the correlation of expression between *NAV2* and *APOE* was tested using Pearson correlation analysis in these ten brain regions.

2.3. Statistical analysis

Departure from Hardy-Weinberg equilibrium (HWE) of each SNP and pairwise linkage disequilibrium (LD) statistic (r2) were assessed for founders using HAPLOVIEW (Barrett et al. 2005). Then, minor allele frequency (MAF) was determined for each SNP. A Family-based association analysis of AD was performed using PBAT 3.6.1 (Van Steen et al., 2005), which has been designed to handle nuclear families with missing parental genotypes, extended pedigrees with missing genotypic information, analysis of SNPs, haplotype analysis, quantitative traits, and time to onset phenotypes. For the affection status of AD, the family-based association test - generalized estimating equations (FBAT-GEE) statistic, adjust for sex, was used to perform family-based association analysis (Lange et al., 2003). For testing time-to-onset trait (AAO), the FBAT-Wilcoxon test was employed (Lange et al., 2004). The AAO values for healthy siblings were censored and age at entry into the study was used. Haplotype analysis was conducted in 2-SNP or 3-SNP sliding windows. To deal with multiple comparison, on the one hand, Bonferroni correction ($\alpha=0.05/317=1.58\times10^{-4}$) was used for statistical significance; on the other hand, we also used software QVALUE (http://faculty.washington.edu/~jstorey/qvalue) to calculate the false discovery rate (FDR) (Storey, 2002; Benjamini and Hochberg, 1995). Descriptive statistics for AAO and survival analyses were conducted with SAS 9.4 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Genotype quality control and descriptive statistics

Out of 317 SNPs in NAV2 gene, 20 SNPs with $p < 10^{-4}$ for HWE test and/or MAF < 5% were removed for further analysis. The demographic characteristics of the subjects in the study are shown in the Table 1. The mean AAO for cases was 76.4 years and the mean age at entry was 75.5 years for unaffected family members.

3.2. Association with the risk of AD

Twenty out of 297 SNPs were associated with the risk of AD ($p < 0.05$) using the FBAT-GEE analysis (Table 2). The most significant SNP was rs7112354 with $p = 8.46 \times 10^{-4}$ and the second and third best SNPs were rs6483627 and rs2034031 with $p = 1.12 \times 10^{-3}$ and 1.40×10^{-3}, respectively. Based on the QVALUE, the FDR of all top 3 SNPs is 14%.

The pairwise LD statistics (r^2) for 15 SNPs were assessed for founders using HAPLOVIEW (Figure 1). Rough rule of thumb, values of r^2 >1/3 might indicate sufficiently strong LD to be used for fine mapping (Ardlie et al., 2002).

Table 3 shows the part of haplotypes associated with the risk of AD ($p < 10^{-3}$). For example, the haplotype G-C-C inferred from rs1209071-rs4757023-rs2034031 ($r^2 = 0.53$ between rs1209071 and rs4757023, $r^2 = 0.72$ between rs4757023 and rs2034031, Figure 1) was mostly significantly associated with the risk of AD ($p = 6.9 \times 10^{-5}$), remained significant after a Bonferroni correction ($p<1.58\times10^{-4}$). The second significant haplotype was T-C-C inferred from rs47557875-rs4757023-rs2034031 ($p = 2.67 \times 10^{-4}$; $r^2 = 0.94$ between rs47557875 and rs4757023 and $r^2 = 0.98$ between rs4757023 and rs2034031).

3.3. Association with AAO of AD

Our results showed that 11 SNPs were associated with AAO ($p < 0.05$) by using the FBAT-Wilcoxon test (Table 4). The most significant SNP was rs1354269 ($p = 2.87 \times 10^{-3}$) followed by rs12364788 ($p = 6.06 \times 10^{-3}$) and 2702733 ($p = 7.34 \times 10^{-3}$). Based on the QVALUE, the FDR of the top 3 SNPs is 64%. Interestingly, two SNPs rs17614100 and rs12364788 were associated with both the risk ($p = 1.7 \times 10^{-2}$ and 2.71×10^{-2}; respectively) and AAO ($p = 1.85 \times 10^{-3}$ and 6.06×10^{-3}; respectively) of AD. Figure 2 shows the location of the SNPs showing association with AD and AAO.

Table 5 shows the top 2 haplotypes associated with AAO. For example, the T-T haplotype from rs1354269-rs4757793 ($r^2 = 0.69$ between 2 SNPs, Figure 1) showed the most significant associations with AAO ($p = 8.11 \times 10^{-4}$) and the second haplotype was T-T-C inferred from rs1354269-rs4757793-rs2702733 ($p = 8.4 \times 10^{-4}$; $r^2 = 0.49$ between rs1354269-rs4757793, $r^2 = 0.65$ between rs4757793 and rs2702733).

3.4. In silico analysis

We evaluated whether these variants were located within the regions of the gene that might have potential functional importance. The sequences containing the associated SNPs were examined for microRNA binding sites, splicing sites, regulatory gene regions, and species-conserved regions using NIH-SNP Function Prediction (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.php). We further found one AAO-associated SNP rs1354269 was located at the gene regulatory region and species-conserved region.

3.5. NAV2 mRNA was expressed across human brains

NAV2 mRNA had significant expression ($6.98\leq$normalized intensity≤7.50) across all ten human brain regions examined (Table 6). It was significantly correlated with APOE expression in CRTX, MEDU, THAL and WHMT ($3.73 \times 10^{-8}\leq p\leq 7.40 \times 10^{-3}$).

4. Discussion

In this article we reported the associations of NAV2 gene with the risk and AAO of AD using a family-based association study. The FBAT-GEE and FBAT-Wilcoxon tests showed that 20 SNPs were significantly associated with AD and 11 SNPs associated with AAO,

respectively. Furthermore, two SNPs rs17614100 and rs12364788 were associated with both the risk and AAO of AD. In addition, haplotype analyses further supported the results of single marker analysis.

Previous studies have shown that the NAV2 gene was highly expressed in brain. Several studies suggested that this gene plays an important role in cellular growth, migration, neuronal elongation and nervous system development (Coy et al., 2002;, Ishiguro et al., 2002; Maes et al., 2002; Merrill et al., 2002; Peeters et al., 2004; Clagett-Dame et al., 2006; McNeill et al., 2010; Marzinke et al., 2013). Furthermore, the NAV2 gene may be associated with episodic memory scores in AD (Yan et al., 2015). Therefore, we hypothesized that NAV2 gene polymorphisms may play a role in AD development. To our best knowledge, this is the first report to detect significant associations between several genetic variants within the NAV2 gene and the risk and AAO of AD. However, the mechanism for the role of NAV2 in AD still remains unclear. An animal model has shown that the NAV2 gene plays an important role in shaping the development of the mammalian nervous system and is involved in blood pressure regulation (McNeill et al., 2010). One human study revealed that the AD associated SNP rs2702663 in the present study was previously associated with both plaque presence and area in Dominican families (Dong et al., 2012), implicating that NAV2 polymorphisms may be involved in the development of atherosclerosis. Through the gene-level main effect analysis, NAV2 gene was recently reported to be associated with seven cognitive scores related to episodic memory in Caucasian AD patients (Yan et al., 2015). This indicates that NAV2 may be one of the mechanisms linking to blood pressure, cardiovascular disease, and neurodegenerative diseases. Another gene expression study showed that NAV2 was overexpressed in human colorectal cancer, indicating that NAV2 could serve as both a prognostic biomarker and a potential therapeutic target for colorectal cancer (Tan et al., 2015). In addition, in the present study, we found rs1354269 was located at species-conserved region, suggesting potential functional importance of the NAV2 gene in AD.

There are a number of strengths in this study. First, we used a family-based design, which can reduce the type 1 error rate arising from population stratification. Especially, the FBAT-GEE approach in the PBAT software can easily be adapted to scenarios with multiple offspring per family and missing parental information, and testing for linkage disequilibrium under the assumption of linkage (Lange et al., 2003). Second, we examined a large number of markers, totally 317 SNPs, within the NAV2 gene. Like most research, some limitations may exist in this study; for example, the sample size is moderate. Furthermore, only one 3-SNP haplotype G-C-C inferred from rs1209071-rs4757023-rs2034031 showed significant association after a Bonferroni correction. Thus, these findings need to be supported by additional large samples in future study.

5. Conclusion

This study firstly provided valuable evidence of several genetic variants within the NAV2 gene influencing the risk and AAO of AD using a family-based association study. The results were further supported by a haplotype analysis. These results will serve as a resource for replication in other populations to elucidate the potential role of these genetic variants in

AD. Further functional studies of these polymorphisms would help us better understand the pathogenesis of AD.

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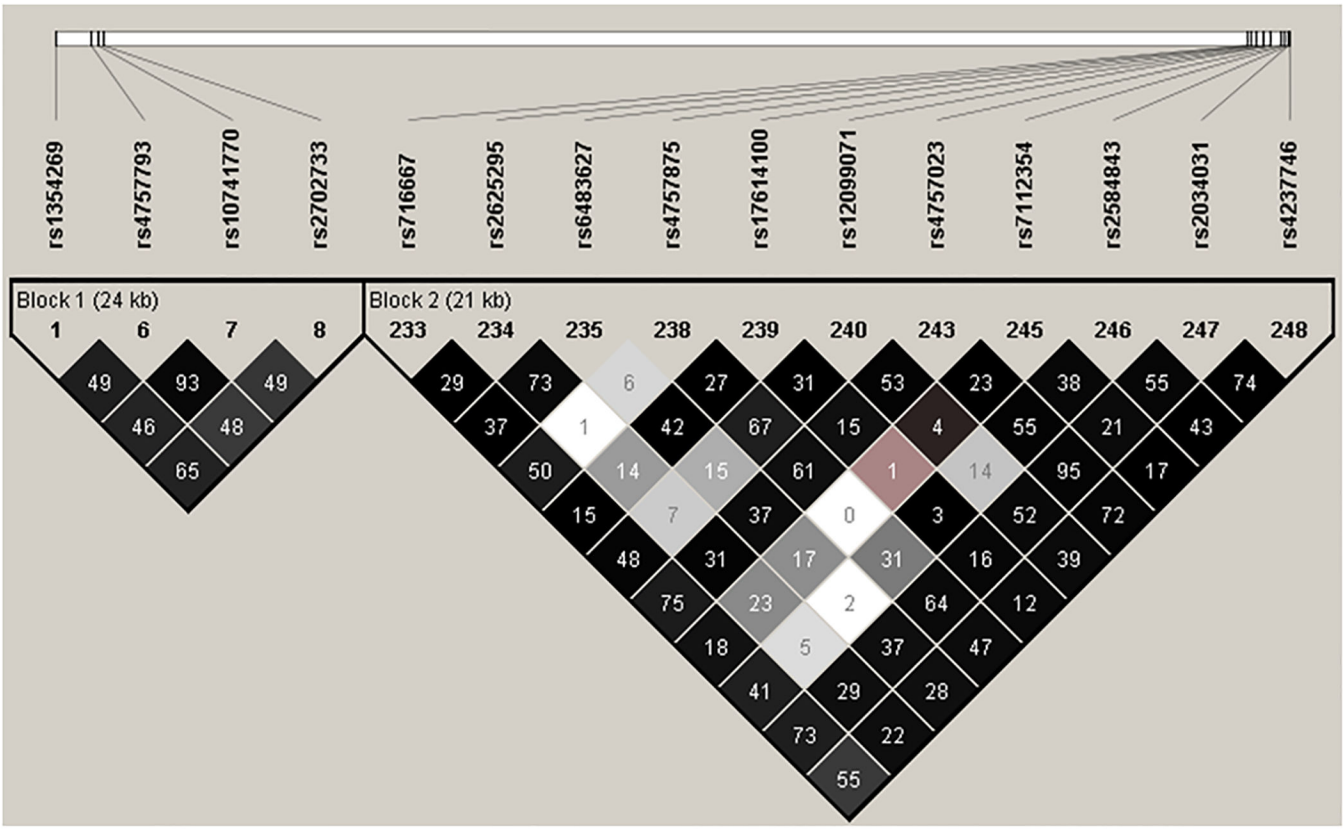

Fig. 1.

LD structure of SNPs within the NAV2 gene. The numbers indicate the r^2 values between the corresponding two SNPs.

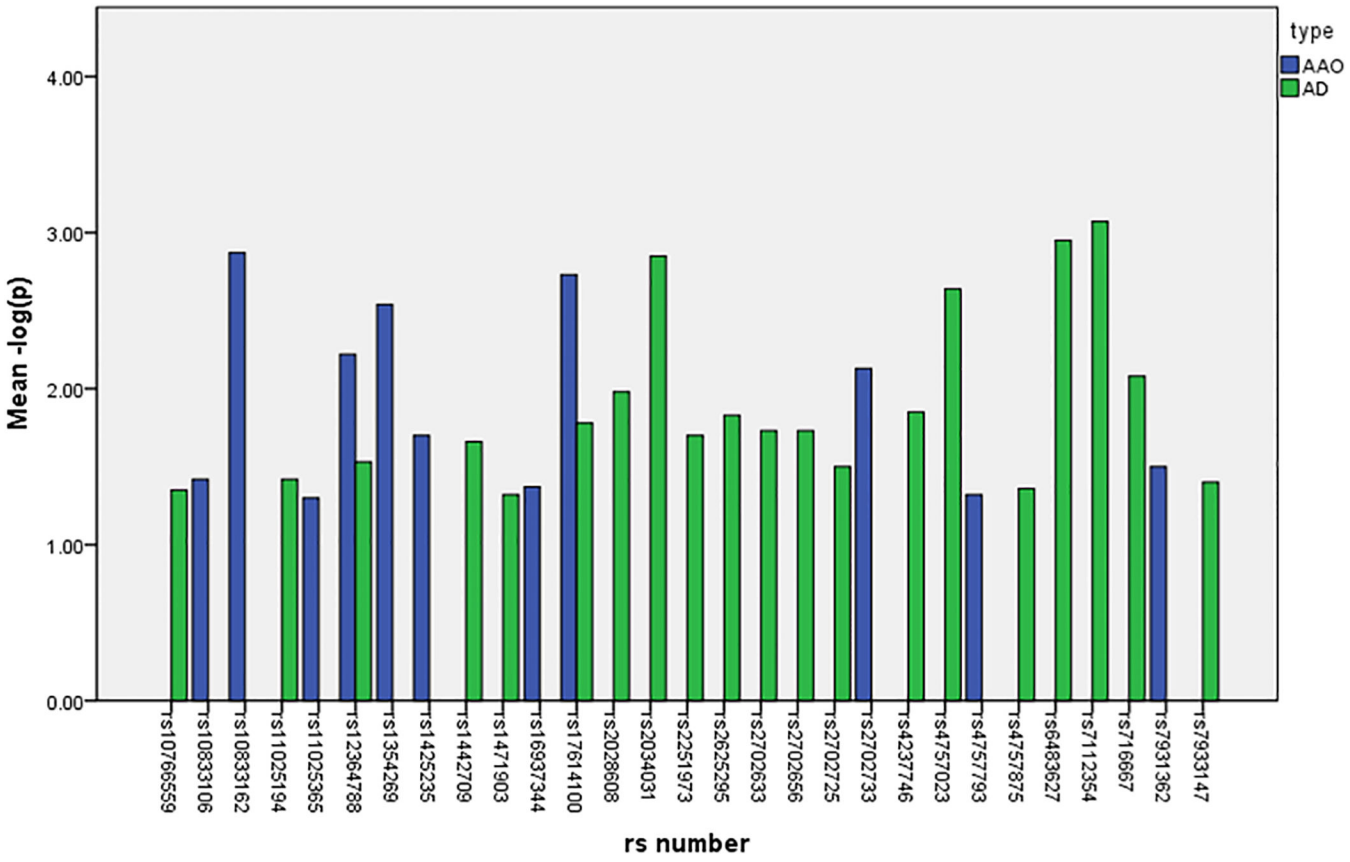

Fig.2.
The results of–log(p) values for a single SNP analysis

Table 1

Descriptive characteristics of cases and unaffected relatives.

Variable	Family Study	
	Patients	**Unaffected relatives**
Sample size (n)	1266	1279
Sex		
Male	435	466
Female	831	813
Mean AAO (years±SD)	76.4 (±6.7)	–
Median AAO (years)	77	–
Range of age at onset (years)	50–98	–
Mean age at entry (years±SD)	–	75.5 (±8.1)
Median age at entry (years)	–	75
Range of age at entry (years)	–	42–103

Table 2

Single marker analysis of risk of AD based on FBAT-GEE ($p < 0.05$).

SNP	Position[a]	Function[b]	AL[c]	MAF[d]	HWE[e]	Fam#[f]	$p_{\text{-FBAT-GEE}}$[g]	FDR[h]
rs7112354	19973194	Intron	A	0.13	0.198	135	8.46E-04	0.14
rs6483627	19958226	Intron	G	0.20	0.831	216	1.12E-03	0.14
rs2034031	11975334	Intron	T	0.37	0.389	278	1.40E-03	0.14
rs4757023	19971796	Intron	T	0.38	0.242	274	2.27E-03	0.21
rs716667	19954380	UTR-5	G	0.39	0.603	287	8.40E-03	0.51
rs2028608	19884920	Intron	G	0.38	0.191	278	1.05E-02	0.51
rs4237746	19975713	Intron	G	0.44	0.286	276	1.42E-02	0.51
rs2625295	19955495	Intron	T	0.17	0.292	170	1.48E-02	0.51
rs17614100	19966021	Intron	G	0.10	0.764	136	1.66E-02	0.51
rs2702656	19479440	Intron	G	0.12	0.031	161	1.88E-02	0.51
rs2702633	19447975	Intron	T	0.11	0.463	154	1.88E-02	0.51
rs2251973	19949373	Intron	T	0.09	0.311	111	1.98E-02	0.51
rs1442709	20046554	Intron	C	0.21	0.795	195	2.19E-02	0.52
rs12364788	19383668	Intron	G	0.31	0.601	264	2.96E-02	0.65
rs2702725	19452187	Intron	G	0.15	0.832	187	3.14E-02	0.65
rs11025194	19593961	Intron	G	0.48	0.153	290	3.78E-02	0.72
rs7933147	19950352	Intron	G	0.43	0.898	282	3.96E-02	0.72
rs4757875	19962468	Intron	G	0.28	0.802	230	4.35E-02	0.72
rs10766559	19420822	Intron	A	0.31	0.65	276	4.45E-02	0.72
rs1471903	19576520	Intron	T	0.39	0.242	282	4.81E-02	0.74

[a] Physical position is based on NCBI Genome Build 36.3;

[b] SNP function;

[c] Minor allele;

[d] MAF refers to the minor allele frequency;

[e] HWE refers to p -value of Hardy-Weinberg equilibrium test;

[f] Fam# refers to the number of informative families;

[g] p-FBAT-GEE refers to p-value based on FBAT-GEE analysis for affection status;

[h] False discovery rate (FDR) for the SNP.

Table 3

Haplotypes associated with risk of AD based on FBAT-GEE ($p < 0.001$).

SNPs	Haplotype[a]	Frequency[b]	Fam#[c]	$p_{\text{-FBAT-GEE}}$[d]
rs2625295-rs6483627	C-A	0.69	224	6.75E-04
rs47557875-rs12099071-rs4757023	T-G-C	0.64	142	4.64E-04
rs47557875-rs12099071-rs2584843	T-G-C	0.62	203	3.85E-04
rs47557875-rs4757023-rs2034031	T-C-C	0.62	131	2.67E-04
rs12099071-rs4757023-rs2034031	G-C-C	0.61	142	6.90E-05
rs12099071-rs4757023-rs4237746	G-C-A	0.54	165	3.08E-04

[a] Haplotype inferred from 2 or 3 SNPs;

[b] Haplotype frequency;

[c] Fam# refers to the number of informative families;

[d] p-FBAT-GEE refers to p-value based on FBAT-GEE analysis for affection status.

Table 4

Single marker analysis of age at onset of AD based on FBAT-Wilcoxon test ($p < 0.05$).

SNP	Position[a]	Function[b]	AL[c]	MAF[d]	HWE[e]	Fam#[f]	$p_{\text{-FBAT-Wilcoxon}}$[g]	FDR[h]
rs1354269	19330820	Regulatory	C	0.38	0.532	210	2.87E-03	0.64
rs12364788	19383668	Intron	G	0.31	0.601	197	6.06E-03	0.64
rs2702733	19355815	Intron	A	0.32	0.17	183	7.33E-03	0.64
rs10833162	19727374	Intron	T	0.11	0.326	93	1.35E-03	0.83
rs17614100	19966021	Intron	G	0.10	0.764	111	1.85E-03	0.83
rs1425235	19795239	Intron	C	0.15	0.741	134	2.01E-02	0.83
rs7931362	19767763	Intron	C	0.10	0.458	83	3.18E-02	0.83
rs10833106	19379204	Regulatory	G	0.22	0.158	169	3.78E-02	0.83
rs16937344	19980715	Intron	G	0.07	0.176	58	4.22E-02	0.83
rs4757793	19349749	Intron	C	0.26	0.655	166	4.83E-02	0.83
rs11025365	20035072	Intron	A	0.17	0.545	130	4.98E-02	0.83

[a] Physical position is based on NCBI Genome Build 36.3;

[b] SNP function;

[c] Minor allele;

[d] MAF refers to the minor allele frequency of the SNP;

[e] HWE refers to *p*-value of Hardy-Weinberg equilibrium test;

[f] Fam# refers to the number of informative families;

[g] $p_{\text{-FBAT-Wilcoxon}}$ refers to *p*-value based on FBAT-Wilcoxon analysis for AAO;

[h] False discovery rate (FDR) for the SNP.

Table 5

Haplotypes associated with age at onset of AD based on FBAT-Wilcoxon test ($p < 0.001$).

SNPs	Haplotype[a]	Frequency[b]	Fam#[c]	p-FBAT-Wilcoxon[d]
rs1354269-rs4757793	T-T	0.51	235	8.11E-04
rs1354269-rs4757793-rs2702733	T-T-C	0.54	102	8.40E-04

[a] Haplotype inferred from 2 or 3 SNPs;

[b] Haplotype frequency;

[c] Fam# refers to the number of informative families;

[d] p-FBAT-GEE refers to p-value based on FBAT-GEE analysis for affection status.

Table 6

The mRNA expression levels of NAV2 in human brains of a UK European sample (n=134).

	Average	CRTX	FCTX	HIPP	MEDU	OCTX	PUTM	SNIG	TCTX	THAL	WHMT
Normalized intensity	7.17	6.98	7.06	7.08	7.50	7.12	7.13	7.32	7.06	7.41	7.16
Correlation with APOE: r^2	–	−0.25	–	–	0.48	–	–	–	–	0.24	0.36
Correlation with APOE: p	–	4.15E-03	–	–	3.73E-08	–	–	–	–	7.40E-03	2.55E-05

r^2, correlation coefficient. CRTX, cerebellar cortex; FCTX, frontal cortex; HIPP, hippocampus; MEDU, medulla (specifically inferior olivary nucleus); OCTX, occipital cortex (specifically primary visual cortex); PUTM, putamen; SNIG, substantia nigra; TCTX, temporal cortex; THAL, thalamus; WHMT, intralobular white matter.