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Genome-wide analysis identified abundant genetic modulators of contributions of the *APOE* alleles to the Alzheimer's disease risk

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Abstract

INTRODUCTION: The *APOE* ε2 and ε4 alleles have beneficial and adverse impacts on Alzheimer's Disease (AD), respectively, with incomplete penetrance, which may be modulated by other genetic variants.

METHODS: We examined whether the associations of the *APOE* alleles with other polymorphisms in the genome can be sensitive to AD-affection status.

RESULTS: We identified associations of the ε2 and ε4 alleles with 314 and 232 polymorphisms, respectively. Of them, 35 and 31 polymorphisms had significantly different effects in AD-affected and unaffected groups, suggesting their potential involvement in the AD pathogenesis by modulating the effects of the ε2 and ε4 alleles, respectively. Our survival-type analysis of the AD risk supported modulating roles of multiple group-specific polymorphisms. Our functional analysis identified gene enrichment in multiple immune-related biological processes, e.g., B cell function.

DISCUSSION: These findings suggest involvement of local and inter-chromosomal modulators of the effects of the *APOE* alleles on the AD risk.

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AUTHOR CONTRIBUTIONS

A.N. and A.M.K. conceived and designed the study and wrote the manuscript; A.N. and I.C. performed statistical and bioinformatics analyses; Y.L. and L.H. downloaded and imputed genotyping data.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Information File: containing *Supporting Acknowledgment*, Table S1, and Figures S1–S4. Tables S2–S16 in Excel format.

Keywords

Neurodegenerative Diseases; Dementia; Aging; GWAS; Cox Regression; Age at the Onset; Sex-Specific Associations; Genetic Modulators; Genetic Polymorphisms

1. BACKGROUND

Apolipoprotein E (*APOE*) gene that encodes a protein involved in lipids transport and metabolism[1] has been widely studied in the past decades due to its broad functional implications and potential roles in various traits[2], such as Alzheimer's disease (AD)[3–5], vascular dementia[6], dementia with Lewy bodies[7], coronary artery diseases[8,9], cerebrovascular accidents[10–12], Parkinson's disease-associated dementia[13], frontotemporal lobar degeneration[14], malignancies[15], immune/inflammatory responses and autoimmune disorders[16–18], and longevity[19,20]. The *APOE* gene has three main alleles, i.e., ε2, ε3, and ε4. The ε4 allele encoded by the minor allele of rs429358 single-nucleotide polymorphisms (SNP) is considered as the strongest single genetic risk factor for AD, which is associated with AD in various populations[4,21]. The ε2 allele encoded by the minor allele of rs7412 shows beneficial associations with AD[4,22], but the understanding of its potential protective role is tempered due to, in part, its small population frequency and the diminished number of AD cases among ε2 carriers.

Despite decades of research, the role of the *APOE* gene and its neighboring region in AD development is not entirely clear because of uncertainty about how to treat genetic variants from this region. For example, while most of the field tends to consider the role of the ε4 allele itself, the role of more complex structures such as haplotypes with variants from different genes in the *APOE* region is also widely emphasized[23–27]. The complex role of the *APOE* region variants, as well as the other variants from the entire genome, in AD has been supported by environmental[28] and evolutionary[27,29] studies.

In this study, we examined the associations of the ε2 and ε4 alleles with other SNPs in the human genome. We leveraged a strategy, which can partly address heterogeneity in genetic predisposition to AD by examining these allele-SNP associations in the AD-affected and unaffected subjects separately. We evaluated whether these associations in the AD-affected and unaffected subjects were different. Our analyses aimed to better understand genetic modulators of contributions of the *APOE* alleles to AD pathogenesis, especially variants outside of the neighboring genes in the *APOE* 19q13.3 region. These analyses identified a large number of promising ε2- and ε4-associated loci, both within and outside the *APOE* region, in the AD-affected, unaffected, or both groups. We found a subset of these loci in which associations of the ε2 and ε4 alleles with the other SNPs were statistically different in the AD-affected and unaffected groups. These findings suggest the roles of interactions of the ε2- and ε4 alleles with SNPs from specific loci spread throughout the entire genome in AD pathogenesis.

2. METHODS

2.1 Study participants

We used data on individuals of European ancestry from five studies: Cardiovascular Health Study (CHS)[30], Framingham Heart Study (FHS)[31,32], Late Onset Alzheimer's Disease Family Study (LOADFS) from the National Institute on Aging (NIA)[33], whole genome sequencing (WGS) data from Alzheimer's Disease Sequencing Project (ADSP-WGS)[34,35], and three cohorts from the NIA Alzheimer's Disease Centers (ADCs), which are a part of the Alzheimer's Disease Genetics Consortium (ADGC)[36]. The overlapping ADSP-WGS participants with the other datasets were excluded. The *APOE* genotypes in CHS and LOADFS were determined based on their genotypes at rs429358 and rs7412 loci. The ADGC, ADSP-WGS, and FHS have directly reported the *APOE* genotypes for recruited subjects. AD patients were directly identified by the ADGC, ADSP-WGS, FHS, and LOADFS researchers primarily based on the neurologic exam criteria[37,38]. In CHS, AD-affected subjects were determined using the International Classification of Disease codes, ninth revision (i.e., code: 331.0). Basic information on 6136 AD-affected and 10555 unaffected subjects is presented in Table S1.

2.2 Genotype data and quality control (QC)

Genetic data in the selected five studies were from the array-based (i.e., ADGC, CHS, FHS, and LOADFS) or whole-genome sequencing (i.e., ADSP-WGS) platforms. First, we imputed SNPs to harmonize about 2.5 million of variants to facilitate cross-platform analyses[39]. Then, we performed QC using *PLINK* package[40] to filter out low-quality data including: imputed SNPs with $r^2<0.7$ (in ADGC, CHS, FHS, and LOADFS), SNPs/subjects with missing rates >5%, SNPs with minor allele frequencies <1% or $P_{Hardy\text{-}Weinberg}<1E\text{-}06$, and SNPs/subjects/families with Mendel error rates >2% in family-based datasets (i.e., ADSP-WGS, FHS, and LOADFS). The QC process resulted in 1904013, 1844347, 1695409, 1541793, 1829245 SNPs in ADGC, ADSP-WGS, CHS, FHS, and LOADFS, respectively.

2.3 Two-stage genetic analysis

Design.—We used two variables as outcomes in our analysis. One outcome included carriers of the ε2ε2 and ε2ε3 AD-protective genotype (herein referred to as the ε2 allele) as cases, and the other included carriers of the ε4ε4 and ε3ε4 AD-risk genotype (herein referred to as the ε4 allele) as cases. The same ε3ε3 genotype was used as a reference in each outcome. The analyses leveraged a two-stage approach. The first stage was designed to examine associations of the ε2 and ε4 alleles with the other SNPs in the genome in AD-affected (AD) and unaffected (NAD) groups of subjects separately. At stage two, we examined group-specific effects by evaluating the differences in associations of the ε2 and ε4 alleles with the other SNPs in the AD-affected and unaffected subjects, which were selected at stage one.

Stage one: Genome-wide association study (GWAS).—Additive genetic models were fitted separately in each dataset to associate the ε2 or ε4 alleles with the other SNPs in the genome. The models were adjusted for fixed-effects covariates, including the top five

principal components of genetic data, sex, age/birth year, and ADC cohorts (in ADGC), as well as random-effects family structure (in ADSP-WGS, FHS, and LOADFS). The logistic regression models were fitted using *GENESIS* R package[41,42]. The GWAS results from these five datasets were combined using a fixed-effects inverse-variance meta-analysis implemented in *GWAMA* package[43].

We used two GWAS strategies. First, following the discovery-replication strategy, we selected two independent sets of data. One set (referred to as nonADGC) included data from ADSP-WGS, CHS, FHS, and LOADFS, and the other set (referred to as ADGC) was represented by the ADGC cohort. These datasets were used as the discovery and replication sets interchangeably. In other words, results of the meta-analysis of the GWAS statistics from the nonADGC studies were used as the discovery set and ADGC as the replication set, and vice versa. The second strategy was to pool the results from all five datasets (i.e., nonADGC+ADGC samples) using meta-regression. We selected promising SNPs for stage two from the associations attained genome-wide (P<5E-08) or suggestive-effect (5E-08≤ P<5E-06) significance in: (i) the discovery dataset and had the same effect direction and P<0.05 in the replication dataset, and vice versa, and (ii) the meta-analysis of all five datasets.

Stage two: Group-specific analysis—Group-specific analysis provides quantitative metric to identify AD or NAD group-specific associations. This metric is necessary because significance of the association in one group and the lack of significance in the other group does not automatically guarantee significant difference of the associations between these groups. We quantified the differences in the associations between these groups by fitting an interaction model with a SNP-by-AD status term in the pooled sample of AD and NAD subjects for each SNP selected at stage one. Significant findings from the interaction model were identified after Bonferroni correction for the number of SNPs selected at stage one.

2.4 Analysis of the role of sex

To examine the potential role of sex as a modulator of the associations of the ε2 and ε4 alleles with group-specific SNPs, we fitted the same models as in our stage-two analysis with an additional SNP-by-sex interaction term using *GENESIS* R package[41,42].

2.5 Analysis of the AD risk

To examine whether the group-specific SNPs identified at stage two can modulate the impact of the *APOE* alleles on the AD risk, we performed survival-type analysis using Cox regression model. We evaluated the main effects of the ε2-coding rs7412 or ε4-coding rs429358 and each group-specific SNP, along with their interactions. We used age at onset (AAO) of AD as a time variable. As in our GWAS analysis, we fitted additive genetic models and included the same fixed- and random-effects adjustments. These analyses were performed using *coxme* (for family-based studies) and *survival* R packages[44,45].

2.6 Functional enrichment analysis

To make biological sense of the observed statistical associations, we examined gene enrichment in bio-functions (defined by “molecular and cellular function” and

"physiological system development and function" categories) using the Ingenuity Pathway Analysis (IPA) tool (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis).

3. RESULTS

Our stage-one (i.e., GWAS) analyses revealed several associations of the ε2 and ε4 alleles with other SNPs across the genome in AD, NAD, or both groups. Figures S1–S4 show Manhattan and QQ plots for these results in nonADGC, ADGC, and nonADGC+ADGC samples. The genomic control values (i.e., lambda) in these analyses were between 0.972–1.002 in nonADGC samples and 1.010–1.023 in ADGC dataset, indicating adequate control of potential confounding effects of population structure. Next, we discuss the results from the promising associations.

3.1 Associations for the ε2 allele.

The ε2 allele showed promising associations with 29 SNPs in 13 loci in the AD group but not in the NAD group (Table S2), and with 191 SNPs in 16 loci in the NAD group but not in the AD group (Table S3). In the AD group, we identified three (of 29) promising SNPs associated with the ε2 allele in the *APOE* 19q13.3 locus. In contrast, the vast majority of SNPs identified in the NAD group, 159 of 191 (83.2%), were in the *APOE* 19q13.3 locus. In addition to these 220 (=29+191) SNPs, the ε2 allele was associated with 94 SNPs (all in the *APOE* 19q13.3 locus) in both AD and NAD groups (Table S4), totaling 314 promising SNPs in all groups combined.

Differences in the detected associations of SNPs with the ε2 allele in the AD and NAD groups suggested potential group-specific effects, i.e., interactions of SNPs with the AD status. The stage-two analysis identified group-specific associations of the ε2 allele with 35 SNPs in 11 loci, which attained a conservative Bonferroni-adjusted (i.e., all selected SNPs for this test were conservatively considered as independent) significance level of $P<1.59\times10^{-4}$ (i.e., 0.05/314) in the interaction analyses (Tables 1 and S5).

Most of these SNPs, 22 of 35, were selected at stage one based on promising associations with the ε2 allele only in the AD group, and one SNP (rs3101357 mapped to *FRMDF4*) was associated with this allele only in the NAD group. These 23 SNPs were mapped to ten genes/loci which were on nine chromosomes outside of the *APOE* 19q13.3 locus. The other 12 SNPs with group-specific effects had promising significant association signals in both AD and NAD groups. These SNPs were mapped to 7 genes within the *APOE* 19q13.3 locus. Figure 1 illustrates the effect sizes of the ε2-associated group-specific SNPs from the analyses of nonADGC+ADGC samples in the AD and NAD groups. In general, the magnitudes of the effects were larger in the *APOE* 19q13.3 locus than in the non-*APOE* loci in both AD and NAD groups. Also, the magnitudes of effect sizes of the associations of the ε2 allele with each of these 35 SNPs were larger in the AD than NAD group, indicating stronger associations between the ε2 allele and alleles of these SNPs in the AD group. For most of these SNPs, 26 of 35, the effect directions were positive in both AD and NAD groups, indicating stronger associations between their minor alleles and the ε2 allele in the AD group. The remaining 9 of 35 SNPs had different directions of effects in the AD and

NAD groups, denoting the opposite patterns of associations between the minor/major alleles of these SNPs and the ε2 allele in the two groups.

3.2 Associations for the ε4 allele.

In the AD group, we identified promising associations of the ε4 allele with 12 SNPs in seven non-*APOE* loci and 86 SNPs in the *APOE* 19q13.3 locus, totaling 98 SNPs in eight loci (Table S6). In the NAD group, there were nine promising associations in six non-*APOE* loci and 14 in the *APOE* 19q13.3 locus, totaling 23 SNPs in seven loci (Table S7). In addition, there were 111 SNPs (all within the *APOE* 19q13.3 locus) with promising associations with the ε4 allele in both AD and NAD groups (Table S8). Overall, this analysis identified 232 promising associations with the ε4 allele.

Of them, the stage-two analysis identified group-specific associations of the ε4 allele with 31 SNPs at a conservative Bonferroni-adjusted significance level of $P<2.16\times10^{-4}$ (i.e., 0.05/232) in the fitted interaction models (Tables 2 and S9). Figure 2 illustrates the effect sizes of the ε4-associated group-specific SNPs from the analyses of nonADGC+ADGC samples in the AD and NAD groups. Six SNPs with group-specific effects were associated with the ε4 allele only in the AD (5 SNPs) or NAD (1 SNP) groups. They were mapped to 3 genes in 3 loci, including *EXOC3L2* gene in the *APOE* 19q13.3 locus. All these 6 SNPs had negative effect directions in the AD group and positive ones in the NAD groups, highlighting the opposite patterns of the associations of the ε4 allele and minor/major alleles of these SNPs in the two groups. The other 25 SNPs with group-specific effects (mapped to 5 genes within the *APOE* 19q13.3 locus) were associated with the ε4 allele in both AD and NAD groups. They had the same directions of effects in both groups (14 positive and 11 negative effects). The effect sizes of SNPs with positive effect directions were larger in the NAD group than the AD group implying stronger associations between the ε4 allele and minor alleles of these SNPs in the NAD group. In contrast, magnitudes of the effects of SNPs with negative directions were larger in the AD group than the NAD group indicating stronger associations between the ε4 allele and major alleles of these SNPs in the AD group. Again, the magnitudes of the effects were mainly larger in the *APOE* 19q13.3 locus than in the non-*APOE* loci.

3.3 The role of sex

We found interactions between sex and each of four and three group-specific SNP in their associations with the ε2 and ε4 alleles, respectively, at P<0.05. Only the interaction of sex with ε2-associated rs445925 (*APOC1* variant), however, attained Bonferroni-adjusted significance (P<1.43E-03=0.05/35). All these seven SNPs were within the *APOE* 19q13.3 locus (Tables S10 and S11).

3.4 Associations with the AD risk

Among 35 ε2-associated group-specific SNPs, our survival-type analysis revealed significant (P<0.05) interaction effects of the ε2-coding rs7412 with 30 SNPs on the AD risk in the ε4-negative sample (Table S12). Twelve of these 30 interactions attained Bonferroni-adjusted significance (P<1.43E-03=0.05/35) (Table 3). All these 12 interactions had positive effect directions and were with SNPs not on chromosome 19. Among 31 ε4-associated

group-specific SNPs, we identified significant (P<0.05) interaction effects of the ε4-coding rs429358 with 16 SNPs on the AD risk in the ε2-negative sample (Table S13). Four of them attained Bonferroni-adjusted significance (P<1.61E-03=0.05/31) (Table 3). All these four interactions had negative effect directions and were with SNPs in the *APOE* locus.

3.5 Functional enrichment analysis

The analysis was performed for 15 and seven protein-coding genes (excluding *CEACAM22P* and *APOC1P1* pseudogenes) harboring group-specific SNPs associated with the ε2 and ε4 alleles, respectively. We found that 32 and four bio-functions were enriched by three or more genes for the ε2- and ε4-associated SNPs, respectively (Figure 3, Tables S14 and S15), at a false discovery rate adjusted P<0.05[46]. One of them, activation of leukocytes, was significantly enriched in both sets (Figure 3).

4. DISCUSSION

In this study, we assumed the *APOE* alleles as proxies for potential biological processes related to the protection against (ε2 allele) or predisposition to (ε4 allele) cognitive decline. We analyzed the associations of the ε2 and ε4 alleles with other SNPs in the genome to identify genetic variants, which may modulate the effects of these alleles. We were particularly interested in dissecting heterogeneous genetic architecture of AD by identifying associations that can be different between the AD-affected and unaffected subjects. Such differences may indicate genetic modulators of *APOE* impacts on AD development and partly explain the incomplete penetrance of the *APOE* alleles[47,48].

Our stage-one analysis revealed promising associations of the ε2 and ε4 alleles with 314 and 232 SNPs, respectively. The associations identified only in the AD or NAD groups were with SNPs both within and outside of the *APOE* 19q13.3 locus (Tables S2, S3, S6, and S7), whereas those identified in both AD and NAD groups were with SNPs within the *APOE* 19q13.3 locus only (Tables S4 and S8).

Among SNPs with significant association signals only in the AD or NAD groups, our stage-two analysis revealed group-specific effects for 23 and six ε2- and ε4-associated SNPs, respectively (Tables 1, 2, S5, and S9). These SNPs were mapped to 13 genes/loci, of which only *EXOC3L2* gene was located within the *APOE* 19q13.3 locus. The magnitude of the effects of all these SNPs, except rs1414663 (*LRRC7* variant), were larger in the AD than NAD group. Almost half of these SNPs had different directions of effects (i.e., opposite patterns of associations between their minor/major alleles and the *APOE* alleles) in the two groups. Accordingly, the alleles of these SNPs may affect the AD risk by modulating the effects of the ε2 or ε4 alleles. A literature review revealed that polymorphisms in most of the genes harboring these group-specific SNPs were implicated in AD pathology. For instance, SNPs mapped to *FRMD4A*[49] and *EXOC3L2*[50,51] were previously associated with AD at the genome-wide significance. Also, a previous study of epistatic associations with AD reported that interactions of SNPs mapped to *VAV3*, *MPDZ*, *FRMD4A*, *DDX10*, *SDK2*, *ZFP64*, and *KCNQ3* with SNPs in the other genes were associated with pathological hallmarks of AD such as paired helical filament tau protein, neurofibrillary tangles, and diffuse brain plaques[52]. Additionally, *LRRC7* was previously associated with cognitive performance[53].

Notably, none of the previously reported SNPs from these genes are in significant LD with SNPs identified in our study in the Caucasian population[54].

Among promising SNPs with significant association signals in both AD and NAD groups, 12 and 25 SNPs exhibited group-specific associations with the ε2 and ε4 alleles, respectively (Tables 1, 2, S5, and S9). They were mapped to *CEACAM22P*, *CEACAM16*, *BCL3*, *NECTIN2*, *TOMM40*, *APOE*, *APOC1*, *APOC1P1* genes within the *APOE* 19q13.3 locus. Four SNPs from the *APOE* (rs75627662 and rs72654473) and *APOC1* (rs445925 and rs483082) genes were associated with both ε2 and ε4 alleles. The magnitudes of the effects for all 12 ε2-associated group-specific SNPs (with positive effects in the AD and NAD groups) and those for 11 of 25 ε4-associated group-specific SNPs (with negative effects in the AD and NAD groups) were larger in the AD than NAD group, indicating stronger associations of the ε2 or ε4 alleles with alleles of these SNPs in the AD group. In contrast, the effect sizes of the remaining 14 of 25 ε4-associated SNPs (with positive effects in the AD and NAD groups) were larger in the NAD than AD group, indicating stronger associations of their minor alleles and the ε4 allele in the NAD group. Hence, the alleles of these 37 SNPs are likely involved in modulating the effects of the ε2 or ε4 alleles on AD risk. Our findings are consistent with the other reports, which emphasize the roles of the complex haplotype structure in the *APOE* 19q13.3 locus in the AD risk and support the importance of more complex analyses to dissect heterogeneity in genetic architecture of AD[26,27,55–59].

Expression quantitative trait loci (eQTLs) that are in high LD (i.e., r^2 and/or D' ≥ 0.8) with several group-specific SNPs were previously reported to alter the expressions *APOC1P1*, *GNPDA2*, *KCNQ3*, *NECTIN2*, and *ZFP64* in brain tissue at P<5E-06[60] (Table S16). Since the group-specific SNPs differentially impacted the AD and NAD groups, we suggest the alterations in these genes' expressions may contribute to the AD pathogenesis.

Our analysis supported the minor role of sex as a modulator of the associations of the ε2 or ε4 allele with group-specific SNPs. Our survival-type analyses revealed a three-time larger number of interactions of the ε2-encoding rs7412, than the ε4-encoding rs429358, with group-specific SNPs (12 vs. 4 interactions) in their associations with the AD risk at Bonferroni-adjusted significance (Table 3). All SNP-ε2 interactions were with 12 SNPs not on chromosome 19, whereas all interactions with the ε4 allele were with four SNPs in the *APOE* 19q13.3 locus. These interactions imply that the beneficial effect of the ε2 allele (i.e., smaller AD risk, or, equivalently, AAO at older ages, compared to the ε3ε3 carriers) can be significantly modulated by alleles from SNPs spread throughout the entire genome. This study also shows that the adverse effect of the ε4 allele (i.e., larger AD risk or, equivalently, AAO at younger ages, compared to the ε3ε3 carriers) can be significantly modulated by alleles from the other *APOE* and *APOC1* SNPs. While genetic linkage may drive the associations of the ε2/ε4 alleles and local variants (i.e., cis modulators), the functional linkage may underline the roles of, particularly, trans-modulators of the effects of these alleles[61].

Our functional enrichment analysis revealed that the genes harboring the group-specific ε2-associated SNPs were mainly enriched in inflammation- and immunity-related processes.

For example, significantly enriched functions highlighted B and T lymphocytes and phagocytes, such as neutrophils and macrophages, which are involved in antigen-specific (adaptive) and nonspecific (innate) immunity. Immune system and inflammatory responses have been implicated in AD pathogenesis[39,62–64]. The top term enriched for the ε2-associated genes was the quantity of marginal-zone B (MZB) lymphocytes (Figure 3A). It is believed that MZB cells mainly produce IgM antibodies and may regulate autoimmunity[65,66]. The MZB cells play their vital role in the early antibody reaction to pathogens by mobilizing an optimal response of the innate and adaptive immune systems[67,68]. Although B cells may have a neuroprotective effect by producing immunoglobulins against amyloid-beta (Aβ)[69], murine AD models show that B cells may also influence the formation of Aβ plaques through deposition of immunoglobulins and appear to be enriched in the AD brains[64]. Interestingly, the inflammatory response-related process, activation of leukocytes, was also at the top for genes harboring the group-specific ε4-associated SNPs (Figure 3B). These results suggest inflammation and immunity as mechanisms modulating penetrance of the *APOE* alleles.

Despite the rigor of this study, we acknowledge its limitations. First, although we analyzed five well-known AD datasets, further validation of our findings in larger samples would provide additional strength. Second, the statistical power of the ε2 allele-related analysis may not be optimal due to the small frequency of this allele in the general population of Caucasians and, especially, in cohorts enriched for AD patients. Third, the functional enrichment analysis had an inherent limitation of a relatively small number of protein-coding genes.

In conclusion, our analyses demonstrated that the associations of the *APOE* ε2 and ε4 alleles with multiple SNPs spread throughout the entire genome are affected by the AD-affection status. We found that 66 SNPs had significantly different effects in the AD-affected and unaffected groups. The group-specific SNPs may modulate the contributions of the ε2 or ε4 alleles to the AD protection or susceptibility. Our survival-type analysis of the AD risk supported modulating roles of multiple group-specific SNPs. Genes harboring the group-specific SNPs were mainly enriched in inflammation- and immune-related biological processes, e.g., B cell function. These findings provide novel insights into the incomplete penetrance of the *APOE* alleles and suggest involvement of local and inter-chromosomal modulators of their effects on the AD risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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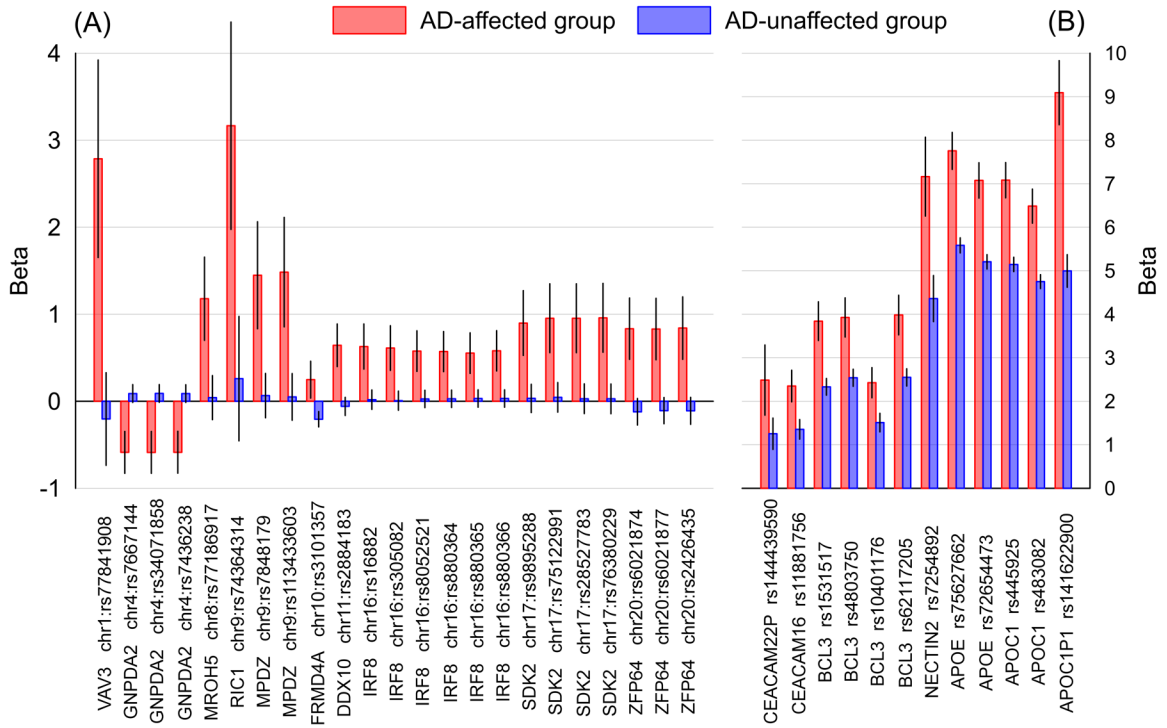

Figure 1.

The effect sizes of the ε2-associated group-specific SNPs in Alzheimer's disease-affected (AD) and unaffected (NAD) groups. (A) SNPs outside of the *APOE* 19q13.3 locus and (B) SNPs within the *APOE* 19q13.3 locus. The x-axis shows SNPs and genes identifiers; the y-axis shows the effect sizes (i.e., beta coefficients), red bars indicate the AD group; blue bars indicate the NAD group. Vertical lines show 95% confidence intervals.

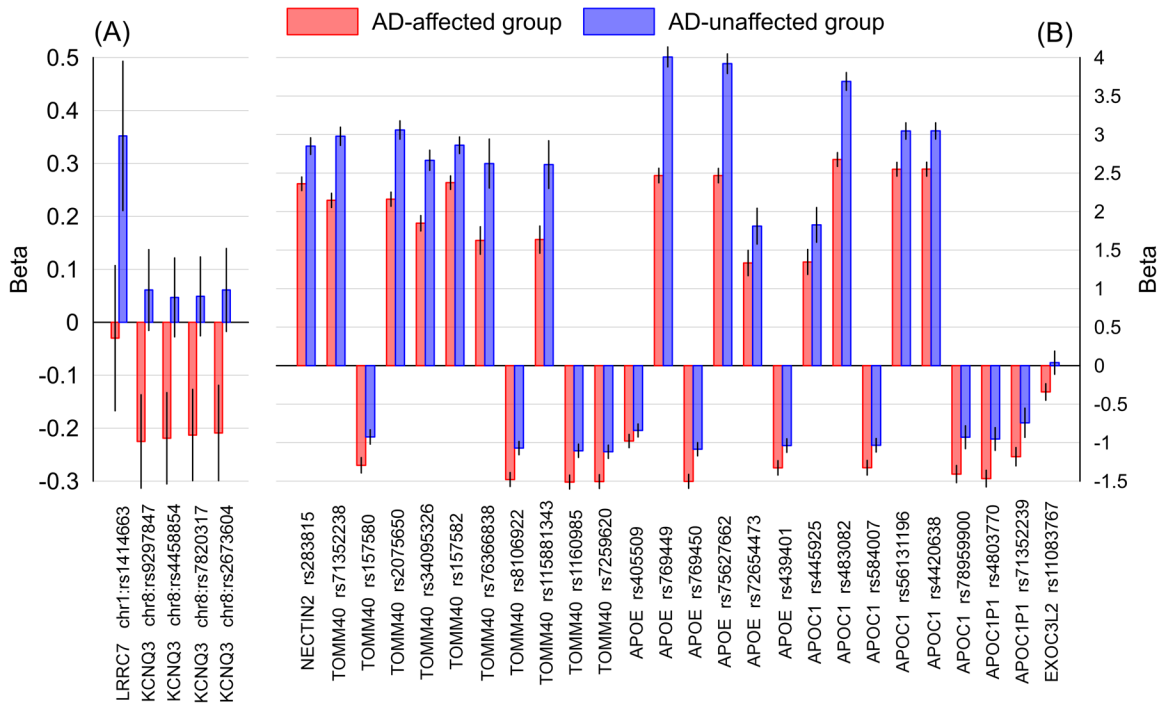

Figure 2.

The effect sizes of the ε4-associated group-specific SNPs in Alzheimer's disease-affected (AD) and unaffected (NAD) groups. (A) SNPs outside of the *APOE* 19q13.3 locus and (B) SNPs within the *APOE* 19q13.3 locus. The x-axis shows SNPs and genes identifiers; the y-axis shows the effect sizes (i.e., beta coefficients), red bars indicate the AD group; blue bars indicate the NAD group. Vertical lines show 95% confidence intervals.

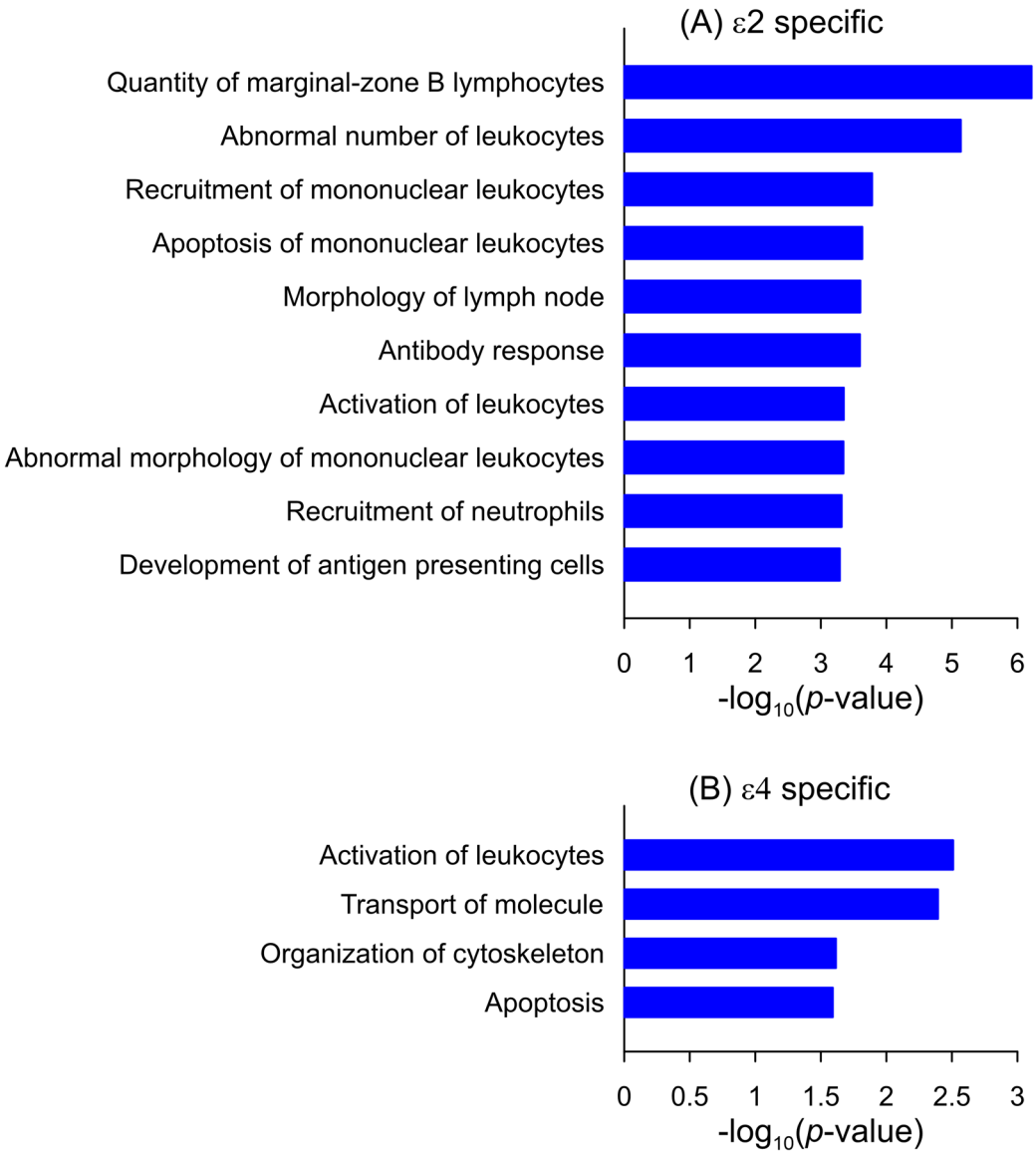

Figure 3.

Enrichment of bio-functions. (A) Top-10 bio-functions enriched for genes harboring the ε2-associated SNPs. (B) Enrichment of bio-functions for genes harboring the ε4-associated SNPs. All bio-functions are significantly enriched at a false discovery rate-adjusted P<0.05.

Table 1.

SNP-by-AD status interaction meta-analysis for ε2-associated group-specific SNPs in the pooled samples of AD and NAD groups.

SNPs									SNP-by-AD Status Interaction Effect			
Rep/Meta	**CHR**	**Locus**	**Gene**	**SNP**	**POS**	**EA**	**EAF**	**N**	**BETA**	**SE**	**P-value**	**Effects**
AD-Meta	1p13.3	1	*VAV3*	rs77841908	107713714	T	0.012	5568	3.268	0.751	1.39E-05	+??++
AD-Meta	4p12	2	*GNPDA2*	rs7667144	44988902	T	0.240	10493	−0.585	0.124	2.22E-06	−−−−−
AD-Meta	4p12	2	*GNPDA2*	rs34071858	44989232	G	0.240	10489	−0.587	0.124	2.12E-06	−−−−−
AD-Meta	4p12	2	*GNPDA2*	rs7436238	44995666	A	0.239	10477	−0.585	0.124	2.35E-06	−−−−−
AD-Meta	8q24.3	3	*MROH5*	rs77186917	141434800	C	0.047	7334	1.317	0.265	6.87E-07	−+?−+
AD-Rep	9p24.1	4	*RIC1*	rs74364314	5640617	G	0.016	3025	2.685	0.701	1.28E-04	+???+
AD-Meta	9p23	5	*MPDZ*	rs7848179	13030037	A	0.028	10493	1.417	0.323	1.16E-05	+++−+
AD-Meta	9p23	5	*MPDZ*	rs113433603	13036364	T	0.026	10493	1.623	0.337	1.53E-06	+++−+
NAD-Meta	10p13	6	*FRMD4A*	rs3101357	14077721	T	0.382	10492	0.459	0.111	3.82E-05	−++++
AD-Meta	11q22.3	7	*DDX10*	rs2884183	109127412	T	0.212	10474	0.736	0.131	1.76E-08	+++++
AD-Meta	16q24.1	8	*IRF8*	rs16882	85901967	C	0.180	10489	0.615	0.136	6.20E-06	+++++
AD-Meta	16q24.1	8	*IRF8*	rs305082	85903372	C	0.183	10463	0.616	0.135	5.27E-06	+++++
AD-Meta	16q24.1	8	*IRF8*	rs8052521	85925123	T	0.248	10473	0.550	0.122	7.26E-06	+++++
AD-Meta	16q24.1	8	*IRF8*	rs880364	85925559	G	0.249	10472	0.543	0.122	9.39E-06	+++++
AD-Meta	16q24.1	8	*IRF8*	rs880365	85925756	T	0.246	10471	0.508	0.123	3.77E-05	+++++
AD-Meta	16q24.1	8	*IRF8*	rs880366	85925832	C	0.248	10472	0.551	0.123	6.96E-06	+++++
AD-Meta	17q25.1	9	*SDK2*	rs9895288	73623974	A	0.075	10490	0.902	0.198	5.16E-06	+++−+
AD-Meta	17q25.1	9	*SDK2*	rs75122991	73639797	G	0.067	10490	0.974	0.209	3.13E-06	+++−+
AD-Meta	17q25.1	9	*SDK2*	rs28527783	73640957	T	0.068	10491	0.983	0.209	2.52E-06	+++−+
AD-Meta	17q25.1	9	*SDK2*	rs76380229	73643396	T	0.067	10489	0.989	0.209	2.23E-06	+++−+
AD-Meta/NAD-Meta	19q13.31	10	*CEACAM22P*	rs144439590	44537330	T	0.026	5568	1.896	0.470	5.56E-05	+??−+
AD-Rep/NAD-Rep	19q13.32	10	*CEACAM16*	rs11881756	44717621	C	0.107	4791	0.964	0.193	5.75E-07	++??+
AD-Rep/NAD-Rep	19q13.32	10	*BCL3*	rs1531517	44738916	A	0.072	7332	1.529	0.221	4.54E-12	++?−+
AD-Rep/NAD-Rep	19q13.32	10	*BCL3*	rs4803750	44744370	G	0.067	7326	1.535	0.224	7.56E-12	++?−+
AD-Rep/NAD-Rep	19q13.32	10	*BCL3*	rs10401176	44750234	T	0.117	4791	0.872	0.183	1.84E-06	++??+
AD-Rep/NAD-Rep	19q13.32	10	*BCL3*	rs62117205	44752009	C	0.066	7334	1.589	0.225	1.74E-12	++?−+
AD-Meta/NAD-Meta	19q13.32	10	*NECTIN2*	rs7254892	44886339	A	0.031	2296	2.952	0.469	3.18E-10	????+

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SNPs									SNP-by-AD Status Interaction Effect			
Rep/Meta	**CHR**	**Locus**	**Gene**	**SNP**	**POS**	**EA**	**EAF**	**N**	**BETA**	**SE**	**P-value**	**Effects**
AD-Rep/NAD-Rep	19q13.32	10	*APOE*	rs75627662	44910319	T	0.082	7334	2.211	0.079	2.07E-171	++?++
AD-Rep/NAD-Rep	19q13.32	10	*APOE*	rs72654473	44911142	A	0.091	7332	2.074	0.101	8.90E-94	+−?++
AD-Rep/NAD-Rep	19q13.32	10	*APOC1*	rs445925	44912383	A	0.092	7333	2.164	0.102	1.58E-99	+−?++
AD-Rep/NAD-Rep	19q13.32	10	*APOC1*	rs483082	44912921	T	0.104	7334	1.894	0.111	9.78E-65	+−?++
AD-Rep/NAD-Rep	19q13.32	10	*APOC1P1*	rs141622900	44923535	A	0.046	3025	4.825	0.320	2.89E-51	+???+
AD-Meta	20q13.2	11	*ZFP64*	rs6021874	52342233	C	0.085	10486	0.865	0.184	2.66E-06	−++++
AD-Meta	20q13.2	11	*ZFP64*	rs6021877	52345128	T	0.085	10492	0.839	0.184	4.98E-06	−++++
AD-Meta	20q13.2	11	*ZFP64*	rs2426435	52357570	A	0.082	10481	0.847	0.186	5.55E-06	−++++

Abbreviations: AD = Alzheimer's disease-affected group; NAD = Alzheimer's disease-unaffected group; SNP = single-nucleotide polymorphism; Rep/Meta = SNP identified at the stage one of our two-stage genetic analysis by the discovery-replication strategy (i.e., nonADGC and ADGC as discovery and replication sets or vice versa) or in the meta-analysis of five datasets under consideration (i.e., combined nonADGC and ADGC); CHR = chromosomal region (i.e., cytogenetic band); POS = SNP position based on Human Genome version 38 (hg38); EA = effect allele; EAF = effect allele frequency; N = Number of subjects; BETA and SE = effect size and its standard error; Effects = directions of effects in the ADSP, LOADFS, FHS, CHS, ADGC datasets, respectively.

Table 2.

SNP-by-AD status interaction meta-analysis for ε4-associated group-specific SNPs in the pooled samples of AD and NAD groups.

SNPs									SNP-by-AD Status Interaction Effect			
Rep/Meta	**CHR**	**Locus**	**Gene**	**SNP**	**POS**	**EA**	**EAF**	**N**	**BETA**	**SE**	**P-value**	**Effects**
NAD-Meta	1p31.2	1	*LRRC7*	rs1414663	69168451	G	0.102	11576	−0.423	0.108	8.60E-05	−−?−−
AD-Meta	8q24.22	2	*KCNQ3*	rs9297847	132351982	T	0.318	15047	−0.292	0.066	8.90E-06	−−−−−
AD-Meta	8q24.22	2	*KCNQ3*	rs4458854	132360860	A	0.338	15048	−0.277	0.065	2.00E-05	−−−−−
AD-Meta	8q24.22	2	*KCNQ3*	rs7820317	132362575	A	0.339	15044	−0.265	0.065	4.58E-05	−−−−−
AD-Meta	8q24.22	2	*KCNQ3*	rs2673604	132399360	C	0.299	15044	−0.281	0.067	2.98E-05	−−−−−
AD-Rep/NAD-Rep	19q13.32	3	*NECTIN2*	rs283815	44887076	G	0.307	11576	−0.274	0.052	1.54E-07	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs71352238	44891079	C	0.245	11573	−0.475	0.061	4.90E-15	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs157580	44892009	G	0.314	10506	−0.316	0.071	9.68E-06	?−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs2075650	44892362	G	0.243	11570	−0.553	0.061	1.17E-19	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs34095326	44892587	A	0.184	11576	−0.542	0.077	1.81E-12	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs157582	44892962	T	0.307	11574	−0.282	0.052	6.53E-08	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs76366838	44896639	A	0.048	8875	−0.937	0.178	1.40E-07	−−??−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs8106922	44898409	G	0.369	11559	−0.395	0.062	2.38E-10	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs115881343	44899959	T	0.050	8874	−0.910	0.172	1.29E-07	−−??−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs1160985	44900155	T	0.391	11576	−0.435	0.061	8.42E-13	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*TOMM40*	rs7259620	44904531	A	0.388	11576	−0.413	0.061	1.18E-11	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOE*	rs405509	44905579	G	0.437	11572	−0.286	0.063	5.06E-06	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOE*	rs769449	44906745	A	0.225	11573	−1.065	0.053	3.18E-91	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOE*	rs769450	44907187	A	0.372	11574	−0.422	0.062	1.18E-11	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOE*	rs75627662	44910319	T	0.228	11576	−0.994	0.053	1.40E-79	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOE*	rs72654473	44911142	A	0.051	11572	−0.770	0.147	1.84E-07	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOE*	rs439401	44911194	T	0.342	11558	−0.270	0.065	3.68E-05	+−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOC1*	rs445925	44912383	A	0.054	11575	−0.741	0.144	2.71E-07	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOC1*	rs483082	44912921	T	0.286	11574	−0.747	0.044	3.31E-65	−−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOC1*	rs584007	44913221	A	0.341	11573	−0.274	0.065	2.81E-05	+−?−−
AD-Rep/NAD-Rep	19q13.32	3	*APOC1*	rs56131196	44919589	A	0.287	12347	−0.447	0.046	1.63E-22	−−−?−
AD-Rep/NAD-Rep	19q13.32	3	*APOC1*	rs4420638	44919689	G	0.287	12332	−0.451	0.046	8.56E-23	−−−?−

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SNPs								SNP-by-AD Status Interaction Effect			
Rep/Meta	**CHR**	**Locus**	**Gene**	**SNP**	**POS**	**EA**	**EAF**	**N**	**BETA**	**SE**	**P-value**	**Effects**
AD-Rep/NAD-Rep	19q13.32	3	*APOC1*	rs78959900	44920379	A	0.298	5508	−0.463	0.090	2.94E-07	−???−
AD-Rep/NAD-Rep	19q13.32	3	*APOC1P1*	rs4803770	44924096	G	0.325	5508	−0.516	0.087	3.36E-09	−???−
AD-Meta/NAD-Meta	19q13.32	3	*APOC1P1*	rs71352239	44926286	T	0.280	4450	−0.434	0.107	5.33E-05	????−
AD-Rep	19q13.32	3	*EXOC3L2*	rs11083767	45212422	C	0.338	5508	−0.418	0.097	1.61E-05	−???−

Please see the description provided below Table 1.

Table 3.

Bonferroni-adjusted significant results from the survival-type analysis of the main and interaction effects of the rs7412 or rs429358 (i.e., ε2- or ε4-encoding SNPs) and the ε2/ε4-associated group-specific SNPs on the AD risks in the pooled samples of AD and NAD groups.

SNPs							SNP Main Effect			rs7412 or rs429358 Main Effect			Interaction Effect		
CHR	**Gene**	**SNP**	**POS**	**EA**	**EAF**	**N**	**BETA**	**SE**	**P-value**	**BETA**	**SE**	**P-value**	**BETA**	**SE**	**P-value**
ε2-associated group-specific SNPs															
1p13.3	*VAV3*	rs77841908	107713714	T	0.012	5430	−0.062	0.225	7.83E-01	−0.655	0.091	7.73E-13	1.418	0.363	9.32E-05
8q24.3	*MROH5*	rs77186917	141434800	C	0.047	6513	−0.038	0.086	6.63E-01	−0.734	0.090	5.35E-16	0.724	0.200	3.04E-04
11q22.3	*DDX10*	rs2884183	109127412	T	0.213	6849	−0.136	0.045	2.54E-03	−0.750	0.099	2.97E-14	0.451	0.112	6.00E-05
16q24.1	*IRF8*	rs16882	85901967	C	0.180	6853	−0.110	0.048	2.09E-02	−0.743	0.095	4.84E-15	0.441	0.115	1.32E-04
16q24.1	*IRF8*	rs305082	85903372	C	0.183	6846	−0.111	0.047	1.93E-02	−0.756	0.095	2.23E-15	0.457	0.116	8.75E-05
17q25.1	*SDK2*	rs9895288	73623974	A	0.078	6854	−0.052	0.070	4.60E-01	−0.638	0.084	3.99E-14	0.598	0.172	5.07E-04
17q25.1	*SDK2*	rs75122991	73639797	G	0.069	6853	−0.057	0.074	4.45E-01	−0.638	0.083	2.06E-14	0.662	0.178	1.99E-04
17q25.1	*SDK2*	rs28527783	73640957	T	0.069	6854	−0.061	0.074	4.10E-01	−0.639	0.083	1.96E-14	0.664	0.178	1.91E-04
17q25.1	*SDK2*	rs76380229	73643396	T	0.069	6852	−0.066	0.074	3.78E-01	−0.640	0.083	1.87E-14	0.667	0.178	1.76E-04
20q13.2	*ZFP64*	rs6021874	52342233	C	0.085	6855	−0.152	0.067	2.34E-02	−0.665	0.086	8.45E-15	0.611	0.155	8.23E-05
20q13.2	*ZFP64*	rs6021877	52345128	T	0.086	6854	−0.147	0.067	2.79E-02	−0.662	0.086	1.13E-14	0.595	0.154	1.20E-04
20q13.2	*ZFP64*	rs2426435	52357570	A	0.083	6854	−0.162	0.068	1.81E-02	−0.660	0.085	1.01E-14	0.609	0.156	9.86E-05
ε4-associated group-specific SNPs															
19q13.32	*APOE*	rs769449	44906745	A	0.217	9932	0.352	0.068	2.19E-07	0.698	0.045	1.17E-54	−0.120	0.037	1.24E-03
19q13.32	*APOE*	rs72654473	44911142	A	0.051	9932	0.337	0.104	1.23E-03	0.875	0.026	1.18E-241	−0.266	0.074	2.95E-04
19q13.32	*APOC1*	rs445925	44912383	A	0.053	9934	0.322	0.103	1.83E-03	0.877	0.026	3.84E-242	−0.257	0.073	4.70E-04
19q13.32	*APOC1*	rs483082	44912921	T	0.277	9933	0.483	0.076	2.27E-10	0.593	0.077	1.17E-14	−0.110	0.033	9.05E-04

Please see the description provided below Table 1. The Bonferroni-adjusted significance thresholds were 1.43E-03 (i.e., 0.05/35) and 1.61E-03 (i.e., 0.05/31) in the ε2 and ε4 analyses, respectively.