Brain APOE expression quantitative trait loci-based association study identified one susceptibility locus for Alzheimer’s disease by interacting with APOE ε4

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Some studies have demonstrated interactions of AD-risk single nucleotide polymorphisms (SNPs) in non-APOE regions with APOE genotype. Nevertheless, no study reported interactions of expression quantitative trait locus (eQTL) for APOE with APOE genotype. In present study, we included 9286 unrelated AD patients and 8479 normal controls from 12 cohorts of NIA Genetics of Alzheimer’s Disease Data Storage Site (NIAGADS) and Alzheimer’s Disease Neuroimaging Initiative (ADNI). 34 unrelated brain eQTLs for APOE were compiled from BRAINEAC and GTEx. We used multi-covariate logistic regression analysis to identify eQTLs interacted with APOE ε4. Adjusted for age and gender, substantia nigra eQTL rs438811 for APOE showed significantly strong interaction with APOE ε4 status (OR, 1.448; CI, 1.124−1.430; P-value = 7.94 × 10−6). APOE ε4-based sub-group analyses revealed that carrying one minor allele T of rs438811 can increase the opportunity of developing to AD by 26.75% in APOE ε4 carriers but not in non-carriers. We revealed substantia nigra eQTL rs438811 for APOE can interact with APOE ε4 and confers risk in APOE ε4 carriers only.

Alzheimer’s Disease (AD) is the most common form of dementia with strong genetic etiology. Apolipoprotein E (APOE) ε4 allele has been universally confirmed as a strong risk factor for Late-Onset Alzheimer’s Disease (LOAD)1,2. APOE4, the isoform of APOE determined by ε4 allele, differs with other two isoforms APOE2 and APOE3 at protein structure, lipid association and receptor binding3. In most, if not all, putative AD pathogenic pathways, APOE4 either diminishes neuroprotection or augments neurotoxicity when compared with other two isoforms. These evidences which could explain the AD pathogenic nature of APOE4 include: APOE4 impairs synaptic repair and plasticity4,5; might be less efficient in transporting brain cholesterol6,7; increases Aβ aggregation and impairs clearance8,9; increases formation of neurofibrillary tangles10; decreases metabolic activity of neurons11.

Although APOE4 is neurotoxic remarkably and miscellaneously, not all APOE ε4 carriers developed to AD in a population, even for carriers of APOE ε4 homozygotes12. Likewise, not all APOE ε4 non-carriers are intact from AD. Except for APOE ε4, other genes were also identified to be associated with AD by genome-wide association studies (GWASs) in recent years13–15. Interestingly, Jun et al. revealed interaction of one of these susceptible genes PICALM with APOE ε4 on AD risk—genotypes at PICALM confer risk predominantly in APOE ε4 non-carriers16. In addition, GAB2 and some other genes can also modify AD risk by interacting with APOE ε417–19. Seemingly, these APOE ε4-interactive genes can give some reasons for the imperfect effect of APOE ε4, nevertheless, all of them are in non-APOE regions. Until now, no study has reported interactions of single nucleotide polymorphisms (SNPs) in APOE regulatory region with APOE genotypes, especially brain expression quantitative trait loci (eQTLs) for APOE. We speculate that some brain eQTLs can be related to AD by potentially regulating expression level of APOE ε4.

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This study was aimed at identifying brain eQTLs for APOE which can interact with APOE ε4 allele to confer AD risk.

**Results**

Characteristics of included GWAS cohorts after related individual removal. Across the 13 GWAS cohorts, 1,320 AD patients and 1,502 healthy controls were identified by KING as duplicate samples or kin with a third degree (e.g. first cousin) or closer relationship. After excluding these samples, 9,286 unrelated AD patients and 8,479 healthy controls were retained.

Description of the 12 GWAS cohorts from NIA Genetics of Alzheimer’s Disease Data Storage Site (NIAGADS) and GWAS data from Alzheimer’s Disease Neuroimaging Initiative (ADNI) after related individual removal is shown in Table 1.

Brain tissue-specific eQTLs for APOE and determination of proxy SNPs. We collected 73 brain tissue-specific eQTLs from BRAINEAC (http://www.braineac.org/) and GTEx (https://www.gtexportal.org/home/). 34 out of the 73 eQTLs were determined by LDproxy (https://analysistools.nci.nih.gov/LDlink/?tab=ldproxy) as proxy eQTLs. After excluding low-quality imputed eQTLs with imputation info score less than 0.9, all 34 proxy eQTLs were retained for further analysis. For the detailed information of the 34 proxy eQTLs, please refer to Table S1.

rs438811 confers risk in APOE ε4 carriers predominantly. We used multivariate logistic regression analysis to identify eQTLs which can confer AD risk in the 34 proxy eQTLs for APOE. After adjustment for age and gender, substantia nigra eQTL rs438811 (odds ratio [OR], 2.343; 95% confidence interval [CI], 2.205–2.490; raw P-value = 7.49 × 10^{-167}) was associated with AD (Table 2). After adjustment for age, gender and APOE ε4 status, rs438811 (OR, 1.049; 95% CI, 0.969–1.135; P-value = 0.237) was not associated with AD (Table 2). After introducing interaction item rs438811 genotype × APOE ε4 status into the model, rs438811 was found can confer AD risk by interacting with APOE ε4 status strongly (OR, 1.448; 95% CI, 1.231–1.704; P-value = 7.94 × 10^{-6}; Table 3). Sub-group analysis showed rs438811 confer risk predominantly in APOE ε4 carriers (OR, 1.267; 95% CI, 1.124–1.430; P-value = 1.12 × 10^{-4}; Table 3), which indicates carrying one minor allele T of rs438811 can increase the opportunity of developing to AD by 26.75% in APOE ε4 carriers. As shown in BRAINEAC, minor allele T of rs438811 was associated with increased APOE expression level in substantia nigra. The APOE eQTL P-values of rs438811 across the ten different brain regions were shown in Table S2.

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### Table 1. Cohort description. *Cohort full names: NIA-LOAD, National Institute on Aging Genetics Initiative for Late-Onset Alzheimer’s Disease; ADC1, Alzheimer’s Disease Center Dataset 1; ADC3, Alzheimer’s Disease Center Dataset 3; UPITT, University of Pittsburgh; TGEN II, Translational Genomics Research Institute II; ROSMAP, Religious Orders Study and Memory and Aging Project; WashU1, Washington University Dataset 1; MIRAGE, Multi Institutional Research on Alzheimer Genetics Epidemiology; ACT, Adult Changes in Thought; UMVUMSSM, University of Miami (UM), Vanderbilt University (VU) and Mount Sinai School of Medicine (MSSM); ADNI, Alzheimer’s Disease Neuroimaging Initiative.

| Abbreviated cohort name | Ancestry | Cases/controls | Female (%) | Presence of APOE ε4 (%) |
|-------------------------|----------|----------------|------------|------------------------|
| NIA-LOAD mixed, 92.33% caucasian | 993/884 | 62.30% | 52% |
| ADC1 caucasian | 1574/527 | 55.30% | 57.90% |
| ADC2 caucasian | 745/165 | 54.20% | 55.10% |
| ADC3 caucasian | 862/618 | 54.20% | 40.70% |
| UPITT mixed, 91.73% caucasian | 1424/996 | 63.80% | 42.20% |
| TGEN II caucasian | 1013/585 | 54.20% | 48.50% |
| ROSMAP caucasian | 368/1326 | 69.10% | 23.30% |
| WashU1 caucasian | 403/235 | 58.10% | 43.60% |
| MIRAGE caucasian | 603/885 | 59.70% | 38.90% |
| ACT unknown | 567/1701 | 57.70% | 26.10% |
| UMVUMSSM unknown | 1240/1230 | 62.50% | 37.90% |
| MHTO caucasian | 841/1253 | 53.40% | 42.70% |
| ADNI mixed, 92.88% caucasian | 2124/325 | 47% | 41.60% |

### Table 2. Substantia nigra eQTL rs438811 for APOE identified as a susceptibility locus for AD. *Weighed-average minor allele frequency.

| SNP | Minor allele | MAF* | Adjusted for age and gender | Adjusted for age, gender and APOE ε4 status |
|-----|--------------|------|-----------------------------|------------------------------------------|
| rs438811 | T | 0.287 | 2.343 (2.205–2.490) 7.49 × 10^{-167} | 1.049 (0.969–1.135) 0.237 |

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**Results**

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By querying Encyclopedia of DNA Elements (ENCODE), rs438811 was found to be target of transcription factors POLR2A and RPC15. rs483082, a brain eQTL for APOE which is in complete linkage with rs438811 and was also identified can interact with APOE ε4 status to confer AD risk in this study, was the target of multiple transcription factors: HNF4G, CEBPB, MX1, HDAC2, SP1, RFXS, MAX, EP300, JUND, FOSL2, ZBTB7A and CEBPD. rs483082 was also found be to located in a DNase I hypersensitivity cluster.

Discussion

APOE has long been a widely-investigated gene since the identification of its association with AD. Many studies have reported the relations between APOE genotypes and AD-related traits, such as cerebral spinal fluid (CSF) biomarkers,26–28 brain morphology changes,23–25 and particular cognitive measures.23,26,27 However, except for APOE ε4, no locus encompassing the APOE region, including brain eQTLs for APOE, were identified as a conferring risk for AD or AD-related traits. This study identified AD risk-associated brain eQTL for APOE by incorporating multiple GWAS cohorts.

The susceptibility locus rs438811 identified is in complete linkage with another brain eQTL for APOE–rs483082—which was associated with AD in summary statistics of International Genomics of Alzheimer’s project (IGAP) and was reported to associate with AD in a Japanese population.15,28 rs483082 was also associated with AD and confer AD risk in APOE ε4 carriers only in this study (data not shown). Furthermore, rs483082 was also reported to associate with lipid level. Abnormal lipid metabolism has long been demonstrated to as being involved in AD pathology.30–32 Our results revealed that the eQTL may influence the progression of AD APOE ε4 carriers by increasing the expression level of APOE. -491A/T, or rs449647, a polymorphism located in APOE transcriptional regulatory region, is the earliest-reported APOE expression-associated variant by luciferase/β-galactosidase activity assay related to AD independent of APOE ε4 dosage.25 In contrast to the earliest AD-associated APOE eQTL, the APOE eQTL identified in this study affect APOE expression in a brain region-specific manner. We also analyzed the association of -491A/T with AD (Table S3) and its interactive effect with APOE ε4 status on AD risk (Table S4). In consistent with the result from the study applied luciferase/β-galactosidase activity assay, -491A/T confer AD risk independent of APOE ε4 status. rs438811 is a substantia nigra-specific APOE eQTL. As a brain substructure dysfunction of which contributes to extrapyramidal signs (EPS), pathological changes of substantia nigra are responsible for EPS and aggravated EPS in AD patients.46

As to how the brain APOE eQTL influence the progression of AD, one most probable explanation is the neurotoxic effect of increased APOE4 expression level in substantia nigra. Nevertheless, pathological mechanism of the brain APOE eQTL still needs to be unveiled by molecular biological experiments.

In summary, this is a pilot study associating brain APOE eQTLs to AD risk. It identified a novel SNPs associated with AD by interacting with APOE ε4 status and potentially regulating expression level of APOE.

Methods

Compiling of brain APOE eQTLs.

Brain APOE eQTLs studied in this multi-cohort gene-wide association study were collected by querying BRAINEAC (http://www.brainexc.org/) and GTEx (https://www.geexporal.org/home/) databases. BRAINEAC provides the gene expression across ten brain tissues (cerebellar cortex, frontal cortex, hippocampus, medulla, occipital cortex, putamen, substantia nigra, thalamus, temporal cortex and intralobular white matter) from 134 healthy control individuals. GTEx provides the gene expression across ten brain tissues (cerebellar cortex, frontal cortex, medulla, occipital cortex, putamen, substantia nigra, thalamus, temporal cortex and intralobular white matter) from 134 healthy control individuals. GTEx provides the gene expression across ten brain tissues (cerebellar cortex, frontal cortex, medulla, occipital cortex, putamen, substantia nigra, thalamus, temporal cortex and intralobular white matter) from 134 healthy control individuals.

Subjects.

In this study, we included a total of 13 AD GWAS cohorts. 12 out of them were from NIAGADS. The criteria for inclusion of cohorts from NIAGADS was carrying covariate information on age, gender and APOE genotype. For detailed information on the 12 cohorts from NIAGADS, please refer to Table S5. The last cohort was from ADNI. GWAS data from ADNI were generated as previously described and obtained from the ADNI database (http://www.loni.ucla.edu/ADNI/). Finally, a total of 10606 AD patients and 9981 healthy controls were included in this study.

Identification and exclusion of related individuals.

We used KING to identify and exclude duplicate samples and kin with a third degree (e.g. first cousin) or closer relationship within and across datasets.

Quality control of chromosome 19.

For the purpose of imputation, we extracted all SNPs in chromosome 19, where APOE is located, from each GWAS dataset. Standard quality control was then applied using Plink.
1.9^{27}. For each dataset, SNPs with a call rate of less than 99%, minor allele frequency of less than 1% and violation of Hardy-Weinberg equilibrium in controls ($P < 1 \times 10^{-3}$) were removed. Samples with a call rate of less than 90% were removed.

**Imputation of chromosome 19.** Because all the brain eQTLs collected in this study are cis eQTLs which are located within up- or down-stream 10 Mb boundary of the APOE gene, only chromosome 19 was imputed to reduce computation burden. The SNPs of chromosome 19 were prephased using SHAPEIT2 for each dataset\(^{38}\). SNPs were then imputed to a reference panel of 1000 Genome Project Phase 3 by IMPUTE2\(^{29}\). An imputation info score cutoff of 0.9 was applied to exclude low-quality imputed SNPs. After imputation, collected eQTLs from the two databases for brain tissues were extracted.

**Identification of population substructure.** To adjust for confounding effect of population substructure in our data, we calculated eigenvectors of individuals through whole genome-wide principal component analysis (PCA) before chromosome 19 SNPs were extracted using Plink 1.9\(^{27}\). All Principal components (PCs, from PC1 to PC20) were used for confounding adjustment.

**Combination of genotyped and imputed data.** In this study, the genotyped and imputed brain eQTLs for APOE were inconsistent among different datasets. In order to combine all the GWAS datasets for AD association analysis, imputed high-quality brain eQTL with high confidence (genotype probability greater than 0.8) were converted to be simulated genotype data by fGENE for each individual\(^{40}\). Simulated genotype data were then combined with originally genotyped data.

**Statistical analysis.** After adjusting for age, gender and all PCs, these proxy brain eQTLs were tested for associations with AD with or without adjustment for APOE ε4 status through multivariate logistic regression analysis. eQTLs were coded as 0, 1, or 2 according to their number of minor alleles. APOE ε4 status was coded as 0 or 1 according to absence (APOE ε2/2, ε2/3 and ε3/3 subjects) or presence (APOE ε2/4, ε3/4 and ε4/4 subjects) of APOE ε4. Interaction item SNP genotype × APOE ε4 status was then introduced into the model to investigate the interactive effect of SNP and APOE ε4 status on AD risk. To account for multiple testing, we used the Bonferroni correction and considered significant only those brain eQTLs for which P-value $< 0.05/34 = 0.05/34 = 1.47 \times 10^{-3}$. All statistical calculations were performed using R\(^{41}\).

**Biological function annotation of identified APOE ε4 status-interactive brain eQTLs for APOE.** Biological function annotation of identified APOE ε4 status-interactive brain eQTLs for APOE was performed via querying Encyclopedia of DNA Elements (ENCODE, [https://www.encodeproject.org/](https://www.encodeproject.org/)) and GTEx ([https://www.gtexportal.org/home/](https://www.gtexportal.org/home/)). The GWAS datasets used in this study can be applied and downloaded from NIAGADS ([https://www.niagads.org/](https://www.niagads.org/)) and ADNI ([http://adni.loni.usc.edu/](http://adni.loni.usc.edu/)).

**Data availability statement.** The brain eQTL data for APOE can be retrieved from Brainiac ([http://www.brainiac.org/](http://www.brainiac.org/)) and GTEx ([https://www.gtexportal.org/home/](https://www.gtexportal.org/home/)). The GWAS datasets used in this study can be applied and downloaded from NIAGADS ([https://www.niagads.org/](https://www.niagads.org/)) and ADNI ([http://adni.loni.usc.edu/](http://adni.loni.usc.edu/)).

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**Author Contributions**

D.X. and S.J. designed the study. A.Z. and D.X. collected the brain eQTL data for APOE. A.Z and S.J. performed the analysis of the GWAS data and interpreted the results. S.J. wrote the manuscript. Q.Z. re-analyzed the GWAS data in the revision of the manuscript.

**Additional Information**

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