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Rao, Shuquan, Ghani, Mahdi, Guo, Zhiyun, Deming, Yuetiva, Wang, Kesheng, Sims, Rebecca, Mao, Canquan, Yao, Yao, Cruchaga, Carlos, Stephan, Dietrich A. and Rogaeva, Ekaterina 2018. An APOE-independent cis-eSNP on chromosome 19q13.32 influences tau levels and late-onset Alzheimer's disease risk. *Neurobiology of Aging* 66, p. 178. [10.1016/j.neurobiolaging.2017.12.027](https://doi.org/10.1016/j.neurobiolaging.2017.12.027)

Publishers page: <http://dx.doi.org/10.1016/j.neurobiolaging.2017.12...>

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An APOE-independent cis-eSNP on chromosome 19q13.32 influences hippocampal structure, tau levels, and late-onset Alzheimer's disease risk

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Abstract

Although multiple susceptibility loci for late-onset Alzheimer's disease (LOAD) have been identified, a large portion of the genetic risk for this disease remains unexplained. LOAD risk may be associated with single-nucleotide polymorphisms responsible for changes in gene expression (eSNPs). To detect eSNPs associated with LOAD, we integrated data from LOAD genome-wide association studies and expression quantitative trait loci using Sherlock (a Bayesian statistical method). We identified a cis-regulatory eSNP (rs2927438) located on chromosome 19q13.32, for which subsequent analyses confirmed the association with both LOAD risk and the expression level of several nearby genes. Importantly, rs2927438 may represent an APOE-independent LOAD eSNP according to the weak linkage disequilibrium of rs2927438 with the 2 polymorphisms (rs7412 and rs429358) defining the APOE- ϵ 2, - ϵ 3, and - ϵ 4 alleles. Furthermore, rs2927438 does not influence chromatin interaction events at the APOE locus or cis-regulation of APOE expression. Further exploratory analysis revealed that rs2927438 is significantly associated with tau levels in the cerebrospinal fluid. Our findings suggest that rs2927438 may confer APOE-independent risk for LOAD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease primarily affecting the elderly that manifests through memory and cognitive decline. The hallmark features of the disease include the accumulation of amyloid plaques, tau neurofibrillary tangles, and neuronal destruction, leading to brain atrophy. Currently, the processes leading to the formation of these neuronal lesions are not well understood (Small and Duff, 2008). Twin studies indicate that susceptibility alleles contribute as much as 79% to late-onset AD (LOAD) cases (Gatz et al., 2006), and genetic variance analyses estimate >53% of the variance in LOAD status can be explained by common variants with a minor allele frequency >1% (Ridge et al., 2016).

With the advance of genome-wide association studies (GWASs), more than 20 loci have been reliably associated with risk for LOAD (Escott-Price et al., 2014; Guerreiro et al., 2013; Harold et al., 2009; Hollingworth et al., 2011; Jonsson et al., 2013; Lambert et al., 2009, 2013; Naj et al., 2011; Seshadri et al., 2010; Sims et al., 2017). APOE on chromosome 19q13.32 showed the strongest and most consistent evidence for association with LOAD. The 2 single-nucleotide polymorphisms (SNPs) in APOE (rs429358, rs7412) are underlying the ϵ 2, ϵ 3, and ϵ 4 alleles. However, these polymorphisms are not reliably detected in the genome-wide arrays due to high GC content, and its genotyping requires special conditions, which complicates the assessment of linkage disequilibrium (LD) structure of APOE region in GWASs (Ghani et al., 2015). The ϵ 4 allele increases LOAD risk in a dose-dependent fashion (odds ratio (OR) \approx 3 for estimate $>53\%$ of the variance in LOAD status can be explained by common variants with a minor allele frequency $>1\%$ (Ridge et al., 2016).

With the advance of genome-wide association studies (GWASs), more than 20 loci have been reliably associated with risk for LOAD (Escott-Price et al., 2014; Guerreiro et al., 2013; Harold et al., 2009; Hollingworth et al., 2011; Jonsson et al., 2013; Lambert et al., 2009, 2013; Naj et al., 2011; Seshadri et al., 2010; Sims et al., 2017). APOE on chromosome 19q13.32 showed the strongest and most consistent evidence for association with LOAD. The 2 single-nucleotide polymorphisms (SNPs) in APOE (rs429358, rs7412) are underlying the ϵ 2, ϵ 3, and ϵ 4 alleles. However, these polymorphisms are not reliably detected in the genome-wide arrays due to high GC content, and its genotyping requires special conditions, which complicates the assessment of linkage disequilibrium (LD) structure of APOE region in GWASs (Ghani et al., 2015). The ϵ 4 allele increases LOAD risk in a dose-dependent fashion (odds ratio (OR) \approx 3 for heterozygotes) and significantly lowers the age at onset (AAO), whereas the ϵ 2 allele confers a protective benefit (Corder et al., 1993, 1994). Previous studies suggested that other genetic variability within the APOE locus may exist, such as modifiers of the level of expression of APOE alleles (Lambert et al., 1997).

Due to the strong impact of APOE, it is difficult to determine if additional loci on chromosome 19q13.32 also contribute to LOAD (Jun et al., 2012; Perez-Palma et al., 2014). Several SNPs located in this region have been shown to be associated with LOAD due to strong LD with the APOE locus (Hollingworth et al., 2011; Naj et al., 2011; Seshadri et al., 2010). In models adjusting for APOE ϵ 4, no SNPs in the extended APOE region were significantly associated with LOAD (Jun et al., 2012). However, it is important to explore whether this genomic region contains functional APOE-independent variation(s) that also confer risk for LOAD.

Although multiple LOAD risk loci have been identified, the genes or DNA functional elements through which the risk variants of LOAD exert their effects on disease remain largely unknown. Accumulating evidence has demonstrated that changes in gene expression may play a key role in the pathogenesis of LOAD, and SNPs that influence LOAD risk may be responsible for changes in gene expression (Webster et al., 2009; Zou et al., 2010). Accordingly, recent studies have used integrative strategies to combine results from GWASs and expression quantitative trait loci (eQTL) data to identify novel potential risk genes (Ramasamy et al., 2014; Schadt et al., 2005). However, there is no study that systematically integrated eQTL data and GWASs of LOAD to identify functional risk variants (called eSNPs). Here, we first used the Sherlock algorithm to integrate eQTL data from lymphoblastoid cell lines (LCLs) derived from peripheral blood samples (N \approx 400) (Dixon et al., 2007) as well as association data from a large GWAS for LOAD (Naj et al., 2011) and reported a novel risk eSNP, rs2927438, on chromosome 19q13.32, acting independently of APOE (Fig. 1). To further characterize the potential role of rs2927438 in LOAD etiology, we conducted follow-up analyses in independent gene expression and large-scale GWAS data sets. Moreover, we assessed the association between rs2927438 and several endophenotypes, including hippocampal volume, as well as Ab42, tau and phosphorylated tau (ptau181) levels in the cerebro-spinal fluid (CSF).

2. Methods

2.1. Sherlock integration analysis

To identify genes with expression changes potentially contributing to the etiology of LOAD, we integrated the eQTL of LCLs and GWAS data of LOAD using a Bayesian statistical framework, named Sherlock (<http://sherlock.ucsf.edu/submit.html>) (He et al., 2013). The statistical model and threshold setting can be found in the original article (He et al., 2013) and Supplementary materials.

2.2. Analysis of eQTL and LOAD GWAS data

The eQTL data set used in the discovery stage was reported previously (Dixon et al., 2007), which consisted of 400 LCL samples. More information about sample description, genotyping, transcriptome profiling, and statistical analyses can be found in the original article (Dixon et al., 2007). The Alzheimer Disease Genetics Consortium (ADGC) reported a GWAS of LOAD investigating 2,046,563 SNPs using a 3-stage design consisting of a discovery stage and 2 replication stages, which identified 5 novel risk loci for LOAD (Naj et al., 2011). In the discovery stage, the ADGC performed a meta-analysis and/or joint analysis using data from 9 case-control cohorts, consisting of 8309 individuals with LOAD and 7366 cognitively normal age-/gender-matched controls. We used the summary statistics (p-values) from the discovery stage as input in our study.

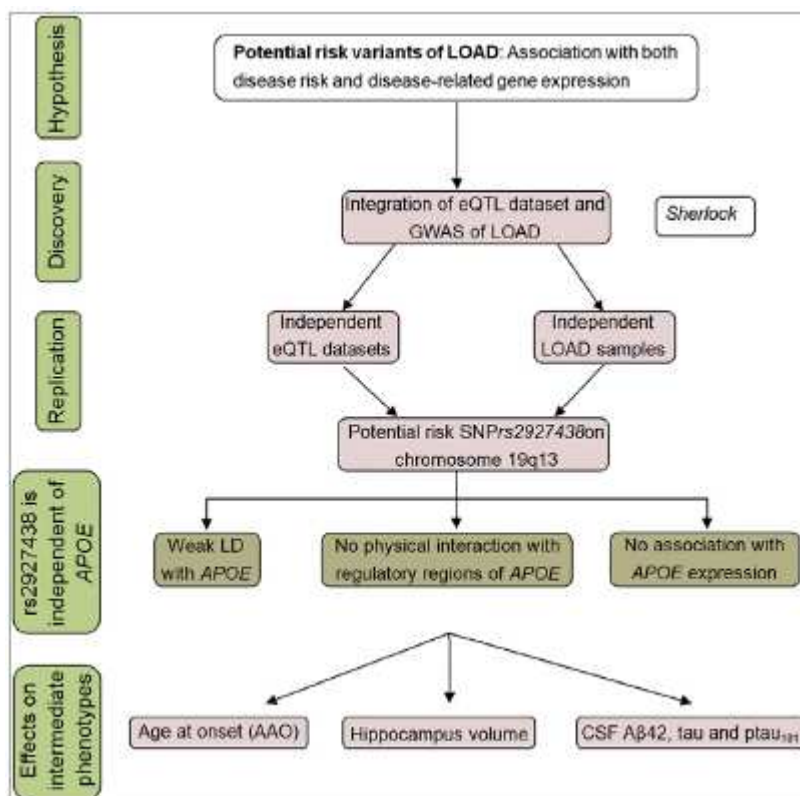


Fig. 1. Overview of research strategy. The strategy is based on the hypothesis that there may be variants that affect LOAD risk by influencing gene expression, and such variants would be associated with both AD risk and gene expression levels. We systematically integrated LOAD GWAS and eQTL data with the Sherlock algorithm. The top signals identified by Sherlock were then replicated in independent LOAD and eQTL data sets. We also tested whether the positive risk SNP, rs2927438, modified LOAD risk independent of APOE ϵ 4 status. Finally, we explored its association with several endophenotypes, including AAO of LOAD, hippocampal volume, and cognitive performance.

Abbreviations: AAO, age at onset; AD, Alzheimer's disease; eQTL, expression quantitative trait loci; LOAD, late-onset Alzheimer's disease; GWAS, genome-wide association study; SNP, single-nucleotide polymorphism.

2.3. The follow-up of eQTL and LOAD GWAS results

The following well-characterized expression data sets were used to validate the gene expression results that contributed to the identification of functional LOAD-related eSNPs: (1) SNPExpress data set (brain frontal cortex, N = 93; peripheral blood mono-nuclear cells [PBMC], N = 80) (Heinzen et al., 2008); (2) meta-analysis of blood eQTL by Westra et al. (N = 5311) (Westra et al., 2013); (3) GTEx data set (53 different tissues, including 12 brain tissues, N = 544) (GTEx-Consortium, 2015); (4) BrainCloud (dorsolateral prefrontal cortex, N = 261) (Colantuoni et al., 2011); and (5) Brain cortex data (Myers et al., 2007) (N = 193). Detailed information can be found in supplementary data and the original articles (Colantuoni et al., 2011; GTEx-Consortium, 2015; Heinzen et al., 2008; Myers et al., 2007; Westra et al., 2013). We used the largest reported GWAS meta-analysis of LOAD as a replication data set, which consisted of 17,008 cases and 37,154 controls (Lambert et al., 2013), including the ADGC data set used in our discovery stage. Detailed information on each data set, including diagnostic assessment, genotyping method, and quality control can be found in the original article (Lambert et al., 2013).

We conducted bioinformatics analyses, including functional prediction, and linkage analysis between the risk SNPs and rs429358 or rs7412 underlying the ϵ 2, ϵ 3, and ϵ 4 APOE alleles. In addition, we analyzed the association of the risk eSNP with hippocampal volume, as well as CSF levels of Ab42, tau, and ptau181. Detailed description of the methodology can be found in the supplementary methods.

3. Results

3.1. Integrative analyses of eQTL and LOAD GWAS Sherlock integrated the eQTL of LCLs (N = 400) and GWAS data (2,046,563 SNPs) from 8309 LOAD cases and 7366 controls (Naj et al., 2011) using a Bayesian statistical framework, and identified 71,323 SNPs showing significant eQTL effects. After implementing genetic signature matching from eQTL data with patterns of association in the GWAS, we ranked potential LOAD risk genes according to their logarithm of Bayes factor (LBF) and p-value. The integrative analysis yielded 10 candidate LOAD genes with both LBF scores >4.0 and p-value <1.0 $\times 10^{-4}$, which is the threshold applied in the original article (He et al., 2013) (Table 1). Among these genes, both bridging integrator 1 (BIN1) on chromosome 2q14.3 and biogenesis of lysosomal organelles complex 1 subunit 3 (BLOC1S3) on chromosome 19q13.32 have been associated with LOAD in previous GWAS and follow-up studies (Chapuis et al., 2013; Cruchaga et al., 2011; Seshadri et al., 2010). We therefore excluded these 2 genes from further analysis. The first-ranked gene was ZNF257 (ZNF257 zinc finger protein 257; LBF = 5.23), which is located on chromosome 19p12. Intriguingly, 5 genes on chromosome 19q13 are influenced by the same eSNP (rs2927438) in a cis-regulatory manner: EML2 (echinoderm microtubule associated protein like 2; LBF = 5.17), ZNF226 (zinc finger protein 226; LBF = 5.00), PVRL2 (also known as NECTIN2, nectin cell adhesion molecule 2; LBF = 4.67); KLC3 (kinesin light chain 3; LBF = 4.61); and ZNF222 (zinc finger protein 222; LBF = 4.56). To the best of our knowledge, this SNP has never been reported in genetic association studies of LOAD. The remaining 2 genes, RFFL (ring finger and FYVE-like domain containing E3 ubiquitin protein ligase; LBF = 5.22) and LRRC46 (leucine-rich repeat containing 46 proteins; LBF = 4.80) are influenced by trans-eSNPs (rs4726618 and rs4838429 for RFFL; rs8103315 and rs7743515 for LRRC46).

3.2. Replication of eQTL effects

Considering the many confounders in a single eQTL database, it is important to validate the eQTL associations in independent samples.

For the cis-eSNP rs417628, we were unable to validate its association with ZNF257 expression in the replication data set due to the absence of either the genotypic data for rs417628 or the expression data of ZNF257 (Supplementary table S1). Nevertheless, as demonstrated by a previous study (Puig et al., 2015), 1 breakpoint may disrupt the ZNF257 locus, causing a significant reduction in the total expression level of this gene in LCLs. We thus excluded the association of rs417628 with ZNF257 expression in further analysis. None of the trans-eQTL associations (RFFL and LRRC46) could be replicated (Supplementary table S1) and were also excluded from further study. Notably, trans-eQTL is more likely to reflect an indirect relationship of the SNP on gene expression. A gene's trans perturbation may come from the mutation of a regulatory RNA, but this mutation may also affect multiple other genes. More importantly, trans-eQTL is usually much weaker than those in cis and suffers from greater multiple testing burden, resulting in lower replication across multiple studies (Cheung et al., 2010).

For rs2927438, we first validated its cis-regulatory effect on the abovementioned candidate genes in SNPExpress, which contains expression data from both brain frontal cortical tissues and PBMC samples (Heinzen et al., 2008). With the exception of EML2, the results confirmed the association of rs2927438 with the expression of ZNF226 (PBMC, $p \approx 0.0084$), PVRL2 (PBMC, $p \approx 0.0024$), KLC3 ($p \approx 0.0037$), and ZNF222 (a trend of association, $p \approx 0.0795$) (Fig. 2), with the risk A-allele predicting higher gene expression for ZNF226, PVRL2, and ZNF222 and lower expression for KLC3. In the brain cortical tissue, rs2927438 was not significantly associated with the expression of ZNF226, PVRL2, KLC3, ZNF222, and EML2 (Supplementary fig. S1). Using another peripheral blood eQTL data set (N = 5311) (Westra et al., 2013), we further validated the association of rs2927438 with PVRL2 mRNA levels ($p \approx 3.66 \times 10^{-5}$); again, the A-allele was associated with higher gene expression.

We also tested whether rs2927438 was associated with the expression of these 5 genes in 13 brain tissues using the GTEx database (GTEx-Consortium, 2015). As shown in Supplementary fig. S2, we observed significant association of rs2927438 with expression of PVRL2 in the accumbens ($p \approx 0.033$) and KLC3 in the hippocampus ($p \approx 0.047$), and a trend of association with EML2 in the caudate ($p \approx 0.093$). Collectively, these data provide strong evidence of the cis-regulatory function of rs2927438.

Table 1
Results of integrative analysis (Sherlock) of LOAD GWAS and eQTL

Gene symbol	Gene LBF ^a	Gene P-value ^b	Supporting SNP	Proximity	eQTL P-value ^c	GWAS P-value ^d	SNP LBF ^e
ZNF257	5.23	2.59 E-05	rs417628	cis	1.10 E-06	8.86 E-06	5.18
RFFL	5.22	2.59 E-05	rs4726618	trans	6.40 E-07	8.94 E-06	5.95
			rs4838429	trans	1.00 E-05	3.13 E-03	0.76
BIN1	5.18	2.59 E-05	rs744373	cis	2.40 E-07	3.00 E-10	5.89
EML2	5.17	2.59 E-05	rs2927438	cis	1.10 E-04	1.05 E-07	5.73
ZNF226	5.00	3.35 E-05	rs2927438	cis	2.90 E-06	1.05 E-07	6.36
LRRC46	4.80	5.02 E-05	rs8103315	trans	5.80 E-06	3.46 E-11	4.63
			rs7743515	trans	5.20 E-06	5.97 E-03	0.86
PVRL2	4.67	7.00 E-05	rs2927438	cis	9.00 E-04	1.05 E-07	5.89
BLOC1S3	4.61	8.06 E-05	rs10416371	cis	5.00 E-05	7.80 E-08	5.12
KLC3	4.61	8.06 E-05	rs2927438	cis	2.00 E-04	1.05 E-07	5.24
ZNF222	4.56	8.52 E-05	rs2927438	cis	3.00 E-04	1.05 E-07	4.90

rs2927438 was shown in bold since it occurred 5 times without any threshold of significance.

Key: eQTL, expression quantitative trait loci; GWAS, genome-wide association study; LBF, logarithm of Bayes factor; LOAD, late-onset Alzheimer's disease; SNP, single-nucleotide polymorphism.

^a Gene LBF (Log Bayes Factors for each gene) evaluates the possibility of the combined evidence from GWAS and eQTL supporting a gene being associated with LOAD. For each gene, Sherlock computes individual LBF for each eSNP in the alignment, and the sum of these constitutes the final LBF score for the gene.

^b p-value for each gene based on Sherlock integrative analysis. High LBFs tend to correlate with lower p-values.

^c p-value for eQTL SNP from the gene expression study.

^d p-value for eQTL SNP from the GWAS of LOAD.

^e SNP LBF, Log Bayes Factors for each SNP.

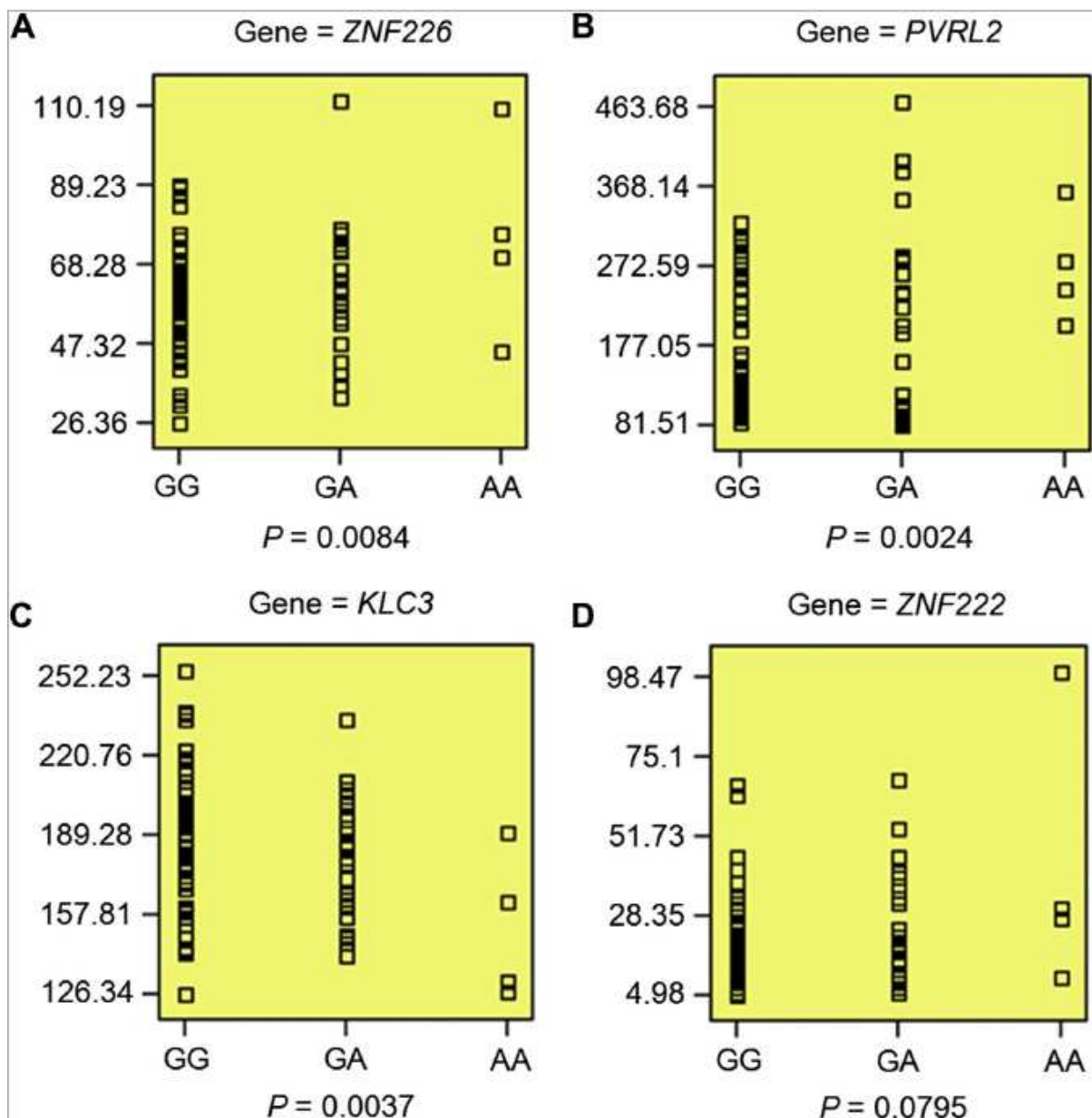


Fig. 2. Validation of regulatory effect of rs2927438 on gene expression in PBMC. (A–D) rs2927438 showed significant association with the expression levels of *ZNF226* ($p = 0.0084$), *PVRL2* ($p = 0.0024$), and *KLC3* ($p = 0.0037$), as well as a trend of association with *ZNF222* expression ($p = 0.0795$). Individuals with an AA genotype had higher expression of *ZNF226* (A), *PVRL2* (B), and *ZNF222* (D), but lower expression of *KLC3* (C). Data were retrieved from SNPExpress (<http://fgm.cumc.columbia.edu/SNPExpress/>; PBMC, $N = 80$) (Heinzen et al., 2008). Abbreviation: PBMC, peripheral blood mononuclear cells.

3.3. Replication of association between rs2927438 and LOAD risk

Similar to our validation of the eQTL results, we attempted to validate the association of rs2927438 with LOAD. Using the largest GWAS meta-analysis of LOAD (17,008 cases and 37,154 controls, partially overlapping with the ADGC discovery data set) (Lambert et al., 2013), we observed significant association between the A-allele of rs2927438 and increased risk of LOAD ($p \approx 5.69 \times 10^{-29}$, OR ≈ 1.236). It might be an authentic LOAD variant because the increase in sample size of the replication cohort generated a much more significant result ($p \approx 5.69 \times 10^{-29}$) than the smaller discovery data set ($p \approx 1.05 \times 10^{-7}$). Of note, the original study did not report this SNP because all SNPs within/flanking the APOE locus were excluded due to the common presumption that the association signal in this region is APOE dependent (Lambert et al., 2013). In contrast, our LD

analyses discussed below revealed that the association of LOAD with rs2927438 is likely APOE independent (Supplementary table S2).

3.4. Pleiotropic analyses for rs2927438

We explored whether rs2927438 is also associated with other human traits. A query of the National Human Genome Research Institute-European Bioinformatics Institute (NHGRI-EBI) catalog of published GWASs for rs2927438 revealed that the only entry listed was a suggestive GWAS signal for the increased risk of Barrett's esophagus and esophageal adenocarcinoma (combined) with the G-allele of rs2927438 ($p \approx 2.0 \times 10^{-6}$, OR ≈ 1.16 , 95% confidence interval ≈ 1.10 – 1.25) (Levine et al., 2013). In contrast, LOAD risk was associated with the A-allele.

We further tested whether rs2927438 showed any association with the expression of nearby genes in the esophagus. Based on the expression data from the GTEx, we found significant association for rs2927438 with PVRL2 expression ($p \approx 0.021$) and marginal association with EML2 expression ($p \approx 0.055$) in esophagus muscularis (Supplementary fig. S3). Again, the G-allele predicted higher expression for both EML2 and PVRL2.

3.5. rs2927438 represents an APOE-independent risk locus of LOAD on 19q13.32

Because rs2927438 maps to an LD region on chromosome 19q13.32 significantly associated with LOAD, and is located ≈ 170 kb upstream of APOE, we tested whether the observed association of rs2927438 with LOAD was APOE dependent. First, we checked whether rs2927438 was in LD with the 2 SNPs responsible for the APOE $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles (rs429358 and rs7412) according to population data from the 1000 Genomes Project (phase 3). Haplo-view analyses of SNPs within a 300-kb region encompassing rs2927438 and the 2 APOE SNPs (Supplementary table S2) showed weak LD of rs2927438 with both rs429358 and rs7412 in all 26 different subpopulations of European, African, Admixed-American, East Asian, and South Asian background ($r^2 \leq 0.07$ and logarithm of the odds (LOD) score ≤ 1.5). Second, we explored whether there is a chromatin interaction between the genomic region containing rs2927438 and APOE using published data (Dixon et al., 2012) because recent studies showed that chromatin interactions play an important role in regulating gene expression (Pope et al., 2014). When using rs2927438 as bait, potential chromatin interactions (topologically associated domains) were observed only between rs2927438 and a 100-kb genomic region not affecting APOE (Supplementary figs. S4 and S5). Third, we tested whether APOE expression was affected by rs2927438 genotype in both blood and brain tissue. No significant association between rs2927438 and APOE expression was observed in any of the expression data sets (Supplementary table S3). Taken together, these lines of evidence strongly suggest that rs2927438 may confer risk for LOAD independent of APOE.

3.6. Effect of rs2927438 on AAO of LOAD, hippocampal volume, Ab42, tau and ptau181 levels

We investigated if there have been any reports on the relationship between rs2927438 and AAO of LOAD. Interestingly, the protective G-allele of this SNP revealed significant association with delayed AD onset ($p \approx 1.39 \times 10^{-12}$), according to a genome-wide survival analysis of 14,046 AD cases and 25,849 controls (Huang et al., 2017). Furthermore, there was a report of an association between rs2927438 and human hippocampal volume, which may modify risk for developing LOAD (Apostolova et al., 2006). Using the ENIGMA 2 data set ($N \approx 33,536$) (Hibar et al., 2017), we also observed a trend of association between the A-allele of rs2927438 and a smaller hippocampal

volume ($p \approx 0.107$, $b \approx -1.61$), consistent with previous observations of hippocampal atrophy in LOAD patients (Supplementary table S4).

Genetic variants that increase risk for LOAD could also modify Ab42, tau and ptau181 levels in the CSF, which are used as endo-phenotypes of LOAD (Deming et al., 2017). Hence, we tested whether rs2927438 was associated with Ab42, tau and ptau181 levels in the CSF using published samples ($N \approx 3146$) (Deming et al., 2017). Significant association was observed only between rs2927438 and tau levels ($b \approx 0.015$, $p \approx 0.037$), with the risk A-allele showing higher CSF tau levels (Supplementary table S5). 9q13 also exhibited significant association with LOAD, that is, rs6859 in PVRL2 (Carrasquillo et al., 2009), rs4420638 in APOC1 (Li et al., 2008), rs59007384 and rs10524523 (poly-T polymorphism) in TOMM40 (Cervantes et al., 2011; Roses et al., 2010). The risk region around the APOE locus may be explained either by the strong LD with the APOE $\epsilon 4$ allele (Jun et al., 2012) or genetic variations in regulatory regions affecting APOE expression. However, this may not be the case for rs2927438, which showed weak LD with APOE across all 26 subpopulations available at the 1000 Genomes Project. Furthermore, no significant association between rs2927438 and APOE expression was observed in either blood or brain tissue across different data sets. Moreover, there were no chromatin interactions between the genomic region containing rs2927438 and the promoter or enhancer region of APOE according to Hi-C chromatin interaction analysis.

In this study, we used the eQTL effect in blood-derived LCLs, which is not ideal as a proxy for the eQTL effect in the brain (Nica et al., 2011; Ramasamy et al., 2014). However, we gain power for genes that are ubiquitously expressed because of the use of a relatively large data set for eQTL analysis in LCLs ($N \approx 400$) (Dixon et al., 2007) compared with the available brain eQTL data set ($N \approx 193$ cortical tissues) (Myers et al., 2007). In addition, the number of eSNPs ($N \approx 71,732$) for the blood-derived samples was much larger than that for cortical brain tissue ($N \approx 21,940$), which can greatly increase power of candidate gene identification. Also, the specific tissue or cell type relevant to a trait or disease is often unknown. For AD, neurons in the brain are seemingly the most relevant cell type; however, several studies have highlighted the involvement of microglia, which originate from peripheral monocytes (El Khoury et al., 2007). Recent genetic and molecular studies have also indicated crucial roles for peripheral monocytes in AD pathogenesis (Tajuddin et al., 2016), and LOAD risk alleles are polarized for cis-eQTL effects in monocytes (Raj et al., 2014). Finally, eQTLs were inconsistently observed even among data sets focusing on the same brain tissue (McKenzie et al., 2014). For example, in 2 studies examining the prefrontal cortex (Colantuoni et al., 2011; Liu et al., 2010), the proportion of overlapping eQTLs was less than 10% (McKenzie et al., 2014).

Furthermore, we observed a trend of association between the rs2927438 A-allele and reduced hippocampal volume in healthy subjects. Previous studies have shown marked reductions in the volume of the hippocampus and amygdala in patients with overt LOAD compared to healthy elderly individuals (Jack et al., 1992). In addition, patients with mild cognitive impairment, who are at high risk of developing AD, also have smaller hippocampal volumes than healthy elderly people (Du et al., 2001). Even before the first occurrence of memory complaints, hippocampal atrophy can predict subsequent development of AD (den Heijer et al., 2006). Although the p-value of association between rs2927438 and hippocampal volume did not achieve nominal statistical significance ($p \approx 0.107$), it is not surprising, given that atrophy in the hippocampus is age dependent; and the mean age of the samples included in the present study was far less than 65 years (Hibar et al., 2017). Further studies are warranted to investigate this association.

There are additional limitations in the present study. First, other genes that did not meet our strict selection criteria for the integrative analysis of GWAS/eQTL data may also contribute to LOAD risk. Second, we cannot exclude the possibility that the association signal was actually caused by the hitchhiking effect of other causative variants, such as rare missense mutations and copy-number variations. The results from whole-genome sequencing of a LOAD data set may provide a more

complete survey. Third, most of the AD samples collected for meta-analysis are of European origin, and whether rs2927438 also contributes to AD susceptibility in other populations remains elusive. Fourth, although blood-related eQTL data sets have been extensively used in AD studies, we cannot exclude the possibility of other missing risk genes specific to brain function regulation, and further studies using high-coverage large brain eQTL data sets are necessary. Notably, during the revision of the current article, the GTEx consortium published a large study on the effect of genomic variability on gene expression across multiple human tissues (GTEx-Consortium et al., 2017), which is an extremely valuable resource for future eQTL studies.

Disclosure statement

The authors have no conflict of interests.

Acknowledgements

This work was in part supported by National Institutes of Health (to ER). The authors are deeply grateful for Christiane Reitz (Department of Neurology & Department of Epidemiology, Columbia University), Laura Cantwell (Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine), Haiyan Wang (Department of Statistics, Kansas State University), and Rachel A. Gibson (GSK Medicines Research Centre) for their valuable information about the LOAD GWAS data. The authors would like to thank Gary Donohoe (Cognitive Genetics and Therapy Group, School of Psychology, National University of Ireland, Galway & Department of Psychiatry and Trinity College Institute of Psychiatry, Trinity College, Dublin) for his willingness to share the cognitive data.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.neurobiolaging.2017.12.027>.

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