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Genome-wide Significant, Replicated and Functional Risk Variants for Alzheimer's disease

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Abstract

Genome-wide association studies (GWASs) have reported numerous associations between risk variants and Alzheimer's disease (AD). However, these associations do not necessarily indicate a causal relationship. If the risk variants can be demonstrated to be biologically functional, the possibility of a causal relationship would be increased. In this article, we reviewed all of the published GWASs to extract the genome-wide significant ($p < 5 \times 10^{-8}$) and replicated associations between risk variants and AD or AD-biomarkers. The regulatory effects of these risk variants on the expression of a novel class of non-coding RNAs (piRNAs) and protein-coding RNAs (mRNAs), the alteration of proteins caused by these variants, the associations between AD and these variants in our own sample, the expression of piRNAs, mRNAs and proteins in human brains targeted by these variants, the expression correlations between the risk genes and *APOE*, the pathways and networks that the risk genes belonged to, and the possible long non-coding RNAs (LncRNAs) that might regulate the risk genes were analyzed, to investigate the potential biological functions of the risk variants and explore the potential mechanisms underlying the SNP-AD associations. We found replicated and significant associations for AD or AD-biomarkers, surprisingly, only at 17 SNPs located in 11 genes/snRNAs/LncRNAs in eight genomic regions. Most of these 17 SNPs enriched some AD-related pathways or networks, and were potentially functional in regulating piRNAs and mRNAs; some SNPs were associated with AD in our sample, and some SNPs altered protein structures. Most of the protein-coding genes regulated by the risk SNPs were expressed in human brain and correlated with *APOE* expression. We conclude that these variants were most robust risk markers for AD, and their contributions to AD risk was likely to be causal. As expected, *APOE* and the lipoprotein metabolism pathway possess the highest weight among these contributions.

Keywords

Alzheimer's disease; *GWAS*; genome-wide significant; replicated; risk variant; gene expression; *APOE*

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia. It affects several millions of people worldwide. It is a primary neurodegenerative cerebral disease in the elderly, characterized by two major histopathologic changes in the brain, i.e., extracellular amyloid plaques that are composed of A β 40 and A β 42 and intracellular neurofibrillary tangles (NFT) that are composed of hyperphosphorylated protein Tau (Masters et al. 1985). The core cerebrospinal fluid (CSF) biomarkers for the diagnosis of AD include A β 42 (amyloid-42 peptide), T-tau (total tau), and P-tau (phosphorylated tau) (Anoop et al. 2010).

From 2007 till July, 2017, 1055 genome-wide association studies (GWASs) and whole genome/exome sequencing studies of AD or AD-biomarkers have been published. However, the vast majority of these studies produced results that were not significant at genome-wide level and/or were not replicated by any other study, and thus might confuse or even mislead the readers. Although some studies reported “replicable” findings at gene level (i.e., different risk markers from the same gene), the replicable findings at individual marker level (i.e., same risk markers) were not common. Here, we only followed up the latter, which we believe can really be called “replicated” and more conservative, robust and reliable than the “gene-level” findings.

In this article, we reviewed all of these published studies, and extracted the most robust, i.e., both genome-wide significant ($p < 5 \times 10^{-8}$) and replicated, associations between risk SNPs and AD or AD-biomarkers. These associations themselves do not necessarily indicate a cause-effect relationship. However, if we can demonstrate that the risk SNPs are biologically functional, these associations are more likely to be causal ones. Thus, in this study, a series of functional analyses were conducted based on the pathway illustrated in Figure 1.

2. Summary of Materials and Methods (much detailed in the Supplementary Materials)

All associations between SNPs and AD/AD-biomarkers screened from the published literatures that are genome-wide significant ($p < 5 \times 10^{-8}$) and replicated across at least two independent studies are summarized in Table 1. These risk SNPs were genotyped in our own small cohort (6 AD and 6 controls) to replicate the SNP-AD association (Qiu et al. 2017), which was approved by the ethics committee of The Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences.

To explore the potential biological functions of these risk SNPs, we predicted their various functions using a series of bioinformatics analyses, and examined the potential regulatory effects of these SNPs on RNA expression using real molecular experiment, including the expression of the protein-coding mRNAs and the largest class of non-coding RNAs, i.e., piRNAs. The whole set of analyses was based on a regulation pathway illustrated in Figure 1.

For the mRNA expression, the genes that these risk SNPs are located were studied and the *cis*-regulatory effects of these SNPs on the mRNAs in human brains of a European cohort ($n=134$) were examined. For the piRNA expression, the transcriptome-wide piRNAs in human brains of our small Chinese cohort (6 AD and 6 controls; see above) were studied (Qiu et al. 2017) and the *trans*-regulatory effects of these SNPs on the piRNAs were examined. Furthermore, the expression of mRNAs and proteins encoding by these risk genes were examined in multiple brain regions in multiple independent European and American cohorts, the correlations between their mRNA expression and the *APOE* expression in brain were tested, and the most possible phenotype that these risk genes can predict for was explored using pathway/network analysis. Finally, the long non-coding RNAs (LncRNAs) proximate to these risk genes were explored. Integrating multiple independent pieces of positive evidence from these analyses would increase the possibility that the SNP-AD

relationships are causal and suggest the potential mechanisms underlying these SNP-AD associations.

3. Results

3.1. Genome-wide Significant and Replicated Associations between SNPs and AD/AD-biomarkers

Although 10,393 candidate gene studies and 1055 GWASs on AD/AD-biomarkers have been published from 2007 till July, 2017, surprisingly, we could only extract the associations of 17 variants located in 11 genes/snRNAs/LncRNAs in eight loci from 28 studies that were genome-wide significant (1.0×10^{-295} p 9.0×10^{-9}) and replicated across at least two independent studies (not only two independent samples in the same study) at single-point level. These eight loci include *APOE* cluster (*NECTIN2-TOMM40-APOE-APOC1*) (Abraham et al. 2008; Logue et al. 2011; Naj et al. 2010; Antunez et al. 2011; Feulner et al. 2010; Kim et al. 2011; Harold et al. 2009; Lambert et al. 2009; Nelson et al. 2014; Heinzen et al. 2010; Seshadri et al. 2010; Perez-Palma et al. 2014; Shen et al. 2010; Meda et al. 2012; Ramirez et al. 2014; Ramanan et al. 2014; Melville et al. 2012; Kamboh et al. 2012b; Li et al. 2008; Webster et al. 2008; Coon et al. 2007; Kamboh et al. 2012a), *APOJ* (Lambert et al. 2009; Jun et al. 2016; Lambert et al. 2013), *SORL1* (Miyashita et al. 2013; Lambert et al. 2013; Jun et al. 2016), *SLC24A4* (Jun et al. 2016; Lambert et al. 2013), *CR1* (Lambert et al. 2013; Lambert et al. 2009), *CD33* (Naj et al. 2011; Lambert et al. 2013), *LOC105373605* (Kamboh et al. 2012b; Naj et al. 2011; Lambert et al. 2013; Jun et al. 2016; Hollingworth et al. 2011; Antunez et al. 2011; Hu et al. 2011) and *RNU6-560P* (Lambert et al. 2013; Jun et al. 2016; Harold et al. 2009) (Table 1), which is highly consistent with the gene list presented in the largest GWAS meta-analysis (Lambert et al. 2013). Among them, the associations between AD and variants (rs6859, rs157580, rs2075650, rs429358 and rs4420638) within *APOE* cluster are most robust. The associations between AD and rs6859 and rs157580 were replicated across three GWASs (8×10^{-89} p 6×10^{-14}) (Abraham et al. 2008; Logue et al. 2011; Naj et al. 2010; Antunez et al. 2011; Feulner et al. 2010; Kim et al. 2011); the associations between either AD or age-of-onset of AD and rs4420638 were replicated across five GWASs (8×10^{-149} p 1×10^{-12}) (Kamboh et al. 2012b; Coon et al. 2007; Li et al. 2008; Kamboh et al. 2012a; Webster et al. 2008); the associations between AD and rs2075650 were replicated across nine GWASs (1×10^{-295} p 3×10^{-11}) (Harold et al. 2009; Lambert et al. 2009; Nelson et al. 2014; Heinzen et al. 2010; Seshadri et al. 2010; Perez-Palma et al. 2014; Naj et al. 2010; Kim et al. 2011; Shen et al. 2010); and the associations between AD or AD biomarkers ($A\beta_{42}$, t-tau, p-tau181p, hippocampal, amygdalar or total cerebral volume) and rs429358+rs7412 (e2/e3/e4) were replicated across eight GWASs (2×10^{-33} p 4×10^{-9}) (Meda et al. 2012; Kim et al. 2011; Ramirez et al. 2014; Ramanan et al. 2014; Melville et al. 2012; Shen et al. 2010). Some of these associations have been validated by previous functional studies. Basically, other surrounding markers that might be in linkage disequilibrium with these 17 SNPs have not been reported to be genome-wide significant, replicated across studies or biologically functional (data not shown). Finally, more other phenotypes associated with these genes and the molecular functions of these genes are presented in Supplementary Table S1.

3.2. Bioinformatics Analysis

Among the 17 risk SNPs in Table 1, the only nonsynonymous variant is rs429358+rs7412 ($\epsilon 2/\epsilon 3/\epsilon 4$; Cys/Arg). It alters the APOE protein structure and has been demonstrated to be pathogenic, playing clinically significant roles in the development of AD. It is located at an 880bp CpG island of *APOE*, may alter the methylation status of this CpG island, and therefore affect the expression of *APOE*. One SNP at *APOE* cluster and one *CD33* SNP at the transcription factor binding sites (TFBS) may affect the local DNA conformation and thereby influence the binding of transcription factors. Three SNPs located at the open chromatin regions are often associated with regulatory factor binding. Two SNPs may affect miRNA binding site activity, and therefore may regulate protein translation. Two SNPs are located in LncRNAs that might regulate the gene expression.

3.3. The Risk Variants May Regulate the Expression of Risk Genes in Human Brains

Cis-eQTL analysis showed that most SNPs had nominal *cis*-acting regulatory effects on mRNA expression ($p < 0.05$). The effect of rs6656401 on *CR1* in HIPPI ($p = 2.7 \times 10^{-5}$) and WHMT ($p = 6.5 \times 10^{-7}$), and the effects of rs2279590 and rs9331896 on *APOJ* in TCTX ($p = 1.3 \times 10^{-4}$ and 4.5×10^{-4} , respectively) and WHMT ($p = 1.4 \times 10^{-4}$ and 1.5×10^{-4} , respectively) remained significant after Bonferroni correction. (Table 2)

3.4. The Risk Variants May Regulate the Expression of piRNAs in brains

We found difference in genotype frequency of rs2075650 at *APOE* cluster (A/G genotype frequency: 0.714 vs. 0.111; $p = 0.029$) and allele frequency (G allele frequency: 0.357 vs. 0.056; $p = 0.055$) between AD cases and controls in our small cohort. The association between this marker and AD or AD-biomarkers has been reported to be the most robust one in literatures. It has been reported to be extremely significant (with lowest p values) and has been replicated across nine (the highest number) independent GWASs (Table 1). Our small cohort had 35% of power (calculated by an R program *pwr.chisq.test*) to detect this association based on the most significant finding reported previously (Seshadri et al. 2010).

eQTL analysis showed that some risk SNPs had transcriptome-wide significant ($p < 5.3 \times 10^{-6}$) regulatory effects on piRNAs (Table 3), including rs10792832 and rs3851179 at *RNU6-560P* and $\epsilon 2/\epsilon 3/\epsilon 4$ at *APOE* (9.4×10^{-8} p 4.7×10^{-6}). (Table 3)

3.5. mRNA/Protein Expression of Risk Genes in Human Brain

Except for *CR1* and *CD33*, other seven protein-coding genes, including *APOJ*, *SORL1*, *SLC24A4*, *NECTIN2*, *TOMM40*, *APOE* and *APOC1*, had significant mRNA expression ($36 < \text{normalized intensity} < 9088$ or $1 < \text{RPKM} < 779$) in 26 human brain regions in the three independent cohorts (Table 4). Specifically, in the first UK European cohort, all of these seven protein-coding genes were expressed in the ten brain regions including CRTX, FCTX, HIPPI, MEDU, OCTX, PUTM, SNIG, TCTX, THAL, and WHMT (all $\log_2(\text{normalized intensity}) > 5.17$), except for the low expression of *SLC24A4* in CRTX. In the American cohort, all seven genes were expressed in CTX, FCTX, ACTX, HPTH, HIPPI, SNIG, NACC, CDNL, PUTM, AMYG, CRHM, CRBL and/or PITT. Among these seven genes, *APOE* (201.40 RPKM 778.30) and *APOJ* (48.90 RPKM 602.00) were expressed in highest density across all 13 brain regions, except for low expression of *APOE* in PITT. In the

second UK European cohort, all seven genes were expressed in at least one of the 17 brain regions, including AMYG, CDNL, CRBL, CRPD, CCTX, DRGL, FTBR, HPTH, MEDU, OCTX, PITT, PONS, PFCX, TCTX, THAL, WHOL and PINL. Among these seven genes, *APOE* (102.58 normalized intensity 1875.35) and *APOJ* (856.65 normalized intensity 9087.92) were expressed in highest density across all 17 brain regions. Finally, in the Germany cohort, proteomic analysis showed that *APOJ*, *TOMM40*, *APOE* and *APOC1* had significant protein expression in WHOL, FCTX or CRTX. Among these four genes, *APOE* (40ppm concentration 267ppm) and *APOJ* (40 concentration 291) were expressed in highest density across all three brain regions.

3.6. The Expression of Eight Protein-Coding Genes Was Correlated with APOE Expression

The mRNA expression of all eight risk genes in brain was significantly correlated with *APOE* expression in at least one of the ten brain regions in the UK European cohort ($0.29 \leq r \leq 0.81$; $2.9 \times 10^{-32} \leq p \leq 6.2 \times 10^{-4}$), and *APOJ*, *SORL1*, *APOE* and *APOC1* belong to the same lipoprotein metabolism pathway (www.reactome.org). Except for the negative correlations ($r < 0$) between *SORL1* and *APOE* in OCTX, FCTX and TCTX and between *SLC24A4* and *APOE* in OCTX, all others were positive ($r > 0$) (Table 5)

3.7. Gene network and pathway analysis

Overrepresentation analysis using IPA identified biological pathways containing a statistically significant excess of these genes. The top 5 canonical pathways found to be overrepresented in these genes using IPA are shown in Table 6. Two canonical pathways related to the retinoid X receptor were the most significant enriched pathways: LXR/RXR Activation ($p = 1.50 \times 10^{-5}$) and FXR/RXR Activation pathways ($p = 1.72 \times 10^{-5}$). These were followed by Atherosclerosis Signaling ($p = 1.74 \times 10^{-5}$), IL-12 Signaling and Production in Macrophages ($p = 2.64 \times 10^{-5}$), and Production of Nitric Oxide and Reactive Oxygen Species in Macrophages ($p = 6.07 \times 10^{-5}$) pathways. Late-onset AD ($p = 1.75 \times 10^{-10}$), efflux of phospholipid ($p = 1.92 \times 10^{-7}$), AD ($p = 2.20 \times 10^{-7}$), familial amyloidosis ($p = 4.48 \times 10^{-7}$), lattice corneal dystrophy type I ($p = 4.84 \times 10^{-7}$) were the top enriched diseases and functions. Only the network for Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry was significantly enriched (Score=23). IPA knowledge base significantly associated this network to both late-onset AD ($p = 1.75 \times 10^{-10}$), and AD ($p = 2.20 \times 10^{-7}$) as shown in Figure 2.

Current network and pathway analysis is limited by the state of current knowledge about gene interactions and functions in the biomedical literature. Thus, well studied genes have more complete, validated interaction and functional data in contrast to less studied ones. In this regard, these results represent our findings with respect to the current state of knowledge.

3.8. The LncRNAs Related to the Replicated Risk Genes

The LncRNAs proximate to each gene are listed in Table 7. One sense LncRNA 2.9 kb to *SLC24A4* is a large intergenic non-coding RNA (LincRNA), with a length of 15 kb; three others overlapping with or close to *SORL1* and *CD33* are antisense LncRNAs, with lengths of 12 kb to 31 kb. The results presented above are also illustrated in Figure 1.

4. Discussion

More than 10,000 genetic studies of AD have been published so far, and over 560 genes have been “associated” with AD risk, which might confuse the readers and some might mislead them due to the non-replicability issue. Our analysis first explicitly showed that, after stringent screening, only 17 SNPs in eight loci were robustly associated with AD; that is, these SNPs were biologically functional and potentially causal, and their associations with AD were genome-wide significant and replicated across independent studies. Most of these eight loci enriched some AD-related pathways or networks. We believe these SNPs/loci should have the highest possibility of contribution to AD risk. *APOE* and the lipoprotein metabolism pathway possess the highest weight among this contribution, as expected though. These risk SNPs/loci contribute to AD risk probably via common or distinct mechanisms. These mechanisms may be related to the direct or indirect regulatory functions of the risk variants. We illustrate possible mechanisms underlying these SNP-AD associations in Figure 1.

The *cis*-eQTL and bioinformatics analyses provided evidence to support that these replicated risk SNPs might be biologically functional in regulating piRNAs and mRNAs, altering protein structures, and influencing the transcription, expression and splicing of the risk genes. Many piRNAs, mRNAs and proteins targeted by these variants were expressed in human brains. This evidence increased the possibility that these risk SNPs might play functional roles in AD development.

As expected, association between *APOE* and AD has been intensively reported and is most robust. *APOE* is highly abundant in human brains. It may be directly involved in AD pathology or indirectly through a β -amyloid (A β) or tau pathway (Puglielli et al. 2003; Namba et al. 1991; Lilius et al. 1999). Six other protein-coding risk genes, including *APOJ*, *SORL1*, *SLC24A4*, *NECTIN2*, *TOMM40* and *APOC1*, were significantly expressed in multiple human brain regions too. They were all correlated with *APOE* in expression. *NECTIN2*, *TOMM40* and *APOC1* are closely located within the same genomic cluster as *APOE*. *APOJ*, *SORL1* and *APOC1* belong to the same lipoprotein metabolism pathway (www.reactome.org) as *APOE* too. This pathway is critical in redistributing cholesterol and phospholipids in brains, responsible for the apoE4-associated neuropathology including the formation of neurotoxic fragments that have been demonstrated to be involved in the development of AD (Mahley 2016; Jones et al. 2010). *APOE* and *APOJ* expression in brains is the most abundant among all risk genes for AD. Apparently, *APOE* and the lipoprotein metabolism pathway possess the highest weight in contribution to the AD risk. Moreover, *SLC24A4* is involved in neural development (Larsson et al. 2011). *RIN3* next to *SLC24A4* interacts with *BIN1* in the early endocytic pathway (Kajiho et al. 2003), and *BIN1* is another well-known risk gene for AD, which is probably the alternative non-*APOE* pathway via which *SLC24A4* is implicated in AD (Tan et al. 2013).

The protein-coding risk gene *CD33* was expressed a little bit lower than the threshold in brains in our four independent brain tissue cohorts (data not shown). However, this low expression was significantly correlated with *APOE* expression in SNIG and TCTX in our cohorts, and literatures have also reported low expression of *CD33* around the threshold

mainly in microglial cells (Jiang et al. 2014), which suggested it might still be able to play a direct functional role in AD too. CD33 can inhibit the uptake and clearance of A β 42 in microglial cells and CD33 inactivation can mitigate A β pathology (Griciuc et al. 2013). CD33 was associated with accumulation of neuritic amyloid pathology and fibrillar amyloid, and increased numbers of activated human microglia (Bradshaw et al. 2013).

The protein-coding risk gene *CR1* (i.e., *CD35*) was expressed much lower than the threshold in brains in our four independent brain cohorts (data not shown), consistent with previous reports (Fonseca et al. 2016). However, this very low expression was also significantly correlated with *APOE* expression in multiple brain regions in our cohorts. Some studies have also reported low expression of *CR1* in some brain regions (Chibnik et al. 2011; Bralten et al. 2011), and *CR1* interacted with *APOE* and participated in the clearance of β amyloid (A β) peptide. A β is the principal constituent of amyloid plaques that is one of the major brain lesions of individuals with AD (Thambisetty et al. 2013; Lambert et al. 2009; Biffi et al. 2012).

Additional functional pathway and network analysis with IPA suggested that more other pathways that these risk genes belong to might also be involved in AD. The top most significant canonical pathways resulting from the enrichment analysis were related to the retinoid X receptors (RXRs). Recent studies have pointed to a role of liver X receptor, a RXR binding partner, in the AD pathophysiology (Sandoval-Hernandez et al. 2015). The third most significant enriched canonical pathway was Atherosclerosis signaling. The association between cerebrovascular atherosclerosis and AD has been examined in many cross-sectional studies (Kim et al. 2016). Both Atherosclerosis and AD involve inflammation and macrophage infiltration (Lathe et al. 2014). Subsequently, two canonical pathways indicative of macrophage activation [production of IL-12 and nitric oxide and reactive oxygen species (NOROS)] were among the top 5 significantly enriched pathways. The NOROS pathway was listed as top 1 by a previous report (Li et al. 2015). Finally, if analyzed using MetaCore, the top 4 significantly enriched canonical pathways were all “immune response” pathways (data not shown), consistent with a previous report (Jones et al. 2010).

Recent evidence suggests that piRNAs are abundant in the brain (Mani and Juliano 2013; Rajasethupathy et al. 2012; Lee et al. 2011; Sharma et al. 2001; Perrat et al. 2013; Yan et al. 2011; Ghildiyal et al. 2008; Dharap et al. 2011; Peng and Lin 2013; Zuo et al. 2016b; Qiu et al. 2017). piRNAs may regulate the mRNAs and translation of the proximate risk genes (Grivna et al. 2006) or interact with neurotransmitters in brains (Ross et al. 2014; Rajasethupathy et al. 2012), and thus may play roles in brain disorders. Our transcriptome-wide eQTL screening demonstrated that the risk SNPs, especially in non-coding loci, might have *trans*-regulatory effects on piRNAs, which may suggest a novel mechanism underlying the SNP-disease associations.

Finally, five risk variants are located within or close to either an antisense lncRNA (i.e., *LOC105373605*) or a snRNA that does not encode any protein. These lncRNA and snRNA are proximate to *BIN1* and *PICALM*, respectively. *BIN1* and *PICALM* are expressed in human brains and have widely been associated with AD by numerous studies (Wang et al.

2016; Holler et al. 2014). The antisense LncRNA and snRNA might use diverse transcriptional and post-transcriptional mechanisms (Faghihi and Wahlestedt 2009; Villegas and Zaphiropoulos 2015; Zuo et al. 2016a) to regulate *BIN1* and *PICALM*, respectively, to play roles in AD. *PICALM* and *BIN1* were modifiers of A β toxicity and they could promote APP internalization, endocytic trafficking, and A β generation in neurons in vitro (Xiao et al. 2012; Treusch et al. 2011). Furthermore, several risk genes, e.g., *SORL1*, *SLC24A4* and *CD33*, overlap with or are close to LncRNAs in genomic location, also suggesting the role of LncRNAs in AD. These hypotheses regarding LncRNAs and snRNAs should be tested in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Illustration for the pathways underlying SNP-AD association

[Solid lines: Directly evidenced by our study; Dash lines: Indirectly evidenced by literatures. ❶ LncRNA expression is regulated by the SNPs (by eQTL analysis); ❷ LncRNAs regulate the expression of the nearest protein-coding genes by sequence complementarity; ❸ mRNA expression of the risk genes is correlated to APOE mRNA expression (by correlation analysis); ❹ mRNA of APOE encodes APOE protein, and both mRNA and protein of APOE are expressed in brain and related to AD; ❺ RNAs/proteins expressed in brain are assumed to have potential brain functions; ❻ many brain functions are assumed to be related to the development of AD; ❼ piRNA expression is regulated by the risk SNPs (by transcriptome-wide eQTL analysis); ❽ piRNAs regulate the expression of the nearest protein-coding genes by sequence complementarity; ❾ mRNAs encode proteins; ❿ proteins are expressed in brains (by mass spectrometry-based proteomics microarray analyses); ⓫ mRNA expression is regulated by the risk SNPs (by cis-eQTL analysis and bioinformatic analysis); ⓬ protein structures are altered by SNPs (by bioinformatics analysis); ⓭ mRNA expression of risk genes is detected in brains (by RNA-Seq or RNA microarray analyses); ⓮ SNPs are associated with AD (by our study or published GWASs); ⓯ piRNAs are detected in brains (by microarray)]

Figure 2. The significantly enriched network Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry

[This figure was generated from a Core Analysis in IPA for the risk genes (green-colored) and their relationship to AD (red-colored)]

Table 1

Replicated associations between genes and Alzheimer's disease

SNP	Location(GRCh38)	Gene (Alias) [Class]	Functional class	p-value	Ref.	p-value	Ref.	p-value	Ref.
rs6656401	chr1:207518704	CR1 (CD35)	Intron; chromatin	6×10 ⁻²⁴	[1]	4×10 ⁻⁹	[2]		
rs7561528	chr2:127132061	LOC105373605 [LncRNA]	Intron	6×10 ⁻¹¹	[3]	4×10 ⁻¹⁴	[4]		
rs6733839	chr2:127135234	LOC105373605 [LncRNA]	Intron; chromatin	7×10 ⁻⁴⁴	[1]	1×10 ⁻⁹ *	[5]		
rs744373	chr2:127137039	LOC105373605 [LncRNA]	3'	3×10 ⁻¹⁴	[6]	2×10 ⁻⁹	[7]	1×10 ⁻¹⁰	[8]
rs2279590	chr8:27598736	APOJ (CLU)	Exon; chromatin	9×10 ⁻⁹	[2]	2×10 ⁻⁶ **	[5]		
rs9331896	chr8:27610169	APOJ (CLU)	Intron; miRNA	3×10 ⁻²⁵	[1]	3×10 ⁻⁹ **	[5]		
rs11218343	chr11:121564878	SORL1 (LRP9)	Intron	2×10 ⁻⁹	[9]	1×10 ⁻¹⁴	[1]	9×10 ⁻⁶ **	[5]
rs10792832	chr11:86156833	RNU6-560P [snRNA]	3'	9×10 ⁻²⁶	[1]	4×10 ⁻⁶ *	[5]		
rs3851179	chr11:86157598	RNU6-560P [snRNA]	3'	1×10 ⁻⁹	[10]	2×10 ⁻⁶ **	[5]		
rs10498633	chr14:92460608	SLC24A4 (SHEP6)	Intron	6×10 ⁻⁹	[1]	4×10 ⁻⁷ **	[5]		
rs6859	chr19:44878777	NECTIN2-TOMM40-APOE-APOC1	Exon of NECTIN2;miRNA	6×10 ⁻¹⁴	[11]	5×10 ⁻⁷	[12]	1×10 ⁻⁷	[13]
rs157580	chr19:44892009	NECTIN2-TOMM40-APOE-APOC1	Intron of TOMM40;TFBS	8×10 ⁻⁸⁹	[7]	1×10 ⁻⁴⁰	[14]	<1×10 ⁻⁶ #	[15]
rs2075650	chr19:44892362	NECTIN2-TOMM40-APOE-APOC1 (<i>CD112-TOMM40-APOE-APOC1</i>) (<i>APOE cluster</i>)	Intron of TOMM40	2×10 ⁻¹⁵⁷	[10]	2×10 ⁻¹⁶	[2]	4×10 ⁻¹³	[16]
				3×10 ⁻¹¹	[17]	1×10 ⁻²⁹⁵	[18]	9×10 ⁻¹¹⁶	[19]
				5×10 ⁻³⁶	[13]	<10 ⁻⁶ ,	[15]	<10 ⁻⁶ ¶	[20]
rs429358	chr19:44908684	NECTIN2-TOMM40-APOE-APOC1	Exon of APOE; 3D;	7×10 ⁻¹⁶	[21]	4×10 ⁻⁹	[21]	<10 ⁻⁶ ####, ,	[15]
+rs7412			Pathogenic; CpG	4×10 ⁻¹⁷ #	[22]	5×10 ⁻¹⁴ #	[23]	2×10 ⁻³³ ¶	[24]
(e2/e3/e4)				1×10 ⁻⁷ ¶	[24]	<10 ⁻⁶ ¶	[20]		
rs4420638	chr19:44919689	NECTIN2-TOMM40-APOE-APOC1	3' to APOC1	8×10 ⁻¹⁴⁹	[3]	2×10 ⁻⁴⁴	[25]	1×10 ⁻³⁹	[26]
				1×10 ⁻³⁹	[27]	1×10 ⁻¹² ø	[28]		
rs3865444	chr19:51224706	CD33	Exon of CD33; TFBS	2×10 ⁻⁹	[4]	3×10 ⁻⁶	[1]		

All target traits are Alzheimer's disease, except for the following.

* in APOE^εe4 carriers (*) or non-APOE^εe4 carriers (**);

ø age of onset of AD;

#/ associated with Aβ42 (amyloid-42 peptide) (#), t-tau (total cerebrospinal fluid tau) (##), p-tau 181p (tau phosphorylated at threonine 181) (###), t-tau/Aβ42 (), p-tau181p/Aβ42 ();

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hippocampal, amygdalar or total cerebral volume.

LncRNA, long non-coding RNA; snRNA, small nuclear RNA. TFBS, these SNPs are located in the transcription factor binding sites; chromatin, this SNP is located in an open chromatin region; miRNA, these SNPs may affect miRNA binding site activity, which may regulate protein translation; pathogenic, this SNP is clinically pathogenic; 3D, this SNP alters the protein structure; CpG, this SNP is located at an 880bp CpG island.

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Table 2
cis-acting expression of quantitative locus (*cis*-eQTL) analysis on the risk markers in human brains of a UK European cohort (n=134)

SNPs	Target	FCTX	TCTX	OCTX	PUTM	THAL	HIPP	SNIG	WHMT
rs6656401	<i>CR1</i>	0.014	4.0×10^{-3}		0.049	0.013	2.7×10^{-5} *		6.5×10^{-7} *
rs2279590	<i>APOJ</i>	3.9×10^{-4}	1.3×10^{-4} *	7.8×10^{-4}			1.5×10^{-3}	0.011	1.4×10^{-4} *
rs9331896	<i>APOJ</i>	1.2×10^{-3}	4.5×10^{-4}	7.7×10^{-4}			6.9×10^{-4}	0.029	1.5×10^{-4} *
rs6859	<i>TOMM40</i>	5.0×10^{-3}							
rs157580	<i>NECTIN2</i>	0.036		8.1×10^{-4}					
rs2075650	<i>NECTIN2</i>	0.034							
rs2075650	<i>TOMM40</i>	0.047							
rs429358	<i>NECTIN2</i>	0.015							
rs429358	<i>APOC1</i>						0.050		
rs4420638	<i>APOC1</i>		0.017						
rs3865444	<i>CD33</i>		0.017						

The SNP list refers to Table 1. Brain regions: frontal cortex (FCTX), temporal cortex (TCTX), occipital cortex (specifically primary visual cortex, OCTX), putamen (PUTM), thalamus (THAL), hippocampus (HIPP), substantia nigra (SNIG), and intralobular white matter (WHMT).

* The effects remaining significant after Bonferroni correction are bold, i.e., $p < \alpha = 2.0 \times 10^{-4} = 0.05 / (10 \text{ brain tissues} \times 25 \text{ regulations})$.

Table 3

The piRNAs significantly correlated with risk SNPs for AD

piRNA	location	RNU6-560P		APOE
		rs10792832	rs3851179	(e2/e3/e4)
DQ571511	SNORD49A	2.8×10^{-7}	2.8×10^{-7}	
DQ570728	SNORD63	4.0×10^{-6}	4.0×10^{-6}	
DQ596993	SNORD110	4.7×10^{-6}	4.7×10^{-6}	
DQ581176	Close to DLG5			4.1×10^{-7}
DQ589995	LRRC37A4P			3.3×10^{-6}
DQ574832	Close to BUB1			5.1×10^{-6}
DQ574527	FAM95B1			9.4×10^{-8}
DQ577188	GOLGA6L9			4.3×10^{-6}
DQ571900	LINC00837			3.0×10^{-6}

 $\alpha = 5.3 \times 10^{-6}$

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Table 4

The mRNA and protein expression of risk genes in human brains in four cohorts

	WHOL	CTX	PFCX	FCTX	TCTX	ACTX	OCTX	CRTX	HPTH	HIPP	SNIG	NACC	CDNL	PUTM	MEDU	AMYG	CRHM	CRBL	PITT	THAL	CRPD	DRGL	FTBR	PONS	PINL	WHMT	
mRNA expression in a UK European cohort (n=134) [log2-transformed normalized intensity values from Affymetrix Human ST 1.0 exon arrays]:																											
APOJ			8.9	8.8		8.7	8.7		9.0	9.1			8.9	9.2						9.1							8.8
SORL1			8.9	8.8		8.9	8.7	8.7		8.7	8.8			8.0	8.6					8.5							8.8
SLC24A4			5.8	5.9		6.1	-		5.5	6.2			5.3	6.5						6.0							5.8
NECTIN2			6.2	6.2		6.1	5.9	5.9		6.4	6.5			6.3	6.5					6.4							6.2
TOMM40			6.3	6.3		6.3	6.4	6.4		6.2	6.3			6.3	6.3					6.2							6.4
APOE			6.7	6.7		6.6	6.6	6.6		7.0	7.8			7.5	7.1					7.4							7.1
APOC1			5.4	5.4		5.5	5.3		5.5	5.8			5.8	5.5						5.6							5.6
mRNA expression in an American cohort (n=2712) [RPKM values from RNA-Seq]:																											
APOJ		585.6		564.4		565.5			391.8	376.8	468.2	540.5	602.0	547.9		475.5	307.6	423.6	48.9								
SORL1		14.1		17.4		11.6			10.0	11.0	13.8	9.0	9.0	6.4		7.8	19.7	20.3	15.3								
SLC24A4		1.8		1.3		-			-	-	1.2	1.1	-	-		-	-	-	-								
NECTIN2		4.6		4.1		4.6			5.8	4.0	5.2	3.9	4.4	4.1		4.7	2.0	3.4	15.6								
TOMM40		11.0		10.7		8.3			9.3	7.4	8.0	7.4	7.4	7.5		6.4	12.5	12.5	10.0								
APOE		252.8		239.0		331.6			519.9	289.6	753.4	778.3	791.1	762.3		556.2	201.4	269.8	-								
APOC1		4.2		4.0		4.7			43.6	11.1	75.1	81.2	78.9	72.6		25.8	-	-	3.4								
mRNA expression in a UK European cohort (n=176; BioGPS) [normalized intensity values from Affymetrix microarray]:																											
APOJ	5671.0		9087.9		2467.2	2637.9	3509.0		5470.7				3132.7		2342.2	6874.6		2878.1	729.9	3995.2	3565.2	1737.8	856.7	2452.2		8721.4	
SORL1	607.9		1144.0		288.1	355.1	523.7		472.8				131.8		415.3	510.0		320.9	420.1	219.1	509.9	-	83.9	198.9		848.6	
SLC24A4	45.5		43.7		47.9	49.5	48.2		47.2				50.9		48.7	45.5		47.9	48.2	48.7	48.3	48.4	47.6	48.4		46.8	
NECTIN2	-		-		-	34.2	-		-				-		-	-		-	32.2	-	-	-	-	-		63.0	
TOMM40	220.1		398.3		449.5	514.7	274.7		303.1				312.2		322.3	245.8		259.6	365.3	697.0	697.0	307.5	334.2	519.9		355.1	
APOE	1139.5		818.9		417.9	461.3	528.3		1769.5				990.9		438.2	1875.4		591.3	102.6	506.2	809.8	158.9	193.5	449.7		403.7	
APOC1	40.8		32.0		32.7	134.5	40.8		150.8				164.8		95.6	56.4		-	65.6	122.7	30.1	33.6	-	100.1		36.0	
protein expression in a Germany cohort (ProteomicsDB) [ppm values from mass spectrometry-based proteomics arrays]:																											

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WHOL	CTX	PFCX	FCTX	TCTX	ACTX	OCTX	CRTX	HPTH	HIPP	SNIG	NACC	CDNL	PUTM	MEDU	AMYG	CRHM	CRBL	PITT	THAL	CRPD	DRGL	FTBR	PONS	PINL	WHMT
APOC1	-		10				19																		

The expression levels less than thresholds are not listed in this table [normalized intensity < 36, i.e., log2(normalized intensity) < 5.17 from microarray; RPKM < 1 from RNA-Seq; or raw protein concentration value < 10ppm from HPLC]. Brain regions: whole brain (WHOL), cortex (CTX), prefrontal cortex (PFCX), anterior cingulate cortex (ACTX), cerebellar cortex (CRTX), hypothalamus (HPTH), nucleus accumbens (NACC), caudate nucleus (CDNL), medulla (specifically inferior olivary nucleus, MEDU), amygdala (AMYG), cerebellar hemisphere (CRHM), pituitary (PITT), cerebellum peduncles (CRPD), dorsal root ganglion (DRGL), fetal brain (FTBR), pons (PONS), and pineal (PINL). Other abbreviations refer to Table 2.

Table 5
Significant expression correlation between *APOE* and other risk genes in human brain (n=134)

	<u>CRI</u>		<u>APOJ</u>		<u>SORL1</u>		<u>SLC24A4</u>		<u>NECTIN2</u>		<u>TOMM40</u>		<u>APOC1</u>		<u>CD33</u>	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
CRBL			.54	3.8×10^{-11}			.30	5.2×10^{-4}	.60	7.2×10^{-14}						
FCTX					-.49	1.6×10^{-9}			.44	1.1×10^{-7}						
HIPP			.34	4.9×10^{-5}					.47	8.2×10^{-9}						
MEDU	.32	2.0×10^{-4}	.51	4.7×10^{-10}			.35	3.6×10^{-5}	.42	8.2×10^{-7}						
OCTX			.58	3.3×10^{-13}	-.52	1.2×10^{-10}	-.40	1.3×10^{-6}	.63	4.0×10^{-16}						
PUTM			.48	3.6×10^{-9}			.37	1.2×10^{-5}	.60	1.1×10^{-14}	.31	2.2×10^{-4}	.29	6.2×10^{-4}		
SNIG	.44	2.1×10^{-7}	.71	4.0×10^{-21}			.73	2.2×10^{-23}	.81	2.9×10^{-32}			.70	1.2×10^{-20}	.70	7.5×10^{-21}
TCTX	.47	9.5×10^{-9}	.56	3.4×10^{-12}	-.55	5.3×10^{-12}			.64	1.0×10^{-16}			.32	1.9×10^{-4}	.33	1.3×10^{-4}
THAL	.39	2.7×10^{-6}	.46	2.2×10^{-8}			.61	5.3×10^{-15}	.54	2.9×10^{-11}			.49	3.1×10^{-9}		
WHMT			.47	1.5×10^{-8}			.39	2.9×10^{-6}	.33	1.1×10^{-4}						

The abbreviations of brain regions refer to Table 4. r, correlation coefficient; $\alpha = 6.3 \times 10^{-4} = 0.05 / (10 \text{ brain tissues} \times 8 \text{ genes})$; any correlation with $p > \alpha$ is not listed in this table.

Table 6

Enrichment analysis for the risk genes using IPA

Pathway/Phenotype/Network	p-value/score
<i>Ingenuity Canonical Pathways</i>	
LXR/RXR Activation	1.50×10^{-5}
FXR/RXR Activation	1.72×10^{-5}
Atherosclerosis Signaling	1.74×10^{-5}
IL-12 Signaling and Production in Macrophages	2.64×10^{-5}
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	6.07×10^{-5}
<i>Diseases or Functions Annotation</i>	
late-onset Alzheimer's disease	1.92×10^{-10}
efflux of phospholipid	1.75×10^{-7}
Alzheimer's disease	2.20×10^{-7}
familial amyloidosis	4.48×10^{-7}
lattice corneal dystrophy type I	4.84×10^{-7}
<i>Networks</i>	
Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	score: 23

Table 7

The LncRNAs or snRNAs proximate to the replicated risk genes for AD

LncRNA name (NCBI Gene)	Alias	Length (nt)	Distance to risk gene	Category
XR_948110.2	LOC105369536	13,932	Overlap with exons 1&2 of <i>SORL1</i>	antisense LncRNA
XP_016874140.1	LOC105369535	11,887	Covered by <i>SORL1</i>	antisense LncRNA
XR_944153.1	LOC105370627	15,237	2,931bp to TSS of <i>SLC24A4</i>	sense LincRNA
XR_001753982.1	LOC107985327	31,013	148bp to 3' of <i>CD33</i>	antisense LncRNA
XR_923311.2	LOC105373605	29,009	99bp to 3' of <i>BIN1</i>	antisense LncRNA
NC_000011.10	RNU6-560P	108	83,453bp to TSS of <i>PICALM</i>	snRNA

Sense, LncRNAs are transcribed from the same genomic strand as the protein-coding mRNAs; Antisense, LncRNAs are transcribed from the antisense strand.