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Citation
Nho, K., S. Kim, E. Horgusluoglu, S. L. Risacher, L. Shen, D. Kim, S. Lee, et al. 2017. “Association analysis of rare variants near the APOE region with CSF and neuroimaging biomarkers of Alzheimer’s disease.” BMC Medical Genomics 10 (Suppl 1): 29. doi:10.1186/s12920-017-0267-0. http://dx.doi.org/10.1186/s12920-017-0267-0.

Published Version
doi:10.1186/s12920-017-0267-0

Citable link
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Association analysis of rare variants near the APOE region with CSF and neuroimaging biomarkers of Alzheimer’s disease

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From The 6th Translational Bioinformatics Conference
Je Ju Island, Korea. 15-17 October 2016

Abstract

Background: The APOE ε4 allele is the most significant common genetic risk factor for late-onset Alzheimer’s disease (LOAD). The region surrounding APOE on chromosome 19 has also shown consistent association with LOAD. However, no common variants in the region remain significant after adjusting for APOE genotype. We report a rare variant association analysis of genes in the vicinity of APOE with cerebrospinal fluid (CSF) and neuroimaging biomarkers of LOAD.

Methods: Whole genome sequencing (WGS) was performed on 817 blood DNA samples from the Alzheimer’s Disease Neuroimaging Initiative (ADNI). Sequence data from 757 non-Hispanic Caucasian participants was used in the present analysis. We extracted all rare variants (MAF (minor allele frequency) < 0.05) within a 312 kb window in APOE’s vicinity encompassing 12 genes. We assessed CSF and neuroimaging (MRI and PET) biomarkers as LOAD-related quantitative endophenotypes. Gene-based analyses of rare variants were performed using the optimal Sequence Kernel Association Test (SKAT-O).

Results: A total of 3,334 rare variants (MAF < 0.05) were found within the APOE region. Among them, 72 rare non-synonymous variants were observed. Eight genes spanning the APOE region were significantly associated with CSF Aβ1-42 (p < 1.0 × 10^{-3}). After controlling for APOE genotype and adjusting for multiple comparisons, 4 genes (CBLC, BCAM, APOE, and RELB) remained significant. Whole-brain surface-based analysis identified highly significant clusters associated with rare variants of CBLC in the temporal lobe region including the entorhinal cortex, as well as frontal lobe regions. Whole-brain voxel-wise analysis of amyloid PET identified significant clusters in the bilateral frontal and parietal lobes showing associations of rare variants of RELB with cortical amyloid burden.

(Continued on next page)
Conclusions: Rare variants within genes spanning the APOE region are significantly associated with LOAD-related CSF Aβ1-42 and neuroimaging biomarkers after adjusting for APOE genotype. These findings warrant further investigation and illustrate the role of next generation sequencing and quantitative endophenotypes in assessing rare variants which may help explain missing heritability in AD and other complex diseases.

Keywords: Whole genome sequencing, Rare variants, Near APOE, ADNI, CSF, Neuroimaging

Background
The number of individuals with late-onset Alzheimer’s disease (LOAD) is rapidly increasing and predicted to triple by 2050 with the increasing population of aging adults [1]. The heritability of LOAD was predicted to be up to 80% based on twin studies [2] and large-scale genome-wide association studies (GWAS) have recently led to the identification and confirmation of approximately 22 LOAD-associated genes including APOE (Apolipoprotein E), the best established and most significant susceptibility gene for LOAD [3]. The association of APOE with LOAD has been replicated and validated in many studies from different populations [4]. The APOE ε4 allele increases an individual’s risk for developing LOAD and also reduces age-at-onset in patients with LOAD in a dose-dependent manner, while the APOE ε2 allele appears to reduce the risk for LOAD [5]. Furthermore, GWAS studies have repeatedly identified several susceptibility loci for LOAD near the 19q13 on the chromosome 19 including APOE and TOMM40 (translocase of outer mitochondrial membrane 40 homolog) [3, 6]. In particular, TOMM40 has the second most significant SNP (single nucleotide polymorphism) associated with LOAD and multiple LOAD-related neuroimaging phenotypes in the 19q13 region [7–9]. However, conditional analyses strongly suggested that this effect is due to APOE [10, 11]. As APOE and TOMM40 are in strong linkage disequilibrium (LD), it is not easy to attribute an APOE-independent role of TOMM40 in the risk of LOAD development, although TOMM40 is essential for protein trafficking into mitochondria and mitochondrial dysfunction has been widely implicated in LOAD pathophysiology. Several groups investigated the association between a variable length poly-T polymorphism (poly-T) at rs10524523 within TOMM40 and LOAD, and yielded contrasting results [12–16]. Recently, Jun et al. comprehensively evaluated the association of risk and age at onset of LOAD with common SNPs (MAF (minor allele frequency) > 5%) and poly-T repeat in the APOE region using approximately 23,000 cases and controls, and found no significant independent association after adjusting for APOE genotype [16]. Highly significant results, after adjusting for APOE genotype, are unlikely in view of the very strong LD in this region.

Up to 50% of LOAD heritability remain unexplained by all of the known LOAD susceptibility genes including APOE and a substantial missing heritability for LOAD remains to be identified [17]. The advent of high throughput next generation sequencing such as whole genome sequencing (WGS) to identify variation in human genes has created unprecedented opportunities to discover genetic factors that influence disease risk in the field of human genetics [18, 19]. Several recent reports show that deep re-sequencing of GWAS-implicated loci and WGS-based association studies can identify independent functional rare variants with large effects on diseases including LOAD pathogenesis [20–22].

Two neuropathological hallmarks of the AD brain are extracellular amyloid-β plaques and intracellular neurofibrillary tangles. Studies have shown decreased concentrations of the CSF Aβ1–42 peptide and increased concentrations of total tau (t-tau) and hyperphosphorylated tau (p-tau) in AD compared with cognitively normal elders [23, 24]. Here we performed a gene-based association analysis of rare variants within genes in the vicinity of APOE with cerebrospinal fluid (CSF) and LOAD-related neuroimaging markers using a WGS data set (N = 757) from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) cohort. Our results strongly suggest rare variants in the region surrounding APOE on chromosome 19 were significantly associated with LOAD-related CSF Aβ1-42 and neuroimaging biomarkers.

Methods
Study participants
All individuals included in this study were participants of the longitudinal Alzheimer’s Disease Neuroimaging Initiative (ADNI) initiated in 2004, especially its subsequent extensions (ADNI-GO/2). Information about ADNI has been published previously and can be found at http://www.adni-info.org [25, 26]. All data were downloaded from the ADNI data repository (http://www.loni.usc.edu/ADNI/). All participants provided written informed consent at the time of enrollment for imaging and genetic sample collection and study protocols were approved by each participating sites’ Institutional Review Board (IRB).
For the control for population substructure, we restricted our analyses to participants with non-Hispanic Caucasian ancestry determined by using HapMap 3 genotype data and the multidimensional scaling (MDS) analysis (http://www.hapmap.org) [18, 19, 27]. Participants aged 55–90 to be used in this analysis include 259 cognitively normal older individuals (CN), 219 individuals with early mild cognitive impairment (MCI), 232 cognitively normal older individuals (CN), 219 individuals with late MCI, and 47 individuals diagnosed with late MCI, and 232 individuals with early mild cognitive impairment (MCI), 232 individuals diagnosed with late MCI, and 47 individuals diagnosed with AD.

**Whole genome sequencing (WGS) analysis**

WGS data from 817 ADNI participants were downloaded from the ADNI data repository (http://www.loni.usc.edu/ADNI/). An established next generation sequencing analysis pipeline based on GATK previously described was used to process ADNI WGS data performed on blood-derived genomic DNA samples and sequenced on the Illumina HiSeq2000 using paired-end read chemistry and read lengths of 100 bp at 30-40X coverage (http://www.illumina.com) [28]. We extracted all variants (SNPs and short indels) within a 312 kb region in APOE's vicinity including 12 genes.

**Neuroimaging analysis**

T1-weighted brain MRI scans were processed using previously described automated MRI analysis techniques [29], whole-brain voxel-based morphometry (VBM) and FreeSurfer software [30, 31]. [18F]Florbetapir PET scans were pre-processed as described [30] and intensity normalized by the whole cerebellum. These normalizations yielded standardized uptake value ratio (SUVR) images [32].

**Statistical analysis**

The SKAT-O software was used to perform a gene-based association analysis of all WGS-identified rare SNPs and short indels (MAF < 0.05) in the APOE cluster region [33]. We performed an association analysis first using only all SNPs and second using all SNPs plus short indels. Baseline CSF measurements (Amyloid-β 1–42 peptide (Aβ1–42), total tau (t-tau), and tau phosphorylated at the threonine 181 (p-tau181p)) were downloaded [34]. GWAS of CSF biomarkers found that several SNPs in TOMM40 and APOE are significantly associated with Aβ1–42 [34]. Thus, for the CSF analysis, we used CSF Aβ1–42 as a quantitative phenotype and age, gender, and APOE genotype as covariates. For the neuroimaging analysis, age, gender, year of education, MRI field strength, total intracranial volume (ICV), and APOE genotype were as covariates. We considered associations with \( p < 0.0042 \) (=0.05/12) to be significant in order to control for multiple comparisons.

### Results

#### Sequencing of chromosome 19q13 region

Within a 312 kb window in APOE's vicinity spanning 12 genes, we found 683 common variants (618 SNPs and 65 indels) and 3,334 rare variants (3,040 SNPs and 294 indels) (Table 1). Among 4,017 variants, there are 147 exonic and 2,159 intronic variants. Of 147 exonic variants, we found 1 frameshift and 3 nonframeshift indels, 72 nonsynonymous and 51 synonymous SNPs, and 20 unknown variants.

#### Association of rare variants near the APOE region with CSF Aβ1–42

Gene-based association analysis of rare SNPs near the APOE region identified three genes (TOMM40, APOE, and APOC1) that achieved a genome-wide significant association with CSF Aβ1–42 (\( p < 5 \times 10^{-7} \)) (Table 2) and the most significant association was between APOC1 and CSF Aβ1–42. After controlling for APOE genotype and adjusting for multiple comparisons based on a Bonferroni threshold (\( p < 0.05/12 = 0.0042 \)), 4 genes (CBLC, BCAM, APOE, and RELB) remain significant. The strongest significant association was observed at the BCAM gene (\( p = 0.0006 \)). There were about 10% short indels of all rare variants near the APOE region. The results of gene-based association of both rare SNPs and short indels near the APOE region with CSF Aβ1–42 were almost same as the association results of only rare SNPs (Table 2).

#### Association of rare variants near the APOE region with neuroimaging (MRI, PET)

To examine the LOAD-related neuroimaging biomarker association of all rare variants in 3 genes (CBLC, BCAM,  

| Table 1 | Number of common and rare variants (SNPs and Indels) of 12 genes near the APOE region |
|---------|---------------------------------|---------------------------------|---------------------------------|
| Gene    | Common variant (MAF ≥ 0.05)     | Rare variant (MAF < 0.05)       |                                 |
|         | SNP    | Indel | SNP    | Indel  |
|---------|--------|-------|--------|-------|
| BCL3    | 67     | 9     | 408    | 42    |
| CBLC    | 28     | 5     | 361    | 52    |
| BCMN    | 41     | 2     | 327    | 25    |
| PVR12   | 190    | 29    | 513    | 65    |
| TOMM40  | 32     | 2     | 154    | 11    |
| APOE    | 13     | 1     | 51     | 3     |
| APOC1   | 23     | 2     | 102    | 7     |
| APOC1P1 | 21     | 3     | 113    | 9     |
| APOC4   | 19     | 4     | 90     | 13    |
| APOC2   | 27     | 2     | 61     | 3     |
| CLIP1M1 | 105    | 7     | 456    | 34    |
| RELB    | 52     | 7     | 404    | 30    |
| Total   | 618    | 65    | 3,040  | 294   |
and RELB) significantly associated with CSF Aβ1-42 after adjusting for APOE genotype, a detailed whole-brain multivariate analysis of cortical thickness (MRI) and amyloid-β burden ([18F]-florbetapir PET) was performed to detect brain regions of associations of a single polygenic risk score. A single polygenic risk score was determined by collapsing all rare variants and counting minor alleles with a dominant genetic model. Figure 1 displays the results of the main effect of all rare variants after adjusting for APOE genotype in a surface-based cortical thickness whole brain analysis. Highly significant clusters associated with the risk score were found in temporal lobes including the entorhinal cortex, where AD pathology primarily begins, frontal lobe regions for

| Gene       | SNP + Indel p-value | p-value adjusted for APOE genotype | SNP p-value | p-value adjusted for APOE genotype |
|------------|---------------------|------------------------------------|------------|------------------------------------|
| BCL3       | 8.38E-04            | 0.0056                             | 7.60E-04   | 0.0054                             |
| CBLC       | 7.07E-05            | 0.0011                             | 7.35E-05   | 0.0013                             |
| BCAM       | 1.97E-04            | 0.0005                             | 2.49E-04   | 0.0006                             |
| PVRL2      | 3.55E-05            | 0.3842                             | 4.40E-05   | 0.4605                             |
| TOMM40     | 6.84E-07            | 0.0922                             | 5.01E-07   | 0.0880                             |
| APOE       | 3.35E-10            | 0.0039                             | 4.08E-07   | 0.0036                             |
| APOC1      | 6.18E-11            | 0.2394                             | 2.85E-11   | 0.1636                             |
| APOC1P1    | 4.43E-02            | 0.0145                             | 6.16E-02   | 0.0097                             |
| APOC4      | 2.11E-02            | 0.0262                             | 1.60E-02   | 0.1642                             |
| APOC2      | 2.23E-01            | 0.5363                             | 1.64E-01   | 0.5102                             |
| CLPTM1     | 1.02E-02            | 0.0438                             | 9.12E-03   | 0.0377                             |
| RELB       | 2.36E-04            | 0.0053                             | 1.51E-04   | 0.0042                             |

**Table 2** Gene-based association results (p-values) of rare variants (MAF < 5%; SNPs and Indels) of genes near the APOE region with CSF biomarker Aβ1-42 with and without adjusting for APOE genotypes

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**Fig. 1** Surface-based whole-brain analysis results. A whole-brain multivariate analysis of cortical thickness was performed on a vertex-by-vertex basis to visualize the topography of genetic association in an unbiased manner. Statistical maps were thresholded using a random field theory adjustment to a corrected significance level of \( p = 0.05 \). a CBLC. b RELB. c BCAM. d CBLC + RELB + BCAM
CBLC, and temporal lobe regions for BCAM and RELB, where subjects having high risk scores showed thinner mean cortical thickness compared with the participants having lower risk scores. A polygenic risk score of all rare variants in 3 genes (CBLC, BCAM, RELB) was associated with multifocal brain atrophy, predominantly in the temporal and bilateral frontal lobes (Fig. 1d). Fig. 2 shows the association of all rare variants in RELB with cortical amyloid burden from voxel-wise analysis of the effect of rare variants on amyloid accumulation measured by [18F]-florbetapir PET at a voxel-wise threshold of \( p < 0.005 \) (uncorrected). The color scale indicates regions where the risk scores were associated with higher amyloid burden after adjusting for APOE genotype. The significant clusters were observed in the bilateral frontal and parietal lobes.

Association of common SNPs near the APOE region with CSF A\( \beta \)_{1-42}

The association analysis of common SNPs near the APOE region was performed using PLINK set-based tests and permutation while considering the linkage disequilibrium structure of SNPs and identified one significant gene (BCL3) passed a Bonferroni threshold after adjusting for APOE genotype \( (p = 0.0005; \text{Table 3}) \). The association results remain almost unchanged when both common SNPs and short indels were used.

Discussion and Conclusions

We show for the first time to our knowledge that rare variants within genes near the APOE region are significantly associated with a LOAD biomarker CSF A\( \beta \)_{1-42} after adjusting for APOE genotype. Our results indicated that four genes (CBLC, BCAM, APOE, and RELB) remained significant after correcting for multiple comparisons. In addition, gene-based association analysis of common variants identified one significant gene BCL3. Whole-brain surface-based analysis identified highly significant clusters associated with rare variants of CBLC in temporal lobe regions including the entorhinal cortex and frontal lobe regions.

BCL3 (B-cell CLL/lymphoma 3) gene functions as a transcriptional co-activator involved in cell replication and apoptosis that activates through its association with NF-\( \kappa \)B homodimers [35]. BCL3 gene is associated with genetic linkage with late-onset Familial Alzheimer’s disease as well as chronic lymphocytic leukemia [36–38]. RELB (RELB proto-oncogene, NF-\( \kappa \)B subunit) gene is a member of NF-\( \kappa \)B family of transcriptional factors. Among its related pathways are immune system and interleukin-3, 5 and GM-CSF signaling. NF-\( \kappa \)B plays a central role in the inflammatory and immune responses and controls cell proliferation and protects the cell from apoptosis [39]. NF-\( \kappa \)B is a major transcription factor and activated in AD patients. Amyloid beta accumulation is a potential activator of NF-\( \kappa \)B in primary neurons [40]. CBLC (Cbl proto-oncogene C, E3 ubiquitin protein ligase) gene is the member of the Cbl family of E3 ubiquitin ligases. Cbl proteins play an important role in cell signaling through the ubiquitination and subsequent downregulation of the tyrosine kinases. BCAM (basal cell adhesion molecule) gene encodes a glycoprotein expressed on cell surfaces [41]. BCAM is a member of the immunoglobulin superfamily and a receptor for the extracellular matrix protein, laminin \( \alpha \)-5. BCAM may play a role in intracellular signaling. BCAM is related to the Lutheran glycoprotein, which is a specific marker of brain capillary endothelium, which forms the blood brain barrier (BBB) in vivo [42, 43].

ADNI is a unique cohort and the only large WGS data set of LOAD with CSF A\( \beta \)_{1-42} and neuroimaging data also available. However, a limitation of the present report is that we used a modest sample size \( (n = 757) \) of whole genome sequencing data for genetic analysis. Therefore, validation in independent and larger cohorts is warranted.

In conclusion, we used whole genome sequencing to perform an association analysis of rare variants within genes near the APOE region with CSF A\( \beta \)_{1-42} and
neuroimaging biomarkers of LOAD. Importantly, our results implicate this region or these genes contain additional explanatory information with regard to LOAD endophenotypes above and beyond that conferred by APOE genotype. Overall, combining whole genome sequencing and LOAD-related quantitative endophenotypes adds to the growing understanding of the genetics of LOAD and holds promise for discovery of rare variants involved in neurodegeneration and other brain disorders, further nominating novel potential diagnostic and therapeutic targets.

Acknowledgements
Data used in preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf. Data collection and sharing for this project was funded by the Alzheimer’s Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer’s Association; Alzheimer’s Drug Discovery Foundation; BioClinica, Inc.; Biogen Idec Inc; Bristol-Myers Squibb Company; Eisai Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, NV; JAVCO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (http://www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer’s Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. Samples from the National Cell Repository for AD (NCRAD), which receives government support under a cooperative agreement grant (U44 AG21886) awarded by the National Institute on Aging (NIA), were used in this study. Funding for the WGS was provided by the Alzheimer’s Association and the Brin Wojcicki Foundation.

Funding
Additional support for data analysis was provided by NLM R00 LM011384, NIA R01 AG19771, NIA P30 AG10133, NLM R01 LM011360, NSF IIS-117335, DOD W81XWH-14-2-0151, NCCAI 14132004, NIGMS P50GM115318, and NCATS UL1 TR001108. Publication of this article has been funded by NLM R00 LM011384.

Availability of data and materials
Demographic information, raw neuroimaging scan data, APOE and whole genome sequencing data, neuropsychological test scores, and diagnostic information are available from the ADNI data repository (http://www.loni.usc.edu/ADNI/).

Authors’ contributions
All authors contributed substantively to this work. KN, EH, SK, SLR, LS, and AJS were involved in study conception and design. KN, EH, DK, SL, and AJS were involved in data organization, whole genome sequencing analysis and statistical analyses. TF, PSA, RCP, RCG, CRJ, LMS, JQT, RCG, AWT, MMW and AJS were involved in coordination and data collection and processing for ADNI. KN and AJS drafted the report and prepared all figures and tables. All authors were involved in reviewing and editing of the manuscript and approved it.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
Written informed consent was obtained at the time of enrollment for imaging and genetic sample collection and protocols of consent forms were approved by each participating site’s Institutional Review Board (IRB).

About this supplement
This article has been published as part of BMC Medical Genomics Volume 10 Supplement 1, 2017: Selected articles from the 6th Translational Bioinformatics Conference (TBC 2016): medical genomics. The full contents of the supplement are available online at https://bmcmedgenomics.biomedcentral.com/articles/supplements/volume-10-supplement-1.

Table 3 Gene-based association results (p-values) of common variants (MAF ≥ 5%; SNPs and Indels) of genes near the APOE region with CSF biomarker Aβ1-42 with and without adjusting for APOE genotypes

| Gene  | SNP + Indel | p-value         | p-value adjusted for APOE genotype | p-value | p-value adjusted for APOE genotype |
|-------|-------------|-----------------|------------------------------------|---------|------------------------------------|
| BCL3  |             | 0.0013          | 0.0005                             |         | 0.0019                             |
| CBLC  |             | 0.0006          | 0.0122                             |         | 0.0005                             |
| BCAM  |             | 0.0014          | 0.0132                             |         | 0.0016                             |
| PVRIL2|             | <1.0E-05        | 0.6852                             | <1.0E-05| 0.6665                             |
| TOMM40|             | <1.0E-05        | 1.0000                             | <1.0E-05| 1.0000                             |
| APOE  |             | <1.0E-05        | 0.1380                             | <1.0E-05| 1.0000                             |
| APOC1 |             | <1.0E-05        | 1.0000                             | <1.0E-05| 1.0000                             |
| APOC1P1|            | <1.0E-05        | 1.0000                             | <1.0E-05| 1.0000                             |
| APOC4 |             | 0.1718          | 1.0000                             | 0.1437  | 1.0000                             |
| APOC2 |             | 0.0406          | 0.0621                             | 0.0152  | 0.0570                             |
| CLPTM1|             | 0.0198          | 0.0467                             | 0.0331  | 0.0464                             |
| RELB  |             | 0.0515          | 0.6551                             | 0.2485  | 0.6905                             |
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Published: 24 May 2017

References
1. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol. 2003;60(8):1119–22.
2. Bergem AL, Engedal K, Kringlen E. The role of heredity in late-onset Alzheimer disease neuroimaging: A study of the ADNI cohort. NeuroImage. 2010;53(3):1051–63.
3. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al. Genetic variation in Alzheimer disease. Arch Neurol. 2010;67(5):536–46.
4. Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, Arrighi HM. Atypical inheritance of the Alzheimer disease susceptibility APOE gene. JAMA. 2005;293(19):2395–401.
5. Roses AD. An inherited variable poly-T repeat genotype in TOMM40 in Alzheimer disease. Pharmacogenomics. 2011;10(5):575–84.
6. Johnson SC, La Rue A, Hermann BP, Xu G, Kosik KL, Jonatlis EM, Bendlin BB, Hogan KJ, Roses AD, Saunders AM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011;7(4):456–65.
7. Cruchaga C, Nowotny P, Kauwe JS, Risacher SL, Shen L, Swaminathan S, Ramanan VK, Liu Y, Foroud T, et al. Identification of functional variants from whole-exome sequencing, combined with neuroimaging genetics. Mol Psychiatry. 2013;18(7):739.
8. Nho K, Corneveaux JJ, Kim S, Lin H, Risacher SL, Shen L, Swaminathan S, Ramanan VK, Liu Y, Foroud T, et al. Whole-exome sequencing and imaging genetics identify functional variants for rate of change in hippocampal volume in mild cognitive impairment. Mol Psychiatry. 2013;18(7):781–7.
9. Guerreiro RJ, Hardy J. TREM2 and neurodegenerative disease. N Engl J Med. 2013;369(16):1569–70.
10. Johnson SC, La Rue A, Hermann BP, Xu G, Kosik KL, Jonatlis EM, Bendlin BB, Hogan KJ, Roses AD, Saunders AM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011;7(4):456–65.
11. Cruchaga C, Nowotny P, Kauwe JS, Ridge PG, Mayk O, Bertelsen S, Hinrichs BB, Hogan KJ, Roses AD, Saunders AM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011;7(4):456–65.
12. Roses AD, Lutz MW, Amrine-Madsen H, Saunders AM, Crenshaw DG, Sundseth SS, Huentelman MJ, Welsh-Bohmer KA, Reiman EM. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. Pharmacogenomics. 2010;11(3):275–84.
13. Johnson SC, La Rue A, Hermann BP, Xu G, Kosik KL, Jonatlis EM, Bendlin BB, Hogan KJ, Roses AD, Saunders AM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011;7(4):456–65.
14. Johnson SC, La Rue A, Hermann BP, Xu G, Kosik KL, Jonatlis EM, Bendlin BB, Hogan KJ, Roses AD, Saunders AM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011;7(4):456–65.
15. Cruchaga C, Nowotny P, Kauwe JS, Ridge PG, Mayk O, Bertelsen S, Hinrichs BB, Hogan KJ, Roses AD, Saunders AM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011;7(4):456–65.
16. Johnson SC, La Rue A, Hermann BP, Xu G, Kosik KL, Jonatlis EM, Bendlin BB, Hogan KJ, Roses AD, Saunders AM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. Alzheimers Dement. 2011;7(4):456–65.
33. Lee S, Emond MJ, Bamshad MJ, Barnes KC, Rieder MJ, Nickerson DA, Team NGESP-ELP, Christians DC, Wurfel MM, Lin X. Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. Am J Hum Genet. 2012;91(2):224–37.

34. Kim S, Swaminathan S, Shen L, Risacher SL, Nho K, Porcine T, Shaw LM, Trojanowski JQ, Potkin SG, Huentelman MJ, et al. Genome-wide association study of CSF biomarkers Abeta1-42, t-tau, and p-tau181p in the ADNI cohort. Neurology. 2011;76(1):69–79.

35. Porcellini E, Carbone L, Ianni M, Licastro F. Alzheimer’s disease gene signature says: beware of brain viral infections. Immun Ageing. 2010;7:16.

36. Pericak-Vance MA, Bebout JL, Gaskell Jr PC, Yamaoka LH, Hung WY, Alberts MJ, Walker AP, Bartlett RJ, Haynes CA, Welsh KA, et al. Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. Am J Hum Genet. 1991;48(6):1034–50.

37. St George-Hyslop PH, Ohno H, Gusella JF, McKeithan T. The BCL3 locus on chromosome 19 displays an informative microsatellite polymorphism. Nucleic Acids Res. 1992;20(4):927.

38. Miller LL, Ploughman LM, Marazita ML. Heterogeneity testing for Alzheimer’s disease within and between data sets. Genet Epidemiol. 1993;10(6):443–7.

39. Baud V, Collares D. Post-Translational Modifications of RelB NF-kappaB Subunit and Associated Functions. Cells. 2016;5(2):22.

40. Kaltschmidt B, Uherek M, Volk B, Baeuerle PA, Kaltschmidt C. Transcription factor NF-kappaB is activated in primary neurons by amyloid beta peptides and in neurons surrounding early plaques from patients with Alzheimer disease. Proc Natl Acad Sci U S A. 1997;94(6):2642–7.

41. Latini FR, Bastos AU, Amorini CP, Muniz JG, Person RM, Baleotti Jr W, Barreto JA, Castilho L, Cerutti JM, DARC (Duffy) and BCAM (Lutheran) reduced expression in thyroid cancer. Blood Cells Mol Dis. 2013;50(3):161–5.

42. Boado RJ, Li JY, Pardridge WM. Selective Lutheran glycoprotein gene expression at the blood-brain barrier in normal brain and in human brain tumors. J Cereb Blood Flow Metab. 2000;20(7):1096–102.

43. Provias J, Jeynes B. The role of the blood-brain barrier in the pathogenesis of senile plaques in Alzheimer’s disease. Int J Alzheimers Dis. 2014;2014:191863.