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Blood and Brain Transcriptome Analysis Reveals APOE Genotype-mediated and Immune-related Pathways Involved in Alzheimer Disease

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Abstract

Background: While Alzheimer disease (AD) is generally considered as a brain disorder, blood biomarkers may be useful for diagnosis and prediction of AD brain pathology. The APOE ε4 allele has shown cerebrovascular effects including acceleration of blood brain barrier breakdown.

Methods: We evaluated differential expression of previously established AD genes in brains from 344 pathologically confirmed AD cases and 232 controls and in blood from 112 pathologically confirmed AD cases and 67 controls from the Religious Orders Study and Memory and Aging Project. Differential gene expression between AD cases and controls was analyzed in the blood and brain jointly using a multivariate approach in the total sample and within APOE genotype groups. Gene set enrichment analysis was performed within APOE genotype groups using the results from the combined blood and brain analyses to identify biologically important pathways. Gene co-expression networks in brain and blood samples were investigated using weighted correlation network analysis. Top ranked genes from networks and pathways were further evaluated with vascular injury traits.

Results: We observed differentially expressed genes with P<0.05 in both brain and blood for established AD genes INPP5D (upregulated) and HLA-DQA1 (downregulated). PIGHP1 and FRAS1 were differentially expressed at the transcriptome-wide level (P<3.3x10^-6) within ε2/ε3 and ε3/ε4 groups, respectively. Gene-set enrichment analysis revealed 21 significant pathways (false discovery rate P<0.05) in at least one APOE genotype group. Ten pathways were significantly enriched in the ε3/ε4 group, and six of these were unique to these subjects. Four pathways were enriched for AD upregulated genes in the ε3/ε4 group and AD downregulated genes in ε4 lacking subjects. We identified a co-expressed gene network in brain that reproduced in blood and showed
higher average expression in ε4 carriers. Twenty-three genes from pathway and network analyses were significantly associated at \( P<0.05 \) with at least one vascular injury trait.

**Conclusion:** These results suggest that *APOE* genotype contributes to unique expression network profiles in both blood and brain. Several genes in these networks are associated with measures of vascular injury and potentially contribute to ε4’s effect on the blood brain barrier.

**Keywords:** Alzheimer’s disease, blood brain barrier, *APOE*, differential expression, co-expression network, vascular injury
Background

Alzheimer disease (AD) is a neurodegenerative disorder characterized by amyloid plaques and neurofibrillary tau tangles in the brain [1]. Because these hallmark proteins are sometimes detectable in blood before clinical symptoms appear, there are on-going efforts to identify blood-based signatures from multi-omics and biomarker data that can facilitate detection of AD preclinically [2,3].

Cerebrovascular AD-related pathology that may affect the blood brain barrier (BBB), such as cerebral amyloid angiopathy (CAA) has been shown to exacerbate neurodegeneration and neuroinflammation [4]. Dysfunction of the BBB, a semi-permeable border separating the extracellular fluid and brain tissue from circulating blood, has been implicated in the accumulation of amyloid-β (Aβ) and hyperphosphorylation of tau protein [5,6]. Apolipoprotein E (APOE) genotype is the strongest genetic risk factor for late onset AD and the ε4 allele has been recently associated with BBB dysfunction leading to cognitive decline [7,8]. Heterozygosity of the APOE ε4 allele confers 3-4 fold increase of AD risk and ε4 homozygotes have a 10-12 fold increased likelihood of a clinical diagnosis of AD among persons of European ancestry [7,9]. By contrast, among clinically and neuropathologically confirmed AD cases and controls of European ancestry, a single copy of the APOE ε2 allele is associated with 61% decreased risk and ε2 homozygotes have an 87% reduced risk for AD compared to individuals with the ε3/ε3 genotype [10]. Cerebrovascular AD-related pathologies have also shown APOE genotype-dependent patterns. Both ε2 and ε4 are significantly associated with CAA [11].

Previous whole transcriptome-wide studies from autopsied brains demonstrate that the classical complement cascade and tau phosphorylation are linked to AD in an APOE genotype-specific manner [12,13]. However, expression profiles associated with AD has not been intensively
investigated in blood and brain from the same individuals, especially separated by APOE genotype. Here, we analyzed gene expression measured in blood and brain tissue obtained from participants of the Religious Orders Study and Rush Memory and Aging Project (ROSMAP) [14] stratified by APOE genotype in order to discern AD-related differential gene expression, biological pathways, and gene networks shared in blood and brain.

Methods

Sources of Blood and Brain Transcriptomic and Phenotypic Data

RNA sequencing (RNA-seq) data generated from blood donated by 614 ROSMAP participants and phenotypic data collected from those subjects were obtained from the Synapse portal [15]. RNA batches were prepared using a SMART-seq2 protocol (batches 1-2) or a SMART-seq2 like protocol (batch 3). Batch 1 (2 x 101bp) and Batch 2 (2 x 76bp) were pooled and sequenced by HiSeq 2500 (Illumina). Batch 3 (2 x 50 bp) was pooled and sequenced on Nova Seq 6000 (Illumina). A post-mortem diagnosis of AD was established for 112 participants using NIA-Reagan criteria including Braak staging for assessing the severity of neurofibrillary tangles and the Consortium to Establish a Registry for Alzheimer Disease (CERAD) semi-quantitative measure for neuritic plaques (CERAD Score). Another 67 participants who were clinically normal showed no pathological evidence of AD and were included in this study as controls (Supplementary Table 1) [14,16]. Age, sex, sequencing batch, and library batch information was available for all subjects. Publicly available prefrontal cortex brain RNA-seq and neuropathological data for 639 ROSMAP participants were obtained from the Synapse portal [15]. Sequencing libraries were prepared using the strand-specific dUTP method with poly-A selection, and all samples were sequenced using an Illumina HiSeq instrument. Of these 639 samples, data
from 576 samples with both RNA integrity number (RIN) and post-mortem interval (PMI) were included in subsequent differential expression analyses (Supplementary Table 1). Previously reported RNA-seq data were also available which were derived from the frontal cortex tissue region of 208 frontal autopsied brains (64 AD and 129 controls) donated to the Framingham Heart Study and Boston University Alzheimer’s Disease Center (FHS/ADRC) [12]. A diagnosis of AD in these brains was established using NIA-Regan criteria including Braak staging and CERAD score [12].

Quality Control, Mapping, and Quantification of Gene Expression Data and Sample

The 614 FASTQ files derived from blood RNA-seq data were processed in batches. Quality control (QC) of the sequence data was performed using FastQC which checked for over-abundance of adaptors and over-represented sequences [17]. Reads passing initial QC were aligned to the human reference genome (GRCh38.95) using STAR (version 2.6.1c), which implements 2-pass mapping to increase the chances of mapping splice reads from novel junctions [18,19]. To account for differences in read-lengths between batches, we created three genomic alignment index files with read lengths of 50bp, 76bp, and 101bp, respectively, for mapping the study samples to the reference genome. The 639 binary alignment map (BAM) files containing brain RNA-seq data required additional processing before alignment and thus were converted to FASTQ files using the FastqTosam function in Picard tools [20]. Samples were checked for adaptor overabundance and overrepresented sequences using FastQC [17]. Paired-end reads were aligned to the human reference genome as described above. In order to map brain samples to the reference genome, genomic index files (read length = 101bp) were created. The resulting BAM files for each brain and blood sample contained mapped paired-end reads and a corresponding alignment report file.
Gene and isoform levels were quantified using RSEM (version 1.3.1) [21] and Bowtie2 (version 2.3.4.1) [22] and then annotated using Homo sapiens GRCh38.95.gtf annotation files. Files generated by this process for each sample contained several variables for each gene including gene id, gene length, effective gene length, expected count, counts per million (CPM), and fragments per kilobase of exon model per million reads mapped (FPKM) reads.

**Gene Expression Analysis in Blood and Brain**

**Differential Expression Analysis:** Genes with less than two reads on average among 80% or more of the samples were excluded from analyses. Blood and brain samples were corrected for between-sample variability using a trimmed mean of M-values normalization method [23]. Differential gene expression analysis between AD and control subjects in blood and brain was performed separately using the VOOM and LIMMA software [24,25]. For differential gene expression analysis in brain, the normalized expression of each gene was compared between AD cases and controls using linear regression models adjusting for sex, age at death, RNA integrity number (RIN), post-mortem interval (PMI), and sequencing batch as covariates. Gene expression analysis of the blood samples included only the 179 individuals who were neuropathologically examined and models included covariates for sex, age at exam, and library batch. Analyses were performed in the total sample and subgroups defined by APOE genotype (ε2/ε3, ε3/ε3, and ε3/ε4). Subjects with genotypes ε2/ε2, ε2/ε4, and ε4/ε4 were excluded from analyses due to small samples sizes (Supplementary Table 1). In blood, only samples containing a neuropathological diagnosis of AD were used. Analyses of gene expression in blood were further stratified by RNA batch due to differences in read-length and sample substructure (Supplementary Figure 1). For the ε3/ε4 subgroup data from batch 2 were only analyzed because the batch 1 sample size was too small (Supplementary Table...
Results from analyses of each batch and APOE genotype group were combined by meta-analysis weighting for the number of AD cases and accounting for effect direction using the METAL program [26].

To evaluate differential gene expression patterns in the joint blood and brain datasets, we combined univariate results from blood and brain using the R package CUMP which incorporates O’Brien’s method [27]. In this method, a combined z-score was calculated using t-value estimates derived from the LIMMA linear regression analyses and/or from z-score estimates from the meta-analysis of the blood batches. All analyses were weighted by the number of samples within APOE genotype groups or in the total sample.

**Single Cell Gene Expression Analysis:** A normalized single cell RNA-sequencing expression matrix from ~2,400 cells collected from the blood of healthy individuals and proportions of dendritic cells and monocytes for each sample was obtained from the Single Cell Portal [28]. FASTQ single nuclei RNA-sequencing data from the prefrontal cortex of 48 brains from ROSMAP participants (24 AD cases, 24 controls) were obtained from the Synapse portal [15] and processed as previously described [12]. The average expression for each cell-type in the blood and brain RNA-seq datasets was calculated for each gene.

**Gene Set Enrichment Analysis:** Differentially expressed genes in the total sample or within APOE genotype groups were ranked by a combined z-score from blood and brain using the O’Brien method. Gene set enrichment analysis was performed using this ranked list and gene set pathway information obtained from the Molecular Signatures Database (MSigDB) as previously described [28,29]. Pathway enrichment scores were determined based on the degree to which a set of genes was overrepresented by the largest positive and smallest negative z-scores. Genes that
contributed the most to the enrichment score of each pathway were designated as leading-edge genes.

**Co-Expressed Gene Network Analysis in Blood and Brain**

Co-expressed genes in networks were identified using 14,456 coding genes in brain and 11,379 coding genes in blood in the ROSMAP RNA-seq dataset using the weighted gene correlation network analysis (WGCNA) algorithm [30]. Analyses of data from blood included only 141 batch 2 samples with and without post-mortem examination to avoid batch effects, and analyses of data from brain comprised 636 samples excluding lack of RIN or batch information (Supplementary Table 2). We used gene expression levels calculated as log-transformed fragments per kilobase of transcript per million (FPKM). Soft-power parameters of 12.0 and 12.5 were selected for analyses of brain and blood data, respectively, as previously described [12]. Expression data were clustered hierarchically by implementing a dissimilatory topological overlap matrix (TOM). Initial modules with a minimal network size of 100 genes were identified and labeled using dynamic tree cutting. Eigengenes were derived from the first principle component for each module and served as representative values of gene expression in a given module [31]. Networks with high eigengene similarity and a height of 0 were merged using the mergeCloseModules function in WGCNA. Fuzzy module membership was assigned using the signedKME function.

Network modules identified in brain were examined for preservation in blood using the modulePreservation function in WGCNA. Brain networks with a Zsummary score > 5 were considered preserved in brain networks [32]. Relevance of the networks to AD pathology was established based on enrichment of AD-related genes that was determined using the userListEnrichment function in WGCNA. For the purpose of this analysis, we defined AD-related genes which included those within 20kb of single nucleotide polymorphisms (SNPs) showing at
least modest evidence (p<0.001) for association with AD risk [33] or AD-related neuropathological measures of Tau and Aβ proteins [34]. We used EnrichR to identify KEGG pathways enriched for AD related genes in the preserved networks [35]. Next, genes in networks contributing to significant pathways were further evaluated using Ingenuity Pathway Analysis software (QIAGEN Inc.) to identify biological subnetworks.

**Measurements and Association with Vascular Injury Related Proteins**

Intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and serum amyloid α (SAA) were detected and measured in fresh tissue lysate from the dorsolateral prefrontal cortex area using the Mesoscale Discovery V-PLEX Plus Vascular Injury Panel Kit (Mesoscale Discovery, K15198G, Rockville MD). Grey matter was separated from frozen brain tissue on dry ice and weighed. Ice-cold RIPA buffer (ThermoScientific, #89901) was added to the grey matter at 5mL RIPA: 1g brain wet weight, and homogenized with Qiagen Tissue Lyser LT at 50Hz for five minutes (Qiagen, Germany) (ThermoScientific, Waltham MA). The homogenate was centrifuged at 17,000g at 4°C for 15 minutes, then the supernatant was aliquoted and stored at -80°C until use. Buffers and immunoassay plates were prepared according to the manufactory instructions and the brain homogenate was further diluted 5-fold. The immunoassay plates were read using the multi-detection SPECTOR 6000 Imager to quantitate protein levels (Mesoscale Discovery).

Additional analyses of 107 top-ranked genes emerging from pathways identified by differential gene expression and network analyses were performed using log-transformed FPKM values obtained previously from these FHS/ADRC donor brains [12]. Levels of ICAM-1, VCAM-1, and SAA proteins were rank-transformed after adjusting for age and sex. We performed
association analyses using the expression levels of the selected genes with the levels of vascular injury related proteins as quantitative outcomes in linear regression models further adjusting for RIN.

**Results**

**Differentially Expressed Genes in Blood and Brain**

Gene expression levels in 179 blood and 576 brain samples from the ROSMAP dataset were compared between AD cases and controls (Figure 1). In the total sample, no genes in the combined data from blood and brain were differentially expressed at the transcriptome-wide significance level (P<3.3x10^-6). Of 78 genes associated with AD at a genome-wide significance level in a recent large genome-wide association study (GWAS) [36], 64 passed QC and were expressed in both brain and blood. Expression of five of these 64 genes (HLA-DQA1, INPP5D, SPDYE3, TSPOAP1, and SIGLEC11) were nominally significant (P<0.05) in the analysis of the combined blood and brain data (Table 1, Supplemental Table 3, Supplementary Figure 2). Differential expression of HLA-DQA1 and INPP5D was nominally significant at P<0.05 in both blood and brain with the same direction of effect. Differentially expressed genes (DEGs) after multiple testing correction at P<6.4x10^-4 were evident only in brain for BCKDK (P=5.1x10^-4), TSPOAP1 (P=2.6x10^-4) and SIGLEC11 (P=1.6x10^-4).

Two genes were differentially expressed between AD cases and controls at the transcriptome-wide level (P<3.3x10^-6) within a particular APOE genotype group. PIGHP1 was significantly upregulated in AD cases in the combined brain and blood samples in the ε2/ε3 group (Z=4.67, P=3.1x10^-6), a pattern predominated by the evidence in brain but also apparent in blood (Table 2, Supplementary Figure 2, Supplementary Figure 3a). Among ε3/ε4 subjects,
expression of \textit{FRASI} was significantly downregulated in AD cases in blood only (Z=-4.66, P=3.2x10^{-6}) (\textbf{Supplementary Figure 3b}). No transcriptome-wide significant DEGs were identified in brain from any \textit{APOE} genotype groups. Among genes previously associated with AD among ε2/ε3 subjects [12], \textit{C4A}, \textit{C4B}, and \textit{HSPA2} were moderately (P<10^{-3}) upregulated in brain but not blood from AD ROSMAP Study participants in the ε2/ε3 subgroup and total sample (\textbf{Supplemental Table 4}). Notably, \textit{C4B} expression trended in the opposite direction (i.e., downregulated in AD cases) in blood from ε2/ε3 subjects (P=0.08).

Examination of cell-level expression profiles of the DEGs in \textbf{Tables 1 and 2} revealed that in blood cell types \textit{HLA-DQA1} and \textit{INPP5D} were more highly expressed in dendritic cells and monocytes compared to other genes in this group (\textbf{Supplementary Figure 4a}). \textit{INPP5D} was the only gene in this group expressed in brain cell types and specifically in microglia (\textbf{Supplementary Figure 4b}).

\textbf{APOE Genotype Dependent Pathways in Combined Blood and Brain Expression Profiles}

We identified 21 pathways that were significantly enriched for upregulated or downregulated genes in the combined blood and brain expression levels in at least one \textit{APOE} genotype group (\textbf{Figure 2a} and \textbf{Supplementary Table 5}). Enrichment scores from significant pathways identified in the ε3/ε4 group were generally downregulated and had the opposite effect direction compared to those for the other \textit{APOE} genotype groups (\textbf{Figure 2a, Table 3}). Six pathways were significantly and uniquely enriched in the ε3/ε4 subgroup including apoptosis, estrogen response late, hypoxia, il6/jak/stat3 signaling, inflammatory response, and p53 pathway. Pathways for allograft rejection, interferon gamma response, peroxisome and TNFA signaling via NFKB were enriched for upregulated AD genes in the ε3/ε4 group but for downregulated AD genes in the ε2/ε3
and ε3/ε3 groups. There was little overlap of the leading-edge genes for these four pathways across APOE genotype groups with at most one overlapping gene per pathway (Table 3). Leading-edge genes in the same pathway shared by different APOE genotypes included HLA-DRA in allograft rejection, CD74 in interferon gamma response, and KYNU in TNFA signaling via NFKB. Among 92 leading-edge genes from the four pathways that had enrichment scores in opposite directions among subjects with and without ε4 (Table 3), expression of 21 genes in FHS/ADRC brains was significantly associated (P<4.67x10\(^{-4}\)) with at least one of the three vascular injury related proteins (Figure 2b, Table 4). Expression of 11 of these 21 genes (52%) was significantly associated with SAA level, and expression of 13 genes (62%) was significantly associated with VCAM-1 level. Expression of only two genes, TRIP10, and FOSL1, which are both involved in signaling via NFKB, was significantly associated with ICAM-1 levels.

Co-Expression Networks Common to Brain and Blood

Four co-expression networks identified in brain were preserved in blood (Supplementary Table 6). The eigengene value (i.e., first principle component of gene expression across the network) in the greenyellow network was significantly higher among ε4 carriers than non-carriers (P=4.7x10\(^{-3}\)) (Figure 3a). The greenyellow network is significantly enriched for genes previously associated with AD risk [33] and plaque score [34] (Supplementary Table 6). The AD-related genes in this network were significantly enriched in nine KEGG pathways (Figure 3b, Supplementary Table 7). Seventeen genes contributing to these significant pathways form a biological subnetwork (Figure 3c). One of these genes, NFKBIA, is a leading-edge gene from the signaling via NFKB pathway and was involved in five out of nine significant pathways in the greenyellow network (Table 3, Supplementary Table 7). HLA-DRA is involved in six of the nine significant pathways
in the greenyellow network and is a leading-edge gene in the *allograft rejection pathway* identified in the ε3/ε3 and ε3/ε4 groups. *INPP5D*, which is differentially expressed in both blood and brain (Table 1, Supplementary Figure 2), is involved in two significant KEGG pathways (*Fc gamma R-mediated phagocytosis* and *B cell receptor signaling*) in the greenyellow network. *C4B*, which is upregulated in brain from AD cases compared to controls in the ε2/ε3 group (Supplementary Table 4), was included in the greenyellow network pathways involved in *staphylococcus aureus infection* and *systemic lupus erythematosus*.

Two of the 17 sub-network genes in the significant pathways enriched for AD genes in the greenyellow network (Supplementary Table 8) were significantly associated with the level of at least one of the three vascular injury proteins after multiple testing correction (Figure 3d). Specifically, *VASP* expression was significantly associated with levels of ICAM-1 (P=3.7x10^-4) and SAA (P=1.0x10^-4), and *C4B* expression was significantly associated with levels of ICAM-1 (P=1.3x10^-5), SAA (P=1.6x10^-4) and VCAM-1 (P=7.0x10^-6).

**Discussion**

The primary purpose of this study was to identify genes previously associated with AD and in biological pathways enriched for AD genes whose expression differs between AD cases and controls in both blood and brain, especially in an *APOE* genotype-specific manner. We observed that two established AD genes, *INPP5D* and *HLA-DQA1*, were differentially expressed in both blood and brain. Among the 21 top-ranked pathways in the combined blood and brain expression profiles, 10 pathways were specific to persons having the *APOE* ε3/ε4 genotype. Additionally, we identified a co-expression network enriched for AD genes in brain that was preserved in blood and showed significantly higher average expression in ε4 carriers than non-carriers. Lastly, several genes from the top-ranked pathways and co-expression networks were significantly associated
with levels of vascular injury proteins. These findings suggest that AD genes that are differentially expressed in both blood and brain and associated with vascular markers may be involved in BBB function, and their effects are dependent on APOE genotypes.

The BBB is a semi-permeable endothelial cell membrane regulating transport between cerebral blood vessels and the central nervous system [37]. The dysregulation of the BBB has been implicated in early cognitive decline and exacerbation of neuroinflammation and neurodegeneration [38]. A recent study showed that APOE ε4 carriers exhibit BBB dysfunction and cognitive decline independent of AD pathology [8]. Our analyses identified six pathways uniquely enriched for DEGs among ε3/ε4 carriers in combined blood and brain expression data. Expression of INPP5D and HLA-DQA-1 was significantly greater in both blood and brain from individuals with AD compared to controls. Increased expression of INPP5D in blood has been previously linked with increased risk of hemorrhagic transformation [39], which is associated also with BBB permeability [40]. INPP5D is highly expressed in microglia and encodes the protein SHIP1 which has been implicated in many neuroinflammatory processes [41]. Additionally, HLA-DQA1 and INPP5D are expressed in dendritic cells and monocytes and involved in immune processes, and the migration of monocytes across an inflamed BBB can cause differentiation into dendritic cells [42]. FRAS1 was significantly downregulated in AD compared to controls in blood from ε3/ε4 AD individuals and a recent study showed that FRAS1 knockdown mice were impaired in memory and learning behaviors [43].

We identified four pathways (allograft rejection, interferon gamma response, peroxisome, and TNFA signaling via NFKB) containing gene sets that, with respect to AD, were significantly upregulated in blood and brain from ε4 carriers and other gene sets from the same pathways that were downregulated in individuals without ε4. The inflammatory cytokine interferon gamma has
been shown to impact directly brain endothelium to cause BBB breakdown [44] and can inhibit ApoE production in macrophages [45]. Peroxisomes synthesize fatty acids which have been implicated in the development of AD [46]. The TNFA via NFKB signaling pathway has been implicated in BBB dysfunction [47], and the TNFA and NFKB pathways have been independently associated with increased neuroinflammation related to APOE ε4 [48,49].

Multiple genes from networks we observed to be preserved in brain and blood transcriptome data and enriched in pathways from combined blood and brain expression profiles showed significant association with the vascular injury proteins ICAM-1, SAA, and VCAM-1. SAA level increases in the presence of BBB dysfunction [50]. ICAM-1 is a cytokine involved regulation of the BBB [51] and increased ICAM-1 level has been associated with BBB damage and neuroinflammation [52]. Under inflammatory conditions, VCAM-1 level is upregulated and the BBB can release soluble VCAM-1 which in turn can compromise BBB function [53]. Our study showed that FOSL1 and TRIP10 were among the genes enriched in the TNFA via NFKB pathway, and their expression was associated with ICAM-1. TRIP10 was previously included in an AD network derived from multi-omic integration [54] and FOSL-1 was identified in conjunction with PIAS1, a protein associated with AD and inflammatory response [55]. We identified VASP and C4B in an APOE genotype-specific co-expressed gene network in brain that was reproduced in blood, and expression of these genes was significantly associated with levels of multiple vascular damage proteins. VASP encodes vasodilator-stimulated phosphoprotein which regulates BBB function [56]. The pattern of C4B expression in brain is dependent on APOE genotype [12] and dysregulation of complement system can cause or exacerbate BBB dysfunction [57]. These genes require further investigation in their role with AD specifically related to BBB function and APOE.
Limitations

Our study has several limitations. First, the sample sizes of the APOE genotype groups in the blood dataset were relatively small which limited statistical power. Additionally, the ROSMAP blood dataset exhibited significant batch effects. However, we were able to account for these batch effects by running each batch separately and meta-analyzing our results. Second, the software WGCNA creates networks based on strong computational correlations but does not account for underlying biological implications. We evaluated biological connections using the IPA software by rebuilding subnetworks of the leading-edge genes. Third, we were able to obtain single-cell data only for dendritic cells and monocytes in blood and therefore could not analyze a wider array of blood cell types.

Conclusions

Our study provides evidence of the importance of evaluating brain and blood transcriptome data together with genetic information derived from the same subjects to identify meaningful correlations of blood biomarkers with AD-related proteins in brain. Future studies are required to investigate further, how the genes and biological pathways identified in this study in the context of APOE genotype influence the BBB and contribute to and/or exacerbate AD-related pathology.

List of abbreviations:

Aβ: amyloid-β, AD: Alzheimer's Disease, BBB: Blood-Brain Barrier, CAA: cerebral amyloid angiopathy, CERAD: Consortium to Establish a Registry for Alzheimer Disease, CPM: counts per million, DE: differential expression, DGE: Differential gene expression, FHS/ADRC: Framingham Heart Study/Boston University Alzheimer’s Disease Research Center, FPKM:
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Availability of data and materials

The datasets supporting the conclusions of this article are available in https://singlecell.broadinstitute.org/ and http://www.synapse.org, as well as from the corresponding author upon request.
Authors’ contributions
R.P., L.A.F., and G.R.J. conceived overall study design. R.P. and G.R.J. perceived statistical analysis. R.P. and J.H. performed data analyses. W.X. and T.D.S. conducted immunoassay experiments using autopsied brains. D.A.B. and T.D.S. provided neuropathological data. R.P., D.A.B., T.D.S., W.X., L.A.F., and G.R.J. reviewed and edited the manuscript. G.R.J. and L.A.F. supervised and obtained funding for the project.

Ethics approval and consent to participate
The study protocol, design, and performance of the current study were approved by the Boston University Institutional Review Board.

Consent for publication
Not applicable

Competing interests
The authors declare no competing interests.

Figure Legends
Figure 1. Analysis design and workflow. RNA-sequencing data were obtained from blood and post-mortem frozen brain of neuropathologically verified AD cases and controls. Data were analyzed in two ways. First, gene co-expression analysis identified networks in brain that reproduced in blood. A second analysis identified genes differentially expressed between AD cases
in controls in the total sample as well as within APOE genotype groups in both blood and brain. Expression of genes in the co-expression networks that were previously associated with AD by GWAS were tested for associated with AD-related traits measured in brain. Next, genes in significant co-expression networks and differentially expressed genes in blood and brain were incorporated as seeds in pathway analysis. Finally, expression of genes from the most significant pathways was tested for association with levels of several vascular damage proteins. Figure created with BioRender.com

**Figure 2. Significant pathways in blood and brain by APOE genotype.** (a) Significant pathways (adjusted P<0.05) within APOE genotype groups that are enriched for differentially expressed genes in blood and brain combined are plotted according to the normalized enrichment score. Enrichment score indicates whether the genes in the pathway are upregulated (positive) or downregulated (negative) in AD. (b) Heatmap shows strength and direction of association of levels of proteins involved in vascular damage with expression of leading-edge genes from significant pathways where enrichment scores are in opposite directions between APOE ε4 carriers (+) and non-carriers (-). Genes whose expression was significantly (P<4.67x10^{-4}) associated with the level of at least one protein (indicated by an asterisk) are shown.

**Figure 3. APOE genotype-specific co-expression networks in blood and brain.** (a) Boxplot for the greenyellow network showing the distribution of eigengene values, which summarize gene expression across a network, among APOE ε4 carriers (+) and non-carriers (-). P-value was calculated using the student’s t-test. (b) Barplot showing significant pathways enriched for established AD genes in the greenyellow network. (c) Biological subnetwork including established
AD genes involved in significant pathways in the greenyellow network. (d) Heatmap showing strength of association of seed-gene expression in brain from the biological subnetwork in (c) with levels of proteins involved in vascular damage. Asterisks indicate significant associations (P<4.67x10^{-4}).

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| Gene    | Dataset | # | Z-score | P-value | # | Z-score | P-value | # | Z-score | P-value | Total       | # | Z-score | P-value |
|---------|---------|---|---------|---------|---|---------|---------|---|---------|---------|-------------|---|---------|---------|
| INPP5D  | Blood   | 26 | -0.62  | 0.53    | 102 | 1.67   | 0.10    | 31 | 2.12   | 0.03    | 179 | 2.16   | 0.03   |
|         | Brain   | 71 | 1.80   | 0.07    | 355 | 0.92   | 0.36    | 126 | 0.66   | 0.51    | 576 | 2.30   | 0.02   |
|         | Combined| 97 | 1.51   | 0.13    | 457 | 1.29   | 0.20    | 157 | 1.31   | 0.19    | 755 | 2.42   | 0.02   |
| HLA-DQA1| Blood   | 26 | -1.49  | 0.14    | 102 | -1.80 | 0.07    | 31 | 0.96   | 0.34    | 179 | -2.26  | 0.03   |
|         | Brain   | 71 | 0.30   | 0.76    | 355 | -3.13 | 1.7x10^{-3} | 126 | -1.01  | 0.31    | 576 | -2.23  | 0.03   |
|         | Combined| 97 | -0.16  | 0.87    | 457 | -2.84 | 4.6x10^{-3} | 157 | -0.66 | 0.51    | 755 | -2.42  | 0.02   |
| SPDYE3  | Blood   | 26 | -0.08  | 0.94    | 102 | -0.76 | 0.45    | 31 | -0.96  | 0.34    | 179 | -1.47  | 0.14   |
|         | Brain   | 71 | -0.79  | 0.43    | 355 | -0.97 | 0.33    | 126 | -2.16  | 0.03    | 576 | -2.73  | 6.4x10^{-3} |
|         | Combined| 97 | -0.77  | 0.44    | 457 | -0.96 | 0.33    | 157 | -2.37  | 0.02    | 755 | -2.26  | 0.02   |
| TSPOAP1 | Blood   | 26 | -1.38  | 0.17    | 102 | 0.30  | 0.77    | 31 | -0.16  | 0.87    | 179 | -0.55  | 0.58   |
|         | Brain   | 71 | -1.04  | 0.30    | 355 | -2.17 | 0.03    | 126 | -1.85  | 0.06    | 576 | -3.65  | 2.6x10^{-4} |
|         | Combined| 97 | -1.39  | 0.16    | 457 | -1.35 | 0.18    | 157 | -1.82  | 0.07    | 755 | -2.24  | 0.03   |
| SIGLEC11| Blood   | 26 | 1.43   | 0.15    | 102 | 0.47  | 0.64    | 31 | 0.24   | 0.81    | 179 | 0.33   | 0.74   |
|         | Brain   | 71 | 1.05   | 0.29    | 355 | 2.46  | 0.01    | 126 | 2.58   | 9.9x10^{-3} | 576 | 3.77   | 1.6x10^{-4} |
|         | Combined| 97 | 1.42   | 0.16    | 457 | 1.86  | 0.06    | 157 | 2.54   | 0.01    | 755 | 2.17   | 0.03   |
Table 2. Novel differentially expressed genes in blood or brain within *APOE* genotype groups

| Gene  | Dataset | *APOE* ε2/ε3 |  | *APOE* ε3/ε3 |  | *APOE* ε3/ε4 |  | Total |  |
|-------|---------|--------------|----------------|--------------|----------------|--------------|----------------|--------|
|       |         | N  | Z-score | P-value | N  | Z-score | P-value | N  | Z-score | P-value | N  | Z-score | P-value |
|       |         |     |         |         |     |         |         |     |         |         |     |         |         |
| FRAS1 | Blood   | NA | NA | NA | 75 | -0.24 | 0.81 | 31 | -4.66 | 3.2x10^{-6} | 132 | -2.26 | 0.02 |
|       | Brain   | NA | NA | NA | 355 | -0.97 | 0.33 | 126 | 0.28 | 0.78 | 576 | -0.49 | 0.62 |
|       | Combined| NA | NA | NA | 430 | -0.75 | 0.45 | 157 | -1.23 | 0.22 | 708 | -1.27 | 0.20 |
|       | Blood   | 26 | 1.67 | 0.09 | 102 | 0.43 | 0.67 | 31 | 1.30 | 0.19 | 179 | 1.14 | 0.25 |
| PIGHP1 | Brain   | 71 | 4.42 | 9.8x10^{-6} | 355 | 0.53 | 0.59 | 126 | 0.47 | 0.64 | 576 | 1.98 | 0.05 |
|       | Combined| 97 | 4.67 | 3.1x10^{-6} | 457 | 0.53 | 0.59 | 157 | 0.87 | 0.39 | 755 | 1.68 | 0.09 |

NA: not available due to low expression. Bolded p-values pass transcriptome-wide multiple testing threshold (3.3x10^{-6}).
Table 3. Significant co-expressed gene pathways in the combined blood and brain datasets

| Hallmark Pathway                          | APOE genotype | NES   | Adjusted P-value | Leading Edge Genes                                                                 |
|-------------------------------------------|---------------|-------|------------------|-----------------------------------------------------------------------------------|
| Allograft rejection                       | $\varepsilon^3/\varepsilon^3$ | -1.89 | 0.02             | C2, HLA-DQA1, FAS, HLA-A, UBE2N, HLA-DOB, LTB, F2R, HLA-DRA, TAP2, B2M, CD1D, CD74, MAP3K7 |
|                                           | $\varepsilon^3/\varepsilon^4$ | 2.16  | $7.4 \times 10^{-3}$ | IRF4, CCL22, IRF7, CD74, HLA-DRA, ELF4, IL16, IFNGR2, IL27RA, IL1B                |
| Interferon gamma response                 | $\varepsilon^3/\varepsilon^3$ | -2.56 | $1.3 \times 10^{-5}$ | HLA-DQA1, FAS, CFB, BPGM, C1S, HLA-A, LAP3, MVP, PSME2, PSMA2, UBE2L6, SERPING1, DHX58, IFITM2, CD38, B2M, CD74 |
|                                           | $\varepsilon^3/\varepsilon^4$ | 1.80  | 0.04             | CD274, CD69, BTG1, ISG20, PML, IRF4, NFKBIA, IRF7, CD74, IL10RA, IRF9             |
| Peroxisome                                | $\varepsilon^3/\varepsilon^3$ | -1.81 | 0.03             | IDH2, EHHADH, MVP, ALDH1A1, SCP2, SOD1, ABCD2, MSH2                              |
|                                           | $\varepsilon^3/\varepsilon^4$ | 1.90  | 0.03             | RDH11, ELOVL5, SLC25A19, CTPS1, SLC23A2, SEMA3C                                  |
| Tnfa signaling via nfkb                   | $\varepsilon^2/\varepsilon^3$ | -2.03 | 0.01             | DUSP4, NR4A1, NR4A3, MARCKS, NFAT5, PHLDA1, DUSP2, KNYU, G0S2, ETS2, PTGS2, GCH1, MSC, SOD2, EGR2 |
|                                           | $\varepsilon^3/\varepsilon^4$ | 2.27  | $2.5 \times 10^{-3}$ | TRIP10, CD69, BTG1, DENND5A, PFKFB3, FOS, NFKBIA, LDLR, IER2, JUN, IL1A, PANX1, PRNC1, DUSP1, IFNGR2, OLR1, MAFF, IL1B, TNIP2, CCL20, BIRC2, IER3, GADD45B, KNYU, LITAF, CCRL2, SPHK1, FOSL1 |

NES = Normalized Enrichment Score
Table 4. Association of expression of leading-edge genes from co-expressed gene networks with vascular damage protein levels

| Gene | APOE Genotype | Pathway(s) | I-CAM1 β | I-CAM1 P-value | SAA β | SAA P-value | V-CAM1 β | V-CAM1 P-value |
|------|---------------|------------|-----------|----------------|--------|--------------|-----------|----------------|
| C1S  | ε3/ε3         | IGR        | 0.11      | 0.13           | 0.24   | 2.5x10⁻³    | 0.30      | 2.0x10⁻⁴     |
| C2   | ε3/ε3         | AR         | 0.05      | 0.53           | 0.17   | 0.04         | 0.30      | 2.2x10⁻⁴     |
| CD1D | ε3/ε3         | AR         | 0.28      | 3.4x10⁻³      | 0.21   | 0.05         | 0.46      | 1.7x10⁻⁵     |
| CD38 | ε3/ε4         | IGR        | 0.09      | 0.25           | 0.07   | 0.41         | 0.39      | 6.1x10⁻⁶     |
| CFB  | ε3/ε3         | IGR        | 0.16      | 0.05           | 0.46   | 2.2x10⁻⁷    | 0.26      | 5.4x10⁻³     |
| DHX58| ε3/ε3         | IGR        | 0.11      | 0.34           | 0.20   | 0.13         | 0.51      | 7.1x10⁻⁵     |
| ELF4 | ε3/ε4         | AR         | 0.32      | 8.7x10⁻⁴      | 0.46   | 1.9x10⁻⁵    | 0.26      | 0.02          |
| FOSL1| ε3/ε4         | TSN        | 0.20      | 3.1x10⁻⁴      | 0.18   | 3.1x10⁻³    | 0.08      | 0.21          |
| HLA-A| ε3/ε3         | AR; IGR    | 0.27      | 0.03           | 0.38   | 3.9x10⁻³    | 0.53      | 7.0x10⁻⁵     |
| IDH2 | ε3/ε3         | P          | 0.09      | 0.43           | 0.24   | 0.05         | 0.61      | 3.6x10⁻⁷     |
| IER3 | ε3/ε4         | TSN        | 0.14      | 0.06           | 0.37   | 3.5x10⁻⁶    | 0.07      | 0.43          |
| IFITM2| ε3/ε3        | IGR        | 0.19      | 0.01           | 0.48   | 2.9x10⁻⁹    | 0.18      | 0.03          |
| IRF7 | ε3/ε4         | AR; IGR    | 0.24      | 9.5x10⁻⁴      | 0.41   | 2.8x10⁻⁷    | 0.20      | 0.02          |
| ISG20| ε3/ε4         | IGR        | 0.21      | 3.6x10⁻³      | 0.34   | 1.9x10⁻⁵    | 0.29      | 4.4x10⁻⁴     |
| LITAF| ε3/ε4         | TSN        | 0.27      | 4.1x10⁻³      | 0.39   | 2.1x10⁻⁴    | 0.43      | 3.9x10⁻⁵     |
| MVP  | ε3/ε3         | IGR; P     | 0.21      | 0.01           | 0.42   | 7.0x10⁻⁶    | 0.45      | 2.4x10⁻⁶     |
| PFKFB3| ε3/ε4        | TSN        | 0.30      | 5.6x10⁻⁴      | 0.30   | 2.6x10⁻³    | 0.35      | 4.0x10⁻⁴     |
| PML  | ε3/ε4         | IGR        | 0.28      | 0.02           | 0.47   | 3.8x10⁻⁴    | 0.50      | 2.0x10⁻⁴     |
| SERPINGI| ε3/ε3      | IGR        | 0.17      | 0.13           | 0.35   | 4.4x10⁻³    | 0.50      | 4.7x10⁻⁵     |
| TNIP2| ε3/ε4         | TSN        | 0.36      | 1.9x10⁻³      | 0.59   | 4.7x10⁻⁶    | 0.19      | 0.15          |
| TRIP10| ε3/ε4        | TSN        | 0.32      | 2.1x10⁻⁴      | 0.35   | 3.4x10⁻⁴    | 0.06      | 0.58          |

I-CAM: Intercellular Adhesion Molecule 1; SAA: Serum Amyloid A; V-CAM1: Vascular Cell Adhesion Molecule 1; AR: Allograft rejection; IGR: Interferon gamma response; P: Peroxisome; TSN: Tnfa signaling via nfkb

Results in bold surpass the multiple-testing threshold (P<4.67x10⁻⁴).
Figure 1

N = 576

Gene Co-expression Networks in Brain Reproduced in Blood

Statify by APOE genotype (ε2/ε3, ε3/ε3, ε3/ε4, total sample)

GWAS Gene Enrichment (AD, Braak Stage, Plaque Score)

Pathway Analysis

Association of Expression of Pathway Genes with Vascular Damage Proteins

N = 179

AD Differentially Expressed Genes in Blood and Brain
Figure 3

(a) Box plot showing Eigengene Value with different groups.

(b) Bar chart displaying -Log10 Adjusted P-value for various conditions.

(c) Network diagram illustrating complex interactions.

(d) Heatmap indicating T-Value for different genes.
Supplementary Files

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