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RNASE6 is a novel modifier of *APOE*-ε4 effects on cognition

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Abstract

Apolipoprotein E4 (*APOE*-ε4), the strongest common genetic risk factor for Alzheimer's disease (AD), contributes to worse cognition in older adults. However, many *APOE*-ε4 carriers remain cognitively normal throughout life, suggesting that neuroprotective factors may be present in these individuals. In this study, we leverage whole-blood RNA sequencing (RNAseq) from 324 older adults to identify genetic modifiers of *APOE*-ε4 effects on cognition. Expression of *RNASE6* interacted with *APOE*-ε4 status ($p=4.35x10^{-8}$) whereby higher *RNASE6* expression was associated with worse memory at baseline among *APOE*-ε4 carriers. This interaction was replicated using RNAseq data from the prefrontal cortex in an independent dataset (N=535; p=0.002), suggesting the peripheral effect of *RNASE6* is also present in brain tissue. *RNASE6* encodes an antimicrobial peptide involved in innate immune response and has been previously observed in a gene co-expression network module with other AD-related inflammatory genes, including *TREM2* and *MS4A*. Together, these data implicate neuroinflammation in cognitive

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Conflicts of Interest

Nothing to report.

Supplementary Materials

Supplementary material is available online.

decline, and suggest that innate immune signaling may be detectable in blood and confer differential susceptibility to AD depending on *APOE-ε4*.

Keywords

Alzheimer's; cognition; gene expression; blood; brain

1. Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder that is the 6th leading cause of death among all adults in the United States (Association, 2021). The most common form of Alzheimer's disease is sporadic late-onset AD (LOAD), which is complex in etiology and heterogeneous in clinical presentation (Bekris et al., 2010; Reitz et al., 2020). Sporadic LOAD is polygenic, and to date, over 40 risk loci for AD have been identified via large genome-wide association studies (Jansen et al., 2019; Kunkle et al., 2019; Lambert et al., 2013; Wightman et al., 2021). One key genetic driver is *APOE* (apolipoprotein E), which has three common polymorphic alleles: ε2, ε3, and ε4. The *APOE*-ε4 allele is the strongest common genetic risk factor for AD (Corder et al., 1993; Saunders et al., 1993). A single allele of *APOE*-ε4 can increase AD risk by up to 3 times compared to *APOE*-ε3, and two *APOE*-ε4 alleles can increase risk by up to 15-fold (Reiman et al., 2020; Wu and Zhao, 2016). In addition to increased AD risk, the *APOE*-ε4 allele is associated with increased brain amyloid and tau burden (Baek et al., 2020), subsequently leading to downstream neurodegeneration and cognitive impairment (Jack et al., 2013; Jack et al., 2010). However, many *APOE*-ε4 carriers remain cognitively normal throughout life despite the increased AD risk (Emrani et al., 2020), suggesting that there may be neuroprotective molecular modifiers of *APOE* effects. For example, mutations within the caspase 7 (*CASP7*) and Klotho (*KL*) genes were suggested to have protective effects (*e.g.,* reduced AD risk, slower cognitive decline) in *APOE*-ε4 carriers in comparison to non-carriers (Seto et al., 2021). In the brain, epigenetic modifiers of *APOE*-ε4 have been observed, such as the recently described epigenomic factor of activated microglia (EFAM; (Ma et al., 2021). Indeed, identifying and describing *APOE* modifiers may provide critical insight into the pathophysiology of AD and provide novel targets for therapeutic intervention.

The primary function of the APOE protein is lipid transport and signaling, which plays important roles in the brain, innate immune system, and vascular system (Husain et al., 2021; Safieh et al., 2019). Given the roles of APOE in both the peripheral and central nervous system (CNS), whole blood transcriptomics may provide an opportunity to identify novel genes and pathways that contribute to neuroprotection by modifying the effect of *APOE*. Blood transcriptomics provide some important advantages over brain transcriptomics alone, particularly when seeking for modifiers of *APOE* effects. While transcriptomic signatures in blood do not perfectly mimic the brain (Sullivan et al., 2006; Tylee et al., 2013), many of the gene networks and molecular pathways that change over the course of AD are measurable in the blood and provide a window into relevant biological cascades such as inflammation (Cullen et al., 2021). Moreover, peripheral inflammation changes very early in AD and contributes to AD progression (King et al., 2018; Tao et al., 2018), with emerging

evidence (Kloske and Wilcock, 2020; Krasemann et al., 2017) suggesting that non-CNS inflammation is particularly relevant among *APOE*-ε4 carriers.

In the present study, we leverage whole blood RNA sequencing (RNAseq) data from the Vanderbilt Memory and Aging Project (VMAP) to identify genes that modify the association between *APOE*-ε4 and cognitive performance. We then extend our analyses to the brain to characterize whether genes identified in blood show comparable modifying effects in the brain. By leveraging blood and brain transcriptomics, we aim to better characterize the molecular modifiers of *APOE* on cognition and potentially uncover novel blood-based biomarkers and targets for future drug discovery efforts.

2. Materials and Methods

2.1 Participants

The Vanderbilt Memory and Aging Project (VMAP) is a longitudinal aging cohort that was established in 2012 to investigate the relationship between vascular health and brain aging. 335 individuals were enrolled; the study preferentially recruited participants with mild cognitive impairment (MCI, N=168) aged 60 and above along with matched counterparts who had normal cognition (N=167). Individuals with cognitive diagnoses other than MCI or normal cognition (NC), history of neurological disease, MRI contraindications, heart failure, major psychiatric illness, or systemic or terminal illness were excluded. At enrollment, participants underwent a comprehensive evaluation including, but not limited to, *APOE* genotyping, neuroimaging, cognitive assessment, blood draw, and optional lumbar puncture (Jefferson et al., 2016).

Independent data for replication were acquired from the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP), known as ROS/MAP collectively. The ROS began in 1994 and enrolls priests and nuns from across the United States. The MAP cohort began in 1997 and enrolls lay persons from northeastern Illinois. Both longitudinal aging studies were launched to better understand risk factors for and the neurobiology of cognitive decline and dementia. Both studies were approved by an Institutional Review Board of Rush University Medical Center. All participants were without known dementia at enrollment, agree to comprehensive neuropsychological evaluations, and sign an Anatomic Gift Act and Repository Consent to allow their data to be shared (Bennett et al., 2018). Additional ROS/MAP data can be requested via the Accelerating Medicines Partnership – Alzheimer's Disease (AMP-AD) Knowledge Portal (https://adknowledgeportal.synapse.org/) as well as the Rush Alzheimer's Disease Center Resource Sharing Hub (https://www.radc.rush.edu/).

2.2 Neuropsychological Assessment

Composite measures for memory and executive function in VMAP were generated following previously described procedures (Crane et al., 2012; Kresge et al., 2018). Briefly, the memory composite leveraged data from the California Verbal Learning Test (2nd edition) and the Biber Figure Learning Test. The executive function composite score in VMAP was derived from the following tasks: Digit Span from the Wechsler Adult Intelligence Scale

(3rd edition), Trail Making Test, Stroop Color Word Inhibition, and Controlled Oral Word Association.

The global cognition variable in ROS/MAP was generated by averaging the Z-scores of 17 neuropsychological tests across five domains of cognition (i.e., episodic, semantic, and working memory, perceptual orientation, and perceptual speed). This composite measurement has been described fully elsewhere (Wilson et al., 2015).

2.3 RNA Extraction, Library Preparation, and Sequencing

2.3.1 Vanderbilt Memory and Aging Project—Blood draws were performed in the morning under fasting conditions. Approximately 2.5 mL of whole blood were kept frozen at −80°C in a PAXgene tube (QIAGEN, 761115) until processing (Jefferson et al., 2016). RNA extraction, library preparation, and RNA sequencing were performed by the VANTAGE Core (Vanderbilt University, TN, USA). Total RNA was extracted from whole blood using the QIASymphony RNA Kit (QIAGEN, 931636), and both ribosomal RNA and hemoglobin were depleted with the NEBNext Globin and rRNA Depletion Kit (New England BioLabs, Inc., E7750). Library preparation was completed using the NEBNext Ultra Directional Library Prep Kit (New England BioLabs, Inc., E7420) before sequencing was performed using 150 base pair (bp) paired end reads on an Illumina NovaSeq 6000 (Illumina), targeting an average of 50 million reads per sample.

2.3.2 Religious Orders Study and Memory and Aging Project—50 mg of frozen brain tissue were dissected and homogenized in DNA/RNA shield buffer (Zymo Research, R1100). RNA was extracted from the dorsolateral prefrontal cortex (DLPFC), posterior cingulate cortex (PCC), and head of the caudate nucleus (CN) using the Chemagic RNA tissue kit (PerkinElmer, Inc. CMG-1212) on a Chemagic 360 instrument. 500 ng of total RNA was used as input for sequencing library generation and rRNA was depleted with RiboGold (Illumina, 20020599). A Zephyr G3 NGS workstation (PerkinElmer, Inc.) was utilized to generate TruSeq stranded sequencing libraries (Illumina, 20020599). Libraries were normalized for molarity and sequenced using 2 x 150 bp paired end reads on a NovaSeq 6000 (Illumina) targeting a total of 40 to 50 million reads. Additional details are previously described (De Jager et al., 2018; Lee et al., 2021; Mostafavi et al., 2018). These data are available on the AMP-AD Knowledge Portal (https://www.synapse.org/#!Synapse:syn3219045).

2.4 RNAseq Alignment and Quality Control

RNAseq alignment and quality control (QC) for both VMAP and ROS/MAP samples largely followed a previously reported procedure used by the AMP-AD Consortium (Logsdon et al., 2019). Alignment was performed using STAR (version 2.5.2b) with twopassMode set to basic (Dobin et al., 2013). Reads were aligned to the Ensembl (GRCh38, version 99) reference genome (Howe et al., 2021), and gene counts were computed using the featureCounts (Liao et al., 2014) command from the Subread package (version 2.0.0). Summary metrics were calculated using Picard (version 2.18.27, http://broadinstitute.github.io/picard/) to evaluate sample quality and for later use as covariates (Institute, 2019).

Before QC of the VMAP whole blood RNAseq, samples with RNA integrity number (RIN) less than 3.0 were excluded. In addition, genes with missing gene length or GC-content were removed, after which all gene counts were quantile normalized using the cqn R package (version 1.30.0) to remove technical variability due to gene length and GC-content (Hansen et al., 2012). At this time, gene expression values greater than three standard deviations from the mean expression for each gene were removed. Additional samples were removed if deemed principal component outliers or if missing RIN, age, sex, other demographic information, or cognition data prior to batch correction. Expression values were adjusted for batch effects using the R package limma (version 3.40.6; (Law et al., 2014; Ritchie et al., 2015). This left 60,669 genes and 324 samples in VMAP for discovery analyses.

QC of the bulk brain RNAseq from ROS/MAP followed the aforementioned pipeline. From these data, samples with RIN less than 4.0 or with post-mortem interval (PMI) greater than 24 hours were excluded. Additional samples were removed if missing covariates or cognitive data resulting in a final dataset of 535 samples.

Sensitivity analyses leveraged RNAseq data from VMAP that was additionally adjusted *(i.e.*, along with quantile normalization and controlling for batch) for the following covariates using limma: sex, race, *APOE*-ε4 allele count, RIN, age, education, percentage pass-filter reads aligned, and percent coding, intergenic, intronic, and ribosomal bases.

2.5 Biomarker Quantification

2.5.1 VMAP Cerebrospinal Fluid Biomarkers—Cerebrospinal fluid (CSF) was collected from 155 individuals enrolled in VMAP. A total of 151 individuals remains when samples missing covariates are removed (Supplementary Table 1). Additional detail on lumbar puncture and collection is described elsewhere (Jefferson et al., 2016). Beta-amyloid ($A\beta_{1\text{-}42}$, Fujirebio, 81583), total tau (Fujirebio, 81579), and tau phosphorylated at threonine 181 (pTau, Fujirebio 81581) were quantified using commercially available immunoassays. The CSF thresholds for pathologic amyloid and tau positivity are as follows: CSF $A\beta_{1\text{-}42}$ less than 530 ng/L (Skillbäck et al., 2015) and CSF total tau levels greater than 400 ng/L (Dorey et al., 2015).

2.5.2 ROS/MAP Brain Neuropathological Measures—Neuropathological outcomes included beta-amyloid (Aβ), phosphorylated tau, neuritic plaques, and neurofibrillary tangles (NFT). Aβ and phosphorylated tau were identified via immunohistochemistry and quantified via image analysis. The overall amyloid level is defined as the mean percent of cortex occupied by Aβ across eight brain regions (hippocampus, angular gyrus, and entorhinal, midfrontal, inferior temporal, calcarine, anterior cingulate, and superior frontal cortices). The overall tangle density is defined as the mean cortical density per mm^2 of the same eight brain regions mentioned above. Neuritic plaque and NFT burden were determined by microscopic examination of silver-stained slides across 5 brain regions (hippocampus and entorhinal, midtemporal, inferior parietal, and midfrontal cortices). These neuropathological measures were previously characterized by ROS/MAP (Bennett et al., 2018). CERAD scores (Definite and/or Probable AD, 1 and 2) and Braak staging (Braak <

4) were used to determine amyloid and tau positivity (Bennett et al., 2006; Braak and Braak, 1991; Mirra et al., 1991).

2.6 Statistical Analyses

All statistical analyses were performed using R (version 3.6.3, https://www.r-project.org/). False-discovery rate (Benjamini and Hochberg, 1995) was used to correct for multiple comparisons in all analyses, with family-wise α set *a priori* to 0.05. Both baseline and longitudinal memory and executive function scores were used as continuous outcome variables. Linear regression was used to assess the interaction between *APOE*-ε4 positivity (*i.e.*, presence of at least one ε4 allele) and gene expression measured by RNAseq on cross-sectional memory performance. Linear-mixed effects regression tested the *APOE* interaction with gene expression on longitudinal memory, where the intercept and interval from baseline were entered as both fixed and random effects. Covariates in both models included baseline age and sex.

Replication analyses were performed using ROS/MAP samples. Specifically, linear regression models were used to examine the interaction between *APOE*-ε4 positivity and bulk brain gene expression in the DLPFC, CN, and PCC (Supplementary Table 2) on the last global cognition composite score before death. Covariates included PMI, sex, and age at death.

2.7 Sensitivity Analyses

Sensitivity analyses included models with more stringent QC for VMAP whole blood RNAseq data (*i.e.*, adjusted for batch, sex, age, RIN, percentage of coding, intronic, and intergenic bases) to determine whether the observed results were due to technical effects. Additional sensitivity analyses included stratifying by cognitive diagnosis and covarying for education because of their effects on cognitive performance.

2.8 Post-hoc Blood to Brain RNASE6 Expression Correlation

To investigate the correlation between peripheral *RNASE6* expression and brain *RNASE6* expression, gene expression data from the NIH Genotype-Tissue Expression (GTEx) Project was leveraged (Lonsdale et al., 2013). Linear regression and Pearson correlation were used to assess the relationship between brain and blood *RNASE6* expression. *RNASE6* expression in the brain cortex (N=137) and hippocampus (N=118) were evaluated against whole blood *RNASE6* expression.

2.9 Post-hoc Biomarker Analyses

Given the relationship between *APOE* and AD biomarkers, we plan to examine the interaction between amyloid and tau positivity and any significant gene hits on cognition to better understand the biological mechanisms behind our cognitive results. For these analyses, we leveraged CSF measurements of amyloid and tau in VMAP and neuropathological measurements from ROS/MAP. Covariates for these analyses included age, sex, and PMI where relevant. Using similar regression models, we also investigated whether the gene x *APOE*-ε4 interaction had any effect on baseline CSF amyloid and tau levels, covarying for age and sex.

3. Results

The characteristics of individuals from VMAP (N=324) and those from ROS/MAP who have DLPFC RNAseq (N=535) are presented in Table 1. Overall, a larger percentage of participants in VMAP are *APOE*-ε4 positive (34.8% versus 23.1%, respectively) and have normal cognition (51.8% versus 34.2%). VMAP participants are also younger than participants from ROS/MAP, on average. In contrast, a higher percentage of participants in ROS/MAP are tau and amyloid positive and they are more highly educated on average. It should be noted that cognition scores cannot be compared across studies because two different cognitive composites are used that are not scaled across studies. The total number of samples from each brain region in ROSMAP can be found in Supplementary Table 2.

3.1 *Gene Expression Interactions with APOE-ε4 on Cognition*

Of the 60,669 genes tested, expression of *RNASE6*, ribonuclease A family member K6, interacted with *APOE*-ε4 status on baseline memory in VMAP (β=−1.16, p.fdr=0.003, p.unadjusted= $4.35x10^{-8}$) whereby higher *RNASE6* expression in whole blood was associated with worse memory performance at baseline among *APOE*-ε4 carriers (β=−0.96, $p=4.43x10^{-8}$). In contrast, higher levels of *RNASE6* expression were nominally associated with better memory among *APOE*-ε4 non-carriers (β=0.22, p=0.09, Figure 1A). We observed similar results for another cognitive domain, executive function (β=−0.54, p=0.008, Figure 1B). We did not observe any significant interactions between whole blood gene expression and *APOE*-ε4 positivity on longitudinal cognition.

The *RNASE6* x *APOE*-ε4 interaction on baseline memory remained significant in sensitivity analyses (see Methods) when leveraging more stringent QC controlling for technical variation in RNA sequencing ($p=2.23x10^{-8}$, Table 2). The interaction also remained significant when stratifying by diagnosis (i.e., normal cognition or MCI, Figure 1C, D) and covarying for education (p-values<0.00657, Table 2).

3.2 Replication in ROS/MAP

Leveraging data from an independent cohort, ROS/MAP, we examined the interaction between *RNASE6* expression in brain tissue and *APOE*-ε4 genotype on global cognition at the final visit prior to death. Using bulk RNAseq data from 3 distinct brain regions: DLPFC, PCC, and head of the CN, we observed replication of the previous interaction on memory (β=−0.35, p=0.002) in the DLPFC (Supplementary Figure 1), though the observed effects were not present in the PCC or the CN (p-values>0.3; Table 3). Similar to VMAP, the *RNASE6* x *APOE*-ε4 interaction was not significant longitudinally in ROS/MAP.

3.3 Correlation of Blood and Brain RNASE6 expression

To further investigate the relationship between blood and brain *RNASE6* expression, we utilized data from the NIH GTEx project (Lonsdale et al., 2013) in which *RNASE6* expression was measured in both whole blood and brain tissue from the same individuals. Whole blood *RNASE6* expression was significantly associated with *RNASE6* expression in both the brain cortex (r=0.30, β=0.39, p=0.0004, Supplementary Figure 3A) and hippocampus (r=0.28, β=0.40, p=0.0024, Supplementary Figure 3B).

3.4 Post-Hoc Analyses with Biomarkers

Like our initial findings within VMAP, we observed an interaction with amyloid positivity whereby higher levels of *RNASE6* in blood were associated with worse baseline memory in VMAP (β=−1.14, p=0.001, Figure 2A). We also observed a similar interaction with tau positivity whereby higher levels of *RNASE6* were associated with worse baseline memory (β=−0.63, p=0.04, Figure 2B). Neither interaction was significantly associated with baseline executive function (p-values>0.1). We replicated the amyloid interaction effect in ROS/MAP DLPFC leveraging an immunohistochemical measurement of amyloid (β=−0.26, p=0.007, Supplementary Figure 2), though the tau interaction did not replicate (p=0.1).

We also wanted to examine whether *RNASE6* expression influenced AD biomarker levels in *APOE*-ε4 carriers and non-carriers. The main effect of *RNASE6* was significantly associated with CSF $A\beta_{1-42}$ (Figure 3A, β=91.8, p=0.02) such that higher *RNASE6* levels in blood were correlated with reduced brain amyloid burden. However, when examining the *RNASE6* x *APOE*-ε4 positivity interaction, this effect appeared to be in *APOE*-ε4 non-carriers only, though the interaction was non-significant (p=0.1, Figure 3A). Though *RNASE6* expression alone was not significantly associated with CSF tau or pTau, it significantly modified the relationship between *APOE*-ε4 and both CSF tau (β=230.1, p=0.003) and CSF pTau (β=22.7, p=0.01) levels such that *APOE*-ε4 carriers expressing higher levels of *RNASE6* in blood have increased tau pathology at baseline (Figure 3B, C). None of these effects were observed using neuropathological measures of amyloid and tau in ROS/MAP.

4. Discussion

In this study, we observed the significant *APOE4*-modifying effect of *RNASE6* expression, in both blood and brain tissues, on cognition. Specifically, *APOE*-ε4 carriers expressing higher levels of *RNASE6* in whole blood had worse baseline memory and executive function performance. We also replicated this novel discovery in an independent sample leveraging transcriptomic data from the dorsolateral prefrontal cortex. We also demonstrated that whole blood *RNASE6* expression significantly correlates with brain *RNASE6* expression. In addition, we found that higher *RNASE6* levels are associated with poorer memory performance in individuals that are amyloid-positive and/or tau-positive in comparison to individuals who are biomarker negative (Figure 2A, B). *RNASE6* also modifies the effect of *APOE*-ε4 on CSF tau and pTau levels, such that *APOE*-ε4 carriers expressing higher levels of *RNASE6* have increased tau burden.

RNASE6 is a fascinating, novel inflammatory risk factor for AD. Excitingly, *RNASE6* expression in whole blood significantly correlates with *RNASE6* expression in the brain when using data measured within the same participants from GTEx (Lonsdale et al., 2013), suggesting that it may have utility as a blood-based biomarker in lieu of brain samples.

RNASE6 protein exhibits antimicrobial activity (Becknell et al., 2015). Overexpression of endogenous *RNASE6* in mice is also associated with increased levels of reactive oxygen species as well as increased inflammatory factor secretion (Fang et al., 2021). In addition, *RNASE6* levels are increased in individuals with AD across several brain regions including

the cerebellum, inferior frontal gyrus, and temporal cortex (AMP-AD Agora; https://agora.adknowledgeportal.org/genes/(genes-router:gene-details/ENSG00000169413). It has been established that immune function plays a role in cognitive decline (King et al., 2018; Shen et al., 2019; Tao et al., 2018), and recent evidence has implicated the importance of neuroinflammation early in the AD cascade (Pascoal et al., 2021) driving downstream neurodegeneration. Furthermore, different microglial pathways appear to be involved in the accumulation of amyloid and tau proteinopathies (Patrick et al., 2021). *RNASE6* was found to be upregulated in neurofibrillary tangle-bearing neurons suggesting that it may play a role in increasing tau pathology burden (Miller et al., 2013). However, we also observe a trend in which higher *RNASE6* expression in *APOE*-ε4 non-carriers correlates to reduced brain amyloid burden (i.e., higher CSF $A\beta_{1-42}$, Figure 3A). Though we cannot conclude it without further study, we hypothesize that *RNASE6* may play two distinct roles in both the neuroinflammatory-related clearance and deposition of AD neuropathology in *APOE*-ε4 carriers. In contrast, the observed effect of *RNASE6* may simply be due to differences in the inflammatory milieu between *APOE*-ε4 carriers and non-carriers (Friedberg et al., 2020).

Our significant results are restricted to cross-sectional cognitive performance. Although we looked at longitudinal outcomes, it should be noted that the *RNASE6* x *APOE*-ε4 interaction on cognitive performance over time is non-significant (p.unadjusted > 0.05) in whole blood and brain tissue suggesting that RNASE6 may play a role in the overall biological state of aging and/or neurodegeneration instead of affecting the rate of cognitive decline during the neuropathological progression of AD. This is further supported by our discovery that the *RNASE6* x *APOE*-ε4 interaction on cognition remains significant despite diagnosis (i.e., individuals with normal cognition or MCI).

Additionally, we note that carrying the *APOE*-ε4 allele does not modify the association between amyloid and cognition in our sample (Supplementary Figure 4). The amyloid x *RNASE6* interaction results and the lack of an *APOE*-ε4 x amyloid interaction in our sample suggests the effects of *APOE*-ε4 observed here are likely reflecting the known effects of *APOE* in driving AD neuropathology, primarily through amyloidosis. Thus, one explanation for the lack of a longitudinal association between baseline RNASE6 and cognitive decline among *APOE*-ε4 carriers (despite a strong association with cross-sectional cognition) may be that the effect of RNASE6 among amyloid-positive individuals occurs very early in disease, but longitudinal analysis of *RNASE6*, amyloid, and cognition would be needed to confirm this hypothesis. If *RNASE6* expression is indeed a surrogate for an immune response, it may be that *APOE*-ε4 carriers have a higher susceptibility to a deleterious immune response to amyloid very early in disease.

However, there are other possibilities as to why we do not see longitudinal effects of the *RNASE6* x *APOE*-ε4 interaction. First, we were unable to deconvolve the relationship between *APOE*-ε4 and amyloid in our analyses, although our results suggest the *APOE*-ε4 interaction effect is largely mediated by amyloid burden. Second, these analyses are limited by small sample sizes and the lack of longitudinal gene expression and biomarker data. Thus, studies utilizing additional RNAseq, cognition, and biomarker timepoints are needed for further examination of *RNASE6* in this context.

There is substantial evidence that neuroinflammatory pathways may be particularly relevant among *APOE*-ε4 carriers (reviewed in (Kloske and Wilcock, 2020), and contribute to impaired amyloid clearance (Qiao et al., 2001), enhanced gliosis (Egensperger et al., 1998), and enhanced brain cytokine levels (Lynch et al., 2003). *APOE*-ε4 carriers have also displayed prolonged inflammatory responses in comparison to non-carriers (Safieh et al., 2019). If *RNASE6* expression is indeed a surrogate for an immune response, it can be hypothesized that *APOE*-ε4 carriers have a higher susceptibility to inflammation than non-carriers and also that our findings in baseline cognition may be due to prolonged inflammation before any changes to cognition.

RNASE6 is also in a brain gene co-expression module with several AD genes including *TREM2* and *MS4A6A* (AMP-AD Agora, see above), which are both highly expressed in microglia (Deming et al., 2019; Hickman and El Khoury, 2014). In publicly available microglia data published previously, both *RNASE6* and *TREM2* are upregulated in an AD-associated microglial cluster (Miller et al., 2013; Olah et al., 2020).

As aforementioned, the report discussing EFAM and its impact on *APOE*-ε4 (Ma et al., 2021) provides an interesting convergence of observations from the peripheral and CNS resident immune systems; future work can explore whether these two factors (EFAM and RNASE6) are independently influencing *APOE*-ε4 or may synergize. In addition, future work on *RNASE6* expression in microglia may help clarify the potential mechanistic pathway of the observed effect. It is also notable that another RNASE family gene, *RNASE13* was previously associated with executive function resilience (Mukherjee et al., 2014), suggesting that this family of proteins may be exciting targets for future investigation, particularly in response to pathology along an inflammatory pathway.

This study has multiple strengths including our multi-modal discovery analyses, independent replication, and the use of comprehensive longitudinal cognitive data from two deeply characterized aging studies. Furthermore, our findings that brain and blood *RNASE6* expression are significantly correlated highlight the potential of blood transcriptomics to identify inflammatory and/or immune factors that have additional effects on the brain. Blood draws are also more accessible to individuals than in comparison to other procedures such as lumbar punctures, making similar analyses using blood transcriptomics increasingly viable in larger or more diverse populations. Also, recent studies not only allude that blood transcriptomics are useful in predicting AD (Lee and Lee, 2020) but also that brain markers of AD replicate in the blood, further supporting that RNASE6 may be an promising target for future study (Iturria-Medina et al., 2020; Panitch et al., 2022).

However, there are limitations in our study that should be considered. The individuals in our sample are largely non-Hispanic white and highly educated, limiting the generalizability of our results. We also lack functional data to support a specific mechanism of action; it is particularly challenging given that RNASE6 biology and the relationship between peripheral and brain *RNASE6* expression is not well-characterized. Along with these considerations, the gene expression and pathology data used in our analyses are both cross-sectional; we cannot make a conclusion on how *RNASE6* expression may affect AD neuropathology over time nor on how a diagnosis of AD may affect *RNASE6* expression longitudinally. Future

work with data captured both peripherally and centrally will be critical to extend these findings.

4.1 Conclusion

To conclude, we identified a gene, *RNASE6,* that modifies the association between *APOE*-ε4 and baseline cognition. In addition to supporting previous analyses implicating neuroinflammation in cognitive decline, our results suggest that data from blood transcriptomics can provide information about AD-relevant biological changes that may be occurring in the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

Aβ/Aβ1-42	amyloid-beta
AD	Alzheimer’s disease
AMP-AD	Accelerating Medicines Partnership - Alzheimer’s Disease
APOE	apolipoprotein E
bp	base pair
CERAD	Consortium to Establish a Registry for AD
CN	head of caudate nucleus
CNS	central nervous system

CSF	cerebrospinal fluid
DLPFC	dorsolateral prefrontal cortex
EFAM	epigenomic factor of activated microglia
GTEx	NIH Genotype-Tissue Expression Project
LOAD	late-onset Alzheimer's disease
MCI	mild cognitive impairment
NC	normal cognition
NFT	neurofibrillary tangles
PCC	posterior cingulate cortex
PMI	post-mortem interval
pTau	phosphorylated tau
QC	quality control
RIN	RNA integrity number
RNAseq	RNA sequencing
ROS/MAP	Religious Orders Study and Rush Memory and Aging Project
VMAP	Vanderbilt Memory and Aging Project

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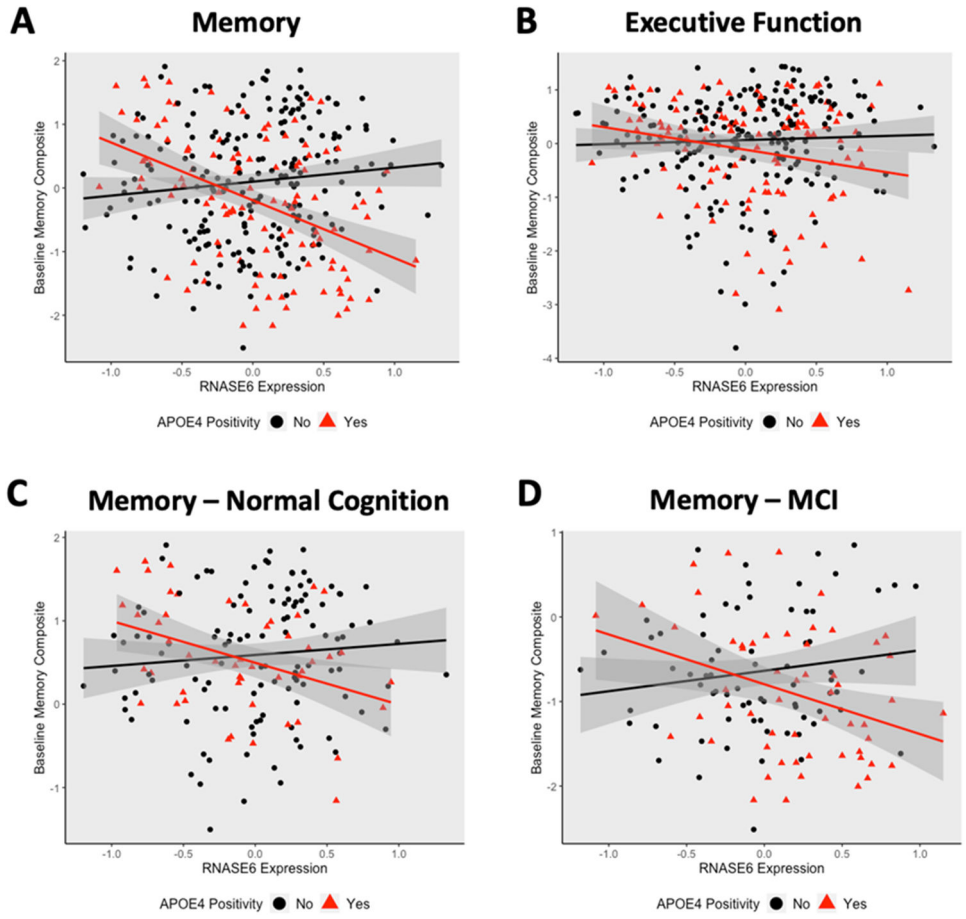

Fig. 1. *APOE*-ε4 allele carriers in VMAP have worse baseline cognition in the presence of higher levels of *RNASE6* expression.

A) A scatterplot demonstrating how *RNASE6* expression modifies the association between *APOE*-ε4 positivity and cognitive performance (β=−1.16, p=4.35x10^{-8}). Baseline composite memory scores are denoted on the y-axis; and the x-axis represents quantile normalized and batch controlled *RNASE6* expression in whole blood. Points and lines are colored by *APOE*-ε4 positivity where *APOE-ε4* carriers are denoted by the color red. B) Baseline executive function scores are denoted on the y-axis. *APOE*-ε4 carriers expressing higher levels of *RNASE6* also have worse baseline executive function (β=−0.54, p=0.008). C) A scatterplot including only individuals with normal cognition (β=−0.58, p=0.007). D) A scatterplot including only individuals with MCI (β=−0.87, p=0.002).

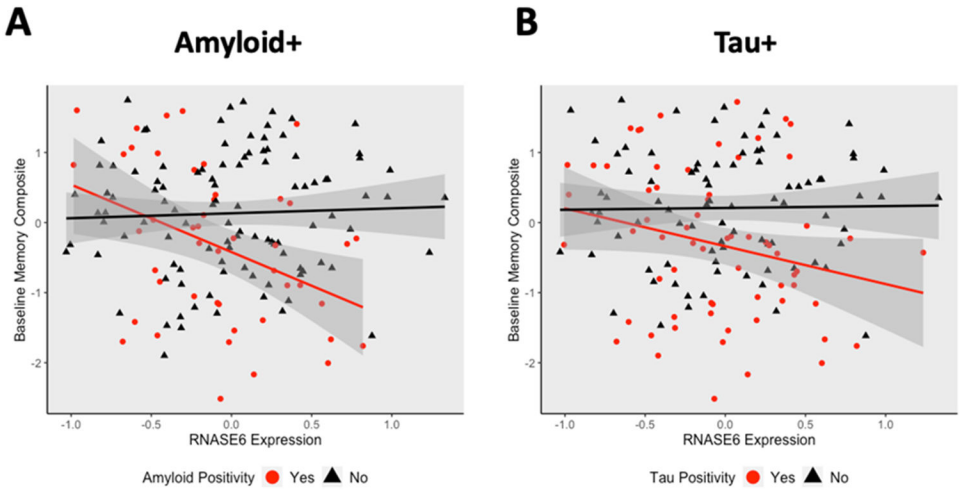

Fig. 2. Amyloid and tau positivity drives poorer cognitive performance at baseline

A) A scatterplot demonstrating how *RNASE6* expression modifies the association between amyloid positivity and baseline memory. Amyloid-positive individuals expressing higher levels of *RNASE6* have worse baseline memory than individuals who are not amyloid-positive (β=−1.14, p=0.001). Baseline composite memory scores are denoted on the y-axis; and the x-axis represents quantile normalized and batch controlled *RNASE6* expression in whole blood. Points and lines are colored by amyloid positivity where amyloid positivity is denoted by the color red. B) Tau-positive individuals expressing higher levels of *RNASE6* also have worse baseline memory than individuals who are not tau-positive (β=−0.63, p=0.04).

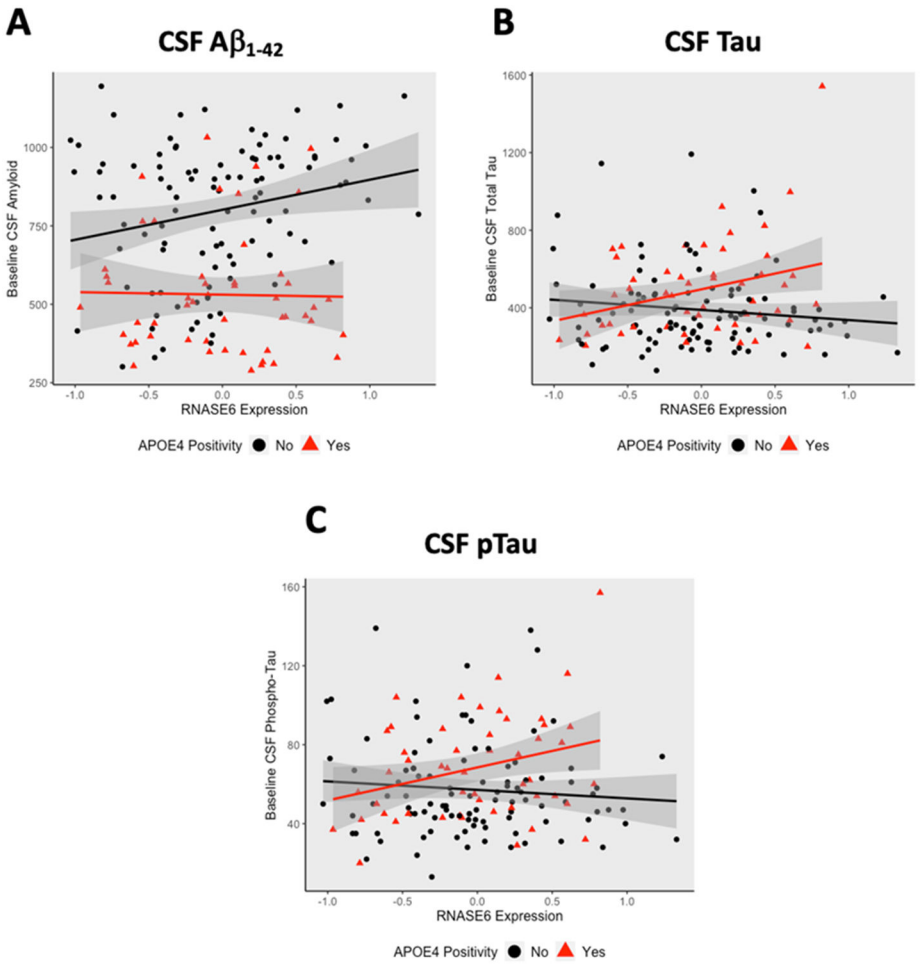

Fig. 3. CSF biomarker levels are modulated by *RNASE6* expression.

A) Brain amyloid burden is reduced in *APOE*-ε4 non-carriers when *RNASE6* expression is high (β= 91.8, p=0.02). Baseline CSF $A\beta_{1\text{-}42}$ levels are denoted on the y-axis and whole blood *RNASE6* expression is on the x-axis. CSF $A\beta_{1\text{-}42}$ levels have an inverse relationship with brain amyloid burden such that higher CSF $A\beta_{1\text{-}42}$ is indicative of lower brain amyloid levels. B) In contrast, CSF tau levels increase as *RNASE6* levels increase in *APOE*-ε4 carriers (β=230.1, p=0.003). C) CSF pTau levels also increase as *RNASE6* levels increase in *APOE*-ε4 carriers (β=22.7, p=0.01). In all plots, CSF biomarker levels are denoted on the y-axis; and the x-axis represents quantile normalized and batch controlled *RNASE6* expression in whole blood. Points and lines are colored by *APOE*-ε4 positivity where *APOE*-ε4 carriers are denoted by the color red.

Table 1:

Participant Demographics

	VMAP	ROS/MAP[a]	P
N	324	535	
Age in Years[b]	72.9 (7.3)	88.5 (6.6)	$< 1x10^{-5}$
Composite Cognition Score	−0.009 (0.97)[c]	−0.78 (1.04)[d]	
% Male	58.0	36.8	$< 1x10^{-5}$
Education in Years	15.8 (2.7)	16.4 (3.5)	0.02
% Normal Cognition	51.8	34.2	$< 1x10^{-5}$
# *APOE*-ε4 Alleles (0/1/2)	211 / 92 / 21	411 / 118 / 6	0.0002
% Amyloid Positive	30.4[e]	60.5	$< 1x10^{-5}$
% Tau Positive	43.0[e]	52.3	0.04

[a] Samples with dorsolateral prefrontal cortex RNAseq; demographics for other brain regions can be found at Supplementary Table 2

[b] VMAP: age at baseline, ROSMAP: age at death

[c] Memory composite score at baseline

[d] Global cognition composite score at last visit before death

[e] CSF measurements only available in 151 participants. Values are given as mean (SD) unless otherwise noted. Analysis of variance (ANOVA) analyses were performed to assess differences between the discovery (VMAP) and replication (ROS/MAP) datasets.

Table 2:

Sensitivity Analysis Results for the RNASE6 x *APOE*-ε4 Interaction on Baseline Memory

Analysis	**N**	**B**	**SE**	**P**
Original *RNASE6* x *APOE*-ε4 Interaction	324	−1.164	0.208	$4.35x10^{-8}$
Using Strict RNAseq QC	324	−1.197	0.209	$2.23x10^{-8}$
Participants with NC	168	−0.580	0.211	$6.57x10^{-3}$
Participants with MCI	128	−0.867	0.278	$2.30x10^{-3}$
Including Education as a Covariate	324	−1.042	0.206	$7.47x10^{-7}$

Abbreviations: NC = normal cognition, MCI = mild cognitive impairment, B = beta, SE = standard error, P = p-value, QC = quality control

Table 3:

Interaction Results of *RNASE6* Expression and *APOE*-ε4 Positivity on Global Cognition at Last Visit in ROS/MAP Brain Regions

Tissue	N	B	SE	P.unadjusted
DLPFC	535	−0.349	0.11	0.002
PCC	322	−0.167	0.18	0.36
CN	435	−0.080	0.17	0.63

Abbreviations: B = beta, SE = standard error, P = p-value, DLPFC = dorsolateral prefrontal cortex, PCC = posterior cingulate cortex, CN = head of the caudate nucleus.