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Association Between Common Variants in *RBFOX1*, an RNA-Binding Protein, and Brain Amyloidosis in Early and Preclinical Alzheimer Disease

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IMPORTANCE Genetic studies of Alzheimer disease have focused on the clinical or pathologic diagnosis as the primary outcome, but little is known about the genetic basis of the preclinical phase of the disease.

OBJECTIVE To examine the underlying genetic basis for brain amyloidosis in the preclinical phase of Alzheimer disease.

DESIGN, SETTING, AND PARTICIPANTS In the first stage of this genetic association study, a meta-analysis was conducted using genetic and imaging data acquired from 6 multicenter cohort studies of healthy older individuals between 1994 and 2019: the Anti-Amyloid Treatment in Asymptomatic Alzheimer Disease Study, the Berkeley Aging Cohort Study, the Wisconsin Registry for Alzheimer's Prevention, the Biomarkers of Cognitive Decline Among Normal Individuals cohort, the Baltimore Longitudinal Study of Aging, and the Alzheimer Disease Neuroimaging Initiative, which included Alzheimer disease and mild cognitive impairment. The second stage was designed to validate genetic observations using pathologic and clinical data from the Religious Orders Study and Rush Memory and Aging Project. Participants older than 50 years with amyloid positron emission tomographic (PET) imaging data and DNA from the 6 cohorts were included. The largest cohort, the Anti-Amyloid Treatment in Asymptomatic Alzheimer Disease Study (n = 3154), was the PET screening cohort used for a secondary prevention trial designed to slow cognitive decline associated with brain amyloidosis. Six smaller, longitudinal cohort studies (n = 1160) provided additional amyloid PET imaging data with existing genetic data. The present study was conducted from March 29, 2019, to February 19, 2020.

MAIN OUTCOMES AND MEASURES A genome-wide association study of PET imaging amyloid levels.

RESULTS From the 4314 analyzed participants (age, 52-96 years; 2478 participants [57%] were women), a novel locus for amyloidosis was noted within *RBFOX1* ($\beta = 0.61$, $P = 3 \times 10^{-9}$) in addition to *APOE*. The RBFOX1 protein localized around plaques, and reduced expression of *RBFOX1* was correlated with higher amyloid-β burden ($\beta = -0.008$, $P = .002$) and worse cognition ($\beta = 0.007$, $P = .006$) during life in the Religious Orders Study and Rush Memory and Aging Project cohort.

CONCLUSIONS AND RELEVANCE *RBFOX1* encodes a neuronal RNA-binding protein known to be expressed in neuronal tissues and may play a role in neuronal development. The findings of this study suggest that *RBFOX1* is a novel locus that may be involved in the pathogenesis of Alzheimer disease.

Supplemental content

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Alzheimer disease (AD) is a complex polygenic disease with high heritability. Genome-wide association studies (GWAS) have identified more than 25 risk loci that highlight amyloid processing, lipid metabolism, endocytosis, and innate immunity as important biological factors in the development of AD.[1,2] While much of the genetic work on AD has focused on clinical diagnosis as the primary outcome, AD is heterogeneous and has a long preclinical phase when brain amyloid deposition accumulates before the onset of cognitive impairment.[3]

The development of amyloid positron emission tomographic (PET) imaging tracers has provided a biomarker for diagnosis and risk assessment enabling in vivo detection of fibrillar amyloid-β before the onset of symptoms.[4] The approval by the US Food and Drug Administration of additional ligands facilitated the application of amyloid PET imaging in clinical practice and in research.[5] Advancing this biomarker, Jack et al[6] proposed a model in which brain amyloid-β deposition precedes the onset of neurodegeneration and cognitive dysfunction. This model also implied that an amyloid-β biomarker, such as PET imaging, could identify individuals at the highest risk for AD long before the diagnosis. Several previous genetic investigations of brain amyloidosis using amyloid PET imaging have found an association with the *APOE* locus.[7-11] However, to our knowledge, there has been no consistent confirmation of other loci.

Therapeutic efforts have begun to shift focus toward identifying and treating individuals in the preclinical phase of disease before onset of neurodegeneration and cognitive decline. Using a PET biomarker of brain amyloidosis to screen participants, the Anti-Amyloid Treatment in Asymptomatic Alzheimer Disease (A4 Study) clinical trial screened more than 4000 asymptomatic older individuals with amyloid PET imaging, of whom 1169 had elevated amyloid levels and were eligible for a prevention trial.[12,13] Clinical information and DNA from these at-risk, asymptomatic study participants provided an opportunity to identify novel genetic associations with brain amyloidosis during the preclinical phase of disease. In addition, the analyses of such data could provide insight into the mechanisms underlying cerebral amyloid accumulation.

Key Points

Question Is *RBFOX1* associated with brain amyloidosis, as measured by positron emission tomographic imaging, in early and preclinical Alzheimer disease?

Findings In this genetic association study, a meta-analysis of amyloid positron emission tomographic imaging data collected on 4314 participants in 6 studies noted genome-wide significant associations with single-nucleotide variants in a novel locus, *RBFOX1*, as well as in *APOE*. In addition, reduced expression of *RBFOX1* appeared to be associated with increased amyloid burden and global cognitive decline during life.

Meaning In this study, *RBFOX1* appeared to be a novel locus associated with positron emission tomographic imaging–derived brain amyloidosis and may be involved in the pathogenesis of Alzheimer disease.

Methods

In this genetic association study, participant data were acquired during the screening process in the A4 Study.[12,13] We also included other cohort studies: the Alzheimer Disease Neuroimaging Initiative (ADNI), the Berkeley Aging Cohort Study, the Wisconsin Registry for Alzheimer's Prevention (WRAP), the Biomarkers of Cognitive Decline Among Normal Individuals: the BIOCARD cohort, and the Baltimore Longitudinal Study of Aging (BLSA). Vanderbilt University and Columbia University institutional review boards approved the data analyses. The present study was conducted from March 29, 2019, to February 19, 2020. This study followed the Strengthening the Reporting of Genetic Association Studies (STREGA) reporting guideline for genotyping, population stratification, haplotype modeling, Hardy-Weinberg equilibrium, and replication.[14]

We also describe how the participant data were selected, how quantitative traits were harmonized before analyses, the statistical methods used, and the sources of data.

The A4 Study clinical trial began screening in 2014, recruiting healthy adults aged 65 to 85 years with amyloid PET imaging.[12,13] The ADNI study was launched in 2003 and has included more than 1500 participants aged 55 to 90 years with normal cognition, mild cognitive impairment, or AD. In 2001, WRAP began recruiting participants aged 40 to 65 years who had a parent with autopsy-confirmed or clinically verified AD.[15,16] The BIOCARD study enrolled middle-aged participants who were cognitively intact; 75% of the participants had a first-degree relative with AD. The study began in 1995, stopped in 2005, and was reestablished in 2009, with annual clinical and cognitive assessments.[17] The neuroimaging substudy of the BLSA began in 1994 and included participants without dementia aged 59 to 85 years who had up to 10 years of prospective data collection at baseline.[18] Amyloid imaging with PET and carbon 11 Pittsburgh Compound B (C^{11}PiB) was introduced into the study in 2005.[19] The Berkeley Aging Cohort Study began enrolling cognitively normal individuals recruited from the local community in 2005. For the amyloid PET imaging GWAS, we filtered each data set to individuals older than 50 years who had amyloid PET imaging (either C^{11}PiB or florbetapir) and genetic data available for analysis. Informed consent was obtained from participants in each study.

To validate genetic findings, we used autopsy data from the Religious Orders Study and Rush Memory and Aging Project (ROS/MAP), which were 2 harmonized longitudinal studies enrolling older adults without dementia who underwent annual clinical evaluations and organ donation at death.[20] Both studies were approved by an institutional review board of Rush University Medical Center. All participants in ROS/MAP signed an informed consent, an Anatomical Gift Act form, and a repository consent that allows their data to be repurposed. The Rush Alzheimer Disease Center resource sharing hub (https://www.radc.rush.edu/) and the Accelerating Medicines Partnership-AD Knowledge Portal (syn3219045) provided access to the data and are available on request with a data use agreement.

Genotyping was performed in each study on different platforms. Data from all cohorts underwent a quality control[21] process to filter variants not successfully genotyped (missing >5%), out of Hardy-Weinberg equilibrium ($P > 1 \times 10^{-6}$), or with low minor allele frequency (<1%). Participants were excluded for poor genotypic efficiency (missing >1% of variants) if reported and genotyped sex differed if cryptic relatedness was identified (removed second-degree or closer relatives) or if large-scale differences in ethnicity/race were identified by principal component detection. After these filters, imputation was performed using the European samples from the HRC r1.1.2016 reference panel (Build 37 Assembly 19) and SHAPEIT phasing on the Michigan imputation server.[22] Postimputation genotype data were filtered for imputation quality (R^2 >0.9) and minor allele frequency (<1%). A summary of the quality control process performed on each data set is reported in eTable 1 in the Supplement.

Amyloid PET Imaging Acquisition

Protocols for amyloid acquisition differed by site (eTable 2 in the Supplement). The A4 Study is a large, multisite trial with florbetapir F 18 (^{18}F) amyloid PET imaging data acquired 50 to 70 minutes postinjection. ADNI ^{18}F-florbetapir and C^{11}PiB data were acquired using a dynamic 3-dimensional scan on various scanner platforms with four 5-minute frames acquired 50 to 70 minutes postinjection. Berkeley C^{11}PiB data were acquired using a full dynamic protocol for 90 minutes (35 total frames) in a scanner (ECAT EXACT HR+ PET; Siemens). BIOCARD and BLSA C^{11}PiB data were acquired on a scanner (GE Advance; GE Healthcare) using a 70-minute dynamic protocol. Similarly, WRAP C^{11}PiB data were acquired on a scanner using a dynamic 70-minute protocol (ECAT EXACT HR+; Siemens). In all studies, images were reconstructed, averaged, spatially aligned, interpolated, and smoothed using study-specific pipelines. Mean standard uptake value ratio and distribution volume ratio calculations varied by site; all sites used whole or gray matter cerebellum as the reference region.

Harmonization of Amyloid Data

Harmonization was performed from composite cortical values within each site. To ensure that all amyloid values were on the same scale, we applied a gaussian mixture model[23] using a modification of a recently developed harmonization algorithm.[24] Gaussian mixture models were estimated among individuals who were cognitively normal within each cohort, and the mean (SD) was applied to the entire sample. In all cases, a 2-component model fit the data, confirming that global amyloid PET imaging followed a bimodal distribution reflecting amyloid-negative and amyloid-positive groups. Mean standard uptake value ratios were scaled and normalized using the mean and SD estimated from the predicted amyloid-negative gaussian distribution. The harmonization appropriately overlaid all data sets onto a common scale (eFigure 1 in the Supplement). As noted in the original harmonization manuscript, C^{11}PiB has a larger dynamic range compared with ^{18}F-florbetapir ligands, including a higher ceiling and wider distribution, particularly among amyloid-positive individuals.[24] Consistently, we observed higher values among the harmonized C^{11}PiB samples. An alternative approach to harmonization is to use the characteristics of both gaussian distributions to transform all C^{11}PiB values to ^{18}F-florbetapir values.[24] As a sensitivity analysis, we performed harmonization using this full transformation and compared results.

Data on RNA sequencing from the dorsolateral prefrontal cortex of individuals participating in ROS/MAP were used for validation of candidate genes from the GWAS analysis. Details of the RNA sequencing methods have been published previously.[25]

Autopsy measures of β-amyloid were quantified in ROS/MAP using immunohistochemistry.[26] Immunohistochemistry estimates of amyloid (anti-Aβ) were quantified from 8 brain regions, including the angular gyrus, hippocampus, entorhinal, inferior temporal, calcarine, middle frontal, superior frontal, and anterior cingulate cortices.

In ROS/MAP, a comprehensive neuropsychological protocol was completed at each study visit. For the present analysis, we leveraged both a global composite measure of cognition, quantified previously based on *z* scores from 17 total tests that assess 5 different cognitive domains (semantic memory, episodic memory, perceptual orientation, perceptual speed, and working memory)[27] and the Mini-Mental State Examination.[28]

Additional human brain tissues from Vanderbilt University Medical Center were obtained from decedents with AD (n = 5) and age-matched controls (n = 5) after approval of the Vanderbilt University Medical Institutional Review Board. Fixed tissue was sectioned at 50 µm on a vibratome (Leica Biosystems) to produce floating sections. Antigen retrieval was performed by heating sections to 95 °C in a borate buffer for 20 minutes. Sections were photobleached for 48 hours using a light-emitting diode microarry (HTG Supply), blocked in bovine serum albumin, 4%, and incubated with the primary antibody (anti-RBFOX1; Atlas, 1:100; Cathepsin B; R&D, 1:500; or pan-neurofilament; Biolegend, 1:150) overnight. After washing, sections were incubated with a conjugated secondary antibody (Alexa Fluor; Abcam, 1:1000) for 4 hours and then were washed, counterstained with methoxy-X04 (100 µM; Tocris) to identify amyloid-β and tau aggregates, and mounted to slides (Prolong Glass Antifade Mountant; Invitrogen). Images were produced on a laser scanning confocal microscope (LSM710; Zeiss) using ×20 or ×63 objectives and a minimum resolution of at least 1024 × 1024 pixels. Images then were processed (ImageJ).[29,30]

Statistical Analysis

Genome-wide association studies were completed using PLINK, version 1.9[31] and R, version 3.6.2 (R Project for Statistical Computing), with additive coding and the harmonized continuous amyloid PET metric set as a quantitative outcome. Genome-wide association studies were completed in each cohort separately. Covariates included age, sex, and the first 3 principal components to account for unmeasured population stratification. Meta-analyses of all results were performed using the inverse-weighted method in METAL.[32]

Table 1. Amyloid PET GWAS Participant Characteristics by Data Set

Characteristic	Mean (SD)[a]								
	A4 NHW	A4 AA	A4 Hispanic	ADNI	ADNI	Berkeley	BIOCARD	BLSA	WRAP
Amyloid acquisition	^{18}F-florbetapir	^{18}F-florbetapir	^{18}F-florbetapir	^{18}F-florbetapir	C^{11}PiB	C^{11}PiB	C^{11}PiB	C^{11}PiB	C^{11}PiB
No. of participants	2960	89	105	623	88	172	44	144	89
Women, No. (%)	1768 (60)	63 (71)	63 (60)	279 (45)	27 (31)	101 (59)	28 (64)	91 (63)	58 (65)
Normal cognition, No. (%)	2960 (100)	89 (100)	105 (100)	217 (33)	63 (72)	172 (100)	44 (100)	138 (96)	87 (98)
Age, y	71.4 (4.8)	70.3 (4.6)	71.9 (4.9)	74.6 (7.6)	76.5 (7.3)	74.4 (6.4)	70.8 (6.1)	77.2 (7.9)	67.3 (6.2)
APOE4 carriers, No. (%)	1057 (36)	33 (37)	33 (31)	255 (41)	45 (51)	48 (28)	14 (32)	39 (27)	35 (39)
Amyloid (standardized)	1.4 (2.5)	0.49 (1.5)	2.2 (4.5)	2.7 (3.4)	3.9 (3.0)	1.8 (4.1)	2.0 (4.5)	3.9 (6.4)	2.7 (5.1)
NC participants only	1.4 (2.5)	0.49 (1.5)	2.2 (4.5)	1.4 (2.8)	2.2 (2.6)	1.8 (4.1)	2.0 (4.5)	3.5 (6.1)	2.3 (4.7)
AD participants only	NA	NA	NA	5.2 (3.1)	5.1 (2.7)	NA	NA	15.3 (11.3)	NA

Abbreviations: A4, Anti-Amyloid Treatment in Asymptomatic Alzheimer Disease screening cohort; AA, African American; AD, Alzheimer disease; ADNI, Alzheimer Disease Neuroimaging Initiative; BIOCARD, Biomarkers of Cognitive Decline Among Normal Individuals cohort; BLSA, Baltimore Longitudinal Study of Aging; GWAS, genome-wide association studies; NA, not applicable; NC, normal cognition; NHW, non-Hispanic white; PET, positron emission tomographic; C^{11}PiB, Pittsburgh Compound B; WRAP, Wisconsin Registry for Alzheimer's Prevention.

[a] Analysis of variance indicated significant differences ($P < .001$) across cohorts for all demographic categories.

Results were restricted to variants present in all cohorts. Significance was set a priori to $P = 5 \times 10^{-8}$. The R packages EasyStrata,[33] qqman,[34] and Metafor[35] were used for data visualization, with additional variant-level visualization completed using LocusZoom.[36]

We used RNA sequencing data from ROS/MAP to validate candidate genes or loci. First, we assessed the association between gene expression and amyloid-β using linear regression. Immunohistochemistry measures of amyloid-β were square root transformed before analysis. Covariates included age at death, sex, and postmortem interval. For analyses of longitudinal cognitive performance, we performed a mixed-effects regression model with the same covariates. The interval (years prior to death) and intercept were entered as both fixed and random effects in all longitudinal models.

Results

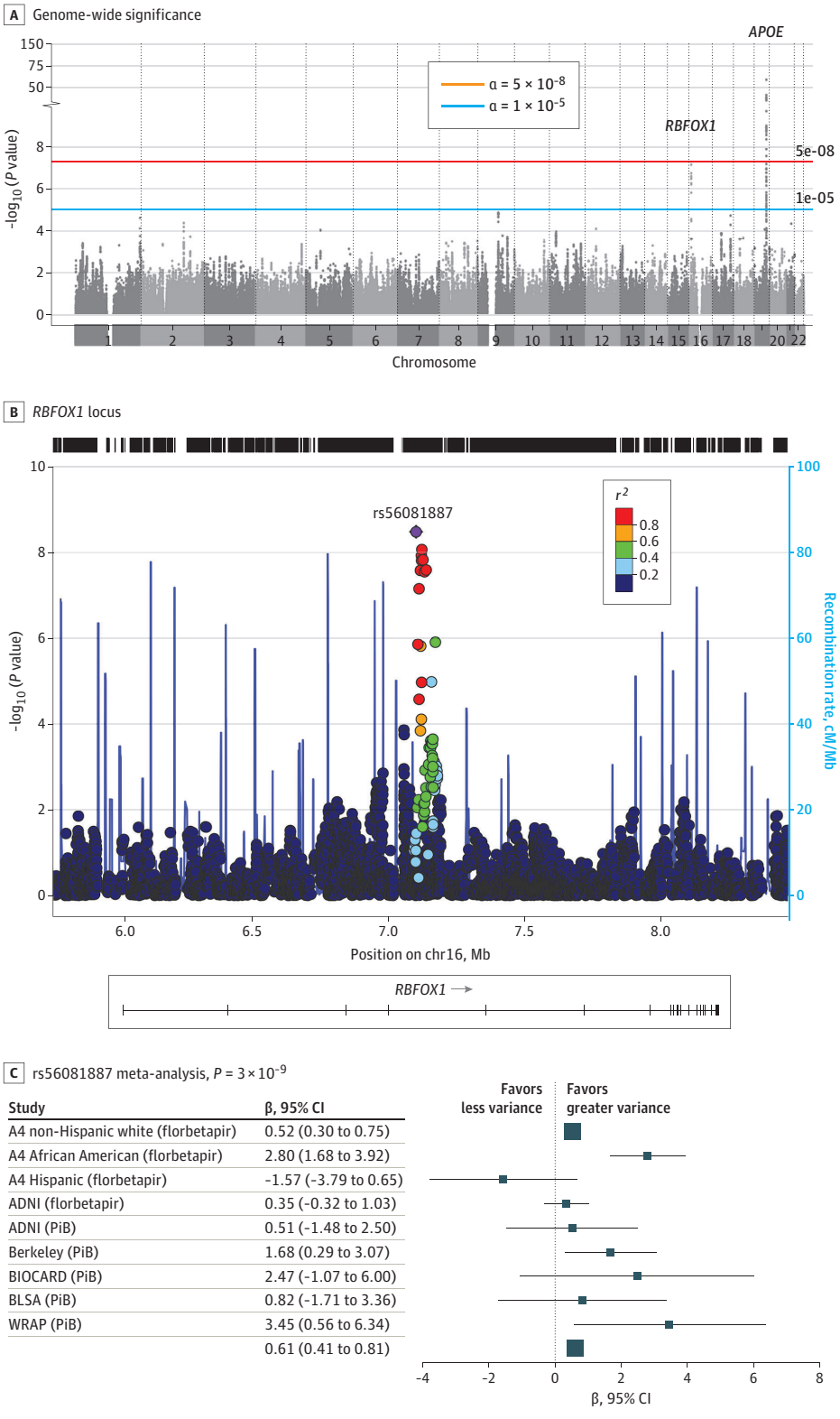
Clinical data for the 3154 individuals in the A4 Study included those whose race/ethnicity was determined genetically to be non-Hispanic white (n = 2960), African American (n = 89), and Hispanic (n = 105). In addition, 6 amyloid PET data sets with participants of non-Hispanic white ethnicity (n = 1160) were analyzed (**Table 1**). Together, the participants ranged from age 52 to 96 years; 2478 of the participants (57%) were women. With the exception of the 2 ADNI cohorts, 99% of the participants had normal cognition; with those cohorts added, cognition was normal in 90% of the participants. Analysis of variance of each demographic variable indicated significant differences across the cohorts (Table 1). For example, percent women ($F_{8,4305} = 10.8, P < .001$), age ($F_{8,4305} = 58.5, P < .001$), and percent *APOE*-positive ($F_{8,4305} = 3.2, P < .001$) were significantly different between groups.

Combining GWAS statistics and harmonized PET imaging amyloid data from each cohort, we completed a meta-analysis of all 6 studies to identify novel genetic associations with brain amyloid levels (n = 4314). We observed a robust association with brain amyloidosis at the *APOE* locus (top single-nucleotide variant [SNV; formerly SNP]: rs6857, β = 1.67, $P = 5.79 \times 10^{-132}$), similar in magnitude to previous reports.[7-11] To determine whether other genes in the *APOE* region contributed to the association, we performed conditional analyses covarying for *APOE ε4* and *APOE ε2* status. All associations in the region were no longer significant (eFigure 2 in the Supplement).

We observed a novel risk locus on chromosome 16p.13.3 (top SNV: rs56081887, β = 0.61, $P = 3 \times 10^{-9}$) that included *RBFOX1* (**Figure 1**A and B). Ten SNVs within *RBFOX1* reached genome-wide significance in meta-analysis; the top 2 are displayed in **Table 2**. *RBFOX1* variants were associated with increased amyloid levels in all data sets except for Hispanic individuals in the A4 Study (Figure 1C); however, the small sample size of the Hispanic cohort and the observation that a higher proportion of amyloid-positive individuals were Hispanic (40%) compared with the African American cohort (16%) precluded firm conclusions. All genome-wide significant SNVs in *RBFOX1* were in moderate to high linkage disequilibrium (non-Hispanic white r^2 all >0.84; African American r^2 all >0.53). Results for all variants with $P < 19 \times 10^{-5}$ are presented in eTable 3 in the Supplement. The corresponding QQ-plot is presented in eFigure 3 in the Supplement. There was no compelling evidence for an interaction with *APOE ε4*. Results were consistent when applying the alternative harmonization algorithm.

To validate and augment genetic findings, we analyzed RNA sequencing data from the prefrontal cortex in 600 individuals from the ROS/MAP study (**Table 3**). Lower levels of *RBFOX1* messenger RNA (mRNA) in prefrontal cortex were as-

Figure 1. Association of 2 Single-Nucleotide Variants in the *RBFOX1* Gene With Amyloid Levels



C rs56081887 meta-analysis, $P = 3 \times 10^{-9}$

Study	β, 95% CI
A4 non-Hispanic white (florbetapir)	0.52 (0.30 to 0.75)
A4 African American (florbetapir)	2.80 (1.68 to 3.92)
A4 Hispanic (florbetapir)	-1.57 (-3.79 to 0.65)
ADNI (florbetapir)	0.35 (-0.32 to 1.03)
ADNI (PiB)	0.51 (-1.48 to 2.50)
Berkeley (PiB)	1.68 (0.29 to 3.07)
BIOCARD (PiB)	2.47 (-1.07 to 6.00)
BLSA (PiB)	0.82 (-1.71 to 3.36)
WRAP (PiB)	3.45 (0.56 to 6.34)
	0.61 (0.41 to 0.81)

A, Genome-wide significance (α = 5 x 10^{-8}), suggestive (α = 1 x 10^{-5}). Gene symbols for suspected genes within locus. B, Regional plots of the *RBFOX1* locus. Points are colored by linkage disequilibrium with the top variant, denoted by the diamond shape. C, Associations across studies. Squares (point estimate) 95% CIs (line segments); size inversely related to the variance. chr, chromosome; cM/Mb, megabase; PiB, Pittsburgh compound B; Other abbreviations are expanded in note to Table 1.

sociated with a higher amyloid β burden (β = −0.008, *P* = .002) (eFigure 4 in the Supplement). Associations remained significant when covarying for differences in cell type composition across samples (eTable 4 in the Supplement). Lower *RBFOX1* mRNA levels were also associated with poorer global cognitive performance at the final visit before death (β = 0.007,

Table 2. Top 2 Genome-Wide *RBFOX1* Variants[a]

Single-nucleotide variant	Chr:BP	MAF	Gene	Function	Meta-analysis		
					No.	β (95% CI)	*P* value
rs56081887	16:6903160	0.09	*RBFOX1*	Intron	4314	0.61 (0.41-0.81)	3×10^{9}
rs34860942	16:6919189	0.09	*RBFOX1*	Intron	4314	0.59 (0.39-0.80)	8×10^{9}

Abbreviations: BP, base pair; Chr, chromosome; MAF, minor allele frequency.

[a] Top 2 outside of *APOE*.

Table 3. ROS/MAP Participant Characteristics

Characteristic	Brain tissue gene expression[a]
No. of participants	600
Age at death, mean (SD), y	88.61 (6.64)
Education, mean (SD), y	16.48 (3.51)
Women, No. (%)	384 (64)
Non-Hispanic white, No. (%)	586 (98)
APOE4 carriers, No. (%)	151 (25)
AD, No. (%)	215 (36)
Postmortem interval, mean (SD), h	6.83 (4.86)

Abbreviations: AD, Alzheimer disease; ROS/MAP, Religious Orders Study and Rush Memory and Aging Project.

[a] Gene expression data were collected from prefrontal cortex tissue of participants from ROS/MAP. Brain amyloid levels were measured using immunohistochemistry.

P = .006) and a faster rate of global cognitive decline across all study visits (β = 0.001, $P = 4 \times 10^{-5}$) (eFigure 5 in the Supplement). Expression of *RBFOX1* explained 1.5% of the variance in cognitive trajectories beyond covariates and remained statistically significant when covarying for amyloid and tau, which explained 5% and 15% of variance in cognitive trajectories, respectively. When assessing the results of the Mini-Mental State Examination for clinical interpretation, an SD decrease in *RBFOX1* was associated with an annual 0.2-point decrease in the Mini-Mental State Examination score.

In the microscopic evaluation, RBFOX1 protein localized to neurons in control brains and colocalized with neuropil threads inside dystrophic neurites surrounding amyloid plaques in AD brains (**Figure 2**). In addition, we observed some colocalization of RBFOX1 with neurofibrillary tangles in AD. Both observations support a potential role for RBFOX1 in AD pathogenesis.

Discussion

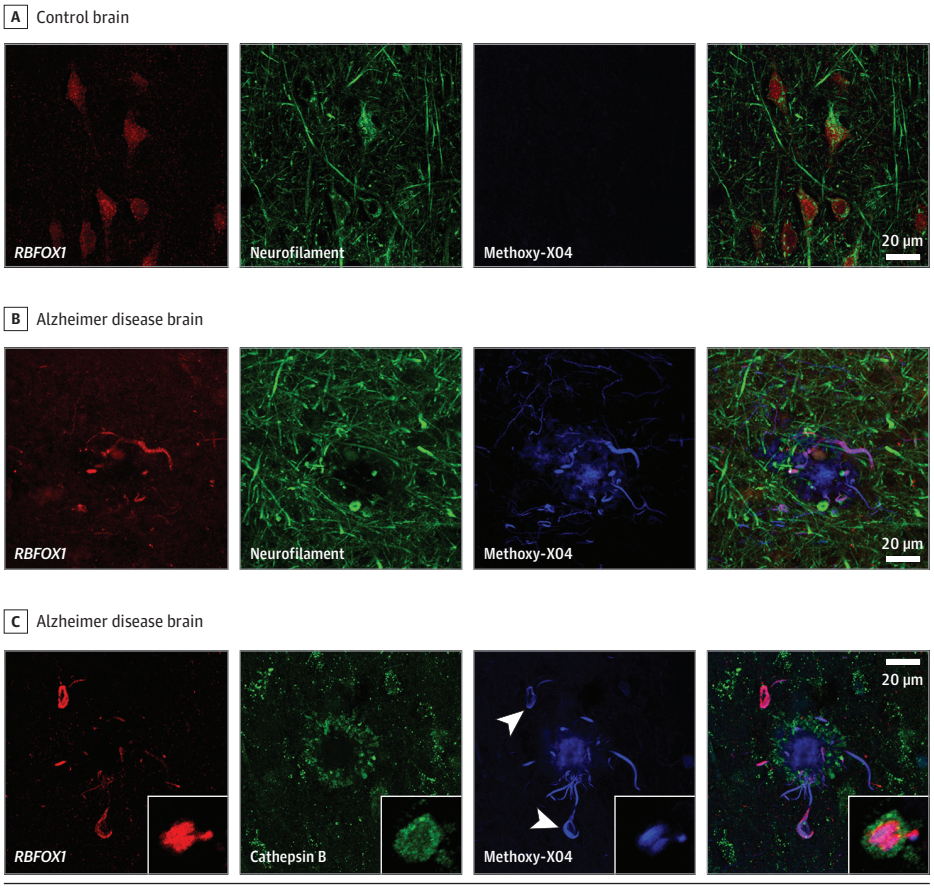
The goal of this investigation was to examine the genetic basis of brain amyloidosis in preclinical AD. Using a collection of 6 publicly available data sets in a meta-analysis, we replicated the previously reported association between *APOE* and brain amyloidosis. In addition, we identified a novel locus on chromosome 16p13.3, *RBFOX1*, which encodes ataxin-2-binding protein, an RNA-binding protein. In support of the genetic findings reported herein, evidence for an association between variants in the *RBFOX1* locus and AD were observed in an African American GWAS of AD (rs79537509, $P = 5.3 \times 10^{-7}$) (B. Kunkle, PhD, written communication, September 19, 2019), in a family-based study,[37] and in a study of cerebral glucose metabolism in ADNI.[38]

Previous studies have used amyloid PET imaging to investigate the genetic basis of brain amyloidosis. A meta-analysis of 3 PET-PiB GWAS (n = 983) showed an association with *APOE* but no other genome-wide significant loci.[8] In contrast, using ^{18}F-florbetapir PET imaging within the ADNI cohort, 2 GWAS studies by Ramanan et al[10,11] reported associations between brain amyloidosis and *APOE* and 2 other loci in a cross-sectional and longitudinal analysis, respectively: *BCHE* (butyrylcholinesterase) and *IL1RAP* (interleukin-1 receptor accessory protein). Although we observed an association for the *BCHE* SNV (rs509208, *P* = .007), the association was solely driven by the ADNI cohort. Therefore, neither previous locus was detected in the present study. The small sample size of previous studies likely limited the ability to detect the association with *RBFOX1*.

RBFOX1 encodes an RNA-binding protein expressed in muscle, heart, and neurons and is a member of the evolutionarily conserved Fox-1 family of RNA-binding proteins that bind to ataxin-2 and regulate alternative splicing.[39] In addition, mammalian RBFOX1 is present in the cytoplasm where it binds to 3 prime untranslated regions of multiple mRNAs, regulating their stability.[40] RBFOX1 is a highly conserved protein that can regulate splicing and transcriptional networks in human neuronal development, particularly in neuronal migration and synapse network formation within the cerebral cortex.[40,41] In addition to a potential role as the binding protein for ataxin-2 in spinocerebellar ataxia type 2, deletions and other structural variants in the *RBFOX1* gene increase the risk of generalized epilepsy, intellectual disability, autism spectrum disorder, and developmental disorders associated with aggression.[42-44]

While the exact mechanisms relating dysfunctional human RBFOX proteins with various neuropsychiatric disorders are not fully understood, there is evidence for multiple possible molecular causal pathways. Downregulation of RBFOX1 leads to destabilization of both nuclear and cytoplasmic mRNAs encoding for synaptic transmission proteins and loss of synaptic function in AD.[45,46] RBFOX1 may regulate al-

Figure 2. Microscopy of *RBFOX1*, Neuropil Threads, and Neurofibrillary Tangles



A, In postmortem control human brain tissue, *RBFOX* 1 (red) is localized to neurons (neurofilament, green). B, In Alzheimer disease brain, *RBFOX1* localizes to neuropil threads around β-amyloid plaques (methoxy-X04, blue). C, In Alzheimer disease brain, *RBFOX1* is present in tau tangles (arrowheads) and neuropil threads running through dystrophic neurites (cathepsin B, green) surrounding β-amyloid plaques (methoxy-X04, blue). Insets: cross-section through a dystrophic neurite showing lysosomes (green) surrounding a core of tau (blue) on which *RBFOX1* (red) is enriched. Scale bar is 20 µm. Width of inset box is 12 µm.

ternative splicing of *APP*,[47] which may be particularly relevant to the amyloid associations observed in the present analysis. Alternatively, downregulation of RBFOX1 in AD may directly affect the stability and abundance of mRNAs that encode synaptic transmission proteins.[45] Furthermore, because FOX1 and ataxin-2 are also present in the trans-Golgi network, a trafficking or recycling mechanism might be implicated. Clearly, additional experimental work will be needed to clarify the potential role of *RBFOX1* in brain amyloidosis and AD dementia. Aberrant colocalization of disease-associated proteins has been previously reported in other neurodegenerative diseases, such as the TDP-43 protein in amyotrophic lateral sclerosis and frontotemporal lobar degeneration.[48] We found colocalization of the RBFOX1 protein not only just around amyloid plaques but also with neurofibrillary tangles. These results imply that the protein may play a general role in AD-related proteinopathy.

We also observed associations between variants in the *APOE* region and brain amyloidosis, consistent with previous reports leveraging autopsy measures of neuropathologic characteristics,[49] cerebrospinal fluid biomarkers of amyloidosis,[50] and PET biomarkers of amyloidosis.[8,10,11] The locus surrounding APOE, chromosome 19q13.32, includes a number of potential genes, such as *TOMM40*, *APOC1*, and *PVRL2* (eFigure 2 in the Supplement), but conditional analyses indicated that the genetic association was driven by *APOE*. *APOE* is thought to relate to AD through an amyloid clearance pathway, with *APOE ε4* associated with earlier deposition of amyloid even during preclinical stages of disease.

Strengths and Limitations

The strengths of this study include the large sample size, the number of asymptomatic individuals allowing a focus on preclinical disease, and comprehensive validation analyses at the RNA and protein level. Study limitations include clinical heterogeneity across studies, overrepresentation of non-Hispanic white women with high levels of education, and our reliance on harmonized data acquired on different scanners and processed in different ways. Although we limited these factors statistically when possible, residual confounding cannot be ruled out.

Conclusions

To our knowledge, this is the largest GWAS of PET amyloid imaging; we report a novel genetic risk locus for brain amyloidosis within *RBFOX1*. Additional evidence at the transcript and protein level may further implicate *RBFOX1* as a novel genetic risk locus for brain amyloidosis and a candidate for early progression in AD.

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REFERENCES

1. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51(3):404-413. doi:10.1038/s41588-018-0311-9

2. Kunkle BW, Grenier-Boley B, Sims R, et al; Alzheimer Disease Genetics Consortium (ADGC); European Alzheimer's Disease Initiative (EADI); Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE); Genetic and Environmental Risk in AD/Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease Consortium (GERAD/PERADES). Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet*. 2019;51(3):414-430. doi:10.1038/s41588-019-0358-2

3. Vickers JC, Mitew S, Woodhouse A, et al. Defining the earliest pathological changes of Alzheimer's disease. *Curr Alzheimer Res*. 2016;13(3):281-287. doi:10.2174/1567205013666151218150322

4. Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. *Ann Neurol*. 2004;55(3):306-319. doi:10.1002/ana.20009

5. Yang L, Rieves D, Ganley C. Brain amyloid imaging—FDA approval of florbetapir F18 injection. *N Engl J Med*. 2012;367(10):885-887. doi:10.1056/NEJMp1208061

6. Jack CR Jr, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013;12(2):207-216. doi:10.1016/S1474-4422(12)70291-0

7. Vacher M, Porter T, Villemagne VL, et al. Validation of a priori candidate Alzheimer's disease SNPs with brain amyloid-beta deposition. *Sci Rep*. 2019;9(1):17069. doi:10.1038/s41598-019-53604-5

8. Yan Q, Nho K, Del-Aguila JL, et al; Alzheimer's Disease Neuroimaging Initiative (ADNI). Genome-wide association study of brain amyloid deposition as measured by Pittsburgh compound-B (PiB)-PET imaging. *Mol Psychiatry*. 2018. Published online October 25, 2018. doi:10.1038/s41380-018-0246-7

9. Li J, Zhang Q, Chen F, et al. Genetic interactions explain variance in cingulate amyloid burden: an AV-45 PET genome-wide association and interaction study in the ADNI Cohort. *Biomed Res Int*. 2015;2015:647389. doi:10.1155/2015/647389

10. Ramanan VK, Risacher SL, Nho K, et al; Alzheimer's Disease Neuroimaging Initiative (ADNI). GWAS of longitudinal amyloid accumulation on 18F-florbetapir PET in Alzheimer's disease implicates microglial activation gene IL1RAP. *Brain*. 2015;138(pt 10):3076-3088. doi:10.1093/brain/awv231

11. Ramanan VK, Risacher SL, Nho K, et al; Alzheimer's Disease Neuroimaging Initiative. *APOE* and *BCHE* as modulators of cerebral amyloid deposition: a florbetapir PET genome-wide association study. *Mol Psychiatry*. 2014;19(3):351-357. doi:10.1038/mp.2013.19

12. Sperling RA, Rentz DM, Johnson KA, et al. The A4 Study: stopping AD before symptoms begin? *Sci Transl Med*. 2014;6(228):228fs13. doi:10.1126/scitranslmed.3007941

13. Sperling RA, Donohue MC, Raman R, et al; A4 Study Team. Association of factors with elevated amyloid burden in clinically normal older individuals. *JAMA Neurol*. Published online April 6, 2020.

14. Little J, Higgins JP, Ioannidis JP, et al; Strengthening the Reporting of Genetic Association Studies. Strengthening the Reporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. *PLoS Med*. 2009;6(2):e22. doi:10.1371/journal.pmed.1000022

15. Sager MA, Hermann B, La Rue A. Middle-aged children of persons with Alzheimer's disease: *APOE* genotypes and cognitive function in the Wisconsin Registry for Alzheimer's Prevention. *J Geriatr Psychiatry Neurol*. 2005;18(4):245-249. doi:10.1177/0891988705281882

16. Johnson SC, Koscik RL, Jonaitis EM, et al. The Wisconsin Registry for Alzheimer's Prevention: a review of findings and current directions. *Alzheimers Dement (Amst)*. 2017;10:130-142. doi:10.1016/j.dadm.2017.11.007

17. Albert M, Soldan A, Gottesman R, et al. Cognitive changes preceding clinical symptom onset of mild cognitive impairment and relationship to ApoE genotype. *Curr Alzheimer Res*. 2014;11(8):773-784. doi:10.2174/1567205011080910121920

18. Resnick SM, Goldszal AF, Davatzikos C, et al. One-year age changes in MRI brain volumes in older adults. *Cereb Cortex*. 2000;10(5):464-472. doi:10.1093/cercor/10.5.464

19. Resnick SM, Sojkova J, Zhou Y, et al. Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [^{11C}]PiB. *Neurology*. 2010;74(10):807-815. doi:10.1212/WNL.0b013e3181d3e3e9

20. Bennett DA, Buchman AS, Boyle PA, Barnes LL, Wilson RS, Schneider JA. Religious Orders Study and Rush Memory and Aging Project. *J Alzheimers Dis*. 2018;64(s1):S161-S189. doi:10.3233/JAD-179939

21. Darst BF, Lu Q, Johnson SC, Engelman CD. Integrated analysis of genomics, longitudinal metabolomics, and Alzheimer's risk factors among 1,111 cohort participants. *Genet Epidemiol*. 2019;43(6):657-674. doi:10.1002/gepi.22211

22. Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and

methods. *Nat Genet*. 2016;48(10):1284-1287. doi:10.1038/ng.3656

23. Mormino EC, Betensky RA, Hedden T, et al; Alzheimer's Disease Neuroimaging Initiative; Australian Imaging Biomarkers and Lifestyle Flagship Study of Ageing; Harvard Aging Brain Study. Amyloid and *APOE* ε4 interact to influence short-term decline in preclinical Alzheimer disease. *Neurology*. 2014;82(20):1760-1767. doi:10.1212/WNL.0000000000000431

24. Properzi MJ, Buckley RF, Chhatwal JP, et al. Nonlinear distributional mapping (NoDiM) for harmonization across amyloid-PET radiotracers. *Neuroimage*. 2019;186:446-454. doi:10.1016/j.neuroimage.2018.11.019

25. De Jager PL, Ma Y, McCabe C, et al. A multi-omic atlas of the human frontal cortex for aging and Alzheimer's disease research. *Sci Data*. 2018;5:180142. doi:10.1038/sdata.2018.142

26. Bennett DA, Schneider JA, Tang Y, Arnold SE, Wilson RS. The effect of social networks on the relation between Alzheimer's disease pathology and level of cognitive function in old people: a longitudinal cohort study. *Lancet Neurol*. 2006;5(5):406-412. doi:10.1016/S1474-4422(06)70417-3

27. Yu L, Petyuk VA, Tasaki S, et al. Association of cortical β-amyloid protein in the absence of insoluble deposits with Alzheimer disease. *JAMA Neurol*. 2019;76(7):818-826. doi:10.1001/jamaneurol.2019.0834

28. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12(3):189-198. doi:10.1016/0022-3956(75)90026-6

29. Rueden CT, Schindelin J, Hiner MC, et al. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*. 2017;18(1):529. doi:10.1186/s12859-017-1934-z

30. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671-675. doi:10.1038/nmeth.2089

31. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559-575. doi:10.1086/519795

32. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26(17):2190-2191. doi:10.1093/bioinformatics/btq340

33. Winkler TW, Kutalik Z, Gorski M, Lottaz C, Kronenberg F, Heid IM. EasyStrata: evaluation and visualization of stratified genome-wide association meta-analysis data. *Bioinformatics*. 2015;31(2):259-261. doi:10.1093/bioinformatics/btu621

34. Turner SD. qqman: an R package for visualizing GWAS results using Q-Q and Manhattan plots. Preprint. bioRxiv 005165. Posted online May 14, 2014. doi:10.1101/005165

35. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw*. 2010;36(3):1-48. doi:10.18637/jss.v036.i03

36. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26(18):2336-2337. doi:10.1093/bioinformatics/btq419

37. Herold C, Hooli BV, Mullin K, et al. Family-based association analyses of imputed genotypes reveal genome-wide significant association of Alzheimer's disease with *OSBPL6*, *PTPRG*, and *PDCL3*. *Mol Psychiatry*. 2016;21(11):1608-1612. doi:10.1038/mp.2015.218

38. Kong LL, Miao D, Tan L, et al; Alzheimer's Disease Neuroimaging Initiative. Genome-wide association study identifies *RBFOX1* locus influencing brain glucose metabolism. *Ann Transl Med*. 2018;6(22):436. doi:10.21037/atm.2018.07.05

39. Auweter SD, Fasan R, Reymond L, et al. Molecular basis of RNA recognition by the human alternative splicing factor Fox-1. *EMBO J*. 2006;25(1):163-173. doi:10.1038/sj.emboj.7600918

40. Hamada N, Ito H, Iwamoto I, Morishita R, Tabata H, Nagata K. Role of the cytoplasmic isoform of RBFOX1/A2BP1 in establishing the architecture of the developing cerebral cortex. *Mol Autism*. 2015;6:56. doi:10.1186/s13229-015-0049-5

41. Fogel BL, Wexler E, Wahnich A, et al. *RBFOX1* regulates both splicing and transcriptional networks in human neuronal development. *Hum Mol Genet*. 2012;21(19):4171-4186. doi:10.1093/hmg/dds240

42. Lal D, Trucks H, Møller RS, et al; EMINet Consortium; EPICURE Consortium. Rare exonic deletions of the *RBFOX1* gene increase risk of idiopathic generalized epilepsy. *Epilepsia*. 2013;54(2):265-271. doi:10.1111/epi.12084

43. Bill BR, Lowe JK, Dybuncio CT, Fogel BL. Orchestration of neurodevelopmental programs by *RBFOX1*: implications for autism spectrum disorder. *Int Rev Neurobiol*. 2013;113:251-267. doi:10.1016/B978-0-12-418700-9.00008-3

44. Fernàndez-Castillo N, Gan G, van Donkelaar MMJ, et al. *RBFOX1*, encoding a splicing regulator, is a candidate gene for aggressive behavior. *Eur Neuropsychopharmacol*. 2020;30:44-55. doi:10.1016/j.euroneuro.2017.11.012

45. Alkallas R, Fish L, Goodarzi H, Najafabadi HS. Inference of RNA decay rate from transcriptional profiling highlights the regulatory programs of Alzheimer's disease. *Nat Commun*. 2017;8(1):909. doi:10.1038/s41467-017-00867-z

46. Lee JA, Damianov A, Lin C-H, et al. Cytoplasmic Rbfox1 regulates the expression of synaptic and autism-related genes. *Neuron*. 2016;89(1):113-128. doi:10.1016/j.neuron.2015.11.025

47. Alam S, Suzuki H, Tsukahara T. Alternative splicing regulation of APP exon 7 by RBFox proteins. *Neurochem Int*. 2014;78:7-17. doi:10.1016/j.neuint.2014.08.001

48. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006;314(5796):130-133. doi:10.1126/science.1134108

49. Beecham GW, Hamilton K, Naj AC, et al; Alzheimer's Disease Genetics Consortium (ADGC). Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. *PLoS Genet*. 2014;10(9):e1004606. doi:10.1371/journal.pgen.1004606

50. Deming Y, Li Z, Kapoor M, et al; Alzheimer's Disease Neuroimaging Initiative (ADNI); Alzheimer Disease Genetic Consortium (ADGC). Genome-wide association study identifies four novel loci associated with Alzheimer's endophenotypes and disease modifiers. *Acta Neuropathol*. 2017;133(5):839-856. doi:10.1007/s00401-017-1685-y