JAMA Neurology | Original Investigation

Analysis of Whole-Exome Sequencing Data for Alzheimer Disease Stratified by *APOE* Genotype

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IMPORTANCE Previous genome-wide association studies of common variants identified associations for Alzheimer disease (AD) loci evident only among individuals with particular *APOE* alleles.

OBJECTIVE To identify *APOE* genotype-dependent associations with infrequent and rare variants using whole-exome sequencing.

DESIGN, SETTING, AND PARTICIPANTS The discovery stage included 10 441 non-Hispanic white participants in the Alzheimer Disease Sequencing Project. Replication was sought in 2 independent, whole-exome sequencing data sets (1766 patients with AD, 2906 without AD [controls]) and a chip-based genotype imputation data set (8728 patients with AD, 9808 controls). Bioinformatics and functional analyses were conducted using clinical, cognitive, neuropathologic, whole-exome sequencing, and gene expression data obtained from a longitudinal cohort sample including 402 patients with AD and 647 controls. Data were analyzed between March 2017 and September 2018.

MAIN OUTCOMES AND MEASURES Score, Firth, and sequence kernel association tests were used to test the association of AD risk with individual variants and genes in subgroups of *APOE* ε4 carriers and noncarriers. Results with $P \leq 1 \times 10^{-5}$ were further evaluated in the replication data sets and combined by meta-analysis.

RESULTS Among 3145 patients with AD and 4213 controls lacking ε4 (mean [SD] age, 83.4 [7.6] years; 4363 [59.3.%] women), novel genome-wide significant associations were obtained in the discovery sample with rs536940594 in AC099552 (odds ratio [OR], 88.0; 95% CI, 9.08-852.0; $P = 2.22 \times 10^{-7}$) and rs138412600 in *GPAA1* (OR, 1.78; 95% CI, 1.44-2.2; meta-$P = 7.81 \times 10^{-8}$). *GPAA1* was also associated with expression in the brain of *GPAA1* (β = −0.08; $P = .03$) and its repressive transcription factor, *FOXG1* (β = 0.13; $P = .003$), and global cognition function (β = −0.53; $P = .009$). Significant gene-wide associations (threshold $P \leq 6.35 \times 10^{-7}$) were observed for *OR8G5* ($P = 4.67 \times 10^{-7}$), *IGHV3-7* ($P = 9.75 \times 10^{-16}$), and *SLC24A3* ($P = 2.67 \times 10^{-12}$) in 2377 patients with AD and 706 controls with ε4 (mean [SD] age, 75.2 [9.6] years; 1668 [54.1%] women).

CONCLUSIONS AND RELEVANCE The study identified multiple possible novel associations for AD with individual and aggregated rare variants in groups of individuals with and without *APOE* ε4 alleles that reinforce known and suggest additional pathways leading to AD.

JAMA Neurol. doi:10.1001/jamaneurol.2019.1456
Published online June 10, 2019.

Supplemental content

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The *APOE* (348 Entrez Gene) ε4 allele is consistently identified as the strongest common genetic factor contributing to the risk of late-onset Alzheimer disease (AD).[1-3] However, drugs targeting *APOE* have proven to be ineffective,[4] suggesting that *APOE* genotype might act as a proxy or biomarker[5] for the causal mechanism. Aleternatively, the influence of *APOE* on AD pathogenesis may have a role in multiple pathways leading to AD,[6] or is dependent on other genetic or nongenetic factors.[7] More than 30 additional AD loci have been identified by genome-wide association studies and bioinformatics approaches,[3,7-13] but their individual contributions to the total heritability of AD are comparatively small,[3] suggesting that many loci have escaped detection even in analyses of very large data sets. A previous study by the Alzheimer's Disease Genetics Consortium identified among individuals lacking the ε4 allele genome-wide significant association of AD with single-nucleotide variants (SNVs) in the region of *MAPT* (4137 Entrez Gene),[7] the gene encoding tau protein, which is central to AD hallmark abnormalities.[14] This finding suggests that other genes may exist whose effects on AD risk are masked by or dependent on particular *APOE* alleles. We applied this *APOE* genotype stratification analysis strategy in a study aimed at identifying novel associations with common and rare variants using a large whole-exome sequence (WES) data set from the Alzheimer Disease Sequencing Project.

Key Points

Question Are there rare variants associated with Alzheimer disease among individuals who possess or lack the *APOE* ε4 allele?

Findings This case-control, whole-exome sequencing study of 10 441 individuals identified a possibly novel association with a *GPAA1* variant among those who lacked the *APOE* ε4 allele, a finding that was replicated in independent data sets and supported by analyses of whole-genome and RNA sequencing data derived from human brain tissue. Novel associations were identified among individuals with the *APOE* ε4 allele for variants in *ISYNA1*, *OR8G5*, *IGHV3-7*, and *SLC24A3*.

Meaning This study supports the apparent involvement of genes in Alzheimer disease whose effects are dependent on *APOE* genotype.

Methods

Subjects

The discovery sample included 10 441 unrelated non-Hispanic white individuals (5522 with AD, 4919 cognitively normal controls) in the Alzheimer's Disease Sequencing Project case-control WES data set. The details of the study design, sequencing, and data quality control procedures were described previously.[15,16] In brief, participants were selected on the basis of a risk score that considers age, sex, and *APOE* genotype in a manner that maximized power for detection of novel AD risk and protective variants, but this ascertainment scheme yielded groups of patients with AD and controls that were not well balanced with respect to age and *APOE* genotype. An independent sample including WES data sets from the Alzheimer's Disease Exome Sequencing–France Project (subset of 1174 patients with late-onset AD, 1101 controls), the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium (592 patients with AD, 1805 controls), and the Alzheimer's Disease Genetics Consortium by genome-wide association studies data set (8728 patients with AD, 9808 controls) imputed to the Haplotype Reference Consortium panel was used for replication. Variants with imputation quality values (R^2) of 0.4 or less were excluded. Additional details of the replication data sets are reported elsewhere.[15] Functional tests of the top variants were conducted using whole-genome sequencing data obtained from participants (402 patients with AD, 647 controls) of the Religious Orders Study (ROS) or the Rush Memory and Aging Project (MAP). Details of the study design, as well as derivation of phenotype, genotype, and gene expression data, were described previously[17,18] and are briefly stated in the eMethods in the Supplement. Salient characteristics of the discovery and replication data sets are summarized in eTable 1 in the Supplement. Data were analyzed between March 2017 and September 2018. Written informed consent was obtained from all participants or their legal guardians. This study was approved by the Boston University Institutional Review Board.

Statistical Analysis

Genome-wide association analyses in the discovery sample were conducted separately in groups of individuals with and without an *APOE* ε4 allele (ie, $\varepsilon4^+$ and $\varepsilon4^-$). To have sufficient statistical power to detect associations with rare variants and minimize the number of tests, we limited our analyses of individual biallelic variants or short indels to those with a minor allele count of 10 or more, yielding 87 405 variants in the $\varepsilon4^+$ group and 123 178 variants in the $\varepsilon4^-$ group (eFigure 1 in the Supplement). Based on a total of 210 583 tests, the Bonferroni-corrected threshold for study-wise significance (SWS) was 2.37×10^{-7}. The association of each variant with AD risk was evaluated using 2 regression models. By design, there was a substantial difference in mean age between patients with AD and controls, so model 1 included only the first 10 ancestry principal components (PCs) to account for population substructure. Covariates for age, sex, and sequencing center, in addition to 10 PCs, were included in model 2 to account for dependencies on age, sex, and batch effects.[15] Analyses of single variants were conducted using the score test that was designed for extremely rare variants[19] implemented in the EPACTS software.[20]

Because the score test may overestimate the significance of some results, associations of the top-ranked variants were reevaluated using the Firth test, which better controls for type I error than the score test.[21] Association of single variants yielding score test *P* values $\leq 1 \times 10^{-5}$ in the discovery sample (a post hoc cutoff level that limits the number of tests in the replication stage and may yield a potentially SWS result after combining data from the discovery and replication samples) was evaluated in each replication data set using a generalized linear model implemented with the UGA tool's glm function[22] to

obtain the logistic regression coefficients and SEs that were input into the meta-analysis using the fixed-effects, inverse-weighted method in METAL.[23]

Gene-based tests were performed by aggregating variants with a minor allele frequency less than 5% (except for singletons) that were annotated as having high or moderate influence on the encoded protein as previously described.[15] In brief, variants with high influence are those classified by variant effect predictor or single-nucleotide polymorphism (SNP) effect as splice acceptor, splice donor, stop gained, frameshift, stop lost, start lost, or transcript amplification, and variants with moderate influence are those annotated as inframe insertion, inframe deletion, missense variant, or protein altering.[24,25] Genes with a cumulative minor allele count of 10 or more were included in the analysis, yielding a total of 78 779 tests across 6 groups with variants aggregated by ε4 status and functional influence (all, high + moderate, high) and a corresponding Bonferroni-corrected SWS threshold of 6.35×10^{-7}. The combined association of multiple variants in each gene with the risk of AD was evaluated using the optimal sequence kernel association test[26] with default rho settings implemented in the seqMeta R package.[27] Gene-based association results with *P* values $< 1 \times 10^{-5}$ in the discovery sample were further evaluated in each replication data set using the same optimal sequence kernel association test settings as in the discovery analysis. Because not all replication data sets were analyzed using seqMeta or EPACTS, gene-based *P* values were combined across data sets using the *z*-score approach in METAL.

Potential functional significance of genome-wide significant variants was investigated by applying multiple analytical approaches using data on gene expression in brain tissue, cognitive performance, and AD-related neuropathologic changes obtained from autopsied patients in the ROSMAP Project.[18] First, we tested the association of a variant on the cell-type gene expression modules–adjusted residuals of expression in brain that were adjusted for source of sample (ROS or MAP studies), postmortem interval, 3 PCs of ancestry, age at death, sex, RNA integrity number, number of ribosomal bases, and number of aligned reads in a manner described previously using a general linear regression model.[18] Next, we evaluated the influence of the variant on a test of global cognition function and neuropathologic measures of neuritic plaques and neurofibrillary tangles adjusted for source of sample, postmortem interval, PCs, age at death, and sex. The association of *GPAA1* variants with transcriptional and post-transcriptional mechanisms was explored using genomic information in the Regulatory Build of Ensembl (Human GRCh38.p12).[28,29] Significance was determined using a 2-tailed unpaired analysis model and a significance threshold of $P < .05$.

Results

After quality control, there remained 2377 AD cases and 706 controls (mean [SD] age, 75.2 [9.6] years; 1668 [54.1%] women) who had the *APOE* ε4 allele (ε4$^+$) and 3145 AD cases and 4213 controls (mean 83.4 [7.6]; years; 4363 [59.3%] women) who lacked the ε4 allele (ε4$^-$). The quantile-quantile plots indicated modest inflation of *P* values for the regression model, including covariates for PCs (model 1) in both the *APOE* ε4$^+$ ($\lambda = 1.083$) and ε4$^-$ ($\lambda = 1.062$) groups (eFigure 1 in the Supplement), whereas there was no evidence of inflation for the model with additional adjustment for age, sex, and sequencing center (model 2) in either the *APOE* ε4$^+$ ($\lambda = 0.994$) or ε4$^-$ ($\lambda = 0.998$) groups. A total of 22 variants (12 in the ε4$^+$ group, 10 in the ε4$^-$ group) showed suggestive evidence of association ($P \le 1 \times 10^{-5}$) in at least 1 model, and these variants were further evaluated in the replication data sets (eFigure 2, eTable 2, and eTable 3 in the Supplement).

Meta-analysis of the results from the discovery and replication cohorts showed that the association of AD risk with several SNVs in 1 novel locus, *ISYNA1*, under model 1 was nearly SWS in the *APOE* ε4$^+$ group (top SNV rs2303697, $P = 4.61 \times 10^{-7}$) (eTable 3, eFigure 3B in the Supplement). The rs2303697 minor (*T*) allele was protective in all cohorts except for CHARGE (eTable 3, eFigure 3B in the Supplement). In the discovery sample, the interaction between rs2303697 and *APOE* ε4 status was significant (interaction $P = 3.88 \times 10^{-5}$) (eTable 2 in the Supplement), and the *T* allele showed a significant protective effect in *APOE* ε4$^+$ participants (odds ratio [OR], 0.73; 95% CI, 0.64-0.84; $P = 3.49 \times 10^{-6}$), but it was not associated with AD risk in ε4$^-$ participants (OR, 1.00; 95% CI, 0.93-1.04; $P = .98$) (eFigure 3A in the Supplement).

In the *APOE* ε4$^-$ group, SWS associations were observed with variants in the established AD loci *TREM2* (54209 Entrez Gene) (top SNV rs75932628: OR, 3.66; 95% CI, 2.36-5.68; $P = 6.84 \times 10^{-9}$) and *MAPT* (top SNV rs62063857: OR, 1.17; 95% CI, 1.1-1.23; $P = 1.59 \times 10^{-7}$) (**Table 1**). The associations for rs75932628 and rs62063857 were only nominally significant in the *APOE* ε4$^+$ group (eTable 2, eFigure 4 and eFigure 5 in the Supplement). The SWS associations were also identified with variants in novel loci including *NSF* (4905 Entrez Gene) (top SNV rs199533, OR, 0.86; 95% CI, 0.82-0.91; $P = 1.66 \times 10^{-7}$), *GPAA1* (8733 Entrez Gene) (top SNV rs138412600: OR, 1.78; 95% CI, 1.44-2.2; $P = 7.81 \times 10^{-8}$), and *AC099552* (18873975 Entrez Gene) (top SNV rs536940594: OR, 88.0; 95% CI, 9.08-852.0; $P = 2.22 \times 10^{-7}$) (Table 1; eTable 2 and eFigures 5, 6, and 7 in the Supplement), noting that the *MAPT* and *NSF* SNPs are in high linkage disequilibrium ($r^2 = 0.85$) (eFigure 5 in the Supplement) and results for rs536940594 were not available in the replication data sets. Among these 4 variants, only *GPAA1* rs138412600 showed a significant interaction with *APOE* ε4 status (interaction $P = 8.12 \times 10^{-4}$; OR, 3.01; 95% CI, 1.58-5.72); the minor A allele was significantly associated with increased AD risk in the ε4$^-$ group (OR, 2.13; 95% CI, 1.59-2.87; $P = 3.91 \times 10^{-7}$) but had a nonsignificant protective effect in the ε4$^+$ group (OR, 0.64; 95% CI, 0.31-1.35; $P = .24$) (eTable 2 in the Supplement). The deleterious effect of the A allele in the ε4$^-$ group was consistently observed in all replication cohorts (**Figure**, B).

The rs138412600 variant is located within the promoter region of *GPAA1*, which is expressed in many cell types. Bioinformatic analysis revealed that the A allele (37th nucleotide within exon 2) binds exclusively to the 13th nucleotide in the motif of the DNA binding site for its repressive transcription

Table 1. Study-Wide Significant ($P \leq 2.37 \times 10^{-7}$) Associations With Individual Variants in the *APOE* ε4⁻ Group

Chr	Position[a]	rsID	EA	Gene	GnomAD MAF (%)	Discovery				Replication[b]		Discovery + Replication[b]	
						MAC	MAF (%)	OR (95% CI)[b]	*P* Value[c]	*P* Value[b]	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
6	41,129,252	rs75932628[d]	T	*TREM2*	0.2	68	0.50	4.85 (2.74-8.60)	2.12×10^{-9}	4.59×10^{-8}	2.40 (1.20-4.81)	.01	3.66 (2.36-5.68)	6.84×10^{-9}
7	154,988,675	rs536940594[e]	A	*AC099552*	0	10	0.10	88.0 (9.08-852.0)	2.22×10^{-7}	9.47×10^{-5}	NA	NA	NA	NA
8	145,138,063	rs138412600[e]	A	*GPAA1*	2	239	2.00	2.13 (1.59-2.87)	2.70×10^{-7}	3.91×10^{-7}	1.47 (1.08-1.98)	.01	1.78 (1.44-2.2)	7.81×10^{-8}
17	44,076,665	rs62063857[d]	A	*MAPT*	18.8	2063	16.33	1.25 (1.14-1.37)	1.83×10^{-6}	4.23×10^{-6}	1.12 (1.04-1.2)	2.03×10^{-3}	1.17 (1.1-1.23)	1.59×10^{-7}
17	448,28,931	rs199533[d]	A	*NSF*	17.8	2983	20.35	0.82 (0.76-0.89)	3.97×10^{-6}	4.28×10^{-6}	0.9 (0.83-0.96)	3.38×10^{-3}	0.86 (0.82-0.91)	1.66×10^{-7}

Abbreviations: AD, Alzheimer disease; Chr, chromosome; EA, effect allele; GnomAD, Genome Aggregation Database; MAC, minor allele count; MAF, minor allele frequency; NA, not available; OR, odds ratio; rsID, rs number.

[a] Position based on genome build GRCh37.

[b] Odds ratios, 95% CIs, and *P* values are based on generalized linear model.

[c] *P* value for score test.

[d] All statistics and estimates based on model 1, which adjusted only for principal components.

[e] All statistics and estimates based on model 2, which adjusted for age, sex, and principal components.

factor, *FOXG1* (2290 Entrez Gene), and inclusion of exon 2 in the transcript is necessary but not sufficient for high expression of *GPAA1* (Figure, A). Functional analysis of whole-genome sequencing data from ROSMAP showed that, particularly among *APOE* ε4⁻ participants, the A allele was significantly associated with higher expression of *FOXG1* (β = 0.13, *P* = .003) (Figure, B) and lower expression of *GPAA1* (β = −0.08, *P* = .03) (Figure, C), but not associated with expression of the *GPAA1-202* isoform, which lacks the second exon. The A allele was associated with lower global cognition function (β = − 0.53, *P* = .009) (Figure, D) in the *APOE* ε4⁻ group, but not in the *APOE* ε4⁺ group (β = 0.70, *P* = .12).

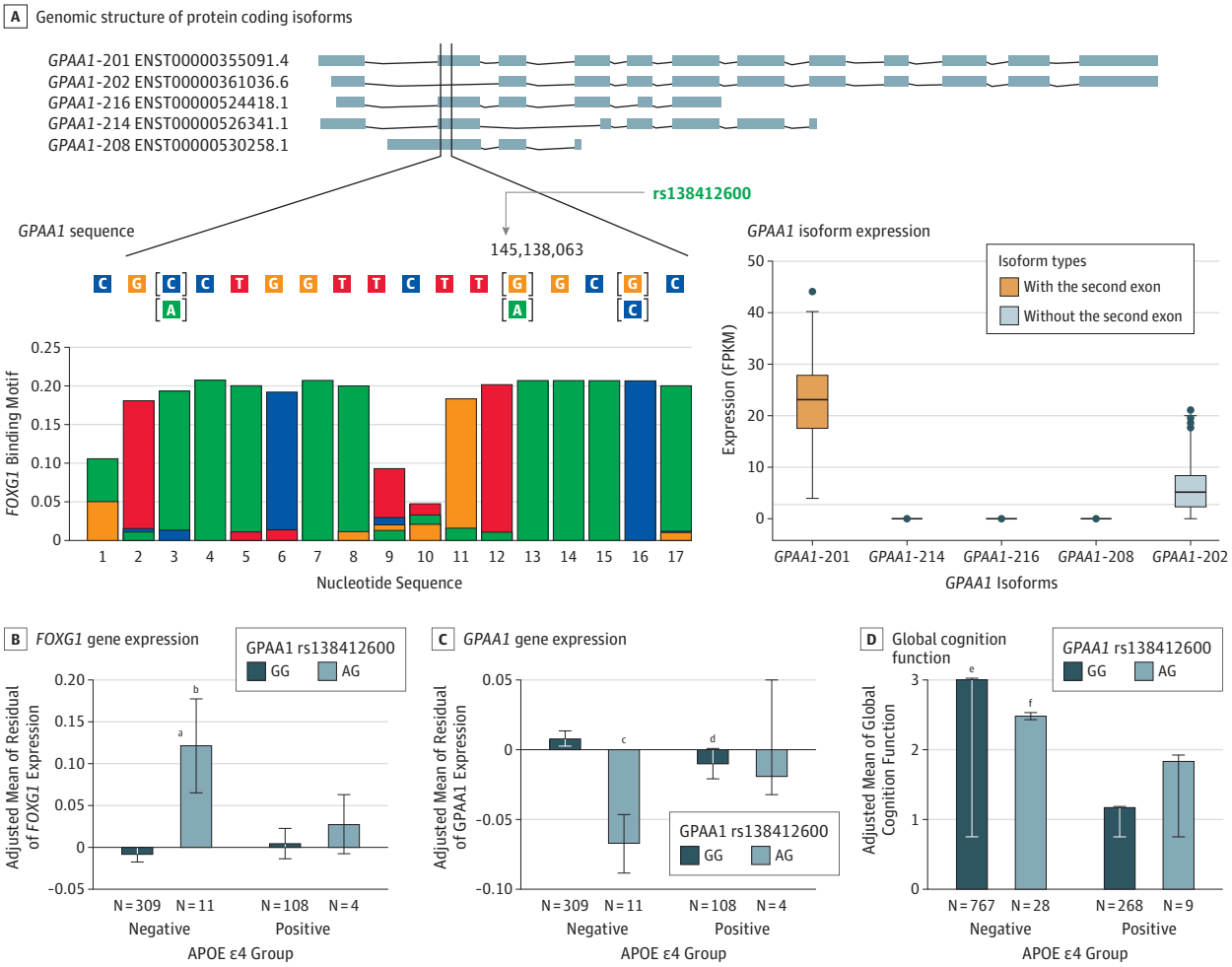
Gene-based analysis conducted in the discovery data set yielded 4 SWS significant ($P \leq 2.37 \times 10^{-7}$) loci in the ε4⁺ group, including *OR8G5* (219865 Entrez Gene; ε4⁺ $P = 4.67 \times 10^{-7}$; ε4⁻ *P* = .93), *IGHV3-7* (28452 Entrez Gene; ε4⁺ $P = 9.75 \times 10^{-16}$; ε4⁻ *P* = .46), and *SLC24A3* (57419 Entrez Gene; ε4⁺ $P = 2.67 \times 10^{-12}$; ε4⁻ *P* = .15) (**Table 2**). Gene-based test results for these loci in the replication sample were not significant in the ε4⁺ group; however, inspection of the counts for each variant included in these tests revealed that the sentinel variants, which primarily accounted for the significance in the discovery sample, were not observed in the replication data sets (eTable 4 in the Supplement). *TREM2* was the only SWS gene in the ε4⁻ group in both the discovery ($P = 2.75 \times 10^{-8}$) and replication ($P = 2.25 \times 10^{-4}$) samples.

Discussion

Our WES study of more than 16 000 patients with AD and 17 500 controls confirmed the association of AD risk with variants in the *MAPT* region among participants lacking ε4, which had been established previously by analysis of common variants.[7] We also identified SWS associations with the well-established AD risk variant *TREM2* R47H[30,31] and variants in several novel loci, including *GPAA1* and *NSF* among ε4⁻ participants. We also showed a possible association of AD with a variant in another novel locus, *ISYNA1* ($P = 4.61 \times 10^{-7}$) that approached the SWS threshold ($P < 2.37 \times 10^{-7}$) among participants with ε4⁺ in the combined discovery and replication samples. Analysis of aggregated rare variants identified possibly novel associations with *OR8G5*, *IGHV3-7*, and *SLC24A3* among ε4⁺ individuals in the discovery sample, but the replication data sets were not suitable for replication testing because the sentinel variants accounting for the associations were either not present or not well imputed in these samples. Thus, these gene-based findings should be considered tentative. This limitation of gene-based tests of rare variants was previously recognized[15] and indicates the need for much larger replication samples or collating and simultaneous recalling variants in deep-sequencing data sets.

GPAA1 encodes glycosylphosphatidylinositol (GPI) anchor attachment 1 protein, which is a subunit of the protein complex of GPI transamidase. GPI transamidase supports the GPI translational modifications of GPI-anchored proteins.[32] The associated variant, rs138412600, encodes amino acid 37 of the protein, which is located within exon 2

Figure. Functional Analysis of *GPAA1* rs138412600



The top panel shows the genomic structure of 5 protein coding isoforms with wild-type full length on the top and the alternative ones below (A). The expression levels of these 5 isoforms in postmortem dorsolateral prefrontal cortex (DLPFC) samples obtained from 423 ROSMAP participants aged 70 years or older (mean age at death, 88.5 years, and 38% patients with AD) are presented in the adjacent box plot. The rs138412600 single-nucleotide polymorphism is located in a 17-nucleotide motif sequence (chr8: 145 138 051-145,138,067) for the DNA binding site of transcription factor *FOXG1*. The reference sequence for this region is presented with genetic variants shown in brackets and highlighted with color according to their functional influence (yellow, missense; green, synonymous). Alternative alleles are shown below the wild-type alleles. The sequence of the corresponding *FOXG1* binding motif was downloaded from the Ensembl website and is presented under the *GPAA1* sequence using color-coding for the 4 nucleotides (green, A; red, T; blue, C; and orange, G). The x-axis indicates the nucleotide position in the motif and the y-axis indicates the bit score that represents the certainty of the enrichment of the nucleotide at each location. *APOE* ε4-dependent effects of rs138412600 on expression of *FOXG1* (B) and *GPAA1* (C), as well as on global cognition function (D) measured in ROSMAP participants. Homozygotes of the major allele (*GG*) and heterozygotes (*AG*) are shown with dark blue and light blue bars, respectively.

[a] $P < .01$ compared with negative GG.

[b] $P < .01$ compared with positive GG.

[c] $P < .05$ compared with negative GG.

[d] $P < .05$ compared with positive GG.

[e] $P < .01$ compared with negative AG.

[f] $P < .05$ compared with positive AG.

that encodes a portion of the functional luminal loop (from start to amino acid 282).[33,34] Although our analyses did not indicate that this variant is associated with expression of the alternative *GPAA1* isoform resulting from splicing of this exon, we demonstrated that the minor allele *A* is significantly associated with increased expression of the transcription factor *FOXG1*, which binds to *GPAA1*. *FOXG1* is a repressive transcription factor with restricted expression in neuronal cells and strong expression in the developing dentate gyrus and hippocampus.[35] *FOXG1* mutations cause the congenital form of Rett syndrome,[36] a severe neurodevelopmental disorder. Our results also suggest that rs138412600 is associated with global cognition function. Expression of *GPAA1* in hippocampus was reported to be upregulated after spatial training in a calcium/calmodulin kinase β mutant mouse model.[37] Studies of this model suggested that calcium/calmodulin kinase β has a male-specific function in hippocampal memory formation.[38]

Table 2. Study-Wide Significant Gene-Based Test Results

APOE ε4 Group	Chr	Start	End	Gene	SNV Influence	Model[a]	No. Variants	cMAC	Sentinel Variant	No.	*P* Value
With ε4	11	124,134,751	124,135,752	*OR8G5*	High + moderate	2	3	267	rs200328143	3082	4.67×10^{-7}
	14	106,518,415	106,518,824	*IGHV3-7*	All	2	4	11	rs188349361	2870	9.75×10^{-16}
	20	19,261,606	19,261,724	*SLC24A3*	All	2	2	68	rs3790174	3082	2.67×10^{-12}
Without ε4	6	41,126,505	41,130,815	*TREM2*	High + moderate	1	14	310	rs75932628	6656	2.75×10^{-8}

Abbreviations: Chr, chromosome; cMAC, cumulative minor allele count; SNV, single-nucleotide variant.

[a] Model 1: adjusted only for principal components; model 2, adjusted for age, sex, and principal components.

Our association finding in the total sample with the *ISYNA1* variant, rs2303697, in the APOE ε4$^+$ group is also noteworthy. *ISYNA1* encodes inositol-3-phosphate synthase 1, a rate-limiting enzyme that catalyzes the conversion from glucose-6-phosphate to myoinositol (MI) 1-phosphate,[39] which is a component of plasma membrane phospholipids and functions as a cell signaling molecule. Glucose is the major energy source for brain and the reduction of brain glucose metabolism is a prominent feature of AD.[40] The level of brain MI detected by magnetic resonance spectroscopy in patients with AD has been shown to be positively correlated with total and phosphorylated tau, but not Aβ in cerebrospinal fluid.[41] A 7-year, longitudinal study of individuals aged 69 to 89 years who were cognitively normal at baseline found that the ratio of *N*-acetyl aspartate to myoinositol in the posterior cingulate cortex was significantly decreased in individuals who subsequently developed AD, mild cognitive impairment, and dementia with Lewy bodies compared with those who remained cognitively normal.[42] This study also showed that the *N*-acetyl aspartate/MI ratio was significantly lower among individuals with vs without *APOE* ε4. Evidence for a connection between myoinositol and *APOE* genotype is also suggested by a cross-sectional study showing that the ratio of MI to creatine was significantly higher in an elderly group of ε4$^+$ compared with ε4$^-$ participants who had normal cerebrospinal fluid Aβ42 levels.[43]

The association of AD with the *NSF* rs199533 variant, which was SWS among ε4$^-$ individuals, may not be an independent signal because of the high linkage disequilibrium between rs199533 and the *MAPT* rs62063857 variant (r^2 = 0.85). Nonetheless, the protein encoded by *NSF* (*N*-ethylmaleimide-sensitive factor) may be functionally related to AD because it is an adenosine triphosphatase that is involved in cellular membrane fusion events, including vesicle-mediated protein transport, exocytosis of neurotransmitters, and reassembly of the Golgi apparatus during mitosis.[44] It has been shown that proteins in the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors complex are essential for neuronal Aβ release at presynaptic terminals.[45] The explanation for the stronger association of rs199533 with AD risk among persons lacking ε4 is unclear, but warrants further study.

The most significant finding was observed with *IGHV3-7* among participants with ε4$^+$ in a gene-based test including 4 aggregated variants ($P = 9.75 \times 10^{-16}$). *IGHV3-7* encodes one of the immunoglobulin heavy variable chains and is a good candidate given its functional similarity to *IGHG3* (3502 Entrez Gene) and *IGHJ6* (28475 Entrez Gene), 2 of the top associated genes in the Alzheimer Disease Sequencing Project WES sample including participants with and without ε4,[15,46] and to *IGHV1-67* (28463 Entrez Gene), which was identified as an AD locus in a large genome-wide association study performed by the International Genomics of Alzheimer Project,[11] as well as evidence that antibodies to IgG cross-react with fibril and oligomer amyloid-β aggregates.[47]

Limitations

Our study has several limitations. First, the WES discovery sample for the present study (n = 10 441) is more than 5 times smaller than that for a previous chip-based *APOE* ε4-stratified analysis (n = 53 771).[7] This disparity is particularly acute for ε4$^+$ controls (n = 706 vs n = 9207). However, the present study based on WES was more uniquely suited than the previous study of imputed genotypes for detecting associations with rare variants, particularly in gene-based tests. Reduced power was also exacerbated by the stratification into *APOE* genotype subgroups. However, this concern is mitigated by the increased ability to detect association with variants whose effects are dependent on interaction with or could be diluted in a sample including individuals with the *APOE* ε4 allele. In addition, the greater than 2-fold sample size for the ε4$^-$ group compared with the ε4$^+$ group may account for the paucity of significant and replicable findings among the ε4$^+$ group. This idea is exemplified by the *TREM2* R47H variant, which had nearly identical ORs in both groups but was 6 orders of magnitude more significant in the ε4$^-$ group. Another concern is that the comparatively small size of the follow-up WES data sets and unreliable imputation of very rare variants in imputed samples limited our ability to replicate findings, especially from gene-based tests, as exemplified by a previous study of this data set without stratification by *APOE* genotype.[15] These limitations underscore the need to replicate our findings in other data sets.

Conclusions

We identified multiple possibly novel associations for AD with individual and aggregated rare variants in groups of individuals with and without *APOE* ε4. Bioinformatics and functional studies of the *GPAA1* rs138412600 variant, which was the most robust novel association signal, demonstrated that it may also be associated with global cognition function and expression in brain of *GPAA1* and its repressive transcription factor, *FOXG1*.

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Accepted for Publication: March 22, 2019.

Published Online: June 10, 2019. doi:10.1001/jamaneurol.2019.1456

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Administrative, technical, or material support: Naj, Nicolas, Bennett, Mayeux, Haines, Schellenberg.
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Conflict of Interest Disclosures: Dr DeStefano reported receiving grants from the National Institutes of Health (NIH) during the conduct of the study that are not related to this study. Dr Haines reported receiving grants from the NIH during the conduct of the study. Dr Schellenberg reported receiving grants from National Institute on Aging (NIA) during the conduct of the study. Dr Farrer reported receiving grants from the NIH during the conduct of the study. No other disclosures were reported.

Funding/Support: This work was supported in part by NIA grants R01-AG048927, P30-AG13846, and RF1-AG057519. The Alzheimer's Disease Sequencing Project comprises 2 Alzheimer disease genetics consortia and 3 National Human Genome Research Institute funded Large-Scale Sequencing and Analysis Centers (LSAC). The 3 LSACs are the Human Genome Sequencing Center at the Baylor College of Medicine (grant U54 HG003273), the Broad Institute Genome Center (grant U54HG003067), and the Washington University Genome Institute (grant U54HG003079). The 2 Alzheimer disease genetics consortia are the Alzheimer's Disease Genetics Consortium funded by NIA grant U01-AG032984, and the Cohorts for Heart and Aging Research in Genomic Epidemiology funded by NIA grant R01-AG033193. This study was also supported by the National Heart, Lung, and Blood Institute, other NIH institutes, and several foreign governmental and nongovernmental organizations. The Discovery Phase analysis of sequence data is supported by NIA grants UF1-AG047133, U01-AG049505, U01-AG049506, U01-AG049507, and U01-AG049508. The Discovery Extension Phase analysis is supported by NIA grants U01-AG052411, U01-AG052410, and U01-AG052409. Data generation and harmonization in the follow-up phases is supported by NIA grant U54-AG052427. ROSMAP data were generated with support from NIA grants R01-AG036836, P30-AG10161, R01-AG15819, R01-AG17917, R01-AG36042, and U01-AG46512. Biological samples and associated phenotypic data used in primary data analyses were stored at study investigator institutions and at the National Cell Repository for Alzheimer's Disease grant U24AG021886) at Indiana University funded by the NIA. Associated phenotypic data used in primary and secondary data analyses were provided by study investigators, the NIA-funded Alzheimer's Disease Centers, and the National Alzheimer's Coordinating Center (grant U01AG016976) and the NIA Genetics of Alzheimer's Disease Data Storage Site (grants NIAGADS and U24AG041689) at the University of Pennsylvania, funded by NIA, and at the Database for Genotypes and Phenotypes funded by the NIH. This research was supported in part by the Intramural Research Program of the NIH National Library of Medicine. Contributors to the genetic analysis data included study investigators on projects that were individually funded by the NIA and other NIH institutes, and by private US organizations or foreign governmental or nongovernmental organizations. The portion of the study conducted in France was funded by grants from the Clinical Research Hospital Program from the French Ministry of Health (PHRC 2008/067), the Centre National de Référence pour les Malades Alzheimer Jeunes, the Joint Programme-Neurodegenerative Disease Research PERADES (defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease, Using Multiple Powerful Cohorts, Focused Epigenetics and Stem Cell Metabolomics), and the FP7 AgedBrainSysBio, France Génomique, Labex GENMED grant ANR-10-LABX-0013, the National Foundation for Alzheimer's Disease and Related Disorders, the Institut Pasteur de Lille, the Centre National de Génotypage, Inserm, Fondation pour la Recherche sur le Cerveau, the Lille Métropole Communauté Urbaine council, and the French government's LABEX (Laboratory of Excellence Program Investment for the Future) program DISTALZ grant (Development of Innovative Strategies for a Transdisciplinary Approach to Alzheimer's Disease). We are indebted to the Banque d'ADN et de cellules-Institut du Cerveau et de la Moelle épinière (ICM-Inserm) for grants U1127-UPMC P6, UMR S 1127-CNRS, and UMR 7225. The 3C Study received support from the Fondation Leducq (Transatlantic Network of Excellence on the Pathogenesis of SVD of the Brain), the EU Joint Programme-Neurodegenerative Disease Research project, the European Research Council, and the European Union's Horizon 2020 research and innovation programme (grant agreements 640643, 643417, and 667375).

Role of the Funder/Sponsor: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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