Rare genetic variation implicated in non-Hispanic white families with Alzheimer disease

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Abstract

Objective

To identify genetic variation influencing late-onset Alzheimer disease (LOAD), we used a large data set of non-Hispanic white (NHW) extended families multiply-affected by LOAD by performing whole genome sequencing (WGS).

Methods

As part of the Alzheimer Disease Sequencing Project, WGS data were generated for 197 NHW participants from 42 families (affected individuals and unaffected, elderly relatives). A two-pronged approach was taken. First, variants were prioritized using heterogeneity logarithm of the odds (HLOD) and family-specific LOD scores as well as annotations based on function, frequency, and segregation with disease. Second, known Alzheimer disease (AD) candidate genes were assessed for rare variation using a family-based association test.

Results

We identified 41 rare, predicted-damaging variants that segregated with disease in the families that contributed to the HLOD or family-specific LOD regions. These included a variant in nitric oxide synthase 1 adaptor protein that segregates with disease in a family with 7 individuals with AD, as well as variants in *RP11-433J8*, *ABCA1*, and *FISIP2*. Rare-variant association identified 2 LOAD candidate genes associated with disease in these families: *FERMT2* (p -values = 0.001) and *SLC24A4* (p -value = 0.009). These genes still showed association while controlling for common index variants, indicating the rare-variant signal is distinct from common variation that initially identified the genes as candidates.

Conclusions

We identified multiple genes with putative damaging rare variants that segregate with disease in multiplex AD families and showed that rare variation may influence AD risk at AD candidate genes. These results identify novel AD candidate genes and show a role for rare variation in LOAD etiology, even at genes previously identified by common variation.

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Glossary

ADSP = Alzheimer Disease Sequencing Project; **CH** = Caribbean Hispanic; **HLOD** = heterogeneity logarithm of the odds; **LOAD** = late-onset Alzheimer disease; **LOD** = logarithm of the odds; **LRP1** = LDL-receptor-related protein 1; **MAF** = Minor allele frequency; **NHGRI** = National Human Genome Research Institute; **NHW** = non-Hispanic white; **NOS1AP** = nitric oxide synthase 1 adaptor protein; **QC** = quality control; **SNP** = single nucleotide polymorphism; **WES** = whole exome sequencing; **WGS** = whole genome sequencing.

Late-onset Alzheimer disease (LOAD) is a neurodegenerative disease, characterized by progressive dementia, and pathologic changes include neuronal loss, neurofibrillary tangles, and amyloid-beta deposits.¹ LOAD is highly heritable (60%–80%), but most of this heritability remains unexplained, despite the identification of genetic factors that influence LOAD.² These factors include the *APOE* gene, as well as other genes identified through genome-wide association studies (GWAS) and a limited number of studies of rare genetic variation.^{3–9} While these factors have replicable association with LOAD, few of the underlying causal variants have been definitively identified.

To identify additional genes influencing LOAD and to better understand known LOAD genes, the Alzheimer Disease Sequencing Project (ADSP) was established.¹⁰ A key component of the ADSP is inclusion of whole genome sequencing (WGS) in large, multiply-affected LOAD families of non-Hispanic white (NHW) and Caribbean Hispanic (CH) ancestry. This family-based design enriches the study for risk variation, making it ideal to identify novel risk variants.¹¹ Family structure facilitates the prioritization of risk variation through linkage and segregation-based approaches. In this study, we report on analyses of the NHW families. Two primary approaches were taken: examination of linkage regions segregating with disease in these families to identify novel genes and a gene-based association analysis to rare variation at known Alzheimer disease (AD) candidate genes. Results indicate that rare variants play a role in LOAD multiplex families, both at novel genes identified through linkage and through rare variation at AD candidate genes.

Methods

The Alzheimer Disease Sequencing Project

Families were assembled as part of the ADSP. The ADSP is a collaboration of the LOAD genetics research community, the National Institutes on Aging, and the National Human Genome Research Institute (NHGRI). The full design is described elsewhere.¹¹ The ADSP includes contributors from the Alzheimer Disease Genetics Consortium, the neurology working group of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE), as well as 3 NHGRI sequencing centers at Baylor University, the Broad Institute, and Washington University. Data are available through dbGaP (phs000572).

Family selection and design

Approximately 1,400 multiplex LOAD families were reviewed for inclusion. Families were derived from the National

Institute on Aging Late Onset of Alzheimer's Disease family study, the National Cell Repository for Alzheimer's Disease, and families contributed by investigators from Columbia University, University of Miami, University of Washington, University of Pennsylvania, Case Western Reserve University, and Erasmus University. Families analyzed here were of NHW (CH descent families analyzed elsewhere)¹² and were required to have multiple members with LOAD, available genomic DNA, and available *APOE* genotypes. We excluded families known to carry mendelian AD mutations or were pathologically confirmed non-Alzheimer dementia.

Families meeting initial criteria were prioritized and chosen based on the number of affected individuals, number of generations affected, age at onset of clinical symptoms, and presence of *APOE* ϵ 4 risk alleles (table 1). Details of criteria and selection process are described elsewhere.¹⁰ No ϵ 4/ ϵ 4 individuals were included. Cognitively intact participants were selected if available and informative for phasing.

All cases met National Institute of Neurological Diseases-Alzheimer's NINCDS-ADRDA criteria for possible, probable or definite AD.¹³ Unaffected individuals were free of clinical AD at the most recent cognitive assessment. In total, 42 NHW families were included. These families included 208 affected individuals and 185 unaffected individuals with array data available, of which 164 affected individuals and 33 unaffected individuals were included in the sequencing experiment.

Standard protocol approvals, registrations, and patient consents

All individuals (or caregivers) provided written informed consent for genomic studies, including broad data sharing, and were assessed with the approval of the relevant institutional review boards.

NGS sequencing

WGS was performed at the NHGRI sequencing centers at the Broad Institute (Boston, MA), Baylor College of Medicine (Houston, TX), and Washington University (St. Louis, MS). Samples were sequenced using Illumina instruments to a minimum average 30× depth. Details of the sequencing experiments are described elsewhere.¹⁴

NGS calling and quality control

Raw NGS data were aligned to hg19 using BWA.¹⁵ Genotype calling was performed using Atlas (v2).¹⁶ Extensive variant-level quality control (QC) was performed (appendix e-1,

Table 1 Priority variants from consensus linkage regions

Chrm	Position	RS ID	Family	Gene	Alleles	Consequence	CADD	MAF (1kGP)
1	162,167,769		LD0254F	NOS1AP	C/T	Intron variant	1.2	NA
1	162,207,390		LD0254F	NOS1AP	A/T	Intron variant	0.2	NA
1	162,223,640		LD0254F	NOS1AP	A/G	Intron variant	13.6	NA
1	162,479,200		UM0464F	UHMK1	T/G	Intron variant	0.4	0
1	162,564,187		LD1223F	UAP1	A/G	Intron variant	8.7	0.008
1	162,564,187		LD1223F	UAP1	A/G	Intron variant	8.7	0.008
1	162,700,025		UM0464F	DDR2	A/C	Intron variant	0.5	0.009
1	162,735,057		UM0464F	DDR2	G/A	Intron variant	0.7	0.009
1	162,739,064		UM0464F	DDR2	G/A	Intron variant	5.4	0.009
1	162,742,651		LD0254F	DDR2	G/A	Intron variant	0.1	0.001
1	162,751,967		LD0254F	DDR2	T/A	3' UTR variant	6.6	0.009
1	162,757,273		LD1223F	DDR2	T/C	Upstream gene variant	2.2	0.009
1	162,928,238		UM0464F		G/A	Intergenic variant	0.9	0.002
1	163,032,461		LD0254F		T/A	Intergenic variant	1.9	0.002
1	163,202,875		UM0464F	RGS5	G/A	Intron variant	8.3	0.002
1	163,578,427		UM0464F		C/A	Intergenic variant	4.7	0.003
1	163,749,571		LD0949F		A/G	Intergenic variant	7.2	0.002
1	163,841,625		UM0464F		C/T	Intergenic variant	7.1	0.010
1	164,034,469	rs187504850	UM0464F		A/G	Intergenic variant	19.6	NA
1	164,448,463		UM0464F		G/A	Intergenic variant	3.2	0.003
1	164,622,647		LD0949F	PBX1	G/A	Intron variant	1.2	0.006
1	164,887,661		UM0464F		C/T	Downstream gene variant	8.2	0.005
1	165,253,949		LD0254F	LMX1A	C/T	Intron variant	14.7	0.002
1	165,342,593		UM0464F		G/A	Intergenic variant	5.9	0.010
1	165,532,785		LD1223F	LRRCS2	G/A	Synonymous variant	18.3	0.004
14	95,913,507	rs191535004	LD0949F	SYNE3	G/A	Intron variant	0.7	0.008
14	95,923,822		UM0464F	SYNE3	C/T	Intron variant	3.5	0.008
14	96,449,175		UM0464F		C/A	Intergenic variant	0.8	NA
14	96,568,984		UM0464F		C/A	Regulatory region variant	1.5	NA
14	96,923,339		LD0254F	AK7	C/T	Noncoding transcript exon variant	0.1	0.008
14	97,029,358		UM0464F	PAPOLA	C/G	Intron variant	10.4	NA
14	97,228,875		LD0949F	RP11-433J8.2	A/G	Intron variant	12.3	0.003

Abbreviations: CADD = Combined Annotation Dependent Depletion score; Chrm = chromosome; MAF (1kGP) = Minor Allele Frequency among the European samples in the 1,000 Genomes Project data; UTR = untranslated region.

links.lww.com/NXG/A117). Principal components analysis was used to assess population substructure, using the EIGENSTRAT.¹⁷ Array data were compared with WGS data to assess and confirm the pedigree structure for all individuals. Additional details of QC are reported elsewhere.¹⁴

Linkage analyses

MERLIN v1.1.2 software¹⁸ was used to perform parametric and nonparametric multipoint linkage analyses on the array data available for the entire family. Nonparametric analyses are described in detail elsewhere.¹⁹ For parametric multipoint

analyses, we first pruned markers to minimize linkage disequilibrium ($r^2 < 0.01$) using PLINK v1.07 software.²⁰ Using this pruned grid of markers, parametric multipoint linkage analyses were performed using a rare disease allele frequency (0.0001) and a dominant model with incomplete penetrance (noncarrier 0.01 and carrier 0.90). Consensus linkage regions (i.e., consistent across multiple families) were defined as peak HLOD ≥ 3.3 per Lander and Kruglyak.²¹ Any family with peak family-specific LOD >0.58 in the consensus region was considered a contributor to the consensus signal. Family-specific linkage regions were defined as regions with a parametric family-specific LOD >2 .

Annotations

Variants were annotated for location, gene (if applicable), putative function (missense, nonsense, splice site, etc.), combined annotation dependent depletion (CADD) score (a quantitative summary of putative function and conservation),²² contextual analysis of transcription factor occupancy (CATO) scores²³ for intergenic variation, and allele frequency in the NHW families and in the 1,000 Genomes Project European-ancestry populations.²⁴ As a QC measure, we used BLAST to interrogate the genome for similar sequence as the high-priority variants, to ensure uniqueness of the relevant sequence.²⁵

Variant filtering and prioritization

Variants were filtered based on complete segregation among affected individuals (and absent from unaffected individuals) and rarity (minor allele frequency [MAF] < 0.05 in our data set, <0.01 in 1,000 Genomes Project data). Additional prioritization was applied to variants with high CADD scores, were observed in multiple families, had CATO predictions, had multiple filtered variants in the same gene, or showed nominal association in the ADSP case-control analyses.

Validation genotyping

High-priority genotypes were validated using Sanger sequencing of whole genome sequenced family members to confirm carrier/noncarrier status. Sequencing was performed using standard protocol on genomic DNA (~ 50 ng). Details of validation typing are in appendix e-1 (links.lww.com/NXG/A117).

Gene-based association tests

Gene-based association tests were performed using the FSKAT v1 software.²⁶ A cutoff of MAF <0.02 was used among the non-Finnish European ancestry populations in the 1,000 Genomes Project data (1kGP)²⁴. Variants were analyzed in 2 sets: (1) damaging rare variants (loss-of-function variants, nonsense, stop-loss, etc and those predicted to be damaging) and (2) damaging variants plus all nonsynonymous variants. Genes with only a single variant were excluded. FSKAT was applied to the remaining genes using 2 models: one adjusted for age, sex, and the top 10 principal components and the other unadjusted.

Candidate gene list

Candidate genes (appendix e-1, links.lww.com/NXG/A117) were selected from replicable population and family AD

genetics studies, mostly from GWAS of LOAD or known early-onset AD genes.⁴⁻⁹

Data availability

Anonymized data are available by request from qualified investigators through dbGaP (phs000572.v1.p1) and through the National Institute on Aging Genetics of Alzheimer Disease Data Storage Site (www.niagads.org).

Results

Consensus linkage regions

Linkage scans identified 2 primary “consensus” linkage regions (peak HLOD ≥ 3.3)²¹: a parametric multipoint peak on chromosome 1q23 (peak HLOD = 3.58; 162.2–165.8 Mb; figure 1A) and a nonparametric multipoint peak on chromosome 14q32 (LOD = 4.18; 98.9–99.6 Mb; figure 1B). The 1q23 region was supported (LOD > 0.58) by 8 families (LD0241F, LD0254F, LD0856F, LD1223F, LD1260F, UM0196F, UM0463F, and UM0464F), while the 14q32 region was supported by 4 families (LD0223F, LD0949F, LD1223F, and UP0004F).¹⁹

In total, there were 86 rare (MAF <0.01 1kGP) variants that segregated with disease in sequenced affected individuals in at least 1 of the 8 families that supported the chromosome 1 peak. Of the 86 variants, 43 were genic (50%) and 43 were intergenic (50%). This initial set of 86 segregating variants was further refined by requiring variants to also be absent from unaffected individuals in the family (if available), have moderate-to-high

Figure Summary of consensus linkage regions on chromosomes 1 and 14

(A) Peak LOD region on chromosome 1. X-axis denotes positions in centimorgans. Y-axis denotes the LOD score at the corresponding position. (B) Peak LOD region on chromosome 14. X-axis denotes positions in centimorgans. Y-axis denotes the LOD score at the corresponding position.

CADD (CADD > 10), CATO predictions, or be seen in multiple families. Of the 86 variants, 24 matched these criteria and were considered “high priority.” At the 14q32 locus, we identified 23 rare variants segregating with disease among affected individuals in at least 1 of the 4 families supported the linkage signal. Of this set, there were 7 variants absent from the unaffected individuals, had high CADD predictions, or were seen in multiple families. In total, 31 variants in the consensus linkage regions were prioritized; of these, 29 variants were validated using Sanger sequencing, 1 was a false positive, and 1 could not have a reliable assay developed.

A number of interesting results come out of this set of 29 confirmed variants (table 1). In the 1q23 region, a variant (chr1:162,223,640 A/G) in nitric oxide synthase 1 adaptor protein (*NOS1AP*) segregates with disease in family UM0464F with 7 individuals with AD (family-specific LOD = 2.62; figure e-1, links.lww.com/NXG/A119); while the variant is intronic, it has a moderate CADD score (13.6) and is completely absent in the 1kGP reference data set. Other variants in *NOS1AP* (chr1:162,167,769 C/T; chr1:162,207,390 A/T) segregate with disease in family LD0254F (figure e-2, links.lww.com/NXG/A120). In the 14q32 region, an intronic variant in ncRNA RP11-433J8 segregates with disease in family LD1223F (6 AD family members; family-specific LOD = 1.45); the variant was also present in a second family (LD0307F) although it was not present in all AD individuals. The variant is rare in 1kGP (MAF = 0.003) and has a moderate CADD score (CADD = 12.2).

Family-specific linkage regions

In addition to the consensus linkage regions, there were 10 family-specific regions identified using parametric multipoint linkage (table 2). These regions showed family-specific LOD scores >2. Among the 10 regions, there were 647 variants that were rare (MAF <0.01 1kGP) and segregated among the

affected individuals in family with the LOD score >2. The 647 variants were further prioritized based on absence in unaffected family members with WGS, high CADD predictions, as well as presence in multiple families, identifying 13 additional variants as high priority (table 3). Twelve of these 13 variants were validated using an orthogonal technology (the last could not have a reliable assay developed).

Among the family-specific regions, a missense variant (rs137854495) in the chromosome 9 *ABCA1* (ATP binding cassette subfamily A member 1) gene segregated with disease in a family with 4 individuals affected with AD (family-specific LOD = 2.04). The variant was absent in the 1kGP reference data set and had a high CADD score (CADD = 34). Two missense variants in *FSIP2* (fibrous sheath interacting protein 2) segregates with disease in a single family with 7 AD family members (family-specific LOD = 2.07). Both variants were rare in 1kGP (MAF < 0.001) and had high CADD scores (CADD = 25.2 and 22.6). This analysis also identified a missense variant with high CADD (CADD = 32) in *TTC3*, from family UM0146F. This variant was previously identified through whole exome sequencing (WES) in the same family and is described elsewhere.²⁷

Candidate genes

FSKAT, a family-based kernel test for association of sets of variants, was used to perform gene-based association in the families²⁷ (Table 4). A list of 31 candidate genes (identified from GWAS and studies of familial AD) was tested for association with LOAD. Two genes showed association with LOAD in the unadjusted analysis that included nonsynonymous variants: *FERMT2* (*p*-value = 0.001) and *SLC24A4* (*p*-value=0.009). The association in *FERMT2* survives a Bonferroni correction for 31 genes tested (*p*-value = 0.05/31 = 0.0016). Both genes still showed association after adjusting for age, sex, and the top 10

Table 2 Family-specific linkage regions

Family ID	Chrm	cM (start)	cM (end)	BP (start)	BP (end)	Peak LOD
UM0458F	12	0.18	41.96	0.38	20.56	2.95
UM0458F	14	6.03	15.44	21.64	24.64	2.7
UM0146F	21	41.07	49	36.55	40.37	2.63
UM0146F	19	76.43	85.05	48.55	51.38	2.23
UP0005F	1	131.84	144.67	103.72	115.74	2.07
UP0005F	2	188.73	210.97	183.63	213.07	2.07
UP0005F	16	78.58	91.44	58.51	73.81	2.07
UM0463F	5	138.9	146.8	131.38	141.03	2.06
UP0001F	9	106.1	116.2	104.11	112.23	2.04
UP0001F	9	95.12	105.57	92.41	103.64	2.04

Abbreviations: BP (start) and BP (end) = position of the region in megabases; Chrm = Chromosome; cM (start) and cM (end) = position of the region in centimorgans; LOD = logarithm of the odds.

Table 3 Priority variants from family-specific linkage regions

Chrm	Position	Gene	Alleles	Family	Consequence	CADD	MAF (1kGP)
2	186,611,520	FSIP2	C/T	UP0005F	Missense variant	25.2	0.001
2	186,611,521	FSIP2	G/T	UP0005F	Missense variant	22.6	0.001
2	199,347,563	PLCL1	A/G	UP0005F	Intron variant	17.7	0.010
2	208,614,446	CCNYL1	C/G	UP0005F	Intron variant	20.1	0.001
9	100,819,143	NANS	C/T	UP0001F	Missense variant	22.5	0.001
9	107,584,795	ABCA1	G/A	UP0001F	Missense variant	34.0	NA
12	16,342,622	SLC15A5	G/A	UM0458F	Missense variant	24.0	0.008
12	17,149,860		T/A	UM0458F	Downstream gene variant	16.4	0.010
12	18,891,317	CAPZA3	C/T	UM0458F	Missense variant	21.4	0.005
16	61,999,830	CDH8	A/C	UP0005F	Intron variant	15.5	0.007
16	70,546,287	COG4	C/T	UP0005F	Missense variant	23.9	NA
16	73,127,644	HCCAT5	A/G	UP0005F	Noncoding transcript exon variant	16.3	0.002
21	38,534,308	TTC3	C/G	UM0146F	Missense variant	32.0	NA

Abbreviations: CADD = Combined Annotation Dependent Depletion score; Chrm = chromosome; MAF (1kGP) = minor allele frequency among European samples in the 1,000 Genomes Project data.

principal components (*FERMT2* p -value = 0.002; *SLC24A4* p -value = 0.023). The *PICALM* gene also showed nominal association after adjusting for age, sex, and principal components (p -value = 0.032; unadjusted p -value = 0.111). *SLC24A4* also showed nominal association in the damaging variant-only analysis (p -value = 0.026). Because these 3 genes were all initially identified using GWAS, we also performed the association analysis with the genotyped GWAS index single nucleotide polymorphisms (SNPs) as covariates in the FSKAT model (rs17125944 for *FERMT2*, rs10498633 for *SLC24A4*, and rs10792832 for *PICALM*). Each gene still showed evidence of association after including index SNP genotypes as covariates (*FERMT2* p -value = 0.002, *SLC24A4* p -value = 0.015, *PICALM* p -value = 0.021).

Because the gene of interest for a particular associated locus may not be the gene physically closest to the index SNP, as a secondary analysis, we expanded the list of 31 candidate genes to include genes near the GWAS index SNPs ($\pm 1,000,000$ bp). In this analysis, an additional 586 genes were tested using FSKAT. Near the *FERMT2* locus (within 100 kb downstream), the genes *STYX*, *PSMC6*, and *GNPNAT1* all showed association in the analysis including nonsynonymous variants (p -values = 0.0011, 0.0012, and 0.0016, respectively). *STYX*, in particular, also showed nominal association in a large case-control WES study conducted by the ADSP (p -value = 0.00119).²⁸ As with *FERMT2*, the p -values did not appreciably change when adjusting for age, sex, and principal components (p -values = 0.0013, 0.0016, and 0.0024, respectively). Additional genes showed association in the nonsynonymous analysis include *MGC45922* (p -value = 0.0030; near *CD33*), *TAP2* (p -value = 0.0043; near *HLA-*

DRB1/DRB5; p -value = 0.0047 in the ADSP WES analysis), and *FAM210B* (p -value = 0.0084; near *CASS4*), when adjusting for age, sex, and principal components. In the analysis of damaging variants, the *CPSF2* gene was associated in the adjusted analysis (p -value = 0.000498), which would survive a Bonferroni multiple testing correction for 586 genes. This gene is located near the *SLC24A4* gene and was also nominally associated in the ADSP WES analyses (p -value = 0.034). The *FIS1* gene also showed evidence of association in the unadjusted analysis (p -value = 0.00748, near *ZCWPW1*; unadjusted analysis p -value = 0.0147) and was also nominally associated in the ADSP WES analyses (p -value = 0.034).

Discussion

To identify rare variation influencing LOAD, we performed analyses of WGS data in NHW families multiply affected for LOAD. A two-pronged approach was taken: examination of linkage regions identified through analysis of genome-wide genotyping array data and a gene-based association analysis of rare coding variants, focusing on AD candidate loci identified in GWAS. These results indicate a potential role for rare variants in LOAD etiology. Numerous rare, predicted damaging rare variants were identified that segregate with disease in multiplex LOAD families and were validated with independent technologies. Additionally, rare variation in LOAD candidate genes was associated with AD in these multiplex families. This association persisted even when the common variant index SNPs were included in the models, indicating the rare variant association is likely distinct from the common variants that initially implicated the genes.

Table 4 Gene-based association test results at known AD candidate genes

Gene	Putative damaging + nonsynonymous		Putative damaging	
	<i>p</i> -Value (unadj)	<i>p</i> -Value (adj)	<i>p</i> -Value (unadj)	<i>p</i> -Value (adj)
ABCA7	0.534	0.414	0.782	0.657
AKAP9	0.226	0.167	0.115	0.145
APOE	0.334	0.228	—	—
APP	0.802	0.651	—	—
BIN1	0.422	0.339	—	—
CASS4	0.159	0.164	0.527	0.732
CD2AP	0.939	0.892	0.110	0.216
CD33	0.321	0.250	0.111	0.122
CELF1	0.353	0.223	—	—
CLU	0.608	0.475	0.465	0.631
CR1	0.349	0.182	0.403	0.629
EPHA1	0.481	0.459	0.842	0.896
FERMT2	0.001	0.002	—	—
GRN	0.310	0.453	—	—
HLA-DRB1	0.262	0.166	—	—
INPP5D	0.140	0.200	—	—
MAPT	0.673	0.668	0.596	0.677
MEF2C	0.266	0.323	—	—
MS4A6A	0.264	0.287	0.751	0.555
NME8	0.228	0.127	—	—
PICALM	0.111	0.032	—	—
PLD3	0.169	0.146	0.348	0.305
PSEN1	0.755	0.418	0.154	0.089
PSEN2	0.725	0.444	0.080	0.173
PTK2B	0.653	0.489	0.622	0.447
RIN3	0.419	0.341	0.935	0.958
SLC24A4	0.009	0.023	0.026	0.076
SORL1	0.642	0.438	0.263	0.172
TREM2	0.678	0.575	0.394	0.358
TREML2	0.381	0.300	0.208	0.143
ZCWPW1	0.560	0.478	—	—

Bold indicates *p*-values < 0.05.

Rare variation in *NOS1AP* was identified. *NOS1AP* lies under one of the HLOD linkage peaks, is expressed in the brain,²⁹ and is known to interact with the LDL-receptor-related protein 1 (LRP1). LRP1 is an APOE receptor that helps bring APOE into neurons^{30,31} and *APP*. In addition, LRP has been associated with AD in the ADSP WES experiment (*p* = 0.00018).²⁸

NOS1AP also interacts with nNOS,³² encoded by *NOS1*, which has been linked to AD³³ as well as other neurologic diseases.

A missense variant (rs137854495) in ATP binding cassette subfamily A member 1 (*ABCA1*) was found to segregate with disease in one family under a family-specific linkage peak on

chromosome 9. The variant was rare, with a very high CADD score (CADD = 34). *ABCA1* is expressed in brain (though not exclusively; *ABCA7* is expressed in many tissues) and is involved in lipid removal pathways.²⁹ Variants in *ABCA1* have been associated with HDL deficiency, familial hypercholesterolemia, and *APOA* deficiency.^{34–37} The rs137854495 variant, in particular, has been noted in a family with Tangier disease as part of a compound heterozygote.³⁸ Dyslipidemias and lipid pathways have long been linked to LOAD,³⁹ starting with the *APOE* gene,³ and more recently *CLU*, *ABCA7*, etc.,^{4,22} although exact mechanisms remain unclear. Tangier disease, in particular, has also been proposed as having links to AD, primarily through amyloid- β pathways, although evidence supporting this is mixed.^{40–44} The ADSP WES project identified nominal association with 2 additional apolipoproteins (*APOA2*, $p = 0.000636$; *APOA5*, $p = 0.0413$).²⁸

Gene-based association tests implicated fermitin family member 2 (*FERMT2*) and surrounding genes *STYX*, *PSMC6*, and *GNPNAT*, all with similar levels of significance ($p = 0.0010$ – 0.0016). *FERMT2* is involved in cell adhesion, is expressed in brain, and is near an SNP with strong association to AD.⁴ *STYX* is likely involved in phosphatase activity and has been associated with diabetes mellitus type 1.⁴⁵ *PSMC6* is likely involved in hydrolase activity; *GNPNAT* is involved in sugar metabolism. *SLC24A4* has been associated with AD through a genome-wide meta-analysis,⁴ and brain methylation in *SLC24A4* region has been associated with AD risk.⁴⁶ Although *FERMT2* and *SLC24A4* were initially identified using common variant approaches, the association observed at these 2 genes was not greatly affected by including the GWAS index SNPs as covariates in the model. If common variants were solely responsible for the association, then we would expect to fail to reject the null hypothesis at the rare variants. This implies the rare variation associated with disease in these families is distinct from the common variant index SNPs initially used to identify the genes.

There are limitations to this study. The sample size was modest relative to GWAS approaches. This of course limits power, particularly for the association-based approaches. However, the use of familial data and linkage and segregation-based approaches mitigates some of these power concerns. Increasing sample sizes and number of multiplex families is an ongoing effort for future studies. Additional limitations include the use of in silico predictions of function (e.g., CADD). While useful as a first pass, these predictions should be seen as a putative,⁴⁷ and function will need to be validated by functional genetic approaches.

These results imply a role for rare variation in familial LOAD. The linkage analysis identified 41 high-priority variants, including variants in *NOS1AP* and *ABCA1*, both with plausible roles in AD and AD-related pathways. The analysis of LOAD candidate genes identified several genes with rare variation associated with AD. The tests were still significant while controlling for the common index SNPs, implying a role for rare variation even at

GWAS-identified loci. Future directions include a thorough analysis on noncoding variation, particularly the role of enhancers and other regulatory elements in the etiology of AD.

Author contributions

All authors contributed to the work presented in this article. Critical revision: Primary manuscript was prepared by G.W. Beecham, with significant contributions from B. Vardarajan, E. Blue, E. Wijsman, and M.A. Pericak-Vance. All authors participated in the revision and editing of the manuscript. Concept and design: There were significant contributions to concept and design from G.W. Beecham, B. Vardarajan, E. Blue, W. Bush, A. DeStefano, E.R. Martin, A. Naj, C. Reitz, C. van Duijn, A. Goate, S. Seshadri, L.A. Farrer, E. Boerwinkle, G. Schellenberg, J.L. Haines, E. Wijsman, R. Mayeux, and M.A. Pericak-Vance. Analysis and interpretation: Review of family data was performed by M. Pericak-Vance, R. Mayeux, E. Boerwinkle, S. Seshadri, and C. van Duijn. Primary statistical analyses were performed by G.W. Beecham, J. Jaworski, E.R. Martin, and K. Hamilton-Nelson, with additions from B. Vardarajan, W. Bush, and E. Blue. All authors participated in the interpretation and discussion of results. Acquisition of data: Sample data were contributed by C. van Duijn, A. DeStefano, L.A. Farrer, A. Goate, J.L. Haines, M.A. Pericak-Vance, E. Boerwinkle, R. Mayeux, S. Seshadri, and G. Schellenberg. Statistical analyses: Statistical analyses were primarily conducted by G.W. Beecham; additional analyses conducted by J.C.B., A.C.N., E.R. Martin, S.H.C., A. DeStefano, and S. Seshadri (affiliations noted above, all academic). Study supervision and coordination: Primary study supervision and coordination was by R. Mayeux, M.A. Pericak-Vance, and E. Wijsman. Funding: Primary funding was by G. Schellenberg, R. Mayeux, E. Boerwinkle, M.A. Pericak-Vance, J.L. Haines, S. Seshadri, A. Goate, L.A. Farrer, and E. Wijsman. A detailed list of funding is noted in the acknowledgements.

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