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

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Neurol Genet 2018;4:e286. doi:10.1212/NXG.0000000000000286

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 FISP2. 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.
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. LOAD is highly heritable (60%–80%), but most of this heritability remains unexplained, despite the identification of genetic factors that influence LOAD. 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. 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. 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. 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. 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) 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 e4 risk alleles (table 1). Details of criteria and selection process are described elsewhere. No e4/e4 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. 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 30x depth. Details of the sequencing experiments are described elsewhere.

NGS calling and quality control

Raw NGS data were aligned to hg19 using BWA. Genotype calling was performed using Atlas (v2). Extensive variant-level quality control (QC) was performed (appendix e-1, 13).
Principal components analysis was used to assess population substructure, using the EIGENSTRAT. Array data were compared with WGS data to assess and confirm the pedigree structure for all individuals. Additional details of QC are reported elsewhere.

**Table 1** Priority variants from consensus linkage regions

| Chr | 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  | UHMK1  | T/G    | Intron variant | 0.4  | 0          |
| 1   | 162,928,238 | UM0464F  | UHMK1  | T/G    | Intron variant | 0.4  | 0          |
| 1   | 162,757,273 | LD1223F  | DDR2   | T/C    | Upstream gene variant | 2.2 | 0.009      |
| 1   | 162,751,967 | LD0254F  |DDR2   | G/A    | Intron variant | 0.7  | 0.009      |
| 1   | 162,757,273 | LD1223F  | DDR2   | T/C    | Upstream gene variant | 2.2 | 0.009      |
| 1   | 162,757,273 | LD1223F  | DDR2   | T/C    | Upstream gene variant | 2.2 | 0.009      |
| 1   | 162,757,273 | LD1223F  | DDR2   | T/C    | Upstream gene variant | 2.2 | 0.009      |
| 1   | 162,757,273 | LD1223F  | DDR2   | T/C    | Upstream gene variant | 2.2 | 0.009      |
| 1   | 162,757,273 | LD1223F  | DDR2   | T/C    | Upstream gene variant | 2.2 | 0.009      |
| 1   | 162,757,273 | LD1223F  | DDR2   | T/C    | Upstream gene variant | 2.2 | 0.009      |

**Linkage analyses**

MERLIN v1.1.2 software 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. For parametric multipoint

Abbreviations: CADD = Combined Annotation Dependent Depletion score; Chr = chromosome; MAF (1kGP) = Minor Allele Frequency among the European samples in the 1,000 Genomes Project data; UTR = untranslated region.
analyses, we first pruned markers to minimize linkage disequilibrium ($r^2 < 0.01$) using PLINK v1.07 software. 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 $\geq 3.3$ per Lander and Kruglyak. 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), contextual analysis of transcription factor occupancy (CATO) scores for intergenic variation, and allele frequency in the NHW families and in the 1,000 Genomes Project European-ancestry populations. 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.

**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 ($\sim 50$ 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. A cutoff of MAF $<$0.02 was used among the non-Finnish European ancestry populations in the 1,000 Genomes Project data (1kGP). 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.

**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 $\geq 3.3$): 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). 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

![Figure](image-url)
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 1q432 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 (rs137584495) 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.  

**Candidate genes**

FSKAT, a family-based kernel test for association of sets of variants, was used to perform gene-based association in the families (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

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**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  | FIS1   | 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.

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 (±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). 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 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.
Rare variation in NOS1AP was identified. NOS1AP lies under one of the HLOD linkage peaks, is expressed in the brain, and is known to interact with the LDL-receptor-related protein 1 (LRP1). LRP1 is an APOE receptor that helps bring APOE into neurons and APP. In addition, LRP has been associated with AD in the ADSP WES experiment ($p = 0.00018$). NOS1AP also interacts with nNOS, encoded by NOS1, which has been linked to AD 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 Table 4: Gene-based association test results at known AD candidate genes

| Gene          | Putative damaging + nonsynonomous | Putative damaging |
|---------------|-----------------------------------|-------------------|
|               | p-Value (unadj) | p-Value (adj)     | p-Value (unadj) | p-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         |
| EPRA1         | 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             | —               | —             |
| MS4AGA        | 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.
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. Variants in ABCA1 have been associated with HDL deficiency, familial hypercholesterolemia, and APOA deficiency. The rs137854495 variant, in particular, has been noted in a family with Tangier disease as part of a compound heterozygote. Dyslipidemias and lipid pathways have long been linked to LOAD, starting with the APOE gene, and more recently CLU, ABCA7, etc., 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. The ADSP WES project identified nominal association with 2 additional apolipoproteins (APOA2, p = 0.000636; APOA5, p = 0.0413). 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. STYX is likely involved in phosphatase activity and has been associated with diabetes mellitus type 1. 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, and brain methylation in SLC24A4 region has been associated with AD risk. 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, 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.

**Acknowledgment**

The Alzheimer’s Disease Sequencing Project (ADSP) comprises 2 Alzheimer disease (AD) genetics consortia and 3 National Human Genome Research Institute–funded Large Scale Sequencing and Analysis Centers (LSAC). The 2 AD genetics consortia are the Alzheimer’s Disease Genetics Consortium (ADGC) funded by the National Institute on Aging (NIA; U01 AG032984), and the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) funded by NIA (R01 AG033193), the National Heart, Lung, and Blood Institute (NHLBI), other NIH institutes and other foreign governmental and nongovernmental organizations. The Discovery Phase analysis of sequence data is supported through U19 AG047133 (to Drs. Farrer, Haines, Mayeux, Pericak-Vance, and Schellenberg); U01 AG049505 to Dr. Seshadri; U01AG049506 to Dr. Boerwinkle; U01AG049507 to Dr. Wijsman; and U01AG049508 to Dr. Goate; and the Discovery Extension Phase analysis is supported through U01AG052411 to Dr. Goate, U01AG052410 to Dr. Pericak-Vance, and U01 AG052409 to Drs. Seshadri and Fornage. Data generation and harmonization in the Follow-up Phases
research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants U01HL080295 and U01HL130114 from the NHLBI with additional contribution from the NINDS. Additional support was provided by R01AG023629, R01AG15928, and R01AG20098 from the NIA. FHS research is supported by NHLBI contracts N01-HC-25195 and HHSN268201500011I. This study was also supported by additional grants from the NIA (R01s AG054076, AG049607, and AG033040) and NINDS (R01 NS017950). The ERF study as a part of EUROSPLAN (European Special Populations Research Network) was supported by European Commission FP6 STRP grant number 018947 (LSHG-CT-2006-01947) and also received funding from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413 by the European Commission under the programme "Quality of Life and Management of the Living Resources" of 5th Framework Programme (no.QLG2-CT-2002-01254). High-throughput analysis of the ERF data was supported by a joint grant from the Netherlands Organization for Scientific Research and the Russian Foundation for Basic Research (NWO-RFBR 047.017.043). The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the municipality of Rotterdam. Genetic data sets are also supported by the Netherlands Organization of Scientific Research NWO Investments (175.010.2005.011, 911-03-012), the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), and the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO), Netherlands Consortium for Healthy Aging (NCHA), project 050-060-810. All studies are grateful to their participants, faculty, and staff. The content of these manuscripts is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the U.S. Department of Health and Human Services. The ADES-FR study was funded by grants from the Clinical Research Hospital Program from the French Ministry of Health (GMAD, PHRC, 2008/067), the CNR-MAJ, the JPND PERADES, the GENMED labex (LABEX GENMED ANR-10-LABX-0013), and the FP7 AgedBrainSysBio. Whole exome sequencing in the 3C-Dijon study was funded by the Fondation Leducq. This work was supported by the France Génomique National infrastructure, funded as part of the Investissements d’Avenir program managed by the Agence Nationale pour la Recherche (ANR-10-INBS-09), the Centre National de Recherche en Génomique Humaine, the National Foundation for Alzheimer’s disease and related disorders, the Institut Pasteur de Lille, Inserm, the Lille Métropole Communauté Urbaine council, and the French government’s LABEX (laboratory of excellence
program investment for the future) DISTALZ grant (Development of Innovative Strategies for a Transdisciplinary approach to Alzheimer’s disease). The 3C Study supports are listed on the Study Website (three-city-study.com). The FinnAD Study at the University of Tampere was supported by The Academy of Finland; grants 286284 (T.L.), Competitive State Research Financing of the Expert Responsibility area of Tampere University Hospitals (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation for Cardiovascular Research; Finnish Cultural Foundation; Tampere Tuberculosis Foundation; Yrjö Jahnsson Foundation; Signe and Ane Gyllenberg Foundation; and Diabetes Research Foundation of Finnish Diabetes Association. The three LSACs are the Human Genome Sequencing Center at the Baylor College of Medicine (U54 HG003273), the Broad Institute Genome Center (U54HG003067), and the Washington University Genome Institute (U54HG003079).

**Study funding**

Supported by the NIH, primarily the National Institute on Aging (NIA), the National Heart, Lung, and Blood Institute, and the National Human Genome Research Institute. Primary support includes the Alzheimer’s Disease Genetics Consortium funded by NIA (U01 AG032984), and the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) funded by NIA (R01 AG033193), the Human Genome Sequencing Center at the Baylor College of Medicine (U54 HG003273), the Broad Institute Genome Center (U54HG003067), and the Washington University Genome Institute (U54HG003079). Additional funding of contributing sites is noted below in the acknowledgements.

**Disclosure**

G. Beecham has received research support from the NIH and the Department of Defense. B. Vardarajan reports no disclosures. E. Blue has received research support from the NIH and the Cystic Fibrosis Foundation and has been a grant reviewer for the NIH. W. Bush has served on the editorial board for BMC Biodata Mining and PLoS One and has received research support from the USDA, the Foundation for Food and Agriculture, NIDDK, and the National Institute on Aging (NIA). J. Jaworski, S. Barral, and A. DeStefano report no disclosures. K. Hamilton-Nelson has received research support from the NIH. B. Kunkle reports no disclosures. E. Martin serves on the editorial board of Frontiers in Statistical Genetics and Methodology; and holds a patent for Test for Linkage and Association in General Pedigrees: The Pedigree Disequilibrium Test. A. Naj, F. Rajabli, and C. Reitz report no disclosures. T. Thornton has received research support from the NIH. C. van Duijn reports no disclosures. A. Goate has served on the scientific advisory boards of Denali Therapeutics, Pfizer, and DZNE; has received travel funding/speaker honoraria from the Rainwater Foundation, the Indiana University ADRC advisory board, and Wellcome Trust; serves on the editorial board of eLife; holds patents for PSEN mutations in AD, Tau mutations in FTD, and TDP43 mutations in ALS\FTD; has been a consultant for Cognition Therapeutics, AbbVie, and Biogen; has received research support from F Prime, NIA, Rainwater Charitable Foundation, and JPB Foundation; and receives royalty payments from Taconic Industries for tau mutation patent, and from Athena Diagnostics for TDP43 mutation testing. S. Seshadri serves on the editorial boards of the Journal of Alzheimer’s Disease, Stroke, and Neurology and has received research support from NIA and NINDS. L. Farrer serves on the editorial boards of the American Journal of Alzheimer’s Disease & Other Dementias, Clinical Genetics, and the Journal of Clinical Medicine; holds a patent (pending) for Use of PLXNA4 as a drug target and biomarker for Alzheimer disease; has been a consultant for Novartis Pharmaceuticals, Gerson Lerman, and Guidepoint Global; has received research support from the NIH, the Fidelity Foundation, and the Thome Memorial Foundation; and was a consultant for legal proceedings involving Finnegan & Associates, LLP. E. Boerwinkle has received travel funding and speaker honoraria from the Harvard School of Public Health and the Metabolomics Forum in Cambridge, United Kingdom; serves on the editorial board of Annals of Epidemiology; is a scientific officer at Codified Genomics, LLC; and has received research support from the NIH. G. Schellenberg has served on scientific advisory boards for the Alzheimer’s Association, the Society of Progressive Supranuclear Palsy, the United Kingdom Parkinson Disease Center, University College London, the Alzheimer’s Disease Sequence Project (co-chair), Structural Variant Work Group, the Alzheimer’s Disease Sequence Project, Mayo Clinic Rochester Uddal Center, University of Miami Uddal Center, Discovery Assessment Panel, and the Oxford Parkinson’s Disease Centre; has received travel funding/speaker honoraria from Alzheimer’s Disease Center, CurePSP, the University of California San Diego, Keystone Symposia, Southern California Alzheimer’s Disease Research Conference, University of California Institute for Memory Impairment and Neurological Disorders, NIH, Novartis, McKnight Brain Institute, University of Florida, Keep Memory Alive Event Center, Cleveland Clinic, Accelerated Medicines Program, PSP/Lewy Body Disease Think-Tank, Alzheimer’s Disease Center, American Association of Neuropathologists, Fusion Conferences, Center for Public Health Genomics, Columbia University, PSP Genetics Consortium, Tetra Institute, Rockefeller University, Blechman Foundation, Niigata University, Xuan Wu Hospital (Capital Medical University), Genetics of Dementia Summit (United Kingdom), Icahn School of Medicine Mount Sinai, and Biogen; has served on the editorial boards of the Journal of Neural Transmission, American Journal of Alzheimer’s Disease and Other Dementias, Alzheimer’s Research, Neurodegenerative Diseases, Current Alzheimer Research, and Pathology and Laboratory Medicine International; is employed by the University of Pennsylvania; has been a consultant for Biogen; and has received research support from the NIH, CurePSP, and CBD Solutions. J. Haines has served on the editorial boards of Neuragenetics, Current Protocols in Human Genetics, and Human Molecular Genetics; receives publishing royalties from John Wiley &
Sons; and has received research support from the NIH. E. Wijsman has served on the editorial boards of BMC Proceedings and Faculty of 1000 and has received research support from the NIH and the Metropolitan Life Foundation. R. Mayeux has received research support from the NIH. R. Pericak-Vance reports no disclosures. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

APPENDIX 1 Co-investigators

| Affiliation                  | Full Name               | Contributions                                                                                                                                 |
|-----------------------------|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Baylor College of Medicine  | Adam English            | Baylor College of Medicine site contributed expertise to the study design, sequencing of samples, bioinformatics analyses, quality control, data management, structural variation working group, as well as input into both case-control and family study working groups. |
|                             | Divya Kalra             |                                                                                                                                               |
|                             | Donna Muzny             |                                                                                                                                               |
|                             | Evette Skinner          |                                                                                                                                               |
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|                             | Richard A. Gibbs        |                                                                                                                                               |
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|                             | Shannon Dugan-Perez     |                                                                                                                                               |
|                             | Simon White             |                                                                                                                                               |
|                             | Viktoria Korchina       |                                                                                                                                               |
|                             | Waleed Nasser           |                                                                                                                                               |
|                             | William Salerno         |                                                                                                                                               |
|                             | Xiuping Liu             |                                                                                                                                               |
|                             | Yi Han                  |                                                                                                                                               |
|                             | Yiming Zhu              |                                                                                                                                               |
|                             | Yue Liu                 |                                                                                                                                               |
|                             | Ziad Khan               |                                                                                                                                               |
| Boston University           | Adrienne Cupples        | The Boston University site contributed expertise to the study design, bioinformatics analyses, quality control, data management, structural variation working group, case-control working group, family study working group, as well as significant sample contributions. |
|                             | Alexa Beiser            |                                                                                                                                               |
|                             | Anita DeStefano*        |                                                                                                                                               |
|                             | Ching Ti Liu            |                                                                                                                                               |
|                             | Chloe Sarnowski         |                                                                                                                                               |
|                             | Claudia Satizabal       |                                                                                                                                               |
|                             | Dan Lancour             |                                                                                                                                               |
|                             | Devanshi Patel          |                                                                                                                                               |

Continued
## APPENDIX 1 Co-investigators (continued)

| Affiliation                        | Full Name            | Contributions                                                                                                                                                                                                 |
|------------------------------------|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                    |                      | **Fangui Jenny Sun**                                                                                                                                                                                         |
|                                    |                      | **Honghuang Lin**                                                                                                                                                                                            |
|                                    |                      | **Jaeyoon Chung**                                                                                                                                                                                             |
|                                    |                      | **John Farrell**                                                                                                                                                                                              |
|                                    |                      | **Josee Dupuis**                                                                                                                                                                                             |
|                                    |                      | **Kathy Lunetta**                                                                                                                                                                                             |
|                                    |                      | **Lindsay Farrer***                                                                                                                                                                                          |
|                                    |                      | **Sudha Seshadri***                                                                                                                                                                                            |
|                                    |                      | **Xiaoling Zhang**                                                                                                                                                                                             |
|                                    |                      | **Yiyi Ma**                                                                                                                                                                                                    |
|                                    |                      | **Yuning Chen**                                                                                                                                                                                                |
| Broad Institute                    | **Eric Banks**       | The Broad Institute site contributed expertise to the study design, sequencing of samples, bioinformatics analyses, quality control, and data management working group                                                  |
|                                    |                      | **Namrata Gupta**                                                                                                                                                                                             |
|                                    |                      | **Seung Hoan Choi**                                                                                                                                                                                            |
|                                    |                      | **Stacey Gabriel**                                                                                                                                                                                             |
| Case Western Reserve University    | **Jonathan Haines*** | The Case Western Reserve University site contributed expertise to the study design, bioinformatics analyses, quality control, data management, structural variation working group, case-control working group, family study working group, annotation working group, as well as sample contributions |
|                                    |                      | **Mariusz Butkiewicz**                                                                                                                                                                                          |
|                                    |                      | **Sandra Smieszek**                                                                                                                                                                                            |
|                                    |                      | **Will Bush***                                                                                                                                                                                                 |
|                                    |                      | **Yeunjoo Song**                                                                                                                                                                                                |
| Columbia University                | **Badri Vardarajan*** | The Columbia University site contributed expertise to the study design, bioinformatics analyses, quality control, data management, structural variation working group, case-control working group, family study working group, annotation working group, as well as sample contributions |
|                                    |                      | **Christiane Reitz***                                                                                                                                                                                          |
|                                    |                      | **Dolly Reyes**                                                                                                                                                                                                 |
|                                    |                      | **Giuseppe Tosto**                                                                                                                                                                                             |
|                                    |                      | **Phillip L De Jager**                                                                                                                                                                                           |
|                                    |                      | **Richard Mayeux***                                                                                                                                                                                            |
|                                    |                      | **Sandra Barrai***                                                                                                                                                                                             |
| Erasmus Medical University/       | **Ashley Vanderspek** | The Erasmus Medical University site contributed expertise to the study design, the family study working group, as well as sample contributions                                                                  |
| Rotterdam                          |                      | **Cornelia van Duijn***                                                                                                                                                                                          |
|                                    |                      | **M Afran Ikram**                                                                                                                                                                                              |
|                                    |                      | **Najaf Amin**                                                                                                                                                                                                  |
|                                    |                      | **Shahzad Amad**                                                                                                                                                                                               |
|                                    |                      | **Sven van der Lee**                                                                                                                                                                                             |

Continued
## APPENDIX 1 Co-investigators (continued)

| Affiliation                                           | Full Name                  | Contributions                                                                                                                                 |
|-------------------------------------------------------|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| **Indiana University**                                 | Kelley Faber               | The Indiana University site contributed expertise to the study design, data and sample collection and management, participated in the family and case-control working groups |
|                                                       | Tatiana Foroud             |                                                                                                                                             |
| **Medical University Graz, Austria**                  | Helena Schmidt             | Medical University Grace contributed data and samples to the study                                                                            |
|                                                       | Reinhold Schmidt           |                                                                                                                                             |
| **Mount Sinai School of Medicine**                    | Alan Renton                | The Mount Sinai site contributed samples as well as expertise to the design of the study, the family and case-control working groups, and the protective variant working group |
|                                                       | Alison Goate*              |                                                                                                                                             |
|                                                       | Edoardo Marcora            |                                                                                                                                             |
|                                                       | Manav Kapoor               |                                                                                                                                             |
| **National Center for Biotechnology Information**     | Adam Stine                 | National Center for Biotechnology Information site contributed expertise to data management                                                |
|                                                       | Michael Feolo              |                                                                                                                                             |
| **National Institutes of Aging**                      | Lenore J. Launer           | National Institute on Aging site contributed expertise to data and study management                                                           |
| **Rush University**                                   | David A Bennett            | Rush University site contributed samples and data to the study                                                                                 |
| **Stanford University**                               | Li Charlie Xia             | The Stanford University site contributed expertise to the structural variation working group                                                  |
| **University of Miami**                               | Brian Kunkle*              | The University of Miami site contributed expertise to the study design, bioinformatics analyses, quality control, data management, structural variation working group, case-control working group, family study working group, as well as significant sample contributions |
|                                                       | Eden Martin*               |                                                                                                                                             |
|                                                       | Farid Rajabi*              |                                                                                                                                             |
|                                                       | Gary Beecham*              |                                                                                                                                             |
|                                                       | James Jaworski*            |                                                                                                                                             |
|                                                       | Kara Hamilton-Nelson*      |                                                                                                                                             |
|                                                       | Margaret Pericak-Vance*    |                                                                                                                                             |
|                                                       | Michael Schmidt            |                                                                                                                                             |
| **University of Mississippi**                         | Thomas H. Mosley           | The University of Mississippi site contributed samples and data to the study                                                                   |
| **University of Pennsylvania**                        | Amanda Kuzma               | The University of Pennsylvania site contributed expertise to the study design, bioinformatics analyses, quality control, data management, structural variation working group, case-control working group, family study working groups |
|                                                       | Han-Jen Lin                |                                                                                                                                             |
|                                                       | Liming Qu                  |                                                                                                                                             |
|                                                       | Li-San Wang                |                                                                                                                                             |
|                                                       | Micah Childress            |                                                                                                                                             |
|                                                       | Otto Valladares            |                                                                                                                                             |
|                                                       | Prabhakaran Gangadharan    |                                                                                                                                             |
|                                                       | Rebecca Cweibel            |                                                                                                                                             |
|                                                       | Yi Zhao                    |                                                                                                                                             |
|                                                       | Yi-Fan Chou                |                                                                                                                                             |

Continued
APPENDIX 1 Co-investigators (continued)

| Affiliation                      | Full Name         | Contributions                                                                                                                                 |
|---------------------------------|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| University of Texas Houston     | Eric Boerwinkle*  | The University of Texas Houston site contributed expertise to the study design, sequencing of samples, bioinformatics analyses, quality control, data management, structural variation working group, as well as both case-control and family study working groups |
|                                 | Jan Bressler      |                                                                                                                                             |
|                                 | Jennifer E. Below |                                                                                                                                             |
|                                 | Myriam Fornage    |                                                                                                                                             |
|                                 | Xiaoming Liu      |                                                                                                                                             |
|                                 | Xueqiu Jian       |                                                                                                                                             |
| University of Washington        | Alejandro Q Nato Jr. | The University of Washington site contributed expertise to the bioinformatics analyses, quality control, data management, structural variation working group, case-control working group, and family study working group |
|                                 | Andrea R Horimoto |                                                                                                                                             |
|                                 | Bowen Wang        |                                                                                                                                             |
|                                 | Bruce Psaty        |                                                                                                                                             |
|                                 | Daniela Witten     |                                                                                                                                             |
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|                                 | Elizabeth Blue*    |                                                                                                                                             |
|                                 | Ellen Wijsman*     |                                                                                                                                             |
|                                 | Harkirat Sohi      |                                                                                                                                             |
|                                 | Hiep Nguyen        |                                                                                                                                             |
|                                 | Joshua C. Bis      |                                                                                                                                             |
|                                 | Kenneth Rice      |                                                                                                                                             |
|                                 | Lisa Brown        |                                                                                                                                             |
|                                 | Michael Dorschner  |                                                                                                                                             |
|                                 | Mohamad Saad       |                                                                                                                                             |
|                                 | Pat Navas          |                                                                                                                                             |
|                                 | Rafael Nafikov     |                                                                                                                                             |
|                                 | Timothy Thornton*  |                                                                                                                                             |
|                                 | Tyler Day          |                                                                                                                                             |
APPENDIX 1 Co-investigators (continued)

| Affiliation                  | Full Name                 | Contributions                                                                 |
|------------------------------|---------------------------|-------------------------------------------------------------------------------|
| Washington University St. Louis | Carlos Cruchaga           | The Washington University St. Louis site contributed expertise to the study design, sequencing of samples, bioinformatics analyses, quality control, data management, structural variation working group, as well as both case-control and family study working groups |
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|                              | David E. Larson           |                                                                                |
|                              | Elizabeth Appelbaum       |                                                                                |
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|                              | Lucinda Antonacci-Fulton  |                                                                                |
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Asterisks (*) indicate coinvestigators whose contributions were sufficient for authorship.

Received February 6, 2018. Accepted in final form October 3, 2018.

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*Neurol Genet* 2018;4;
DOI 10.1212/NXG.0000000000000286

This information is current as of November 26, 2018
