Whole genome sequencing of Caribbean Hispanic families with late-onset Alzheimer’s disease

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Abstract
Objective: To identify rare causal variants underlying known loci that segregate with late-onset Alzheimer’s disease (LOAD) in multiplex families. Methods: We analyzed whole genome sequences (WGS) from 351 members of 67 Caribbean Hispanic (CH) families from Dominican Republic and New York multiply affected by LOAD. Members of 67 CH and additional 47 Caucasian families underwent WGS as a part of the Alzheimer’s Disease Sequencing Project (ADSP). All members of 67 CH families, an additional 48 CH families and an independent CH case-control cohort were subsequently genotyped for validation. Patients met criteria for LOAD, and controls were determined to be dementia free. We investigated rare variants segregating within families and gene-based associations with disease within LOAD GWAS loci. Results: A variant in AKAP9, p.R434W, segregated significantly with LOAD in two large families (OR = 5.77, 95% CI: 1.07–30.9, P = 0.041). In addition, missense mutations in MYRF and ASRG1 under previously reported linkage peaks at 7q14.3 and 11q12.3 segregated completely in one family and in follow-up genotyping both were nominally significant (P < 0.05). We also identified rare variants in a number of genes associated with LOAD in prior genome wide association studies, including CR1 (P = 0.049), BIN1 (P = 0.0098) and SLC24A4 (P = 0.040). Conclusions and Relevance: Rare variants in multiple genes influence the risk of LOAD disease in multiplex families. These results suggest that rare variants may underlie loci identified in genome wide association studies.
Introduction

Late-onset Alzheimer’s disease (LOAD) is the most common form of dementia in older adults, it lacks an effective treatment and represents an enormous societal burden. The disease is characterized by progressive deterioration of memory and cognitive functions, leading to loss of autonomy and ultimately requiring full-time medical care. Pathologically, LOAD is defined by severe neuronal loss, aggregation of amyloid β (Aβ) in extracellular senile plaques, and formation of intra-neuronal neurofibrillary tangles consisting of hyper-phosphorylated tau protein.

Over the past decade, genetic research in LOAD has been dominated by genome-wide association studies (GWAS), a hypothesis-free scan of the genome using dense genotyping arrays based on common variants (single nucleotide polymorphisms, SNPs). Several genes within LOAD susceptibility loci cluster in specific pathways,1–6 including amyloid processing, oxidative stress and immune or inflammatory pathways. Collectively, GWAS demonstrates that apart from the strongest risk factor, APOE-ε4 a large number of loci with modest effect size also contribute to LOAD risk. Common variants identified through GWAS may not have functional consequences, simply reflecting linkage disequilibrium with the unobserved causal variants. It is also possible that these causal variants are rare and have large effects, such as TREM2,7–13 and are not covered by commercially available GWAS platforms. In fact, putatively damaging variants have already been identified (for example TREM2, SORL1, and ABCA7) in some of these LOAD susceptibility loci, advancing our understanding of disease risk.14–16

Whole genome sequencing (WGS) provides a comprehensive and detailed investigation of human genetic variation allowing interrogation of coding and noncoding regions of the genome. Increasingly, WGS studies have provided the strongest evidence that rare genetic variants can have large cumulative effects on human diseases.17–20 Family-based studies represent an implicit enrichment strategy for identifying rare variants.21 Transmission of variants from parents to offspring maximizes the chance that multiple copies of rare variants exist in the pedigree. Moreover, compared with analyses using unrelated samples, sequencing in families provides data concerning identical-by-descent or sharing among relatives, greatly reducing false positives and permitting detection of sequencing errors, while facilitating the identification of alleles that cause genetic disorders.22,23

In the current report, we focused on Caribbean Hispanic families multiply affected by LOAD. The frequency of LOAD among multiplex families from the Dominican Republic is known to be significantly higher (5-fold) than expected for similarly aged individuals in a non-Hispanic white population from the United States.24 Furthermore, we found that a moderate degree of inbreeding was present and a predictor of LOAD risk in this population.25 As part of the national Alzheimer’s Disease Sequencing Project (ADSP), we identified several chromosomal regions with strong evidence for linkage in Caribbean Hispanic families with multiple LOAD cases.26 In the present study, we used WGS data (Data S1) from 67 families as discovery to detect rare variants in previously identified linkage regions and in previously reported LOAD candidate genes. Genotyping in additional WGS on additional 47 Caucasian families were used to replicate the findings from the CH families.

Material and Methods

Family characteristics

All participants (Table 1) were recruited after providing informed consent and with approval by the relevant institutional review boards both in the United States and in

| Pedigrees Sequenced in ADSP discovery |
|--------------------------------------|
| Number of pedigrees                    | 67 |
| Total number of subjects sampled       | 860 |
| Average subjects sampled per pedigree  | 12.84 ± 7.28 |
| Total number of subjects sequenced     | 351 |
| Average samples sequenced in each pedigree | 5.24 ± 1.67 |
| Affected                               | 302 |
| Unaffected                             | 49 |
| Average affected per pedigree          | 7.42 ± 3.61 |
| Age (sequenced individuals)            | 73.02 ± 10.0 |
| Women (%)                              | 58.72 |
| APOE                                   | 17.03% |

Additional pedigrees used in validation genotyping

| Number of pedigrees | 48 |
|---------------------|----|
| Total number of subjects sampled | 404 |
| Average subjects sampled per pedigree | 8.41 ± 4.8 |
| Average affected per pedigree | 5.08 ± 2.3 |
| Age                    | 70.76 ± 10.11 |
| Women (%)              | 65 |
| APOE                   | 29.90% |

Unrelated case–control set

| Total number of subjects sampled | 450 |
| No of affected individuals      | 152 |
| Age (affecteds)                 | 85.5 ± 6.5 |
| Age                              | 79.3 ± 6.7 |
| Women (%)                       | 68.7 |
| APOE                            | 12.80% |
the Dominican Republic. Patients, unaffected family members and healthy unrelated controls were required to have had standard neuropsychological tests and neurological examinations to verify their clinical status and diagnosis. Most individuals have been evaluated on multiple occasions over the past 10 years. Families in which patients had known early-onset disease mutations in APP, PSEN1, PSEN2, GRN, or MAPT were excluded from this analysis to increase power of discovery variants predisposing to LOAD. All selected probands came from families with three or more affected individuals recruited in the Dominican Republic and New York. Recruitment for this family study began in 1998, and was restricted to Caribbean Hispanics, predominately from the Dominican Republic. As a part of the ADSP, a set of 67 CH families and 47 Caucasian families were selected for whole genome sequencing from approximately 1400 families reviewed. Selection was based on the number of affected individuals, the number of generations affected, age at onset of clinical symptoms, and presence of APOEε4 alleles. 29

**Whole Genome Sequence analysis of GWAS linkage peaks**

Details about WGS methodology and variant calling pipelines can be found in Data S1. Three regions demonstrating linkage previously identified using GWAS data were prioritized for WGS data analysis. Results from genome-wide linkage analysis revealed a strong linkage signal (HLOD = 3.6) on chromosomal region 3q29. 26 We previously reported microsatellite linkage analyses in this region as strongly associated with LOAD in Caribbean Hispanics, including a single family that achieved a LOD score of 1.28. WGS data was generated for four of the family members with LOAD. Analysis of the WGS data was limited to an approximate 2 Mb region encompassing the 1-LOD interval around linkage peak (3q29: 197,052,973 bp–199,212,658 bp). We also selected two chromosomal regions where we observed genome-wide significant linkage and association signals across the families: 7p14.3 and 11q12.3. 26 Annotation of the identified variants in these linkage regions was performed using the ANNOVAR software. 31

**Whole genome sequence variants prioritization**

**Consensus linkage regions**

We followed a principled filtering strategy (Fig. 1, Table S4) to test the segregation of rare variants with LOAD status in the three linkage regions: 7p14.3 and 11q12.3 and 3q29 (Table S6). The first criterion used to prioritize the identified variants was based on familial segregation of cases. We required that at least 75% of the patients with LOAD and WGS data in the families were carriers. Additionally we also prioritized variants that were observed in at least five affected individuals in multiple Caribbean Hispanic families.

We filtered variants with minor allele frequency (MAF) of less than 0.10 in the ADSP families and with an MAF of 0.02 in Caucasian and African American populations in ExAC database (for exonic variants) 32 and 1000 genomes project (for intronic and intergenic variants) 33 to restrict our analyses to rare variants in the population. Functional annotations were also used as filtering strategy, that is, variants were analyzed in order of increasing priority: exonic, inter-genic, intronic and others (non-coding RNA, 5’/3’ UTR, upstream/downstream gene). We also included any intronic or intergenic variants with a Combined Annotation Dependent Depletion (CADD) score of greater than 30 to include highly conserved and putative non-exonic regulatory variants in the analyses. Further criteria for variant prioritization included, additional LOAD patients from different families carrying the variant and that the variant was either not present or present in very low frequency in the non-demented family members.

**Follow-up genotyping validation**

To confirm putative variants that segregated in 67 discovery families and candidate GWAS loci, we genotyped all of the family members in the discovery families, an additional 48 families and unrelated, unaffected controls of the same ancestry and age (Table 1). The validation cohort consisted of Caribbean Hispanic individuals from 115 multiplex families and 450 unrelated cases and controls from the same ethnic background (Table 1). Population allele frequencies for novel variants were estimated from unaffected persons in the Caribbean Hispanic population from samples/families that we have amassed over 15 years. Genotypes were generated using the KASP™ genotyping technology which uses allele-specific polymerase chain reaction for accurate calling of single nucleotide variants (SNVs) and indels. 35

**Single variant association analyses**

SNV genetic associations were restricted to individuals 60 years of age or older and were tested using General Linear Models in generalized estimating equations (GEE) to adjust for familial correlation in the data. The family ID was used as a repeat measure to account for correlation in the genotype data within family members. All analyses were adjusted for sex, age at last evaluation,
education, APOE-ε4 genotype and the first ten principal components.

LOAD GWAS and candidate loci

We tested genes associated with LOAD in large GWAS studies with multiple replication reports. We also included known early-onset AD genes and genes implicated in earlier sequencing efforts in LOAD.¹ ⁶-¹⁶ Candidate genes evaluated included: APP, PSEN1, PSEN2, GRN, MAPT, TREM2, PLD3, APOE, ABCA7, SORL1, CR1, BIN1, CD2AP, EPHA1, CLU, MS4A6A, PICALM, CD33, HLA-DRB5, HLA-DRB1, PTK2B, SLC24A4, RIN3, INPP5D, MF2C, NME8, ZCWPW1, CELF1, FERMT2, CASS4, TREML2, and AKAP9.

Gene-based association analyses

Gene-based F-SKAT analyses were conducted for genes in the three consensus linkage regions. The F-SKAT test was restricted to variants defined as exonic and rare (<2% in Europeans, African Americans and overall in the ExAC database). To define a testable-set, we used two sets of variants in the gene-based test based on functional annotation: (1) Stop-loss, stop gain and missense damaging variants (as defined by SIFT or PolyPhen) and (2) including all non-synonymous variants. We also tested gene-sets that included both loss of function variants and variants that had a high CADD score (>30). The CADD score filter captures putatively non-functional variants in the non-coding regions. For most genes (Table S5), we did not observe variants in non-coding regions with a high CADD score. In addition functional characterization of GWAS loci have indicated that cis-regulation is a common mechanism underlying these associations. The most frequent elements affected are transcriptional enhancers and silencers that regulate transcription through long-range interactions, typically located more than 1 Kb from their target genes. Therefore, to examine the top GWAS signals, we have considered a chromosomal region of 1 Mb upstream and 1 Mb downstream the reported GWAS loci. We conducted F-SKAT analyses of

Figure 1. Filtering strategy to prioritize variants for follow-up analyses in the validation sample.
genes within ±1 Mb encompassing the reported LOAD-GWAS SNPs. Each gene was independently assessed for association with LOAD using F-SKAT with the modified R code to adjust for family structure. We tested models of association also adjusting for (1) age, sex, population substructure, ancestry proportions for African-American and native American ancestry (Data S1) and (2) including APOE-ε4 dosage with other covariates. Analyses were subsequently repeated adjusting for the possible effect of the reported GWAS SNP to assess the additional contribution of rare variants in conferring LOAD risk. We also conducted region-based FSKAT test combining variants from all the genes in the 7p14.3 and 11q12.3 regions to assess the joint burden of all variants together in the region.

Results

Families

As detailed in Table 1, 67 Caribbean-Hispanic families underwent WGS. The families consisted of 351 individuals (302 individuals with LOAD and 49 unaffected family members) with average age of 73.02 ± 10 years, 59% were women. Seventeen percent were carriers of APOE-ε4 allele. On average, approximately five individuals were sequenced within each of the families. For validation genotyping, we genotyped all the members of the 67 families, an additional 48 Hispanic families and an independent elderly case control cohort of the same ethnic background. The additional 48 Hispanic families had similar characteristics to the discovery families (Table 1). These families also had, on average, five affected individuals, but had a higher frequency for the APOE ε4 allele (29.9%).

Linkage region in a single Caribbean-Hispanic family at 3q29

After applying quality control metrics, we identified a total of 6551 sequence variants under the 3q29 linkage peak. Applying the filters in the prioritization pipeline, we identified 11 rare SNVs: six intronic and five inter-genic variants. The 11 identified SNVs were genotyped in 1,720 Caribbean Hispanic unrelated individuals. The strongest association with LOAD ($\beta = 0.83$, SE = 0.45, $P = 0.064$) was found for an intronic variant rs186972238 in the LRCH3 gene. A complex pattern of inheritance with incomplete penetrance emerged when the variant segregation pattern was evaluated within each of these families.

Locus 7q14.3

A total of 35,376 high quality SNVs were observed in 67 families from the WGS experiment and subsequent QC, of which 26,654 SNVs were observed a frequency of <0.10 in the ADSP families. Applying the filtering criteria described above (Fig. 1), we observed 12 non-synonymous and one synonymous mutation that were observed in at least two affected individuals and absent in unaffected members (Table S1).

Locus 11q12.3

A total of 19,106 SNVs were observed in the 11q12.3 linkage regions in the 67 ADSP families of which 149 SNVs were putatively damaging (stop-loss, stop-gain or damaging classified by SIFT or PolyPhen) and novel or rare (Fig. 1). Forty variants were observed to be perfectly or imperfectly segregating in at least one family. Remarkably, p. V707I in TMEM132A was observed in two families with complete segregation in one family. (Table S2)

Analyses of loci under linkage peaks

We selected 14 variants under the linkage peaks (10 from 11q12.3 and 4 from 7q14.3) for follow-up genotyping in additional family members and unrelated controls. We chose variants that fulfilled at least one of the following criteria: (1) variants observed in two or more Caribbean Hispanic families in at least five affected LOAD individuals, and further prioritized if observed in affected individuals in Caucasian families showing segregation with LOAD and (2) variants observed in at least four affected LOAD individuals and no unaffected individuals from one Caribbean Hispanic family with CADD score $>15$. We tested SNV association with LOAD using a GEE model to adjust for familial correlation. We found nominally significant association ($P < 0.05$) for two SNVs each in MYRF and ASRGLI, respectively, (Table 2).

Segregation of variants in established GWAS loci

We evaluated the segregation with LOAD of the 147 observed rare functional variants in 30 candidate genes implicated in LOAD by GWAS, next-generation sequencing or associated with early-onset Alzheimer’s disease. Sixteen of these variants were segregating in at least one family (Table S3). Remarkably, five missense variants in AKAP9 segregated with LOAD status in one or more families, p.R434W (Figs. 2 and 3, Tables S9 and S10) and p.I1448V (Fig. S1) were subsequently genotyped in the all members of these families, and in an additional 48 families and an independent case–control set to determine complete segregation and test association with LOAD. We also genotyped two missense
variants in \textit{CR1} and one variant each, from \textit{EPHA1}, \textit{RIN3}, and \textit{ABCA7} (Table 2) and used GEE adjusting for familial correlation. \textit{p.R434W} was nominally associated with \textit{LOAD} adjusting for age and sex (OR = 5.77; 95% confidence interval [CI] 1.07–30.9, \(P = 0.041\)) and \textit{APOE-e4} (OR = 6.3; CI: 1.11–35.54, \(P = 0.038\)).
p.R434W also segregated with LOAD status in two large families (Fig. 2). p.R434W was predicted to be damaging by SIFT and Polyphen and had a highly deleterious CADD score of 32. Previously implicated variants in AKAP9 (rs144662445 and rs149979685) were each observed in one LOAD patient.

**F-SKAT gene based tests**

Nominal association was found for CR1 \((P = 0.049)\), SLC24A4 \((P = 0.040)\) and BIN1 \((P = 0.0098)\). CR1 and SLC24A4 were also significant in replication study of the Caucasian families \((P = 0.040 \text{ and } 0.002, \text{ respectively})\). (Table 3). However, only rare variants in BIN1 \((P = 0.026)\) remained significant after adjusting for the significant GWAS-SNP that was previously associated with LOAD (Table 4).

As described in Table 4, the strongest LOAD associations after adjusting for the effect of the corresponding common variants were found in genes located in three loci: HLA-DRB5, ZCWPW1 and BIN1. MSH5 \((P = 0.0026)\) and HLA-DQA2 \((P = 0.006)\) located in HLA-DRB5 region, and CYP3A43 gene \((5.09E-05)\) located in ZCWPW1 region were significantly associated with LOAD. LGMN in the SLC24A4 region was modestly associated with LOAD in a model adjusted for APOE dosage \((P = 0.033)\).

**Discussion**

A variant in AKAP9, (p.R434W), a gene previously associated with LOAD in a case–control study among African Americans,\(^{40}\) segregated in two large families and was nominally associated with LOAD, with fivefold increased risk adjusted for age, sex, and APOE-ε4. The two different variants in AKAP9 were previously identified, were considered rare in populations African-descent, and were not present in European-descent or East Asian-descent individuals in the 1000 Genomes database. AKAP9 is located on chromosome 7q21–22, and it encodes a member of the A kinase anchoring protein (AKAP) family. The A-kinase anchor proteins (AKAPs) are structurally diverse proteins that bind to the regulatory subunits of protein kinase A (PKA), confining the holoenzyme to discrete locations within the cell. Alternate splicing of this gene

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**Table 3. F-SKAT analyses of AD candidate GWAS loci.**

| GENE | N_LOF | No APOE | APOE² |
|------|-------|---------|-------|
| ABCA7| 12    | 0.6655  | 0.7486|
| AKAP9| 9     | 0.6659  | 0.5895|
| BIN1 | 3     | 0.0098  | 0.0139|
| CASS4| 2     | 0.3814  | 0.3871|
| CIT  | 6     | 0.4767  | 0.5128|
| CR1  | 9     | 0.0490  | 0.0830|
| DSG2 | 5     | 0.4623  | 0.3587|
| EPHA1| 3     | 0.8159  | 0.8659|
| FRMD4A| 2    | 0.1807  | 0.1418|
| INPP5D| 4    | 0.5328  | 0.6067|
| MAPT | 5     | 0.7163  | 0.7605|
| PSEN1| 2     | 0.5914  | 0.7302|
| RIN3 | 5     | 0.8367  | 0.8526|
| SLC24A4| 3   | 0.0433  | 0.0505|
| SORL1| 3     | 0.4285  | 0.4552|
| Trem2| 2     | 0.9846  | 0.9619|

¹Model- LOAD – Gene + Age + Sex + proportion of African ancestry + proportion of Native American Ancestry + First ten principal components.

²Model- LOAD – Gene + Age + Sex + number of APOE-ε4 alleles + proportion of African ancestry + proportion of Native American Ancestry + First ten principal components.
results in at least two isoforms that localize to the centrosome and the Golgi apparatus, and interact with numerous signaling proteins from multiple signal transduction pathways.41 AKAP9 is also expressed in the cerebral cortex, hippocampus, and cerebellum, and is involved in the cytoskeletal attachment of NMDA receptors, important for controlling synaptic plasticity and memory function.42

In addition, missense mutations in Myelin Gene Regulatory Factor (MYRF) and Asparaginase-Like Protein 1 (ASRGL1) genes under linkage peak on chromosome 11q12.3 segregated completely in one LOAD family and tested nominally significant (P < 0.05) in association with LOAD in follow-up genotyping. The MYRF gene encodes a transcription factor that is required for central nervous system myelination and may regulate oligodendrocyte differentiation. It increases expression of genes that affect myelin production. Mutant huntingtin mice show progressive neurological symptoms and early death, as well as age-dependent demyelination and reduced expression of myelin genes that are downstream of MYRF.43 A missense mutation in ASRGL1 has been associated with autosomal recessive retinal degeneration,44 but it has not been previously related to Alzheimer’s disease or neurodegenerative disorders. In patients with retinal degeneration, variants in ASRGL1 are thought to be the cause of protein misfolding, intracellular mis-routing and aggregation of misfolded proteins. It is expressed in brain, specifically in astrocytes and oligodendrocytes.45

Examination of known LOAD genes (e.g., from large GWAS-based meta-analyses) confirmed the role of rare functional variation in CR1 (P = 0.049), BIN1 (P = 0.0098), and SLC24A4 (P = 0.040). CR1 encodes a transmembrane glycoprotein in the innate immune system. It is a receptor for the complement components C3b and C4b. It has been consistently identified as a risk factor in Alzheimer’s disease.3,4,46 BIN1 encodes the bridging integrator 1 gene, which has also been consistently associated with Alzheimer’s disease in GWAS studies.4,47 BIN1 is present in neurons, oligodendrocytes and microglia, and its primary role is thought to be in endocytosis and trafficking in neurons and immune response in glia cells.48 SLC24A4 is a solute carrier that has been associated with pigmentation traits in European populations. However, more relevant to LOAD is its association with blood pressure in African Americans, as LOAD may be influenced by vascular disease.49

Of the genes identified within the 2 Mb region surrounding the previously identified GWAS loci from the IGAP study,1 several were nominally significant. CYP3A43 is a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids, and are part of a cluster of cytochrome P450 genes on chromosome 7q21.1. MSH5 encodes a member of the mutS family of proteins that are involved in DNA mismatch repair or meiotic recombination processes. Women with premature ovarian failure were found to carry a mutation in MSH5 suggesting a role in meiotic recombination. Genetic variants in a gene within the major histocompatibility complex, HLA-DQA2, were associated with LOAD in this study. However, this gene has low expression in brain and has been associated with multiple inflammatory disorders.

Table 4. Results from FSKAT gene-based analyses of all the genes within 1 Mb of the AD loci implicated in the Lambert et al. report.

| GENE | Chr | N_NS | NO APOE | APOE | N_NS | NO APOE | APOE | Top IGAP SNP | Nearest Gene |
|------|-----|------|---------|------|------|---------|------|--------------|--------------|
| MSH5 | 6   | 5    | 1.77e-03| 0.00246474| 5    | 1.81e-03| 2.61e-03| nrs271192    | HLA-DRB5     |
| HLA-DQA2 | 6  | 3    | 7.37e-03| 6.50e-03| 3    | 6.64e-03| 6.01e-03| nrs271192    | HLA-DRB5     |
| CYP3A43 | 7  | 6    | 5.69e-03| 4.09e-03| 6    | 6.92e-03| 5.09e-05| rs1476679    | ZCWPW1       |
| TAF6  | 7   | 4    | 9.37e-02| 3.11e-02| 4    | 1.44e-02| 1.67e-04| rs1476679    | ZCWPW1       |
| MAP3K2 | 7   | 2    | 1.56e-02| 1.48e-02| 3    | 1.46e-02| 1.28e-02| rs6733839    | BIN1         |
| ZSCAN21 | 7  | 2    | 8.40e-02| 1.06e-01| 2    | 1.80e-02| 4.10e-03| rs1476679    | ZCWPW1       |
| BIN1  | 2   | 4    | 2.77e-02| 3.36e-02| 4    | 3.46e-02| 3.56e-02| rs10498633   | SLC24A4      |
| CHGA  | 14  | 4    | 3.39e-02| 3.31e-02| 4    | 3.46e-02| 3.56e-02| rs10498633   | SLC24A4      |
| NAT16 | 7   | 2    | 7.30e-02| 8.31e-02| 2    | 4.55e-02| 1.05e-01| rs1476679    | ZCWPW1       |
| LGMN  | 14  | 4    | 5.24e-02| 2.74e-02| 4    | 4.70e-02| 2.29e-02| rs10498633   | SLC24A4      |
| TNXB  | 6   | 25   | 4.79e-02| 5.19e-02| 25   | 4.91e-02| 5.38e-02| nrs271192    | HLA-DRB5     |
| TFR2  | 7   | 6    | 1.04e-01| 8.82e-02| 6    | 1.12e-01| 1.14e-02| rs1476679    | ZCWPW1       |

1Model- LOAD = Gene + Age + Sex + proportion of African ancestry + proportion of Native American Ancestry + First ten principal components (without and without APOE ε4 adjustment).
2Model- LOAD = Gene + Age + Sex + number of APOE ε4 alleles + proportion of African ancestry + proportion of Native American Ancestry + First ten principal components + Corresponding IGAP SNP (without and without APOE ε4 adjustment).
Family-based WGS on previously localized linkage regions remains a particularly powerful strategy for causal variant identification. Several new disease susceptibility genes have been successfully identified using linkage analysis coupled with WGS, in complex phenotype disorders such as hearing impairment, familial goiters, and familial hypertension. Taken together the results here imply that rare variants in multiple genes are likely to increase the risk of LOAD. Large families multiply affected by LOAD are extremely helpful in identifying novel rare variants even in genes previously investigated by other means. For example, loci identified by genome wide array studies have pointed to a large number of genes many of which have rare variants suggesting that these loci were in linkage disequilibrium with one or more of the mutations. How variants in these multiple genes lead to a common phenotype of LOAD needs to be investigated. However, investigations of gene functions may point to potential targets for new treatments or preventive measures.

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**Conflict of Interest**

The authors have no conflicts of interest to disclose.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Data S1. Methods.
Figure S1. Details of carriers of pV707I variant in TMEM132A in Caribbean Hispanic family 860.
Table S1. WGS rare exonic variants segregating with LOAD in chromosome 7q14.3 linkage region.
Table S2. Interesting variants segregating with affection status in Chr 11 Linkage region.
Table S3. Rare missense variants in candidate LOAD genes segregating with AD in Caribbean Hispanic families.
Table S4. Variant count across the chromosomes and linkage regions at each filtering step (corresponding to Fig. 1).
Table S5. Gene-based test for variants sets that including variants with CADD>30.
Table S6. Caribbean Hispanic families contributing to the linkage signals on chromosomes 7 and 11.
Table S7. Details of carriers of pv707I variant in TMEM132A.
Table S8. For family 284, individual and family IDs are displayed as EFIGSA IDs.
Table S9. For family 348, individual and family IDs are displayed as EFIGA IDs.