Identification of Novel Loci for Alzheimer Disease and Replication of CLU, PICALM, and BIN1 in Caribbean Hispanic Individuals

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Objectives: To identify novel loci for late-onset Alzheimer disease (LOAD) in Caribbean Hispanic individuals and to replicate the findings in a publicly available data set from the National Institute on Aging Late-Onset Alzheimer’s Disease Family Study.

Design: Nested case-control genome-wide association study.


Participants: Five hundred forty-nine affected and 544 unaffected individuals of Caribbean Hispanic ancestry.

Intervention: The Illumina HumanHap 650Y chip for genotyping.

Main Outcome Measure: Clinical diagnosis or pathologically confirmed diagnosis of LOAD.

Results: The strongest support for allelic association was for rs9945493 on 18q23 (P = 1.7 × 10^{-7}), but 22 additional single-nucleotide polymorphisms (SNPs) had a P value less than 9 × 10^{-6} under 3 different analyses: unadjusted and stratified by the presence or absence of the APOE ε4 allele. Of these SNPs, 5 SNPs (rs4669573 and rs10197851 on 2p25.1; rs11711889 on 3q25.2; rs1117750 on 7p21.1; and rs7908652 on 10q23.1) were associated with LOAD in an independent cohort from the National Institute on Aging Late-Onset Alzheimer’s Disease Family Study. We also replicated genetic associations for CLU, PICALM, and BIN1.

Conclusions: Our genome-wide search of Caribbean Hispanic individuals identified several novel genetic variants associated with LOAD and replicated these associations in a white cohort. We also replicated associations in CLU, PICALM, and BIN1 in the Caribbean Hispanic cohort.

multiply affected by LOAD. We first examined unrelat-
ed cases and controls in the Caribbean Hispanic indi-
viduals and then replicated the associations using the pub-
licly available GWAS data from the National Institute
on Aging Late-Onset Alzheimer’s Disease (NIA-LOAD)
Family Study (E. M. Wijsman, PhD, N. Pankratz, PhD,
Y. Choi, PhD, J. H. Rothstein, MS, K. Faber, MS,
R.C., J.H.L., T. D. Bird, MD, D. A. Bennett, MD, R. Dia-
z-Arrastia, MD, A. M. Goate, DPhil, M. Farlow, MD,
B. Ghetti, MD, R.A. Sweet, MD, T. M. Foroud, PhD, and
R.P.M.; for the NIA-LOAD/NCRAD Family Study Group.
“Genome-wide Association Study of Familial Late-Onset Al-
zheimer’s Disease Replicates BIN1 and CLU and Nomini-
tates CLU/GBP2 in Interaction with APOE,” unpublished
data). This approach allowed us to further assess the role
of genetic admixture in the Caribbean Hispanic popu-
lion. To our knowledge, this is the only GWAS of Al-
zheimer disease that focuses exclusively on a Caribbean
Hispanic population.

METHODS

SAMPLES OF
CARIBBEAN HISPANIC INDIVIDUALS

We studied 1093 unrelated Caribbean Hispanic individuals com-
prising 549 cases and 544 controls (Table 1). These partici-
ants were selected from the Washington Heights–Inwood Col-
bria Aging Project (WHICAP) study and the Estudio Familiar de
Influencia Genetica de Alzheimer (EFIGA) study. The WHICAP
study is a population-based epidemiologic study of randomly
selected elderly individuals residing in northern Manhattan, New
York, comprising 3 ethnic groups: non-Hispanic white, Carib-
bean Hispanic, and African American. For the current study,
we restricted the study inclusion to individuals who were self-
reported Hispanic of Caribbean origin and did not include non-
Hispanic white or African American individuals. In addition, we
selected 1 affected individual from each family participating in
the EFIGA study of Caribbean Hispanic families with LOAD. Both
studies followed the same clinical diagnostic methods.

The participants originated from the Dominican Republic and
Puerto Rico. Approximately 60.3% of the affected individuals were
participants in the WHICAP epidemiologic study, and the re-
main ing 39.7% of the participants were from the EFIGA study.
All unaffected individuals were participants in the WHICAP epi-
demiologic study. For the familial cases, we selected 1 proband
from each family to create a cohort of unrelated individuals. We
selected persons with definite or probable LOAD over those with
possible LOAD to limit the effects of comorbidity.

CLINICAL ASSESSMENTS

Data were available from medical, neurological, and neuropsy-
chological evaluations collected from 1999 through 2007. The
standardized neuropsychological test battery covered mul-
tiple domains and included the Mini-Mental State Examina-
tion,24 the Boston Naming Test,25 the Controlled Word Asso-
ciation Test26 from the Boston Diagnostic Aphasia Evaluation,27
the Wechsler Adult Intelligence Scale–Revised similarities sub-
test,28 the Mattis Dementia Rating Scale,29 the Rosen Drawing
Test,30 the Benton Visual Retention Test,31 the multiple-
choice version of the Benton Visual Retention Test,32 and the
Selective Reminding Test.33


Table 1. Characteristics of Subjects in the Caribbean
Hispanic Genome-Wide Association Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>WHICAP</th>
<th>EFIGA Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At onset (affected)</td>
<td>79.98 (8.0)</td>
<td>82.61 (7.3)</td>
<td>76.46 (7.7)</td>
</tr>
<tr>
<td>At last examination (unaffected)</td>
<td>78.87 (6.4)</td>
<td>78.94 (6.2)</td>
<td></td>
</tr>
<tr>
<td>Female, %</td>
<td>69.7</td>
<td>68.4</td>
<td>74.2</td>
</tr>
</tbody>
</table>

| APOE allele frequency, %          |       |          |             |
| ε4                               | 18.16 | 23.41    | 12.87       |
| ε3                               | 75.07 | 70.58    | 79.60       |
| ε2                               | 6.77  | 6.01     | 7.54        |

Abbreviations: AD, Alzheimer disease; EFIGA, Estudio Familiar de
Influencia Genetica de Alzheimer; WHICAP, Washington Heights–Inwood Columbia Aging Project.

aDescriptive demographic and clinical characteristics of the participating subjects from the WHICAP epidemiologic study and from the EFIGA Family Study are presented. To maintain a cohort of unrelated individuals, we selected 1 subject with definite/probable AD from each family for the EFIGA Family Study participants.

bAllele frequency was significantly different in affected vs unaffected individuals.

DIAGNOSIS OF DEMENTIA

The diagnosis of dementia was established on the basis of all available information gathered from the initial and follow-up assessments and medical records. The diagnosis of LOAD was based on the National Institute of Neurological Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria.34

GENOTYPING

Single-nucleotide polymorphisms (SNPs) were genotyped at the Illumina Genotyping Service Center, San Diego, California, using Illumina HumanHap 650Y chips. From the 650Y chips, 658 610 SNP markers were originally genotyped. Quality control mea-
sures for SNP genotype were performed using PLINK (http://pngu.
mgh.harvard.edu/~purcell/plink/). We excluded SNPs with the following characteristics: missing genotype rate more than 20%; minimum allele frequency less than 1%; Hardy-Weinberg equi-
librium test at a P value less than .0001 in controls. Although the 650Y chip includes additional SNPs for Yoruban individu-
als, we initially used less stringent criteria for quality control than others because the Illumina SNP chips are optimized for white populations. Furthermore, we wanted to reduce the likelihood of false-negative results. To limit the possibility that positive sig-
als were caused by SNPs with poor calling rate, we lowered the threshold for the missing genotype rate to 5%. This screen reduced the total number of analyzed SNPs by 0.26%. None of the SNPs of main interest (ie, P value <9×10−8 shown in Table 2) had low genotype rates. Following all quality control measures, we analyzed 627 380 autosomal SNPs.

POPULATION STRATIFICATION

We applied 2 methods to estimate ancestry proportion in each subject, and thus population stratification, in this case-control data set: STRUCTURE version 2.235 and identity-by-state–
based clustering method using PLINK version 1.05c (cAppendix, http://www.cogeneurol.com). Briefly, we used 500 unlinked SNPs for the STRUCTURE analysisd and all available SNPs (n=627 380 autosomal SNPs) for the PLINK analysis to assess underlying population structure. To see better representation of the geographic separation from source populations, we augmented the 1093 Hispanic samples with 210 subjects from the HapMap Web site (http://www.hapmap.org), which included 60 European American, 60 Yoruban, and 90 East Asian individuals. Our analyses revealed that the assignment of cluster from the STRUCTURE program was comparable with that from the PLINK program (data not shown). For all subsequent association analyses, we used the cluster information obtained from the PLINK analysis to correct for population stratification. The genomic inflation factor was not inflated (1.0378 after population stratification correction, eFigure 1).

**STATISTICAL ANALYSIS**

We conducted single-point allelic association analysis using the Mantel-Haenszel χ² test statistic, which tests for SNP-disease association conditional on population subcluster estimated from the PLINK analysis described earlier (Table 2). In addition, we performed a multivariate logistic regression analysis, adjusted for age, sex, education, and population stratification, using PLINK (Table 3). For the analysis of all subjects only, we adjusted for the presence or absence of APOE along with the earlier-mentioned 4 covariates. To determine whether the associations were caused by statistical artifact, we computed the P value for 1 million replications to derive empirical P values for the top 23 SNPs that showed the strongest support for association with LOAD. For this purpose, we randomly shuffled affection status for each subject to create the null distribution and assess the likelihood of false-positive results for each SNP.

**REPLICATION DATASETS**

We prioritized candidate SNPs by selecting SNPs that had a nominal P value of 9 × 10⁻⁶ or lower. While this cut point does not reach the Bonferroni-corrected genome-wide P value of 0.05, this cut point helped us to prioritize SNPs of importance. To determine whether the findings from the Caribbean Hispanic ins...
individuals could be replicated in an independent data set, we examined the publicly available GWAS data from the NIA-LOAD study (Wijsman et al, unpublished data [full citation on page 321]). Briefly, the study first examined the publicly available GWAS data from the NIA-LOAD study and 325 individuals from the NIA-LOAD study and 325 individuals from the National Cell Repository for Alzheimer's Disease (NCRAD) (Table 2). The details of the demographic and clinical characteristics of the NIA-LOAD participants who were included in the study (Wijsman et al, unpublished data [full citation on page 321]). In the present study, we specifically compared the results from this GWAS in Caribbean Hispanic individuals from the NIA-LOAD study and 325 individuals from the National Cell Repository for Alzheimer's Disease (NCRAD) database. These self-reported European American individuals were subsequently clustered into 3 groups (northern European, Ashkenazi Jewish, and southern European) based on a principle component analysis. Subsequent analyses took ethnic background into consideration. In the present study, we specifically compared the results from this GWAS in Caribbean Hispanic individuals against the results from 3 subanalyses in the NIA-LOAD GWAS: case-control analysis of unrelated individuals; family-based analysis stratified by APOE; and family-based analysis stratified by ethnicity. Table 2 presents the P values for each SNP. We also list SNPs located within 5 kilobases that have a nominal P value less than .05.

We subsequently identified a set of self-reported Caribbean Hispanic individuals from the NIA-LOAD data set. These include an additional 116 unrelated patients with LOAD and 70 unrelated controls who were not included in previous analyses. To check comparability between the 2 Caribbean Hispanic data sets and to check SNP calling between the Illumina 650Y and 610K SNP chips, we compared allele frequencies for common randomly selected SNPs. Allele frequencies between the 2 data sets did not differ significantly.

### Table 3. ORs Associated With Minor Allele in the Caribbean Hispanic GWAS

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>OR (95% CI)</th>
<th>P Emp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs752939</td>
<td>T</td>
<td>0.165</td>
<td>0.582 (0.460-0.736)</td>
<td>.00141</td>
</tr>
<tr>
<td>2</td>
<td>rs4666573</td>
<td>G</td>
<td>0.465</td>
<td>1.421 (1.198-1.658)</td>
<td>.01183</td>
</tr>
<tr>
<td>3</td>
<td>rs1017651</td>
<td>A</td>
<td>0.488</td>
<td>0.713 (0.602-0.846)</td>
<td>.00229</td>
</tr>
<tr>
<td>4</td>
<td>rs1402752</td>
<td>C</td>
<td>0.172</td>
<td>1.480 (1.180-1.855)</td>
<td>.01500</td>
</tr>
<tr>
<td>5</td>
<td>rs1711899</td>
<td>A</td>
<td>0.087</td>
<td>0.472 (0.383-0.614)</td>
<td>.00016</td>
</tr>
<tr>
<td>6</td>
<td>rs119287</td>
<td>G</td>
<td>0.268</td>
<td>0.708 (0.584-0.860)</td>
<td>.00162</td>
</tr>
<tr>
<td>7</td>
<td>rs11876092</td>
<td>G</td>
<td>0.279</td>
<td>0.694 (0.578-0.838)</td>
<td>.00311</td>
</tr>
<tr>
<td>8</td>
<td>rs976728</td>
<td>G</td>
<td>0.231</td>
<td>1.511 (1.261-1.811)</td>
<td>.00017</td>
</tr>
<tr>
<td>9</td>
<td>rs10578359</td>
<td>G</td>
<td>0.480</td>
<td>0.671 (0.566-0.797)</td>
<td>.00124</td>
</tr>
<tr>
<td>10</td>
<td>rs7908622</td>
<td>G</td>
<td>0.420</td>
<td>1.466 (1.261-1.773)</td>
<td>.00389</td>
</tr>
<tr>
<td>11</td>
<td>rs978770</td>
<td>T</td>
<td>0.384</td>
<td>0.661 (0.555-0.805)</td>
<td>.00018</td>
</tr>
<tr>
<td>12</td>
<td>rs11213703</td>
<td>C</td>
<td>0.368</td>
<td>0.628 (0.535-0.733)</td>
<td>.00149</td>
</tr>
<tr>
<td>13</td>
<td>rs11671026</td>
<td>A</td>
<td>0.090</td>
<td>0.541 (0.403-0.727)</td>
<td>.00192</td>
</tr>
<tr>
<td>14</td>
<td>rs15633092</td>
<td>A</td>
<td>0.360</td>
<td>0.661 (0.540-0.800)</td>
<td>.00000</td>
</tr>
<tr>
<td>15</td>
<td>rs6434359</td>
<td>A</td>
<td>0.140</td>
<td>1.692 (1.272-2.251)</td>
<td>.00356</td>
</tr>
<tr>
<td>16</td>
<td>rs9945493</td>
<td>G</td>
<td>0.375</td>
<td>0.726 (0.588-0.989)</td>
<td>.00527</td>
</tr>
</tbody>
</table>

Abbreviations: Chr, chromosome; CI, confidence interval; Emp, empirical; GWAS, genome-wide association study; MAF, minor allele frequency; NIA-LOAD, National Institute on Aging Late-Onset Alzheimer's Disease; OR, odds ratio; SNP, single-nucleotide polymorphism.

CANDIDATE GENE ANALYSES

We performed separate analyses focusing on SNPs in the candidate genes that were identified from previous GWAS, including CRI, CLU, PICALM, and BIN1, for the significant genetic associations reported and replicated in 3 previous studies.7,9,13 For these genes, we performed 4 analyses: Mantel-Haenszel χ² test taking into account population stratification, APOE ε4–restricted analysis (ie, restricted to individuals with at least 1 copy of ε4 compared with those without), and Mantel-Haenszel χ² test taking into account the presence or absence of APOE ε4. In addition to these 4 genes, we followed up the novel genetic association identified from the NIA-LOAD GWAS (Wijsman et al, unpublished data [full citation on page 321]). The NIA-LOAD GWAS identified the CUBGP2 gene to be significantly associated with LOAD among a subset of samples with homozygous APOE ε4 carriers. Herein, we evaluated the association using 2 different models to account for its association with the APOE ε4 genotype (Table 4). Under model 1, homozygous APOE ε4 carriers were considered to have the putative genotype and all others do not. Under model 2, homozygous APOE ε4 carriers were considered to have the putative genotype, while homozygous APOE ε3 carriers, the most common isoform, were considered to have a wild type. The remaining subjects were excluded in the analysis.

RESULTS

SUBJECTS

Seventy percent of the participants were women. The mean (SD) age at onset of LOAD was 79.98 (8.0) years,
None of the SNPs reached genome-wide statistical significance at a nominal P value of 7.97 × 10^{-6} or lower. The results from the population stratification–adjusted single-point analysis are shown in a Manhattan plot (Figure 2). Twenty-three SNPs had P values less than 9 × 10^{-6} in at least 1 of the 3 analyses, including all combined subjects, carriers of the APOE ε4 allele, and non-carriers of the APOE ε4 allele (Table 2). Of those, the strongest evidence for association was observed for rs9945493 (P = 1.7 × 10^{-7}; OR, 0.33; 95% confidence interval, 0.21-0.51) on 18q23. For each SNP, we calculated ORs and 95% confidence intervals as well as empirical P values based on 1 million replicates (Table 3). As observed in other GWAS, ORs ranged from 0.33 for rs9945493 to 1.87 for rs1117750 for all subjects.

We then examined the same 23 SNPs from Table 2 in an independent data set by comparing the results from each of our 3 analyses against data from the NIA-LOAD GWAS, which was restricted to self-reported European American individuals (Wijsman et al, unpublished data [full citation on page 321]). Five SNPs (rs4669573 and rs10197851 on 2p25.1, rs11711889 on 3q25.2, rs1117750 on 7p21.1, and rs7908652 on 10q23.1) from the list of 23 had a nominal P value less than .05 in at least 1 of the 3 analyses in the NIA-LOAD GWAS (Table 2, footnote c); rs4669573 is located within the HPCAL1 (hippocalcin-like) gene, and the ODC1 gene is located 100 kilobases away, and rs1117750 and several flanking SNPs that supported allelic association were located within the DGKB (diacylglycerol kinase, β 90 kDa) gene. Lastly, rs7908652 is located proximal to multiple genes, including GHITM (growth hormone inducible transmembrane protein), C10orf99 (chromosome 10 open reading frame 99), PCDH21 (protocadherin 21), LRIT2 (leucine-rich repeat, immunoglobulin-like, and transmembrane domains 2), LRIT1 (leucine-rich repeat, immunoglobulin-like, and transmembrane domains 1), and RGR (retinal G protein-coupled receptor) (Figure 2).

### Table 4. Replication of Candidate SNPs (CLU, PICALM, and BIN1) From Previous GWAS: Examination in Caribbean Hispanic Individuals

<table>
<thead>
<tr>
<th>SNP</th>
<th>bp</th>
<th>Ethnicity: Population-Stratified Analysis</th>
<th>APOE Stratified</th>
<th>ε4*</th>
<th>ε4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLU (8p21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs881146</td>
<td>27 500 194</td>
<td>0.03740</td>
<td>0.06170</td>
<td>0.67570</td>
<td>0.00213</td>
</tr>
<tr>
<td>rs70120100</td>
<td>27 504 646</td>
<td>0.15900</td>
<td>0.12540</td>
<td>0.42890</td>
<td>0.1210</td>
</tr>
<tr>
<td>rs11136000</td>
<td>27 520 436</td>
<td>0.04170</td>
<td>0.07797</td>
<td>0.01770</td>
<td>0.67560</td>
</tr>
<tr>
<td>PICALM (11q14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs17159904</td>
<td>85 463 935</td>
<td>0.04243</td>
<td>0.04951</td>
<td>0.04270</td>
<td>0.65250</td>
</tr>
<tr>
<td>rs541458</td>
<td>85 465 999</td>
<td>0.36300</td>
<td>0.44710</td>
<td>0.04710</td>
<td>0.79400</td>
</tr>
<tr>
<td>rs543293</td>
<td>85 497 725</td>
<td>0.72240</td>
<td>0.93160</td>
<td>0.40560</td>
<td>0.27150</td>
</tr>
<tr>
<td>rs7941541</td>
<td>85 536 186</td>
<td>0.73180</td>
<td>0.92010</td>
<td>0.38990</td>
<td>0.27920</td>
</tr>
<tr>
<td>BIN1 (2q14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3851179</td>
<td>85 546 288</td>
<td>0.32050</td>
<td>0.44610</td>
<td>0.08170</td>
<td>0.21020</td>
</tr>
</tbody>
</table>

Abbreviations: bp, base pair; GWAS, genome-wide association studies; SNP, single-nucleotide polymorphism.

a Association with SNPs in Caribbean Hispanic samples for the 4 known genes, including CR1, CLU, PICALM, and BIN1. However, none of the SNPs were associated nominally for CR1.

b Allelic association analysis, stratified by ethnicity.

c P values <.05.

d Originally noted as genome-wide significant in Harold et al' and Lambert et al.©2011 American Medical Association. All rights reserved.
ε BIN1 adjusted analyses. For APOE LOAD in population stratification–adjusted and PICALM individuals, was not associated with LOAD herein. For association with LOAD in European and American white LOAD in population-stratified analysis and among Table 4, footnote c) was significantly associated with ε We evaluated an interaction model between APOE and CUGBP2 to follow up the putative gene × gene interaction finding in the NIA-LOAD study (Wijsman et al, unpublished data [full citation on page 321]) (Figure 3). In that study, rs201119 in the CUGBP2 gene was significantly associated with LOAD only among individuals with a homozygous ε4 genotype ($P_{\text{nominal}} = 1.52 \times 10^{-6}$), but this SNP was not significantly associated with LOAD when all subjects were considered ($P_{\text{nominal}} = .726$ for allelic association and $P = .2607$ for genotype association). Because we had a smaller sample size than the NIA-LOAD GWAS, we applied 2 somewhat different models to test whether the allelic association between CUGBP2 and LOAD was restricted to carriers of APOE ε4 and absent in non–APOE ε4s carriers. For this purpose, we performed an interaction model using PLINK in both the Caribbean Hispanic and NIA-LOAD samples. As shown in Figure 3, in the Caribbean Hispanic individuals, we observed a modest interaction between the genotype at rs201119 in the CUGBP2 gene and APOE ε4 genotype ($P_{\text{nominal}} = .04898$ under model 2). This is the SNP that showed the original allelic association in the NIA-LOAD GWAS samples. For the same SNP, the NIA-LOAD samples had a $P$ value of .00012 under model 1 and .00016 under model 2, supporting the association under our models for both data sets. When we examined all SNPs in CUGBP2 in both data sets, however, we
observed 2 different regions with strongest signals (Figure 2). The SNP rs2242451 showed the strongest support under model 2 ($P_{\text{nominal}} = 0.00324$) in the Caribbean Hispanic samples, while in the NIA-LOAD samples, the strongest signal came from rs201119 and adjacent SNPs.

**COMMENT**

We report several novel candidate loci that may harbor putative disease variants in Caribbean Hispanic individuals with LOAD and confirmed associations between LOAD and the 4 genes that have been previously reported. These 4 novel loci (5 SNPs) include multiple genes, and further examination is necessary to verify their involvement in LOAD. We replicated the allelic association between LOAD and CUGBP2 in homozygous carriers of the APOE ε4 allele reported by Wijsman and colleagues (Wijsman et al, unpublished data [full citation on page 321]). This gene was studied because the strongest signal was observed in homozygous ε4 carriers and this region on chromosome 10p14 contains the gene CUGBP2. CUGBP2 has 1 isoform that is expressed predominantly in neurons, with experimental evidence suggesting involvement in apoptosis in the hippocampus. Further, it is involved in posttranscriptional RNA binding activities as well as pre-messenger RNA alternative splicing. Based on structural similarity, it is speculated that this gene may be involved in increasing COX2 messenger RNA. Although the current study does support association with LOAD, the pattern of the associated SNPs differed between the 2 populations. The difference in genetic architecture between non-Hispanic and Hispanic populations is the most likely explanation for the fact that the associated SNPs differed between the 2 populations.

We found that the 4 candidate loci that were strongly associated with LOAD and were replicated in the NIA-LOAD cohort are located near genes that could be biologically relevant to LOAD. HPCAL1 on 2p25.1 is a calcium-binding protein expressed in the brain and has been associated with hypertension in Japanese individuals, which in turn is associated with LOAD risk. The region 10q23.1 includes 3 genes that are expressed in the brain and have been reported by Grupe et al, including PCDH21 (believed to be involved in the neuronal maintenance), LRRTM1, and RGR.

We replicated associations between LOAD and SNPs in 3 of the 4 genes that were previously reported to be significant at the genome-wide level, namely CLU, PICALM, and BIN1. However, the associated SNPs between these candidate genes and LOAD were not necessarily identical in the Caribbean Hispanic individuals compared with a European American data set. Nonetheless, the overall support for the 3 genes is enhanced by the observation that the allelic association extends to an ethnically distinct population.

CLU, believed to be involved in modulation of inflammation and lipid metabolism, was associated with LOAD in carriers of ε4 ($P = 0.00213$). More than a decade ago, we examined CLU (also known as APOJ) as a risk factor for LOAD because it shares similar functional roles as APOE, including cholesterol binding and involvement in inflammation or injury. Based on a small set of coding polymorphisms in APOJ, Tycko and colleagues did observe a positive association in 1 homozygous polymorphism, but this association was no longer significant when all subjects with at least 1 copy of the APOE ε4 allele were excluded. Further, they observed a significant difference in allele frequencies by race, and the present study also shows different linkage disequilibrium patterns between the Caribbean Hispanic individuals and the NIA-LOAD cohorts (eFigure 3). Thus, the inconsistent findings across studies could be attributed to an interaction between APOE and APOJ, small sample size, different distribution of ethnic background in the participants, or any combination of these factors. The present study observed an association between CLU and LOAD in the presence of APOE ε4 (Table 4). This is consistent with the much larger study by Lambert and colleagues but not with the study by Harold et al.

BIN1, a gene expressed in the central nervous system and reported to activate a caspase-independent apoptotic process, was also associated with LOAD in only carriers of ε4 ($P = 0.00536$). PICALM is reported to be involved in the neurotransmitter release processes, thereby affecting memory functions.

Together these 3 genes suggest that they contribute to the overall LOAD phenotype. However, the measures of association are unlikely to be consistent across data sets, since in addition to allelic differences among race groups, significant differences in the distribution of vascular and inflammation risk factors can also alter the observed genotype-phenotype relations, even after adjusting for other known risk factors including age, sex, and education.

The current study has some limitations. First, this study, based on a modest sample size of Caribbean Hispanic individuals, does not have power to detect rare variants with weak effects; thus, some risk variants may have been missed. Based on the original GWAS set, the current study has 80% power, genome-wide, to detect alleles with a frequency of 0.35 or higher when the OR is 1.5. When the OR for SNPs is 1.7, this study has 80% power to detect SNPs with an allele frequency of 0.25 or higher. When we combined both Caribbean Hispanic data sets (specifically, one from our GWAS along with the Caribbean Hispanic subset that is part of the NIA-LOAD GWAS), the current study has 80% power genome-wide to detect SNPs with somewhat lower allele frequencies. For a SNP with an OR of 1.5, 80% power can be achieved for SNPs with an allele frequency of 0.3 or higher. For a SNP with an OR of 1.7, 80% power can be achieved for SNPs with an allele frequency of 0.2 or higher. Power calculation was carried out assuming an additive model with SNP minor allele frequency being comparable with the allele frequency of the putative variant (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). Second, independent replication of the candidate SNPs in Caribbean Hispanic individuals who share comparable genetic architecture would have further strengthened the validity of the findings because the likelihood of replicating the same allele within the same SNP would be higher in other ethnic groups. For this reason, we added a small set of Caribbean Hispanic individuals from the NIA-LOAD GWAS data set who were evaluated using the same
diagnostic tools. However, the sample size remained relatively modest. When we evaluated the candidate SNPs in an independent sample of European American individuals with different genetic background (NIA-LOAD GWAS), often allelic associations for the same SNPs were modest, but different SNPs within the gene supported allelic association. However, genetic associations using a cohort with a different ethnic background strengthen the observed association since (1) it is not unexpected to have multiple variants within a gene associated with a disease (eg, PSEN1) and (2) the findings may be generalizable to a wider set of populations. These findings need to be further evaluated using functional genetics approaches to evaluate the validity of observed association.

We used a dense set of SNPs to survey the genome to identify novel loci and to assess support for allelic association with BIN1, CLU, and PICALM. The current cohort extends previous GWAS of non-Hispanic white populations by exploring allelic association in an admixed cohort with a different set of genetic and environmental risk factors. The confirmation in the present study further strengthens the associations between variants in these genes and LOAD. It also supports the role of other genetic (eg, APOE) and environment factors modulating the genetic variant, especially when each variant may only have a small effect size. We also identified novel candidate genes (eg, HPCAL1, DGKB) in a Caribbean Hispanic cohort and replicated the association in an independent ethnically different data set. These genes need to be examined further in independent data sets.

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**Announcement**

“What is Your Diagnosis?” is a new quarterly online feature of the Archives of Neurology edited by Lawrence S. Honig, MD, PhD, of Columbia University. A case history including an image will be presented, followed by the request for your diagnosis from a list of 4 possible choices. The correct diagnosis will then be presented with a commentary as to why it is correct. We believe it will become a popular and anticipated new feature and welcome your comments and suggestions.