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 (\(P = 1.7 \times 10^{-7}\)), but 22 additional single-nucleotide polymorphisms (SNPs) had a \(P\) value less than \(9 \times 10^{-6}\) under 3 different analyses: unadjusted and stratified by the presence or absence of the APOE \(\varepsilon4\) 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 unrel-
related 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-
Arrastia, MD, A. M. Goate, DPphil, 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 of Familial Late-Onset Alz-
heimer’s Disease Replicates BIN1 and CLU and Nomi-
nates CUGBP2 in Interaction with APOE,” unpublished
data). This approach allowed us to further assess the role
of genetic admixture in the Caribbean Hispanic popu-
lation. To our knowledge, this is the only GWAS of Alz-
heimer’s 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 Co-
lumbia 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.22
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-
maining 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 evaluations23 collected from 1999 through 2007. The stan-
dardized neuropsychological test battery covered mul-
tiple domains and included the Mini-Mental State Exami-
nation,24 the Boston Naming Test,25 the Controlled Word Assos-
tion 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.32

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 Associa-
tion criteria.31

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://pangu.
.mgh.harvard.edu/~purcell/plink/). We excluded SNPs with the fol-
lowing characteristics: missing genotype rate more than 20%;
minimum allele frequency less than 1%; Hardy-Weinberg equi-
librium test33 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-
nals were caused by SNPs with poor calling rate, we lowered the
threshold for the missing genotype rate to 5%. This screen re-
duced the total number of analyzed SNPs by 0.26%. None of the
SNPs of main interest (ie, P value < .05 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 2.233 and identity-by-state–
Based clustering method using PLINK version 1.0536 (eAppendix, http://www.archneurol.com). Briefly, we used 500 unlinked SNPs for the STRUCTURE analysis35 and all available SNPs (n=627380 autosomal SNPs) for the PLINK analysis to assess 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 had 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 .05, this cut point helped us to prioritize SNPs of importance. To determine whether the findings from the Caribbean Hispanic in-

### Table 2. Candidate SNPs From the Caribbean Hispanic GWAS and Replication in the NIA-LOAD GWAS³

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>bp</th>
<th>Cyto</th>
<th>All</th>
<th>ε 4⁺</th>
<th>ε 4⁻</th>
<th>Unrelated</th>
<th>Family-Based</th>
<th>Flanking SNP</th>
<th>Candidate Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs7525939</td>
<td>224822594</td>
<td>1q42.1</td>
<td>5.29 × 10⁻⁴</td>
<td>.000462</td>
<td>.01300</td>
<td>.48650</td>
<td>.67635</td>
<td>.16364</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>rs4669573³</td>
<td>10396387</td>
<td>2p25.1</td>
<td>5.26 × 10⁻⁵</td>
<td>.00587</td>
<td>.03628</td>
<td>.29032</td>
<td>.26800</td>
<td>.45349</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>rs10197851²</td>
<td>10402860</td>
<td>2p25.1</td>
<td>.001002</td>
<td>7.13 × 10⁻⁶</td>
<td>.54250</td>
<td>.20623</td>
<td>.71710</td>
<td>.10894</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>rs1402752</td>
<td>44662304</td>
<td>3p21.3</td>
<td>.000663</td>
<td>6.42500</td>
<td>.83480</td>
<td>.86980</td>
<td>.88940</td>
<td>.82175</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>rs11711889²</td>
<td>15485356</td>
<td>3q25.2</td>
<td>6.95 × 10⁻⁴</td>
<td>.000175</td>
<td>.02247</td>
<td>.03814</td>
<td>.49350</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>rs919289</td>
<td>12059322</td>
<td>5q23.1</td>
<td>.000468</td>
<td>3.20 × 10⁻⁵</td>
<td>.74380</td>
<td>.79070</td>
<td>.34351</td>
<td>.95958</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>rs4895298</td>
<td>12059707</td>
<td>5q23.1</td>
<td>.000477</td>
<td>2.00 × 10⁻⁵</td>
<td>.65480</td>
<td>.63350</td>
<td>.22174</td>
<td>.14639</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>rs2973413</td>
<td>120597210</td>
<td>5q23.1</td>
<td>.000571</td>
<td>3.53 × 10⁻⁴</td>
<td>.91730</td>
<td>.53150</td>
<td>.21149</td>
<td>.15152</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>rs10271466</td>
<td>10084597</td>
<td>7q22.1</td>
<td>.004105</td>
<td>.001305</td>
<td>.95270</td>
<td>.04630</td>
<td>.26433</td>
<td>.12643</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>rs1117750²</td>
<td>14854943</td>
<td>7q22.1</td>
<td>8.02 × 10⁻⁴</td>
<td>.052861</td>
<td>.01305</td>
<td>.95270</td>
<td>.04630</td>
<td>.26433</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: bp, base pair; Chr, chromosome; Cyto, cytogenetic location; GWAS, genome-wide association study; NA, not available; NIA-LOAD, National Institute on Aging Late-Onset Alzheimer’s Disease; SNP, single-nucleotide polymorphism.

³The SNPs with the most significant P values (P < 9 × 10⁻⁶) in at least 1 of the 3 analyses (overall, APOE ε4 carriers, and APOE ε4 noncarriers) are presented. P values for the SNPs of interest in the Hispanic GWAS were compared with those in the NIA-LOAD GWAS.

b Family-based allelic association stratified by APOE status.

c Family-based allelic association taking into account population substructure.

d Flanking SNP within 5 kilobases on either side with a nominal P < .05 in the NIA-LOAD data set.

⁴These SNPs have 1 or more SNPs with a nominal P < .05 in the NIA-LOAD data set.
The 2 data sets did not differ significantly. To check comparability between the 2 Caribbean Hispanic data sets and to check SNP calling between the Illumina database. These self-reported European American individuals were examined self-reported European American individuals: 2124 in the National Cell Repository for Alzheimer's Disease (NCRAD) examined the publicly available GWAS data from the NIA-LOAD Institute on Aging Late-Onset Alzheimer's Disease; OR, odds ratio; SNP, single-nucleotide polymorphism.

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.

For the same 23 candidate SNPs, we provide age-, sex-, education-, and population stratification-adjusted ORs from a multivariate logistic regression. Empirical P values are based on 1 million replicates.

These SNPs have 1 or more SNPs with a nominal P < .05 in the NIA-LOAD data set.

### 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>
<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></td>
<td>0.165</td>
<td>0.582 (0.460-0.736)</td>
<td>.00241</td>
<td>T</td>
<td>0.171</td>
<td>0.601 (0.451-0.800)</td>
<td>.00249</td>
</tr>
<tr>
<td>2</td>
<td>rs4669573</td>
<td>G</td>
<td>0.465</td>
<td>1.421 (1.198-1.685)</td>
<td>.01837</td>
<td>G</td>
<td>0.463</td>
<td>1.437 (1.302-1.624)</td>
<td>.00005</td>
</tr>
<tr>
<td>2</td>
<td>rs10197851</td>
<td>A</td>
<td>0.388</td>
<td>1.073 (0.602-0.846)</td>
<td>.01032</td>
<td>A</td>
<td>0.489</td>
<td>0.619 (0.502-0.784)</td>
<td>.00015</td>
</tr>
<tr>
<td>3</td>
<td>rs1402752</td>
<td>C</td>
<td>0.172</td>
<td>1.480 (1.180-1.855)</td>
<td>.01503</td>
<td>C</td>
<td>0.159</td>
<td>1.069 (0.807-1.417)</td>
<td>.00000</td>
</tr>
<tr>
<td>3</td>
<td>rs1171889</td>
<td>A</td>
<td>0.087</td>
<td>0.496 (0.383-0.676)</td>
<td>.000165</td>
<td>A</td>
<td>0.092</td>
<td>0.485 (0.330-0.712)</td>
<td>.000615</td>
</tr>
<tr>
<td>5</td>
<td>rs192989</td>
<td>G</td>
<td>0.268</td>
<td>0.708 (0.584-0.860)</td>
<td>.01052</td>
<td>G</td>
<td>0.262</td>
<td>0.616 (0.440-0.717)</td>
<td>.00014</td>
</tr>
<tr>
<td>5</td>
<td>rs4898528</td>
<td>T</td>
<td>0.254</td>
<td>0.705 (0.579-0.858)</td>
<td>.01078</td>
<td>G</td>
<td>0.247</td>
<td>0.548 (0.427-0.704)</td>
<td>.02002</td>
</tr>
<tr>
<td>5</td>
<td>rs2837413</td>
<td>T</td>
<td>0.242</td>
<td>0.705 (0.577-0.860)</td>
<td>.01244</td>
<td>T</td>
<td>0.233</td>
<td>0.556 (0.431-0.717)</td>
<td>.00011</td>
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<tr>
<td>7</td>
<td>rs10274466</td>
<td>G</td>
<td>0.199</td>
<td>1.520 (1.231-1.885)</td>
<td>.00232</td>
<td>G</td>
<td>0.191</td>
<td>1.824 (1.404-2.369)</td>
<td>.00072</td>
</tr>
<tr>
<td>7</td>
<td>rs1171750</td>
<td>T</td>
<td>0.115</td>
<td>1.871 (1.418-2.470)</td>
<td>.01091</td>
<td>T</td>
<td>0.112</td>
<td>1.871 (1.418-2.470)</td>
<td>.01011</td>
</tr>
<tr>
<td>7</td>
<td>rs11878902</td>
<td>G</td>
<td>0.279</td>
<td>0.694 (0.573-0.838)</td>
<td>.00211</td>
<td>T</td>
<td>0.287</td>
<td>0.593 (0.470-0.748)</td>
<td>.00019</td>
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<td>8</td>
<td>rs977656</td>
<td>T</td>
<td>0.321</td>
<td>1.511 (1.261-1.811)</td>
<td>.00013</td>
<td>T</td>
<td>0.311</td>
<td>1.404 (1.126-1.751)</td>
<td>.00014</td>
</tr>
<tr>
<td>9</td>
<td>rs1058393</td>
<td>G</td>
<td>0.480</td>
<td>0.671 (0.585-0.790)</td>
<td>.00012</td>
<td>G</td>
<td>0.494</td>
<td>0.689 (0.535-0.850)</td>
<td>.00007</td>
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<tr>
<td>10</td>
<td>rs9098652</td>
<td>T</td>
<td>0.427</td>
<td>1.466 (1.261-1.773)</td>
<td>.01060</td>
<td>T</td>
<td>0.417</td>
<td>1.738 (1.119-1.697)</td>
<td>.00045</td>
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<tr>
<td>11</td>
<td>rs1177869</td>
<td>T</td>
<td>0.304</td>
<td>0.661 (0.555-0.796)</td>
<td>7.10E-05</td>
<td>T</td>
<td>0.300</td>
<td>0.661 (0.555-0.796)</td>
<td>7.10E-05</td>
</tr>
<tr>
<td>11</td>
<td>rs978770</td>
<td>T</td>
<td>0.383</td>
<td>0.653 (0.548-0.777)</td>
<td>4.10E-04</td>
<td>T</td>
<td>0.394</td>
<td>0.717 (0.580-0.886)</td>
<td>0.04560</td>
</tr>
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<td>11</td>
<td>rs11213703</td>
<td>C</td>
<td>0.348</td>
<td>0.655 (0.573-0.783)</td>
<td>6.30E-10</td>
<td>C</td>
<td>0.396</td>
<td>0.723 (0.585-0.893)</td>
<td>0.03930</td>
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<tr>
<td>11</td>
<td>rs1617026</td>
<td>A</td>
<td>0.090</td>
<td>0.541 (0.375-0.781)</td>
<td>0.09990</td>
<td>A</td>
<td>0.061</td>
<td>0.823 (0.532-1.272)</td>
<td>0.00000</td>
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<tr>
<td>11</td>
<td>rs1633802</td>
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<td>0.339</td>
<td>0.661 (0.544-0.790)</td>
<td>0.00017</td>
<td>A</td>
<td>0.364</td>
<td>0.708 (0.570-0.880)</td>
<td>0.00026</td>
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<tr>
<td>12</td>
<td>rs6434359</td>
<td>A</td>
<td>0.104</td>
<td>1.692 (1.227-2.521)</td>
<td>0.00000</td>
<td>A</td>
<td>0.105</td>
<td>2.087 (1.553-2.702)</td>
<td>0.00000</td>
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<tr>
<td>18</td>
<td>rs9945493</td>
<td>A</td>
<td>0.050</td>
<td>0.329 (0.213-0.508)</td>
<td>5.00E-04</td>
<td>A</td>
<td>0.047</td>
<td>0.383 (0.219-0.669)</td>
<td>0.00030</td>
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<tr>
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<td>rs6157052</td>
<td>C</td>
<td>0.092</td>
<td>0.503 (0.372-0.681)</td>
<td>0.00018</td>
<td>C</td>
<td>0.100</td>
<td>0.563 (0.393-0.808)</td>
<td>0.00030</td>
</tr>
<tr>
<td>21</td>
<td>rs2403731</td>
<td>A</td>
<td>0.117</td>
<td>0.542 (0.414-0.700)</td>
<td>0.00018</td>
<td>A</td>
<td>0.123</td>
<td>0.525 (0.377-0.732)</td>
<td>0.00026</td>
</tr>
</tbody>
</table>

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and 18.2% of the subjects were carriers of an APOE ε4 allele. The mean (SD) age at last examination of the controls was 78.87 (6.4) years. The analysis testing for population stratification revealed that the 1093 Hispanic individuals comprised 658 individuals (60.2%) who were likely to be of European white ancestry, 401 (36.7%) who were likely to be of African ancestry, and 34 (3.1%) who were unrelated to the prior 2 groups and from other Latin American countries (Figure 1).

STATISTICAL ANALYSIS

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 1q23.1) 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). 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.

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REPLICATION OF THE PUBLISHED CANDIDATE GENES

For CLU, we observed that rs881146 ($P_{\text{nominal}}=.00213$; Table 4, footnote c) was significantly associated with LOAD in population-stratified analysis and among APOE ε4 carriers (Table 4). However, rs11136000 in CLU, reported both by Harold et al and Lambert et al to be associated with LOAD in European and American white individuals, was not associated with LOAD herein. For PICALM, rs17159904 was marginally associated with LOAD in population stratification–adjusted and APOE-adjusted analyses. For BIN1, we observed a positive association in ε4 carriers for rs7561528 ($P_{\text{nominal}}=.00536$).

GENE × GENE INTERACTION

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 ($P_{\text{nominal}}=1.52 \times 10^{-8}$), 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 ($P_{\text{nominal}} = .00324$) in the Caribbean Hispanic samples, while in the NIA-LOAD samples, the strongest signal came from rs201119 and adjacent SNPs.

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 cohorts. 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), LRIT1, 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 ($P = .00213$). 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 ($P = .00536$). 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 than 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.