

ONLINE FIRST

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.

Setting: The Washington Heights–Inwood Columbia Aging Project and the Estudio Familiar de Influencia Genética de Alzheimer 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 9×10^{-6} under 3 different analyses: unadjusted and stratified by the presence or absence of the *APOE* $\epsilon 4$ 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.

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NUMEROUS GENOME-WIDE association studies (GWAS) have been published for late-onset Alzheimer disease (LOAD).¹⁻¹³ Aside from *APOE*, additional candidate susceptibility genes identified using GWAS methods for LOAD have included *GAB2*, *GALP*, *14q32.13*, *LOC651924*, *PGBD1*, *TNK1*, *CR1*, *CLU*, *PICALM*, and *BIN1*.^{14,15} In addition, variants in *SORL1* identified by Rogaeva et al¹⁶ have been replicated in several independent cohorts and were significantly associated with LOAD in a meta-analysis.¹⁷ Difficulties inherent to the genetics of complex diseases (eg, etiologic heterogeneity, gene \times environment and gene \times gene interactions, and methylation) remain with these studies, and much work needs to be done. For example, the strength of association, or effect size, as measured by odds ratios (ORs) varies widely across studies

and is generally small. Yet, these GWAS have identified a number of candidate genes that need to be replicated and their functional roles determined. Despite the increasing number of identified susceptibility genetic variants, a relatively large proportion of genetic variance remains unexplained.¹⁸ This has much to do with both the complexity of the genetics and inadequacy of heritability as a measure of genetic contribution. Similar phenomena have been observed in other common, complex genetic diseases and invoked a term, *genetic dark matter*, in GWAS.^{19,20}

In the current study, we report the results of a GWAS in unrelated patients with LOAD and controls of Caribbean Hispanic ancestry. This population was selected because the prevalence and incidence rate of LOAD is higher than in white, non-Hispanic individuals living in the same community²¹ and because we had previously identified numerous large families

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multiply affected by LOAD. We first examined unrelated cases and controls in the Caribbean Hispanic individuals and then replicated the associations using the publicly 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. Diaz-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 of Familial Late-Onset Alzheimer's Disease Replicates *BIN1* and *CLU* and Nominates *CUGBP2* in Interaction with *APOE*," unpublished data). This approach allowed us to further assess the role of genetic admixture in the Caribbean Hispanic population. To our knowledge, this is the only GWAS of Alzheimer disease that focuses exclusively on a Caribbean Hispanic population.

METHODS

SAMPLES OF CARIBBEAN HISPANIC INDIVIDUALS

We studied 1093 unrelated Caribbean Hispanic individuals comprising 549 cases and 544 controls (**Table 1**). These participants were selected from the Washington Heights–Inwood Columbia Aging Project (WHICAP) study and the Estudio Familiar de Influencia Genética 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, Caribbean Hispanic, and African American.²¹ 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 remaining 39.7% of the participants were from the EFIGA study. All unaffected individuals were participants in the WHICAP epidemiologic 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 neuropsychological evaluations²³ collected from 1999 through 2007. The standardized neuropsychological test battery covered multiple domains and included the Mini-Mental State Examination,²⁴ the Boston Naming Test,²⁵ the Controlled Word Association Test²⁶ from the Boston Diagnostic Aphasia Evaluation,²⁷ the Wechsler Adult Intelligence Scale–Revised similarities subtest,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,³¹ and the Selective Reminding Test.³²

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

Characteristic	Total	WHICAP	EFIGA Family Study
Affected with AD, No.			
Definite/probable/possible	549	311	238
Definite/probable	400	173	227
Unaffected	544	543	1
Age, y, mean (SD)			
At onset (affected)	79.98 (8.0)	82.61 (7.3)	76.46 (7.7)
At last examination (unaffected)	78.87 (6.4)	78.94 (6.2)	
Female, %	69.7	68.4	74.2
<i>APOE</i> allele frequency, %	Total	Affected	Unaffected
$\epsilon 4^b$	18.16	23.41	12.87
$\epsilon 3$	75.07	70.58	79.60
$\epsilon 2$	6.77	6.01	7.54

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

^a Descriptive 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.

^b Allele 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.³³

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 measures 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 equilibrium test³⁴ at a *P* value less than .0001 in controls. Although the 650Y chip includes additional SNPs for Yoruban individuals, 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 signals 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 $< 9 \times 10^{-6}$ 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–

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

Chr	SNP	bp	Cyto	P Value						Flanking SNP ^d	Candidate Genes
				Caribbean Hispanic GWAS			NIA-LOAD GWAS				
				All	$\epsilon 4^-$	$\epsilon 4^+$	Unrelated	Family-Based Association			
								<i>APOE</i> ^b	Ethnicity ^c		
1	rs7525939	224 822 594	1q42.12	5.29×10^{-6}	.000462	.01300	.48650	.67635	.16364		
2	rs4669573 ^e	10 396 387	2p25.1	5.26×10^{-5}	3.67×10^{-6}	.52320	.00628	.38310	.25040	<i>HPCAL1</i> , <i>ODC1</i>	
2	rs10197851 ^e	10 402 860	2p25.1	.000102	7.13×10^{-6}	.54250	.02023	.71710	.10894		
3	rs1402752	44 662 304	3p21.31	.000663	.642500	8.69×10^{-6}	.80860	.88493	.81715		
3	rs11711889 ^e	154 853 356	3q25.2	6.95×10^{-6}	.000175	.02247	.03814	.49535	NA		
5	rs919289	120 593 422	5q23.1	.000468	3.20×10^{-6}	.74380	.79070	.34351	.09558		
5	rs4895298	120 597 007	5q23.1	.000477	2.00×10^{-6}	.65480	.63350	.22174	.14639		
5	rs2973413	120 597 210	5q23.1	.000571	5.35×10^{-6}	.91730	.53150	.21149	.15152		
7	rs10271466	10 090 002	7p21.3	.000105	5.98×10^{-6}	.92640	NA	NA	NA	rs10224072	
7	rs1117750 ^e	14 854 943	7p21.1	8.02×10^{-6}	.002861	.001305	.95270	.04563	.26433	<i>DGKB</i>	
8	rs11786902	4 388 532	8p22	.000134	8.67×10^{-6}	.97670	.60000	.43359	.09129		
9	rs6477258	8 085 638	9p24.1	7.19×10^{-6}	.002497	.001142	NA	NA	NA	rs7867126	
9	rs10758939	8 115 410	9p24.1	4.75×10^{-6}	.000478	.004208	.68920	.25155	.36560	rs16927158	
10	rs7908652 ^e	85 781 903	10q23.1	3.59×10^{-6}	.002532	.000451	.02650	.13064	.03457	<i>GHITM</i> , <i>C10orf99</i> , <i>PCDH21</i> , <i>LRT2</i> , <i>LRT1</i> , <i>RGR</i>	
11	rs978769	110 393 694	11q23.1	2.90×10^{-6}	.003084	.000287	.67400	.05720	.25093		
11	rs978770	110 393 802	11q23.1	1.49×10^{-6}	.002075	.000223	.68270	.05678	.25242		
11	rs11213703	110 395 150	11q23.1	2.35×10^{-6}	.002643	.000287	.63300	.06128	.30704		
13	rs11617026	100 843 480	13q33.1	.000912	.380700	1.70×10^{-6}	.44530	.81524	.29264		
15	rs11630802	80 028 079	15q25.2	4.88×10^{-6}	.001851	.000706	NA	NA	NA		
16	rs4843359	84 834 942	16q24.1	.000277	5.81×10^{-6}	.77970	.88660	.39124	.09346	rs17245059	
18	rs9945493	74 604 241	18q23	1.71×10^{-7}	.000494	8.97×10^{-6}	.91430	.94111	NA	rs2931024	
20	rs6135782	1 652 114	20p13	6.18×10^{-6}	.001629	.002194	.70880	.15025	.61181	rs202516	
21	rs2403771	14 650 668	21q11.2	6.20×10^{-6}	.000120	.03668	.12620	.47516	.51869	rs2822618	

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.

^aThe SNPs with the most significant *P* values ($P < 9 \times 10^{-6}$) in at least 1 of the 3 analyses (overall, *APOE* $\epsilon 4$ carriers, and *APOE* $\epsilon 4$ noncarriers) are presented. *P* values for the SNPs of interest in the Hispanic GWAS were compared with those in the NIA-LOAD GWAS.

^bFamily-based allelic association stratified by *APOE* status.

^cFamily-based allelic association taking into account population substructure.

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

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

based clustering method using PLINK version 1.05³⁶ (eAppendix, <http://www.archneuro.com>). Briefly, we used 500 unlinked SNPs for the STRUCTURE analysis³⁷ 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 λ 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 χ^2 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 3. ORs Associated With Minor Allele in the Caribbean Hispanic GWAS^a

Caribbean Hispanic GWAS														
All														
Chr	SNP	Minor Allele	MAF	All			APOE ε4 Noncarriers				APOE ε4 Carriers			
				OR (95% CI)	P Emp	Minor Allele	MAF	OR (95% CI)	P Emp	Minor Allele	MAF	OR (95% CI)	P Emp	
1	rs7525939	T	0.165	0.582 (0.460-0.736)	.000141	T	0.171	0.601 (0.451-0.800)	.009299	T	0.152	0.589 (0.385-0.899)	.253700	
2	rs4669573 ^b	G	0.465	1.421 (1.198-1.685)	.001183	G	0.463	1.637 (1.328-2.019)	8.50 × 10 ⁻⁵	G	0.470	1.106 (0.812-1.508)	1.000000	
2	rs10197851 ^b	A	0.488	0.713 (0.602-0.846)	.002209	A	0.489	0.619 (0.502-0.764)	.000160	A	0.484	0.908 (0.666-1.238)	1.000000	
3	rs1402752	C	0.172	1.480 (1.180-1.855)	.015030	C	0.159	1.069 (0.807-1.417)	1.000000	C	0.199	2.643 (1.703-4.104)	.000251	
3	rs11711889 ^b	A	0.087	0.495 (0.363-0.676)	.000165	A	0.092	0.485 (0.330-0.712)	.003654	A	0.077	0.523 (0.298-0.917)	.382600	
5	rs919289	G	0.268	0.708 (0.584-0.860)	.010520	G	0.262	0.561 (0.440-0.717)	7.50 × 10 ⁻⁵	G	0.283	1.060 (0.749-1.499)	1.000000	
5	rs4895298	G	0.254	0.705 (0.579-0.858)	.010780	G	0.247	0.548 (0.427-0.704)	5.20 × 10 ⁻⁵	G	0.269	1.084 (0.762-1.541)	1.000000	
5	rs2973413	T	0.242	0.705 (0.577-0.860)	.012440	T	0.233	0.556 (0.431-0.717)	.000114	T	0.260	1.019 (0.716-1.451)	1.000000	
7	rs10271466	G	0.199	1.523 (1.231-1.885)	.002321	G	0.191	1.824 (1.404-2.369)	.000129	G	0.215	1.018 (0.700-1.480)	1.000000	
7	rs1117750 ^b	T	0.115	1.871 (1.418-2.470)	.000192	T	0.106	1.673 (1.191-2.351)	.057500	T	0.135	2.290 (1.367-3.835)	.027880	
8	rs11786902	G	0.279	0.694 (0.575-0.838)	.003110	G	0.287	0.593 (0.470-0.748)	.000190	G	0.261	1.005 (0.709-1.425)	1.000000	
9	rs6477258	T	0.321	1.511 (1.261-1.811)	.000173	T	0.311	1.404 (1.126-1.751)	.051040	T	0.341	1.744 (1.246-2.441)	.025510	
9	rs10758939	G	0.480	0.671 (0.566-0.797)	.000124	G	0.494	0.689 (0.559-0.850)	.009761	G	0.452	0.637 (0.467-0.868)	.086790	
10	rs7908652 ^b	C	0.427	1.495 (1.261-1.773)	9.20 × 10 ⁻⁵	C	0.417	1.378 (1.119-1.697)	.051450	C	0.447	1.740 (1.273-2.377)	.010600	
11	rs978769	T	0.384	0.661 (0.555-0.786)	7.40 × 10 ⁻⁵	T	0.395	0.726 (0.588-0.898)	.060520	T	0.361	0.561 (0.409-0.768)	.006810	
11	rs978770	T	0.383	0.653 (0.548-0.777)	4.10 × 10 ⁻⁵	T	0.394	0.717 (0.580-0.886)	.040560	T	0.359	0.555 (0.405-0.761)	.005272	
11	rs11213703	C	0.384	0.658 (0.553-0.783)	6.30 × 10 ⁻⁵	C	0.396	0.723 (0.585-0.893)	.053390	C	0.361	0.561 (0.409-0.768)	.006810	
13	rs11617026	A	0.060	0.541 (0.375-0.781)	.019920	A	0.061	0.823 (0.532-1.272)	.999900	A	0.060	0.210 (0.106-0.419)	5.50 × 10 ⁻⁵	
15	rs11630802	A	0.359	0.661 (0.554-0.790)	.000127	A	0.364	0.708 (0.570-0.880)	.038260	A	0.348	0.575 (0.418-0.793)	.015640	
16	rs4843359	A	0.104	1.692 (1.272-2.251)	.006356	A	0.105	2.193 (1.553-3.098)	.000122	A	0.103	0.930 (0.560-1.544)	1.000000	
18	rs9945493	A	0.050	0.329 (0.213-0.508)	5.00 × 10 ⁻⁶	A	0.047	0.383 (0.219-0.669)	.010350	A	0.057	0.229 (0.114-0.460)	.000263	
20	rs6135782	C	0.092	0.503 (0.372-0.681)	.000158	C	0.100	0.563 (0.393-0.808)	.033470	C	0.075	0.420 (0.239-0.741)	.049810	
21	rs2403771	A	0.117	0.542 (0.414-0.709)	.000158	A	0.123	0.525 (0.377-0.732)	.002671	A	0.104	0.598 (0.367-0.973)	.534400	

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.

^aFor 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.

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

dividuals 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]) (Table 2). The details of the demographic and clinical characteristics of the NIA-LOAD participants who were included in the GWAS are provided in their report (Wijsman et al, unpublished data [full citation on page 321]). Briefly, the study first examined self-reported European American individuals: 2124 individuals from the NIA-LOAD study and 325 individuals from the National Cell Repository for Alzheimer's Disease (NCRAD) study. Those were augmented with 1186 unrelated individuals from the NIA-LOAD study and 204 individuals from the 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.

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 χ^2 test taking into account population stratification, *APOE* $\epsilon 4$ -restricted analysis (ie, restricted to individuals with at least 1 copy of $\epsilon 4$ compared with those without), and Mantel-Haenszel χ^2 test taking into account the presence or absence of *APOE* $\epsilon 4$ (Table 4). In addition to those 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 *CUGBP2* gene to be significantly associated with LOAD among a subset of samples with homozygous *APOE* $\epsilon 4$ carriers. Herein, we evaluated the association using 2 different models to account for its association with the *APOE* $\epsilon 4$ genotype (Table 4). Under model 1, homozygous *APOE* $\epsilon 4$ carriers were considered to have the putative genotype and all others do not. Under model 2, homozygous *APOE* $\epsilon 4$ carriers were considered to have the putative genotype, while homozygous *APOE* $\epsilon 3$ 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,

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

	SNP	bp	Ethnicity: Population-Stratified Analysis ^b	<i>APOE</i>		
				<i>APOE</i> Stratified	$\epsilon 4^-$	$\epsilon 4^+$
<i>CLU</i> (8p21)	rs881146	27 500 194	0.03740 ^c	0.06170	0.67570	0.00213 ^c
	rs70120100	27 504 646	0.15900	0.12540	0.42890	0.12210
	rs17057441	27 508 939	0.04517 ^c	0.07797	0.01770 ^c	0.67650
	rs11136000 ^{c,d}	27 520 436	0.58190	0.37320	0.96180	0.12230
<i>PICALM</i> (11q14)	rs17159904	85 463 935	0.04243 ^c	0.04951 ^c	0.04270 ^c	0.65250
	rs541458	85 465 999	0.36300	0.44710	0.28270	0.79400
	rs543293	85 497 725	0.72240	0.93160	0.40560	0.27150
	rs7941541	85 536 186	0.73180	0.90210	0.38990	0.27920
	rs3851179 ^{c,d}	85 546 288	0.32050	0.44610	0.08170	0.21020
	rs10194375 ^c	127 556 011	0.33590	0.38940	0.65870	0.02755 ^c
<i>BIN1</i> (2q14)	rs13426725 ^c	127 557 567	0.04159 ^c	0.07147	0.32890	0.08500
	rs4663098 ^c	127 589 265	0.71350	0.64590	0.36830	0.02851 ^c
	rs11685593	127 604 351	0.52240	0.45390	0.50050	0.01471 ^c
	rs7561528	127 606 107	0.49870	0.46270	0.32670	0.00536 ^c

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 al⁷ and Lambert et al.⁹

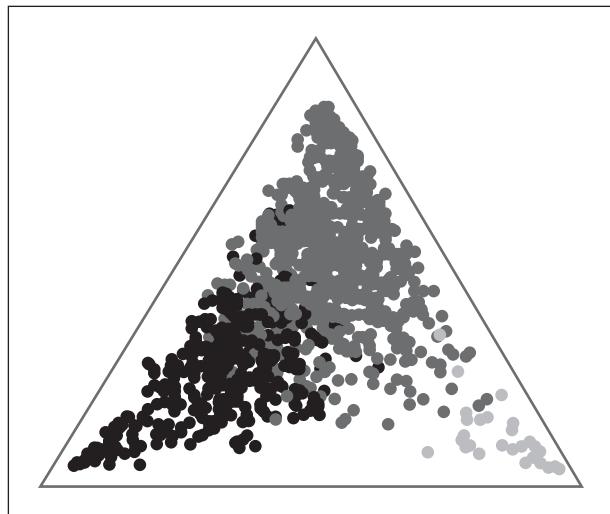


Figure 1. Population structure of a Caribbean Hispanic population. The dark gray dots represent Hispanic white individuals, while the black dots represent Hispanic African individuals. The light gray dots represent individuals from other Central American countries. The Figure was generated using STRUCTURE.³⁵

and 18.2% of the subjects were carriers of an *APOE* $\epsilon 4$ 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^{-8} 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* $\epsilon 4$ allele, and non-carriers of the *APOE* $\epsilon 4$ allele (Table 2). Of those, the strongest evidence for association was observed for rs9945493 ($P = 1.7 \times 10^{-7}$; 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 e); rs4669573 is located within the *HPCAL1* (hippocampal-like 1) 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) (eFigure 2).

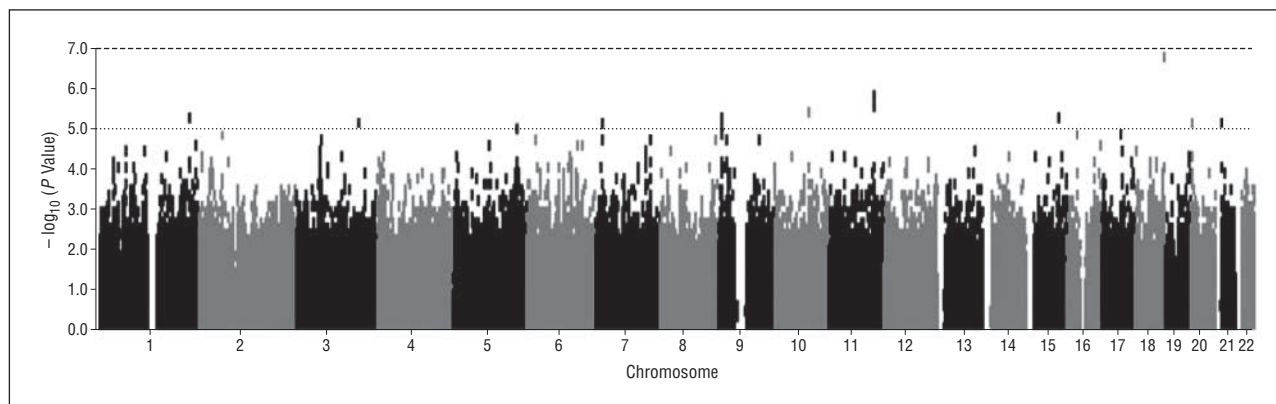


Figure 2. Manhattan plot of allelic association analysis in a Caribbean Hispanic population. The results of genome-wide association analysis are presented. One single-nucleotide polymorphism has a P value less than 9×10^{-6} and multiple single-nucleotide polymorphisms have P values less than 9×10^{-6} .

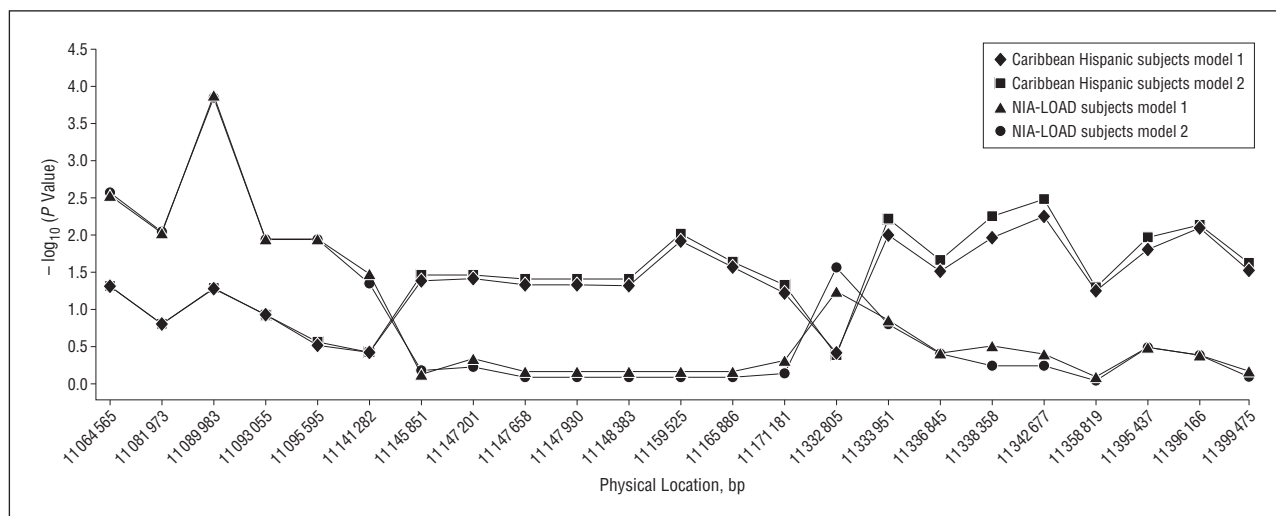


Figure 3. Association between *CUGBP2* and late-onset Alzheimer disease (LOAD) among homozygous *APOE* $\epsilon 4$ carriers in Caribbean Hispanic subjects vs National Institute on Aging Late-Onset Alzheimer's Disease study European American subjects. Two models were used to examine the relation between *CUGBP2* and LOAD, conditional on *APOE* $\epsilon 4$ status. Model 1 is homozygous *APOE* $\epsilon 4$ carriers vs others; model 2 is homozygous *APOE* $\epsilon 4$ carriers vs homozygous *APOE* $\epsilon 3$ carriers. The remaining subjects were excluded from the analysis. bp Indicates base pair. The base pair location on the x-axis is not in scale.

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* $\epsilon 4$ carriers (Table 4). However, rs11136000 in *CLU*, reported both by Harold et al⁷ and Lambert et al⁹ 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 $\epsilon 4$ carriers for rs7561528 ($P_{\text{nominal}} = .00536$).

GENE \times GENE INTERACTION

We evaluated an interaction model between *APOE* and *CUGBP2* to follow up the putative gene \times 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 signifi-

cantly associated with LOAD only among individuals with a homozygous $\epsilon 4$ 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* $\epsilon 4$ and absent in non-*APOE* $\epsilon 4$ s 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 rs20119 in the *CUGBP2* gene and *APOE* $\epsilon 4$ 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.

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* $\epsilon 4$ 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 $\epsilon 4$ 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.³⁷ 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,³⁸ 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,³⁹ 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 $\epsilon 4$ ($P = .00213$). 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.⁴⁰ Based on a small set of coding polymorphisms in *APOJ*, Tycko and colleagues⁴⁰ 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* $\epsilon 4$ 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* $\epsilon 4$ (Table 4). This is consistent with the much larger study by Lambert and colleagues⁹ but not with the study by Harold et al.⁷

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 $\epsilon 4$ ($P = .00536$). *PICALM* is reported to be involved in the neurotransmitter release processes, thereby affecting memory functions.^{41,42}

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.^{43,44}

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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Online-Only Material: The eAppendix and eFigures are available at <http://www.archneur.com>.

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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.