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Effect of Genetic Variation in *LRRTM3* on Risk of Alzheimer Disease

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Abstract

Objective—To explore the role of leucine-rich repeat transmembrane 3 (LRRTM3) in late-onset Alzheimer disease (AD) by independent genetic epidemiologic and functional studies.

Methods—First, we explored associations between *LRRTM3* single-nucleotide polymorphisms and AD in the National Institute on Aging Late-Onset Alzheimer's Disease case-control data set (993 patients and 884 control subjects) and a cohort of Caribbean Hispanics (549 patients and 544 controls) using single-marker and haplo-type analyses. Then we explored the effect of LRRTM3 small-hairpin RNAs on amyloid precursor protein processing.

Results—One single-nucleotide polymorphism in the promoter region (rs16923760; C allele: odds ratio,−0.74, P=.03), and a block of 4 single-nucleotide polymorphisms in intron 2 (rs1925608, C allele: 0.84, P=.04; rs7082306, A allele: 0.75, P=.04; rs1925609, T allele: 1.2, P=.03; and rs10997477, T allele: 0.88, P=.05) were associated with AD in the National Institute on Aging Late-Onset Alzheimer's Disease data set or the Caribbean His-panic data set. The corresponding haplotypes were also associated with AD risk (.01< P<.05). In addition, LRRTM3 knockdown with small-hairpin RNAs caused a significant decrease in amyloid precursor protein processing (P<.05 to P<.01) compared with the scrambled small-hairpin RNA condition.

Conclusions—These complementary findings support the notions that genetic variation in *LRRTM3* is associated with AD risk and that LRRTM3 may modulate γ-secretase processing of amyloid precursor protein. Additional studies are needed to determine whether the specific alleles

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associated with differential risk for AD indeed confer this risk through an effect of LRRTM3 expression levels that in turn modulates amyloid precursor protein processing.

Late-onset Alzheimer disease (AD) is the most common form of dementia in Western societies. The putative culprit is β-amyloid (Aβ), which is produced through β-secre-tase cleavage of the amyloid precursor protein (APP) at the N-terminus of the Aβ peptide followed by γ-secretase cleavage of the membrane-bound C-terminal APP fragment.[1] Although it is clear that in early-onset familial AD, mutations in the *APP*,[2] presenilin 1 (*PSEN1*),[3] and presenilin 2 (*PSEN2*)[4] genes lead to altered APP processing and accumulation of Aβ, the genetic factors affecting β- and γ-secretase cleavage in the common late-onset form remain largely unknown.

In 2006, Majercak et al[5] assessed 15 200 genes for their role in Aβ42 secretion using high-throughput small-interfering RNA screening technology. They identified the leucine-rich repeat transmembrane 3 gene (*LRRTM3*; OMIM 610869) as a neuronal gene promoting APP processing by β-secretase 1. They showed that small-interfering RNAs targeting *LRRTM3* inhibit the secretion of Aβ40, Aβ42, and sAPPβ (the N-terminal APP fragment produced by β-secretase 1 cleavage), whereas overexpression increases the secretion of Aβ. The gene *LRRTM3* is expressed nearly exclusively in the nervous system, including regions predominantly affected in AD, such as the dentate gyrus. It is nested in the alpha-3 catenin gene (*CTNNA3*) on chromosome 10q22.2 that in turn binds presenilin 1. These data suggest that *LRRTM3* may be a functional and positional candidate gene for AD. In several genetic linkage and association studies,[6–11] the CTNNA3 and LRRTM3 locus has been linked to AD and elevated plasma Aβ42, alone and in interaction with apolipopro-tein E, with different disease-associated of variants identified in the individual data sets. However, other studies[12] did not find an association.

The goal of the present study was to investigate whether genetic variation in *LRRTM3* is associated with AD risk in 2 independent data sets that have sufficient power to detect modest effect sizes (odds ratio [OR], ≥1.18). In independent functional studies, we also explored the effect of LRRTM3 small-hairpin RNAs (shRNAs) on APP processing.

METHODS

PARTICIPANTS

Written informed consent was obtained from all participants. Recruitment for the Caribbean Hispanic study was approved by the institutional review board of the Columbia University Medical Center. Recruitment for the National Institute on Aging Late-Onset Alzheimer's Disease (NIALOAD) study was approved by the relevant institutional review boards of the participating centers (Boston University, Columbia University, Duke University, Indiana University, Massachusetts General Hospital, Mayo Clinic, Mount Sinai School of Medicine, Oregon Health & Science University, Rush University Medical Center, University of Alabama at Birmingham, UCLA [University of Cali-fornia, Los Angeles], University of Kentucky, University of Penn-sylvania, University of Pittsburgh, University of Southern California, University of Texas Southwestern Medical Center at Dallas, University of Washington, Washington University Medical Center, University of Miami, Northwestern University, and Emory University). The study was conducted in accordance with principles expressed in the Declaration of Helsinki.

The 2 data sets comprised 993 patients and 884 control subjects from the NIALOAD study[13] (white participants) and 549 patients and 544 controls from a Caribbean Hispanic data set[14] that have been described in detail elsewhere. The diagnoses of *probable* or *possible* AD were defined in accordance with the diagnostic criteria of the National Institute of Neurological

and Communication Disorders and Stroke–Alzheimer Disease and Related Disorders Association at clinics specializing in memory disorders or in clinical investigations. Persons were classified as *controls* when they were without cognitive impairment or dementia at the last visit. Cognitive impairment was determined using the neuropsychologic test battery described hereafter. Informed consent was obtained from all participants using procedures approved by institutional review boards at each of the clinical research centers collecting human subjects.

COGNITIVE ASSESSMENTS

For both studies, all participants (patients and controls) underwent a standardized neuropsychologic test battery that examined multiple domains.[15] In the Caribbean Hispanic study, orientation was evaluated using parts of the modified Mini-Mental State Examination.[16] Language was assessed using the Boston Naming Test,[17] the Controlled Word Association Test,[18] category naming, and the complex ideational material and phrase repetition subtests from the Boston Diagnostic Aphasia Evaluation.[19] Abstract reasoning was evaluated using the Wechsler Adult Intelligence Scale–Revised similarities subtest[20] and the nonverbal identities and oddities subtest of the Mattis Dementia Rating Scale.[21] Visuospatial ability was examined using the Rosen Drawing Test[22] and a matching version of the Benton Visual Retention Test.[23] Memory was evaluated using the multiple-choice version of the Benton Visual Retention Test[23] and the 7 subtests of the Selective Reminding Test[24]: total recall, long-term recall, long-term storage, continuous long-term storage, words recalled on last trial, delayed recall, and delayed recognition. This neuropsychologic test battery has established norms for the same community.[25] In the NIALOAD study, cognition was measured with a battery of 7 brief tests.[26] Working memory was assessed with Digit Span Forward,[27] Digit Span Backward,[27] and Digit Ordering.[28] Two measures of episodic memory were included: immediate and delayed recall of story A from the Wechsler Memory Scale–Revised.[27] Semantic memory was assessed by asking persons to name members of 2 semantic categories (animals and vegetables) in separate 1-minute trials.[26,28,29]

GENOTYPING

Both study sites provided the results from genotyping of *LRRTM3* single-nucleotide polymorphisms (SNPs) that were part of the genome-wide studies described previously.[13,14] For the NIALOAD study, SNPs were genotyped using the Human610Quadv1_B BeadChips (Illumina). For the Caribbean Hispanic study, SNPs were genotyped using the HumanHap 650Y chip (Illumina). Genotyping of *APOE* polymorphisms (based on SNPs rs7412 and rs429358) for all samples was performed at Prevention-Genetics. The base pair (bp) locations of genotyped SNPs correspond to Genome hg19, dbSNP build 131.

ASSAY DETAILS

The γ-secretase activity and nuclear translocation of the APP/Fe65/TIP60 protein complex was monitored with a luciferase-based assay[30] consisting of the APP gene's C-terminus (AICD) fused to a transcription factor composed of the GAL4 DNA binding domain with VP16 transcriptional activator (GV) and called the APP-GV assay. In addition, the AICD fragment is fused to the GV domains as a positive control of AICD generation and allows for the evaluation of the AICD-specific contribution to the observed modulation in the APP-GV assay. Briefly, LRRTM3 complementary DNA or LRRTM3 shRNAs transiently transfected were evaluated in the APP-GV assay, as previously described[30] in the HEK293 cell lines.

STATISTICAL ANALYSIS

Single-nucleotide polymorphism marker data were assessed for deviations from the Hardy-Weinberg equilibrium. Independently for each data set, multivariate linear regression analyses as implemented in PLINK, version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/), were used to assess genotypic and allelic associations with AD, adjusting for sex, apo-lipoprotein E–ε4, and age at onset or age at examination. In addition, all analyses were adjusted for population substructure (determined by STRUCTURE [pritch.bsd.uchicago.edu/structure.html] and EIGENSTRAT [genepath.med.harvard.edu/~reich/EIGENSTRAT.htm]). The false discovery rate,[31] which controls the expected proportion of incorrectly rejected null hypotheses (type I errors), was used to correct for multiple testing.

We used Haploview (http://www.broad.mit.edu/mpg/haploview/index.php) to assess linkage disequilibrium (LD). Haplotype blocks were defined using the CI algorithm. The default settings were used in these analyses, which create 95% CIs on D′ to define SNP pairs in strong LD. Then we carried out 3-SNP sliding window haplotype analyses using PLINK.

We also performed a meta-analysis of all data sets. To determine the strength of associations between the individual *LRRTM3* SNPs and AD, we calculated a pooled OR for each marker using fixed and random effects models using PLINK. We first performed meta-analyses of unadjusted results from the individual data sets; we then repeated the meta-analyses using the results from the individual data sets adjusted for *APOEε4*, sex, and age at onset or age at examination. The *P* values for each SNP were corrected for multiple testing using the false discovery rate.[31] Between–data set heterogeneity was quantified using the I^2 metric for inconsistency,[32] and its statistical significance was tested with the χ^2 distributed Q statistic.[33] The I^2 value is calculated by (Q-*df*)/Q; it is considered large for values higher than 50%, and Q is considered statistically significant for P=.10.[32,33]

For statistical analysis for the cell biology assay, an analysis of variance with post hoc correction was performed using GraphPad statistical software (GraphPad, Inc) to compare mean expression levels. All data were normalized to transfection efficiency (eg, green fluorescent protein) and then to the control values on each plate for every assay to allow for comparisons across experiments.

RESULTS

Table 1 shows the characteristics of the study populations. In the single-marker analyses, 1 SNP that is located in intron 2 at 68 760719 bp was significantly associated with AD in the NIALOAD data set (rs10997477, P=.04; Table 2). Four SNPs were associated in the Caribbean Hispanic data set (rs16923760, rs1925608, rs7082306, and rs1925609; P ranging from .03 to .04). Of note, SNPs rs1925608, rs7082306, and rs1925609 are 4.5 kilobase pairs apart and occur in a distinct LD block in intron 2 (Figure 1) close to regulatory regions, and rs16923760 is located in the promoter of the gene. Although none of these SNPs were significant in the NIALOAD data set, 3 showed ORs that were in the same direction as in the Caribbean Hispanic data set. The rs1925608 showed an OR with a tendency in the opposite direction. However, the 95% CIs of both data sets for this SNP overlapped.

Consistent with the single-marker analyses, the 3 SNP haplotypes that include the T allele of rs10997477 were less frequent in patients than in controls in the NIALOAD data set (Table 3). In the Caribbean Hispanic data set, the haplotypes that include the C alleles of rs16923760 or rs1925608 were less frequent in patients than in controls, and the haplotypes that included the T allele of rs1925609 were more frequent in patients than they were in controls. In addition, in both data sets, several additional haplotypes were associated with

AD risk, including haplotype rs10822970|rs2619652|rs2764813 ($P = .04$) in the Caribbean Hispanic data set, which is located in the same LD block as SNP rs10997477 that was significant in the NIALOAD data set. In a meta-analysis of both data sets, the strength of the association of rs10997477 with AD increased, indicated by both the OR and the *P* value (OR, 0.85; $P = .009$). Corresponding to the separate analyses of the NIALOAD data set, the T allele was associated with a decreased risk (OR, 0.87; $P = .01$; $I^2 = 0.0$). All results remained unchanged when we stratified by apolipoprotein E–ε4 carrier status.

We used the APP-GV assay to monitor γ-secretase activity and nuclear translocation of the APP/Fe65/TIP60 protein complex.[30] The shRNAs against LRRTM3 were also used to further investigate the role of LRRTM3 in APP processing. The APP-GV assay is a luciferase-based assay[30] consisting of the APP gene's C-terminus (AICD) fused to a transcription factor composed of the GAL4 DNA binding domain with VP16 transcriptional activator (GV). In addition, the AICD fragment is fused to the GV domains as a positive control of AICD generation and allows for the evaluation of the AICD-specific contribution to the observed modulation in the APP-GV assay. Briefly, LRRTM3 shRNAs transiently transfected were evaluated in the APP-GV assay, as previously described[30] in the HEK293 cell lines (Figure 2). Four of the 5 LRRTM3-shRNAs caused a significant decrease in APP processing ($P < .05$ to $< .01$) compared with the scrambled shRNA condition (analysis of variance with Bonferroni correction [GraphPad software]).

COMMENT

The accumulated findings reported herein suggest that variation in the *LRRTM3* sequence, expression, and function may influence the development of AD. Although the identity of the specific AD-associated sequence variations in *LRRTM3* remains to be determined, our results from the association studies imply (1) that there are different AD-associated allelic variants in the *LRRTM3* gene in different populations and (2) that some of these variants are likely to be in intronic regulatory sequences that affect cell type–specific or tissue-specific expression of *LRRTM3.* The results from our γ-secretase assays suggest that genetic variation in *LRRTM3* might affect AD risk by altering the physiologic role of LRRTM3 in the processing of APP holoprotein. These 2 findings are complementary but independent. We could not examine, and therefore could not conclude, that the specific polymorphisms associated with AD in our data sets affect γ-secretase processing of APP or LRRTM3 levels in any direction. We could only demonstrate independent effects of genetic variants in LRRTM3 on AD risk and of LRRTM3 knockdown on γ-secretase processing. However, both independent findings are in line with a role of LRRTM3 in AD as has been suggested before—namely, that genetic variations in *LRRTM3* or *CTNNA3* are associated with AD[6–10] and that LRRTM3 may promote APP processing through an effect on APP cleavage.[5,10]

Several issues diminish the possibility that the association between *LRRTM3* and AD is spurious. First, several alleles and their corresponding haplotypes were associated with altered AD risk in 2 unrelated data sets from different ethnic groups. Second, the strength of the association (OR and *P* value) of rs10997477 with AD increased in a meta-analysis of both data sets. Although in the Caribbean Hispanic data set a haplotype (rs10822970|rs2619652|rs2764813) that was significantly associated with AD was located in the same LD block as SNP rs10997477, which was significant in the NIALOAD data set, our results are consistent with the notion that there are different disease-associated variants in different ethnic groups. The occurrence of pathogenic mutations across multiple domains of disease genes (allelic heterogeneity) and the absence of these variants in some data sets or ethnic groups (locus heterogeneity) are frequently observed in both mono-genic and complex traits.[34,35] An alternative explanation for the fact that different SNPs are associated with AD

in the 2 ethnic groups may be differences in LD patterns. It is likely that the genotyped variants are not the disease-causing variants but rather are in LD with causative protective or harmful disease-modifying variations in *LRRTM3*.

As previously described, rs1925608, rs7082306, and rs1925609 belong to a distinct LD block in intron 2 containing several regulatory regions or transcription-factor binding sites. Thus, it seems likely that these 3 SNPs point to the same disease-associated variant. The fact that this block is not in LD with regions outside *LRRTM3* supports the notion that the genetic association of *LRRTM3* with AD is independent of a potential association of *CTNNA3* with AD. Our finding of a role of *LRRTM3* in AD is also supported by the results of our cell experiments demonstrating an effect of LRRTM3 on γ-secretase processing of APP. These observations are consistent with the findings by Majercak et al[5] that small-interfering RNAs targeting LRRTM3 inhibit the secretion of Aβ40 and Aβ42.

The fact that the effect sizes of associated SNPs were small (OR, 1.1–1.2) is expected for a common disease and in line with the recently detected novel AD susceptibility loci identified by large genome-wide association studies.[36–40]

It has to be acknowledged that the sample sizes of both individual data sets were modest and we had 80% power to detect effect sizes of OR of 1.18 or larger. Thus, it remains possible that larger individual data sets would have detected additional genotype-phenotype associations with smaller effect sizes or allele frequencies. It is also possible that additional polymorphisms in nontagged regions of the gene are associated with AD risk. A second limitation is that we were not able to directly examine the effect of specific disease-causing mutations on γ-secretase processing of APP or LRRTM3 levels. That would require resequencing of LRRTM3 because the polymorphisms identified in the present study likely are not the disease-causing variants but rather are in LD with causative protective or harmful disease-modifying variations.

In summary, our findings from genetic epidemio-logic and functional analyses provide modest support for a role of *LRRTM3* in AD. Sequencing studies are needed to identify the specific disease-causing mutations and to examine whether they are associated with differences in APP processing through effects on function or level of LRRTM3. If our findings are confirmed, this would hold the promise of an LRRTM3 as a therapeutic target for AD and other related amyloid disorders.

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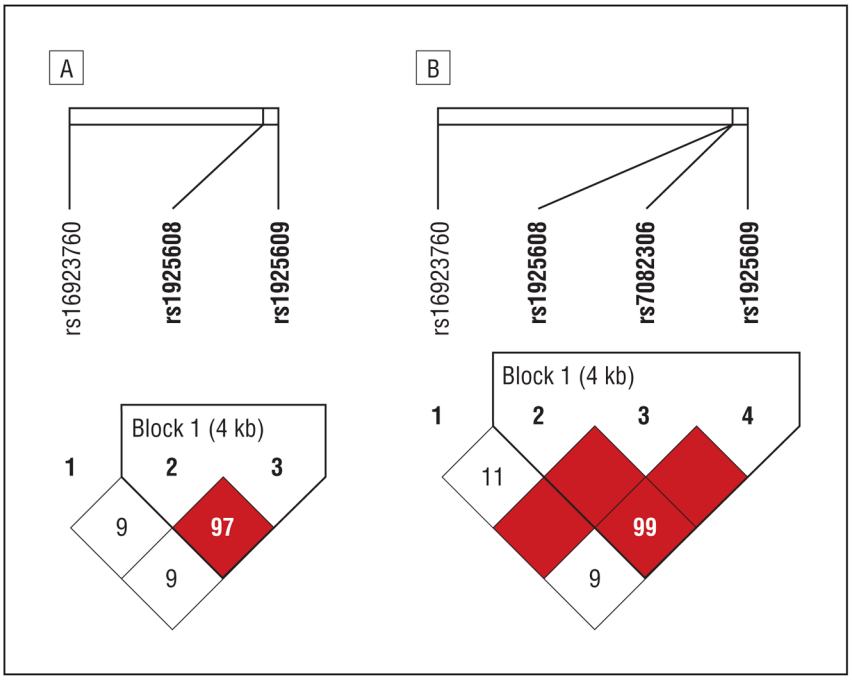

Figure 1.
Linkage disequilibrium (LD) patterns of single-nucleotide polymorphisms (SNPs) rs16923760, rs1925608, rs7082306, and rs1925609 in *LLRTM3*. A, National Institute on Aging Late-Onset Alzheimer's Disease data set (control subjects); B, Caribbean Hispanic data set (controls). Bold font indicates SNPs that are in high LD.

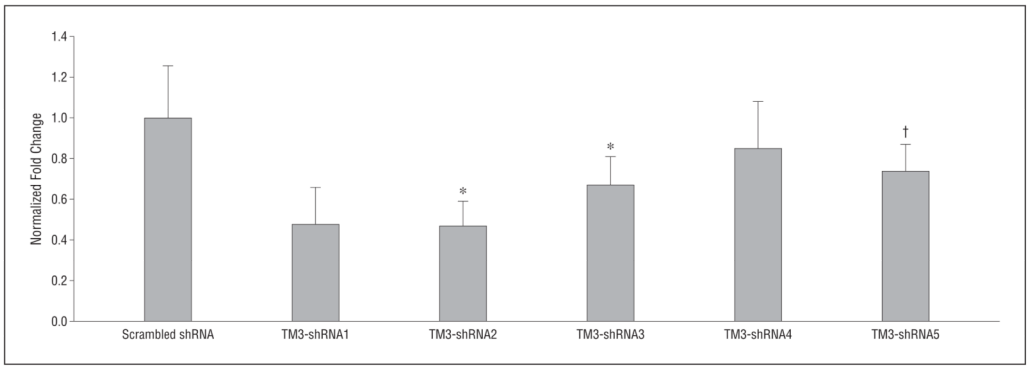

Figure 2.

γ-Secretase activity and nuclear translocation of amyloid precursor protein (APP) assays with leucine-rich repeat transmembrane 3 (LRRTM3) small-hairpin RNAs (shRNAs). The luciferase-based assay[30] consisting of the APP gene's C-terminus (AICD) fused to a transcription factor composed of the GAL4 DNA binding domain with VP16 transcriptional activator (GV) and called the APP-GV assay was performed in the HEK293 cell lines. The data from 5 shRNA LRRTM3 (TM3-shRNA) were normalized to APP-GV with the scrambled sequence shRNA that was included as a negative control. The data are representative for the APP-GV assays, and the assay has been performed in at least 3 separate experiments in replicates of 8 samples per condition (96-well format). Error bars represent SDs. *$P < .01$ compared with scrambled shRNA/APP-GV only (analysis of variance [GraphPad software]). †$P < .05$.

Table 1

Characteristics of the Study Samples

Characteristic	Caribbean Hispanic Study (n = 1093)	NIALOAD Case-Control Study (n = 1877)
Affected with AD, No.	549	993
Unaffected with AD, No.	544	884
Age, mean (SD), y		
At onset: patients	79.98 (8.0)	71.6 (6.9)
At last examination: controls	78.87 (6.4)	76.1 (8.4)
Female sex, %	69.7	62.3
APOE allele frequency, %		
ε4	18.2	31.2
ε3	75.1	63.3
ε2	6.8	5.5

Abbreviations: AD, Alzheimer disease; APOE, apolipoprotein E; NIALOAD, National Institute on Aging Late-Onset Alzheimer's Disease.

Table 2

Single-Marker Associations of *LRRTM3* SNPs and AD[a]

		NIALOAD (n = 1877)				Caribbean Hispanics (n = 1093)			
				P Value			OR (95% CI)	*P* Value	
SNP	bp	Minor allele	OR (95% CI)	Nominal	FDR	Minor allele		Nominal	FDR
rs16923760	68,680,128	C	1.00 (0.83–1.22)	.02	.97	**C**	**0.74 (0.57–0.97)**	**<.001**	**.03**
rs1925608	68,750,412	C	1.10 (0.97–1.25)	.002	.14	**C**	**0.84 (0.71–0.99)**	**<.001**	**.04**
rs7082306	68,750,755	Not genotyped				**A**	**0.75 (0.56–0.98)**	**<.001**	**.04**
rs1925609	68,754,907	C	1.10 (0.96–1.25)	.003	.17	**T**	**1.20 (1.03–1.42)**	**<.001**	**.03**
rs10997477	68,760,719	**T**	**0.88 (0.76–0.99)**	**.001**	**.04**	T	0.88 (0.73–1.06)	.002	.17

Abbreviations: AD, Alzheimer disease; bp, base pair; FDR, false discovery rate; NIALOAD, National Institute on Aging Late-Onset Alzheimer's Disease; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a]All models are adjusted for sex, apolipoprotein E–ε4, and age at onset or age at examination. The FDR was used to correct for multiple testing. Data about the SNPs significant at an α of .05 in FDR-corrected analyses are in bold. The bp locations of genotyped SNPs correspond to Genome hg19, dbSNP build 131.

Table 3

Haplotype Associations of *LRRTM3* SNPs and AD[a]

SNPS, by Study	Haplotype	FA	FU	χ^2	*P* Value	
					Nominal	FDR
NIALOAD						
rs2140372|rs6480246|rs10997477	**CTT**	**0.20**	**0.38**	**3.93**	**<.001**	**.05**
rs2140372|rs6480246|rs10997477	TCC	0.47	0.36	1.40	<.001	.24
rs2140372|rs6480246|rs10997477	TTC	0.07	0.06	0.01	<.001	.94
rs2140372|rs6480246|rs10997477	CTC	0.27	0.20	0.86	.001	.35
rs6480246|rs10997477|rs1925613	CCC	0.47	0.36	1.41	<.001	.24
rs6480246|rs10997477|rs1925613	TCC	0.07	0.06	0.02	.003	.88
rs6480246|rs10997477|rs1925613	**TTA**	**0.20**	**0.38**	**3.94**	**<.001**	**.05**
rs6480246|rs10997477|rs1925613	TCA	0.27	0.20	0.78	.001	.38
rs10997477|rs1925613|rs10997481	CCT	0.53	0.42	1.51	<.001	.22
rs10997477|rs1925613|rs10997481	**TAC**	**0.20**	**0.38**	**3.93**	**<.001**	**.05**
rs10997477|rs1925613|rs10997481	CAC	0.27	0.20	0.76	.001	.38
Caribbean Hispanics						
rs2140380|rs16923760|rs4746648	CTT	0.20	0.21	1.02	<.001	.31
rs2140380|rs16923760|rs4746648	**CCC**	**0.10**	**0.13**	**5.03**	**<.001**	**.02**
rs2140380|rs16923760|rs4746648	ATC	0.10	0.10	0	.003	3.99
rs2140380|rs16923760|rs4746648	**CTC**	**0.60**	**0.56**	**5.13**	**<.001**	**.02**
rs16923760|rs4746648|rs4746649	**CCC**	**0.10**	**0.13**	**5.03**	**<.001**	**.02**
rs16923760|rs4746648|rs4746649	TCC	0.22	0.20	2.00	<.001	.16
rs16923760|rs4746648|rs4746649	TTT	0.20	0.21	1.01	<.001	.32
rs16923760|rs4746648|rs4746649	TCT	0.48	0.46	1.16	<.001	.28
rs12266823|rs4746652|rs1925608	AGC	0.11	0.10	0.31	.002	.58
rs12266823|rs4746652|rs1925608	**CGC**	**0.36**	**0.41**	**6.46**	**<.001**	**.01**
rs12266823|rs4746652|rs1925608	CAA	0.16	0.15	0.76	.001	.38
rs12266823|rs4746652|rs1925608	CGA	0.37	0.34	2.47	<.001	.12
rs4746652|rs1925608|rs7082306	**GCA**	**0.09**	**0.11**	**3.63**	**<.001**	**.05**
rs4746652|rs1925608|rs7082306	GCG	0.38	0.40	1.00	<.001	.32

SNPS, by Study	Haplotype	FA	FU	χ^2	*P* Value	
					Nominal	FDR
rs4746652|rs1925608|rs7082306	AAG	0.16	0.15	0.76	.001	.38
rs4746652|rs1925608|rs7082306	GAG	0.37	0.34	2.46	<.001	.12
rs1925608|rs7082306|rs10740265	**CAC**	**0.09**	**0.11**	**3.58**	**<.001**	**.05**
rs1925608|rs7082306|rs10740265	CGC	0.37	0.38	0.56	.001	.45
rs1925608|rs7082306|rs10740265	CGT	0.01	0.02	1.35	<.001	.25
rs1925608|rs7082306|rs10740265	**AGT**	**0.53**	**0.49**	**4.57**	**<.001**	**.03**
rs10740265|rs716547|rs1925609	**TGT**	**0.52**	**0.47**	**4.58**	**<.001**	**.03**
rs10740265|rs716547|rs1925609	CAC	0.20	0.22	1.31	<.001	.25
rs10740265|rs716547|rs1925609	CGC	0.26	0.28	1.14	<.001	.29
rs10740265|rs716547|rs1925609	TGC	0.02	0.03	0.71	.001	.40
rs716547|rs1925609|rs9971243	GCC	0.14	0.13	0.31	.002	.58
rs716547|rs1925609|rs9971243	**GTT**	**0.52**	**0.47**	**4.45**	**<.001**	**.03**
rs716547|rs1925609|rs9971243	ACT	0.20	0.22	1.22	<.001	.27
rs716547|rs1925609|rs9971243	**GCT**	**0.15**	**0.18**	**4.59**	**<.001**	**.03**
rs1925609|rs9971243|rs1925611	**TTG**	**0.25**	**0.21**	**4.17**	**<.001**	**.04**
rs1925609|rs9971243|rs1925611	CTG	0.21	0.24	2.04	<.001	.15
rs1925609|rs9971243|rs1925611	CCA	0.14	0.13	0.25	.002	.61
rs1925609|rs9971243|rs1925611	TTA	0.27	0.26	0.18	.002	.67
rs1925609|rs9971243|rs1925611	CTA	0.13	0.16	3.08	<.001	.08
rs10822970|rs2619652|rs2764813	**CTC**	**0.02**	**0.01**	**4.07**	**<.001**	**.04**
rs10822970|rs2619652|rs2764813	CCC	0.40	0.40	0.05	.002	.82
rs10822970|rs2619652|rs2764813	CTT	0.34	0.36	1.64	<.001	.20
rs10822970|rs2619652|rs2764813	TCT	0.14	0.13	0.30	.002	.59
rs10822970|rs2619652|rs2764813	CCT	0.10	0.09	0.88	.001	.35

Abbreviations: AD, Alzheimer disease; FA, frequency in affecteds (patients); FDR, false discovery rate; FU, frequency in unaffecteds (controls); NIALOAD, National Institute on Aging Late-Onset Alzheimer's Disease; SNP, single-nucleotide polymorphism.

[a]All models are adjusted for sex, apolipoprotein E–ε4, and age at onset or age at examination. Data about the haplotypes significant at an α of .05 in FDR-corrected analyses are in bold.