

# A Comprehensive Genetic Association Study of Alzheimer Disease in African Americans

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**Objectives:** To evaluate the association of genetic variation with late-onset Alzheimer disease (AD) in African Americans, including genes implicated in recent genome-wide association studies of whites.

**Design:** We analyzed a genome-wide set of 2.5 million imputed markers to evaluate the genetic basis of AD in an African American population.

**Subjects:** Five hundred thirteen well-characterized African American AD cases and 496 cognitively normal African American control subjects.

**Setting:** Data were collected from multiple sites as part of the Multi-Institutional Research on Alzheimer Genetic Epidemiology (MIRAGE) Study and the Henry Ford Health System as part of the Genetic and Environmental Risk Factors for Alzheimer Disease Among African Americans (GenerAAtions) Study.

**Results:** Several significant single-nucleotide polymorphisms (SNPs) were observed in the region of the apo-

lipoprotein E gene (*APOE*). After adjusting for the confounding effects of *APOE* genotype, one of these SNPs, rs6859 in *PVRL2*, remained significantly associated with AD ( $P = .0087$ ). Association was also observed with SNPs in *CLU*, *PICALM*, *BIN1*, *EPHA1*, *MS4A*, *ABCA7*, and *CD33*, although the effect direction for some SNPs and the most significant SNPs differed from findings in data sets consisting of whites. Finally, using the African American genome-wide association study data set as a discovery sample, we obtained suggestive evidence of association with SNPs for several novel candidate genes.

**Conclusions:** Some genes contribute to AD pathogenesis in both white and African American cohorts, although it is unclear whether the causal variants are the same. A larger African American sample will be needed to confirm novel gene associations, which may be population specific.

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**A**LZHEIMER DISEASE (AD) IS the most common form of dementia. Environmental and host risk factors for common late-onset AD (LOAD) include low educational level, diabetes mellitus, hypertension, and head trauma. Genetic factors also influence LOAD risk, evidenced by heritability estimates as high as 75%<sup>1</sup> and analyses showing transmission of a major gene for the disease in families.<sup>2</sup> Until recently, the apolipoprotein E gene (*APOE* [OMIM +107741]) was the only one generally recognized to influence LOAD risk.<sup>3</sup> In whites, homozygosity for the  $\epsilon 4$  variant is associated with an increased risk by as much as 15 times that of the most common *APOE* genotype ( $\epsilon 3/\epsilon 3$ ).<sup>4</sup>

Genome-wide association studies (GWASs) have reported genome-wide sig-

nificant single-nucleotide polymorphisms (SNPs) across a 70-kilobase (kb) region that includes *APOE* and several neighboring genes,<sup>5</sup> namely, poliovirus receptor-related 2 (*PVRL2* [OMIM \*600798]), translocase of outer mitochondrial membrane 40 yeast homologue (*TOMM40* [OMIM \*608061]), and apolipoprotein C-I (*APOC1* [OMIM \*107710]). Both the *TOMM40* and *APOC1* genes have been considered possible risk factors for AD independent of *APOE*. Several lines of inquiry have implicated *TOMM40* as having an effect on AD risk, including evidence of a role of mitochondria in AD pathogenesis,<sup>6</sup> association of an intronic *TOMM40* repeat polymorphism with age at the onset of AD symptoms among subjects lacking the  $\epsilon 4$  allele,<sup>7</sup> and association of a haplotype spanning *TOMM40* with expression of

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**Table 1. Sample Sizes of African American (Discovery) and White (Replication) Data Sets**

	Data Sets								
	African American			White					
	MIRAGE	GenerAAtions	Total	MIRAGE	ADNI	GenADA	NIA-LOAD	FHS	Total
No. of cases	267	246	513	609	254	669	1839	197	3568
No. of controls	292	204	496	875	169	713	1983	2465	6205
<b>Total</b>	<b>559</b>	<b>450</b>	<b>1009</b>	<b>1484</b>	<b>423</b>	<b>1382</b>	<b>3822</b>	<b>2662</b>	<b>9773</b>

Abbreviations: ADNI, Alzheimer Disease Neuroimaging Initiative; FHS, Framingham Heart Study; GenADA, Canadian study on genetics of Alzheimer disease associations; GenerAAtions, Genetic and Environmental Risk Factors for Alzheimer Disease Among African Americans; MIRAGE, Multi-Institutional Research on Alzheimer Genetic Epidemiology; NIA-LOAD, National Institute on Aging–Late-onset Alzheimer’s Disease Family Study.

*APOE*.<sup>8</sup> However, other studies did not find an effect of *TOMM40* after adjusting for *APOE*.<sup>9,10</sup> A polymorphism immediately upstream of the *APOC1* gene has also been proposed as a possible risk locus for AD.<sup>11,12</sup> This polymorphism is in strong linkage disequilibrium (LD) with the *APOE* risk locus, but this pattern varies substantially by population.<sup>13</sup> Studies in mice and humans indicate that *APOC1* expression has an effect on memory.<sup>14-16</sup> Other studies reported that *APOC1* modifies the risk of AD independent of or through interaction with *APOE*.<sup>17,18</sup>

The GWASs conducted by several large consortia have identified robust evidence of an association with genes outside the *APOE* region, including clusterin (*CLU* [OMIM \*185430]),<sup>19,21</sup> phosphatidylinositol-binding clathrin assembly protein (*PICALM* [OMIM \*603025]),<sup>20,21</sup> complement component (3b/4b) receptor 1 (*CR1* [OMIM \*120620]),<sup>19</sup> bridging integrator 1 (*BIN1* [OMIM \*601248]),<sup>20</sup> CD2-associated protein (*CD2AP* [OMIM \*604241]),<sup>22</sup> ephrin type-A receptor 1 (*EPHA1* [OMIM \*179610]),<sup>20,22</sup> the membrane-spanning 4A (*MS4A*) gene cluster,<sup>22,23</sup> myeloid-associated antigen CD33 (*CD33* [OMIM \*159590]),<sup>22</sup> and ATP-binding cassette, subfamily A (*ABCI*), member 7 (*ABCA7* [OMIM \*605414]).<sup>23</sup> Findings with *CLU*, *PICALM*, *CR1*, and *ABCA7* have been replicated.<sup>22-24</sup>

Because there are population differences in LD and allele frequencies, most association studies have focused on a single population to decrease genetic background noise and reduce the likelihood of false-positive findings due to confounding. Thus, confirmation in other populations is required to determine the generalizability of the contribution of each gene to AD risk and the possibility of population-specific causative variants. Although the effect of *APOE* has been investigated extensively in multiple populations,<sup>4,25,26</sup> few African American cohorts have been included in GWASs for AD.

In the present study, we genotyped more than 1000 African American cases and controls for more than 600 000 SNPs covering the entire genome. Genotypes for 2.5 million SNPs imputed from HapMap reference panels were used to investigate the contribution of genes previously implicated in whites to AD risk and to identify novel AD risk variants in this population. We also analyzed a comparable set of SNPs in 5 white AD GWAS data sets containing more than 9700 subjects to replicate novel findings and for comparison with previously obtained results.

## METHODS

Subjects were ascertained from 2 genetic studies of AD focused on African Americans. One subject group includes participants of the Multi-Institutional Research on Alzheimer Genetic Epidemiology (MIRAGE) Study, which contains primarily discordant sibling pairs. Enrollment, data collection, and diagnostic procedures in the MIRAGE Study are explained in detail elsewhere.<sup>27</sup> A second group of primarily unrelated individuals includes participants of the Genetic and Environmental Risk Factors for Alzheimer Disease Among African Americans (GenerAAtions) Study, who were identified through the electronic claims database of the Henry Ford Health System. Community-dwelling African Americans 65 years or older who had at least 1 encounter with the Henry Ford Health System in the 3 years before their recruitment and who had an available proxy informant were eligible for this study. Cases met criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association for possible or probable AD, determined in a consensus conference that included a behavioral neurologist (R.S.), psychiatrist, neuropsychologist, and a behavioral neurology nurse practitioner.

For comparison, we also examined 5 white AD GWAS data sets containing 3568 cases and 6205 controls, namely, the MIRAGE Study white families, and 4 data sets obtained from a public database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap>), including the Alzheimer Disease Neuroimaging Initiative (ADNI),<sup>28,29</sup> a Canadian study on genetics of Alzheimer disease associations (GenADA),<sup>30</sup> the National Institute on Aging–Late-Onset Alzheimer’s Disease Family Study (NIA-LOAD),<sup>31</sup> and the Framingham Heart Study.<sup>32-34</sup> The numbers of cases and controls in each data set are shown in **Table 1**.

Genotyping methods, procedures for data cleaning and imputation, and statistical methods are described in detail in the supplementary material ([http://www.bumc.bu.edu/genetics/results/aa\\_alzheimer](http://www.bumc.bu.edu/genetics/results/aa_alzheimer)). Briefly, the *APOE* genotyping method varied by study. Imputation of autosomal SNP genotypes was performed using the Markov Chain Haplotyping (MaCH) software<sup>35</sup> based on the HapMap 2 and 3 reference SNP panels (<http://hapmap.ncbi.nlm.nih.gov/>). Imputed SNPs were tested for association with AD in the family-based data sets using generalized estimating equations (GEE)<sup>36,37</sup> to account for nonindependence of family members. Analysis of the case-control data sets was performed using logistic regression models. All tests of association were adjusted for sex and age at examination. Two models were evaluated for each SNP, one with and the other without a term for *APOE* genotype coded as the number of *APOE*  $\epsilon 4$  alleles. Unless otherwise noted, all results are from the  $\epsilon 4$ -unadjusted model. An additional analysis of SNPs in the *APOE* region included an adjustment for *APOE* genotype classified into

**Table 2. APOE Genotype Frequency by Population and Alzheimer Disease Status**

Population	Genotype, No. (%) of Subjects <sup>a</sup>						Missing
	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	
African American							
Cases	0	25 (4.9)	19 (3.7)	147 (28.7)	227 (44.2)	78 (15.2)	17 (3.3)
Controls <sup>b</sup>	2 (0.4)	67 (13.3)	12 (2.4)	210 (41.7)	172 (34.1)	18 (3.6)	15 (3.0)
White							
Cases	9 (0.3)	112 (3.1)	93 (2.6)	1045 (29.3)	1743 (48.9)	552 (15.5)	14 (0.4)
Controls	21 (0.3)	673 (10.8)	136 (2.2)	3562 (57.4)	1489 (24.0)	182 (2.9)	139 (2.2)

Abbreviation: APOE, apolipoprotein E.

<sup>a</sup>Percentages have been rounded and might not total 100.

<sup>b</sup>The total number of controls sums to 6202 rather than 6205 owing to the presence of 3 rare APOE isoforms observed in the Framingham Heart Study subjects.

one of the following 4 categories: ε2/ε2 and ε2/ε3; ε3/ε3; ε2/ε4 and ε3/ε4; and ε4/ε4. The SNP association results obtained from individual data sets were combined by meta-analysis using the inverse variance method implemented in the software package METAL.<sup>38</sup> Nominal (uncorrected for multiple testing) *P* values are reported throughout. In the genome-wide analysis, a *P* value of  $5 \times 10^{-8}$  was used as the threshold for significance, and a threshold of  $P < 10^{-5}$  was considered suggestive evidence of association.

## RESULTS

### APOE REGION

The frequency distributions of APOE genotypes for African American and white cohorts are shown in **Table 2**. In the African American cohort, the ε4 allele is very significantly associated with AD ( $P = 9.69 \times 10^{-23}$ ). Analysis of individual genotypes showed evidence of a significant protective effect of ε2 (ε2/ε2 and ε2/ε3 genotypes) compared with the ε3/ε3 genotype and an exponential increase in risk associated with the dose of ε4 (**Table 3**). The odds ratio (OR) estimates and APOE allele frequencies, showing a higher rate of ε4 alleles in African American controls compared with white controls, are in agreement with a previous study of the APOE association in the MIRAGE Study African American cohort.<sup>26</sup>

Analyses of the APOE region in the African American data sets revealed a highly significant association with 3 markers within 25 kb of APOE, including PVRL2 SNP rs6859 ( $P = 5.39 \times 10^{-7}$ ) and TOMM4 SNPs rs157582 ( $P = 3.26 \times 10^{-6}$ ) and rs10119 ( $P = 5.95 \times 10^{-7}$ ) (**Table 4** lists top SNPs in the region; see eTable 1 in the supplementary material for all nominally significant SNPs). Only rs6859 remained significant after adjustment for APOE genotype ( $P = .0087$ ). **Figure 1** shows the unadjusted and APOE genotype-adjusted results for all SNPs in the region immediately flanking APOE. **Figure 2** shows the estimated LD in the region for the African American and MIRAGE Study white data sets. In the white cohorts, ε4 was strongly associated with AD ( $P = 6.80 \times 10^{-147}$ ). In addition, without adjustment for the ε4 allele, 19% of SNPs in this region were very significantly associated with AD ( $P < 10^{-3}$ ). The top-ranked SNPs in this group are rs4420638 in APOC1 ( $P = 1.07 \times 10^{-144}$ ), rs6857 in PVRL2 ( $P = 1.49 \times 10^{-108}$ ), and rs2075650 in TOMM40 ( $P = 1.70 \times 10^{-94}$ ). After adjustment for APOE genotype,

**Table 3. Odds of Alzheimer Disease for APOE Genotypes Relative to ε3/ε3**

Genotypes	OR (95% CI)	<i>P</i> Value
ε2/ε2 or ε2/ε3	0.43 (0.26-0.71)	.0094
ε2/ε4 or ε3/ε4	2.08 (1.58-2.74)	$1.96 \times 10^{-7}$
ε4/ε4	8.23 (4.78-14.15)	$2.62 \times 10^{-14}$

Abbreviations: APOE, apolipoprotein E; OR, odds ratio.

only 7 SNPs remained significant at  $P < .05$ , including rs6857 ( $P = 4.98 \times 10^{-7}$ ), rs4420638 ( $P = 1.54 \times 10^{-7}$ ), and rs2075650 in TOMM40 ( $P = 1.25 \times 10^{-6}$ ).

### PREVIOUSLY IMPLICATED REGIONS

Results for African Americans in the regions of AD associations from the white GWAS are summarized in **Table 5**. There was no evidence of association in African Americans with 2 of these 3 CLU SNPs, including rs11136000, which was consistently significant across multiple studies in white samples. A nominally significant association ( $P = .034$ ) was observed with rs2279590 that had been previously reported in whites. However, the minor allele (T) was associated with increased AD risk in this African American sample (OR, 1.41), whereas the T allele is protective in the white sample (OR, 0.87). Two additional nominally significant SNPs were observed in CLU, the most significant of which was rs9331926 ( $P = .020$ ); complete results including all nominally significant SNPs in previously implicated regions are summarized in eTable 2 in the supplementary material.

Harold et al<sup>21</sup> found genome-wide significant evidence of association with rs3851179, located 88 kb upstream from PICALM. This SNP was not associated with AD in our African American sample ( $P = .16$ ), although the estimated OR (0.85) is nearly identical to the OR reported in the white sample (0.87). However, we observed nominally significant association with 8 of 287 other SNPs tested in the region, including rs12795381 ( $P = .0086$ ) and rs17148827 ( $P = .0089$ ), which is monomorphic in whites. The rs12795381 finding is consistent with modest evidence of association with multiple SNPs in the PICALM coding region.<sup>20</sup> We also evaluated the interaction of PICALM SNPs with APOE as reported

**Table 4. Most Significantly Associated Markers in the APOE Region in African American and White Cohorts**

Marker	Position, dbSNP Build 129. bp	Gene	Effect Allele	Unadjusted for APOE			APOE Genotype Adjusted		
				OR	P Value	Direction <sup>a</sup>	OR	P Value	Direction <sup>a</sup>
African American									
rs6859	50 073 874	PVRL2	A	1.58	5.39 × 10 <sup>-7</sup>	++	1.30	.0087	++
rs3852861	50 074 901	PVRL2	T	0.64	5.99 × 10 <sup>-5</sup>	--	0.78	.038	--
rs157582	50 088 059	TOMM40	T	1.60	3.26 × 10 <sup>-6</sup>	++	1.15	.222	-+
rs157583	50 088 513	TOMM40	T	2.08	2.48 × 10 <sup>-5</sup>	++	0.92	.700	--
rs8106922	50 093 506	TOMM40	A	1.48	9.73 × 10 <sup>-5</sup>	++	1.18	.144	-+
rs1160985	50 095 252	TOMM40	T	0.65	4.92 × 10 <sup>-5</sup>	--	1.04	.730	+-
rs10119	50 098 513	TOMM40	A	1.80	5.95 × 10 <sup>-7</sup>	++	1.07	.614	-+
rs445925	50 107 480	5' APOC1	A	1.57	3.02 × 10 <sup>-4</sup>	++	1.13	.443	++
White									
rs6857	50 084 094	PVRL2	T	3.23	1.49 × 10 <sup>-108</sup>	++ ?? +	1.50	4.98 × 10 <sup>-7</sup>	++ ?? +
rs157580	50 087 106	TOMM40	A	1.70	2.77 × 10 <sup>-29</sup>	++ ?? +	1.09	.130	++ ?? +
rs2075650	50 087 459	TOMM40	A	0.35	1.70 × 10 <sup>-94</sup>	-- ?? -	0.71	1.25 × 10 <sup>-6</sup>	-- ?? -
rs157582	50 088 059	TOMM40	T	2.73	2.54 × 10 <sup>-91</sup>	++ ?? +	1.30	.0011	+ - ?? +
rs8106922	50 093 506	TOMM40	A	1.66	2.06 × 10 <sup>-28</sup>	++ ?? +	1.02	.679	+ - ? -
rs1160985	50 095 252	TOMM40	T	0.58	2.78 × 10 <sup>-33</sup>	-- ?? -	0.95	.326	- + ?? +
rs10119	50 098 513	TOMM40	A	2.48	8.15 × 10 <sup>-70</sup>	++ ?? +	1.22	.0051	+ - ?? +
rs4420638	50 114 786	APOC1	A	0.23	1.07 × 10 <sup>-144</sup>	-- ? --	0.61	1.54 × 10 <sup>-7</sup>	-- ? --

Abbreviations: APOC1, apolipoprotein C-I; APOE, apolipoprotein E; bp, base pairs; dbSNP, database single-nucleotide polymorphism; OR, odds ratio; PVRL2, poliovirus receptor-related 2; TOMM40, translocase of outer mitochondrial membrane 40 yeast homologue.

<sup>a</sup>For the African American cohort, symbols indicate increased (+) or decreased (-) risk in the Genetic and Environmental Risk Factors for Alzheimer Disease Among African Americans and Multi-Institutional Research on Alzheimer Genetic Epidemiology (MIRAGE) data sets; for the white cohort, increased (+) or decreased (-) risk in the MIRAGE, Alzheimer Disease Neuroimaging Initiative, Framingham Heart Study, Canadian Study on Genetics of Alzheimer Disease Associations, and National Institute on Aging-Late-Onset Alzheimer's Disease Family Study data sets.

in a large white sample.<sup>24</sup> Stratified analysis revealed evidence of association with rs12795381 in subjects with the APOE ε4 allele ( $P = .04$ ) but not in those without it ( $P = .61$ ). However, we were unable to perform a formal test of interaction owing to the relatively small sample size and low minor allele frequency.

None of the 88 tested *CRI* SNPs (including the 2 reported as significant by Lambert et al<sup>19</sup>) and none of the 112 tested *CD2AP* SNPs (including rs9349407, which was reported as significant by Naj et al<sup>22</sup>) were associated with AD in African Americans. The most promising result among these SNPs was obtained with rs12734030 in *CRI* ( $P = .09$ ).

Seshadri et al<sup>20</sup> proposed *BIN1* as a candidate gene for AD on the basis of a genome-wide significant  $P$  value observed for rs744373 located approximately 30 kb from the *BIN1* coding region. Although this result was not replicated in our African American sample ( $P > .99$ ), several adjacent SNPs were nominally significant, including rs11685593 ( $P = .0098$ ). Association was also observed with multiple SNPs within the *BIN1* coding region, the most significant of which was rs11691237 ( $P = .0098$ ). The most significant association in the region was observed with rs7585314 ( $P = .0030$ ), which is 68 kb from rs744373 in *CYP27C1*.

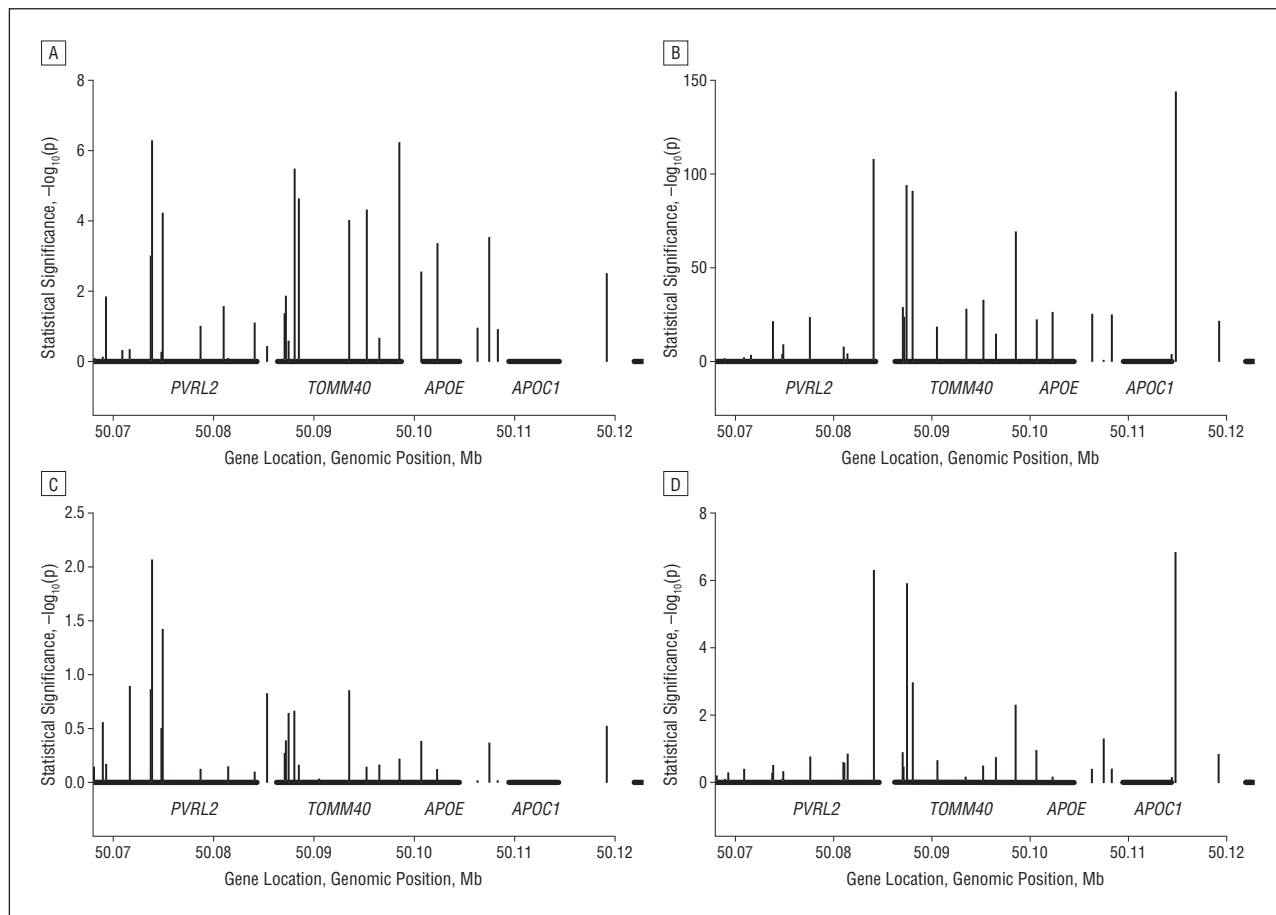
Located approximately 6 kb from *EPHA1*, rs11767557 is the only genome-wide significant result in this region reported by Naj et al<sup>22</sup> and was not associated with AD in African Americans ( $P = .59$ ). However, rs11762262, which is approximately 1260 bp closer to *EPHA1* than rs11767557, was nominally significant ( $P = .034$ ). We observed multiple nominally significant SNPs spread throughout the *EPHA1* region in the African American sample. The strongest evidence for association in this re-

gion was obtained with rs4595035 ( $P = .0094$ ), which is 32 kb upstream from rs11767557.

Naj et al<sup>22</sup> also observed genome-wide significant association with many SNPs in the *MS4A* cluster. We evaluated association with all SNPs across this cluster, which spans about 500 kb. The most significant finding in the *MS4A* region was observed with rs10792258 ( $P = .010$ ). This SNP is 394 bp distal from rs1582763 and 253 bp proximal to rs1562990, both of which were strongly associated with AD ( $P = 5.92 \times 10^{-11}$  and  $P = 2.47 \times 10^{-9}$ , respectively) in the meta-analysis of large white data sets by Naj et al.<sup>22</sup> A similar level of significance was observed with rs3802957 ( $P = .011$ ) in the 3' untranslated region of *MS4A1*, 203 kb from rs10792258.

We did not see association ( $P = .38$ ) with an SNP in *ABCA7* (rs3752246), which approached genome-wide significance in the study by Naj et al.<sup>22</sup> We did, however, observe nominally significant association with rs3764650 ( $P = .019$ ), which was reported as genome-wide significant in the study by Hollingworth et al.<sup>23</sup> The effect of this SNP on risk of AD in our study (OR, 1.27) was very similar to that observed previously (OR, 1.23). Several other nominally significant SNPs were observed in the region, of which the most significant, synonymous coding SNP rs376647 ( $P = .0087$ ), is located 11 kb from rs3752246.

We also did not observe an association with *CD33* SNP rs3865444 ( $P = .73$ ), which was found to have genome-wide significance in the GWAS by Naj et al.<sup>22</sup> The most significant result in the *CD33* region in the African American sample was obtained with rs10419982 ( $P = .0005$ ), 69 kb away from rs3865444. This SNP almost survives correction for the 200 SNPs examined in the region. However, given the great distance of this SNP



**Figure 1.** Association and linkage disequilibrium in the apolipoprotein E (*APOE*) region. A and B, Unadjusted findings in African Americans and whites, respectively; C and D, *APOE*-genotype adjusted findings in African Americans and whites, respectively. *APOC1* indicates apolipoprotein C-I; Mb, megabase; *PVRL2*, poliovirus receptor-related 2; and *TOMM40*, translocase of outer mitochondrial membrane 40 yeast homologue.

from *CD33*, there is not adequate evidence to consider this result a replication.

#### NOVEL GENE DISCOVERY

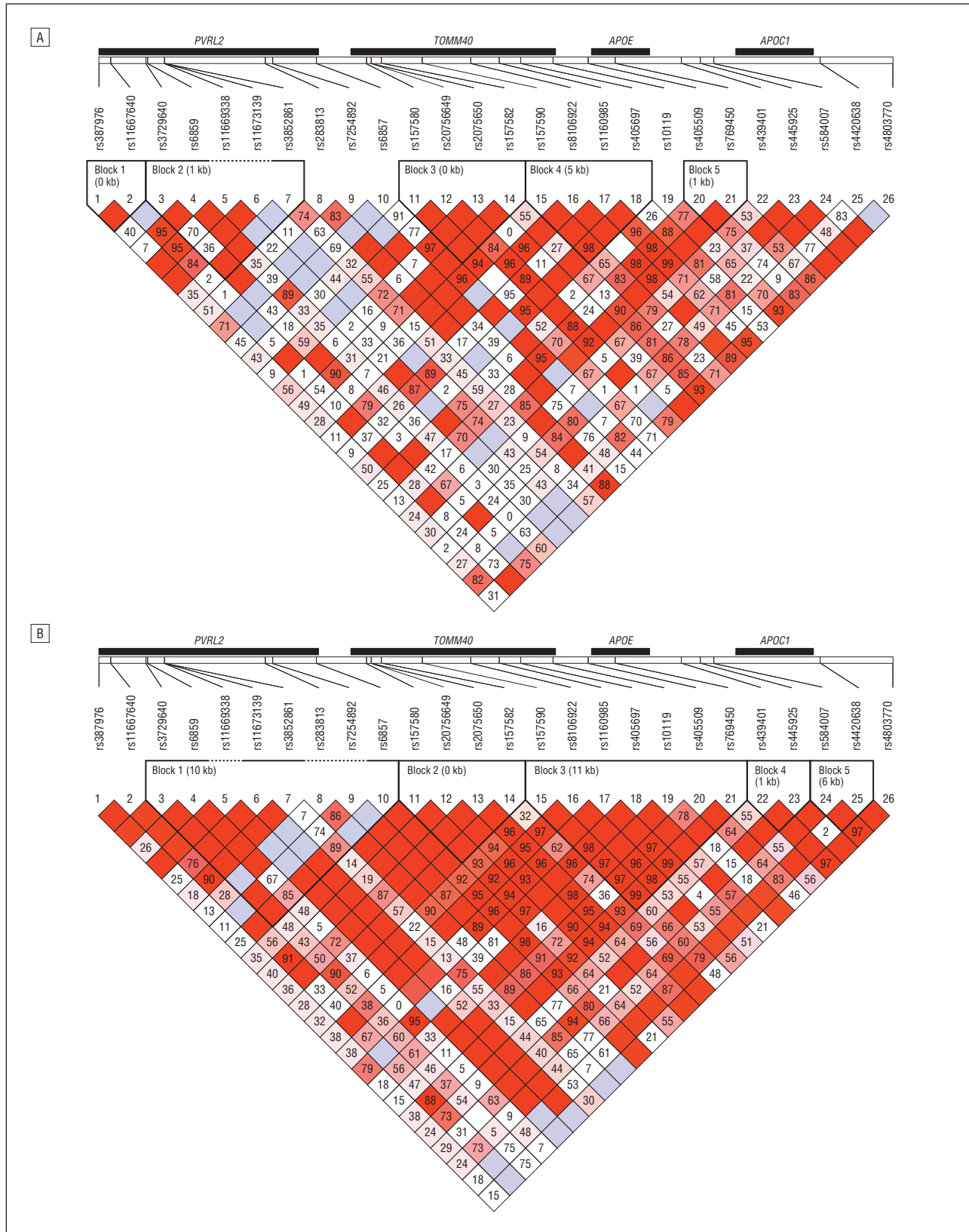
Genotypes were evaluated for 2 505 093 imputed SNPs that passed minor allele frequency criteria and imputation quality thresholds. A Manhattan plot of the results of the genome screen is presented in **Figure 3**. No SNP achieved a genome-wide level of significance. Eleven SNPs achieved suggestive levels of association ( $P < 10^{-5}$ ) (**Table 6**). The direction of effect for these SNPs was consistent across the African American cohorts. Four of these SNPs (rs11889338, rs2221154, rs956225, and rs10850408) are more than 50 kb from the nearest characterized gene. Strong evidence of association was obtained with rs340849 ( $P = 7.52 \times 10^{-6}$ ), located 34 kb from *PROX1*, and with rs3888908 ( $P = 9.52 \times 10^{-6}$ ), located 19 kb upstream from *P4HA3*. The most significant finding was obtained for rs10850408 ( $P = 9.25 \times 10^{-7}$ ), a chromosome 12 SNP more than 250 kb from the nearest gene. The SNPs in *ZC3H3*, *TMTC1*, and *ENOX1* showed suggestive evidence of an association in analyses adjusting for *APOE*ε4 (see eTable 3 in the supplementary material for all SNPs with  $P < 10^{-5}$ ). None of these findings were replicated in the white meta-

analysis (details are provided in the eAppendix in the supplementary material).

#### COMMENT

This is, to our knowledge, the first comprehensive genetic association study of AD in African Americans. This study is timely and important for several reasons. African Americans are about twice as likely as non-Hispanic whites to have AD.<sup>39</sup> Although differences in AD etiology across populations have been widely studied, they are still poorly understood. The occurrence of multiple demented individuals in African American families is significantly higher than in white families, although the genetic risk of AD is similar in these 2 populations.<sup>27</sup> The increased familial risk in African Americans is likely a result of higher rates of risk factors, such as poor education, diabetes mellitus, and smoking.<sup>39</sup> However, comparisons of risk in African American and white cohorts are complicated by differences in assessment of cognitive decline across studies and by population differences in willingness to participate in medical research.<sup>40-42</sup>

We obtained incontrovertible evidence of an association with the *APOE* ε4 allele, thus confirming find-



**Figure 2.** Linkage disequilibrium in the apolipoprotein E (*APOE*) region. A, African American cohort data sets. B, White cohort data sets. Other gene names are described in the legend to Figure 1.

ings from several smaller genetic studies of African Americans.<sup>4,26</sup> In non-Hispanic whites, homozygosity for  $\epsilon 4$  is associated with a 13- to 15-fold increased

odds of developing AD compared with those with the most common genotype,  $\epsilon 3/\epsilon 3$ .<sup>4</sup> We showed previously in a set of 308 African American AD cases and

**Table 5. Association of Alzheimer Disease With Genome-wide Significant Regions in White GWASs**

Gene, SNP	Effect Allele	AF <sub>CEU</sub>	Previously Reported Results in White Cohort				Results in African American Cohort		
			Source	AF	P Value	OR (95% CI)	AF	P Value	OR (95% CI)
<i>CLU</i>									
rs2279590	T	0.31	Lambert et al, <sup>19</sup> 2009	NR	$9.2 \times 10^{-9}$	0.87 (0.83-0.92)	0.12	.034	1.41 <sup>a</sup> (1.03-1.95)
rs11136000	T	0.35	Lambert et al, <sup>19</sup> 2009	NR	$2.0 \times 10^{-9}$	0.86 (0.82-0.91)	0.56	.951	1.01 (0.84-1.20)
			Seshadri et al, <sup>20</sup> 2010	0.39	$1.62 \times 10^{-16}$	0.85 (0.82-0.88)			
			Harold et al, <sup>21</sup> 2009	0.40	$8.5 \times 10^{-10}$	0.86 (0.82-0.90)			
rs9331888	G	0.32	Lambert et al, <sup>19</sup> 2009	NR	$1.4 \times 10^{-7}$	1.15 (1.09-1.22)	0.20	>.99	1.00 (0.76-1.32)
rs9331926 <sup>b</sup>	G	0.07	...	...	...	...	0.04	.020	1.96 (1.11-3.48)
<i>PICALM</i>									
rs3851179	T	0.41	Seshadri et al, <sup>20</sup> 2010	0.37	$3.16 \times 10^{-12}$	0.87 (0.84-0.91)	0.16	.157	0.85 (0.68-1.07)
			Harold et al, <sup>21</sup> 2009	0.37	$1.3 \times 10^{-9}$	0.86 (0.82-0.90)			
rs17148827 <sup>c</sup>	C	0.00	...	...	...	...	0.04	.0089	2.01 (1.19-3.40)
rs12795381 <sup>d</sup>	C	0.14	...	...	...	...	0.04	.0086	0.49 (0.29-0.84)
<i>CR1</i>									
rs6656401	A	0.24	Lambert et al, <sup>19</sup> 2009	NR	$7.9 \times 10^{-9}$	1.20 (1.13-1.28)	0.07	.227	0.79 (0.54-1.16)
rs3818361	A	0.26	Lambert et al, <sup>19</sup> 2009	NR	$1.4 \times 10^{-7}$	1.18 (1.11-1.25)	0.43	.466	0.94 (0.79-1.11)
<i>BIN1</i>									
rs744373	G	0.31	Seshadri et al, <sup>20</sup> 2010	0.29	$1.59 \times 10^{-11}$	1.15 (1.11-1.20)	0.48	.999	1.00 (0.84-1.20)
rs11685593 <sup>e</sup>	T	0.21	...	...	...	...	0.06	.0098	1.66 (1.13-2.45)
rs11691237 <sup>f</sup>	T	0.27	...	...	...	...	0.10	.0098	1.52 (1.11-2.09)
rs7585314 <sup>g</sup>	T	0.85	...	...	...	...	0.33	.0030	0.75 (0.62-0.91)
<i>CD2AP</i>									
rs9349407	C	0.28	Naj et al, <sup>22</sup> 2011	0.27	$8.6 \times 10^{-9}$	1.11 (1.07-1.15)	0.22	.854	0.98 (0.78-1.22)
<i>EPHA1</i>									
rs11767557	C	0.20	Naj et al, <sup>22</sup> 2011	0.19	$6.0 \times 10^{-10}$	0.90 (0.86-0.93)	0.18	.586	1.06 (0.86-1.31)
rs11762262 <sup>h</sup>	T	0.20	...	...	...	...	0.23	.034	1.27 (1.02-1.59)
rs4595035 <sup>i</sup>	T	0.37	...	...	...	...	0.43	.0094	1.25 (1.06-1.47)

(continued)

409 ethnically matched controls that persons with the ε3/ε4 and ε4/ε4 genotypes had 2.6- and 10.5-fold increased odds of AD, respectively, compared with persons with the ε3/ε3 genotype.<sup>26</sup> These risks decreased substantially after 68 years of age. The risk of AD was lower among individuals with the ε2/ε3 genotype. We observed similar risks in the present study. Approximately one-third of the African American sample in this study overlaps with the sample in our earlier report.

Our present study and previous studies in white populations identified highly significant evidence of an association with genes adjacent to *APOE*, most notably *TOMM40* and *APOC1* (reviewed by Ertekin-Taner<sup>5</sup>). Arguably, the distinction of such findings from confounding with *APOE* is intractable because of the tight

LD spanning the genes in this region.<sup>9,10</sup> However, we identified highly significant evidence of an association with several SNPs in the *APOE* region in African Americans and whites after adjustment for *APOE* genotype. This finding is consistent with an AD risk locus distinct from *APOE*. The observation that different SNPs in this region are significant in African Americans and whites after adjustment for *APOE* may reflect differences in LD structure in this region (Figure 2). The residual association in these other genes may also represent unmeasured effects of variants in regulatory regions of *APOE*.<sup>43-45</sup> Additional studies in larger African American samples are needed to determine which of these explanations is more likely.

Among the African Americans in the present study, a subset of 180 cases and 200 controls from the MIRAGE

**Table 5. Association of Alzheimer Disease With Genome-wide Significant Regions in White GWASs (continued)**

Gene, SNP	Effect Allele	AF <sub>CEU</sub>	Previously Reported Results in White Cohort				Results in African American Cohort		
			Source	AF	P Value	OR (95% CI)	AF	P Value	OR (95% CI)
<i>MS4A</i>									
rs4938933	C	0.50	Naj et al, <sup>22</sup> 2011	0.39	1.7 × 10 <sup>-9</sup>	0.88 (0.85-0.92)	0.30	.493	1.06 (0.88-1.29)
rs610932	T	0.54	Hollingsworth et al, <sup>23</sup> 2011	...	1.8 × 10 <sup>-14</sup>	0.90 (0.87-0.92)	0.49	.299	0.91 (0.77-1.08)
rs10792258 <sup>l</sup>	T	0.20	...	...	...	...	0.37	.010	0.79 (0.66-0.95)
<i>ABCA7</i>									
rs3752246	G	0.19	Naj et al, <sup>22</sup> 2011	0.19	5.8 × 10 <sup>-7</sup>	1.15 (1.09-1.21)	0.04	.375	0.82 (0.53-1.27)
rs3764650	G	0.11	Hollingsworth et al, <sup>23</sup> 2011	...	4.5 × 10 <sup>-17</sup>	1.23 (1.18-1.30)	0.28	.019	1.27 (1.04-1.55)
rs3764647 <sup>k</sup>	G	0.04	...	...	...	...	0.25	.0087	1.32 (1.07-1.63)
<i>CD33</i>									
rs3865444	A	0.32	Naj et al, <sup>22</sup> 2011	0.30	1.6 × 10 <sup>-9</sup>	0.91 (0.88-0.93)	0.09	.732	0.95 (0.70-1.29)
rs10419982 <sup>l</sup>	A	0.45	...	...	...	...	0.40	.00054	1.38 (1.15-1.65)

Abbreviations: *ABCA7*, ATP-binding cassette, subfamily A (*ABC1*), member 7; AF, effect allele frequency; *BIN1*, bridging integrator 1; *CD2AP*, CD2-associated protein; *CD33*, myeloid-associated antigen CD33; CEU, HapMap's CEPH (Utah residents with ancestry from Northern and Western Europe) Population; *CLU*, clusterin; *CR1*, complement component (3b/4b) receptor 1; ellipses, missing information; *EPHA1*, ephrin type-A receptor 1; GWAS, genome-wide association study; kb, kilobase; *MS4A*, the membrane-spanning 4A gene cluster; NPR, not previously reported as associated with Alzheimer disease in whites; NR, not reported; OR, odds ratio; *PICALM*, phosphatidylinositol binding clathrin assembly protein; SNP, single-nucleotide polymorphism.

<sup>a</sup>Effect estimate directions differ between whites and African Americans.

<sup>b</sup>This SNP was not previously reported as associated with Alzheimer disease in whites (NPR). The lowest *P* value is in *CLU*.

<sup>c</sup>This SNP was NPR and is located 15 kb from rs3851179.

<sup>d</sup>This SNP was NPR. This represents the lowest *P* value observed in *PICALM*.

<sup>e</sup>This SNP was NPR and flanks rs744375, 6.5 kb away from rs744373.

<sup>f</sup>This SNP was NPR. This represents the most significant *P* value observed in *BIN1*.

<sup>g</sup>This SNP was NPR. This represents the lowest *P* value observed in the *BIN1* region.

<sup>h</sup>This SNP was NPR and flanks rs11767557.

<sup>i</sup>This SNP was NPR. This represents the smallest *P* value observed in the region of *EPHA1*.

<sup>j</sup>This SNP was NPR. This represents the smallest *P* value observed in the *MS4A* region.

<sup>k</sup>This SNP was NPR. This represents the smallest *P* value observed in *ABCA7*.

<sup>l</sup>This SNP was NPR and is 63 kb from rs3865444. This represents the lowest *P* value observed in the *CD33* region.

Study and 221 cases and 186 controls from the Generations Study was included in another recent study<sup>24</sup> that evaluated the association of AD with *PICALM*, *CLU*, and *CRI* SNPs highlighted in the original studies reports.<sup>19,21</sup> The authors did not find evidence of an association with any of the SNPs examined in the African American data sets. We did not replicate the genome-wide significant associations with these loci in a larger set of African American cases and controls, even at a nominal significance level. However, we observed an association in African Americans with other previously unreported variants in each of these regions and in the most recently reported regions of genome-wide significant association,<sup>22,23</sup> except *CRI* and *CD2AP*. Only one previously reported genome-wide significant association (rs3764650 in *ABCA7*) was confirmed in our African American sample.<sup>23</sup> Discordance in the association patterns between whites and African Americans could be related to population differences in allele frequencies or LD patterns. This explanation is consistent with our findings of association in the African Americans between SNPs, which have very low frequency in whites (eg, rs17148827 in *PICALM*), and one of the previously reported AD-associated *CLU* SNPs (rs2279590), but with an opposite pattern of effect. Alternatively, the AD risk variants in these genes may differ

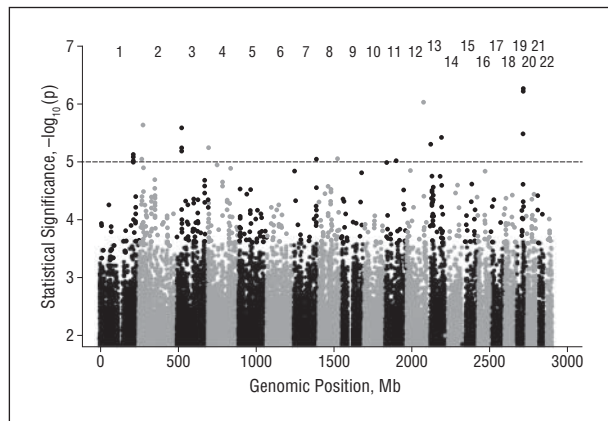
across populations (ie, allelic heterogeneity), as we observed previously in *SORL1*.<sup>46</sup> However, lack of replication might also be a result of small sample size when compared with recent consortium-based GWASs.<sup>19-23</sup> In most instances, the confidence intervals for the effect estimates in African Americans included the point estimates in whites.

Analysis of the entire autosomal genome revealed evidence suggestive of an association with several novel candidate genes that may play a role in AD pathogenesis. *PROX1* (OMIM \*601546) is a prospero-related transcription factor that plays a critical role in the development of various organs, including the mammalian lymphatic and central nervous systems.<sup>47,48</sup> This transcription factor has recently been shown to play a key role in adult neurogenesis, suggesting it may be involved in memory development.<sup>49</sup> The contactin-associated protein-like 2 gene (*CNTNAP2* [OMIM \*604569]) is involved in brain development and has been implicated in susceptibility to autism and language disorders.<sup>50-53</sup> In 2009, Harold et al<sup>21</sup> reported a SNP in the contactin gene, *CNTN5* (OMIM \*607219), to have a GWAS *P* value of 2 × 10<sup>-5</sup>. Subsequently, the same SNP was shown to be associated with a variety of magnetic resonance imaging measures in the Alzheimer Disease Neuroimaging Initiative cohort.<sup>54</sup> Serine/threonine kinase 24 (*STK24* [OMIM \*604984]) is ex-



pressed in the brain.<sup>55,56</sup> An isoform of *STK24* has been shown to be a regulator of axon growth and axon regeneration after injury.<sup>57,58</sup> However, we did not observe association in these regions in a meta-analysis of a replication sample of 5 white AD data sets containing 3568 cases and 6205 controls. A study of a larger independent sample of African American and possibly white samples will be needed to determine whether these associations are spurious or reflect population-specific variants or variable LD patterns among populations.

This study represents an important step in elucidating the genetic basis of AD in African Americans. Our results suggest that African Americans share some but not all AD genetic risk factors with whites. Further research would not only lead to a more accurate understanding of the genetic risk factors that could be incorporated in diagnostic and predictive testing protocols specific for African Americans but may also yield new gene discovery and clues for subsequent interventions useful to all populations at risk for AD.



**Figure 3.** Manhattan plot of genome-wide association study results for the meta-analysis of the African American cohorts. The dotted line indicates suggestive evidence of association ( $P < 10^{-5}$ ).

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**Table 6. Top-Ranked Genetic Association Findings From Genome-wide Survey in African Americans**

CHR	Position, dbSNP Build 129, bp	Gene	SNP	P Value	Effect Allele	AF	OR (95% CI)
1	212 184 713 <sup>a</sup>	–	rs340849 <sup>a</sup>	$7.52 \times 10^{-6}$	A	0.20	0.59 (0.47-0.75)
2	17 291 066	–	rs11889338	$8.94 \times 10^{-6}$	A	0.26	1.55 (1.28-1.88)
2	27 760 977	<i>SLC4A1AP</i>	rs17006206	$2.30 \times 10^{-6}$	G	0.10	2.05 (1.52-2.76)
3	28 903 864	–	rs2221154	$2.58 \times 10^{-6}$	T	0.19	0.57 (0.45-0.72)
4	2 072 894	<i>POLN</i>	rs1923775	$5.61 \times 10^{-6}$	T	0.25	1.60 (1.30-1.95)
7	146 528 336	<i>CNTNAP2</i>	rs10273775	$8.94 \times 10^{-6}$	G	0.42	1.52 (1.27-1.84)
8	122 978 868	–	rs956225	$8.71 \times 10^{-6}$	G	0.03	0.30 (0.18-0.51)
11	73 710 714	–	rs3888908	$9.52 \times 10^{-6}$	A	0.15	1.72 (1.36-2.20)
12	113 864 776	–	rs10850408	$9.25 \times 10^{-7}$	T	0.34	0.63 (0.52-0.76)
13	25 622 328	–	rs17511627	$5.01 \times 10^{-6}$	C	0.17	1.75 (1.37-2.22)
13	97 929 295	<i>STK24</i>	rs912330	$3.79 \times 10^{-6}$	T	0.14	0.54 (0.41-0.70)
<b>Other SNPs of Interest From APOE ε4 Adjusted Analysis</b>							
8	144 692 178	<i>ZC3H3</i>	rs3750208	$7.28 \times 10^{-6}$	A	0.04	0.37 (0.24-0.57)
12	29 812 934	<i>TMTC1</i>	rs302318	$1.97 \times 10^{-6}$	C	0.26	0.59 (0.48-0.74)
13	43 064 019	<i>ENOX1</i>	rs17460623	$9.37 \times 10^{-6}$	C	0.10	0.49 (0.36-0.67)

Abbreviations: AF, effect allele frequency; bp, base pairs; CHR, chromosome; *CNTNAP2*, contactin-associated protein-like 2; dbSNP, database single-nucleotide polymorphism; *ENOX1*, ecto-NOX disulfide-thiol exchanger 1; OR, odds ratio; *POLN*, polymerase (DNA directed) nu; *SLC4A1AP*, solute carrier family 4 (anion exchanger), member 1, adaptor protein; SNP, single-nucleotide polymorphism; *STK24*, serine/threonine kinase 24; *TMTC1*, transmembrane and tetratricopeptide repeat containing 1; *ZC3H3*, zinc finger CCCH-type containing 3; –, indicates that the SNP is more than 50 kilobases from the nearest characterized gene.

<sup>a</sup>Does not include multiple SNPs on CHR 1 that were redundant owing to strong linkage disequilibrium (pairwise  $R^2 > 0.8$ ) and CHR 3 ( $R^2 > 0.7$ ) and 1 SNP that did not meet minor allele frequency criteria in subjects from the Genetic and Environmental Risk Factors for Alzheimer Disease Among African Americans Study.

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