A Comprehensive Genetic Association Study of Alzheimer Disease in African Americans

Mark W. Logue, PhD; Matthew Schu, MS; Badri N. Vardarajan, MS; Jacki Buros, BA; Robert C. Green, MD, MPH; Rodney C. P. Go, PhD; Patrick Griffith, MD; Thomas O. Obisesan, MD; Rhonna Shatz, MD; Amy Borenstein, PhD; L. Adrienne Cupples, PhD; Kathryn L. Lunetta, PhD; M. Daniele Fallin, PhD; Clinton T. Baldwin, PhD; Lindsay A. Farrer, PhD; for the Multi-Institutional Research on Alzheimer Genetic Epidemiology (MIRAGE) Study Group

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 apolipoprotein 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.

Arch Neurol. 2011;68(12):1569-1579

A Alzheimer 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%1 and analyses showing transmission of a major gene for the disease in families.2 Until recently, the apolipoprotein E gene (APOE) was the only one generally recognized to influence LOAD risk.3 In whites, homozygosity for the ε4 variant is associated with an increased risk by as much as 15 times that of the most common APOE genotype (ε3/ε3).4 Genome-wide association studies (GWASs) have reported genome-wide significant single-nucleotide polymorphisms (SNPs) across a 70-kilobase (kb) region that includes APOE and several neighboring genes,5 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,6 association of an intronic TOMM40 repeat polymorphism with age at the onset of AD symptoms among subjects lacking the ε4 allele,7 and association of a haplotype spanning TOMM40 with expression of
APOE. However, other studies did not find an effect of TOMM40 after adjusting for APOE. A polymorphism immediately upstream of the APOCI gene has also been proposed as a possible risk locus for AD. This polymorphism is in strong linkage disequilibrium (LD) with APOE and is being investigated as a possible risk locus for AD. Studies in mice and humans indicate that APOCI expression has an effect on memory. Other studies reported that APOCI modifies the risk of AD independent of or through interaction with APOE.

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]), phosphatidylinositol-binding clathrin assembly protein (PICALM [OMIM *603025]), complement component (3b/4b) receptor 1 (CRI [OMIM *120620]), bridging integrator 1 (BIN1 [OMIM *601248]), CD2-associated protein (CD2AP [OMIM *604241]), ephrin type-A receptor 1 (EPHA1 [OMIM *179610]), the membrane-spanning 4A (MS4A) gene cluster, bridging integrator 1 (BIN1), member 7 (ABCA7 [OMIM *605414]), and ATP-binding cassette, subfamily A (ABC1), member 7 (ABCA7). Findings with CLU, PICALM, CRI, and ABCA7 have been replicated.

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, 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. A second group of primarily unrelated individuals includes participants of the Genetic and Environmental Risk Factors for Alzheimer Disease Among African Americans (GenADA) 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 Diseases 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 6203 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), a Canadian study on genetics of Alzheimer disease associations (GenADA), the National Institute on Aging–Late-Onset Alzheimer’s Disease Family Study (NIA-LOAD), and the Framingham Heart Study. 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). Briefly, the APOE genotyping method varied by study. Imputation of autosomal SNP genotypes was performed using the Markov Chain Haplotyping (MaCH) software 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) 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 ε4 alleles. Unless otherwise noted, all results are from the ε4-unadjusted model. An additional analysis of SNPs in the APOE region included an adjustment for APOE genotype classified into.

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

<table>
<thead>
<tr>
<th></th>
<th>African American</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIRAGE</td>
<td>GenerAAtions</td>
</tr>
<tr>
<td>No. of cases</td>
<td>267</td>
<td>246</td>
</tr>
<tr>
<td>No. of controls</td>
<td>292</td>
<td>204</td>
</tr>
<tr>
<td>Total</td>
<td>559</td>
<td>450</td>
</tr>
</tbody>
</table>

**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.
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.38 Nominal (uncorrected for multiple testing) P values are reported throughout. In the genome-wide analysis, a P value of $10^{-5}$ was used as the threshold for significance, and a threshold of $P < 10^{-3}$ 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.26

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 PVR12 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-adj usted 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 ($P = 8.60 \times 10^{-14}$). 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 rs420638 in APOCI ($P = 1.07 \times 10^{-144}$), rs6857 in PVR12 ($P = 1.49 \times 10^{-138}$), and rs2075650 in TOMM40 ($P = 1.70 \times 10^{-98}$). After adjustment for APOE genotype, only 7 SNPs remained significant at $P < .05$, including rs6857 ($P = 4.98 \times 10^{-7}$), rs420638 ($P = 1.54 \times 10^{-7}$), 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 al21 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.20 We also evaluated the interaction of PICALM SNPs with APOE as reported...
in a large white sample. 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 CR1 SNPs (including the 2 reported as significant by Lambert et al) and none of the 112 tested CD2AP SNPs (including rs9349407, which was reported as significant by Naj et al) were associated with AD in African Americans. The most promising result among these SNPs was obtained with rs12734030 in the AD region. However, given the great distance of this SNP on risk of AD in our study (OR, 1.27) was very small.

Seshadri et al 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 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 region was obtained with rs4595035 (P = .0094), which is 32 kb upstream from rs11767557. Naj et al 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 × 10^-11 and P = 2.47 × 10^-9, respectively) in the meta-analysis of large white data sets by Naj et al. 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. 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. 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. 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...
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 rs3889008 ($P = 9.52 \times 10^{-5}$), 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). None of these findings were replicated in the white meta-analysis (details are provided in the eAppendix in the supplementary material).

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. 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. 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. 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.

We obtained incontrovertible evidence of an association with the APOE ε4 allele, thus confirming find-
ings from several smaller genetic studies of African Americans. In non-Hispanic whites, homozygosity for ε4 is associated with a 13- to 15-fold increased odds of developing AD compared with those with the most common genotype, ε3/ε3. We showed previously in a set of 308 African American AD cases and

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.
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. 26 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). 5 Arguably, the distinction of such findings from confounding with APOE is intractable because of the tight LD spanning the genes in this region. 9,10 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. 43-45 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

<table>
<thead>
<tr>
<th>Gene, SNP</th>
<th>Effect Allele</th>
<th>AF in CEU</th>
<th>Previously Reported Results in White Cohort</th>
<th>Results in African American Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>AF</td>
<td>P Value</td>
<td>OR (95% CI)</td>
<td>AF</td>
</tr>
<tr>
<td>Lambert et al, 2009</td>
<td>NR</td>
<td>9.2 × 10^-8</td>
<td>0.87 (0.83-0.92)</td>
<td>0.12</td>
</tr>
<tr>
<td>Seshadri et al, 2010</td>
<td>0.39</td>
<td>1.62 × 10^-16</td>
<td>0.85 (0.82-0.88)</td>
<td>0.56</td>
</tr>
<tr>
<td>Harold et al, 2009</td>
<td>0.40</td>
<td>8.5 × 10^-10</td>
<td>0.86 (0.82-0.90)</td>
<td>0.20</td>
</tr>
<tr>
<td>Lambert et al, 2009</td>
<td>0.32</td>
<td>1.4 × 10^-7</td>
<td>1.15 (1.09-1.22)</td>
<td>0.20</td>
</tr>
<tr>
<td>Seshadri et al, 2010</td>
<td>0.37</td>
<td>3.16 × 10^-32</td>
<td>0.87 (0.84-0.91)</td>
<td>0.16</td>
</tr>
<tr>
<td>Harold et al, 2009</td>
<td>0.37</td>
<td>1.3 × 10^-4</td>
<td>0.86 (0.82-0.90)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lambert et al, 2009</td>
<td>0.27</td>
<td>7.9 × 10^-8</td>
<td>1.20 (1.13-1.28)</td>
<td>0.07</td>
</tr>
<tr>
<td>Lambert et al, 2009</td>
<td>0.26</td>
<td>1.4 × 10^-7</td>
<td>1.18 (1.11-1.25)</td>
<td>0.43</td>
</tr>
<tr>
<td>Seshadri et al, 2010</td>
<td>0.00</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Seshadri et al, 2010</td>
<td>0.14</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Harold et al, 2009</td>
<td>0.24</td>
<td>1.59 × 10^-31</td>
<td>1.15 (1.11-1.20)</td>
<td>0.48</td>
</tr>
<tr>
<td>Lambert et al, 2009</td>
<td>0.21</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Lambert et al, 2009</td>
<td>0.27</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Lambert et al, 2009</td>
<td>0.85</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Naj et al, 2011</td>
<td>0.27</td>
<td>8.6 × 10^-8</td>
<td>1.11 (1.07-1.15)</td>
<td>0.22</td>
</tr>
<tr>
<td>Naj et al, 2011</td>
<td>0.20</td>
<td>6.0 × 10^-10</td>
<td>0.90 (0.86-0.93)</td>
<td>0.18</td>
</tr>
<tr>
<td>Naj et al, 2011</td>
<td>0.20</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Naj et al, 2011</td>
<td>0.37</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Table 5. Association of Alzheimer Disease With Genome-wide Significant Regions in White GWASs (continued)

<table>
<thead>
<tr>
<th>Gene, SNP</th>
<th>Effect</th>
<th>Source</th>
<th>AF</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>Value</th>
<th>Source</th>
<th>AF</th>
<th>P Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MS4A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4939833</td>
<td>C</td>
<td>Naj et al, 2011</td>
<td>0.39</td>
<td>0.88</td>
<td>0.00054</td>
<td>1.38</td>
<td>(1.15-1.65)</td>
<td>0.03</td>
<td>.493</td>
<td>1.06 (0.88-1.29)</td>
</tr>
<tr>
<td>rs610932</td>
<td>T</td>
<td>Hollingworth et al, 2011</td>
<td>0.50</td>
<td>0.87</td>
<td>0.29</td>
<td>0.90</td>
<td>(0.87-0.92)</td>
<td>0.49</td>
<td>.299</td>
<td>0.91 (0.77-1.08)</td>
</tr>
<tr>
<td>rs10792258</td>
<td>T</td>
<td>Hollingworth et al, 2011</td>
<td>0.39</td>
<td>0.0000001</td>
<td>1.31</td>
<td>(1.27-1.55)</td>
<td>0.37</td>
<td>.010</td>
<td>0.79 (0.66-0.95)</td>
<td></td>
</tr>
<tr>
<td>CD33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3752246</td>
<td>G</td>
<td>Naj et al, 2011</td>
<td>0.19</td>
<td>0.15</td>
<td>0.0012</td>
<td>1.31</td>
<td>(1.09-1.59)</td>
<td>0.04</td>
<td>.379</td>
<td>0.82 (0.53-1.27)</td>
</tr>
<tr>
<td>rs3764650</td>
<td>G</td>
<td>Hollingworth et al, 2011</td>
<td>0.11</td>
<td>0.0000001</td>
<td>1.31</td>
<td>(1.27-1.55)</td>
<td>0.04</td>
<td>.0087</td>
<td>1.32 (1.07-1.63)</td>
<td></td>
</tr>
<tr>
<td>rs3764647</td>
<td>G</td>
<td>Naj et al, 2011</td>
<td>0.32</td>
<td>0.0000001</td>
<td>1.31</td>
<td>(1.27-1.55)</td>
<td>0.37</td>
<td>.010</td>
<td>0.79 (0.66-0.95)</td>
<td></td>
</tr>
<tr>
<td>rs10419982</td>
<td>A</td>
<td>Naj et al, 2011</td>
<td>0.40</td>
<td>0.0000001</td>
<td>1.31</td>
<td>(1.27-1.55)</td>
<td>0.40</td>
<td>.00504</td>
<td>1.38 (1.15-1.65)</td>
<td></td>
</tr>
</tbody>
</table>

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.

a Effect estimate directions differ between whites and African Americans.

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

c This SNP was NPR and is located 15 kb from rs3851179.

d This SNP was NPR. This represents the lowest P value observed in PICALM.

e This SNP was NPR and flanks rs744375, 6.5 kb away from rs744373.

f This SNP was NPR. This represents the most significant P value observed in BIN1.

g This SNP was NPR and flanks rs11767557.

h This SNP was NPR and is located 15 kb from rs3851179.

i This SNP was NPR. This represents the smallest P value observed in the CD33 region.

j This SNP was NPR. This represents the smallest P value observed in the CNTNAP2 region.

k This SNP was NPR and flanks rs11767557.

l This SNP was NPR. This represents the smallest P value observed in ABCA7.

m This SNP was NPR. This represents the smallest P value observed in the CNTNAP2 region.

n This SNP was NPR and flanks rs11767557.

o This SNP was NPR. This represents the smallest P value observed in the CD33 region.

p This SNP was NPR and flanks rs11767557.

q This SNP was NPR. This represents the smallest P value observed in the ABCA7 region.

r This SNP was NPR. This represents the smallest P value observed in the CNTNAP2 region.

s This SNP was NPR and is located 15 kb from rs3851179.

Features of the African American Cohort

- 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.22,23 Except CR1 and CD2AP. Only one previously reported genome-wide significant association (rs3764650 in ABCA7) was confirmed in our African American sample.23 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 (e.g., rs17148827 in PICALM), and one of the previously reported AD-associated CLU SNPs (rs2279990), but with an opposite pattern of effect. Alternatively, the AD risk variants in these genes may differ across populations (i.e., allelic heterogeneity), as we observed previously in SORL1.40 However, lack of replication might also be a result of small sample size when compared with recent consortium-based GWASs.19-23 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.37,40 This transcription factor has recently been shown to play a key role in adult neurogenesis, suggesting it may be involved in memory development.49 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.22,23 In 2009, Harold et al22 reported a SNP in the contactin gene, CNTN5 (OMIM *607219), to have a GWAS P value of $10^{-5}$. Subsequently, the same SNP was shown to be associated with a variety of magnetic resonance imaging measures in the Alzheimer Disease Neuroimaging Initiative cohort.49 Serine/threonine kinase 24 (STK24 [OMIM *604984]) is ex-
pressed in the brain.33,36 An isoform of STK24 has been shown to be a regulator of axon growth and axon regeneration after injury.57,58 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.

Accepted for Publication: March 14, 2011.

Author Affiliations: Departments of Medicine (Biomedical Genetics) (Drs Logue, Green, Baldwin, and Farrer; Messrs Schu and Vardarajan; and Ms Buros), Neurology (Drs Green and Farrer), Ophthalmology (Dr Farrer), and Genetics and Genomics (Dr Farrer), and Center for Human Genetics (Dr Baldwin), Boston University School of Medicine and Public Health, and Departments of Epidemiology (Drs Green and Farrer) and Biostatistics (Drs Logue, Cupples, Lunetta, and Farrer), Boston University School of Public Health, Boston, Massachusetts; Department of Epidemiology and International Health, University of Alabama–Birmingham School of Public Health (Dr Go); Department of Medicine, Morehouse School of Medicine, Atlanta, Georgia (Dr Griffith); Department of Medicine, Howard University, Washington, DC (Dr Obisesan); Department of Neurology, Henry Ford Hospital, Detroit, Michigan (Dr Shatz); Department of Epidemiology and Biostatistics, University of South Florida, Tampa (Dr Borenstein); and Department of Epidemiology, Johns Hopkins University School of Public Health, Baltimore, Maryland (Dr Fallin).

Correspondence: Lindsay A. Farrer, PhD, Department of Medicine, Boston University School of Medicine, Biomedical Genetics L320, 72 East Concord St, Boston, MA 02118 (farrer@bu.edu).

Author Contributions: Study concept and design: Logue, Griffith, Obisesan, Shatz, Borenstein, Cupples, Lunetta, Fallin, Baldwin, and Farrer. Acquisition of data: Green, Go, Obisesan, Shatz, Borenstein, Fallin, Baldwin, and Farrer. Analysis and interpretation of data: Logue, Schu, Vardarajan, Buros, Green, Go, Cupples, Lunetta, and Farrer. Drafting of the manuscript: Logue, Schu, Buros, Green, Baldwin, and Farrer. Critical revision of the manuscript for important intellectual content: Logue, Vardarajan, Go, Griffith, Obisesan, Shatz, Borenstein, Cupples, Lunetta, Fallin, Baldwin, and Farrer. Statistical analysis: Logue, Schu, Vardarajan, Buros, Green, Cupples, Lunetta, Fallin, Baldwin, and Farrer.

Statistical Significance, –log10 (p)

<table>
<thead>
<tr>
<th>CHR</th>
<th>Position, dbSNP Build 129, bp</th>
<th>Gene</th>
<th>SNP</th>
<th>P Value</th>
<th>Effect Allele</th>
<th>AF</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>212184713</td>
<td>–</td>
<td>rs340849</td>
<td>7.52 × 10⁻⁶</td>
<td>A</td>
<td>0.20</td>
<td>0.59 (0.47-0.75)</td>
</tr>
<tr>
<td>2</td>
<td>17291066</td>
<td>–</td>
<td>rs1189338</td>
<td>8.94 × 10⁻⁶</td>
<td>T</td>
<td>0.26</td>
<td>1.55 (1.28-1.88)</td>
</tr>
<tr>
<td>3</td>
<td>27760977</td>
<td>SLC4A1AP</td>
<td>rs7006206</td>
<td>2.30 × 10⁻⁶</td>
<td>G</td>
<td>0.10</td>
<td>2.05 (1.52-2.76)</td>
</tr>
<tr>
<td>4</td>
<td>28903564</td>
<td>–</td>
<td>rs2221154</td>
<td>2.58 × 10⁻⁶</td>
<td>T</td>
<td>0.19</td>
<td>0.57 (0.45-0.72)</td>
</tr>
<tr>
<td>7</td>
<td>14652836</td>
<td>POLN</td>
<td>rs1923775</td>
<td>5.61 × 10⁻⁶</td>
<td>T</td>
<td>0.25</td>
<td>1.60 (1.30-1.95)</td>
</tr>
<tr>
<td>11</td>
<td>12298868</td>
<td>–</td>
<td>rs956225</td>
<td>8.71 × 10⁻⁴</td>
<td>T</td>
<td>0.03</td>
<td>0.30 (0.18-0.51)</td>
</tr>
<tr>
<td>11</td>
<td>73710714</td>
<td>–</td>
<td>rs3889908</td>
<td>9.52 × 10⁻⁴</td>
<td>A</td>
<td>0.15</td>
<td>1.72 (1.36-2.20)</td>
</tr>
<tr>
<td>11</td>
<td>11368477</td>
<td>–</td>
<td>rs1085408</td>
<td>9.25 × 10⁻²</td>
<td>T</td>
<td>0.34</td>
<td>0.63 (0.52-0.76)</td>
</tr>
<tr>
<td>12</td>
<td>2562228</td>
<td>–</td>
<td>rs17511267</td>
<td>5.01 × 10⁻⁴</td>
<td>C</td>
<td>0.17</td>
<td>1.75 (1.37-2.22)</td>
</tr>
<tr>
<td>13</td>
<td>97929295</td>
<td>STK24</td>
<td>rs912330</td>
<td>3.79 × 10⁻⁴</td>
<td>C</td>
<td>0.14</td>
<td>0.54 (0.41-0.70)</td>
</tr>
</tbody>
</table>

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

Other SNPs of Interest From APoe e4 Adjusted Analysis

<table>
<thead>
<tr>
<th>CHR</th>
<th>Position, dbSNP Build 129, bp</th>
<th>Gene</th>
<th>SNP</th>
<th>P Value</th>
<th>Effect Allele</th>
<th>AF</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>144692178</td>
<td>ZC3H3</td>
<td>rs3750208</td>
<td>7.28 × 10⁻⁴</td>
<td>A</td>
<td>0.04</td>
<td>0.37 (0.24-0.57)</td>
</tr>
<tr>
<td>12</td>
<td>29812934</td>
<td>TMTCT1</td>
<td>rs302318</td>
<td>1.97 × 10⁻⁴</td>
<td>C</td>
<td>0.26</td>
<td>0.59 (0.48-0.74)</td>
</tr>
<tr>
<td>13</td>
<td>43064019</td>
<td>ENOX1</td>
<td>rs17460623</td>
<td>9.37 × 10⁻⁴</td>
<td>C</td>
<td>0.10</td>
<td>0.49 (0.36-0.67)</td>
</tr>
</tbody>
</table>

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; TMTCT1, transmembrane and tetracontapeptide repeat containing 1; ZC3H3, zinc finger CCCH-type containing 3; –, indicates that the SNP is more than 50 kilobases from the nearest characterized gene.

a Does not include multiple SNPs on CHR 1 that were redundant owing to strong linkage disequilibrium (pairwise R² > 0.8) and CHR 3 (R² > 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.
lin, and Farrer. Obtained funding: Go, Shatz, Borenstein, and Farrer. Administrative, technical, and material support: Schu, Buros, Green, Obisesan, Borenstein, Baldwin, and Farrer. Study supervision: Logue, Obisesan, Borenstein, Lunetta, Fallin, Baldwin, and Farrer.

Group Members: Members of the MIRAGE Study Group include Drs. Farrer, Green, Baldwin, Cupples, Lunetta, and Logue (Boston University); Dr. Griffith, Abimbola Akomolafe, MD, MPH, Angela Ashley, MD, Lorin Freedman, MD, and Elizabeth Ofili, MD (Morehouse School of Medicine); Helena Chui, MD (University of Southern California, Los Angeles); Ranjan Duara, MD (Mt Sinai Medical Center, Miami, Florida); Tatiana Foroud, PhD, and Martin Farlow, MD (Indiana University School of Medicine, Indianapolis); Robert Friedland, MD (University of Louisville, Louisville, Kentucky); Drs. Go and Linda Harrell, MD, PhD (University of Alabama–Birmingham); Alexander Kurz, MD (Technical University, Munich, Germany); Dr Obisesan (Howard University); Helen Petrovitch, MD, and Lon White, MD (Pacific Health Research Institute, Honolulu, Hawaii); Marwan Sabbagh, MD (Sun Health Research Institute, Sun City, Arizona); Dessa Sadavnick, PhD (University of British Columbia, Vancouver); and Magda Tsoi, MD (University of Aristotle, Thessaloniki, Greece).

Financial Disclosure: None reported.

Funding/Support: This study was supported by grants R01 AG09029, R01 AG022539, R01 HG02213, R01 HG005092, 5R01 AG020688, K24 AG027841, P30 AG13846, P30 AG10129, and K01 MH076100 from the National Institutes of Health.

Online-Only Material: The eTables, eMethods, and eAppendix are available at http://www.bumc.bu.edu/genetics/results/aaa_alzheimer.

Additional Contributions: The GeneralAotions Study Group provided input in design, implementation, and consensus determination. Additional contributions, including Hugh Hendrie, MD, and Fred Unverzagt, PhD (Indiana University), were provided project coordination.

REFERENCES

33. Splansky GL, Corey D, Yang Q, et al.; The Third Generation Cohort of the Na-