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Genetic variants associated with risk of Alzheimer's disease contribute to cognitive change in midlife: The Atherosclerosis Risk in Communities Study

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Abstract

Alzheimer's disease (AD) is the most common form of dementia and is characterized by impairment in memory, behavioral changes, and gradual loss of autonomy. Since there is a long latent period prior to diagnosis, the aim of this study was to determine whether twenty single nucleotide polymorphisms identified in genome-wide association analyses of AD are associated with cognitive change in 8,320 white and 2,039 African-American middle-aged adults enrolled in the prospective Atherosclerosis Risk in Communities (ARIC) study. Cognition was evaluated using the Delayed Word Recall Test (DWRT; verbal memory), Digit Symbol Substitution Test (DSST; processing speed), and Word Fluency Test (WFT; executive function). General linear models were used to assess mean differences in 6-year change in test scores among individuals categorized by genotype after adjusting for age, gender, and years of education. Addition of the minor allele for rs670139 (*MS4A4E*), rs9331896 (*CLU*), and rs12155159 (*NME8*) was nominally associated with change on the DWRT, DSST, and WFT, respectively, in whites. The *ZCWPW1* (rs1476679) and *CDS33* (rs3865444) variants were nominally associated with change on the DWRT and WFT in African-Americans. For rs670139 and rs9331896 the association was only significant in individuals bearing at least one *APOE* ϵ 4 allele in stratified analyses. An unweighted

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CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

genetic risk score aggregating the risk alleles for 15 polymorphisms was not associated with change in cognitive function. Although the AD-associated genetic variants appear to have small effects on early cognitive change, replication will be required to establish whether there is a discernible influence on cognitive status in midlife.

Keywords

Genetic epidemiology; Cognition; Alzheimer's disease; Association study

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia [Alzheimer's Association 2015] and is characterized by significant impairment in memory, behavioral changes, and gradual loss of autonomy. Despite relatively high heritability estimates [Gatz et al. 2006], until recently the only genetic variant shown to reproducibly confer increased risk of common late-onset AD was the apolipoprotein E (*APOE*) $\epsilon 4$ allele [Corder et al. 1993; Strittmatter et al. 1993]. The current availability of high-density genotyping arrays and the haplotype map of the human genome generated by the International HapMap Project [International HapMap Consortium 2005] has facilitated genome-wide association studies (GWAS) of the relationships between large numbers of single nucleotide polymorphisms (SNPs) measured simultaneously and risk for common diseases to identify novel genes or loci influencing a given phenotype. Twenty novel loci for late-onset AD have recently been identified in six large-scale GWAS [Harold et al. 2009; Hollingworth et al. 2011; Lambert et al. 2009; Lambert et al. 2013; Naj et al. 2011; Seshadri et al. 2010].

Low or declining scores on cognitive tests, particularly those that assess episodic memory, have been found to predict the later development of AD in epidemiological studies [Backman et al. 2005; Elias et al. 2000; Rubin et al. 1998; Small et al. 2000; Tierney et al. 2005]. For example, a preclinical phase of reduced cognitive functioning in dementia-free individuals as assessed by a battery of neuropsychological tests was shown to precede the appearance of probable AD by more than 20 years in a large prospective study carried out in the Framingham Cohort [Elias et al. 2000]. Similarly, when the rate of cognitive decline was compared in Swedish participants in the Kungsholmen Project who developed Alzheimer's disease and those who remained unimpaired, there was an accelerated period of change observed prior to diagnosis based on a global screening test of cognitive status [Small et al. 2000]. Although there have been several previous investigations of the association between individual AD loci and cognitive function [Carrasquillo et al. 2015; Chibnik et al. 2011; Hamilton et al. 2011; Marden et al. 2016; Mengel-From et al. 2011; Pedraza et al. 2014; Sweet et al. 2012; Thambisetty et al. 2013; Verhaaren et al. 2013; Vivot et al. 2015], few have been carried out in populations of non-European descent [Pedraza et al. 2014] [Marden et al. 2016] or have examined whether the variants that mediate dementia risk at older ages already exert their effects in midlife before diagnostic criteria for AD are met [Marden et al. 2016; Verhaaren et al. 2013]. The aim of this study was to determine whether the twenty SNPs identified in GWAS of AD were also associated with baseline cognitive function and cognitive change in middle age in the large biracial prospective Atherosclerosis Risk in

Communities (ARIC) cohort study. The cognitive battery used in the ARIC study was originally selected to sample cognitive domains involved in later life neurodegenerative and cerebrovascular diseases, and includes three tests (Delayed Word Recall Test (verbal memory); Digit Symbol Substitution Test (processing speed); and the Word Fluency Test (executive function) that were administered to all participants at the second (1990-1992) and fourth (1996-1998) clinical examinations.

MATERIALS AND METHODS

The Atherosclerosis Risk in Communities Study

The ARIC Study is a prospective longitudinal investigation of the development of atherosclerosis and its clinical sequelae in which 15,792 individuals aged 45 to 64 years were enrolled at baseline. A detailed description of the ARIC study has been reported previously [ARIC investigators 1989]. At the inception of the study in 1987-1989, participants were selected by probability sampling from four communities in the United States: Forsyth County, North Carolina; Jackson, Mississippi (African-Americans only); the suburbs of Minneapolis, Minnesota; and Washington County, Maryland. Four examinations were carried out at three-year intervals (exam 1, 1987-1989; exam 2, 1990-1992; exam 3, 1993-1995; exam 4, 1996-1998). Subjects were contacted annually to update their medical histories between examinations. Cognitive testing was performed at exams 2 and 4. Individuals were not included in this analysis if they were neither African-American nor white ($n = 48$), were African-Americans from the Minnesota or Maryland field centers due to the small numbers of individuals recruited from these sites ($n = 55$), restricted use of their DNA ($n = 45$), had a history of physician-diagnosed stroke ($n = 369$) or unknown history of stroke ($n = 31$), or did not attend the second ($n = 1,431$) or fourth ($n = 2,650$) clinical examination. Additional exclusions were made for missing all genotype data ($n = 34$), missing cognitive data for any of the three neuropsychological tests at either visit 2 ($n = 182$) or visit 4 ($n = 299$), having test scores out of range ($n = 2$), or for missing information concerning the highest level of education completed ($n = 19$) or other covariates used in the regression models ($n = 268$). The final study sample consisted of 8,320 white and 2,039 African-American men and women. Written informed consent was provided by all study participants, and the study design and methods were approved by institutional review boards at the collaborating medical institutions. The research with human subjects was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Cognitive Tests

Cognitive function was assessed by three neuropsychological tests at the second and fourth clinical examinations and have been described previously [Cerhan et al. 1998]: 1) The Delayed Word Recall Test (DWRT) is a measure of verbal learning and recent memory in which the participant is required to use each of 10 common nouns in a sentence. After a 5-minute delay (during which another non-verbal test is given), the participant is asked to recall the 10 nouns. The DWRT score is the number of correct words recalled (range 0 – 10) [Knopman and Ryberg 1989]; 2) The Digit Symbol Substitution Test (DSST) is a subtest of the Wechsler Adult Intelligence Scale-Revised that tests processing speed and requires timed

translation of numbers to symbols using a key with paired symbols and digits. The total number of correct translations within 90 seconds determines the score (range 0 – 93) [Wechsler 1981]; and 3) The Word Fluency Test (WFT) is a measure of executive function. In three separate 1-minute trials, the subject is asked to generate as many words as possible beginning with the letters F, A, and S. The score is the combined total of correct words produced [Lezak 1995; Tombaugh et al. 1999]. The tests were administered by trained interviewers in a standardized order and were given in a single session. For all of the neuropsychological tests, lower scores indicate a lower measure of cognition. Six-year change in cognitive function was analyzed as the difference between the test score obtained at the later clinic visit and the test score obtained at the earlier visit for each neuropsychological test.

Clinical and Laboratory Measurements

The clinical and laboratory measurements for this study were assessed during the second clinical examination with the exception of education which was evaluated at the baseline examination and have been described previously [Bressler et al. 2015]. Education was included as a covariate in regression models as an ordinal variable based on the highest level attained (< 11 years; 11 -16 years; > 16 years). Plasma total cholesterol and triglycerides were measured by enzymatic methods [Nagele et al. 1984; Siedel et al. 1983], and low density lipoprotein (LDL) cholesterol was calculated [Friedewald et al. 1972]. Hypercholesterolemia was defined as LDL cholesterol ≥ 130 mg/dL (3.36 mmol/L) [Adult Treatment Panel III 2002]. High density lipoprotein (HDL) cholesterol was measured after dextran-magnesium precipitation of non-HDL [Warnick et al. 1982]. Blood pressure was measured three times while seated using a random-zero sphygmomanometer and the last two measurements were averaged for analysis. Hypertension was defined by diastolic blood pressure of ≥ 90 mm Hg, systolic blood pressure of ≥ 140 mm Hg, or use of antihypertensive medication. Fasting serum glucose was measured by a standard hexokinase method on a Coulter DACOS chemistry analyzer (Coulter Instruments, Fullerton, CA, USA). The prevalence of diabetes was defined using a fasting glucose level ≥ 7.0 mmol/L, a nonfasting glucose level ≥ 11.1 mmol/L, and/or self-reported physician diagnosis or treatment for diabetes. Body weight and other anthropometric variables were measured by trained technicians according to standardized protocols. Body mass index (BMI) was calculated as weight in kilograms/(height in meters)². Information on cigarette smoking and alcohol consumption was obtained using an interviewer-administered questionnaire and was classified as current, former, or never.

Genotyping of AD GWAS variants

In addition to the *APOE* ε2/ε3/ε4 polymorphism [Weisgraber et al. 1981], twenty genetic variants identified in GWAS of AD [Hollingsworth et al. 2011; Lambert et al. 2009; Lambert et al. 2013; Naj et al. 2011] were examined in this study. Genotyping of the *BIN1* (rs744373) [Seshadri et al. 2010] and *APOE* polymorphisms was performed using the TaqMan system (Applied Biosystems, Foster City, CA, USA). The sequences for primers and probes are available upon request. Since the *APOE* ε2/ε3/ε4 polymorphism is based on genetic variation at codons 112 (rs429358) and 158 (rs7412), two separate TaqMan assays were performed. The data for these two SNPs were combined to generate the six *APOE*

genotypes. Allele detection was performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). The genotype call rate, or the percentage of samples to which a genotype was assigned, was determined prior to exclusion of individuals from the analysis and was 95.6 % for rs744373 and 92.3% for *APOE*. After the application of all exclusion criteria, the proportion of missing data for the *BIN1* SNP was 4.2% and was 6.6% for *APOE*.

The genotyping data for 15 of the remaining polymorphisms (see Table 1) were extracted from a genome-wide scan performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA) at the Broad Institute of MIT and Harvard, Cambridge, MA as previously described [Levy et al. 2009]. The quality control criteria for exclusion of individual SNPs were if no chromosomal location could be determined, if a SNP was monomorphic in both whites and African-Americans, if the call rate was < 95%, or the Hardy-Weinberg equilibrium p -value was < 10^{-5} . In addition, scans for study participants were removed from the analysis if the subject had not consented to use of their DNA, if there was genotype mismatch with 39 previously genotyped SNPs, if there was discordance between phenotypic sex and X-chromosome analysis, if the individual appeared to be a genetic outlier based on average identity by state statistics and principal components analysis using EIGENSTRAT [Price et al. 2006], or if there was suspected first-degree relationship with any other participant. After the application of all exclusion criteria, the study population included 9,345 white and 3,182 African-Americans. Imputation to approximately 2.5 million autosomal SNPs identified in HapMap Phase II CEU samples (release 22, NCBI build 36), or to 2.74×10^6 SNPs in a combined HapMap Phase II CEU and YRI reference panel (release 21, build 35) [Lettre et al. 2011] was performed using MACH v1.0.16 (<http://www.sph.umich.edu/csg/abecasis/MaCH/>) for whites and African-Americans, respectively. A threshold for R_{sq} , an estimate of the correlation between imputed and true genotypes, was set at 0.3 to filter imputation results.

The reported allele frequencies for the polymorphisms located near *CD2AP*, *CRI*, *MS4A4E*, *PICALM*, and *PKT2B* were calculated from measured genotyping data, while gene dosage for all other variants was imputed. Four additional SNPs (rs4147929 (*ABCA7*), rs35349669 (*INPP5D*), rs9271174 (*HLA-DRB5-HLA-DRB1*), and rs7274581 (*CASS4*) [Lambert et al. 2013] were derived after the genotyping data was imputed to the 1000 Genomes reference panel Phase I integrated variant set release (v3) haplotypes in NCBI build 37 (hg19). R_{sq} for all imputed SNPs was > 0.75 except for rs11767557 (*EPHA1*) (R_{sq} white = 0.63, R_{sq} African-American = 0.59) and rs9331896 (*CLU*) (R_{sq} white = 0.68). When the index AD GWAS SNP (rs10838725 (*CELF1*), rs28834970 (*PTK2B*), rs2718058 (*NME8*), and rs9271192 (*HLA-DRB5-HLA-DRB1*) [Lambert et al. 2013] was not available, a proxy SNP was selected at each locus ($r^2 > 0.7$ in the 1000 Genomes Project CEU) using the annotation tool HaploReg version 3 [Ward and Kellis 2012].

Statistical Analysis

All statistical analyses were performed using Stata 9 software (StataCorp, College Station, TX, USA). Hardy-Weinberg equilibrium was tested using a χ^2 goodness-of-fit test for individuals in each racial group. The proportions, means, and standard deviations were

calculated for cardiovascular risk factors for individuals categorized by self-reported race. Comparisons were performed using chi square tests for categorical variables and t-tests for continuous variables. General linear models were used to assess mean differences in cognitive function at baseline or 6-year cognitive change among groups of individuals categorized by genotype for each SNP assuming an additive genetic model and adjusting for age, gender, and years of education (model 1), or for age, gender, education and potential confounding variables including BMI, current smoking, current alcohol intake, diabetes, hypertension, and hypercholesterolemia (model 2) [Cerhan et al. 1998]. All variables except for education which was assessed at visit 1 were measured at the visit 2 examination. Analyses of rs7274581 were only carried out for African-American study participants since this variant did not meet Hardy-Weinberg equilibrium expectations in whites. Tests of interaction between the AD GWAS variants and carriage of at least one *APOE* ε4 allele were conducted by including the main effects for each SNP and *APOE* ε4 carrier status in the general linear models, and introducing a multiplicative 2-way interaction term. In a secondary analysis, an unweighted genetic risk score was constructed for each individual by adding the number of risk alleles for each of 15 index SNPs identified in GWAS of AD for which the effect allele and direction of effect were known and that were in Hardy-Weinberg equilibrium in both whites and African-Americans in order to evaluate the loci in aggregate in the general linear models. The SNPs were coded by assigning 1 to the risk-raising homozygous genotype, 0 to the heterozygote, and -1 to the risk-lowering homozygous genotype. The SNPs were not weighted by their effect sizes since the variants comprising the score were drawn from different meta-analyses [Hollingworth et al. 2011; Lambert et al. 2009; Lambert et al. 2013; Naj et al. 2011; Seshadri et al. 2010]. The results of all statistical analyses are reported separately by racial group. In addition, the summary statistics for the race-specific analyses were combined by performing inverse-variance meta-analyses using the program METAL [Willer et al. 2010]. A two-sided *p*-value of < 0.05 was considered statistically significant, and the Bonferroni correction was used to adjust for multiple comparisons ($0.05/20 \text{ variants} \times \text{scores for 3 cognitive tests} \times \text{change in scores for 3 cognitive tests} \times 2 \text{ racial groups} = 0.00014$).

RESULTS

The allele and genotype frequencies for twenty polymorphisms identified in GWAS of AD, shown in Table I, were in accordance with Hardy-Weinberg expectations for both white and African-American study subjects ($p > 0.05$) with the exception of rs7274581 (*CASS4*) in whites. Since the minor allele frequency for all of the polymorphisms examined with the exception of rs670139 differed significantly when the two racial groups were compared, all results are reported separately by race. The clinical and demographic characteristics of the study population, mean cognitive test scores, and mean 6-year change in cognitive test scores are presented in Table II. There were higher percentages of males, current alcohol drinkers, and individuals with 11-16 years of education, and lower percentages of current smokers and individuals with hypertension, diabetes, or obesity among white study participants than among African-American participants. White participants were also older with lower LDL and HDL cholesterol, and a higher level of triglycerides. The mean age for

white and African-American study participants was 57.0 ± 5.6 years and 55.8 ± 5.7 years, respectively, at the time of the baseline cognitive examination.

The results of the primary analysis of the association between mean 6-year change in three domain-specific cognitive test scores (DWRT, DSST, and WFT) and each of twenty AD-associated SNPs is shown for whites in Table III and for African-Americans in Table IV. The genotypes for each SNP examined were coded with respect to the minor allele identified in the GWAS of AD so that the direction of effect could be easily compared. After adjustment for age, gender, and years of education, addition of the minor allele for rs670139 (*MS4A4E*), rs9331896 (*CLU*), and rs12155159 (*NME8*) was significantly associated with less cognitive decline on the DWRT ($p = 0.026$), DSST ($p = 0.053$), and WFT ($p = 0.039$), respectively, in white participants. In African-American participants, rs1476679 (*ZCWPW1*) was significantly associated with greater decline in scores on the DWRT ($p = 0.030$), while rs38655444 (*CD33*) was associated with less decline on the WFT ($p = 0.045$). While most of these associations were largely independent of demographic and biological factors that can influence cognition including BMI, diabetes, hypertension, current smoking, current alcohol intake, and high midlife LDL cholesterol when these were added to the regression models, the association between addition of the rs9331896 C allele and performance on the DSST in whites was no longer nominally significant (p -value minimally adjusted model = 0.053, p -value fully adjusted model = 0.065) (Tables SI and SII). When *APOE* $\epsilon 4$ carrier status was examined, having at least one *APOE* $\epsilon 4$ allele was significantly associated with greater cognitive decline as measured by the DWRT and DSST in whites, and the DWRT in African-Americans in both the minimally and fully adjusted models.

In a secondary analysis, the relationship between 6-year change in cognitive test scores and the five SNPs for which the association was found to be significant in the primary analysis was further examined after stratifying by *APOE* $\epsilon 4$ carrier status. While interaction between the *MS4A4E* rs670139 variant and *APOE* had not previously been described [Hollingworth et al. 2011], the association was only significant in those individuals bearing at least one $\epsilon 4$ allele for rs670139 and rs9331896 in whites in stratified analyses (Tables SIII and SIV). The five AD GWAS variants were then further tested for multiplicative interaction with *APOE* $\epsilon 4$ carrier status in regression models where the appropriate change in test score was evaluated as the outcome variable; no significant interaction between *APOE* $\epsilon 4$ and any of the AD GWAS SNPs was found (whites: DWRT, p interaction rs670139 = 0.307; DSST, p interaction rs9331896 = 0.068; WFT, p interaction rs12155159 = 0.480; African-Americans: DWRT, p interaction rs1476679 = 0.815; WFT, p interaction rs38655444 = 0.750).

The relationship between the AD GWAS variants including carriage of the *APOE* $\epsilon 4$ allele and baseline cognitive function was also evaluated after adjustment for age, gender, and years of education. Five of the AD GWAS SNPs were significantly associated with scores for at least one of the cognitive tests in whites (rs4147929 (*ABCA7*): DWRT $p = 0.013$; rs6485758 (*CELF1*): DWRT $p = 0.042$, DSST $p = 0.011$, and WFT $p = 0.002$; rs9331896 (*CLU*): DWRT $p = 0.047$; rs17125944 (*FERMT2*): WFT $p = 0.001$; rs35349669 (*INPP5D*): WFT $p = 0.010$), and two were significantly associated with cognitive performance in African-Americans (rs3865444 (*CD33*): WFT $p = 0.037$; rs6656401 (*CRI*): DSST $p = 0.029$) (Tables V and VI). Only rs38655444 (*CD33*) was nominally significantly associated

with both baseline performance and 6-year change in scores on the same test among African-Americans (p -value 6-year WFT score change = 0.045; p -value WFT baseline score = 0.037), while rs9331896 (*CLU*) was associated with six-year change in DSST scores but with DWRT scores at baseline for whites. When cognitive function at baseline was analyzed further using the fully adjusted regression model, all seven of the associations identified using the minimally adjusted model remained nominally significant, and an additional association between rs190982 (*MEF2C*) and decreased baseline DSST scores was detected in whites ($p = 0.042$) (Tables SV and SVI).

Meta-analyses with increased sample sizes achieved by combining the race-specific results for both 6-year cognitive change and baseline cognitive function were carried out. In the meta-analysis for 6-year cognitive change, one nominally significant association with less decline on the DSST was identified for *CLU* rs9331896 using both the minimally and fully adjusted models (p -value minimally adjusted model = 0.035, p -value fully adjusted model = 0.044) (Tables SVII and SIX). The meta-analysis for baseline cognitive function revealed seven nominal associations with the AD GWAS SNPs after adjustment for age, gender, and years of education (rs4147929 (*ABCA7*): DWRT $p = 0.011$; rs6485758 (*CELF1*): DSST $p = 0.008$, WFT $p = 0.002$; rs9331896 (*CLU*): DWRT $p = 0.029$; rs17125944 (*FERMT2*): WFT: $p = 0.001$; rs35349669 (*INPP5D*): WFT $p = 0.004$; rs10498633 (*SLC24A4*): WFT: $p = 0.051$) (Table SVIII), and these were essentially similar after further adjustment for risk factors related to cognition (Table SX).

A genetic risk score was calculated by combining the number of risk alleles for each of the 15 loci for which the AD risk allele was known from the GWAS to examine the effect of the genetic variants in aggregate on cognitive function. In this exploratory analysis, no association between the genetic risk score and either baseline cognitive status or 6-year score change for any of the cognitive tests was observed (Tables III, IV, V, and VI).

None of the associations described above remained significant after correction for multiple comparisons when the number of genetic variants and phenotypes tested in the two racial groups were considered ($p < 0.00014$).

DISCUSSION

Neurocognitive test scores and six-year change in scores were evaluated in the ARIC study as a possible endophenotype for the loss of short-term memory and reduced executive function characteristic of AD. Information processing speed is also known to decline with age [Der and Deary 2006; Salthouse 1996], and low scores on the DSST have been associated with both incident mild cognitive impairment (MCI) and dementia [Lopez et al. 2003a; Twamley et al. 2006]. Because there is no biomarker that can be used to confirm the clinical diagnosis and there is often a mixture of vascular and AD pathology found on autopsy [Fernando et al. 2004; Lopez et al. 2003b; Schneider et al. 2007], genetic studies of AD based on case status may be complicated by substantial phenotypic heterogeneity. Moreover, the long latent period prior to diagnosis can result in misclassification of controls who may later develop dementia [Elias et al. 2000; Snowdon et al. 1997; Sperling et al. 2011], and lead to survivor bias where individuals who would have been classified as cases

have died from other causes. Accordingly, genetic analysis of heritable quantitative endophenotypes such as cognitive function that are often considered as intermediates in the causal chain leading from risk allele to clinical disease may help to uncover loci that increase or reduce susceptibility to dementia in its preclinical phase [Bennett et al. 2009; Carmelli et al. 2002; Gottesman and Gould 2003; McClearn et al. 1997; Shulman et al. 2010; Swan et al. 1990].

Significant associations between variants in or near three different genes identified in GWAS of late-onset AD and mean change in scores on tests of cognitive function over a six-year period were found for white middle-aged participants in the ARIC study (*MS4A4E*, *CLU*, and *NME8*), and two variants were identified in African-Americans (*ZCWPW1* and *CD33*) in the primary analysis after adjustment for age, gender, and education. Examination of the association of the AD GWAS variants and baseline cognitive function also revealed significant associations with seven of the polymorphisms (*ABCA7*, *CELF1*, *CLU*, *FERMT2*, and *INPP5D* in whites, and *CR1* and *CD33* in African-Americans). For both 6-year cognitive change and cognitive performance at baseline, there was no overlap between the significantly associated variants identified in whites and those detected in African-Americans. In addition, only one of the SNPs (rs6485758 (*CELF1*)) was associated with higher scores on all three cognitive tests at baseline in whites while none of the variants appeared to influence more than one cognitive domain in African-Americans. A meta-analysis combining race-specific results for 6-year cognitive change revealed an association between *CLU*rs9331896 and performance on the DSST; genetic variants in *ABCA7*, *CELF1*, *CLU*, *FERMT2*, *INPP5D*, and *SLC24A4* were found to be possible determinants of baseline cognitive function in a second meta-analysis. However, none of the associations observed between cognitive function and the AD GWAS loci remained significant after Bonferroni correction for multiple comparisons.

Unexpectedly, the association detected for the sequence variant nearest to *MS4A4E* (rs670139) and a test of delayed word recall in whites was in the opposite direction from expectation based on the association with AD in the GWAS. In these reports, the *MS4A4E* SNP was a risk allele for AD [Hollingworth et al. 2011; Naj et al. 2011] while there was an association with less cognitive decline for subjects in the ARIC cohort. *MS4A4E*, a gene with no previously identified function, is part of a gene cluster whose members have a common genomic structure including a transmembrane domain found in cell surface proteins [Liang et al. 2001], and has been associated with Braak tangle and plaque scores in the parietal lobe of AD cases and cognitively normal controls [Karch et al. 2012]. Since cognitive function was assessed in the ARIC study in midlife in individuals without dementia, it is possible that the relationship between the *MS4A4E* SNP and AD, and the polymorphism and cognitive change may actually be different. In this view, this region may harbor susceptibility loci for clinical features of AD, or its risk factors, but is protective for early change in memory. Another possibility is that since the cognitive tests were administered to middle-aged adults whereas only 4% of those with AD are estimated to be younger than 65 years of age [Hebert et al. 2003], the relationship between rs670139 and cognitive function in particular may change over the life course so that *MS4A4E* is beneficial in early life and has different effects at older ages. This could potentially occur in response to a specific environmental exposure, or in the context of expression of other genes

such as the *APOE* ϵ 4 allele as suggested by the results of the stratified analysis for rs670139. Alternatively, the *MS4A4E* variant may be associated with an aspect of cognitive function in AD patients that is not captured by the DWRT. It also cannot be excluded that the apparent reversal in the direction of associations between the SNP near *MS4A4E* and change in scores for the DWRT may be due to residual confounding or to chance. Similar considerations may apply to rs1476679 (*ZCWPW1*) and to rs3865444 (*CD33*) where addition of the minor allele was associated with greater 6-year decline on the DWRT and decreased scores at baseline on the WFT, respectively, in African-Americans but decreased susceptibility to AD [Lambert et al. 2013], and to rs4147929 (*ABCA7*) and rs6656401 (*CRI*) where addition of the minor allele was associated with higher scores in whites on the DWRT and in African-Americans on the DSST but with increased risk of AD.

The association between individual AD loci or a polygenic risk score constructed from these markers and cognitive function has been investigated previously, but only a few of these studies have included individuals who were of non-European descent [Marden et al. 2016; Pedraza et al. 2014] or assessed their impact in midlife [Marden et al. 2016; Verhaaren et al. 2013]. In the ARIC study, the addition of the minor allele for *CLU* rs9331896 was associated with less cognitive decline as assessed by the DSST and with higher scores at baseline for the DWRT in whites, and *CRI* rs6656401 was associated with higher DSST scores at baseline in African-Americans. The association between cognitive function and these two loci as well as *PICALM* was examined in 1,666 subjects enrolled in the Religious Orders Study (ROS) and in the Rush Memory and Aging Project (MAP). ROS and MAP are two longitudinal studies of non-demented individuals whose mean age at the baseline examination was 75.5 and 81.0 years, respectively. Only *CRI* rs6656401 was associated with a global measure of cognitive decline while genetic variants in *CLU* (rs11136000) and *PICALM* (rs7110631) were not found to predict cognitive change in these cohorts [Chibnik et al. 2011]. The *CLU* rs1113600 T allele is associated with reduced risk of AD [Harold et al. 2009; Lambert et al. 2009], and was associated with a higher composite cognitive score for both men and women 92-93 years of age at enrollment in a Danish birth cohort study, while the effect of variation in *PICALM* (rs3851179) [Harold et al. 2009] was restricted to males [Mengel-From et al. 2011]. The *CLU* rs11136000 C risk allele, was related to a more rapid trajectory of cognitive decline as measured by the 3MS global test of cognition in 1,831 subjects initially free of dementia in the Cardiovascular Health Study (mean age at baseline 71.7 years). *CRI* (rs3818361) [Hollingworth et al. 2011] was associated with more rapid decline on the DSST in the same study, while *PICALM* (rs3851179 and rs541458) [Harold et al. 2009] was associated with an earlier age at midpoint of decline of the 3MS [Sweet et al. 2012]. The *CLU* rs11136000 risk allele was also associated with faster decline in memory as measured by the California Verbal Learning Test of word list delayed recall in 95 cognitively normal participants in the Baltimore Longitudinal Study of Aging who converted to MCI or AD (mean age 75.9 years at first assessment [Thambisetty et al. 2013]. Finally, the association between genetic variants in *CLU* (rs11136000), *CRI* (rs6656401 and rs3818361), and *PICALM* (rs3851179) and several tests of memory was analyzed cross-sectionally in 2 groups of 268 African-Americans and 2,651 whites that included both AD cases and controls. This study provided nominally significant or suggestive evidence for an influence of *CRI* on immediate recall in African-Americans, and of *CLU* on paragraph

delayed recall in whites using the Logical Memory subtests from the Wechsler Memory Scale-Revised [Pedraza et al. 2014].

There have been several more recent reports in which the AD GWAS loci have been evaluated more comprehensively and either eight [Vivot et al. 2015] or nine [Carrasquillo et al. 2015] AD-associated loci were tested for association with cognitive decline, including *ABCA7*, *CD33*, *CLU*, *CR1*, and *MS4A4E* that were nominally associated with cognitive phenotypes in the ARIC study. In a study of more than 2,000 older white participants (median age at first assessment 77 years) who were assessed longitudinally for progression to MCI or AD the *CLU*rs11136000 risk allele was associated with lower scores at baseline on a test of paragraph delayed recall from the Wechsler Memory Scale-Revised Logical Memory subtest but not with 5-year decline in verbal memory while no associations were found for the other eight loci [Carrasquillo et al. 2015]. Only *BIN1* (rs744373) and *CR1* (rs3818361) out of the 8 non-*APOE* genetic polymorphisms examined were associated with rate of change over more than 10 years in global cognition and verbal fluency, respectively, in a study of 4,931 participants in the 3-C Dijon study (median age 74.0 years), and none were associated with decline in processing speed, visual memory, or premorbid mental abilities [Vivot et al. 2015]. In contrast, analyses of 158 SNPs in 11 genes involved in AD including *BIN1*, *CLU*, *CR1*, and *PICALM* showed no significant association with cognitive phenotypes such as a test of verbal fluency similar to the WFT in 505 participants from the Lothian Birth Cohort of 1921 (mean age 79.1 years) and 998 participants from the Lothian Birth Cohort of 1936 (mean age 69.6 years) [Hamilton et al. 2011]. A genetic risk score that included 10 variants detected in GWAS of AD but excluded *APOE* ϵ 4 was also marginally associated with a compound test score for memory in 5,171 non-demented persons from the population-based Rotterdam Study (mean age 66.2 years) [Verhaaren et al. 2013], and a genetic risk score constructed from 22 AD-associated loci was associated with significantly faster decline in a composite memory score in 7,172 non-Hispanic white (mean age 63.0 years) but not in 1,081 non-Hispanic black participants (mean age 61.6 years) in the nationally representative Health and Retirement Study [Marden et al. 2016].

As outlined above, multiple studies have implicated *CLU* in analyses of cognitive function providing increased plausibility for the findings reported here. In support of our observations, Pedraza et al. found that the *CLU*rs11136000 T allele associated with decreased risk of AD was associated with higher scores on the Logical Memory delayed paragraph recall subtest from the Wechsler Memory Scale-Revised in white study participants, while Carrasquillo et al. reported that the *CLU*rs11136000 C risk allele was associated with lower scores at baseline on the same test [Carrasquillo et al. 2015; Pedraza et al. 2014]. In the ARIC study, addition of the minor rs9331896 C allele associated with reduced risk of AD was associated with higher scores at baseline on the DWRT, a test of word list delayed recall in which a list of 10 unrelated words are presented visually to the participant. *CLU* encodes an apolipoprotein that is highly expressed in the brain, and is present in amyloid plaques and cerebrospinal fluid from individuals with AD [Calero et al. 2000; Liang et al. 2008; McGeer et al. 1992; Roheim et al. 1979]. Clusterin can bind reversibly to A β [Ghiso et al. 1993; Zlokovic et al. 1996], and has been implicated in A β clearance across the blood-brain barrier [Bell et al. 2007; DeMattos et al. 2004]. Plasma concentrations of clusterin have been reported to be associated with atrophy of the

entorhinal cortex and more rapid global cognitive decline in AD patients [Thambisetty et al. 2010], and were also negatively correlated with global cognitive function and with attention-processing speed as measured by the DSST in 664 participants in the Sydney Memory and Aging Study (mean age 78 years) [Song et al. 2012]. The nominal associations in whites between rs9331896 (*CLU*) and reduced risk of decline on the DSST as well as higher scores on a test of verbal memory at baseline that were also identified in the meta-analyses combining race-specific results may suggest that the rate of clearance of amyloid deposits is a physiological mechanism affecting cognitive status as early as middle age.

This study has a number of strengths including the prospective design and well-phenotyped study population in which individuals of both European and African ancestry are represented, the large sample size, the survey of 20 recently identified AD-associated loci, and the availability of longitudinal as well as cross-sectional measures of cognition acquired in midlife but there are also limitations. There was only one cognitive test used to assess each cognitive domain, and the tests were administered at a stage of life at which most individuals would not have experienced extensive cognitive decline particularly during the relatively short 6-year follow-up period. In addition, the common SNPs analyzed in this study may not be the causal variant even in individuals of European descent, and differences in linkage disequilibrium and allele frequency between whites and African-Americans could also result in variation in the ability to detect associations in non-European individuals as well as in the direction of effect. However, although there was no overlap with the variants identified in whites, there was nominal evidence for association of rs1476679 (*ZCWPW1*) with greater decline in verbal memory and of rs3865444 (*CD33*) with reduced decline in verbal fluency in the smaller sample of African-Americans. The variants tested in this study also had small effect sizes even in the large-scale meta-analyses in which they were discovered that may help to explain the absence of even nominal associations with cognition for ten of the AD GWAS SNPs examined in either racial group. Replication in additional population-based samples with participants drawn from a range of different age and ethnic groups will be required to confirm and extend these observations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Genetic variants identified in GWAS of AD – allele frequencies by race. ARIC Study (1990 – 1992)

Table 1

GWAS SNP dbSNP ID	Major/Minor Allele	Risk Allele	Chrom	Nearest Gene	AA MAF	White MAF	p*	Ref.
rs4147929	G/A	A	19	<i>ABCA7</i>	4.7	16.6	<0.001	[Lambert et al. 2013]
rs744373	A/G	G	2	<i>BIN1</i>	46.8	29.9	<0.001	[Seshadri et al. 2010]
rs7274581	T/C	T	20	<i>CASS4</i>	21.7	NA		[Lambert et al. 2013]
rs9349407	G/C	C	6	<i>CD2AP</i>	19.4	25.7	<0.001	[Naj et al. 2011]
rs3865444	C/A	C	19	<i>CD33</i>	7.4	32.6	<0.001	[Naj et al. 2011]
rs6485758	G/A	NA	11	<i>CELF1</i>	5.6	29.8	<0.001	[Lambert et al. 2013]
rs9331896	T/C	T	8	<i>CLU</i>	55.2	37.0	<0.001	[Lambert et al. 2013]
rs6656401	G/A	A	1	<i>CR1</i>	6.6	18.6	<0.001	[Lambert et al. 2009]
rs11767557	T/C	T	7	<i>EPHA1</i>	13.6	17.8	<0.001	[Naj et al. 2011]
rs17125944	T/C	C	14	<i>FERMT2</i>	5.1	9.1	<0.001	[Lambert et al. 2013]
rs9271174	G/C	NA	6	<i>HLA-DRB1</i>	21.7	27.1	<0.001	[Lambert et al. 2013]
rs35349669	C/T	T	2	<i>INPP5D</i>	15.2	49.0	<0.001	[Lambert et al. 2013]
rs190982	A/G	A	5	<i>MEF2C</i>	8.9	37.6	<0.001	[Lambert et al. 2013]
rs670139	G/T	T	11	<i>MS4A4E</i>	41.6	41.6	0.787	[Hollingworth et al. 2011]
rs12155159	A/G	NA	7	<i>NME8</i>	57.5	34.5	<0.001	[Lambert et al. 2013]
rs561655	A/G	A	11	<i>PICALM</i>	16.3	34.0	<0.001	[Naj et al. 2011]
rs17057043	G/A	NA	8	<i>PTK2B</i>	46.3	36.0	<0.001	[Lambert et al. 2013]
rs10498633	G/T	G	14	<i>SLC24A4</i>	12.8	22.6	<0.001	[Lambert et al. 2013]
rs11218343	T/C	T	11	<i>SORL1</i>	7.5	2.1	<0.001	[Lambert et al. 2013]
rs1476679	T/C	T	7	<i>ZCWPW1</i>	5.6	30.2	<0.001	[Lambert et al. 2013]

GWAS, genome-wide association study; AD, Alzheimer's disease, dbSNP, The National Center for Biotechnology's Information SNP database; SNP, single nucleotide polymorphism; ID, identification; Risk allele, allele associated with AD risk in genome-wide association study; Chrom., chromosome; AA, African-American; MAF, minor allele frequency; p*, p-value for comparison of genotype frequencies between races; Ref., reference; rs, reference SNP

Table II

Clinical and demographic characteristics by race. ARIC Study (1990-1992)

	<u>White (N =8,320)</u>		<u>African-American (N = 2,039)</u>	
	N	N (%)	N	N (%)
Gender	8,320		2,039	
Male		3,835 (46.1)		688 (33.7)
Female		4,485 (53.9)		1,351 (66.3)
Education	8,320		2,039	
11 years		1,158 (13.9)		648 (31.8)
>11 years - 16 years		3,836 (46.1)		616 (30.2)
>16 years		3,326 (40.0)		775 (38.0)
Current smokers	8,320	1,560 (18.8)	2,039	437 (21.4)
Current alcohol use	8,320	5,437 (65.4)	2,039	691 (33.9)
Hypertension	8,320	2,278 (27.4)	2,039	1,021 (50.1)
Diabetes	8,320	827 (9.9)	2,039	411 (20.2)
Obese (BMI ≥ 30 kg/m ²)	8,320	1,988 (23.9)	2,039	880 (43.2)
Hypercholesterolemia	8,320	4,197 (50.4)	2,039	1,065 (52.2)
<i>APOE</i> ε4 allele carriers	7,883	2,166 (27.5)	1,793	734 (40.9)
		Mean (SD)		Mean (SD)
Age (years)	8,320	57.0 (5.6)	2,039	55.8 (5.7)
DBP, mm Hg	8,320	71.0 (9.6)	2,039	74.8 (10.2)
SBP, mm Hg	8,320	119.0 (17.1)	2,039	124.2 (18.8)
Glucose (mmol/L)	8,320	6.0 (1.6)	2,039	6.7 (2.9)
BMI (kg/m ²)	8,320	27.3 (4.8)	2,039	29.9 (6.0)
Total cholesterol, mmol/L	8,320	5.39 (0.96)	2,039	5.43 (1.04)
LDL cholesterol, mmol/L	8,320	3.42 (0.91)	2,039	3.47 (1.01)
HDL cholesterol, mmol/L	8,320	1.27 (0.42)	2,039	1.40 (0.45)
Triglycerides, mmol/L	8,320	1.51 (0.75)	2,039	1.22 (0.61)
Genetic risk score	6,485	0.52 (2.32)	1,287	1.25 (1.87)
Cognitive Tests				
Baseline				
DWRT (words)	8,320	6.8 (1.4)	2,039	6.3 (1.6)
DSST (digit-symbol pairs)	8,320	49.8 (11.2)	2,039	33.3 (13.0)
WFT (words)	8,320	35.3 (11.7)	2,039	29.9 (12.8)
6-year change				
DWRT (words)	8,320	-0.11 (1.50)	2,039	-0.24 (1.70)
DSST (digit-symbol pairs)	8,320	-2.68 (6.42)	2,039	-1.90 (8.58)
WFT (words)	8,320	-0.37 (7.87)	2,039	-1.00 (8.24)

N, number; BMI, body mass index; *APOE*, apolipoprotein E; SD, standard deviation; DBP, diastolic blood pressure; SBP, systolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; DWRT, Delayed Word Recall Test; DSST, Digit Symbol Substitution Test; WFT, Word Fluency Test

Table III

Six-year cognitive change (White) ARIC Study (1990-1992 – 1996-1998)

dbSNP ID	N	β	$\frac{DWRT}{SE}$	p^*	β	$\frac{DSST}{SE}$	p^*	β	$\frac{WFT}{SE}$	p^*
<i>ABCA7</i>	7,212	-0.017	0.034	0.617	-0.120	0.144	0.406	0.024	0.178	0.892
rs4147929										
<i>BIN1</i>	7,986	-0.012	0.026	0.653	-0.063	0.109	0.564	0.001	0.135	0.997
rs744373										
<i>CASS4</i>	NA									
rs7274581										
<i>CD2AP</i>	7,008	0.003	0.029	0.910	-0.033	0.124	0.789	0.104	0.154	0.497
rs9349407										
<i>CD33</i>	7,376	-0.038	0.026	0.147	0.010	0.112	0.926	-0.070	0.138	0.610
rs3865444										
<i>CELF1</i>	7,376	-0.007	0.027	0.801	-0.130	0.117	0.267	-0.142	0.144	0.324
rs6485758										
<i>CLU</i>	7,376	-0.003	0.026	0.908	0.212	0.110	0.053	-0.102	0.135	0.451
rs9331896										
<i>CRI</i>	7,035	0.015	0.033	0.636	0.113	0.140	0.419	0.022	0.172	0.898
rs6656401										
<i>EPHA1</i>	7,376	0.022	0.032	0.503	0.024	0.139	0.861	-0.169	0.171	0.323
rs11767557										
<i>FERMT2</i>	7,376	0.031	0.042	0.463	0.018	0.182	0.920	0.215	0.224	0.336
rs17125944										
<i>HLA-DRB1</i>	7,212	0.006	0.028	0.826	0.025	0.120	0.834	0.019	0.148	0.900
rs9271174										
<i>INPP5D</i>	7,212	0.032	0.025	0.198	-0.033	0.107	0.760	0.162	0.132	0.222
rs35349669										
<i>MEF2C</i>	7,376	-0.001	0.025	0.973	0.005	0.109	0.964	0.107	0.134	0.427
rs190982										
<i>MS4A4E</i>	7,110	0.056	0.025	0.026	-0.134	0.108	0.217	-0.150	0.133	0.259
rs670139										

dbSNPID	N	β	SE	p^*	β	SE	p^*	β	SE	p^*
<i>NME8</i>	7,376	0.003	0.026	0.919	-0.122	0.111	0.269	0.281	0.136	0.039
rs12155159										
<i>PICALM</i>	7,105	-0.012	0.026	0.663	-0.085	0.113	0.456	0.012	0.139	0.932
rs561655										
<i>PTK2B</i>	7,101	-0.016	0.026	0.552	-0.055	0.113	0.627	0.182	0.139	0.190
rs17057043										
<i>SLC24A4</i>	7,376	0.003	0.030	0.910	-0.011	0.127	0.931	0.097	0.156	0.532
rs10498633										
<i>SORL1</i>	7,376	-0.033	0.086	0.703	-0.210	0.367	0.566	-0.180	0.451	0.689
rs11218343										
<i>ZCWPW1</i>	7,376	0.021	0.027	0.437	-0.024	0.115	0.836	-0.134	0.142	0.345
rs1476679										
<i>APOE</i> ϵ 4	7,883	-0.095	0.038	0.012	-0.548	0.160	0.001	-0.024	0.199	0.902
GRS	6,485	0.004	0.008	0.644	0.000	0.036	0.995	0.035	0.044	0.428

β , beta coefficient; SE, standard error; p^* , results were adjusted for age, gender, and years of education; GRS, genetic risk score; NA, not available, variant did not meet Hardy-Weinberg equilibrium expectations

Table IV
Six-year cognitive change (African-American) ARIC Study (1990-1992 – 1996-1998)

dbSNP ID	N	β	$\frac{DWRT}{SE}$	p^*	β	$\frac{DSST}{SE}$	p^*	β	$\frac{WFT}{SE}$	p^*
<i>ABCA7</i>	1,594	0.060	0.140	0.669	0.952	0.716	0.184	-0.026	0.671	0.969
rs4147929										
<i>BIN1</i>	1,934	0.032	0.055	0.563	-0.412	0.281	0.142	-0.414	0.265	0.119
rs744373										
<i>CASS4</i>	1,594	-0.096	0.072	0.184	-0.562	0.368	0.127	-0.250	0.345	0.470
rs7274581										
<i>CD2AP</i>	1,762	-0.090	0.074	0.223	-0.490	0.371	0.187	-0.129	0.352	0.713
rs9349407										
<i>CD33</i>	1,597	-0.127	0.116	0.273	0.324	0.589	0.582	1.109	0.553	0.045
rs3865444										
<i>CELF1</i>	1,597	0.141	0.130	0.276	-0.179	0.657	0.786	0.444	0.617	0.472
rs6485758										
<i>CLU</i>	1,597	0.105	0.060	0.081	0.263	0.306	0.389	-0.096	0.288	0.739
rs9331896										
<i>CRI</i>	1,685	0.004	0.117	0.971	-0.776	0.588	0.187	0.036	0.561	0.948
rs6656401										
<i>EPHA1</i>	1,597	0.064	0.090	0.477	-0.049	0.454	0.914	-0.288	0.427	0.500
rs11767557										
<i>FERMT2</i>	1,597	-0.131	0.135	0.333	-0.638	0.685	0.352	0.480	0.643	0.456
rs17125944										
<i>HLA-DRB1</i>	1,594	0.083	0.072	0.255	-0.251	0.371	0.498	0.453	0.347	0.192
rs9271174										
<i>INPP5D</i>	1,594	0.006	0.083	0.941	0.190	0.424	0.654	0.124	0.397	0.754
rs35349669										
<i>MEF2C</i>	1,597	-0.113	0.106	0.286	0.163	0.538	0.762	-0.350	0.506	0.489
rs190982										
<i>MS4A4E</i>	1,774	-0.089	0.058	0.124	0.464	0.291	0.111	0.172	0.276	0.534
rs670139										

	N	β	SE	p^*	β	SE	p^*	β	SE	p^*
dbSNP										
NME8	1,597	0.046	0.060	0.445	-0.205	0.306	0.502	-0.213	0.288	0.458
rs12155159										
PICALM	1,773	0.045	0.078	0.563	0.266	0.393	0.499	0.200	0.373	0.593
rs561655										
PTK2B	1,774	0.014	0.058	0.811	0.082	0.291	0.780	-0.433	0.276	0.117
rs17057043										
SLC24A4	1,597	-0.125	0.090	0.168	-0.722	0.459	0.115	0.117	0.431	0.786
rs10498633										
SORL1	1,597	0.014	0.112	0.903	0.482	0.568	0.396	-0.440	0.534	0.409
rs11218343										
ZCWPW1	1,589	-0.283	0.130	0.030	0.216	0.662	0.744	0.487	0.623	0.435
rs1476679										
APOE e4	1,793	-0.170	0.082	0.037	-0.719	0.415	0.084	0.383	0.399	0.337
GRS	1,287	-0.015	0.026	0.561	-0.109	0.136	0.423	0.030	0.125	0.813

p^* , results were adjusted for age, gender, and years of education

Table V

Baseline cognitive test scores (White) ARIC Study (1990 - 1992)

dbSNPID	N	β	$\frac{DWRT}{SE}$	p^*	β	$\frac{DSST}{SE}$	p^*	β	$\frac{WFT}{SE}$	p^*
<i>ABCA7</i>	7,212	0.075	0.030	0.013	0.218	0.212	0.306	0.328	0.242	0.175
rs4147929										
<i>BIN1</i>	7,986	0.011	0.023	0.638	-0.109	0.162	0.502	0.080	0.185	0.663
rs744373										
<i>CASS4</i>	NA									
rs7274581										
<i>CD2AP</i>	7,008	0.003	0.026	0.897	-0.201	0.183	0.273	0.084	0.210	0.689
rs9349407										
<i>CD33</i>	7,376	0.005	0.023	0.837	-0.090	0.165	0.586	-0.241	0.188	0.199
rs3865444										
<i>CELF1</i>	7,376	0.050	0.024	0.042	0.439	0.172	0.011	0.620	0.196	0.002
rs6485758										
<i>CLU</i>	7,376	0.045	0.023	0.047	-0.031	0.162	0.849	-0.024	0.184	0.894
rs9331896										
<i>CRI</i>	7,035	0.015	0.029	0.605	0.115	0.206	0.576	0.172	0.235	0.466
rs6656401										
<i>EPHA1</i>	7,376	0.012	0.029	0.689	-0.186	0.205	0.365	0.256	0.233	0.274
rs11767557										
<i>FERMT2</i>	7,376	-0.058	0.038	0.129	-0.421	0.268	0.116	-1.034	0.305	0.001
rs17125944										
<i>HLA-DRB1</i>	7,212	0.008	0.025	0.741	0.104	0.176	0.557	0.330	0.201	0.100
rs9271174										
<i>INPP5D</i>	7,212	-0.030	0.022	0.187	-0.164	0.158	0.299	-0.466	0.180	0.010
rs35349669										
<i>MEF2C</i>	7,376	0.031	0.023	0.170	-0.307	0.161	0.057	0.141	0.183	0.443
rs190982										
<i>MS4A4E</i>	7,110	0.003	0.023	0.893	0.222	0.159	0.163	0.210	0.182	0.249
rs670139										

	N	β	SE	p^*	β	SE	p^*	β	SE	p^*
dbSNP		DWRT			DSST			WFT		
<i>NME8</i>	7,376	0.010	0.023	0.666	0.186	0.163	0.255	-0.118	0.186	0.527
rs12155159										
<i>PICALM</i>	7,105	0.008	0.024	0.748	0.113	0.167	0.497	-0.029	0.190	0.881
rs561655										
<i>PTK2B</i>	7,101	-0.017	0.024	0.464	-0.220	0.166	0.184	-0.140	0.190	0.461
rs17057043										
<i>SLC24A4</i>	7,376	0.018	0.026	0.486	0.317	0.186	0.089	0.362	0.212	0.089
rs10498633										
<i>SORL1</i>	7,376	-0.038	0.077	0.625	0.586	0.540	0.278	-0.792	0.616	0.199
rs11218343										
<i>ZCWPW1</i>	7,376	0.014	0.024	0.551	0.293	0.169	0.083	0.265	0.193	0.171
rs1476679										
<i>APOE $\epsilon 4$</i>	7,883	-0.019	0.034	0.576	0.001	0.238	0.997	-0.054	0.271	0.842
GRS	6,485	-0.005	0.008	0.485	-0.006	0.053	0.906	-0.027	0.060	0.650

p^* , results were adjusted for age, gender, and years of education

Table VI

Baseline cognitive test scores (African-American) ARIC Study (1990 - 1992)

dbSNP ID	N	β	$\frac{DWRT}{SE}$	p^*	β	$\frac{DSST}{SE}$	p^*	β	$\frac{WFT}{SE}$	p^*
<i>ABCA7</i>	1,594	0.051	0.122	0.675	0.824	0.850	0.333	0.747	0.878	0.395
rs4147929										
<i>BIN1</i>	1,934	-0.027	0.048	0.573	0.056	0.332	0.865	0.214	0.345	0.535
rs744373										
<i>CASS4</i>	1,594	-0.046	0.063	0.465	0.431	0.438	0.325	0.121	0.452	0.790
rs7274581										
<i>CD2AP</i>	1,762	0.041	0.064	0.520	0.164	0.440	0.709	-0.161	0.464	0.729
rs9349407										
<i>CD33</i>	1,597	0.099	0.101	0.329	-0.637	0.701	0.363	-1.522	0.730	0.037
rs3865444										
<i>CELF1</i>	1,597	-0.074	0.113	0.513	0.563	0.782	0.472	0.077	0.815	0.925
rs6485758										
<i>CLU</i>	1,597	0.051	0.053	0.330	-0.256	0.364	0.483	0.014	0.380	0.971
rs9331896										
<i>CRI</i>	1,685	-0.137	0.102	0.180	1.518	0.696	0.029	-1.265	0.736	0.086
rs6656401										
<i>EPHA1</i>	1,597	-0.033	0.078	0.673	0.005	0.541	0.993	-0.694	0.564	0.218
rs11767557										
<i>FERMT2</i>	1,597	0.157	0.118	0.182	0.188	0.816	0.818	-0.459	0.850	0.589
rs17125944										
<i>HLA-DRB1</i>	1,594	-0.016	0.063	0.799	0.820	0.440	0.062	0.348	0.454	0.444
rs9271174										
<i>INPP5D</i>	1,594	0.009	0.072	0.905	0.274	0.503	0.586	-0.733	0.519	0.158
rs35349669										
<i>MEF2C</i>	1,597	0.033	0.092	0.719	0.213	0.641	0.740	-0.942	0.668	0.159
rs190982										
<i>MS4A4E</i>	1,774	0.044	0.050	0.376	-0.219	0.346	0.527	0.546	0.363	0.133
rs670139										

	N	β	SE	p^*	β	SE	p^*	β	SE	p^*
dbSNPID										
<i>NME8</i>	1,597	-0.011	0.053	0.832	0.178	0.364	0.625	0.073	0.380	0.848
rs12155159										
<i>PICALM</i>	1,773	0.010	0.068	0.888	0.189	0.468	0.687	-0.264	0.492	0.592
rs561655										
<i>PTK2B</i>	1,774	-0.039	0.050	0.437	-0.267	0.346	0.441	-0.232	0.364	0.523
rs17057043										
<i>SLC24A4</i>	1,597	0.116	0.079	0.140	0.329	0.546	0.548	0.575	0.569	0.313
rs10498633										
<i>SORL1</i>	1,597	-0.024	0.098	0.803	0.016	0.677	0.981	0.372	0.705	0.598
rs11218343										
<i>ZCWPW1</i>	1,589	0.016	0.114	0.887	0.736	0.790	0.351	-0.089	0.823	0.914
rs1476679										
<i>APOE ε4</i>	1,793	0.054	0.070	0.445	-0.367	0.489	0.453	-0.428	0.506	0.398
GRS	1,287	-0.016	0.023	0.476	0.080	0.161	0.618	0.157	0.165	0.344

p^* , results were adjusted for age, gender, and years of education