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Interaction between two cholesterol metabolism genes influences memory: findings from the Wisconsin Registry for Alzheimer's Prevention

Corinne D. Engelman^{a,*}, Rebecca L. Kosci^b, Erin M. Jonaitis^b, Ozioma C. Okonkwo^c, Bruce P. Hermann^{b,d}, Asenath La Rue^b, and Mark A. Sager^{b,c}

^aDepartment of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI 53726, USA

^bWisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison, WI 53719, USA

^cDepartment of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792, USA

^dDepartment of Neurology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA

Abstract

The strongest genetic factor for late-onset Alzheimer's disease (AD) is *APOE*; nine additional susceptibility genes have recently been identified. The effect of these genes is often assumed to be additive and polygenic scores are formed as a summary measure of risk. However, interactions between these genes are likely to be important. We sought to examine the role of interactions between the nine recently identified AD susceptibility genes and *APOE* in cognitive function and decline in 1,153 participants from the Wisconsin Registry for Alzheimer's Prevention, a longitudinal study of middle-aged adults enriched for a parental history of AD. Participants underwent extensive cognitive testing at baseline and up to two additional visits approximately 4 and 6 years later. The influence of the interaction between *APOE* and each of 14 single nucleotide polymorphisms (SNPs) in the nine recently identified genes on three cognitive factor scores (Verbal Learning and Memory, Working Memory, and Immediate Memory) was examined using linear mixed models adjusting for age, gender and ancestry. Interactions between the *APOE* ε4 allele and both of the genotyped *ABCA7* SNPs, rs3764650 and rs3752246, were associated with all three cognitive factor scores (*P*-values < 0.01). Both of these genes are in the cholesterol metabolism pathway leading to AD. This research supports the importance of considering non-additive effects of AD susceptibility genes.

Keywords

gene-gene interaction; memory; cognition; Alzheimer's disease; cholesterol

Address correspondence to: Corinne D. Engelman, Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, 610 Walnut Street, 1007A WARF, Madison, WI 53726-2397, (608) 265-5491, (608) 263-2820 (fax), cengelma@wisc.edu.

INTRODUCTION

Over 5 million Americans have Alzheimer's disease (AD; OMIM 104300) and that number is expected to increase to nearly 14 million by 2050, costing an estimated \$1.2 trillion for health and long-term care and hospice [1]. Among the top 10 causes of death in America, AD is the only one with no way to prevent, cure, or impede its progression [1]. Although the genetic causes of autosomal dominant, early-onset AD are well established, those of the late-onset, non-Mendelian form of AD remain elusive. While heritability estimates of non-Mendelian AD range from 60–80% [2, 3] and apolipoprotein E (*APOE*) ϵ 4 carrier status is well established as the most significant genetic risk factor, it accounts for only 4% of the variance in late-onset AD prevalence [3]. Recent large-scale genome-wide association studies (GWAS) of late-onset AD have identified nine susceptibility genes beyond *APOE* [4–9]. These genes implicate three new pathways in AD: immune system function, cholesterol metabolism, and synaptic dysfunction and cell membrane processes [10]. The effect of these genes is often assumed to be additive and polygenic scores that add all risk alleles across genes, often weighted by the effect size, have been formed as a summary measure of risk for AD, brain alterations, and cognitive dysfunction [11–13]. However, these scores are only marginally associated with AD and barely improve the prediction of AD beyond age, sex, and *APOE* ϵ 4 carrier status [13]. It is possible that the effect of these genes is non-additive and interactions between these genes may contribute to AD and cognitive function and decline. Some evidence in support of this has recently emerged. A functional fine-mapping study focused on a linkage disequilibrium block in the complement component (3b/4b) receptor 1 gene (*CR1*) that contained the single nucleotide polymorphism (SNP) identified by GWAS of AD [14]. The study found an interaction between a novel coding variant in *CR1* and the *APOE* ϵ 4 allele that significantly influenced longitudinal episodic memory decline in 1,458 non-demented subjects from the Religious Orders Study (mean age at enrollment = 76 years) and the Rush Memory and Aging Project (mean age at enrollment = 81 years). Another study examined potential interactions between five AD susceptibility genes, *CR1*, bridging integrator 1 (*BINT1*), clusterin (*CLU*), phosphatidylinositol binding clathrin assembly protein (*PICALM*), and *APOE*, in 1,028 non-demented subjects in the National Institute on Aging Late-Onset AD Family Study (mean age = 72 years) [15]. Results from this study showed that a pattern including *PICALM* and *CLU* was the strongest genotypic profile for cross-sectional episodic memory performance and the effect was stronger after the addition of *APOE*. However, to our knowledge, no study has comprehensively examined all nine of the recently identified AD susceptibility single nucleotide polymorphisms (SNPs) for potential interactions with the *APOE* ϵ 4 allele.

The purpose of the current study was to examine the association between the nine recently identified AD susceptibility SNPs, *APOE*, and cognitive function, considering SNP x *APOE* interactions before evaluating SNP main effects, in a longitudinal study of asymptomatic late middle-aged adults who have undergone extensive neuropsychological testing.

MATERIALS AND METHODS

Study population

Study participants were from the Wisconsin Registry for Alzheimer's Prevention (WRAP), a longitudinal study of middle-aged adults enriched for a parental history of AD (i.e., a biological parent with either autopsy-confirmed or probable AD as defined by NINCDS-ADRDA research criteria [16]). Details of the study design and methods have been previously described [17, 18]. WRAP participants were required to be English-speaking and were predominantly between the ages of 40 and 65 years at the baseline study visit. The study design allowed participation of multiple siblings from any given family. Baseline recruitment began in 2001; the study protocol targeted a window of 4 years between the

baseline and wave 2 visits with 2 years between subsequent visits. The present analyses were limited to self-reported non-Hispanic white participants, due to the small sample size of other racial/ethnic groups, to obtain a more genetically homogenous study population. Participants with diseases or comorbidities that might be expected to influence cognitive test performance (e.g., multiple sclerosis, Parkinson's disease, stroke, epilepsy/seizures, or meningitis) were excluded (N=4), as were those who developed AD on or before the second visit (N=2), as WRAP was designed to study predictors of cognitive function and decline in individuals who are initially free of AD or other types of dementia. A total of 1,170 participants met these inclusion/exclusion criteria. This study was conducted with the approval of the University of Wisconsin Institutional Review Board and all subjects provided signed informed consent before participation.

Neuropsychological assessment

The WRAP cognitive test battery consists of standardized, widely used clinical neuropsychological tests, which were selected to provide a comprehensive estimate of cognitive abilities, with an emphasis on abilities most likely to be affected in early-stage AD. A complete list of the tests administered has been published elsewhere [17]. Factor analysis was conducted to reduce the number of outcome measures to a small number of reliable cognitive factors and obtain weights used to combine the measures within each factor [19]. The resulting weighted factor scores were then standardized ($\sim N[0, 1]$) into z-scores, using means and standard deviations obtained from the whole baseline sample. Cognitive factor z-scores associated with a higher risk for change in the earliest stages of AD and for which heritability estimates from previous studies were relatively high [20–23] were included in the current study: Verbal Learning and Memory, Working Memory, and Immediate Memory (Supplementary Table 1). The Wechsler Abbreviated Scale of Intelligence™ (WASI™) was administered to obtain an estimate of general cognitive ability. The WASI full-scale IQ score was used in secondary analyses to ensure that the results were not due to underlying differences in general cognitive ability between genotypic subgroups.

DNA collection, SNP selection, genotyping, and quality assurance

DNA was extracted from whole blood samples using PUREGENE® DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MN). DNA concentrations were quantified using UV spectrophotometry (DU® 530 Spectrophotometer, Beckman Coulter, Fullerton, CA).

SNPs were selected based on a review of all published large-scale GWAS and meta-analyses of AD, including the meta-analysis of over 50,000 individuals performed by the Alzheimer Disease Genetics Consortium [4–8, 24]. The AlzGene Top Results list (<http://www.alzgene.org/TopResults.asp>) was also consulted. Fourteen SNPs in nine genes were selected for genotyping: *ABCA7* (rs3752246 and rs3764650), *BIN1* (rs744373 and rs7561528), *CD2AP* (rs9349407), *CD33* (rs3865444), *CLU* (rs1532278 and rs11136000), *CR1* (rs6656401 and rs6701713), *EPHA1* (rs11767557), *MS4A* gene cluster (rs4938933), and *PICALM* (rs561655 and rs3851179). Genotyping for the two *APOE* SNPs that determine the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles, rs429358 and rs7412, was done previously by WRAP and has been described in detail [25]. A panel of 100 validated ancestry informative markers (AIMs), developed to discern the northwest to southeast cline in European ancestry [26], was also genotyped.

Genotyping was performed by PreventionGenetics (Marshfield, WI) using small volume PCR reactions on ArrayTape™ technology (Douglas Scientific, Alexandria, MN) and the InvaderPlus® assay (Third Wave Technologies, Madison, WI). Duplicate quality control samples from 62 individuals were placed randomly throughout each of the 96-well plates. The genotype discordance rate was 0.09%. All discordant genotypes were set to missing.

Four samples were excluded from subsequent analyses because the self-reported gender did not match the gender indicated by a gender marker (rs25601). Genotype quality assurance checks were performed using the PLINK software v1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>) [27]. Thirteen samples had call rates <90% and were excluded from subsequent analyses. Therefore, the total sample size for all subsequent analyses was 1,153 (98.5% of the 1,170 eligible participants). The SNP call rates ranged from 96.5% to 99.4% and there was no deviation from Hardy-Weinberg equilibrium at a Bonferroni-corrected global significance level $\alpha = 0.05$ in a subset of 927 unrelated participants who met all inclusion and quality control criteria.

Statistical analysis

To minimize the risk of confounding due to population stratification, we used the genotypes from the panel of AIMs and principal components analysis (PCA), implemented in EIGENSTRAT [28] in the EIGENSOFT package, to detect genetic outliers (none were found) and estimate principal components (eigenvalues) that capture genotypic variation due to ancestry. The first two principal components explained 2.1% and 1.8% of the genetic variance and were then used to correct for population stratification in the genetic association analyses.

Genetic associations with each of the three cognitive factor scores at up to three visits were tested using linear mixed models implemented in the SAS MIXED procedure to account for the within-family and within-subject correlations while allowing for missing data [29]. The dependent variables, the cognitive factor scores for Verbal Learning and Memory, Working Memory, and Immediate Memory, were standardized ($\sim N[0, 1]$) continuous variables. The independent variables were counts of the number of copies of the minor allele. For each factor score, a series of models were fit for each of the 14 recently identified AD susceptibility SNPs. All statistical models were adjusted for age, gender, the top two principal components of ancestry, and *APOE* $\epsilon 4$ allele count. When testing for genetic association with each of the SNPs, we took a stepwise backward elimination approach in which we initially included a 3-way interaction term between *APOE* $\epsilon 4$ allele count, the susceptibility SNP, and age to examine the *APOE**SNP effect on longitudinal cognitive decline. Since 14 SNPs were being examined, a Bonferroni adjusted *P*-value cut-off of 0.0036 was applied for each cognitive factor score. When the *APOE**SNP*age interaction term was not significant at this *P*-value threshold, the 3-way interaction term was removed and a model with the three corresponding 2-way interactions was examined to test the effect of 1) an interaction between *APOE* and the susceptibility SNP on repeated measures of cognitive function (*APOE**SNP), 2) *APOE* on longitudinal cognitive decline (*APOE**age), and 3) each susceptibility SNP on longitudinal cognitive decline (SNP*age). We removed non-significant 2-way interaction terms from the model until only significant 2-way interaction terms remained in the model or the model was reduced to only the *APOE* and SNP main effects along with the previously mentioned covariates.

When a SNP**APOE* interaction was significant, secondary analyses were performed to ensure that the results were robust. These analyses included: 1) repeating the mixed model analysis while including the number of visits completed per participant as a covariate, 2) repeating the mixed model analysis using the subset with at least two visits; and 3) testing for a SNP**APOE* effect on the baseline assessment of general cognitive abilities, using the WASI full-scale IQ score, to evaluate the possibility that the effect of the interaction may be due to underlying differences in general cognitive ability between genotypic subgroups.

RESULTS

Cognitive and genetic data were analyzed for 1,153 WRAP participants from 953 families. Of these, all had completed baseline cognitive testing, 892 had completed at least a second wave of testing, and 513 had completed a third wave of testing. The mean age of the WRAP sample at baseline was 53.6 years. Additional characteristics are shown in Table 1.

Characteristics of the 14 newly genotyped AD susceptibility SNPs in the WRAP sample are displayed in chromosomal order in Supplementary Table 2. Linkage disequilibrium, calculated as r^2 , between SNPs in the same gene is shown where applicable.

There were no *APOE**SNP*age, *APOE**age or SNP*age interactions that met the Bonferroni adjusted threshold of .0036. There were only two *APOE**SNP interactions that met the threshold for one or more cognitive factors: *APOE* interactions with rs3764650 and rs3752246, both in the *ABCA7* (ATP-binding cassette, sub-family A, member 7) gene (r^2 between the two SNPs was 0.20). The effect and significance of these two interaction terms for each of the three cognitive factor scores are displayed in Table 2. The interaction between *APOE* and *ABCA7* SNP rs3764650 was significant at the Bonferroni-adjusted threshold for all three cognitive factor scores. The interaction between *APOE* and *ABCA7* SNP rs3752246 was significant for Verbal Learning and Memory and Working Memory, with marginal significance ($P=0.01$) for Immediate Memory.

For the remaining 12 SNPs with no significant interaction terms, the main effects of the SNPs were examined while adjusting for *APOE* $\epsilon 4$ allele count, as well as the other covariates and random effects adjusted for in previous models. There were no significant main effects observed for these 12 SNPs (Table 3). As a point of reference, *APOE* is displayed in the first row of Table 3, since its effect on AD and cognitive function and decline is well established and much greater than that of the other susceptibility genes.

Figure 1 depicts the pattern of cognitive performance across *APOE* and *ABCA7* genotype combinations. Individuals with one or two copies of the *ABCA7* minor allele have been combined to increase the sample size and obtain more stable least squares means estimates. The pattern is consistent across all three cognitive factor scores and is as follows: in the absence of an *ABCA7* minor allele, each additional $\epsilon 4$ allele is associated with lower memory scores; in contrast, in the presence of one or two *ABCA7* minor alleles, each additional $\epsilon 4$ allele is generally associated with better memory scores. The sole exception is rs3764650 (plot E); for this SNP, in the presence of one or two *ABCA7* minor alleles, Immediate Memory scores for one or two copies of the $\epsilon 4$ allele were nearly the same, but still higher than for no copies, consistent with the other plots.

The results of the secondary analyses described in the methods section showed no evidence that the effect of the *APOE***ABCA7* interactions on memory scores could be explained by participants with varying numbers of study visits (e.g., that the results were driven by individuals with only the baseline visit) or by underlying general cognitive ability.

DISCUSSION

In this study of 1,153 non-Hispanic white WRAP participants from 953 families, we conducted a comprehensive evaluation of all recently identified AD susceptibility SNPs for potential interactions with the *APOE* $\epsilon 4$ allele that influence cognitive function or decline. We observed a significant interaction between the *APOE* $\epsilon 4$ allele and both of the genotyped *ABCA7* SNPs for all three cognitive factor scores: Verbal Learning and Memory, Working Memory, and Immediate Memory. Both of these genes are in the cholesterol

metabolism pathway leading to AD [10]. Moreover, the three cognitive domains pertain to learning and memory, the first area of cognitive function to decline in incipient AD [30, 31].

The interaction between the *APOE* $\epsilon 4$ allele and both *ABCA7* SNPs was not only statistically significant, but was consistent across two SNPs in the *ABCA7* gene that are not strongly correlated with each other ($r^2 = 0.20$) and three related, but distinct, cognitive domains (r^2 0.22 to 0.65); this consistency lends credibility to the findings. Moreover, one of the *ABCA7* SNPs, rs3752246, is a non-synonymous SNP that results in an amino acid change from glycine to alanine. Finally, the fact that both of these genes are involved in the cholesterol metabolism pathway leading to AD makes biologic interaction between them plausible.

The direction of the interaction is consistent with a flip-flop effect [32] where the effect of a particular allele of one gene (e.g., the *APOE* $\epsilon 4$ allele) is in opposite directions, depending on the genotype at another gene (e.g., *ABCA7*). In the absence of an *ABCA7* minor allele, each additional $\epsilon 4$ allele is associated with lower memory scores; in contrast, in the presence of one or two *ABCA7* minor alleles, each additional $\epsilon 4$ allele is generally associated with better memory scores (Figure 1). If this interaction were confirmed in AD cases and controls, the *ABCA7* minor alleles could, in part, explain why some individuals with one or two copies of the *APOE* $\epsilon 4$ allele do not develop AD, or develop it at a later age. For these individuals, having one or two copies of the *ABCA7* minor allele may help preserve their memory.

The interaction between *APOE* and *ABCA7* did not appear to influence the longitudinal trajectory of the cognitive factor scores. It is possible that the follow-up period of approximately 4 to 6 years, in the subset of 892 participants who have been seen for a follow-up visit thus far, was too short for these late middle-aged participants to demonstrate significantly differing rates of decline during the study window. Moreover, we were only able to analyze data on up to three visits so the stability of the cognitive factor scores may not be optimal to examine longitudinal decline in cognition. WRAP follow-up visits are ongoing, allowing us to address the potential role of the interaction in cognitive decline as additional data are collected. In the mean time, follow-up of these findings in additional cohorts with more than three follow-up visits or an older study population may resolve this issue.

We did not find an association between the other 12 recently identified susceptibility SNPs and cognitive function or decline. This may be due to a small effect size for these SNPs. Additionally, it is possible that these SNPs do not substantially affect cognitive function or decline at the relatively young age of our sample (mean age of 54 years at the baseline visit). In the Rotterdam Study, the genetic effect sizes for the AD susceptibility SNPs increased with increasing age group and were greatest in individuals over 70 years of age [13]. In the future, data from the ongoing follow-up visits may resolve this issue.

The fact that we did not find a significant interaction between *CRI* and *APOE*, as in a previous study [14], is not surprising given that the two SNPs we genotyped in *CRI* were those identified by GWAS, whereas the interaction identified previously was with a novel coding variant in *CRI* identified through fine mapping, not the GWAS SNPs. In the Late-Onset AD Family Study there was evidence for an interaction between *PICALM* and *CLU* that was stronger after the addition of *APOE* [15]. We chose to focus on interactions with *APOE*, the strongest genetic factor for AD, and did not test for interactions between other genes (e.g., *PICALM* and *CLU*). However, we did detect a marginally significant ($P < 0.05$) interaction between *CLU*, *APOE*, and age for both Verbal Learning and Memory and Working Memory, and between *PICALM*, *APOE*, and age for both Verbal Learning and

Memory and Immediate Memory (data not shown), providing some support for the Late-Onset AD Family Study findings.

In conclusion, we conducted a comprehensive evaluation of all recently identified AD susceptibility SNPs for potential interactions with the *APOE* ϵ 4 allele and found significant interactions between the *APOE* ϵ 4 allele and two *ABCA7* SNPs for all three cognitive factor scores: Verbal Learning and Memory, Working Memory, and Immediate Memory. Future research directions include: 1) studies examining AD, in addition to cognitive function, to determine if this interaction influences risk for AD, 2) replication in both non-Hispanic white and other racial/ethnic samples, and 3) a comprehensive examination of all pair-wise interactions within the identified pathways leading to AD. For the latter, a sample larger than the WRAP sample may be necessary to achieve adequate power while appropriately adjusting for multiple comparisons of all pair-wise interactions; alternately, machine learning approaches may be utilized to identify potential interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cognitive performance across *APOE* and *ABCA7* genotype combinations

Plots of the least squares means for Verbal Learning and Memory (A and B), Working Memory (C and D), and Immediate Memory (E and F) stratified by *APOE* $\epsilon 4$ allele count and presence or absence of the minor allele for *ABCA7* SNPs rs3764650 (C allele; plots A, C, and E) and rs3752246 (G allele; plots B, D, and F). Least squares means are adjusted for age, gender, and the top two principal components of ancestry.

Table 1

Characteristics of the WRAP study population.

Characteristic	<i>N</i>	Percent or mean $\pm$ SD
Gender		
Male	359	31%
Female	794	69%
Age at baseline	1153	53.6 $\pm$ 6.6
Age at 4-year follow-up	892	57.7 $\pm$ 6.6
Age at 6-year follow-up	513	59.8 $\pm$ 6.5
Education		
High school diploma or GED	111	10%
Some college or training after high school	331	29%
College graduate	344	30%
Post-college degree	367	32%
WASI full-scale IQ score	1,153	113.3 $\pm$ 9.4
<i>APOE</i> ϵ 4 allele count:		
0 ϵ 4 alleles	698	61%
1 ϵ 4 alleles	404	35%
2 ϵ 4 alleles	51	4%

Abbreviations: GED, General Equivalency Diploma; WASITM, Wechsler Abbreviated Scale of IntelligenceTM

Table 2

Significant 2-way interactions for cognitive factor scores.

Interaction term	Verbal Learning and Memory	Working Memory	Immediate Memory
<i>APOE</i> ε4	-0.168 ± 0.050 (0.001)	-0.181 ± 0.054 (0.001)	-0.146 ± 0.049 (0.003)
<i>ABCA7</i> rs3764650	-0.098 ± 0.079 (0.22)	-0.131 ± 0.085 (0.12)	-0.169 ± 0.076 (0.03)
<i>APOE</i> ε4 x <i>ABCA7</i> rs3764650	0.342 ± 0.108 (0.002)	0.421 ± 0.115 (<0.001)	0.329 ± 0.104 (0.002)
<i>APOE</i> ε4	-0.199 ± 0.055 (<0.001)	-0.229 ± 0.059 (<0.001)	-0.164 ± 0.053 (0.002)
<i>ABCA7</i> rs3752246	-0.138 ± 0.062 (0.03)	-0.161 ± 0.066 (0.01)	-0.136 ± 0.059 (0.02)
<i>APOE</i> ε4 x <i>ABCA7</i> rs3752246	0.244 ± 0.079 (0.002)	0.322 ± 0.085 (<0.001)	0.199 ± 0.077 (0.01)

$\beta \pm \text{SE}$ and *P*-value are for the main effects and interaction between *APOE* ε4 allele count (0,1 or 2) and the minor allele count for each of the two *ABCA7* SNPs on the cognitive factor score from a linear mixed model adjusting for age, gender, and the top two principal components of ancestry and accounting for within-family and within-subject correlations.

Table 3

Association between remaining 12 SNPs and cognitive factor scores.

Nearest gene	SNP	Verbal Learning and Memory	Working Memory	Immediate Memory
		β (<i>P</i> -value)	β (<i>P</i> -value)	β (<i>P</i> -value)
<i>APOE</i>	$\epsilon 4$ (rs429358 and rs7412)	−0.109 (0.02)	−0.111 (0.03)	−0.093 (0.04)
<i>CR1</i>	rs6656401	0.003 (0.94)	−0.0004 (0.99)	−0.028 (0.53)
	rs6701713	−0.001 (0.98)	−0.024 (0.62)	−0.007 (0.86)
<i>BIN1</i>	rs7561528	−0.042 (0.29)	0.060 (0.16)	−0.034 (0.38)
	rs744373	−0.028 (0.49)	0.057 (0.20)	0.016 (0.68)
<i>CD2AP</i>	rs9349407	0.047 (0.27)	0.006 (0.90)	0.028 (0.50)
<i>EPHA1</i>	rs11767557	0.026 (0.59)	−0.081 (0.11)	0.063 (0.16)
<i>CLU</i>	rs11136000	0.001 (0.98)	0.041 (0.32)	0.018 (0.63)
	rs1532278	−0.003 (0.93)	0.047 (0.26)	0.019 (0.61)
<i>MS4A4A</i>	rs4938933	−0.019 (0.63)	−0.054 (0.19)	−0.049 (0.19)
<i>PICALM</i>	rs561655	−0.001 (0.98)	0.046 (0.28)	0.020 (0.60)
	rs3851179	−0.027 (0.50)	0.0005 (0.99)	0.009 (0.82)
<i>CD33</i>	rs3865444	−0.008 (0.84)	−0.068 (0.13)	0.005 (0.90)

β and *P*-value are for each additional copy of the minor allele on the cognitive factor score from a linear mixed model assuming an additive genetic model, adjusting for age, gender, the top two principal components of ancestry, and *APOE* $\epsilon 4$ allele count (0,1 or 2), and accounting for within-family and within-subject correlations. *APOE* is displayed in the first row as a point of reference, since its effect on AD and cognitive function and decline is much greater than that of the other susceptibility genes.