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Alzheimer's disease risk genes and the age-at-onset phenotype

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ABSTRACT

Despite the recent identification of several novel risk genes for Alzheimer's disease (AD), little is known about their influence on the age at onset (AAO) of AD. The AAO is a phenotype with a heritable component distinct from disease risk and may be a useful trait to study in the context of developing interventions for delaying the onset of AD. We studied the influence of 10 recently identified AD risk genes and *APOE* in relation to AAO in a large cohort of AD patients (N = 2569). We find that the novel AD risk gene, *PICALM*, exerts a small effect on the AAO of AD with earlier disease onset in risk allele carriers. In addition, we confirmed the previously reported association between the *APOE* ε4 allele and earlier disease onset. None of the other AD risk genes influenced AAO of AD. Our results suggest that besides *APOE*, other genes associated with AD risk do not exert large effects on the AAO phenotype of AD.

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1. Introduction

Recent large-scale genome-wide association studies (GWAS) have identified several novel risk variants for Alzheimer's disease (AD) (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011). However, these single nucleotide polymorphisms (SNPs) occur commonly in the general population and exert small effect sizes, making it unlikely that they will be of clinical use in predicting disease risk in older individuals (Seshadri et al., 2010). As the primary outcome in conventional GWAS in AD is the identification of variants associated with increased disease risk, the case versus control design in these studies largely ignores heritable variations in several other disease-related phenotypes that may be revealing of pathogenesis and of clinical use. Examples of such potentially heritable phenotypes include clinical measures such as rates of cognitive decline (Ruiz et al., 2013) and those related to disease pathology such as brain atrophy (Furney et al., 2011; Meda et al., 2013; Potkin et al., 2009), amyloid deposition, and tau accumulation/phosphorylation (Bekris et al., 2012; Cruchaga et al., 2013; Han et al., 2010; Kim et al., 2011). Some recent studies have examined whether SNPs associated with AD risk were also related to other phenotypes such as rates of decline in memory performance and cerebrospinal fluid measures of AD pathology (Alexopoulos et al., 2011; Kauwe et al., 2011; Sweet et al., 2012).

Age at onset (AAO) of AD is a phenotype that is believed to be mediated by a heritable component distinct from disease risk (Dickson et al., 2008; Holmans et al., 2005). Modeling studies have suggested that upto 40% of variability in AAO of AD may be heritable (Daw et al., 1999, 2000; Li et al., 2002). *APOE* genotype, the most robust genetic factor associated with risk for AD, only explains about 10% of variation in AAO of AD (Slooter et al., 1998), and it has been suggested that there might be several other loci with effect sizes on AAO comparable with that of *APOE*. In this study, we examined whether AD risk variants identified by recent large-scale GWAS also influenced the AAO phenotype in AD patients. We also compared the relative contributions of these novel AD risk genes with *APOE* in explaining variation in AAO.

2. Materials and methods

2.1. Subjects

Data used in this analysis were derived from "Alzheimer's Disease Center (ADC) cohorts 1–3" from the 29 National Institute on Aging (NIA)–funded ADCs, with data coordinated by the National Alzheimer's Coordinating Center. Access to the data was facilitated by the National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site, a national genetic data repository that facilitates

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Table 1
Demographic details of subjects included in this analysis

	N	Sex, n (%)	AAO (SD) range
Total	2569	Male, 1182 (46%)	72.6 (7.9)
		Female, 1387 (54%)	43–97
Phase 1	1708	Male, 746 (44%)	72.2 (7.6)
		Female, 962 (56%)	45–97
Phase 2	861	Male, 436 (51%)	73.3 (8.3)
		Female, 425 (49%)	43–96
Difference *p* value		0.0008	0.0021

Key: AAO, age at onset; SD, standard deviation.

access of genotypic data to qualified investigators for the study of the genetics of late-onset AD. Detailed descriptions of the ADC1, 2, and 3 cohorts are available at https://www.alz.washington.edu/ and have previously been described in several publications (Beekly et al., 2004, 2007; Morris et al., 2006; Weintraub et al., 2009).

2.2. Genotyping and SNPs of interest

Methodological details on genotyping, data cleaning, and quality control in the ADC samples have been described by Naj et al. (2011) in their recent publication reporting the identification of several novel AD risk variants in a large GWAS. Briefly, genotyping in the ADC1 and ADC2 samples was performed on the Illumina 660 high-density SNP microarrays and in the ADC3 samples on the Illumina OmniExpress platform. APOE genotyping was performed using SNPs rs7412 and rs429358. In this analysis, we selected the AD risk variant SNPs reported in recent large GWAS to examine their effect on AAO of AD. These included SNPs in the following genes: *CLU* (rs11136000), *PICALM* (rs3851179), *BIN1* (rs744373), *CR1* (rs3818361), *ABCA7* (rs3764650), *MS4A6A* (rs610932), *MS4A4E* (rs670139), *EPHA1* (rs11767557), *CD33* (rs3865444), and *EXOC3L2* (rs597668).

The criteria we adopted for the selection of these specific SNPs in the current report were as follows:

(1) Significant association with AD risk in a large index GWAS (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011) and
(2) Replication of the reported SNP's association with AD risk by independent GWAS and/or by meta-analysis of other GWAS data (Carrasquillo et al., 2011; Hu et al., 2011; Jun et al., 2010; Shang et al., 2013).

Where such independent replication for individual SNPs was not available in the case of *ABCA7* (rs3764640) and *MS4A6A/MS4A4E* (rs610932, rs670139), we selected these SNPs based solely on their reported association with AD risk in the index GWAS.

2.3. AAO of AD

Data on the AAO of AD were collected in the ADC1–3 cohorts in two phases, as described in: https://www.alz.washington.edu/ and in previous publications (Beekly et al., 2004, 2007; Morris et al., 2006; Weintraub et al., 2009). Phase 1 data were collected from ADC enrollees between 1984 and 2005. Phase 2 data were collected between 2005 and the present. Demographic details of subjects included in the analysis are shown in Table 1. This analysis was restricted to Caucasian subjects with a diagnosis of AD.

2.4. Statistical analysis

General linear models were used with AAO of AD as the dependent variable and the number of AD risk alleles of each gene as the main predictor in separate models. Other covariates included sex and the data collection phase. As the analysis was restricted to SNPs associated with increased risk of AD, our a priori hypothesis was that the number of risk alleles of each gene would be negatively correlated with the AAO of AD, that is, the presence of a greater number of AD risk alleles would be associated with an earlier AAO. We report our results as 1-sided *p* values for significance and after adjusting for multiple comparisons using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995).

3. Results

Data on AAO of AD were available in 2569 subjects (Table 1). There were significant differences in the sex distribution between phase 1 and phase 2 samples ($p = 0.0008$) with a slightly earlier AAO for males relative to females (1.92 ± 0.31 years, $p < 0.0001$). The mean AAO of AD in phase 1 and phase 2 samples were 72.2 ± 7.6 and 73.3 ± 8.3 years, respectively ($p = 0.0021$). We therefore entered sex and data collection phase as covariates in our analysis on the effect of AD risk genes on AAO.

Table 2 shows the AD risk allele frequencies in each of the genes studied. *APOE* ε4 carriers had a significantly lower AAO of AD than ε4 noncarriers with a decrease in 3.02 years in AAO for each unit increase in the number of ε4 alleles ($p_{\text{FDR adjusted}} < 0.0001$). Moreover, APOE genotype explained 6.7% of variance in AAO of AD. Among the recently discovered AD risk genes, risk allele carriers of the AD variant of *PICALM* (rs3851179) showed a significantly lower AAO than noncarriers, with a decrease in 0.55 years in AAO of AD for each unit increase in the number of risk alleles ($p_{\text{FDR adjusted}} = 0.0473$). Variation at the PICALM gene explained 0.24% of variance in AAO of AD. None of the SNPs in the other genes tested showed a significant effect on AAO of AD (Table 3).

Table 2
Characteristics of the AD risk genes analyzed in the study

Gene	SNP	RAF	0 RA, n (%)	1 RA, n (%)	2 RA, n (%)	N missing
APOE	—	0.41	854 (33.9%)	1272 (50.5%)	394 (15.6%)	49
CLU	rs11136000	0.62	361 (14.2%)	1217 (47.7%)	974 (38.2%)	17
PICALM	rs3851179	0.66	287 (11.2%)	1169 (45.5%)	1112 (43.3%)	1
BIN1	rs744373	0.33	1144 (44.5%)	1132 (44.1%)	293 (11.4%)	0
CR1	rs3818361	0.21	1599 (62.4%)	841 (32.8%)	124 (4.8%)	5
ABCA7	rs3764650	0.10	2059 (80.2%)	483 (18.8%)	27 (1.1%)	0
MS4A6A	rs610932	0.59	414 (16.1%)	1283 (50%)	869 (33.9%)	3
MS4A4E	rs670139	0.41	638 (33.8%)	944 (50.0%)	305 (16.2%)	682
EPHA1	rs11767557	0.83	87 (3.4%)	724 (28.2%)	1758 (68.4%)	0
CD33	rs3865444	0.70	227 (8.8%)	1103 (42.9%)	1239 (48.2%)	0
EXOC3L2	rs597668	0.20	1225 (64.9%)	585 (31.0%)	77 (4.1%)	682

Key: RA, risk allele; RAF, risk allele frequency; SNP, single nucleotide polymorphism.