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No Association Between *CALHM1* Variation and Risk of Alzheimer Disease

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Abstract

A polymorphism in the calcium homeostasis modulator 1 gene (*CALHM1*) has recently been associated with risk of late-onset Alzheimer disease. We examined this variant (*rs2986017*) in 945 Caucasian Americans with late-onset Alzheimer disease and 875 age-matched Caucasian American controls. No association with risk of late-onset Alzheimer disease ($p = 0.368$ for genotypes; $p = 0.796$ for alleles) was observed in our sample. However, a potential modest association of minor allele homozygosity (TT) with an earlier age-at-onset was seen ($p = 0.034$).

Keywords

Alzheimer disease; *CALHM1*; genetic; association

Introduction

Late-onset Alzheimer disease (LOAD) is a neurodegenerative disease of complex etiology. Although it has an estimated heritability of 0.79 [Gatz et al. 2006], only the *APOE* gene (MIM# 107741) has been definitively associated with risk of LOAD. An association with a polymorphism in the calcium homeostasis modulator 1 gene (*CALHM1*; MIM# 612234), *rs2986017* (c.257T>C [p.L86P]), has recently been reported in four European and one US Caucasian populations totaling 2043 cases and 1361 controls [Dreses-Werringloer et al. 2008]. This candidate gene is located under a linkage peak on chromosome 10 [Kamboh 2004], and the induction of its expression was shown to trigger a decrease in amyloid β levels and an increase in secreted amyloid precursor protein α levels via a Ca^{2+} -dependent mechanism [Dreses-Werringloer et al. 2008]. The p.L86P variant did not appear to have a major impact on *CALHM1* expression though it was reported to significantly impair the function of *CALHM1* in vitro including its effect on amyloid precursor protein proteolysis [Dreses-Werringloer et al. 2008].

To replicate the association of the *CALHM1 rs2986017* polymorphism with LOAD, we have examined this polymorphism in 945 LOAD cases and 875 age-matched older controls from the U.S.

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Materials and Methods

The LOAD subjects were Caucasian Americans ($n = 945$; mean age-at-onset [AAO] 72.8 ± 6.2 [S.D.] years; 67.4% female; 7.7% autopsy-confirmed) recruited by the University of Pittsburgh Alzheimer's Disease Research Center. All cases were evaluated clinically and met criteria for probable or possible AD [McKhann et al. 1984] or by autopsy and met neuropathological criteria for definite AD [Mirra et al. 1991; National Institute on Aging 1997]. Controls were Caucasian Americans of age 60 or above with no psychiatric or neurological disorders ($n = 875$; mean age-at-baseline 73.8 ± 6.3 [S.D.] years; 59.3% female; 1.4% autopsy-confirmed). All experiments on human subjects were conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written consent of the subjects. The genetic study was approved by the University of Pittsburgh Institutional Review Board.

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. The GenBank nucleotide reference sequence for *CALHMI* is NM_001001412.3, and the protein reference sequence is NP_001001412.3.

The DNA samples were genotyped for *rs2986017* through direct sequencing of a 690-bp-long polymerase chain reaction product amplified by using previously reported primers for exon 1 of *CALHMI* [Dreses-Werringloer et al. 2008]. Automated cycle sequencing and capillary electrophoresis were performed in a commercial sequencing facility (Agencourt Bioscience, Beverly, MA). The sequence chromatograms were reviewed independently by two researchers for the assignment of *rs2986017* genotypes. *APOE* genotypes were either determined as previously described [Kamboh et al. 1995] or by TaqMan fluorogenic 5'-nuclease assays (Applied Biosystems, Foster City, CA).

Allele and genotype frequencies were calculated by the direct counting method. Goodness of fit to Hardy-Weinberg equilibrium (HWE) was tested using the χ^2 test. Differences between genotype and allele frequencies in cases and controls were tested with the χ^2 test. The subjects were also stratified by *APOE**4 carrier status, and the cases and controls in each subgroup were compared with the χ^2 test. Association with age-at-onset was tested with ANOVA in the LOAD sample with sex and *APOE* genotype as covariates. Statistics were calculated using R 2.2.0 [R Development Core Team 2005].

Results

Genotype and allele frequencies for *rs2986017* can be found in Table 1. The genotype frequencies did not differ from HWE in cases ($p = 0.451$), but did in the controls ($p = 0.007$). There was no statistically significant difference between cases and controls for either genotypes ($p = 0.368$) or alleles ($p = 0.796$). No statistically significant differences were observed between cases and controls stratified by *APOE**4 carrier status (data not shown). Our power to detect the odds ratio of 1.44 reported by Dreses-Werringloer et al. [2008] was 0.998 at $\alpha = 0.05$ and 0.988 at $\alpha = 0.01$.

The adjusted mean age-at-onset for individuals with *rs2986017* genotypes CC, CT and TT were 72.8 ± 6.1 , 73.1 ± 6.1 and 71.4 ± 6.1 , respectively ($p = 0.088$). When CC and CT were grouped together and compared to TT (recessive model), the adjusted means were 73.0 ± 6.1 versus 71.4 ± 6.1 ($p = 0.034$).

Discussion

Chromosome 10 has repeatedly been reported to contain a linkage peak for Alzheimer disease [Kamboh 2004], but no gene on chromosome 10 has so far been consistently associated with LOAD, despite many promising candidates. Our results fail to lend support to the hypothesis that *CALHM1* at 10q24 is associated with the risk of LOAD, despite our excellent power for detecting the previously reported effect size of *rs2986017*. Although our sample size was smaller than the combined size of five different samples included in the prior report [Dreses-Werringloer et al. 2008], it was larger than each of those five separate collections. While our study was in progress, recently Bertram et al. [2008] have also reported no association of *CALHM1 rs2986017* with AD risk.

Dreses-Werringloer et al. [2008] examined *rs2986017* in five Caucasian AD case-control samples, of which two case samples, the France I and U.K. samples, were not in HWE for *rs2986017* ($p = 3.98 \times 10^{-5}$ and $p = 3.46 \times 10^{-5}$, respectively). However in their combined sample, both the controls and the cases significantly deviated from HWE ($p = 0.012$ and $p = 1.10 \times 10^{-5}$, respectively). In our sample, the controls were not in HWE ($p = 0.007$), but our cases were ($p = 0.451$). One possible source for this disequilibrium could be due to the presence of a length polymorphism in the region around *rs2986017*. Dreses-Werringloer et al. [2008] examined the possibility of a common copy number variant and concluded against the presence of one, but could not preclude the existence of a short sequence variation near the SNP. In addition to the sequencing-based assay we also used a chip-based assay to genotype this SNP and found comparable results. Thus a possible genotyping error is not an explanation of departure from HWE. It is noteworthy that the frequency of the less common allele in our U.S. Caucasian cohort (0.288) was similar to the reported frequency in multiple U.S. white family-based samples (0.28–0.31) [Bertram et al. 2008].

Dreses-Werringloer et al. [2008] also reported evidence for the association of *CALHM1* with age-at-onset of LOAD significant only in combined analysis ($p = 0.03$), with homozygotes for the putative risk allele (T) of *rs2986017* developing LOAD earlier than those with other genotypes (recessive model). In our LOAD sample, the cases who had TT genotypes also presented with an earlier onset of disease than those with the other two genotypes ($p = 0.034$). However, these modest associations would not stand if adjusted for multiple comparisons, and they warrant further consideration.

Although *CALHM1* shows promise as a candidate gene in its initial report of association, its effects on the risk and age-at-onset of LOAD need to be studied further to shed light into its possible role in Alzheimer disease. Additional studies will determine whether *CALHM1* will also join a growing list of genes that have failed consistent efforts at replication following an initial, exciting association with LOAD.

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Table 1

Genotype and allele frequencies for *CALHMI* rs2986017

		AD		Control	
		<i>n</i>	freq.	<i>n</i>	freq.
Genotypes:	CC	489	0.517	460	0.526
	CT	375	0.397	326	0.373
	TT	81	0.086	89	0.102
		945	1.000	875	1.000
Alleles:	C	1353	0.716	1246	0.712
	T	537	0.284	504	0.288