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CALHM1 polymorphism is not associated with late-onset Alzheimer Disease

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Abstract

Data suggests that the P86L polymorphism (rs2986017) in the calcium homeostasis modulator 1 (CALHM1) gene interferes with CALHM1 functionality, increases Aβ levels, and is associated with late-onset Alzheimer's disease (LOAD). Dreses-Werringloer et al (2008) demonstrate association with P86L and LOAD in three of five case-control cohorts, and a joint analysis of all datasets showed association with a p-value of 2×10^{-10} and an allele-specific odds ratio of 1.44 (2,043 cases, 1,361 controls total).

In this short communication we attempt to replicate these results in our case-control cohort (510 cases, 524 controls). We show no association between P86L and LOAD despite having sufficient power to detect at the reported odds ratios, and briefly discuss potential reasons for the discrepancy.

Introduction

Alzheimer disease (AD) is a neurodegenerative disorder and is the leading cause of dementia in the elderly. AD is characterized by neuronal atrophy and the formation of amyloid-β (Aβ) plaques. Aβ is generated by processing of the amyloid-β precursor protein (APP). Functional data suggests that the *calcium homeostasis modulator 1* (*CALHM1*) gene plays a role in controlling cytosolic Ca^{2+} levels and in controlling APP processing. The *CALHM1* P86L polymorphism (rs2986017) was shown to hinder membrane Ca^{2+} permeability, increasing Aβ levels and was associated with Late Onset Alzheimer Disease (LOAD) in three case-control populations ranging in size from 365 cases and 205 controls to 710 cases and 565 controls (U. Dreses-Werringloer et al, 2008). Two additional populations had non-significant results that trended in the same direction. A joint analysis across all datasets for rs2986017 showed association with a p-value of $2x10^{-10}$ and an allele-specific odds ratio of 1.44 (2,043 cases, 1,361 controls). To validate this result, we genotyped the functional SNP rs2986017 in 510 cases and 524 controls and tested for association between the SNP and LOAD.

Methods

Ascertainment and Samples

Our sample set is derived from the Collaborative Alzheimer Project (CAP: The Miami Institute for Human Genomics at the University of Miami Medical Center and The Center for Human Genetics Research at Vanderbilt University Medical Center). After a complete description of the study to the subjects, written informed consent was obtained from all

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participants in agreement with protocols approved by the institutional review board at each contributing center. For inclusion, each LOAD affected individual met the NINCDS-ADRDA criteria for definite or probable AD and had an age at onset greater than 60 years of age (McKhann, Drachman and Folstein 1984). Age at onset (AAO) for LOAD was determined from specific probe questions within the clinical history provided by a reliable family informant or documented significant impairment in the medical record. Cognitive controls were spouses, friends, and other biologically unrelated individuals who were frequency age and gender matched to the cases, and all were from within the same clinical catchment areas. All cognitive controls were examined and none showed signs of dementia by history and upon interview. Additionally, cognitive controls each have a documented Mini-Mental State Exam (MMSE) ≥ 27 or Modified Mini-Mental State Exam (3MS) ≥ 87 (Teng and Chui 1987). Genotypes were obtained for a total of 1,034 individuals of mixed European ancestry. There were 510 LOAD cases with average aged at onset of 73 years, and 524 cognitive controls with average age at exam of 74 years.

Genotyping

The rs2986017 assay was designed using Sequenom SpectroDESIGNER software. 5 ng genomic DNA was amplified following the manufacturer recommendations. Single primer extension over the SNP was carried out in a final concentration of 1.25 uM of the extension primer. The extension step followed the manufacturer's procedure. The reaction was then desalted by addition of 6 mg of resin followed by 15 min mixing and centrifugation (3000rpm) to settle the contents of the plate. The extension product was then spotted onto a 384 well spectroCHIP before being flown in the MALDI-TOF mass spectrometer. Data was collected in real time using SpectroTYPER Analyzer, SpectraAQUIRE and SpectroCALLER (Sequenom). Additionally, to ensure data quality, genotypes for each subject were also checked manually, and genotyping of 96 individuals was checked by direct sequencing of a 207 bp fragment amplified using the forward primer 5′-CCTGGTGCTCTTTCTGCTTG-3′ and reverse primer 5′-CAGAAGGCAGAGGAAGCA-3′ (U. Dreses-Werringloer et al, 2008).

Statistical Analysis

Power calculations were performed using the Genetic Power Calculator (Purcell, Cherny, and Sham, 2003). Hardy-Weinberg Equilibrium was calculated in controls using the exact test in Plink (Purcell et al, 2007). Association was tested using the Armitage trend test in Plink.

Results and Discussion

We found no association between the *CALHM1* SNP, rs2986017 and LOAD. The T allele, the risk allele in Dreses-Werringloer et al, had a frequency of 0.27 among cases and 0.26 among controls (p=0.63; odds ratio=1.05, [95% CI: 0.86, 1.27]; Table 1). For the previously reported odds ratios [U. Dreses-Werringloer et al, 2008] (CT odds ratio = 1.4, TT odds ratio = 2.0) our sample has 95% power to detect an effect. The genotypes for all 96 sequenced samples were in agreement with the results from the Sequenom genotyping. Genotypes were obtained on 1,034 individuals out of 1,042 total individuals, an efficiency of over 99%. The SNP was in Hardy-Weinberg equilibrium within both the control cohort (p=0.11) and case cohort (p=0.22).

Our results do not confirm the risk of LOAD associated with this CALHM1 polymorphism. We have 95% power to detect the reported effect and excellent genotyping results, but the 95% confidence interval does not overlap with their combined confidence interval. This strongly implies that the Dreses-Werringloer genetic association at rs2986017 is a false

positive. However, we cannot completely rule out a true association. Although we had sufficient power to detect the effects reported by U Dreses-Werringloer et al (2008), it is likely that any true odds ratios are less, as initial results often over-estimate effects sizes. Subsequent follow-up studies then fail to detect association since the actual effect size is much smaller, a phenomenon known as “Winner's curse” (HH Goring et al, 2001; P Kraft, 2008). We had 80% power to detect an odds ratio of 1.3, suggesting that any effect of the CALHM1 polymorphism, if it exists, will be smaller than this.

It is possible that variation in CALHM1 may confer risk only in individuals with particular genetic backgrounds or environments. If the Dreses-Werringloer cohorts (2008) were enriched for the susceptibility backgrounds or environments and our cohorts were not, then they would detect association and we would not. However, this potential reason for failure to replicate is highly unlikely. Dreses-Werringloer reported association in multiple populations in the original report, implying that there is no population specific susceptibility background. Our finding of no association shows that if CALHM1 does confer risk to LOAD, its effect is small or is limited to a subset of the population.

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Table 1

rs2986017 allele and genotype counts

		Allele		Genotype		
		C	**T**	**CC**	**CT**	**TT**
Dreses-Werringloer	Cases (n=2,043)	2881 (0.71)	1205 (0.29)	1057 (0.52)	767 (0.37)	219 (0.11)
	Controls (n=1,361)	2110 (0.77)	612 (0.23)	834 (0.61)	442 (0.32)	85 (0.06)
CAP	Cases (n=510)	746 (0.73)	274 (0.27)	267 (0.52)	212 (0.42)	31 (0.06)
	Controls (n=524)	776 (0.74)	272 (0.26)	280 (0.53)	216 (0.41)	28 (0.06)

Allele and genotype counts are displayed with frequencies in parentheses.