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Lack of association of two chromosome 10q24 SNPs with Alzheimer's disease

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Abstract

Several groups have reported evidence of linkage on chromosome 10 to late-onset Alzheimer's disease (LOAD). In a recent scan of single nucleotide polymorphisms (SNPs) on chromosome 10, significant associations between the *rs498055* and *rs4417206* SNPs and risk of LOAD were observed. We examined the association of these two SNPs with LOAD risk in a large Caucasian American cohort comprising about 2000 cases and controls. Neither SNP revealed significant association with LOAD risk or age-at-onset.

Keywords: Association; Chromosome 10; Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder with complex environmental and genetic involvement. Sequence variation in a few genes explains many cases of familial early-onset (age-at-onset [AAO] <60 years) AD, but these cases account for a small fraction of total cases [14]. Only the association between *APOE* genetic variation and risk of late-onset AD (LOAD) has been replicated consistently. However, the *APOE* gene explains only part of the genetic variation associated with LOAD risk.

Several groups have observed linkage with risk of AD or related measures on chromosome 10 [6], but the identification of the LOAD gene on this chromosome remains elusive. A recent scan of chromosome 10 identified several potential single nucleotide polymorphisms (SNPs) associated with LOAD in four Caucasian case–control samples. Two SNPs, *rs498055* and *rs4417206*, revealed the most significant association in the combined sample ($p = 0.00004$ and $p = 0.0019$, respectively). SNP *rs498055* is located in an *RPS3A* homologue, and *rs4417206* is located in the *ALDH18A1* gene. They are approximately 41 kb apart, but in strong linkage disequilibrium [3]. The purpose of this study was to replicate the reported association in a fairly large case–control Caucasian American sample.

Case subjects for this study were Caucasian Americans with LOAD ($n = 1138$; mean AAO 72.5 ± 6.3 [S.D.] years; 67.7% female; 24.1% autopsy-confirmed) recruited through the University of Pittsburgh Alzheimer's Disease Research Center. All cases were either evaluated clinically and satisfied criteria for probable or possible AD [7] or by autopsy and satisfied neuropathological criteria for definite AD [8–9]. The control subjects ($n = 945$, mean age at baseline 74.1 ± 6.2 years, 59.5% female, 1.7% autopsy-confirmed) were Caucasian Americans age 60 or above with no psychiatric or neurological disorders. This study was approved by the University of Pittsburgh Institutional Review Board.

The *rs498055* and *rs4417206* SNPs were genotyped by template-directed dye terminator incorporation assays detected with fluorescence polarization (TDI-FP). Polymerase chain reaction (PCR) primers for *rs498055* were 5′-CGCCAAATCC-AGAAGATGA-3′ and 5′-TTGGCAAGCCTTTTCTATGTC-3′; the (reverse) internal primer for the dye terminator incorporation was 5′-CCTTTTCTATGTCTTTTCCAATGCT-3′. PCR primers for *rs4417206* were 5′-ATTTTTGGAAGGGCTCAGTG-3′ and 5′-CAACCAGGTGCAAAGAGAAG-3′; the (forward) internal primer was 5′-CACTGGCTTCTTTGATTTCAGG-3′. Previous publications' nomenclature for *rs498055* gives the alleles as they appear on the reverse strand of chromosome 10 (G/A) [2–4]; here we give the alleles as they appear on the forward strand of the *RPS3A* homologue (C/T). PCR was completed with 2 ng template DNA. *APOE* was genotyped as described previously [5].

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Both case and control samples were present on every plate. To assess genotyping error rate, 10% of samples were selected at random and repeated.

Allele frequencies were determined by direct allele counting. Goodness of fit to Hardy–Weinberg equilibrium and differences in genotype and allele frequencies between cases and controls were examined using the χ^2 test. Genotype and allele frequencies for each SNP were also stratified by the presence of *APOE*4*, by age at baseline in controls and AAO in cases and by diagnosis method (that is, clinically assessed or by autopsy); the frequencies of cases and controls were compared in each subgroup using the χ^2 test. The mean AAO for different genotypes was compared using one-way analysis of variance (ANOVA) and was adjusted for sex and *APOE*4* carrier status. To assess linkage disequilibrium (LD) between the two SNPs, we calculated D' and r^2. We compared haplotype frequencies in cases and controls and examined the effect of haplotype on AAO, adjusted for sex and *APOE*4*. All statistical calculations were completed using R 2.2.0 [13] with the genetics and haplo.stats packages attached.

Genotyping efficiency was 92.4% for *rs498055* and 88.2% for *rs4417206* with genotyping error about 1.2%. Genotype and allele frequencies in cases and controls for both SNPs are shown in Table 1. Genotype frequencies for *rs498055* did not deviate with statistical significance from those expected under Hardy–Weinberg assumptions. However, *rs4417206* did deviate in cases ($p = 0.024$), but not in controls ($p = 0.730$). The genotype frequencies of the various subgroups defined by *APOE*4* carrier status, AAO/age and diagnosis method did not significantly differ from Hardy–Weinberg equilibrium for either SNP. LD between the two SNPs was estimated as $D' = 0.87$ and $r^2 = 0.45$.

For neither SNP did genotype or allele frequencies differ with statistical significance between cases and controls. The minor allele frequency (MAF) of *rs498055* was 46.2% in cases and 46.9% in controls ($p = 0.900$). The MAF of *rs4417206* was 34.7% in cases and 33.3% in controls ($p = 0.147$). Differences within the *APOE*4*-, AAO/age- and diagnosis-method – stratified subgroups were also non-significant (data not shown). No statistical association between haplotype frequencies and risk of LOAD was observed ($p = 0.773$).

Neither SNP had a statistically significant effect on AAO (*rs498055*, $p = 0.947$; *rs4417206*, $p = 0.780$), nor did the any of the haplotypes defined by the two SNPs (p-value range: 0.867–0.581).

Table 1
Genotype and allele frequencies in cases and controls

	rs498055					*rs4417206*			
	Cases		Controls			Cases		Controls	
	n	%	*n*	%		*n*	%	*n*	%
CC[a]	300	28.3	237	27.4	TT[a]	441	44.3	372	44.2
CT[a]	541	51.0	445	51.4	TG[a]	419	42.1	378	44.9
TT[a]	219	20.7	183	21.2	GG[a]	136	13.7	91	10.8
Total	1060	100.0	865	100.0	Total	996	100.0	841	100.0
C[b]	1141	53.8	919	53.1	T[b]	1301	65.3	1122	66.7
T[b]	979	46.2	811	46.9	G[b]	691	34.7	560	33.3

[a] Genotype.
[b] Allele.

Of the 1412 SNPs examined by Grupe et al. [3], *rs498055* and *rs4417206* yielded the highest statistically significant results. Although there is strong LD between the two SNPs, the correlation between them is not very high. Our measures of LD ($D' = 0.87$; $r^2 = 0.45$) were similar to those determined by Grupe et al. ($D' = 0.98$; $r^2 = 0.43$) [3]. We were unable to verify the reported associations between *rs498055* and *rs4417206* and the risk of LOAD despite our sample of approximately 2000 cases and controls from a single geographical location, a sample comparable to the combined approximately 2000 cases and controls from three locations reported on by Grupe et al. While the MAF of *rs498055* in our LOAD cases is very similar to the reported average frequency in U.S. cases (53.8% in our sample versus 54.3% in Grupe et al.; $p = 0.779$), it is higher in our controls than the U.S. controls reported by Grupe et al. (53.1% versus 47.3%; $p = 0.0014$). On the other hand, the MAF of *rs4417206* is similar in both control groups (33.5% versus 35.6%; $p = 0.180$), but slightly different in case groups (34.7% versus 31.2%; $p = 0.0197$). There is no clear explanation of this odd observation where the frequencies of one SNP were similar between the two case groups while the frequencies of the second SNP were similar between the two control groups. More importantly, the frequencies of the established risk factor – *APOE* – are similar between our case–control cohort (*APOE*4*: 33.5% in cases and 11.2% in controls) and Grupe et al. (35.6% and 12.5%) [4]. Thus, the difference in chromosome 10 SNPs does not appear to be due to population heterogeneity. The other possibility is non-random genotyping errors in cases or controls. However, our error rate was only 1.2% and randomly distributed in cases and controls.

While our study was in progress, Bertram et al. [2] completed an examination of *rs498055* in two family samples. They also did not see any association between this SNP and risk or AAO of AD. In a reply, Grupe et al. [4] proposed that the discrepant findings between their study and Bertram et al. [2] could be due to reduced statistical power in studies involving a family-based sample. They provided evidence for this by comparing the risk allele frequencies of *APOE* and *rs498055* between cases and controls in their case–control series and in their linkage family sample; indeed the differences in allele frequencies were much greater in the case–control series than in the family sample. However, our case–control cohort has *rs498055* frequencies quite similar to Grupe et al. family sample (and to Bertram et al. LOAD families), but our *APOE* frequencies parallel their case–control series. Furthermore, our case–control sample is equally powered to that reported by Grupe et al. Our study has 98% power to detect the reported odds ratio (OR) of 1.3 for the *rs498055* SNP and 83% power to detect the reported OR of 0.81 for the *rs4417206* SNP. Thus, the lack of association in our sample is not likely due to insufficient power.

The lack of association of *rs498055* in our large case–control sample and in two family samples [2] suggests that the sought chromosome 10 gene may not be present in this region. Notably, no significant association of *rs498055* was found in autopsied confirmed cases and controls by Grupe et al. [3], which would

have been more convincing. Additionally we have examined five SNPs around the insulin degrading enzyme gene (IDE) located about 3 Mb proximal to the two SNPs examined in this study, and found no statistical evidence of association [10]. On the other hand, we have found suggestive associations with SNPs located in the *CHAT* and *PLAU* genes [11–12], which mark boundaries of an about 24 Mb region between 10q11.23 and 10q22.23, about 22 Mb proximal to the two SNPs studied here. Compelling evidence of the existence of a LOAD gene between 10q11.23 and 10q22.23 has also recently been provided using parent-of-origin information [1]. Additional comprehensive association studies are needed to identify the elusive chromosome 10 gene for LOAD.

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