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Association of *ABCA1* with late-onset Alzheimer's disease is not observed in a case-control study

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Abstract

Genetic association of *ABCA1* or the ATP-binding cassette A1 transporter with late-onset Alzheimer's disease (LOAD) has recently been proposed for a haplotype comprised of three single nucleotide polymorphisms (SNPs). We have genotyped these and other *ABCA1* SNPs in a LOAD case-control series of 796 individuals (419 cases versus 377 controls) collected at Washington University. While our sample series is larger and thus presumably has greater power than any of the series used to implicate *ABCA1*, we were unable to replicate the published association, using either single markers or multiple marker haplotypes. Further, we did not observe significant and replicated association of other *ABCA1* SNPs we examined with the disease, thus these *ABCA1* variants do not appear to influence the risk of LOAD in this study.
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Genetic studies have provided substantial evidence implicating several genes in the etiology of late-onset Alzheimer's disease (LOAD), a neurodegenerative disease affecting memory and cognition in the elderly (see, e.g., [4,8,12]). With the exception of the *apolipoprotein E* (*APOE*) gene [10], however, other genetic risk factors have not been identified or, at the best, remain controversial, as most initially observed associations between genetic variation and LOAD have not been consistently replicated (see, e.g., [1]). As genetic association studies in case-control series are prone to produce type I errors, replication studies are essential to differentiate true associations from a spurious discovery.

Very recently, genetic variation of *ABCA1* or the ATP-binding cassette A1 transporter has been proposed to modify the risk of Alzheimer's disease (AD) [3,13]. In AD case-control series of Swedish and British origin, Katzov et al. [3] reported an association between several missense SNPs in *ABCA1* and both early and late-onset AD. The association was strongest between AD and haplotypes constructed with three of the tested missense SNPs. The ABCA1 transporter is a membrane-associated protein that functions as a cholesterol efflux pump in the cellular lipid removal pathway, thus making *ABCA1* a potential AD candidate gene. The gene encoding *ABCA1* is located near the previously detected AD linkage peak on chromosome 9 [6].

We have genotyped a LOAD case-control series of 796 Caucasian individuals (419 cases versus 377 controls) collected at Washington University in St. Louis (WashU) to identify SNPs significantly associated with LOAD.

Table 1
Allelic tests of *ABCA1* SNPs with LOAD

SNP ID	SNP type	Sample	Stratum	Case[a]					Control[a]					*P*-value[b]
				11	12	22	Sum	MAF	11	12	22	Sum	MAF	
hCV2741108	Intron	WashU	ALL	0	22	367	389	0.028	0	22	331	353	0.031	0.743
rs2777799	Intron	WashU	ALL	8	84	299	391	0.128	10	73	270	353	0.132	0.825
<u>rs2230808</u>	Missense	WashU	ALL	37	128	254	419	0.241	22	145	209	376	0.251	0.635
<u>rs4149313</u>	Missense	WashU	ALL	8	89	321	418	0.126	3	87	287	377	0.123	0.890
rs2066715	Missense	WashU	ALL	0	37	382	419	0.044	0	42	335	377	0.056	0.289
rs2066718	Missense	WashU	ALL	1	29	389	419	0.037	2	17	358	377	0.028	0.306
			E4−	1	17	160	178	0.053	2	11	275	288	0.026	0.031
			E4+	0	11	229	240	0.023	0	6	81	87	0.035	0.411
		UCSD	ALL	0	17	186	203	0.042	1	28	365	394	0.038	0.749
			E4−	0	8	65	73	0.055	1	21	277	299	0.039	0.376
			E4+	0	8	119	127	0.032	0	7	86	93	0.038	0.726
		UK	ALL	1	26	331	358	0.039	0	16	379	395	0.020	0.030
			E4−	1	7	133	141	0.032	0	12	288	300	0.020	0.279
			E4+	0	19	198	217	0.044	0	4	91	95	0.021	0.166
rs6479283	Intron	WashU	ALL	8	103	278	389	0.153	12	94	240	346	0.171	0.361
			E4−	2	37	124	163	0.126	10	75	179	264	0.180	0.036
			E4+	6	66	154	226	0.173	2	19	60	81	0.142	0.368
		UCSD	ALL	6	51	130	187	0.168	13	86	258	357	0.157	0.621
			E4−	3	17	47	67	0.172	11	67	196	274	0.162	0.796
			E4+	3	33	81	117	0.167	2	19	60	81	0.142	0.506
<u>rs2230806</u>	Missense	WashU	ALL	33	163	222	418	0.274	32	142	202	376	0.274	0.962
rs1883025	Intron	WashU	ALL	25	154	211	390	0.262	25	144	186	355	0.273	0.610

The underlined SNPs are those reported in the Katzov et al. publication [3].

[a] Counts of genotypes 11, 12, and 22 and minor allele frequency (MAF) are presented.

[b] Allele 1 vs. allele 2 *P*-value is presented.

Significant associations were then followed up in one or two additional case-control series collected near San Diego (UCSD, 203 cases versus 394 controls) and in the UK (358 cases versus 395 controls), respectively. Cases have a clinical diagnosis of dementia of the Alzheimer's type according to NINCDS-ADRDA [5] or similar criteria with an age of disease onset of 65 years or more. Demographic information are as follows: Age at assessment, 77.5 ± 7.5 years (WashU), 78.8 ± 7.4 years (UCSD), and 75.7 ± 7.0 years (UK); average MMSE score ranges from 10.9 to 18.8 for cases and is 29 for controls. Other detailed characteristics of the samples including gender, age, and *APOE* status have been described elsewhere [7], but we note here that the series has an expected *APOE* genotype distribution and that there was no evidence of population stratification among the three case-control series using the program STRUCTURE [9].

Genotyping of SNPs was done by allele specific real time PCR for individual samples [2]. Cases and controls were always run on the same plate in a blinded fashion. Overall, genotyping accuracy is expected to be better than 99%, as determined by internal and across group comparisons.

For quality control of genotyping results, an exact test was used to determine whether the observed genotype frequency deviated from Hardy–Weinberg equilibrium in case or control samples. Chi-square tests were then used to assess allelic and genotypic associations. Odds ratios and 95% confidence intervals were calculated for allelic and genotypic association tests. The program haplo.score [11], which uses an expectation maximization algorithm, was used to infer haplotype frequencies and assess the significance of the association between haplotypes and susceptibility to LOAD.

Nine SNPs in the *ABCA1* gene were genotyped in the WashU series. Three of these SNPs were identical to the reported 'haplotype tagging' SNPs from the study by Katzov et al. [3]. None of the SNPs showed significant allelic association with LOAD in the WashU series (Table 1). Two markers (rs2066718, rs6479283) showed a significant interaction with APOE4 in the WashU series, but did not replicate upon follow up genotyping in either one or two other independent case-control series. A meta analysis of the combined data from the three sample series showed a marginal significant association between rs2066718 and LOAD in the absence of ApoE4 (Mantel–Haenszel $P = 0.022$). Using a model free genotypic test, we were also unable to replicate the significant genotypic association of rs2230806 reported by Katzov et al. [3] ($P = 0.912$). In another paper by Wollmer et al. [13] association of rs2230806 with LOAD was not detected either, although they reported that this SNP affects the age at onset of LOAD. rs2230808 reached marginal significance in our sample set with this test ($P = 0.031$; homozygous odds ratio: 1.38, 95% confidence interval: 0.79–2.42; heterozygous odds ratio: 0.73; 95% confidence interval: 0.54–0.98), but this marker lacked significance in any of the LOAD sample sets from the Katzov et al. [3] or the Wollmer et al. [13] publications. Testing of Hardy–Weinberg equilibrium for rs2230808 failed in cases ($P = 0.001$), but we did not find any questionable genotype calls. rs2230808 showed significant genotypic association in the Katzov study with an

Table 2
Measures of pairwise D' and r^2 in the WashU controls

	hCV2741108	rs2777799	rs2230808	rs4149313	rs2066715	rs2066718	rs6479283	rs2230806	rs1883025	Distance[a]
hCV2741108		0.16	1.00	0.18	0.14	0.04	0.21	0.04	0.05	0.00
rs2777799	0.01		0.90	0.01	0.50	0.10	0.07	0.15	0.06	6.44
rs2230808	0.10	0.37		0.10	0.03	0.26	0.21	0.24	0.07	3.75
rs4149313	0.01	0.00	0.01		1.00	0.96	0.45	0.29	0.08	23.95
rs2066715	0.01	0.00	0.00	0.42		0.30	0.96	1.00	0.02	1.28
rs2066718	0.00	0.00	0.01	0.00	0.00		0.77	0.77	0.33	1.22
rs6479283	0.01	0.00	0.03	0.14	0.26	0.00		0.32	0.30	12.54
rs2230806	0.00	0.01	0.05	0.03	0.02	0.05	0.05		0.21	19.06
rs1883025	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.04		43.43

D' is shown in the upper left half, and r^2 is shown in the lower right half.

[a] Distance between markers in kbp.

Table 3
Haplotypic tests of *ABCA1* SNPs with LOAD

Haplotype[a]	rs2230806	rs4149313	rs2230808	Case frequency	Control frequency	P-value[b]
H1	G	T	G	0.545	0.541	0.814
H2	G	T	A	0.131	0.121	0.881
H3	A	T	G	0.134	0.125	0.745
H4	A	T	A	0.065	0.089	0.205
H5	A	C	G	0.043	0.036	0.500
H6	G	C	G	0.038	0.046	0.495
H7	A	C	A	0.032	0.024	0.578
H8	G	C	A	0.012	0.018	0.583

Global P-value = 0.822.

[a] Haplotypes H1–H6 correspond to those reported in the Katzov et al. publication [3].

[b] P-values are calculated according to Schaid et al. publication [11].

early-onset AD case-control series, but the odds ratio effect was in the opposite direction when compared to the WashU series results.

Prompted by the Katzov et al. [3] results, we re-analyzed their reported significant 3-marker haplotype in our WashU set. Pairwise linkage disequilibrium (LD) measures for all nine markers were calculated (Table 2). In the WashU set, all three reported tagging markers appear to be in different LD-blocks and show comparable LD as reported by Katzov et al. [3]. The overall haplotype frequencies between the two studies are comparable, but were nearly identical between cases and controls in the WashU set, thus making them statistically insignificant when testing for an association with LOAD (Table 3). Therefore, we could not replicate the LOAD association with the *ABCA1* SNPs or haplotypes reported by Katzov et al. [3], neither did any of our other six SNPs show significant and replicated association with LOAD. It is worth noting that the size of the WashU case-control series provides 93% power, assuming a type 1 error of 0.05, to replicate the haplotype association of H5 reported by Katzov et al. [3] when the combined results of all their LOAD sets are assumed to be representative of LOAD and control haplotype frequencies.

In summary, we analyzed nine SNPs in the *ABCA1* gene but were unable to find significant and replicated association with LOAD, using either single marker or haplotype analyses. Overall, we failed to replicate the results of a previously published study by Katzov et al. [3], which reported genetic association of *ABCA1* variants with LOAD.

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