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ABCA1 polymorphisms and Alzheimer's disease

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Abstract

In our search for genetic factors related to the development of Alzheimer's disease, we have genotyped 332 pedigrees for three coding polymorphisms in the *ABCA1* gene, two of which are known to alter plasma cholesterol levels, as well as a non-coding polymorphism within the promoter. We show an apparent weak association of rs2230806 (p-value = 0.01) with the disease in a sibpair series of Alzheimer's disease that had shown previously evidence for linkage to the chromosome 9 locus where *ABCA1* maps.

Keywords: *ABCA1*; Alzheimer's disease; Polymorphisms

Despite intensive effort, the only universally acknowledged risk factor locus for late onset Alzheimer's disease is the apolipoprotein E gene on chromosome 19 [6,11]. The known biological function of apolipoprotein E is in cholesterol metabolism and there has been much interest about the role of this metabolism in Alzheimer pathogenesis [4]. The ABCA1 transporter is a membrane-associated protein that functions as a cholesterol efflux pump in the cellular lipid removal pathway. The gene encoding ABCA1 is located near the previously detected AD linkage peak on chromosome 9 at position q22 [1,13,17,18]. With this background, *ABCA1* makes a good positional and functional candidate gene for Alzheimer's disease though previous studies at this locus in Alzheimer's disease show some confliction. Some articles found positive association [12,22,26], while others reported a negative association [15], or even had suggested that it influenced the age at onset, but not the risk of disease [12,26]. Nevertheless, most of those studies being done on case-controls series, we decided to investigate the impact of this gene in our sibpairs series of Alzheimer's disease.

Homozygous mutations in the *ABCA1* gene are associated with Tangier's disease [2,3,21] and heterozygosity for mutant alleles has been reported to influence peripheral cholesterol metabolism [5]. With this in mind, we analyzed the effect that genetic variability, both frequent coding and promoter variants, at the *ABCA1* gene had on the risk for Alzheimer's disease. We selected variants which were shown as potential risk factors for coronary atherosclerosis in the general population [16] further reinforcing our hypothesis as atherosclerosis and cerebrovascular disease have been related to the pathogenesis of Alzheimer disease because of a strong association with frequent neuritic plaques [10].

We selected 4 SNPS among the most informative ones for which restriction digest assays could be easily designed and ran those through the sample series in which we had observed linkage to markers on chromosome 9 [1,13,17]; in addition to supplementary families that became available subsequent to this analysis. Genotypes were generated for 332 pedigrees, represented by 899 persons, which are largely described in our genetic linkage study [17]. In summary, sibpairs affected with the late onset form of AD (680 confirmed affected samples with 71.8% female, mean age at onset = 73.7 ± 8.26 y) were selected from the National Institute of Mental Health (NIMH) and the National Cell Repository for Alzheimer's Disease (NCRAD; grant number, U24 AG21886); as well as their unaffected siblings when available (125 individuals with 55.2% female, mean age = 75.8 ± 6.77 y).

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The SNPs used were rs2230806 (also designated as rs2234884) which encodes variant R219K (c.969A > G, numbering according to GenBank reference NM_005502) with a reported ~25% minor allele frequency in Caucasian populations [5,7], rs2066718 which is a V771M (c.2624A > G) variant with a reported ~3% minor allele frequency [5,7], rs2230808 which encodes a R1587K (c.5073A > G) variant with a reported 26% minor allele frequency [5,7] and finally rs2422493 which is a 5′UTR polymorphism located at −477 in the promoter with a reported 46% minor allele frequency [23,25]. Subsequent to us choosing these SNPs, rs2230808 and rs2066718 variants were reported to be associated with variability in plasma HDL levels [7–9] and with plasma cholesterol levels; which further validated their use in this association study. Further information on those polymorphisms can be found at Alzforum website (http://www.alzforum.org/res/com/gen/alzgene/geneoverview.asp?geneid=88).

Genotyping was performed by RFLP method using the primers described [5]. Tests of association were performed using FBAT program (Version 1.5.1) with an additive model (http://www.biostat.harvard.edu/~fbat/default.html) for all four polymorphisms. The genotype frequencies were consistent with Hardy–Weinberg equilibrium. Given that there have been several reports of linkage in this region on chromosome 9 [1,17], we used the empirical variance option in FBAT (test distribution under the null hypothesis of linkage but no association) that adjusts for the correlation among sibling marker genotypes and for different nuclear families within a single pedigree.

Only T/T genotype of rs2422493 showed an association with the disease in the whole series ($p = 0.02$), while both A/G and G/G genotypes of rs2230806 were associated with the disease only in the ApoE-ε4 non-carriers ($p = 0.01$ and $p = 0.02$, respectively, Table 1). None of the analyzed polymorphisms showed significant linkage disequilibrium with the exception of rs2230806 and rs2230808 which showed evidence of linkage disequilibrium in the cases group ($D' = 0.237$; $p = 1.99 \times 10^{-5}$).

Subsidiary analyses of the different haplotypes in either the presence or absence of the apolipoprotein E4 allele gave different results (Table 2). The haplotypes were created with the following SNPs order: rs2422493–rs2230806–rs2066718–rs2230808. A weak association of the haplotype C-A-G-G ($p = 0.02$) with the disease is present in the ApoE-ε4 carrier; while haplotypes T-G-G-A ($p = 0.01$) and T-A-G-G ($p = 0.01$) showed an association with the disease in the ApoE-ε4 non-carrier; though, the unimpressive overall p-value for this analysis ($p = 0.03$) suggests that the result cannot be relied on because the number of informative families for the second haplotype is too low (2.6). However, the fact, that the genotypes G/G and A/G of rs2230806 also show an association with the disease in the same sub-group (E4 negative), and that the first haplotype occurs in a reasonable number of informative families (7.1), might support the existence of an association for the haplotype T-G-G-A with the disease. It is of interest to note that this result is concordant with the findings of Shibata et al. who also observed a significant association with the G allele of rs2230806 in the absence of the ApoE-ε4 allele [22]. The overall result suggests there might be a genetic influence of the *ABCA1* polymorphisms on Alzheimer's disease, as shown by other groups [12]. The results shown here have not been corrected for multiple testing. Indeed, since the tests performed were done under the null hypothesis, reducing the type I error for null association increases the type II errors for those associations that are not null [19,20]. A correction such as the Bonferroni test would make those results non-significant at the $p = 0.05$ level, while this is a very conservative correction it highlights that the data here should be considered as preliminary and further studies are necessary in order to make any conclusion.

Nevertheless, these results are of interest since it was recently showed that targeted disruption of ABCA1 considerably decreases brain ApoE level and increases amyloid deposition in APP transgenic mice [14]; while lack of ABCA1 causes poor lipidation of ApoE, which become strongly amyloidogenic *in vivo* [24]. These previous findings emphasize the

Table 1
Genotype frequency of *ABCA1* polymorphisms in USA sibpair series

SNP ID (function)		Total (Nf = 331)			ApoE-ε4− (Nf = 76)			ApoE-ε4+ (Nf = 227)		
		Fam	Freq	p-Value	Fam	Freq	p-Value	Fam	Freq	p-Value
rs2422493 (promoter)	C/C	45	0.266	0.62	5	0.245	N/A	25	0.284	0.62
	T/C	79	0.494	0.17	14	0.494	0.29	38	0.489	0.35
	T/T	44	0.241	0.02*	10	0.261	0.30	21	0.227	0.08
rs2230806 (R219K)	A/A	17	0.095	0.93	2	0.058	N/A	10	0.111	0.86
	A/G	66	0.399	0.23	12	0.483	0.01*	31	0.388	0.70
	G/G	56	0.507	0.21	11	0.459	0.02*	25	0.500	0.59
rs2066718 (V771M)	A/A	0	0	N/A	0	0	N/A	0	0	N/A
	A/G	14	0.100	0.69	2	0.162	N/A	6	0.102	N/A
	G/G	14	0.900	0.69	2	0.838	N/A	6	0.898	N/A
rs2230808 (R1587K)	A/A	15	0.066	0.06	5	0.044	N/A	8	0.064	N/A
	A/G	62	0.426	0.69	14	0.505	0.64	32	0.420	0.90
	G/G	56	0.509	0.55	13	0.451	0.57	29	0.515	0.67

Fam, number of informative families; Freq, frequency; Nf, number of nuclear families; N/A, non-applicable.
* Significant p-value.

Table 2
Haplotype permutation test of *ABCA1* polymorphisms (rs2422493–rs2230806–rs2066718–rs2230808) in the USA sibpair series

Haplotype	Total (Nf = 331)			ApoE-ε4− (Nf = 76)			ApoE-ε4+ (Nf = 227)		
	Fam	Freq	*p*-Value	Fam	Freq	*p*-Value	Fam	Freq	*p*-Value
T-G-G-G	63.8	0.318	0.65	13.1	0.332	0.89	32.5	0.307	0.54
C-G-G-G	64.1	0.294	0.77	9.1	0.211	0.40	35.6	0.338	0.47
C-G-G-A	28.3	0.082	0.81	6.1	0.134	0.93	15.1	0.069	0.90
C-A-G-G	22.4	0.071	0.19	4.6	0.076	0.27	10.1	0.059	0.02*
T-A-G-G	23.4	0.069	0.59	2.6	0.062	0.01*	12.7	0.069	0.52
T-G-G-A	27.7	0.061	0.06	7.1	0.08	0.01*	14.9	0.047	0.08
C-A-G-A	13.1	0.044	0.31	3.3	0.054	0.12	8.4	0.04	0.80
T-A-G-A	11.7	0.038	0.63	2.3	0.029	0.76	6.2	0.045	0.83
C-A-A-G	6.1	0.011	0.88	1	0.004	0.50	3	0.015	0.69
T-A-A-G	1.9	0.004	0.72		0.004	N/A	1	0.004	0.49
C-G-A-A	2	0.003	0.43	1	0.004	0.34	1	0.003	0.17
T-A-A-A	Few	0.002	N/A	Few	0.004	N/A		N/A	
C-G-A-G	Few	0.002	N/A		N/A		Few	0.002	N/A
C-A-A-A	1	0.002	0.16	Few	0.004	N/A		N/A	
T-G-A-A	Few	0.001	N/A		N/A		Few	0.002	N/A

Fam, number of informative families; Freq, frequency; Nf, number of nuclear families.
* Significant *p*-value.

role of cholesterol in AD pathology which has been described as a risk factor for several years.

Our data are most consistent with the view that ABCA1 variability has a weak contribution to Alzheimer risk, but even though our study is reasonably large, a study of the order of one magnitude larger than we describe would be required to demonstrate or refute this possibility completely.

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