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Association studies of cholesterol metabolism genes (CH25H, ABCA1 and CH24H) in Alzheimer's disease

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Abstract

Recent studies have demonstrated that cholesterol metabolism has an important role in Alzheimer's disease (AD) pathogenesis, suggesting that cholesterol-related genes may be significant genetic risk factors for AD. Based on the results of genome-wide screens, along with biological studies, we selected three genes as candidates for AD risk factors: ATP-binding cassette transporter A1 (ABCA1), cholesterol 25-hydroxylase (CH25H) and cholesterol 24-hydroxylase (CH24H). Case–control of North American Caucasians and AD families of Caribbean Hispanic origin were examined. Although excellent biological candidates, the case–control dataset did not support the hypothesis that these three genes were associated with susceptibility to AD. Similarly, no association was found in the Caribbean Hispanic families for CH25H. However, we did observe a possible interaction between ABCA1 and APOE in the Hispanics.

Keywords: Alzheimer's disease; Cholesterol; Single nucleotide polymorphism; Risk factor

Alzheimer's disease (AD) is the most common type of dementia associated with over-production or reduced clearance of the Aβ peptides—neurotoxic proteolytic derivatives of the amyloid precursor protein (APP). Up to 5% of AD cases have early-onset familial AD (FAD), genetic analyses of which have found three causal genes (APP, presenilin 1 and presenilin 2; reviewed in [14]). The only well-replicated risk factor for the common late-onset form of AD (>65 years) is the apolipoprotein E (APOE) gene [15], the product of which is involved in cholesterol and APP/Aβ metabolism. Together the known AD genes account for about half of the genetic risk factors. Recent studies have further confirmed the genetic heterogeneity of AD [7,10].

Several lines of evidence converge on the concept that variations in cholesterol metabolism and APP processing may be related to increased risk for AD. Thus, the risk of AD and the level of β-amyloid deposition are altered by factors associated with high cholesterol level, including the APOE ε4 allele [13]. Furthermore, brain cholesterol has been shown to directly affect APP metabolism [4]. Clinical trials using statin treatment have been performed to reduce cholesterol biosynthesis in the brain with the aim of reducing β-amyloid deposition [16].

We considered the following three genes as candidate genes for AD based upon their functional association with cholesterol metabolism. ATP-binding cassette transporter A1 (ABCA1) is a member of the ATP-binding cassette transporters family and plays a role in the cholesterol efflux pump [13]. Cholesterol 24-hydroxylase (CH24H) and cholesterol 25-hydroxylase (CH25H) convert cholesterol to oxsterols,

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24-hydroxycholesterol and 25-hydroxycholesterol, respectively [13,8]. In addition, the ABCA1 (9q31.1) and CH25H (10q23) are positional gene candidates, based on their locations within reported AD loci [10]. In support for a genetic link between AD and cholesterol metabolism, recent results indicate that polymorphisms in ABCA1 and CH24H may affect the risk of AD and/or modify the age-at-onset [2,5,6,17].

We therefore conducted both case–control and family-based association studies to test the hypothesis that variations in the ABCA1, CH25H and CH24H genes might be associated with increased susceptibility to AD.

Informed consent for research purposes was obtained from all individuals involved in the study approved by a research ethics board. Clinical diagnosis of probable or possible AD was defined according to the NINCDS–ADRDA diagnosis criteria [9]. The case–control dataset was composed of North American Caucasian samples recruited and characterized at clinics specializing in memory disorders in Toronto and Miami: 195 normal controls (mean age-at-examination 73.1 ± 9.3 years; women 52%) and 218 sporadic AD cases (mean age-at-onset 75.0 ± 8.4 years; women 53%). The dataset for the family-based association study consists of 118 late-onset AD families of Caribbean Hispanic origin (564 family members), recruited primarily from clinics in the Dominican Republic, Puerto Rico and in New York City. Each family included at least two living AD patients. The Clinical characteristics of the FAD dataset are described elsewhere [7].

Genomic DNA was isolated from blood samples using the QIAGEN kit. From public databases (www.ncbi.nlm.nih.gov/SNP/) we identified 13 single nucleotide polymorphisms (SNPs) with minor allele frequencies >10% that were located in the sequence of the CH25H, ABCA1 and CH24H genes. Table 1 summarizes the characteristics of the selected SNPs and genotyping assays used in the association studies. Genotypes were determined using restriction digest assay or SNAPshot (PE Biosystems, Foster City, CA). The APOE genotypes were determined as described previously [15].

Linkage disequilibrium (LD) between SNP pairs was calculated using the GOLD/Idmax software package. Pairwise Lewontin's disequilibrium coefficient (D') and more conservative delta-squared measure of disequilibrium (Δ^2) were employed to estimate the strength of LD between SNPs [1,3]. Case–control analyses were computed using the software StatView release 5.01. For each SNP a test for deviation from Hardy–Weinberg equilibrium was performed. Differences in genotype or allele frequencies between cases and controls were analyzed by χ^2 or by Fisher's exact test. Logistic regression models were used to consider such variables as age and APOE $\varepsilon 4$ dose. Potential effects of each genotype upon the age-at-onset in the entire dataset were evaluated by ANOVA

Table 1
Characteristics of the genotyped single nucleotide polymorphisms (SNPs), PCR primers and genotyping assays used in the association studies

Gene	SNP ID	SNP function	Forward/Reverse primer (5′–3′)	Method	Extension primer (5′–3′) or fragment's size
CH25H	rs1892336	Downstream from 3′	F-GCTGGGAAACTGGAGGCACAG R-TTCAAACTCAGGCCTAACTCC	Digest (RsaI)	A uncut (333 bp), G cut (157 bp + 176 bp)
	rs2841031	Downstream from 3′	F-TAAAATCCAATAAAGCCATAC R-GAGTGGTATTTATGATGGGAG	Digest (AvaII)	G cut (112 bp + 158 bp), A uncut (270 bp)
	rs17382301	Exon 1 (N219N)	F-CGTGGTCAACATCTGGCTTTC R-CCCAGTATTTTGTCCCAGTGT	SNaPshot	CCGTGGAGGACCACTCCGGCTACAA
	rs4078488	Exon 1 (A168A)	F-TGTTCTGCCTGCTACTCTTCG R-GAAAGCCAGATGTTGACCACG	SNaPshot	CCACCAGAACTCGTCCTCGTTCGC
	rs10887932	Exon 1 (L18L)	F-TTATTCAAGCTTCGCATCGCC R-CAGGAGCACAGGATATCCAGG	SNaPshot	GGTCCTTTGCAGCTCCGGGCAGCT
	rs4417181	5′ UTR (promoter)	F-TTATTCAAGCTTCGCATCGCC R-CAGGAGCACAGGATATCCAGG	SNaPshot	GGCCGTGAGTCCTGTGAAAAGTCG
	rs13500	Upstream of 5′	F-ATGCATCCTTATTTTTAATAC R-ATTCGCCCTGCCTATATTAAC	Digest (MspI)	C cut (101 bp + 100 bp), T uncut (201 bp)
ABCA1	rs2230808	Exon 34 (R1587K)	F-CTGTTGCCCCTTATCTATGTG R-GAATGCCCCTGCCAACTTTAC	SNaPshot	GATTTATGACAGGACTGGACACCA
	rs4149313	Exon 17 (M883I)	F-GGCGAGGAAAGTGATGAGAAG R-CAGTTAGCAGAGGCAGCAGCA	SNaPshot	GACTGACTGACTGACCCTGGTTCCAAC-CAGAAGAGAAT
	rs2230806	Exon 6 (K219R)	F-ATCCTCTTGTGCTTGTCTCTC R-TATCACAAACTCCCAGCCAGC	SNaPshot	GATTTCTGAGCTTTGTGGCCTACCAA
	rs2422493	5′ UTR (promoter)	F-CTCGGGTCCTCTGAGGGACCT R-CCGCAGACTCTCTAGTCCAC	SNaPshot	CAGCCCATTACCCAGAGGACTGTC
CH24H	rs754203	Intron 2	F-CCCGTCCCAGAAGGTGCTCAA R-ATCTGTAAACTGGGTGGGGGG	SNaPshot	GACTGACTTGCTGTCCTGGGGCCCAGG-AGCCC
	rs4900442	Intron 3	F-CCCATCTACTCCCCACATCAA R-CACAAAACCTCAGTCATCGTC	SNaPshot	GACTGACTGACTGACAGGCTTCACTCC-CCCAACCCAAG

Table 2
Results of the pair-wise disequilibrium test between all site pairs, with D' values in cases above the diagonal and D' value in controls below the diagonal

CH25H	rs1892336	rs2841031	rs17382301	rs4078488	rs10887932	rs4417181	rs13500
rs1892336		0.37	0.47	0.61	0.57	0.66	0.52
rs2841031	0.63		1	1	1	1	1
rs17382301	0.03	0.28		0.99	0.88	0.73	0.01
rs4078488	0.3	0.11	1		0.94	1	1
rs10887932	0.21	0.05	1	1		1	1
rs4417181	0.21	0.05	1	1	1		1
rs13500	0.06	0.06	0.16	0.12	0.09	0.1	

ABCA1	rs2230808	rs4149313	rs2230806	rs2422493
rs2230808		0.17	0.24	0.02
rs4149313	0.1		0.12	0.04
rs2230806	0.3	0.2		0.04
rs2422493	0.18	0.11	0.03	

CH24H	rs754203	rs4900442
rs754203		1
rs4900442	0.98	

The gray squares represent the site pairs that were found to be significant ($p < 0.001$) with D' values exceeding 0.9.

analysis (Turkey–Karmer's analysis). Differences in allelic and/or genotypic frequency were considered statistically significant when the p-value was less than 0.05. The statistical power of case–control dataset was evaluated using the Genetic Power Calculator [11] assuming a genetic model as follows: risk allele frequency of 0.10; prevalence of the risk allele in the population of 0.001; and genotype relative risk of 4.0.

A family-based association analysis was conducted using the FBAT program version 1.5 [12]. The data was analyzed under the additive model. The association analysis was performed only when there were at least ten informative families to minimize the likelihood of potential false positive findings arising from rare alleles. To evaluate the potential interaction with APOE, we conducted a conditional analysis. For the APOE *dependent* model, we considered a person affected when an affected individual has at least one copy of APOE ε4 allele, and affection status was considered unknown if the affected individual did not have the ε4 allele. An unaffected person was considered unaffected irrespective of APOE ε4 dosage. Conversely, for the APOE *independent* model, affected individuals without the ε4 allele were considered affected, and affected individuals with at least one copy of the ε4 allele were considered unknown.

Our dataset has a power of 89% ($\alpha = 0.01$) for detecting association under the genetic model described in the methods. As expected, we confirmed the association between the APOE ε4 allele and risk of AD in our dataset (odds ratio 4.32, $CI = 2.65 < OR < 7.07$, $p < 0.001$). All the SNPs studied were in Hardy–Weinberg equilibrium in both the AD and control groups ($p > 0.3$). Measurement of LD (Table 2) demonstrated strong haplotype blocks in the CH25H gene (most of the D' values exceeded 0.9). The results of the case–control analyses of ABCA1, CH25H and CH24H genes are summarized in Table 3. We did not find significant differences in the genotype or allele frequencies between AD and control subjects for any of the SNPs studied ($p > 0.11$). Stratification of the data by APOE ε4 status did not change the results. Furthermore, we did not detect a modifying effect for any genotype on the age-at-onset in the AD group ($p > 0.23$).

Table 3
The results of the case–control study of the North American Caucasian dataset

Gene	SNP ID	Distance (kb)	Minor allele frequency		OVERALL (p)		APOE ε4 positive (p)		APOE ε4 negative (p)	
			Controls	Cases	Genotypic	Allelic	Genotypic	Allelic	Genotypic	Allelic
CH25H	rs1892336		0.20	0.20	0.12	0.97	0.40	0.45	0.90	0.87
	rs2841031	34.4	0.33	0.28	0.41	0.21	0.21	0.24	0.70	0.26
	rs17382301	26.9	0.12	0.09	0.39	0.20	0.16	0.51	0.18	0.11
	rs4078488	0.2	0.13	0.16	0.66	0.44	0.14	0.27	0.17	0.88
	rs10887932	0.5	0.14	0.16	0.71	0.46	0.15	0.41	0.71	0.60
	rs4417181	0.1	0.14	0.16	0.50	0.56	0.17	0.82	0.92	0.79
	rs13500	6.4	0.13	0.16	0.84	0.94	0.72	0.77	0.52	0.82
ABCA1	rs2230808		0.18	0.17	0.25	0.86	0.07	0.51	0.38	0.26
	rs4149313	23.9	0.26	0.29	0.47	0.50	0.51	0.62	0.92	0.78
	rs2230806	34.1	0.22	0.26	0.55	0.27	0.46	0.80	0.71	0.31
	rs2422493	70.1	0.45	0.44	0.19	0.77	0.20	0.90	0.76	0.65
CH24H	rs754203		0.28	0.30	0.60	0.63	0.76	0.58	0.49	0.67
	rs4900442	0.3	0.42	0.46	0.11	0.37	0.11	0.19	0.21	0.78

Table 4
Single-point family-based association analysis of the Caribbean Hispanic AD families

Gene	SNP ID (function)	Allele (frequency)	Overall		APOE varepsilon4 positive		APOE varepsilon4 negative	
			Z-score[a]	*p*-value	*Z*-score	*p*-value	*Z*-score	*p*-value
ABCA1	rs2230808 (R1587K)	A (0.461)	1.07	0.284	**2.07**	**0.038**	−0.91	0.363
		G(0.539)	−1.07		**−2.07**		0.91	
	rs4149313 (M883I)	*A*(0.670)	−0.35	0.726	−0.58	0.561	−0.01	0.999
		G(0.330)	0.35		0.58		0.01	
	rs2230806 (K219R)	*A*(0.365)	−1.63	0.104	0.62	0.534	**−2.69**	**0.007**
		G(0.635)	1.63		−0.62		**2.69**	
	rs2422493 (promoter)	*T*(0.518)	−0.2	0.845	−0.1	0.916	−0.13	0.900
		C(0.482)	0.2		0.1		0.13	
CH25H	rs4078488 (A168A)	A (0.132)	−1.35	0.177	−0.39	0.697	−0.99	0.322
		G(0.868)	1.35		0.39		0.99	
	rs17382301 (N219N)	*T*(0.114)	−0.34	0.735	−0.83	0.408	0.55	0.585
		C(0.886)	0.34		0.83		−0.55	

Significant results are in bold.
[a] Additive model was employed.

To evaluate the cholesterol-related genes in the familial dataset we selected six SNPs prioritized based on their potential functional significance (three coding and one promoter ABCA1 SNPs and two coding CH25H SNPs). Single-point family-based association analysis failed to reveal significant association with age-at-onset or with risk of AD in the overall dataset for any genotyped SNP (Table 4). However, the APOE conditional models demonstrated association between AD and two non-synonymous SNPs in the ABCA1 gene (Table 4): association with the G-allele of rs2230806 was significant in the absence of the APOE $\varepsilon 4$ allele ($Z = 2.69$, $p = 0.007$) while the association with the A-allele of rs2230808 was significant in presence of the APOE $\varepsilon 4$ allele ($Z = 2.07$, $p = 0.038$).

Our study did not reveal any association between AD and the SNPs in the CH25H and CH24H genes. The intronic CH24H SNPs (rs754203 and rs4900442) were previously investigated in a German case–control dataset [6]. Our results do not confirm the association between AD and rs4900442 found in that study (even though our dataset has a statistical power >99% to replicate the reported effect). The frequency of the minor allele in our controls was similar to that reported in the previous study (0.42 versus 0.49), ruling out allelic heterogeneity as the reason for lack of association with AD in our case–control dataset.

None of the four ABCA1 SNPs investigated in the current study were associated with risk of sporadic AD. In a previous study, investigating sporadic AD patients, the A-allele of the non-synonymous R219K SNP (rs2230806) in ABCA1 was associated with a reduction of total cholesterol in cerebrospinal fluid and a delay in age-at-onset [17]. However, the current study failed to detect a significant effect of the R219K variation on age-at-onset. Again, the frequency of the minor allele in the control populations is similar to each other (0.30 versus 0.26). Nevertheless, in the family-based association study using an independent set of Caribbean Hispanics, the APOE conditional models demonstrated an association between AD and the two non-synonymous ABCA1 SNPs (rs2230806 and rs2230808) (Table 4). Given the moderate number of families studied and limited SNP coverage, this finding should be considered preliminary.

Certainly, the genetic background of the two populations – North American Caucasians and Caribbean Hispanics – are different. The minor allele frequency for rs2230808 (R1587K) was substantially different between the Hispanics and the North American Caucasians (0.46 versus 0.18). Nevertheless, the *p*-value for rs2230808 in the case–control analysis of the North American Caucasians was the lowest (0.07). Power calculation for the case–control set excluded insufficient power to detect the previously reported associations, however a more dense coverage with additional SNPs is needed to rule these genes out with certainty, especially given the strong LD between the CH25H SNPs genotyped in the current study.

In conclusion, our study does not support the notion that genetic variability in the set of cholesterol metabolism genes (CH25H, ABCA1 and CH24H) influence the development of AD. However, there may be some influence on AD when ABCA1 interacts with APOE.

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