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Research paper

ABCA1 rs2230805 and rs2230806 common gene variants are associated with Alzheimer's disease

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ABSTRACT

The ATP-binding cassette, sub-family A, member 1 gene (*ABCA1*) is a relevant positional and functional candidate gene for Alzheimer's disease (AD). A case-control association study of genetic variations covering the *ABCA1* locus was performed in relation to AD risk in a Hungarian sample. Five single nucleotide polymorphisms (rs2422493: C-477T, rs2740483: G-17C, rs2230805: G474A/L158L, rs2230806: G656A/R219 K and rs2066718: G2311A/V771 M) were genotyped in 431 AD patients and 302 cognitively healthy, elderly controls. In single marker analysis, significant associations were found in the case of rs2230805 and rs2230806 polymorphisms: the minor A allele containing genotypes for both polymorphisms were more frequent in the control compared to the AD group. Haplotype analysis revealed that rs2230805, rs2230806 and rs2066718 polymorphisms created a linkage disequilibrium (LD) block with a strong LD between rs2230805 and rs2230806 polymorphisms. In the haplotype risk association tests, A-A-G haplotype of the rs2230805-rs2230806-rs2066718 polymorphisms was found to be nominally significantly more frequent in the control group. After correcting *p* values for multiple testing, only the effects of the rs2230805 and rs2230806 polymorphisms remained significant in the recessive model suggesting a modest protective effect of their minor alleles in AD, which should be interpreted with considerable caution, until further studies elucidate their role in AD pathology.

1. Introduction

The non-familial late-onset (> 65 years of age at-onset) form of Alzheimer's disease (AD) is most likely caused by a complex interaction of genetic and environmental factors. The most predictive genetic risk factor for late-onset AD is the ε4 variant of the apolipoprotein E (*APOE*; MIM#107741) gene. The *APOE* ε2/ε3/ε4 isoforms (defined by rs429358 and rs7412 polymorphisms) influence the lipid compounds of the cell membrane and thereby the cleavage of the amyloid-β protein precursor (AβPP) creates aggregation prone amyloid-ß (Aß) peptides [25]. The common genetic variants that have been associated with late-onset AD other than *APOE* ε4 allele have small effect sizes and still a great portion of the predicted heritability for AD remains unrevealed [14].

The ATP-binding cassette, sub-family A, member 1 gene (*ABCA1*; MIM# 600046) is a positional and functional candidate gene for AD. The *ABCA1* gene is located near to an AD linkage peak on chromosome 9 at position q22 [2,18,19]. The encoded product of the *ABCA1* gene is a membrane-associated transporter that functions as an efflux pump for cholesterol and phospholipids from cell membranes to lipid-free apolipoprotein A-I (apoA-I) and apoE in the cellular lipid removal pathway [13,28].

Controlling apoE lipidation, ABCA1 likely has a role in the pathogenesis of AD, which is supported by a number of studies reporting significant impact of ABCA1 on Aβ deposition and clearance in AD model mice [11]. AβPP transgenic mice lacking the *ABCA1* gene have increased Aβ deposition and cognitive decline with diminished levels of soluble apoE, while transgenic mice overexpressing *ABCA1* in the brain have fewer Aβ plaques [11].

In humans, genetic studies further support the involvement of *ABCA1* in AD. The strongest indication for investigating the relationship between lipid metabolism and neurodegeneration came from the association of *APOE* gene variants and the risk for late-onset AD. Genetic variations of the *ABCA1* gene have been studied extensively in relation to cholesterol metabolism and neurodegeneration after two important discoveries. Mutations in the *ABCA1* gene were reported as the primary cause of Tangier disease characterized by extremely low levels of high density lipoprotein [3,4,24]. Non-synonymous common genetic variation in the *ABCA1* gene was found to be associated with altered lipoprotein levels and a modified risk for coronary artery disease [6].

Several case-control association studies attempted to reveal the

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relationship between *ABCA1* common polymorphisms and AD susceptibility; however, despite the biological plausibility of this locus as a modulator of AD risk, contradictory results were reported (*positive findings:* [10,20,22,26,27,31–33] *negative results:* [5,12,15] *meta-analyses:* [9,30]). In a recent study all *ABCA1* coding regions were sequenced and found to have a significantly higher proportion of rare non-synonymous variants in the control compared the AD group suggesting a protective effect [16].

The present association study was designed to give further data on five potentially relevant single nucleotide polymorphisms (SNPs) in a well-defined Hungarian sample. SNPs selected for this study consisted of two promoter polymorphisms (rs2422493: C-477T and rs2740483: G-17C), and three gene variants – synonymous (rs2230805: G474A/L158L) and non-synonymous (rs2230806: G656A/R219 K and rs2066718: G2311A/V771 M) – in the coding region. Applying single-marker and haplotype analyses, we aimed to evaluate the possible association of the above mentioned *ABCA1* genetic variations with the susceptibility to late-onset AD either alone or in epistasis with *APOE* ε2/ε3/ε4 polymorphism.

2. Subjects and methods

Our case-control study population consisted of 431 patients with late-onset AD (74.6 ± 6.8 years of age (mean ± SD), men 34.5%) and 302 cognitively healthy, elderly control individuals (74.1 ± 7.2 years of age (mean ± SD), men 35.5%) of Hungarian Caucasian descent. Demographic characteristics of the investigated groups are presented in Table 1. The AD patients were recruited from the Memory Clinic of the Department of Psychiatry, University of Szeged, Hungary. All diagnoses of late-onset AD were set according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria [17]. No patient had a family history raising suspicion of familial AD and the minimum age at onset was 65 years.

Global cognitive performance was measured by the Mini-Mental State Examination (MMSE). The mean MMSE score in the AD group was 17.5 ± 5.6 (mean ± SD), while in the control group MMSE scores were higher than 28 points and none of the control individuals had any verified symptoms of dementia. The clinical evaluation of all study participants was set without any prior knowledge of genetic background. All recruitment and protocols were conducted with written informed consent and with the approval of the Ethics Committee of the Hungarian Council on Science and Health (ETT-TUKEB).

Blood was drawn by venous puncture, and genomic DNA was extracted from whole blood by standard procedures using the Roche High Pure PCR Template Preparation Kit (Roche Holding AG, Basel, Switzerland). Genotyping of the investigated polymorphisms was performed by applying commercial TaqMan single-nucleotide polymorphism assays (Thermo Fisher Scientific/Applied Biosystems/, Waltham, Massachusetts, USA). The polymerase chain reaction amplification was conducted in single-plex reactions in 96-well plates with a total volume of 20 µl using the following amplification protocol: 95 °C for 10 min, and 40 cycles of 92 °C for 15 s, and 60 °C for 1 min. Fluorescence measurements were performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA).

Table 1
Demographic characteristics and APOE genotype frequencies of the investigated groups.

	AD patients	Controls
age (years; mean ± SD)	74.6 ± 6.8	74.1 ± 7.2
sex (male/female (%))	34.5%/65.5%	35.5%/64.5%
MMSE (scores; mean ± SD)	17.5 ± 5.6	> 28
APOE genotypes*		
ε2/ε2 n (%)	0 (0.0%)	4 (1.3%)
ε2/ε3 n (%)	19 (4.4%)	28 (9.3%)
ε2/ε4 n (%)	10 (2.3%)	6 (2.0%)
ε3/ε3 n (%)	200 (46.4%)	209 (69.2%)
ε3/ε4 n (%)	169 (39.2%)	54 (17.9%)
ε4/ε4 n (%)	33 (7.7%)	1 (0.3%)

AD: Alzheimer's disease, MMSE: Mini Mental State Examination, APOE genotypes(*): apolipoprotein E genotypes defined by rs429358 and rs7412 polymorphisms.

Controls and AD patients were analyzed using *t*-test for continuous parameters (MMSE and age), whereas Fisher's exact and Pearson chi-square tests were used when categorical parameters (gender, allele and genotype frequencies) were compared. Binary logistic regression model was used to test for interaction between the *ABCA1* and *APOE* polymorphisms, and to calculate odds ratios (ORs) with 95% confidence intervals (CIs). To exclude Type I errors, we carried out Bonferroni's correction for multiple testing for 5 single-marker genotype comparisons in the genotypic model, 2 single-marker genotype comparisons in the recessive model for rs2230805 and rs2230806, and 5 haplotype comparisons.

All SNPs were tested for deviation from Hardy-Weinberg equilibrium (HWE) by Pearson chi-square test. The software Haploview 4.2 was used to conduct linkage disequilibrium (LD) calculations, haplotype analyses and to create LD blocks [1]. Power analysis was performed using G*Power 3.0 software [8], and the effect size was determined according to the method published by Cohen [7]. Based on the calculated effect sizes (w = 0.207 for rs2230805 and w = 0.173 for rs2230806), our study sample has 99% power at the significance level of 0.05 to detect differences in rs2230805 and rs2230806 genotype frequencies between AD and control groups. Given the calculated effect sizes (w = 0.106 for rs2422493, w = 0.124 for rs2740483, w = 0.1181 for rs2066718) comparing the different genotype frequencies between the two investigated groups, our study population has a power of 73% for rs2422493, 86% for rs2740483 and 82% for rs2066718 at the significance level of 0.05.

3. Results

A total of 733 samples were analyzed, including 431 AD patients and 302 elderly, cognitively healthy controls. Comparing the background parameters, no significant differences were found between AD cases and controls in mean age or in the distribution of genders, while MMSE mean scores were shown to differ significantly. Genotype frequencies were in agreement with HWE for both AD patients and controls. As shown in Table 1, the APOE ε3/ε4 and ε4/ε4 genotypes were significantly over-represented in the AD group as compared to the control group (χ^2 = 75.995 (5) p < 0.001).

Genotype frequencies of the investigated *ABCA1* gene variants are summarized in Table 2. Genotype distributions of the rs2422493, rs2740483 and rs2066718 polymorphisms did not differ significantly between the AD and control groups (rs2422493: χ^2 = 1.954 (2) p = 0.376; rs2740483: χ^2 = 2.958 (2) p = 0.228; rs2066718: χ^2 = 2.358 (2) p = 0.308). The frequency of the rs2230805 G/G genotype was significantly higher in the AD than in the control group (χ^2 = 7.787 (2) p = 0.020, corrected: p = 0.100). Compared to the controls, the rs2230806 G/G genotype was more frequent in the AD group (χ^2 = 5.245 (2) p = 0.073, corrected: p = 0.365).

As the minor A allele containing genotypes (A+) occurred more frequently in the control group for both rs2230805 and rs2230806 polymorphisms, a recessive model was also applied, and significant differences were found when G/G and A+ genotype frequencies were compared between cases and controls (Fisher's exact test: rs2230805: p = 0.011, corrected: p = 0.022; rs2230806: p = 0.024, corrected: p = 0.048).

The presence of the rs2230805 A allele significantly decreased the risk for AD considering G/G genotype carriers as reference category (OR = 0.674; 95% CI: 0.501-0.906; p = 0.009). Similarly, the A+ genotype of the rs2230806 polymorphism also showed a significantly