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ABCB1 C3435T Polymorphism Influences the Risk for Alzheimer's Disease

Ágnes Fehér · Anna Juhász · Magdolna Pákáski · János Kálmán · Zoltán Janka

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Abstract To evaluate the association of ATP-binding cassette subfamily B member 1 (*ABCB1*) genetic variants with the susceptibility to Alzheimer's disease (AD), we genotyped the rs1128503 (C1236T), rs2032582 (G2677T/A), and rs1045642 (C3435T) polymorphisms in a case–control sample (234 AD patients, 225 controls). Single-marker analyses revealed a significant association solely for the rs1045642 polymorphism (C/C genotype carriers had increased risk for AD), which remains significant after correction for multiple testing. Haplotype analyses indicated three nominally significant associations which were lost after applying multiple test correction.

Keywords Alzheimer's disease · ATP-binding cassette subfamily B member 1 (ABCB1) · P-glycoprotein (P-gp) · Single nucleotide polymorphism (SNP) · rs1128503 (C1236T) · rs2032582 (G2677T/A) · rs1045642 (C3435T)

Background

Late-onset Alzheimer's disease (AD), the most common cause of dementia, is a complex neurodegenerative disorder with a strong genetic predisposition; however, in spite of the extensive research, the larger part of its heritable background is still an open question. At the molecular level, accumulation and aggregation of the amyloid-β (Aβ) peptides, leading to senile plaque formation, and hyperphosphorylation of the tau protein, resulting in neurofibrillary tangle generation, are the major features of AD pathology.

Adenosine triphosphate (ATP)-binding cassette subfamily B member 1 gene (*ABCB1*; MIM# 171050) at locus 7q21.1 encodes P-glycoprotein (P-gp), an efflux transporter that is located at the luminal side of the cerebral endothelial cells at the blood–brain barrier (Pahnke et al. 2008). P-gp effectively prevents a number of structurally different drugs and toxicants from entering the brain and removes metabolic waste products from the brain; hence, P-gp has an essential role in neuroprotection and overall brain homeostasis (Wolf et al. 2012). With respect to AD, the involvement of P-gp in Aβ transport is particularly important. Compared to healthy controls, Aβ clearance from the brain is significantly decreased in AD patients (Mawuenyega et al. 2010).

In vitro experiments provided evidence that P-gp is capable of transporting Aβ (Lam et al. 2001; Kuhnke et al. 2007), and consistent with these findings, reduced P-gp activity via inhibition or genetic modification was reported to reduce Aβ clearance from the brain in an AD mouse model (Cirrito et al. 2005). Additionally, the link between P-gp and AD is also supported by results on the inverse correlation between cerebral Aβ deposition and vascular P-gp expression at the blood–brain barrier (Vogelgesang et al. 2002; Jeynes and Provias 2011).

The *ABCB1* gene is highly polymorphic. The rs1128503 single nucleotide polymorphism (SNP) is a nucleotide change in exon 12 (C1236T) that does not alter the glycine at position 412. The tri-allelic rs2032582 polymorphism in exon 21 (G2677T/A) leads to an alanine to threonine or serine amino acid substitution (Ala893Thr/Ser). The rs1045642 synonymous polymorphism in exon 26 (C3435T) does not affect the ileucine at position 1145. To evaluate the possible association of *ABCB1* genetic variations with the susceptibility to

Á. Fehér (✉) · A. Juhász · M. Pákáski · J. Kálmán · Z. Janka
Department of Psychiatry, University of Szeged, 57 Kálvária Ave, Szeged 6724, Hungary
e-mail: feher.agnes@med.u-szeged.hu

Á. Fehér
e-mail: feherag@gmail.com

late-onset AD, we performed a case–control study of a Hungarian sample genotyping the rs1128503, rs2032582, and rs1045642 polymorphisms.

Subjects and Methods

A total of 234 Hungarian patients with late-onset AD (age 75.6±6.8 years (mean±SD), men 32.1 %) and 225 Hungarian, elderly, cognitively intact, healthy controls (age 74.8± 7.2 years (mean±SD), men 33.3 %) were involved in the study. The AD patients were recruited from the Memory Clinic of the Department of Psychiatry, University of Szeged. A consensus clinical diagnosis of probable late-onset AD was established according to the National Institute of Neurological and Communicative Disorders and Stroke/ Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria (McKhann et al. 1984). 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 17.3± 5.7 (mean±SD), while in the control group, MMSE scores were higher than 28 points and none of the control probands had any verified symptoms of dementia. All recruitment and protocols were conducted with written informed consent and with the explicit approval of the Ethics Committee of the Hungarian Council on Science and Health (ETT-TUKEB).

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. The investigated polymorphisms were genotyped using the method of PCR and enzyme digestion (PCR-RFLP). *ABCB1* rs1128503 genotypes were determined by a formerly described procedure with the restriction enzyme *BsuR*I (Sipeky et al. 2011). Genotyping of the *ABCB1* rs2032582 polymorphism was carried out as previously described with the restriction enzymes *BseY*I and *Rsa*I (Cascorbi et al. 2004). Genotyping of the *ABCB1* rs1045642 polymorphism was assessed by the same method described by Cascorbi et al. (2004) with the restriction enzyme *Mbo*I.

Genotype and allele frequencies were compared between patients and controls using Pearson's chi-square and Fisher's exact tests. To exclude type I errors, Bonferroni's correction for multiple testing was applied for three single-marker and eight haplotype comparisons. The gender distribution of the AD and control groups was compared with Fisher's exact test, and the mean age was compared by using the *t* test for independent samples. No statistically significant difference was found in the distribution of genders or in the mean age between AD and control groups ($p>0.05$). Hardy–Weinberg equilibrium (HWE) testing, linkage disequilibrium (LD) calculations, and haplotype analyses were conducted using Haploview 4.2 (Barrett et al., 2005). Power analysis was performed using G*Power 3.0 software (Faul et al. 2007), and the effect size was determined according to the method published by Cohen (1988). Our study sample ($n=459$) has 81 % power at the significance level of 0.05 to detect differences in rs1045642 genotypes between AD patients and controls (effect size: $w=0.146$).

Results

Genotype frequencies of the investigated polymorphisms in the AD and control groups applied to HWE ($p>0.05$). LD and haplotype data were inferred from genotype data only for subjects not carrying the rs2032582 A allele because of its very low occurrence in both AD and control groups. Table 1 presents the LD characteristics of the genotyped polymorphisms. Moderate LD was observed between the rs1128503 and rs2032582 ($D'=0.749$, $r^2=0.434$) and between the rs2032582 and rs1045642 polymorphisms ($D'=0.729$, $r^2=0.376$), and weak LD was detected between the rs1128503 and rs1045642 polymorphisms ($D'=0.474$, $r^2=0.203$).

The genotype and haplotype frequencies of the *ABCB1* polymorphisms are summarized in Table 2. The ratio of the different rs1128503 genotypes was similar in the AD and in the control groups and showed no statistically significant difference ($\chi^2=0.506$ (2) $p=0.776$). Regarding the rs2032582 polymorphism, no A/A genotype carriers were found; besides, the G/A and the T/A genotypes had a very low frequency in both investigated groups. The G/G and G/T genotypes occurred at a slightly higher frequency in the AD than in the control group; however, the difference was not statistically significant ($\chi^2=3.951$ (4) $p=0.413$). Compared with the controls, the rs1045642 C/C genotype was significantly overrepresented in the AD group ($\chi^2=9.840$ (2) $p=0.007$, corrected: $p=0.021$). The C/C genotype carriers had a significantly increased risk for AD (OR=2.29, 95 % CI 1.35–3.88, $p=0.002$) considering the T/T genotype carriers as reference category.

The predominant haplotypes of the markers rs1128503, rs2032582, and rs1045642 were the C–G–C and T–T–T in both AD and control groups. Our haplotype analyses revealed three nominally significant associations (Table 2). The T–T–T and T–T–C haplotypes were more frequent in the control group than in the AD group (T–T–T: $p=0.040$; corrected $p=0.320$; T–T–C: $p=0.016$; corrected $p=0.128$), while the C–T–T haplotype had a higher occurrence in the AD group ($p=0.032$; corrected $p=0.256$).

Discussion

In single-marker case–control analyses, we found a significant association only for the rs1045642 polymorphism out of the three investigated SNPs. Even after correcting *p* values using Bonferroni's method for multiple tests, this significant

Table 1 Linkage disequilibrium characteristics of the investigated *ABCB1* polymorphisms

Polymorphisms		MAF (%)		*p* value	Linkage disequilibrium		
		AD patients	Controls		With	*D′*	r^2
rs1128503	1236C>T	48.3	49.6	0.702	rs2032582	0.749	0.434
rs2032582[a]	2677G>T	41.2	45.8	0.170	rs1045642	0.729	0.376
rs1045642	3435C>T	46.4	56.7	0.002	rs1128503	0.474	0.203

ABCB1 ATP-binding cassette subfamily B member 1, *MAF* minor allele frequency, *AD* Alzheimer's disease

[a] Linkage disequilibrium data were inferred from genotype data only for subjects not carrying the A allele of the rs2032582 polymorphism

correlation was still found. Significantly increased susceptibility to AD associated with the rs1045642 C/C genotype was found considering the T/T genotype as the reference category. The genotype distributions of the rs1128503 and rs2032582 polymorphisms were similar in the AD and control groups without a statistically significant difference.

Table 2 Genotype and haplotype frequencies of the investigated *ABCB1* polymorphisms

	AD patients	Controls	Chi-square	*p* value
rs1128503			0.506	0.776
C/C	56 (23.9 %)	54 (24.0 %)		
C/T	130 (55.6 %)	119 (52.9 %)		
T/T	48 (20.5 %)	52 (23.1 %)		
rs2032582			3.951	0.413
G/G	69 (29.5 %)	62 (27.6 %)		
G/T	123 (52.6 %)	109 (48.4 %)		
T/T	30 (12.8 %)	44 (19.6 %)		
G/A	9 (3.8 %)	8 (3.5 %)		
T/A	3 (1.3 %)	2 (0.9 %)		
A/A	ND	ND		
rs1045642			9.840	0.007
C/C	66 (28.2 %)	43 (19.1 %)		
C/T	119 (50.9 %)	109 (48.4 %)		
T/T	49 (20.9 %)	73 (32.5 %)		
Haplotypes[a]				
C–G–C	172 (36.9 %)	146 (32.4 %)	2.008	0.157
T–T–T	141 (30.1 %)	164 (36.5 %)	4.227	0.040
C–G–T	46 (9.8 %)	54 (11.9 %)	1.070	0.301
T–G–C	41 (8.8 %)	35 (7.7 %)	0.384	0.536
T–T–C	25 (5.4 %)	10 (2.3 %)	5.780	0.016
C–T–T	12 (2.5 %)	23 (5.2 %)	4.582	0.032
T–G–T	19 (4.0 %)	14 (3.1 %)	0.575	0.448
C–T–C	12 (2.5 %)	4 (0.9 %)	3.625	0.057

ABCB1 ATP-binding cassette subfamily B member 1, *AD* Alzheimer's disease, *ND* not detected

[a] Haplotypes of the rs1128503, rs2032582, and rs1045642 polymorphisms. Haplotype data were inferred from genotype data only for subjects not carrying the A allele of the rs2032582 polymorphism. Chi-squares and *p* values for comparisons of the haplotype frequencies were determined by using the Haploview 4.2 program

Even though the synonymous rs1045642 polymorphism does not produce an altered coding sequence, it seems to have functional consequences, since it was reported to result in a lower intestinal P-gp expression and an increased oral bioavailability of the P-gp substrate digoxin, decreased mRNA stability and expression, and reduced protein function (Hoffmeyer et al. 2000; Wang et al. 2005; Van Assema et al. 2012). On the other hand, reports of conflicting results can also be found (Sakaeda et al. 2001; Nakamura et al. 2002).

The frequencies of the investigated *ABCB1* genotypes in our control sample are comparable to earlier reports on other control populations of Caucasian origin (Cascorbi et al. 2004; Sipeky et al. 2011). Two case–control studies with small and medium sample sizes reported no association between the rs2032582 or rs1045642 polymorphism and the risk for AD (Frankfort et al. 2006; Kohen et al. 2011), and two genome-wide association studies also did not detect a correlation between the rs1045642 polymorphism and the genetic susceptibility to AD (Reiman et al. 2007; Li et al. 2008). The ABCB1 polymorphisms were also studied in a histopathologically confirmed AD case–control sample, and no effect of ABCB1 variants was found in the entire cohort; however, a significant association was detected between rs2032582 genotypes and AD risk among females and individuals older than 65 years (Cascorbi et al. 2013).

Frankfort and co-workers showed evidence for the correlation between ABCB1 rs1128503 and rs2032582 genotypes and Aβ serum profile, and therefore suggested further research on the potential involvement of ABCB1 genetic variations in AD (Frankfort et al. 2008). Another investigation revealed significant differences in peripheral leukocyte gene expression profiles between AD patients and non-demented controls and found that the *ABCB1* gene had decreased expression in AD and the ABCB1 expression levels were positively correlated with MMSE score (Chen et al. 2011).

The investigated polymorphisms are not strictly allelic, and they showed LD; hence, we also performed haplotype estimation between cases and controls and found moderate (between the rs1128503 and rs2032582 and between the

rs2032582 and rs1045642 polymorphisms) and weak LD (between the rs1128503 and rs1045642 polymorphisms) in our sample. Consistent with findings of previous studies, the C–G–C and T–T–T were the predominant haplotypes of the rs1128503, rs2032582, and rs1045642 polymorphisms in both investigated groups. Haplotype analyses revealed three nominally significant associations for the T–T–T, T–T–C, and C–T–T haplotypes; however, they did not remain significant after applying Bonferroni's correction for multiple tests.

The present study led to the conclusion that the *ABCB1* rs1045642 polymorphism may have a role in the genetic risk for developing AD. Subjects carrying the C/C genotype of the *ABCB1* gene appeared to exhibit AD significantly more frequently. The medium size of our sample, an important limitation, should be mentioned, and further investigations with considerably larger samples and in detail using more markers are required.

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