

Case-control association study of ABCB1 gene and major depressive disorder in a local Chinese Han population

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Background: Human P-glycoprotein encoded by the ATP-binding cassette sub-family B member 1 (*ABCB1*) gene is expressed in the blood–brain barrier. *ABCB1* protects the brain from many drugs and toxins such as glucocorticoids through the efflux pump. Recent evidence suggests that a specific allele of the *ABCB1* gene confers susceptibility to major depressive disorder (MDD) in the Japanese population. The aim of this study was to explore the association of *ABCB1* gene polymorphisms with MDD in a local Chinese Han population.

Methods: Two hundred and ninety-two MDD patients and 208 unrelated individuals were matched by age and sex and examined using a case-control design. Six single nucleotide polymorphisms (SNPs) of the *ABCB1* gene, including rs1045642, rs2032583, rs2032582, rs2235040, rs1128503, and rs2235015, were genotyped by ligase detection reaction and multiplex polymerase chain reaction. Linkage disequilibrium and haplotype analysis were investigated in the two study groups.

Results: Significant protection for MDD individuals carrying the TG haplotype of rs1045642–rs2032582 was observed (odds ratio 0.470, 95% confidence interval 0.251–0.897, $P=0.01$). The rs2032582 (G2677T) and rs1128503 (C1236T) SNPs of *ABCB1* showed nominal associations with MDD; the other four SNPs of the *ABCB1* gene were not associated with MDD.

Conclusion: Chinese individuals carrying the TG haplotype of rs1045642–rs2032582 had a nearly 53% lower risk of developing MDD. To the best of our knowledge, this is the first report to analyze the effect of *ABCB1* polymorphism on the risk of MDD in a Chinese population.

Keywords: major depressive disorder, *ABCB1* gene, single nucleotide polymorphism, pharmacogenetics

Introduction

Major depressive disorder (MDD) is a significant contributor to the global burden of disease and affects people in all communities worldwide.¹ According to a disability-adjusted life years survey from the World Health Organization and the World Bank, unipolar depression was ranked fourth in 2012, and is expected to rank second in 2020 and first in 2030.²

The majority of depressed patients have abnormal hypothalamus-pituitary-adrenal (HPA) axis function, and it is believed that dysregulation of the HPA axis can induce depression. Glucocorticoids are end products and stress response factors in the HPA axis. Stressors can activate the HPA axis and increase glucocorticoid levels in the blood.³ High blood glucocorticoid levels are believed to be toxic to neurons in the brain and to cause depression.⁴

Human ATP-binding cassette sub-family B member 1 (*ABCB1*), also known as multidrug resistance protein 1 (*MDR1*), P-glycoprotein 1, and cluster of differentiation

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243 (CD243), is an important cell membrane protein. As one of the ATP-binding cassette transporters, it pumps foreign substances out of cells. This protein is expressed by endothelial cells at the blood–brain barrier, predominantly gathering on the luminal side of brain capillaries and functioning as an ATP-driven efflux pump. ABCB1 has an unusually broad ability to recognize foreign substances and expels hundreds of molecules from the brain, including many types of drugs and glucocorticoids.⁵

The *ABCB1* gene contains single nucleotide polymorphisms (SNPs) in the encoding regions. Variants such as C3435T (rs1045642), G2677T/A (rs2032582), and rs2032583 have been the most commonly studied by previous researchers. Most studies indicate that *ABCB1* haplotypes, the SNPs rs1045642, rs2032582, and rs2032583 affect the response to treatment with antidepressants.^{5–10}

Recently, a study in Japanese MDD patients reported that the A-41G, T-129C, C1236T, and G2677A/T haplotypes were significantly less common and that the C3435T allele was more common when compared with controls.⁴ The results suggested that a specific allele of the *ABCB1* gene confers susceptibility to MDD in the Japanese population.

In the present study, we studied six SNPs in the *ABCB1* gene and examined whether *ABCB1* is associated with MDD, using 292 Chinese MDD patients and 208 unrelated control individuals from a local Chinese Han population. Haplotype-based association analyses were also performed to investigate the role of specific genotype combinations.

Materials and methods

Subjects

Two hundred and ninety-two patients with single or recurrent depression and 208 healthy controls were included in the study. All individuals were of Chinese Han ethnicity but not biologically related. Patients were recruited from the Second Xiangya Hospital of Central South University between May 2006 and November 2012. All patients had more than 9 years of education, ranged in age between 18 and 65 years, and had

a diagnosis of MDD as defined in Axis I of the *DSM-IV-TR* (*Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision) and by a 17-item Hamilton Depression Rating Scale score ≥ 18 . Patients were excluded if they met *DSM-IV-TR* Axis I criteria for any other mental disorder, have other serious illness, or sequelae of serious illness. Patients were also excluded if they had serious suicide attempts and behavior. Each MDD patient underwent a medical assessment by at least two psychiatrists according to the *DSM-IV-TR*. Of the 292 MDD patients, 71.6% belonged to the single-episode category and 28.4% to the recurrent-episode category. The 208 controls (101 males, 107 females) were healthy volunteers recruited from the same geographical area and were matched with the patient group for sex, age, and level of education; they were interviewed using the Mini International Neuropsychiatric Interview by a research psychiatrist to rule out any Axis I psychiatric disorders, and individuals with a current or past history of psychiatric treatment were excluded. The study protocol was approved by the ethics committee at the Second Xiangya Hospital of Central South University and written informed consent was obtained from each patient before enrollment. The clinical characteristics of the two groups are shown in Table 1.

Genotyping

Six SNPs of the *ABCB1* gene were investigated in this study. They were SNP1 (rs1045642) in exon 27, SNP2 (rs2032583) in intron 22, SNP3 (rs2032582) in exon 22, SNP4 (rs2235040) in intron boundary exon 21, SNP5 (rs1128503) in exon 13, and SNP6 (rs2235015) in intron 5 of the *ABCB1* gene. All the genotyping experiments were carried out by Shanghai BioWing Applied Biotechnology Company (<http://www.biowing.com.cn>) using ligase detection reaction (LDR). The target DNA sequences were amplified using a multiplex polymerase chain reaction method. The LDR was performed using 30 cycles of 95°C for 2 minutes, 94°C for 15 seconds, and 50°C for 25 seconds. The fluorescent products of LDR were differentiated using a 3730 ABI sequencer.

Table 1 Clinical characteristics of the study subjects

	Patients (n=292)	Controls (n=208)	t(χ^2)	P-value
Age (years)	30.89 (10.92)	31.71 (8.25)	0.954	0.341
Sex				
Male	143	101	0.008	0.927
Female	149	107		
Total disease course (months)				
Single episode	14.26			
Recurrent episode	38.48			

Haplotype and statistical analysis

Distributions of genotype and allele frequencies were compared between the patients and controls using the χ^2 test for independence. The observed genotype frequencies were compared with the predicted frequencies to investigate the concordance with Hardy–Weinberg equilibrium. Pair-wise linkage disequilibrium, and haplotype analysis were conducted online using <http://analysis.bio-x.cn/myAnalysis.php>, a robust and user-friendly software platform that has a series of highly efficient analytical tools for use in association studies. Logistic regression analysis was used to estimate the risk of MDD associated with each genotype; odds ratios (ORs) with 95% confidence intervals (CIs) were obtained. A *P*-value less than 0.05 was considered to be statistically significant. Logistic regression analyses were performed using Statistical Package for the Social Sciences version 17.0 for Windows software (SPSS Inc, Chicago, IL, USA).

Adjustment for multiple comparisons was performed by Bonferroni correction.

Results

The distributions of genotypes and alleles for the examined *ABCBI* gene SNPs in patients and controls are shown in Table 2. Multivariate logistic analysis was used to adjust for potential risk factors, including sex and age, of the six SNPs in the *ABCBI* gene. No significant difference was observed in the allelic distributions of the rs1045642, rs2032583, rs2235040, and rs2235015 SNPs between patients and controls. The rs2032582 and rs1128503 SNPs, both of which are located in the *ABCBI* gene coding region (exon 22 and exon 13, respectively) were associated with MDD. For the rs2032582 and rs1128503 polymorphisms, the minor T allele was significantly increased in patients when compared with controls (OR 1.325, 95% CI 1.028–1.707 and adjusted OR

Table 2 Genetic association of six single nucleotide polymorphisms in the *ABCBI* gene

Genotype/allele	Case (%)	HWE-P	Controls (%)	HWE-P	OR (95% CI)	Adjusted OR (95% CI)	P-value
Rs1045642	C3435T						
CC	103 (35.3)		86 (41.3)				
CT	151 (51.7)		89 (43.3)		1.417 (0.961–2.088)	1.420 (0.963–2.094)	0.413
TT	38 (13.0)	0.267	33 (15.9)	0.132	0.961 (0.556–1.662)	0.976 (0.564–1.691)	
C allele	357 (61.1)		261 (62.7)				
T allele	227 (38.9)		155 (37.3)		1.071 (0.467–1.378)	1.078 (0.832–1.398)	0.605
Rs2032583							
CT	38 (13)		22 (10.6)				
TT	254 (87.0)	0.42	186 (89.4)	0.234	0.791 (0.452–1.381)	0.785 (0.448–1.373)	0.409
C allele	38 (6.5)		22 (10.6)				
T allele	544 (93.5)		394 (89.4)		0.802 (0.467–1.378)	1.797 (0.463–1.37)	0.424
Rs2032582	G2677T						
GG	86 (29.5)		78 (37.5)				
GT	130 (44.5)	0.065	88 (42.3)	0.06	1.340 (0.89–2.016)	1.345 (0.893–2.205)	
TT	76 (26.0)		42 (20.2)		1.671 (1.01–2.668)	1.674 (1.028–2.728)	0.117
C allele	302 (51.7)		244 (58.7)				
T allele	282 (48.3)		172 (41.3)		1.325 (1.028–1.707)	1.338 (1.037–1.726)	0.03*
Rs2235040							
AG	40 (13.7)		22 (10.6)				
GG	252 (86.3)	0.42	186 (89.4)	0.21	0.745 (0.428–1.296)	0.741 (0.425–1.291)	0.297
A allele	40 (6.8)		22 (5.3)				
G allele	544 (93.2)		394 (94.7)		0.759 (0.444–1.298)	0.756 (0.442–1.293)	0.313
Rs1128503	C1236T						
CC	40 (13.7)		40 (19.2)				
CT	121 (41.4)	0.18	90 (43.3)	0.161	1.344 (0.802–2.253)	1.344 (0.801–2.252)	
TT	131 (44.9)		78 (37.5)		1.679 (0.998–2.826)	1.69 (1.004–2.846)	0.136
C allele	201 (34.4)		170 (40.9)				
T allele	383 (65.6)		246 (59.1)		1.317 (1.016–1.707)	1.322 (1.02–1.715)	0.037*
Rs2235015							
GG	255 (87.6)		187 (89.9)				
GT	37 (12.7)	0.44	21 (10.1)	0.23	1.292 (0.732–2.28)	1.295 (0.733–2.288)	0.375
G allele	547 (93.7)		395 (95.0)				
T allele	37 (6.3)		21 (5.0)		1.272 (0.733–2.207)	1.275 (0.734–2.214)	0.391

Note: *Statistically significant difference between the groups ($P < 0.05$).

Abbreviations: CI, confidence interval; OR, odds ratio; HWE-P, Hardy–Weinberg equilibrium *P*-value.

Table 3 Frequencies of inferred haplotypes of rs1045642–rs2032582 in the two groups

	Case (%)	Controls (%)	OR (95% CI)	χ^2	P-value
CG	218.83 (0.490)	218.83 (0.526)	0.858 (0.835–1.866)	1.29	0.256
CT	71.07 (0.122)	41.17 (0.099)	1.248 (0.835–1.866)	1.26	0.26
TG	16.07 (0.028)	25.17 (0.060)	0.470 (0.251–0.897)	6.69	0.01*
TT	210.93 (0.361)	130.83 (0.315)	1.237 (0.946–1.616)	2.35	0.13

Note: *Statistically significant difference between the groups ($P < 0.05$).

Abbreviations: CI, confidence interval; OR, odds ratio.

1.338, 95% CI 1.037–1.726 for rs2032582; OR 1.317, 95% CI 1.016–1.707, adjusted OR 1.322, 95% CI 1.02–1.715 for rs1128503), but the statistical significance of this difference disappeared after Bonferroni correction ($P > 0.05$). Using haplotype analysis, rs1045642 and rs2032582 were in strong linkage disequilibrium ($D' = 0.8$, $r^2 = 0.47$). Although haplotype analysis did not reveal any significant association with MDD, the TG haplotype of rs1045642–rs2032582 was associated with a lower risk of developing the disorder (OR 0.470, 95% CI 0.251–0.8897, $P = 0.01$; Table 3). The association remained statistically significant after Bonferroni correction ($P = 0.04$).

Discussion

The results of a genetic study of the association of six SNPs of the *ABCB1* gene with MDD in a local Chinese Han population demonstrated significant protection against MDD in individuals carrying the TG haplotype of rs1045642–rs2032582. Chinese individuals carrying the TG haplotype of rs1045642–rs2032582 had a nearly 53% decreased risk of developing MDD. The rs1045642 and rs2032582 cover a region about 21,973 kb between exon 27 and exon 22. Among the six SNPs studied, no association between risk of MDD and the presence of a specific allele or genotype was observed. To our knowledge, this is the first report on the genetic association of *ABCB1* gene polymorphism with MDD in a case-control design in Chinese Han people in Mainland China.

To date, over 100 polymorphisms that occur at a frequency of greater than 5% have been identified in the *ABCB1* gene in Caucasians. It is known that rs2032582 (G2677T/A), a missense mutation in exon 21 that results in an amino acid change from Ala 893 to Ser or Thr, is associated with altered P-glycoprotein 1 expression, while rs1128503, a synonymous variant within exon 12 of the gene, occurring at position 1236 (C>T) and amino acid position 412 (Gly), may be in linkage disequilibrium with other functional variants.^{11,12} However, the functional effect of many common polymorphisms in the *ABCB1* gene remains unknown.⁶

The association of *ABCB1* (*MDR1*) gene with the risk of mood disorders was inconsistent in previous studies. Qian et al

were the first to report that mood disorder was associated with possible alteration of P-glycoprotein 1 activity corresponding to *ABCB1* gene polymorphism.¹³ Sixty-two patients with mood disorder and 160 controls were investigated in a Japanese study, and the results showed that the frequencies of *MDR1* 2677 G/A, the A/A genotype, and the A allele in mood disorders were significantly higher than in controls. Another Japanese study that included 631 MDD patients and 1,100 controls⁴ suggested that rs1045642 (C3435T) T allele or TT genotype carriers are susceptible to development of MDD. With respect to the other four SNPs (rs2188524, rs3213619, rs1128503, rs2032582), there was no significant difference in genotype or allele distribution. Ozbey et al reported that the rs1045642 C allele or CC genotype, but not the T allele, was associated with susceptibility to MDD in a Turkish population.¹⁴ Santos et al compared the distribution of allele and genotype frequencies for *ABCB1* C1236T, G2677TA, and C3435T in 80 MDD patients and 160 controls. The results did not reveal any statistically significant association between the *ABCB1* gene and MDD, but a significant protection against MDD in males carrying the T allele of the three polymorphisms was observed.¹⁵ Similarly, Crisafulli et al did not find any susceptible polymorphisms within the *ABCB1* gene; however, as these authors mentioned, their limited sample size (145 MDD patients) may be one reason.¹⁶ The impact of ethnic variation on genetic distribution may explain the above inconsistency, and further studies with larger samples in different ethnic groups are needed.

Regarding our negative findings, we conducted power calculations using PASS 11 software to assess the power of our study, and determined that the power of the six SNPs in our study varied from 8.1% to 58.4%. Our samples may have been too small and lacked the statistical power to detect differences likely to be associated with single SNPs. Another limitation was that the A allele for rs2032582 was not detected in our study, and has a frequency of about 15% in the Chinese population.¹⁷ Larger and more homogenous samples will be included in our future research to improve the statistical power.

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Disclosure

The authors report no conflicts of interest in this work.

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