Common Genetic Polymorphisms in the *ABCB1* Gene Are Associated with Risk of Major Depressive Disorder in Male Portuguese Individuals

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Major depressive disorder (MDD) is a highly prevalent disorder, which has been associated with an abnormal response of the hypothalamus–pituitary–adrenal (HPA) axis. Reports have argued that an abnormal HPA axis response can be due to an altered P-Glycoprotein (P-GP) function. This argument suggests that genetic polymorphisms in *ABCB1* may have an effect on the HPA axis activity; however, it is still not clear if this influences the risk of MDD. Our study aims to evaluate the effect of *ABCB1 C1236T*, *G2677TA* and *C3435T* genetic polymorphisms on MDD risk in a subset of Portuguese patients. DNA samples from 80 MDD patients and 160 control subjects were genotyped using TaqMan® SNP Genotyping assays. A significant protection for MDD males carrying the T allele was observed (*C1236T*: odds ratio (OR) = 0.360, 95% confidence interval [CI]: [0.140–0.950], $p = 0.022$; *C3435T*: OR = 0.306, 95% CI: [0.096–0.980], $p = 0.042$; and *G2677TA*: OR = 0.300, 95% CI: [0.100–0.870], $p = 0.013$). Male Portuguese individuals carrying the 1236T/2677T/3435T haplotype had nearly 70% less risk of developing MDD (OR = 0.313, 95% CI: [0.118–0.832], $p = 0.016$, FDR $p = 0.032$). No significant differences were observed regarding the overall subjects. Our results suggest that genetic variability of the *ABCB1* is associated with MDD development in male Portuguese patients. To the best of our knowledge, this is the first report in Caucasian samples to analyze the effect of these *ABCB1* genetic polymorphisms on MDD risk.

Introduction

Major depressive disorder (MDD) is a highly prevalent disorder, with a lifetime risk of 10–25% in women and 5–12% in males (Compton *et al.*, 2006). Depression has been associated with an abnormal response of the hypothalamus–pituitary–adrenal (HPA) axis and some authors suggest that the normalization of HPA axis hyperactivity is a crucial step in remission (Holsboer, 2000; Binder *et al.*, 2009). Glucocorticoid hormones, secreted by the adrenal cortex, are potent modulators of neuronal activity and are involved in the maintenance of the basal activity of the HPA axis (Binder *et al.*, 2009). The physiological access of endogenous glucocorticoids to the brain is important for HPA homeostasis. Studies in animal models have proved that the absence of P-Glycoprotein (P-GP) function at the blood–brain barrier (BBB), leads to deep changes in the activity and regulation of the HPA system, suggesting that P-GP may regulate the HPA axis activity (Muller *et al.*, 2003).

P-GP is an ATP-driven efflux pump involved in the pharmacokinetics of P-GP substrate drugs and in the efflux of toxic substances and metabolites, such as glucocorticoids (Kimchi-

Sarfaty *et al.*, 2007b). This transporter molecule is encoded by the *ABCB1* gene (also called *MDR1*), and is expressed on the epithelial cells of the intestine, canalicular membrane of hepatocytes, and on the endothelial cells of the BBB (Ambudkar *et al.*, 1999).

The overall *ABCB1* activity depends on two parameters: the level of expression of the *ABCB1* gene, which controls the amount of protein that is synthesized, and the functionality of the P-GP that determines transporter effectiveness and substrate recognition (Hoffmeyer *et al.*, 2000). Several single-nucleotide polymorphisms (SNPs) have been studied in the *ABCB1* gene. Among these, polymorphisms in exon 12 (*C1236T*; rs1128503), in exon 21 (*G2677TA*; rs2032582), and exon 26 (*C3435T*; rs1045642) are the ones most reported (Hoffmeyer *et al.*, 2000; Leschziner *et al.*, 2007). Studies regarding the effect of these *ABCB1* polymorphisms on P-GP expression and function are not consensual (Borst and Elferink, 2002). Regarding *ABCB1 C1236T* synonymous polymorphism, some reports found altered drug responses associated with 1236CC (Schaich *et al.*, 2009) and 1236TT variants (Zhang *et al.*, 2008), while others were unable to find any relationship (Estrela Rde *et al.*, 2009). Reports regarding the influence of synonymous *C3435T* polymorphism are inconclusive (Hodges *et al.*, 2011), and the exact influence of this polymorphism is not known. Several lines of evidence have indicated that this polymorphism affects the expression (Hoffmeyer *et al.*, 2000) and function (Compton *et al.*, 2006) of P-GP, whereas others reported an altered P-GP conformation despite the fact that levels of mRNA and protein were similar (Kimchi-Sarfaty *et al.*, 2007b). Tri-allelic *G2677TA* nonsynonymous polymorphism has been also widely reported, since its variants are known to induce amino acid modification (Ala893 to Ser/Thr893) (Leschziner *et al.*, 2007). This polymorphism is also known to affect P-GP functionality (Hodges *et al.*, 2011).

Despite functional studies, investigations focusing on the influence of P-GP polymorphisms and the risk of depression are scarce and have only been conducted in Asian (Qian *et al.*, 2006; Fujii *et al.*, 2012) and Mexican American patients (Dong *et al.*, 2009). This article examines whether common genetic polymorphisms in the *ABCB1* gene, previously reported to be associated with antidepressant responses, might be a predictor of risk for MDD in a subset of Portuguese patients.

Materials and Methods

Patients

From a total of 92 Portuguese MDD patients followed in a 18-month follow-up study at Magalhães Lemos Hospital, 80 patients agreed to participate in the genetic study (21 males and 59 females), aged 18–60, with a median age of 41.5 years (mean age 40.48; standard deviation 11.06). Participants in the clinical sample were recruited in the outpatient clinic at the Magalhães Lemos Hospital and as part of a broader research on the social-cognitive predictors of pharmacological responses to depression (Carvalho, 2012). Patients with the suspicion of depression were sent to the clinic by their family doctor and those whose clinical interview confirmed the diagnosis of depression were submitted to further evaluation for major depression using the Structured Clinical Interview for DSM Axis I Disorders (SCID-I), and for personality disorders using the Axis II Disorders (SCID-II). Subjects with more than one previous depressive episode, chronic depression, severe physical illness, psychiatric disorders with psychotic symptoms, substance dependence, or personality disorders were excluded from the study. Age- and gender-matched healthy control individuals were recruited from the same city. These healthy controls consisted of 160 individuals (42 males and 118 females) with median age of 41 years (mean age 40.48; standard deviation 11.08), collected among the blood donors of the Portuguese Institute of Oncology–Porto. The blood donors completed a health questionnaire, and were interviewed by a physician about their medications and possible chronic illnesses. The study was approved by the ethics committee of Magalhães Lemos Hospital. Written informed consent according to *The Code of Ethics of the World Medical Association* (Declaration of Helsinki) was obtained from each individual after explanation of the study.

Sample collection and DNA extraction

Peripheral blood samples were collected following the standard venipuncture technique in EDTA-containing tubes, and the genomic DNA was extracted from the whole blood with a commercial kit (E.Z.N.A.–Omega Bio-tek, Norcross, USA), according to the manufacturer's instructions and stored at −20°C.

C1236T, G2677TA, C3435T ABCB1 *polymorphism analysis*

The *C1236T, G2677TA, C3435T ABCB1* polymorphism analysis was carried using TaqMan® SNP Genotyping Assays C___7586662_10; C_11711720C_30 and C_11711720D_40; C___7586657_20, respectively (Applied Biosystems, Foster City, CA). Reactions were performed on an Applied Biosystems 7300 Real Time PCR System (Applied Biosystems) with a 5 μL final volume mixture containing 1 × TaqMan Genotyping Master Mix (Applied Biosystems), 900 nM of each primer, 200 nM of probes labeled with either FAM or VIC, and 10 ng of extracted DNA. Thermal cycling conditions were 10 min at 95°C followed by 45 cycles (or 50 cycles for *G2677TA)* of 15 s at 95°C and 1 min at 60°C. Allelic discrimination was performed by measuring endpoint fluorescence using an ABI PRISM® 7300 Sequence Detection System (Applied Biosystems). Genotyping data were read blind to the clinical course of illness and, in case of ambiguous genotypic data, experiments were repeated for determining the genotype of every individual.

Statistical analysis

Data preparation and analysis were carried out using the computer software PAWS Statistics 18 (release 18.0.0) and Epi Info (version 6.04a). A 5% level of significance was used in the chi-square (χ^2) analysis to compare the categorical variables. The odds ratio (OR) and 95% confidence interval (CI) were calculated as a measure of association between genotypes and MDD risk. The Fisher's exact test was used for tables containing cells, where values are less than five individuals. The Hardy–Weinberg equilibrium was tested by a χ^2 test to compare the observed versus the expected genotype frequencies. Measures of linkage disequilibrium (LD), represented as D' and r^2, were calculated from the haplotype frequency using the expectation-maximization (EM) algorithm with SNP & Variation Suite 7 *free trial* (Golden Helix) software. In addition, to correct for multiple comparison testing, adjusted *p*-values were determined using the false

discovery rate (FDR) method. A posteriori power analysis was performed using Quanto software (version 1.0; http://hydra.usc.edu/gxe) with input of the following variables: case–control study design, significance level (α) < 0.05 (two sided), model of inheritance = log additive, minor allele frequency = 0.406, and genetic effect for OR (≥1.75 and ≤0.55). This study achieved a statistical power of 80% for the minor allele of *ABCB1 C1236T*, which exhibited the lowest allele frequency among the three polymorphisms.

Results

Allele and genotype frequencies of ABCB1 *gene*

The genotype and allele frequencies of the *ABCB1 C1236T*, *C3435T* and *C2677TA* polymorphisms are shown in Table 1. No significant deviations from the Hardy–Weinberg equilibrium proportions were observed both in controls and cases (Table 1). LD pairwise analysis revealed a strong LD between *C1236T* and *G2677TA* (D' = 0.88, r^2 = 0.75) and *C3435T* and *G2677TA* (D' 0.83, p = 0.59), while *C1236*T and *C3435T* loci were in moderate LD (D' = 0.67, r^2 = 0.40).

Allele and genotype association with MDD risk

To estimate the influence of *ABCB1* polymorphisms in the development of MDD, allele and genotype frequencies were compared between MDD patients and controls. Our results did not reveal any statistically significant association (Table 1). However, a tendency of a lower MDD risk was verified for 1236TT genotype carriers (OR = 0.699; 95% CI: [0.309–1.582]; p = 0.390). Similar results were found for individuals carrying the 2677TT variant (OR = 0.615; 95% CI: [0.248–1.525]; p = 0.291).

Nevertheless, when data were stratified according to gender, a significant association was verified among male patients (Table 2). Carriers of the variant T allele in *C1236T*, *C3435T*, and *G2677TA* polymorphisms had lower risk of developing MDD (OR = 0.360, 95% CI: [0.140–0.950], p = 0.022; OR = 0.306, 95% CI: [0.096–0.980], p = 0.042; OR = 0.300, 95% CI: [0.100–0.870], p = 0.013, respectively).

Haplotype association with MDD risk

To estimate the combined influence of *ABCB1* polymorphisms in the risk of MDD development, haplotype analysis was carried out. A total of seven different haplotypes were identified using the SVS software (Table 3). Although haplotype analysis did not reveal any significant association with MDD diagnosis regarding the overall subjects, TTT haplotype was associated to a lower risk of MDD development in male subjects (OR = 0.313, 95% CI: [0.118–0.832], p = 0.016). The association remained statistically significant after multiple comparisons adjustment (FDR p = 0.032).

Comparison of ABCB1 *genotype frequencies with reports from the literature*

To verify if our control subjects are similar to other Caucasian populations, we compared the frequencies of the genetic polymorphisms evaluated in this study with the observed frequencies in the control samples from other reports. Genotype and allele frequencies of our controls were not different from what is reported in the literature, regarding the Portuguese population (*C1236T*: χ^2 = 4.81, DF = 2, p = 0.09; *G2677TA*: χ^2 = 4.84, DF = 2, p = 0.089; *C3435T*: χ^2 = 4.79; DF = 2; p = 0.091).

Table 1. Allele and Genotype Frequencies of *ABCB1* Polymorphisms and Risk for Major Depressive Disorder

		Controls		*Cases*		*HWE* p				
SNP	*Alleles/genotypes*	N	%	N	%	*Controls*	*Cases*	*OR*	*95% CI*	p-*Value*
C1236T	C	190	59.4	101	63.0	0.395	0.953		Referent	
	T	130	40.6	59	37.0			0.850	[0.570–1.290]	0.428
	CC	59	36.9	32	40.0				Referent	
	CT	72	45.0	37	46.2			0.947	[0.528–1.701]	0.857
	TT	29	18.1	11	13.8			0.699	[0.309–1.582]	0.390
	T Carrier	101	63.1	48	60.0			0.638	[0.505–1.520]	0.638
C3435T	C	185	57.8	91	56.9	0.413	0.956		Referent	
	T	135	42.2	69	43.1			1.040	[0.700–1.550]	0.845
	CC	56	35.0	26	32.5				Referent	
	CT	73	45.6	39	48.8			1.151	[0.628–2.110]	0.650
	TT	31	19.4	15	18.8			1.042	[0.481–2.256]	0.916
	T Carrier	104	65.0	54	67.5			1.118	[0.633–1.977]	0.700
G2677TA	G	195	60.9	101	63.1	0.891	0.953		Referent	
	T	120	37.5	55	34.4			0.880	[0.580–1.350]	0.548
	A	5	1.6	4	2.5			1.540	[0.340–6.800]	0.500*
	GG	59	36.9	32	40.0				Referent	
	GT	72	45.0	36	45.0			0.922	[0.512–1.659]	0.786
	GA	5	3.1	1	1.2			0.369	[0.041–3.294]	0.661*
	TT	24	15.0	8	10.0			0.615	[0.248–1.525]	0.291
	AA	ND	-	ND	-			**	**	**
	TA	ND	-	3	3.8			**	**	**0.049***
	Variant Carrier	101	63.1	48	60.0			0.876	[0.505–1.520]	0.638

Bold values indicate $p < 0.05$.
*One cell count is less than 5, Fisher's exact test was used.
**One cell count is 0, unable to calculate OR.
OR, odds ratio; ND, not detected; HWE, Hardy–Weinberg equilibrium; CI, confidence interval; SNP, single-nucleotide polymorphisms.

Table 2. Allele and Genotype Frequencies of *ABCB1* Polymorphisms and Risk for Major Depressive Disorder in Males

		Controls		*Cases*		*HWE* p				
SNP	*Alleles/genotypes*	N	%	N	%	*Controls*	*Cases*	*OR*	*95% CI*	p-*Value*
C1236T	C	51	60.7	34	81.0	0.755	0.736		Referent	
	T	33	39.3	8	19.0			0.360	[0.140–0.950]	**0.022**
	CC	15	35.7	14	66.7				Referent	
	CT	21	50.0	6	28.6			0.306	[0.096–0.980]	**0.042**
	TT	6	14.3	1	4.8			0.179	[0.019–1.675]	0.200*
	T Carrier	27	64.3	7	33.3			0.278	[0.092–0.839]	**0.020**
C3435T	C	48	57.1	33	78.6	0.418	0.211		Referent	
	T	36	42.9	9	21.4			0.36	[0.140–0.920]	**0.018**
	CC	15	35.7	12	57.1				Referent	
	CT	18	42.9	9	42.9			0.625	[0.207–1.883]	0.402
	TT	9	21.4	ND	-			**	**	**0.016***
	T Carrier	27	64.3	9	42.9			0.417	[0.143–1.215]	0.105
G2677TA	G	53	63.1	35	81.0	0.633	0.359		Referent	
	T	30	35.7	6	14.3			0.300	[0.100–0.870]	**0.013**
	A	1	1.2	1	2.4			1.510	[0.000–57.66]	1.000*
	GG	16	38.1	14	66.7				Referent	
	GT	20	47.6	6	28.6			0.343	[0.107–1.094]	0.066
	GA	1	2.4	1	4.8			1.143	[0.065–20.018]	1.000*
	TT	5	11.9	ND	-			**	**	0.069*
	AA	ND	-	ND	-			**	**	**
	TA	ND	-	ND	-			**	**	**
	Variant Carrier	20	47.6	6	28.6			0.308	[0.102–0.925]	0.032

Bold values indicate $p < 0.05$.
*One cell count is less than 5, Fisher's exact test was used.
**One cell count is 0, unable to calculate OR.

Genotype frequency distribution of the *C1236T* SNP in our study group was not significantly different than other Caucasian populations, such as Czech, French, German, and Hungarian. However, significant differences were found with other ethnic groups, such as Chinese, Indian, Japanese, Turkish, and Malay populations (Table 4). Similar results were found for *G2677TA* (Table 4). Concerning *C3435T* polymorphism, no significant differences were found between our study and the Mediterranean populations. However, significant differences were found for other Caucasian populations such as British, German, Hungarian, Czech, and Polish (Table 4).

Discussion

We investigated three common SNPs in the *ABCB1* gene (*C1236T*, *G2677TA* and *C3435T*) in a subset of Portuguese patients with MDD. To the best of our knowledge, no reports have been published in Caucasian populations regarding the effect of these *ABCB1* genetic polymorphisms on MDD risk.

Table 3. Haplotype Frequencies Derived from *ABCB1 C1236T*, *G2677TA* and *C3435T* Polymorphisms in Control Subjects and in Patients with Major Depressive Disorder

Haplotype	*Controls frequency*	*Cases frequency*	*OR*	*95% CI*	p-*Value*	*FDR* p-*Value*
CGC vs. Non CGC						
Overall	0.481	0.514	1.153	[0.786–1.691]	0.468	0.818
Males	0.511	0.735	2.769	[1.205–6.363]	**0.015**	0.058
TTT vs Non TTT						
Overall	0.316	0.327	1.060	[0.705–1.593]	0.778	0.908
Males	0.345	0.142	0.313	[0.118–0.832]	**0.016**	**0.032**
CGT vs. Non CGT						
Overall	0.072	0.082	1.160	[0.573–2.349]	0.679	0.950
Males	0.071	0.051	0.692	[0.138–3.468]	0.653	0.870
TGC vs. Non TGC						
Overall	0.049	0.031	0.627	[0.225–1.749]	0.368	0.860
Males	0.048	0.048	1.000	[0.175–5.699]	1.000	1.000
TTC vs. Non TTC	0.034	0.007	0.196	[0.027–1.398]	0.071	0.497
CTT vs. Non CTT	0.022	0.006	0.282	[0.034–2.314]	0.209	0.731
CAC vs. Non CAC	0.011	0.013	1.241	[0.219–7.021]	0.807	0.807

Bold values indicate $p < 0.05$.
FDR, false discovery rate.

Table 4. Allele and Genotype Frequencies for the *ABCB1 C1236T*, *G2677TA*, and *C3435T* SNPs in Portuguese Subjects and in Other European and Asian Populations

	Population	*N*	*T%*	*CC% (N)*	*CT% (N)*	*TT% (N)*	p-*Value*	*Ref.*
C1236T	Portuguese	160	40.6	36.9 (59)	45.0 (72)	18.1 (29)	-	This study
	Portuguese	100	46.0	25.0 (25)	58.0 (58)	17.0 (17)	0.090	(Correia *et al.*, 2009)
	French	223	42.0	33.0 (74)	49.0 (110)	17.5 (39)	0.686	(Jeannesson *et al.*, 2007)
	German	461	41.0	34.3 (158)	49.2 (227)	16.5 (76)	0.650	(Cascorbi *et al.*, 2001)
	Hungarian	503	44.3	33.2 (167)	44.9 (226)	21.9 (110)	0.524	(Sipeky *et al.*, 2011)
	Czech	189	44.5	31.7 (60)	47.1 (89)	21.2 (40)	0.561	(Pechandova *et al.*, 2006)
	Polish	139	41.4	35.3 (49)	46.8 (65)	18.0 (25)	0.949	(Tan *et al.*, 2004)
	Chinese	206	63.8	15.5 (32)	41.3 (85)	43.2 (89)	**<0.001**	(Tan *et al.*, 2005)
	Indian	87	67.2	13.8 (12)	37.9 (33)	48.3 (42)	**<0.001**	(Chowbay *et al.*, 2003)
	Japanese	1100	60.0	16.3 (179)	46.7 (514)	37.0 (407)	**<0.001**	(Fujii *et al.*, 2012)
	Malay	92	62.5	12.0 (11)	44.6 (41)	43.5 (40)	**<0.001**	(Chowbay *et al.*, 2003)
	Korean	232	61.9	11.6 (27)	53.0 (123)	35.4 (82)	**<0.001**	(Lee *et al.*, 2005)
	Turkish	100	54.5	20.0 (20)	51.0 (51)	29.0 (29)	**0.009**	(Gumus-Akay *et al.*, 2008)

	Population	*N*	*T%*	*CC% (N)*	*CT% (N)*	*TT% (N)*	p-*Value*	*Ref.*
C3435T	Portuguese	160	42.2	35.0 (56)	45.6 (73)	19.4 (31)	-	This study
	Portuguese	100	57.0	22.8 (23)	57.9 (58)	19.3 (19)	0.091	(Correia *et al.*, 2009)
	Spanish	408	48.0	26.0 (106)	52.0 (212)	22.0 (90)	0.101	(Bernal *et al.*, 2003)
	French	222	46.0	28.0 (61)	52.0 (116)	20.0 (45)	0.275	(Jeannesson *et al.*, 2007)
	Italian	450	47.8	25.6 (115)	53.3 (240)	21.1 (95)	0.071	(Palmieri *et al.*, 2005)
	British	280	53.9	21.8 (61)	48.6 (136)	29.6 (83)	**0.004**	(Onnie *et al.*, 2006)
	German	461	53.9	20.8 (96)	50.5 (233)	28.6 (132)	**<0.001**	(Cascorbi *et al.*, 2001)
	Hungarian	503	52.7	22.3 (112)	50.1 (252)	27.6 (139)	**0.003**	(Sipeky *et al.*, 2011)
	Czech	189	56.5	21.2 (40)	44.9 (85)	33.9 (64)	**<0.001**	(Pechandova *et al.*, 2006)
	Polish	173	52.2	21.6 (30)	52.5 (73)	25.9 (36)	**0.021**	(Tan *et al.*, 2004)
	Chinese	206	34.7	42.2 (87)	46.1 (95)	11.7 (24)	0.091	(Tan *et al.*, 2005)
	Indian	87	62.3	18.4 (16)	36.8 (32)	44.8 (39)	**<0.001**	(Chowbay *et al.*, 2003)
	Japanese	1100	40.0	35.0 (386)	50.0 (552)	15.0 (162)	0.279	(Fujii *et al.*, 2012)

	Population	*N*	*A%*	*T%*	*GG% (N)*	*GT + GA% (N)*	*TT + TA + AA% (N)*	p-*Value*	*Ref.*
G2677TA	Portuguese	160	1.6	37.5	36.9 (59)	48.1 (77)	15.0 (24)	-	This study
	Portuguese	100	NE	47.5	31.0 (31)	43.0 (43)	26.0 (26)	0.089	(Cavaco *et al.*, 2003)
	French	225	45.0		28.4 (64)	52.4 (118)	19.1 (43)	0.189	(Jeannesson *et al.*, 2007)
	Italian	450	2.0	43.6	28.0 (126)	55.1 (248)	16.9 (76)	0.111	(Palmieri *et al.*, 2005)
	British	285	2.5	39.6	33.0 (94)	49.8 (142)	17.2 (49)	0.601	(Onnie *et al.*, 2006)
	German	461	1.9	41.6	31.0 (143)	51.2 (236)	17.8 (82)	0.365	(Cascorbi *et al.*, 2001)
	Hungarian	503	1.1	44.1	30.6 (154)	48.3 (243)	21.1 (106)	0.152	(Sipeky *et al.*, 2011)
	Czech	189	0.5	46.0	29.6 (56)	48.1 (91)	22.2 (42)	0.152	(Pechandova *et al.*, 2006)
	Polish	139	1.1	41.4	33.8 (47)	47.5 (66)	18.7 (26)	0.668	(Tan *et al.*, 2004)
	Chinese	206	11.4	36.4	27.7 (57)	49.0 (101)	23.3 (48)	0.062	(Tan *et al.*, 2005)
	Indian	87	6.9	59.8	13.8 (12)	39.1 (34)	47.1 (41)	**<0.001**	(Chowbay *et al.*, 2003)
	Japanese	1100	18.0	39.0	17.5 (193)	50.9 (560)	31.5 (347)	**<0.001**	(Fujii *et al.*, 2012)
	Korean	632	17.1	39.1	19.1 (121)	49.4 (312)	31.5 (199)	**<0.001**	(Lee *et al.*, 2005)
	Turkish	70	ND	47.0	30.0 (21)	44.3 (31)	25.7 (18)	0.144	(Sapmaz *et al.*, 2008)

Bold values indicate $p < 0.05$.

Furthermore, the previous reports associating the risk of MDD and functional polymorphisms in *ABCB1* were conducted in Asian populations, and have only evaluated the effect of *C1236T* and *C3435T* polymorphisms in haplotype analysis. We included in the study, a third polymorphism in the *ABCB1* haplotype analysis, the triallelic *G2677TA* functional genetic polymorphism.

We demonstrated a significant protection for MDD only in males carrying the T allele in the evaluated polymorphisms. Moreover, the haplotype analysis revealed that male individuals carrying the TTT haplotype have nearly 70% less risk of developing MDD. Overall, the case–control analysis showed no association between the risk of MDD and the presence of a specific allele, genotype, or haplotype. Our results seem to indicate that the influence of *ABCB1* functional polymorphism in the risk of MDD is gender specific.

Although several reports addressed the influence of these *ABCB1* genetic polymorphisms in antidepressant responses, only two studies have evaluated the association of these common polymorphisms in the *ABCB1* gene and the risk of depression (Qian *et al.*, 2006; Fujii *et al.*, 2012). Fujii *et al.* (2012) observed, in a large group of Japanese subjects that, carriers of the 3435T allele or 1236T-3435T haplotype were more common in patients than in controls, suggesting that *C3435T* may

have a role in MDD development risk. However, regarding the triallelic SNP, *G2677TA*, the authors did not find any significant differences in allelic and genotypic frequencies between cases and controls. In another study, Qian *et al.* (2006) evaluated the effect of *C3435T* and *G2677TA* polymorphisms in mood disorders, including depression. No significant association between this *C3435T* polymorphism and the risk of developing mood disorder was found, but a higher frequency of 2677A was observed in patients with mood disorders than in controls ($p < 0.05$) (Qian *et al.*, 2006). The putative reasons for these discrepancies are widely discussed in the article by Fujii *et al.* (2012), and are related to the sample size and the heterogeneity of the patients used in the Qian *et al.* (2006) study.

We observed that the TT genotype in *C3435T* was less common in male cases than in controls and this points to a different direction when compared to the reports of Fujii *et al.* (2012) and Qian *et al.* (2006). These nonconcordant results may be explained by two lines of evidence. First, both studies evaluated MDD risk in Asian subjects. The genetic variability of the *ABCB1* polymorphisms is known to diverge among ethnic groups (Ameyaw *et al.*, 2001; Sipeky *et al.*, 2011), as shown in Table 4. Second, no stratification on gender was present in these studies. As proposed by Becker *et al.*, (2009) gender stratification is essential when evaluating the effect of these *ABCB1* polymorphisms, since they are found to be associated with the cholesterol-lowering effect of simvastatin only in males. Additionally, it has been proposed by several authors that different susceptibility of depression is under the major influence of gender (Unschuld *et al.*, 2010).

Haplotype analysis in *ABCB1* polymorphisms, rather than a single SNP, may provide an advantage in the diagnosis of complex diseases, such as MDD. In fact, there are reports of significant correlations found using *ABCB1* 1236T-2677T-3435T haplotype analysis and lack of association when each SNP was analyzed separately (Potocnik *et al.*, 2004; Kimchi-Sarfaty *et al.*, 2007a). We observed that the *ABCB1* 1236T-2677T-3435T haplotype presents a protective effect since the male carriers of this profile have a lower risk of developing MDD (OR = 0.313, 95% CI: [0.118–0.832], $p = 0.016$). Clinical studies have shown that the 1236T-2677T-3435T haplotype is also associated with reduced P-GP activity (Salama *et al.*, 2006). Indeed, recombinant cells expressing *ABCB1* 1236T-2677T-3435T variants displayed only 0–22.7% transporter activity, which suggests that this haplotype significantly reduces P-GP functionality *in vitro*, losing about 80%-100% of its efflux capability (Salama *et al.*, 2006). Although some authors defend that the lack of P-GP function could lead to the accumulation of deleterious substances, such as neurotoxins, and represent a risk of MDD development (Fujii *et al.*, 2012), we hypothesized that male carriers of the 1236T-2677T-3435T haplotype may have a less active transporter and this might be protective for the risk of MDD. P-GP is known to *in vivo* regulate brain access of endogenous steroid hormones (Uhr *et al.*, 2002; Binder *et al.*, 2009). In normal subjects, the HPA axis is regulated by a negative feedback control, whereby glucocorticoids secreted from the adrenal gland suppress the hypothalamus to secret the corticotrophin releasing hormone (CRH) and arginine–vasopressin, which *per se* limits the release of corticotrophin (ACTH) from the anterior pituitary, and thus promotes less glucocorticoid release from adrenal glands (O'Brien *et al.*, 2012). In depressed patients, this negative feedback control is impaired and patients display HPA axis hyperactivity, evident by an increased cortisol concentration in serum, urine, and the cerebrospinal fluid (Holsboer, 2000; Pariante and Miller, 2001).

Our hypothesis is that the putative lower P-GP function, found in carriers of the 1236T/2677T/3435T haplotype, may restrain HPA system activation after stressful events, and induce a chronically increased negative feedback at the hypothalamic level due to an enhanced penetration of glucocorticoid hormones into the central nervous system, and promote a less active HPA response under stress conditions. Interestingly, *in vivo* studies using *abcb1ab* (−/−) mice mutants prove that the absence of P-GP leads to an increased penetration of corticosteroid hormones in the central nervous system, which in turn, enhanced central negative feedback inhibition of stress hormone secretion, with lower plasma ACTH levels in both at basal and under stress conditions (Muller *et al.*, 2003). Moreover, antidepressants have also inhibited P-GP *in vitro*, and decreased the HPA axis activity *in vivo*, leading to the hypothesis that antidepressants inhibit P-GP at the BBB and increase glucocorticoid access to the brain (Yau *et al.*, 2007).

The gender-specific trait of our results can be explained by the differential expression of P-GP in male and female (Suzuki *et al.*, 2006; Bebawy and Chetty, 2009). Sex hormones can regulate the expression of P-GP in systemic organs, contributing to the differences observed in metabolic disposition (Compton *et al.*, 2006). Interestingly, the male androgens suppress P-GP expression and function, contrary to what is reported for the female sex hormones, but the precise mechanism for testosterone repression of P-GP expression remains undefined (Suzuki *et al.*, 2006). Moreover, a distinct HPA axis activation among male and female might explain our results. Binder *et al.*, (2009) observed a normalization of HPA axis reactivity, illustrated by the dexamethasone-resistant test, only among male patients with remission of depressive symptoms. Another report showed that knockdown of glucocorticoid receptors in the medial prefrontal cortex, hippocampus, and basolateral amygdala induces depression-like behaviors in male mice, but not in females (Solomon *et al.*, 2012). This author suggests that this mechanism for development of mood disorders holds only in males and provable symptoms of depression are mediated by an alternative brain circuitry and/or different molecular substrates in comparison with female gender individuals (Solomon *et al.*, 2012). Additionally, several reports in the literature regarding depression and antidepressant responses, point out for differences among male and female patients (Schaich *et al.*, 2009; Kurita *et al.*, 2012). The absence of a significant association in women found in our study does not exclude the fact that genetic variation in *ABCB1* does affect the risk of MDD development in women, but the effect is most likely weaker in women than in males.

The main limitation of our study is the relatively low number of subjects genotyped. Major concerns of small sample studies are false-positive results and over-estimation of magnitude association. To exclude false-positive results, FDR correction was included for multiple comparisons, and our results remain statistically significant for the TTT haplotype. In this study, high magnitude associations (OR ≥ 1.75 and ≤ 0.55) are detected with an 80% statistical power, like the one we found for the male TTT haplotype. However, it is possible that small effects are not detected with our sample. Control selection bias was excluded by comparing genotypic

frequencies of our controls and other southern Caucasian samples, including Portuguese. Although our study was the first to analyze the influence of *ABCB1 C1236T*, *C3435T* and *G2677TA* polymorphisms in a Caucasian subset of MDD patients, independent association studies with larger sample sizes are needed to confirm the influence of these SNPs in MDD development risk.

In conclusion, this article adds clinical evidence that genetic variability of the *ABCB1* is associated with MDD in males, and that this may influence the HPA system activity, through a less active P-GP. The observed sex differences in *ABCB1* polymorphisms and risk of MDD may explain a gender dimorphic sensitivity of the HPA axis and hypothetically reflect a gender-specific pathophysiology of depression.

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