EVALUATION OF THE ROLE OF ABCB1GENE POLYMORPHIC VARIANTS ON PSYCHIATRIC DISORDERS PREDISPOSITION IN MACEDONIAN POPULATION

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ABSTRACT

The psychiatric and other CNS disorders are characterized with unregulated neuro-inflammatory processes and chronic microglia cell activation resulting with detrimental effect. ABCB1gene polymorphismsC1236T, G2677T/Aand C3435T are associated with P-glycoprotein expression and function andare linked with predisposition to psychiatric disorders such as schizophrenia and bipolar disorders. The relationship between mood disorders and glucocorticoids has been confirmed and ABCB1 SNPs influence the glucocorticoids access to the brain.

The aim of the study is evaluation of the influence of the three most common ABCB1SNPs on predisposition to psychiatric disorders in Macedonian population.

In the study 107 unrelated healthy Macedonians of both sexes were enrolled as a control group and patient population of 54 patients (22 to 65 years old) diagnosed with schizophrenia or bipolar disorder. ABCB1 for three polymorphisms were analyzed by Real-Time PCR in both groups.

The results have confirmed the role of the ABCB1 gene in predisposition to psychiatric disorders and increased risk of developing bipolar disorder in carriers of the heterozygotes and mutant homozygotes for polymorphic variations in 1236 and 2677 in comparison to the normal genotype carriers. Three-fold higher risk was estimated for psychiatric illness in women that are 1236 and 2677 heterozygous carrier (heterozygous and mutant homozygous) compared to healthy control (men and women) population and four-fold higher risk in comparison only to healthy women population. Mutant allele carriers for 1236 and 2677 polymorphisms that are 35 years and below in patients population have almost three-fold higher risk for development of psychiatric illness.

Keywords: P-glycoprotein, ABCB1, schizophrenia, bipolar disorders, pharmacotherapy, neuro-inflammation, ABCB1 and glucocorticoids access to brain

INTRODUCTION

The psychiatric and other CNS disorders are characterized with unregulated neuro-inflammatory processes and chronic microglia cell activation resulting with detrimental effect. The microglia cells are highly associated with neu-rogenesis, neuronal function and programmed neuronal cell death in various CNS regions like hippocampus, cerebellum, retina and spinal cord [1]. The macrophage (microglia)/T cell theory of mood disorders and schizophrenia was initially

adopted in 1995 [2]. According to this theory cytokines and inflammatory compounds produced by microglia and T-cells impact the brain development and make it more liable to genetic and environmental influences in order to present the characteristic psychiatric symptoms.

Schizophrenia is a long-lasting, disabling brain disease involving progressive loss of gray matter which may be explained by neuronal damage arousedsecondary to psychosis when microglia cells and macrophages of the brain are activated [3]. Focal neuro-inflammation is a feature of psychosis and is not necessarily present in stable schizophrenic patients, but it may evolve in more widespread process in time. Confirmed hippocampal inflammation during psychosis may be a result of increased vulnerability of this brain region in this state. MRI studies have confirmed a decrease of hippocampal size in schizophrenic patients, even in prodromal and first-episode patients, which is strong evidence that this change is not a secondary reaction caused by the treatment [4].The decreased size of this region in schizophrenic patients is not a result of change in the total number of neurons in hippocampus but is rather a result of changed neuronal morphology, size, shape and organization [5-7].Abnormal inflammatory activation of microglia is confirmed in a postmortem studies on brains from schizophrenic patients, who had committed suicide during acute psychosis, results in increased level of cytokines (IL-6, TNF-α, IL-8) that directly affects the neuronal proliferation, survival and gene expression [8-13]. On the other hand, three other studies failed to detect activation state of microglia [14-16]. These results are also supported by in vivo PET studies that proved microglial activation in hippocampal area of schizophrenic patients with acute psychosis in which cognitive impairment is most protruding, but not in patients that recovered from psychosis. Beside microglia activation, there is also strong evidence for increased activation of circulating monocytes in patients with psychiatric diseases, especially schizophrenia. In this patients' population there is accumulation of monocytes and macrophages during acute psychosis in cerebrospinal fluid. On the contrary, higher monocyte number were not found in patients with bipolar disorder [1,17,18].

The pathophysiology of bipolar disorder (BD) is complex and its neurobiology remains unknown [19], but it is confirmed that there are complex interactions among stress and immune systems in the brain, and environmental factors.

Inflammatory monocyte gene expression is enhancing during the course of bipolar disorder, suggesting that the progression of the illness goes together with a raised monocyte inflammatory deregulation [20]. Activated monocyte gene expression of pro-inflammatory cytokines (IL-1β, IL-6, TNF) and adhesion/motility factors and chemokines was detected in patients with bipolar disorder, whereas the schizophrenic patients showed only activated gene expression of pro-inflammatory cytokines. Post-mortem studies in humans demonstrated an increased expression of inflammation-related pro-apoptosis genes [21] and oxidative damage [22] to the RNA in the hippocampus of BD patients, as well as decrease of growth-associated protein [23] that has been proposed to be related to neuro-inflammation [24]. Haarmman et al., 2014, revealed the neuro-inflammation in vivo in BD and they confirmed statistically significant increased binding of radiopharmaceutical (11C)-(R)-PK11195, used for visualization of microglia activation with PET, in the right hippocampus of BD-I patients in comparison with healthy controls. This finding was in line with previously confirmed increased peripheral blood monocyte gene expression which is related to hemodynamic changes measured by functional MRI in the hippocampus of combined sample of unipolar and bipolar depressed patients [25].

While ABC transporters actively interfere in immune processes by influencing the inflammatory molecules secretion of IL-1, IL-6, TNF-α, leptin, INF-γ through BBB they could be treated like important factors in pathophysiology of neuro-inflammatory disorders. After crossing the BBB, these cytokines bind to their receptors on glia cells and brain neurons where they trigger and initiate destabilization of major neurotransmitter and neurodevelopment systems and facilitate development of psychiatric symptoms [26].The role of ABC transporters could be considered in two different ways. First some authors suggest that ABC transporters are capable of transporting inflammatory molecules, while others suggest that these transporters mediate the secretion of other relevant physiological substances, such as bioactive lipids that affect the cytokine secretion as secondary effect [27,28].

ABCB1 (MDR1, P-glycoprotein) gene is the first identified and the best characterized gene from ABC transporter family. This gene encodes transmembrane protein, mediates ATP-dependent transport of various molecules and is

located in the chromosome 7q21.1 and consists of 28 translated exons and 27 introns with over 100kb. P-gpis a "gate keeper" of the brain and is expressed in the luminal surface of capillary endothelial cells of the blood-brain barrier (BBB) transport the toxic compounds out of the brain and effectively prevent the uptake [29,30].Beside the huge number of identified SNPs in ABCB1 gene, most studies are focused on three SNPs, namely 1236 C>T in exon 21 and 3435C>T in exon 26 that are synonymous, and 2677G>T/A in exon 21 that is a non-synonymous tri-allelic polymorphism responsible for Ser to Ala/Thr amino acid substitution at position 893. They are in high linkage disequilibrium [31]. The polymorphic variants of ABCB1 are important for mRNA levels and stability [32], protein folding [33], P-gp activity and efflux capacity of this important transporter in the organism.

The relationship between mood disorders and glucocorticoids has been confirmed in some studies [34, 35].Control of access of endogenous corticosteroids regulated by P-gp influences the activity and regulation of HPA system during stress when peripheral glucocorticoid level rapidly increases. Polymorphic variations of ABCB1 (MDR1) gene influence the P-gp activity and influence the glucocorticoid, corticosterone and cortisol, access to the brain. Enchained P-gp expression and function could play pivotal role in the neuroendocrine regulation of this system, by decreased penetration of endogenous corticosteroids into the CNS and lowered central negative feedback inhibition of stress hormone secretion, which is commonly observed in psychiatric disorders. Studies in the animal models confirmed that absence of P-gp function has serious influence to the activity and regulation of HPA system, suggesting regulative role of this transporter on HPA-axis activity. Increased expression of P-gp on BBB, which could be associated with ABCB1 polymorphic variants and its influence on functionality and expression [36], may reduce the penetration of peripheral corticosteroids to the brain resulting in poor suppressive activity of peripheral cortisol levels and prolonged secretion of endogenous stress hormones, condition that is commonly observed in psychiatric disorders [37].

The aim of our study is to evaluate the influence of polymorphic variants of the three most common SNPs C1236T, G2677T and C3435T on pathogenesis and pathological development on schizophrenia and mood disorders.

MATERIALS AND METHODS

The study included 54 patients (27males and 27 females) who aged from 22 to 65 years (36.23±12.457) recruited in the Clinic of Psychiatry in the University Clinical Center "Mother Theresa"-Skopje, Republic of Macedonia. All subjects were adult patients hospitalized in the Acute Psychiatric Unit, unrelated and Caucasian. The diagnosis of the patients was concluded according to the Diagnostic and Statistical Manuel of Mental Disorders Fourth Edition (DSM-IV, American Psychiatric Association 1994). Written informed consent was obtained for each patient. All patients were with variable length of hospital stay and were treated with risperidone. Blood samples were obtained from patients with schizophrenia and mood disorders and genomic DNA was extracted from peripheral lymphocytes. Totally, 107 unrelated healthy ethnical Macedonians of both sexes (76 males and 31 females) from Republic of Macedonia were included as control group. DNA samples of control group of patients were selected from the DNA bank in the Centre for Bimolecular and Pharmaceutical Analysis (CBPA) in the Faculty of Pharmacy in Skopje from individuals that were previously enrolled in other research studies. These research studies that were approved by the Ethical committee of the Faculty of Pharmacy University "Ss. Cyril and Methodius", Skopje, Republic of Macedonia. DNA samples were tested anonymously with previously removed personal data. All procedures were conducted in accordance with the Declaration of Helsinki.

DNA isolation

The genomic DNA was extracted from peripheral lymphocytes in the blood samples obtained in EDTA vacutainers, using Proteinase K digestion, phenol chloroform extraction and ethanol precipitation. DNA yields and purity were measured at 260 nm and 260/280nm respectively (NanoDrop 2000, Thermo Scientific) and DNA integrity was confirmed with electrophoresis on 1% agarose gels, stained with ethidium bromide.

Genotyping

The genotyping was performed with Real-Time PCR based on the allelic discrimination method (MxPRo 3005P, Staratgene, La Jolla, CA, USA) using TaqMan SNP genotyping assay for C1236T (rs1128503 assay ID C___7586662_10), G2677A/T (rs2032582 assay ID C__11711720_C_30 and C__11711720_C_40) and C3435T (rs1045642 assay ID C__7586657_20) according to the guidelines of the manufacturer (Life Technologies, USA).

Data Analysis

Statistical analysis was performed using SPSS software (v.22). The genotype distributions were assessed for the Hardy-Weinberg equilibrium (HWE) with χ2 test using an online calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1. pl). Statistical analysis for allele and genotype frequencies between our and other ethnic populations was evaluated with Chi-squared analysis and Fisher exact probability test. Odds ratios (OR) were calculated with 95% confidence interval limits (95%CI). The level of statistical significance was defined as p≤0.05. Linkage disequilibrium (LD) between SNP pairs in the population was estimated by Lewontin's coefficient (D') and Pearson's correlation (r2) (Lewontin and Kojima, 1960; Lewontin 1964). The statistical analyses were performed with SHSsis software platform for analysis of LD, haplotype and genetic association at polymorphism loci (http://analysis2.bio-x.cn/myAnalysis.php) [38,39].

RESULTS

The allele and genotype frequencies in the patient population (schizophrenic and bipolar disorders) and control group forABCB1 C1236T, G2677T and C3435T polymorphisms are presented in the Table 1. All of the determined distributions in both investigated populations were in agreement with those predicted by the Hardy-Weinberg equilibrium.

According to our results, the frequencies of wild type alleles for these three polymorphisms are lower in patient population. It is estimated that frequencies for 1236C allele are 56% vs. 49%; 2677G are 55% vs. 48% and 3435C are 51% vs. 46% in control vs. patient population. One interesting finding is that the CT genotype for all three allele is more common in patient population (33 patients, 61.1 % for1236; 34 patients 63% for 2677 and 64.8% for 3435 SNPs). The frequency of homozygotes for the wild type CC genotype for 1236, 2677, 3435 in patient population was 18.52%, 16.67% and 12.96%, respectively compared to 33.64%, 32.71% and 25.23% for the control group. The significant differences were confirmed for 1236CT (p=0.026; OR 2.475; CI 95% 1.08-5.67) and 1236CT+TT (p=0.044; OR 2.2; CI95% 0.994-4.87) genotypes in psychiatric patients in comparison to the control group. Even more significant difference was observed in patient population for the non-synonymous 2677 polymorphism; 2677GT (p=0.017; OR 2.755; CI 95% 1.172-6.472) and 2677GT+TT (p=0.0285; OR 2.465; CI95% 1.08-5.61) compared to control group. Our results didn't confirm influence of C3435T polymorphism on psychiatric disorders.

Pairwise LD profile for the three SNPs using D' and r2 values are summarized in (Table 2). All three SNPs in Macedonian patient population are in high Linkage Disequilibrium. The strongest correlation was observed between C1236T and G2677T (D'= 0.9.1, r2=0.76) followed by G2677T and C3435T (D'=0.706, r2=0.385) and C1236T and C3435T (D'=0.645, r2=0.414). These results have confirmed previously published data for haplotype inheriting in healthy Macedonian population where the same trend for high LD was established.

Table 1. *Allele and genotype distribution of polymorphic variants C1236T, G2677T and C3435T of ABCB1 gene in control group, patents population with diagnosed psychiatric disorder and subdivided patient population based on the diagnosis (schizophrenia and bipolar disorder)*

Allele distribution

SNP	Allele	Control group n=214 (chromosomes)	Allele freq	p	Patient population n=108 (chromosomes)	Allele freq	p	Schizophrenia patient population n=76 (chromosomes)	Allele freq	p	Bipolar disorder patient population n=32 (chromosomes)	Allele freq	p
rs.1128503	C	120	0,56 +/- 0.035	p=0.355	53	0,49 +/-0.042	p=0.101	40	0,53 +/- 0.052	p=0.321	13	0,41 +/- 0.066	p=0.09
	T	94			55			36			19		
rs.2032583	G	118	0,55 +/- 0.036	p=0.335	52	0,48 +/-0.041	p=0.054	39	0,51 +/- 0.051	p=0.192	13	0,41 +/- 0.066	p=0.09
	W (T/A)	96			56			37			19		
rs.1045642	C	110	0,51 +/- 0.033	p=0.623	49	0,45 +/-0.04	p=0.038	38	0,50 +/- 0.046	p=0.043	11	0,34 +/- 0.073	p=0.32
	T	104			59			38			21		

Genotype distribution

SNP	Genotype	Control group n=107 (individuals)	Freq. (%) obs	exp	p	Patient population n=54(Patients)	Freq. (%) obs	exp	p	Schizophrenia n=38(patients)	Freq. (%) obs	exp	p	Bipolar disorder n=16(patients)	Freq. (%) obs	exp
rs.1128503	CC	36	33,64	31,36	p=...	10	18,52	24,01	p=...	9	23,68	28,09	p=...	1	6,25	16,81
	CT	48	44,86	49,26		33	61,11	49,98		22	57,89	49,82		11	68,75	48,38
	TT	23	21,50	19,30		11	20,37	25,94		7	18,42	22,09		4	25,00	34,81
	CC	36	33,64			10	18,52			9	23,68			1	6,25	
	CT +TT	71	66,36			44	81,48			29	76,32			15	93,75	
rs.2032583	GG	35	32,71	30,40		9	16,67	23,17		8	21,05	26,01		1	6,25	16,81
	GT	48	44,86	49,47		34	62,96	49,93		23	60,53	49,98		11	68,75	48,38
	TT	24	22,43	20,12		11	20,37	26,89		7	18,42	24,01		4	25,00	34,81
	GG	35	32,71			9	16,67			8	21,05			1	6,25	
	GG+TT	83	77,57			45	83,33			30	78,95			15	93,75	
rs.1045642	CC	27	25,23	26,42		7	12,96	20,25		6	15,79	25,00		1	6,25	11,56
	CT	56	52,34	49,96		35	64,81	49,50		26	68,42	50,00		9	56,25	44,88
	TT	24	22,43	23,62		12	22,22	30,25		6	15,79	25,00		6	37,50	43,56
	CC	27	25,23			7	12,96			6	15,38			1	6,25	
	CC+TT	80	74,77			47	87,04			33	84,62			15	93,75	

Table 2. *Risk evaluation of allele and genotype distribution of polymorphic variants C1236T, G2677T and C3435T of ABCB1 gene in patients population with diagnosed psychiatric disorder and subdivided patient population based on the diagnosis (schizophrenia and bipolar disorder) vs control group*

SNP	Allele	Control group n=214 chromosomes	Control group Allele freq	Patient population n=108 chromosomes	Patient Allele freq	Patient OR 95%CI	Patient p (psihiatry disorder vs. control)	Schizophrenia n=76 chromosomes	Sch Allele freq	Sch OR 95%CI	p (sch vs.control)	Bipolar n=32 chromosomes	Bipolar Allele freq	Bipolar OR 95%CI	p (bd vs.control)	sch vs. bd OR 95%CI	p (sch vs. bd)
rs.1128503	C	120 (56.07)	0.56 +/- 0.035	53 (49.07)	0.49 +/- 0.042	1.00		40 (52.63)	0.53 +/- 0.052	1.00		13 (41)	0.41 +/- 0.066	1.00		1.00	
	T	94 (43.93)		55 (50.93)		1.325 [0.833-2.107]	0.234	36 (47.37)	47.37	1.149 [0.680-1.945]	0.604	19 (59)		1.866 [0.877-3.971]	0.102	1.624 [0.703-3.7501]	0.254
rs.2032583	G	118 (55.14)	0.55 +/- 0.036	52 (48.14)	0.48 +/- 0.041	1.00		39 (51.13)	0.51 +/- 0.051	1.00		13 (41)	0.41 +/- 0.066	1.00		1.00	
	W (T/A)	96 (44.86)		56 (51.86)		1.324 [0.833-2.105]	0.235	37 (48.68)	48.68	1.166 [0.69-1.97]	0.565	19 (59)		1.796 [0.844-3.823]	0.125	1.541 [0.667-3.557]	0.310
rs.1045642	C	110 (51.4)	0.51 +/- 0.033	49 (45.37)	0.45 +/- 0.04	1.00		38 (50)	0.50 +/- 0.046	1.00		11 (34)	0.34 +/- 0.073	1.00		1.00	
	T	104 (48.6)		59 (54.63)		1.274 [0.801-2.026]	0.307	38 (50)	50	1.058 [0.627-1.785]	0.834	21 (66)		2.019 [0.928-4.392]	0.072	1.909 [0.810-4.498]	0.136

SNP	Genotype	Control n=107 (individuals)	Control Freq. (%)	Patient n=54 (Patients)	Patient Freq. (%)	Patient OR 95%CI	p (psihiatry disorder vs. control)	Schizophrenia n=38 (patients)	Sch Freq. (%)	Sch OR 95%CI	p (sch vs.control)	Bipolar disorder n=38 (patients)	Bipolar Freq. (%)	Bipolar OR 95%CI	p (bd vs.control)	sch vs. bd OR 95%CI	p (sch vs. bd)
rs.1128503	CC	36	33.64	10	18.52	1.00		9	23.68	1.00		1	6.25	1		1	
	CT	48	44.86	33	61.11	2.475 [1.08-5.67]	0.030	22	57.89	1.833 [0.755-4.455]	1.777	11	68.75	8.25 [1.018-66.849]	0.022	4.500 [0.504-40.172]	0.150
	TT	23	21.50	11	20.37	1.722 [0.631-4.697]	0.286	7	18.42	1.217 [0.398-3.723]	0.730	4	25.00	6.261 [0.658-59.574]	0.075	5.143 [0.465-56.897]	0.157
	CT+TT	71	66.36	44	81.48	2.231 [1.007-4.941]	0.045	29	76.32	1.634 [0.699-3.817]	0.254	15	93.75	7.606 [0.966-59.88]	0.026	4.655 [0.538-40.284]	0.132
rs.2032583	GG	35	32.71	9	16.67	1.00		8	21.05	1.00		1	6.25	1		1	
	GT	48	44.86	34	62.96	2.755 [1.172-6.472]	0.018	23	60.53	2.096 [0.840-5.233]	0.109	11	68.75	8.021 [0.989-65.040]	0.024	3.826 [0.424-34.514]	0.206
	TT	24	22.43	11	20.37	1.782 [0.641-4.956]	0.265	7	18.42	1.276 [0.408-3.988]	0.675	4	25.00	5.833 [0.614-55.458]	0.089	4.571 [0.409-51.138]	0.194
	GG+TT	83	77.57	45	83.33	2.431 [1.069-5.528]	0.031	30	78.95	1.823 [0.757-4.387]	0.177	15	93.75	7.292 [0.926-57.447]	0.030	4.000 [0.457-35.008]	0.183
rs.1045642	CC	27	25.234	7	12.96	1.00		6	15.79	1.00		1	6.25	1		1	
	CT	56	52.34	35	64.81	2.411 [0.949-6.125]	0.060	26	68.42	2.089 [0.769-5.676]	0.143	9	56.25	4.339 [0.523-36.021]	0.142	2.077 [0.219-19.678]	0.517
	TT	24	22.43	12	22.22	1.929 [0.653-5.692]	0.231	6	15.79	1.125 [0.320-3.959]	0.854	6	37.50	6.75 [0.758-60.147]	0.054	6.000 [0.544-66.169]	0.120
	CC+TT	80	74.766	47	87.04	2.266 [0.916-5.607]	0.072	33	84.62	1.800 [0.679-4.772]	0.233	15	93.75	5.062 [0.638-40.148]	0.091	2.812 [0.310-25.486]	0.341

In table 3 are presented genotype combinations and haplotype frequencies for polymorphisms C1236T, G2677T and C3435T forABCB1gene in control group and group of patients with psychiatric disorder

Table 3. *LD profile for C1236T, G2677T and C3435T SNPs in patient population with psychiatric disorder*

	C1236T	G2677T	C3435T
C1236T		0,901	0,706 D′
G2677T	0,76		0,645
C3435T	0,414	0,358	
	r²		

In our study 12 different genotype combinations among C1236, G2677T and C3435T SNPs were found, so far we have not identified carrier of 2677A mutant allele. Genotype combinations that were observed with frequencies higher than 3% were the following CT-GT-CT (48.15%), TT-TT-TT (12.96%), CC-GG-CC(7.41%), CT-GT-TT (7.41%) and CC-GT-CT (5.56%) (Table3). The haplotype analyses have shown that 1236CT-2677GT -3435TT is the most common genotype combination confirmed in 48.15% of patient population, whereas in control group it estimates only 31.78%. The mutant genotype combination carriers were presented with 12.96% of the patients and 15.89% in control group, but more important is the finding that this is the second most common combination in patient population. The wild type 1236CC-2677GG -3435CC genotype combination is confirmed in only 5.56% of the psychiatric patients, while it is found in 21.5% of controls. Additionally, it is very significant that only in one patient with bipolar disorder the wild type genotype combination was found (presented in table 3). In a population of patient population of Macedonian ethnicity these three SNPs were structured in eight different haplotypes. The haplotype combinations TTT with45.3%, CGC with 37.85% and CGT with 8.33% in our examined population were the most prominent.We confirmed significantly higher presence of mutant TTT haplotype (45.37% vs. 37.04%) in patients compared to haplotypes identified in control groups. The wild type haplotype is found in 37.04% of the patients while it is estimated in 45.33% of controls. Therefore, our findings are in line with many other studies that prefer haplotype analysis as more relevant for evaluation of influences of polymorphic variants, rather than the single SNPs.

Although the frequencies of mutant allele for the three polymorphic variants of ABCB1 gene are higher in psychiatric patient population compared to the control group, the differences didn't reach the statistical significance. Only non-significant tendency was observed for 3435C allele (p=0.072 OR 2.019; CI 95% 0.529–4.392) (table4). Evaluating the genotype distribution influence on psychiatric disorder predisposition in patient population compared to the control group following statistically significant differences were obtained 1236CT (p=0,03; OR 2.475; CI 95% 1.08-5.67);1236CT+TT (p=0.045; OR 2.231; CI 95% 1.007-4.941) and 2677GT (p=0.0117; OR 2.755; CI 95% 1.172-6.472) and 2677CT+TT (p=0.031; OR 2.431; CI95% 1.069-5.528) and 3435CT(p=0.05; OR 2.411 ; CI 95% 0.949-6.125);3435CT+TT (p=0.071; OR 2.266; CI 95% 0.916-5.607). These results confirmed that heterozygous genotypes for all three polymorphisms are statistically significantly more present in patient population in comparison to the control group. Heterozygous carries have two-fold increased risk for psychiatric disease occurrence. Highest difference was estimated for G2677T, followed by C1236T and 3435 polymorphism. These results were also confirmed when dominant model for evaluation was used. The obtained results suggest that the normal alleles might have protective role in development of psychiatric diseases for the three SNPs ofAB-CB1 gene (presented in table 4).

As the patients in the study were diagnosed with schizophrenia or bipolar disorder, we subdivided the investigated individuals in two separate groups according to their illness. In group of schizophrenic patients (38 patients) the statistical significance in comparison with the control group was lost for all three ABCB1 polymorphisms (presented in table 4). According to the results obtained in our study only one patient with wild type haplotype (1236CC, 2677CC and 3435CC) was genotyped in the group of bipolar disorder patients.All the other were carriers of at least one mutant allele for three SNPs. We have estimated the following differences with the controls 1236CC (p=0.0215; OR 8.25; CI 95% 1.08-66.849), 1236CT (p=0.0746; OR 6.261; CI 95% 0.658-59.574); 1236CT+TT (p=0.0258; OR 7.606; CI95% 0.996-59.88) and 2677GG (p=0.0239; OR 8.021; CI 95% 0.989-65.04)

Table 4. *Genotype combinations and haplotype frequencies for polymorphisms C1236T, G2677T and C3435T for ABCB1 gene in control group and group of patients with psychiatric disorder*

genotype combination

C1236T	G2677T	C3435T	Freqences(%)
CT	GT	CT	31,78
CC	GG	CC	21,50
TT	TT	TT	15,89
CC	GG	CT	7,48
CT	GT	TT	5,61
CC	GT	CT	3,74
CT	TT	CT	2,80
TT	TT	CT	2,80
CT	GG	CT	1,87
CT	GT	CC	1,87
CC	GT	CC	0,93
CT	TT	TT	0,93
TT	GG	CC	0,93
TT	GG	CT	0,93
TT	GT	CT	0,93
Total			100,00

C1236T	G2677T	C3435T	Freqences(%)
CT	GT	CT	48,15
TT	TT	TT	12,96
CC	GG	CC	5,56
CT	GT	TT	7,41
CC	GG	CT	5,56
CC	GT	CT	3,70
CT	GG	CT	3,70
TT	TT	CC	3,70
CT	GG	TT	1,85
CC	GT	CC	1,85
CC	TT	CC	1,85
TT	CT	CT	1,85
TT	TT	CT	1,85
Total			100,00

haplotype combination

haplotype	Freqences(%) control group	Freqences(%) patients with psychiatric disorder
CGC	45,33	37,04
TTT	37,85	42,59
CGT	7,48	8,33
CTT	2,34	1,85
CTC	1,87	1,85
TTC	2,34	5,56
TGC	1,40	0,93
TGT	1,40	1,85
Total	100,00	100,00

2677GT (p=0.0889; OR 5.833; CI 95% 0.614-55.458) and 2677CT+TT (p=0.03; OR 7.292; CI95% 0.926-57.447). Although, the p values for C3435T polymorphism were lower compared to the analysis where the patients were not subdivided, they still didn't reach the statistical significance.

In the further step, the influence of the gender and the polymorphic variations of ABCB1 gene were evaluated in order to estimate the risk for psychiatric disorders. Frequencies' differences weren't confirmed for ABCB1 polymorphisms for both and in-between the gender (27men and 27 women) (Table 5). There is no statistically significant differences in genotype frequencies for ABCB1 gene polymorphic variants in man with psychiatric disorder in comparison with the control group (both men and women) nor with the control group including only men (Table 6).

When the women's population with psychiatric disorders was assessed, statistically sig-nificant differences were confirmed compared to the control group (men and women) for 1236CT (p=0.044; OR 3.188; CI 95% 0.988-610.288) and 1236CT+TT (p=0.05; OR 2.915; CI95% 0.937-9.070) and 2677GT (p=0.0018; OR 4.375; CI 95% 1.195-16.014) and 2677CT+TT (p=0.026; OR 3.889; CI95% 1.096-13.797). These results suggest that women carrying the heterogeneous alleles for 1236 and 2677 have almost four-fold higher risk for development of psychiatric disorder in comparison to wild type carriers. Additionally when we compared the women with psychiatric disorder with control group only comprised of women we observed three-fold higher risk for development of schizophrenia or bipolar disorder in carriers of mutant allele for 1236 and 2677 (data presented in table 7).

Because the literature results propose that bipolar disorders and schizophrenia incidence is highest in the period between 20 and 35 years, we have conducted an analysis in patients divided in two groups; first group aged between 20 and 35

Table 5. *Comparison of allele and genotype distribution of polymorphic variants C1236T, G2677T and C3435T of ABCB1 gene between men and women patents population with diagnosed psychiatric disorder.*

SNP	Allele	N=27 women (50%)	Freq. (%) obs	Pearson (p) exp	N=27 men (50%)	Freq. (%) obs	Pearson (p) exp	OR 95%CI	p
rs.1128503	C	25	0.46 +/-0.058	0,167	28	0.52 +/-0.061	0,330	1,00	
	T	29			26			0,800 [0.376-1.704]	0,564
rs.2032583	G	24	0.44 +/-0.055	0,069	28	0.52 +/-0.061	0,332	1,00	
	W (T/A)	30			26			0,743 [0.348-1.5841]	0,441
rs.1045642	C	24	0.44 +/-0.055	0,069	25	0.46 +/-0.058	0,167	1,00	
	T	30			29			0,928 [0.435-1.980]	0,847

SNP	Genotype	N=27 women (50%)	Freq. (%) obs	exp	N=27 men (50%)	Freq. (%) obs	exp	OR 95%CI	p
rs.1128503	CC	4	14,81	21,16	6	22,22	27,04	1,00	
	CT	17	62,96	49,68	16	59,26	49,92	0,627 [0.149-2.642]	0,523
	TT	6	22,22	29,16	5	18,52	23,04	0,556 [0.098-3.148]	0,505
	CC	4	14,81		6	22,22		1,00	
	CT+TT	23	85,19		21	77,78		0,609 [0.151-2.460]	0,484
rs.2032583	GG	3	11,11	19,36	6	22,22	27,04	1,00	
	GT	18	66,67	49,28	16	59,26	49,92	0,444 [0.095-2.075]	0,295
	TT	6	22,22	31,36	5	18,52	23,04	0,417 [0.067-2.584]	0,343
	GG	3	11,11		6	22,22		1,00	
	GG+TT	24	88,89		21	77,78		0,438 [0.097-1.970]	0,273
rs.1045642	CC	3	11,11	19,36	4	14,81	21,16	1,00	
	CT	18	66,67	49,28	17	62,96	49,68	0,708 [0.138-3.641]	0,679
	TT	6	22,22	31,36	6	22,22	21,16	0,750 [0.115-4.898]	0,764
	CC	3	11,11		4	14,81		1,00	
	CC+TT	24	88,89		23	85,19		0,719 [0.145-3.569]	0,685

Table 6. *Evaluation of allele and genotype distribution of polymorphic variants C1236T, G2677T and C3435T of ABCB1 gene in male patents population with diagnosed psychiatric disorder vs control group and sub-classified control group based on gander*

SNP	Allele	Control group vs. patients men		Control group men		Patients men		Control group men vs. patients men	
		OR 95%CI	p	n=76 (men)	Freq. (%)	N=27 men (50%)	Freq. (%)	OR 95%CI	p
rs.1128503	C	1,00		83	0.55 +/-0.042	28	0.52 +/-0.061	1,00	
	T	1.185 [0.652-2.156]	0,577	69		26		1.117 [0.600-2.081]	0,727
rs.2032583	G	1,00		81	0.53 +/-0.044	28	0.52 +/-0.061	1,00	
	W (T/A)	1.141 [0.628-2.075]	0,665	71		26		1.059 [0.569-1.973]	0,856
rs.1045642	C	1,00		81	0.54 +/-0.042	25	0.46 +/-0.058	1,00	
	T	1.227 [0.674-2.232]	0,503	71		29		1.323 [0.710-2.467]	0,377

SNP	Genotype	Control group vs. patients men		Control group men		Patients men		Control group men vs. patients men	
		OR 95%CI	p	n=76 (men)	Freq. (%)	N=27 men (50%)	Freq. (%)	OR 95%CI	p
rs.1128503	CC	1,00		24	31,58	6	22,22	1,00	
	CT	2.000 [0.712-5.619]	0,183	35	46,05	16	59,26	1.829 [0.626-5.344]	0,266
	TT	1.304 [0.357-4.772]	0,688	17	22,37	5	18,52	1.176 [0.308-4.491]	0,812
	CC	1,00		24	33,64	6	22,22	1,00	
	CT+TT	1.775 [0.658-4.785]	0,253	52	66,36	21	77,78	1.615 [0.578-4.516]	0,358
rs.2032583	GG	1,00		24	31,58	6	22,22	1,00	
	GT	1.944 [0.691-5.471]	0,203	33	43,42	16	59,26	1.939 [0.662-5.686]	0,223
	TT	1.215 [0.333-4.439]	0,768	19	25,00	5	18,52	1.053 [0.278-3.983]	0,940
	GG	1,00		24	26,67	6	22,22	1,00	
	GG+TT	1.701 [0.630-4.593]	0,291	43	68,83	21	77,78	1.615 [0.578-4.516]	0,358
rs.1045642	CC	1,00		23	30,26	4	14,81	1,00	
	CT	2.049 [0.628-6.682]	0,228	35	46,05	17	62,96	2.793 [0.833-9.362]	0,088
	TT	1.688 [0.425-6.704]	0,454	18	23,68	6	22,22	1.917 [0.469-7.831]	0,360
	CC	1,00		23	30,263	4	14,81	1,00	
	CC+TT	1.941 [0.616-6.116]	0,251	53	69,737	23	85,19	2.495 [0.775-8.033]	0,117

years (63%), and second group aged 35 years or above (37%). Our results confirmed significant differences in the patients in the former group (under 35 years) compared to control population for 1236 and 2677 polymorphic variants. Approximately three-fold increased risk for development of psychiatric disease in heterozygous or homozygous mutant carriers for both polymorphisms was established (data presented in table 8).

DISCUSSION

The influence of three common SNPs in the ABCB1 gene (C1236T, G2677T and C3435T) in a subset of Macedonian patients with psychiatric disorders diagnosed with schizophrenia or bipolar disorders was investigated in our study. The impacts on the whole psychiatric patient population

Table 7. *Evaluation of allele and genotype distribution of polymorphic variants C1236T, G2677T and C3435T of ABCB1 gene in female patents population with diagnosed psychiatric disorder vs control group and sub-classified control group based on gander*

SNP	Allele	Control group vs. patients women OR 95%CI	p	Control group women n=31 (women)	Freq. (%)	Patients women N=27 women (50%)	Freq. (%)	Control group women vs. patients women OR 95%CI	p
rs.1128503	C	1.00		37	0.60 +/-0.066	25	0.46 +/-0.058	1.00	
	T	1.481 [0.813-2.696]	0,198	25		29		1.717 [0.821-3.589]	0,150
rs.2032583	G	1.00		36	0.58 +/-0.065	24	0.44 +/-0.055	1.00	
	W (T/A)	1.536 [0.843-2.801]	0,159	26		30		1.731 [0.829-3.615]	0,143
rs.1045642	C	1.00		35	0.56 +/-0.059	24	0.44 +/-0.055	1.00	
	T	1.322 [0.726-2.409]	0,361	27		30		1.620 [0.777-3.379]	0,197

SNP	Genotype	Control group vs. patients women OR 95%CI	p	Control group women n=31 (women)	Freq. (%)	Patients women N=27 women (50%)	Freq. (%)	Control group women vs. patients women OR 95%CI	p
rs.1128503	CC	1.00		12	38,71	4	14,81	1.00	
	CT	3.188 [0.988-10.288]	0,044	13	41,94	17	62,96	3.923 [1.025-15.018]	0,040
	TT	2.348 [0.597-9.231]	0,213	6	19,35	6	22,22	3.000 [0.606-14.864]	0,172
	CC	1.00		12	38,71	4	14,81	1.00	
	CT +TT	2.915 [0.937-9.070]	0,056	19	61,29	23	85,19	3.632 [1.005-13.119]	0,042
rs.2032583	GG	1.00		11	35,48	3	11,11	1.00	
	GT	4.375 [1.195-16.014]	0,018	14	45,16	18	66,67	4.714 [1.100-20.202]	0,029
	TT	2.917 [0.664-12.813]	0,144	6	19,35	6	22,22	3.667 [0.666-20.191]	0,127
	GG	1.00		11	35,48	3	11,11	1.00	
	GG+TT	3.889 [1.096-13.797]	0,026	20	64,52	24	88,89	4.400 [1.077-17.982]	0,030
rs.1045642	CC	1.00		9	29,03	3	11,11	1.00	
	CT	2.893 [0.784-10.675]	0,099	17	54,84	18	66,67	3.176 [0.734-13.749]	0,112
	TT	2.250 [0.507-9.993]	0,278	5	16,13	6	22,22	3.600 [0.616-21.033]	0,147
	CC	1.00		9	29,03	3	11,11	1.00	
	CC+TT	2.700 [0.753-9.682]	0,116	23	74,194	24	88,89	3.273 [0.784-13.662]	0,093

Table 8. *Evaluation of allele and genotype distribution of polymorphic variants C1236T, G2677T and C3435T of ABCB1 gene in patents population with diagnosed psychiatric disorder sub-classifed based on age (35 and below vs. above 35 years) and control group*

SNP	Allele	N=33 35 years and below (37%)	Freq. (%)	Pearson (p)	N=21 35 years and above (63%)	Freq. (%)	Pearson (p)	OR 95%CI	p	OR 95%CI	p	OR 95%CI	p
rs.1128503	C	29	0.44 +/-0.056	0,333	24	0.57 +/-0.061	0,092	1,00		1,00		1,00	
	T	37			18			0.588 [0.269-1.283]	0,181	1.629 [0.934-2.840]	0,084	0.957 [0.491-1.868]	0,898
rs.2032583	G	29	0.44 +/-0.051	0,094	23	0.55 +/-0.067	0,250	1,00		1,00		1,00	
	W (T/A)	37			19			0.647 [0.297-1.410]	0,272	1.568 [0.900-2.734]	0,111	1.015 [0.522-1.974]	0,964
rs.1045642	C	28	0.42 +/-0.053	0,167	21	0.50 +/-0.058	0,050	1,00		1,00		1,00	
	T	36			21			0.737 [0.339-1.603]	0,441	1.435 [0.822-2.505]	0,202	1.058 [0.546-2.050]	0,868

| | | | Age 35 and below vs. above 35 | | | | | | controls vs. 35years and below | | controls vs. 35 years and above | |

SNP	Genotype	N=33 35 years and below (37%)	Freq. (%) obs	Pearson (p) exp	N=21 35 years and above (63%)	Freq. (%) obs	Pearson (p) exp	OR 95%CI	p	OR 95%CI	p	OR 95%CI	p
rs.1128503	CC	5	15,15	19,36	5	23,81	32,49	1,00		1,00		1,00	
	CT	19	57,58	49,28	14	66,67	49,02	0.737 [0.178-3.045]	0,673	2.850 [0.972-8.357]	0,050	2.100 [0.693-6.364]	0,183
	TT	9	27,27	31,36	2	9,52	18,49	0.222 [0.031-1.595]	0,122	2.817 [0.838-9.467]	0,086	0.626 [0.112-3.501]	0,591
	CC	5	15,15		5	23,81		1,00		1,00		1,00	
	CT+TT	28	84,85		16	76,19		0.571 [0.143-2.279]	0,425	2.839 [1.011-7.974]	0,041	1.623 [0.550-4.784]	0,377
rs.2032583	GG	4	12,12	19,36	5	23,81	30,25	1,00		1,00		1,00	
	GT	21	63,64	49,28	13	61,90	49,50	0.495 [0.112-2.188]	0,349	3.828 [1.207-12.146]	0,017	1.896 [0.619-5.808]	0,258
	TT	8	24,24	31,36	3	8,82	20,25	0.300 [0.046-1.943]	0,199	2.917 [0.789-10.786]	0,099	0.875 [0.191-4.011]	0,863
	GG	4	12,12		5	23,81		1,00		1,00		1,00	
	GG+TT	29	87,88		16	76,19		0.441 [0.104-1.881]	0,261	3.524 [1.149-10.809]	0,021	1.556 [0.527-4.591]	0,421
rs.1045642	CC	4	12,12	17,64	3	14,29	25,00	1,00		1,00		1,00	
	CT	20	60,61	48,72	15	71,43	40,00	1 [0.194-5.154]	1,000	2.411 [0.750-7.749]	0,131	2.411 [0.643-9.042]	0,182
	TT	9	27,27	33,64	3	14,29	25,00	0.444 [0.061-3.242]	0,419	2.531 [0.690-9.286]	0,153	1.125 [0.207-6.110]	0,891
	CC	4	12,12		3	14,29		1		1		1	
	CC+TT	29	87,88		18	85,71		0.828 [0.166-4.133]	0,817	2.447 [0.788-7.596]	0,113	2.025 [0.553-7.414]	0,279

were analyzed, and further the patients were divided in two separate sub-groups according to the established diagnosis (i.e. schizophrenia and bipolar disorders), gender (men and women) or age (in-between 20 and 35, and 35 years or above). In line with already published data high-linkage disequilibrium correlation was confirmed for all three polymorphic variants in our patent population, but the highest values were identified for C1236T and G2677T (D'=0.901, r2=0.76). Obtained results suggest that there is an evident association between mutant ABCB1 gene variants and predisposition for psychiatric disorders. The activity of P-glycoprotein associated with ABCB1 SNPs is inconstant and sometimes even inconclusive. The impact of ABCB1 and P-gp on predisposition and pathogenesis of psychiatric disorders is still big challenge, while its influence on CNS drug response is already well established. ABCB1 gene variations have been associated with treatment response to antipsychotics commonly used in schizophrenia and bipolar disorder treatment (olansepine and rispereidone). Mutant allele carriers for 2677SNP in female schizophrenic population are associated with better treatment response [40].

Our results suggest that ABCB1 C1236T and G2677T polymorphisms might be one of the genetic factors that increase the risk for bipolar disorder onset and progression, and this risk was not confirmed for schizophrenia. Such difference in these two psychiatric disorders was also confirmed in several recent studies such as the one from Qian et al. [41] The results from this study confirmed higher frequency for the mutant 2677A allele in bipolar disorders, but not in the patient population with diagnosed schizophrenia. The results from our study have confirmed four-fold increased risk for psychiatric disorder in women that are heterozygous for 1236 and 2677 polymorphisms in comparison with the whole control group (men and women), and three-fold higher risk in comparison to control group only with women and such difference wasn't seen in male population. Tovilla-Zárate et al., 2014 confirmed significant association of 2677GG polymorphic variant with severity of particular symptoms in hallucinations and delusions in male patients with schizophrenia [42] .Although, the mechanism by which this non-synonymous polymorphism influence the predisposition for psychiatric illnesses or the severity of the symptoms is still not determined, it could be assumed that mutant P-glycoprotein with replaced hydro-

philic Ser with more lipophilic Thr/Ala is less substrate-specific [43].

Additionally mutant allele carriers for 1236 and 2677 polymorphisms that are 35 years and below in our evaluated patients population have almost three-fold higher risk for development of psychiatric illness. This is very important finding having in consideration the fact that age between 20 and 35 is critical for development of schizophrenia and bipolar disorders.

Polymorphic variants in the promotor region of the ABCB1 gene might be related to ABCB1 mRNA or P-gp expression. It has been also confirmed that the gene expression is independent of the C3435T polymorphism (44). Our study was focused on polymorphic variants on the coding region. In line with evidence of Hoffmayer et al, Tanabe et al, confirmed association of 3435TT and 2677TT/TA with P-gp expression level in placenta. Sakaeda et al reported completely different result with higher duodenal expression of ABCB1 in 3435TT homozygote carriers. [36, 45-47]. In our study we couldn't find any differences in C3435T SNP among control group, schizophrenic or bipolar disorder patients.

Qian et al. confirmed higher frequency of mutant 2677A allele in bipolar disorder, and failed to replicate this result in schizophrenic patient group. They suggested higher mRNA expression or activity of ABCB1 influenced by 2677A allele in bipolar disorder patients in comparison with the control group. On the other hand, they didn't confirm allelic of genotype difference for C3435T SNP in control group and patients with schizophrenia or bipolar disorder. This evidence is in line with previously obtained results from two other studies that have shown no statistically significant difference between schizophrenia and the control group [40, 41]. Turgutet al. confirmed statistically significant difference between the bipolar disorder group and the control group for non-synonymous C3435T genotypes in Turkish population. Only two out of 104 patients with bipolar disorder wild type genotype carriers and 7 were monozygotic for the mutant allele. All the other patients have the heterozygous genotype [48]. ThereforeABCB1 gene could be considered as one of the candidate genes that can be responsible for particular psychiatric disorders. Fujii et al. reveal that carriers of 3435T allele and 1236T-3435T haplotype are more common in patients with MDD then in the control group suggesting an association between P-gp polymor-

phism and MD development risk. In this study no significant differences were confirmed for allelic and genotype frequencies for C2677T/A SNP [49]. The haplotype analysis performed by Santos et al, 2014 confirmed lower risk for development of MDD in mutant haplotype carriers' 1236T-2677T-3435T [50]. Our haplotype analysis suggested higher risk for schizophrenia and bipolar disorders for TTT haplotype. This haplotype is estimated as the highest in patients study group with 45%. Salama et al 2006 have shown that mutant haplotype genetic profile is related to reduced activity of P-gp, as recombinant cells expressing 1236T-2677T-3435T have presented 0-22.7% of transporter activity and lost transport functionality [51]. Therefore, functionally related polymorphisms of ABCB1 might be related with susceptibility to stress induced psychiatric disorders [52]. It should be taken in consideration that P-gp activity is influenced by variable inhibitors and inducers that may influence the transport activity additionally [53, 54].

This influences of ABCB1 gene polymorphic variants on psychiatric disorders could be hypothesized in two directions. First, the importance of the pro-inflammatory cytokines released in these CNS disorders and their influences on P-glycoprotein function on BBB. Second hypothesis refers to the significant role of P-gp and ABCB1 polymorphic variants on gene expression and control of access of endogenous corticosteroids for regulation of HPA system during stress when peripheral glucocorticoid level rapidly increases.

Growing body of evidence suggest that activated pro-inflammatory cytokine network induces psychopathological symptoms and is involved in the pathogenesis and pathology of schizophrenia and mood disorders. Brain inflammation is involved in many CNS disorders such multiple sclerosis, stroke, brain cancer, epilepsy, AD and Parkinson's disease. All these studies confirmed that the inflammation at the BBB leads to big changes of ABC transporters expression and activity but the exact signaling pathways responsible for that are still not determined. Their identification will help in drug development and could improve drug delivery into the brain [1].

The pro-inflammatory cytokines, like TNF-α, IL-1β, IL-6, INF-γ and endotheiln -1 (ETY-1), released in these CNS disorders triggers profound changes in genes expression in the BBB and most particularly changes in expression of ABC transporters. Many studies confirmed that these cytokines decrease the P-gp expression [26] and influence the drug transport, but they are time-, dose- and location-dependent. It is confirmed that P-gp transport activity is reduced after short exposure to TNF-α, and increased after long-term exposure, including a complex and time-dependent regulatory mechanism [55]. A study by von Wedwl-Parow et al. showed that TNF-α and IL-β decrease BCRP protein expression on BBB. Chronic inflammation reduces P-gp activity in brain tissue in general, but most particularly in the brain capillary endothelial cells [56].

The role of neuro-inflammation in schizophrenic patients is confirmed also with other studies where patients with chronic schizophrenia [57], and patients with an acute exacerbation of schizophrenia [58] treated with celecoxib or other NSAID like aspirin [59], in addition to risperidone, had a significantly greater improvement on the PANSS in comparison to patients that use risperidone as monotherapy. Additionally, a growing body of evidence confirms the anti-inflammatory effects of antipsychotic drugs in such a way that they decrease the microglia cell activation. Neuro-inflammation and antipsychotic treatment could influence treatment resistance by affecting P-gp activity by enhancing its activity in synergistic manner. Doordin et al 2014 confirmed that neuro-inflammation increases P-gp activity at the BBB while treatment with risperidone causes a decrease in P-gp activity, whereas clozapine does not affect P-gp. This is the first study that has shown that sub chronic treatment with antipsychotics can affect P-gp activity. The fact that risperidone lowered the P-p activity suggest that long-term treatment with risperidone becomes more effective due to the self-induced increase in availability of the drug for the brain. This effect of risperidone could also be attributed to the active metabolite 9-hydroxyrisperidone which is also P-gp substrate and is used as antipsychotic.

Cumulative evidence support the fact that impairment of corticosteroid receptor signaling and feedback inhibition is crucial for development of neuroendocrine changes associated with schizophrenia and mood disorders. Decreased activity of negative feedback activity of HPA axis is confirmed, with increased concentration of cortisol in serum, urine and cerebrospinal fluid in this patient population [34,58,60].

As endogenous stress hormones are substrates for P-gp, changed expression or functional activity of this transport protein may significantly influence the HPA system regulation during stress when glucocorticoid levels are severely increased. Decreased function of P-gp in mutant 1236T-2677T-3435T haplotype, might diminish the HPA-axis activation during stress, as a result of increased penetration of glucocorticoid hormones in CNS, corticosteroid receptor activation and enhances negative feedback inhibition of stress hormone secretion. The ABCB1 gene variability may influence the normal glucocorticoid secretion and function particularly during stress and could be associated with susceptibility to psychiatric disorders, as corticosteroids play pivotal role in development of neurodegenerative changes associated to this pathology [34, 41, 58].

CONCLUSION

Our study suggested influence of ABCB1 genetic variation on P-gp activity or expression and predisposition for psychiatric disorders (bipolar disorder and schizophrenia) in Macedonian population. Statistically significant higher frequency and three-fold increased risk for development of psychiatric disorder in heterozygous carriers for C1236T and G2677T polymorphisms was observed. We estimated three-fold higher risk for psychiatric illness in women that are heterozygous carrier (heterozygous and mutant homozygous for both alleles) compared to healthy control population (both men and women) and four-fold higher risk in comparison only to healthy women's population. Additionally, mutant allele carriers for C1236T and G2677T/A polymorphisms that are 35 years and younger population have almost three-fold higher risk for development of psychiatric disease. Most important limiting factor in the study was the restricted number of the patients, but the obtained results are in agreement with most of the findings from other relevant studies. Therefore, additional research is inevitable for confirmation of these preliminary results. Polymorphic variants in the promoter region on ABCB1 gene, determination of their influence on gene and transporter expression, as well as estimation of the influence of predisposition for certain psychiatric disorders is considered as interesting field for further studies.

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Резиме

ЕВАЛУАЦИЈА НА УЛОГАТА НА ПОЛИМОРФНИТЕ ВАРИЈАЦИИ НА АБЦБ1 ГЕНОТ ВРЗ ПРЕДИЗПОЗИЦИЈАТА КОН ПСИХИЈАТРИСКИ ЗАБОЛУВАЊА ВО МАКЕДОНСКА ПОПУЛАЦИЈА

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Психијатриските и други ЦНС заболувања се карактеризираат со нерегулиран невроинфламаторен процес и хронична активација на микроглиа клетките што резултира со оштетување на мозочното ткиво. Полиморфните варијации на ABCB1 генот C1236T, G2677T/Aи C3435T влијаат врз експресијата на Р-гликопротеинот и неговата функција, како и со предизпозицијата за развој на психијатриски заболувања (биполарни растројства и шизофренија). Асоцираноста на психијатриските заболувања со гликокортикоидите е веќе добро етаблирана и ABCB1 еднонуклеотидните полиморфизми влијаат врз гликокорикоидниот пристап во мозокот.

Целта на студијата е да се евалуира влијанието на трите најдобро проучени полиморфизми на ABCB1 генот врз предизпозицијата кон пихијатриски нарушувања кај македонската популација.

Во студијата се вклучени 107 здрави Македонци од двата пола како контролна група и 54 пациенти (22 до 65 години) со дијагностицирана шизофренија и биполарни растројства. Трите полиморфни варијации на генот ABCB1 се анализиран со Real-Time PCR метода во двете групи.

Резултатите потврдија дека генот ABCB1 игра значајна улога за предиспозицијата за психијатриски заболувања и зголемен ризик за развој на биполарни растројства кај носителите на хетерозиготи и мутирани хмозиготни алели за полиморфизмите 1236 и 2677, во споредба со носителите на нормалниот гнотип. Определен е трипати зголемен ризик за развој на психијатриско заболување кај жените што се хетерозиготи во споредба со здравата контролна популација (мажи и жени) и четрипати поголем ризик во споредба само со жените како контролна група. Носителите на мутираните алелели за 1236 и 2677 полиморфизмите на возраст до 35 години имаат трипати поголем ризик за развој на психијатриско заболување.

Клучни зборови: Р-гликопротеин, ABCB1, шизофренија, биполарно растројство, невроинфламација, ABCB1 и гликокотрикоиден пристап во мозокот