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HUMAN GENETICS

Association of Several Polymorphic Loci of Serotoninergic Genes with Unipolar Depression

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Abstract—Serotoninergic system is one of the major brain neurotransmitter systems that is involved in the development of depressive spectrum disorders. Regulatory genes of this system are the principle candidate genes predisposing to unipolar depression. Using PCR-RFLP analysis, we have conducted a study of polymorphic loci of several genes of this system: *C1019G* of serotonin receptor 1A gene,(*HTR1A*); *A-1438G* of serotonin receptor 2A gene, (*HTR2A*); *G861C* of serotonin receptor 1B gene, (*HTR1B*); *Stin2VNTR* and *5-HTTLPR* of serotonin transporter gene (*SLC6A4*) in patients with unipolar depression from Tatar and Russian population. The results of the study suggest that genotype *10*/*10* of the *SLC6A4* gene as well as genotype *G*/*G* and allele *G* of the *HTR2A* gene can predispose to increased risk of unipolar depression development in ethnic Russians. In contrast, genotype *12*/*10* of the *SLC6A4* gene is a marker of low risk of the disease in both groups.

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INTRODUCTION

According to World Health Organization 2003 year report, depression is becoming a most common psychic disorder, which involves about 121 million people worldwide. An increase in the incidence of depressive disorders has been recently observed at all ages [1]. Depression is associated with distress and high lethality in patients mainly due to suicide (15%). It is expected by 2020 that depression will be the second cause of disability after ischemic heart disease [2]. In spite of high socio-medical importance of this disease, to date there is no clear understanding of the reasons and mechanism of its development. Unipolar depression (UD) is a clinically heterogenous psychic disorder, caused by gene–gene and gene–environment interaction. The role of genetic factors was documented by studies of adopted children as well as family and twin studies. These studies have shown that UD hereditability is 40–70% [3–5].

Functional deficiency of serotoninergic system and complex deregulation of noradrenergic system (associated mainly with its hyperactivity) underlie key pathogenic mechanisms of depression development [6]. Therefore, genes controlling serotonin concentration in the brain may be candidate genes of UD development. Serotonin transporter plays an important role both in serotonin neurotransmission and in neurogenesis [7, 8]. Serotonin receptors 1A, 2A, and 1B, that represent principle brain sites, assumed to be the main molecular targets for drugs used in the treatment of various neuropsychiatric disorders, were shown to have the major interest in depression studies [9].

The aim of our work was study the involvement of polymorphic loci of four genes of the serotoninergic system in the UD development: the *C–1019G* locus of the *HTR1A* gene, the *A–1438G* locus of the *HTR2A* gene, the *G861C* locus of the *HTR1B* gene, and the *Stin2VNTR* and *5-HTTLPR* loci of the *SLC6A4* gene.

MATERIALS AND METHODS

In this study, we used 174 DNA samples of 99 Tatar and 75 Russian UD patients aged 14–72. UD was diagnosed according to the International Classification of Diseases (ICD-10). Control group included 331 age-matched volunteers of the same ethnicity (170 Tatars and 161 Russians), without any familial or individual history of psychic disorders. DNA isolation was conducted using phenol–chloroform extraction from whole venous blood [10]. Polymorphic markers of genes *HTR2A*, *HTR1A*, *HTR1B*, and *SLC6A4*, were analyzed using polymerase chain reaction of DNA synthesis with primers described previously [11–15]. PCR-RFLP analysis was performed for identification of single nucleotide polymorphisms in the *HTR1A*, *HTR2A*, *HTR1B*, and *SLC6A4* gene loci. For this purpose, 10 μl of the reaction mixture was treated by 5 units of the corresponding restriction endonuclease. The PCR prod-

Table 1. Polymorphism, primer sequences of analyzed DNA loci

Gene	Locus	Primer sequences	Alleles
HTR1A 5q11.2-q13	*C–1019G* (*Bse*GI)	5'-GGCTGGACTGTTAGATGATAACG-3' 5'-GGAAGAAGACCGAGTGTGTCAT-3' [11]	*C* (163 bp), *G* (146 + 17 bp)
HTR1B 6q13	*G861C* (*Hinc*II)	5'-GAAACAGACGCCCAACAGGAC-3' 5'-CCAGAAACCGCGAAAGAAGAT-3' [12]	*G* (452 + 96 bp), *C* (142 + 310 + 96 bp), intact 548 bp
HTR2A 13q14-q21	*A–1438G* (*Msp*I)	5'-AAGCTGCAAGGTAGCAACAGC-3' 5'-AACCAACTTATTTCCTACCAC-3' [13]	*A* (468 bp), *G* (244 + 224 bp)
SLC6A4 17q11.2-12	*5-HTTLPR*	5'-GGCGTTGCCGCTCTGAATTGC-3' 5'-GAGGGACTGAGCTGGACAACCCAC-3' [14]	*S* (480 bp), *L* (520 bp)
	Stin2VNTR	5'-GTCAGTATCACAGGCTGCGAG-3' 5'-TGTTCCTAGTCTTACGCCAGTG-3' [15]	*9* (250 bp), *10* (267 bp), *12* (300 bp)

ucts were fractionated by electrophoresis in 7% polyacrylamide gel. The list of amplified loci, primer sequences, and sizes of amplified products are presented in Table 1.

The comparison of genotype frequencies in various ethnic groups was conducted using χ^2 Pearson's test under the interactive SISA Table (http://www.quantitative skills.com). In case of using contigency table, 2×2 Pearson's χ^2 test was applied with Yates correction for continuity, provided that the expected number in any cell exceeded 5. The differences were considered significant at $P < 0.05$. The odds ratio (OR) and corresponding 95% confidential interval was calculated using SISA Tables (http://www.quantitative skills.com).

RESULTS AND DISCUSSION

It is known that ethnic differences can affect allele and genotype distributions [2]. Therefore we have analyzed the allele and genotype distributions at five polymorphic loci of four genes in patients with unipolar depression and healthy donors, taking into account the ethnicity of the subjects. The results of our study are presented in Tables 2 and 3. Distribution of genotype frequencies of all gene polymorphisms was in accordance with Hardy–Weinberg equilibrium in all groups.

Serotonin receptor 1A is involved in pathophysiology of anxiety and depression. It also serves as a target for antidepressants such as selective serotonin reuptake in inhibitors. Functional mononuleotide exchange *C–1019G* in the promoter region of the serotonin receptor gene (*HTR1A*) (5q11.2-q13) is associated with changes in its transcription level [11, 16, 17]. Association of UD with *C–1019G* polymorphic locus of the *HTR1A* gene was observed in some earlier studies. It was shown previously that this polymorphic marker can affect serotonin receptor type A1 density as well as the presynaptic membranes of raphe nucleus neurons but not the postsynaptic membranes. This may produce downregulation of the serotonin activity in the patients with depression [16]. The association of the *C–1019G G* allele with suicide attempts [18] and with the anxiety-traits in Germans has been also reported [11].

The studies of the allele and genotype frequency distribution in polymorphic locus *C–1019G* of the *HTR1A* gene in Tatars have shown the prevalence of the *C*/*G* genotype both in the groups of healthy individuals (52%) and UD patients (55%). The *G*/*G* genotype had the lowest frequency in the both groups: 19% in control and 12% in the UD patients. The frequencies of the homozygous *C*/*C* genotype were 29% and 33%, respectively. The allele *C* frequency was highest both in the depression patients (60%) and in controls (56%). The frequency of *G* allele was 40% and 44%, respectively. No significant differences between the UD patients and controls in genotype ($\chi^2 = 2.19$, *d.f.* = 2, $P = 0.33$) and allele ($\chi^2 = 0.91$, *d.f.* = 1, $P = 0.34$) frequency in polymorphic locus *C–1019G* of *HTR1A* gene in Tatar sample were observed.

The heterozygous *C*/*G* genotype of polymorphic locus *C–1019G* of the *HTR1A* gene was observed with higher frequency both in the UD patients (55%) and healthy (60%) individuals in Russians. The rarest among UD patients was the homozygous *C*/*C* (20%) genotype while homozygous *G*/*G* genotype (18%) was the rarest in healthy individuals. The UD patients exhibited frequency prevalence of allele *G* (52%) over allele *C* (48%). In contrast, the frequency of *G* allele (48%) was lower than *C* allele (52%) in control group. The differences were not significant both for genotype ($\chi^2 = 1.69$, *d.f.* = 2, $P = 0.43$) and allele ($\chi^2 = 0.34$, *d.f.* = 1, $P = 0.55$) frequencies distribution. The

Table 2. Genotype and allele frequency distribution at polymorphic loci of the serotonin receptor genes in UD patients and healthy individuals

Group		*n*	Genotypes (%)			Alleles (%)	
HTR1A (C–1019G)			***C/C***	***C/G***	***G/G***	***C***	***G***
Tatars	Patients	99	33 (33)	54 (55)	12 (12)	120 (60)	78 (40)
	Control	170	49 (29)	89 (52)	32 (19)	190 (56)	150 (44)
			$\chi^2 = 2.192$, $P = 0.33$			$\chi^2 = 0.91$, $P = 0.34$	
Russians	Patients	75	15 (20)	41 (55)	19 (25)	72 (48)	78 (52)
	Control	161	36 (22)	96 (60)	29 (18)	168 (52)	154 (48)
			$\chi^2 = 1.69$, $P = 0.43$			$\chi^2 = 0.34$, $P = 0.55$	
HTR2A (A–1438G)			***A/A***	***A/G***	***G/G***	***A***	***G***
Tatars	Patients	99	9 (9)	47 (48)	43 (43)	65 (33)	133 (67)
	Control	168	25 (15)	71 (42)	72 (43)	121 (36)	215 (64)
			$\chi^2 = 2.03$, $P = 0.36$			$\chi^2 = 0.56$, $P = 0.46$	
Russians	Patients	75	8 (11)	28 (37)	39 (52)	44 (29)	106 (71)
	Control	160	24 (15)	83 (52)	53 (33)	131 (41)	189 (59)
			$\chi^2 = 7.64$, $P = 0.02$			$\chi^2 = 5.88$, $P = 0.01$	
HTR1B (G861C)			***G/G***	***G/C***	***C/C***	***G***	***C***
Tatars	Patients	99	34 (34)	50 (51)	15 (15)	119 (60)	79 (40)
	Control	159	70 (44)	72 (45)	17 (11)	210 (66)	108 (34)
			$\chi^2 = 2.75$, $P = 0.25$			$\chi^2 = 1.86$, $P = 0.17$	
Russians	Patients	75	39 (52)	31 (41)	5 (7)	109 (73)	41 (27)
	Control	154	82 (53)	65 (42)	8 (5)	228 (74)	80 (26)
			$\chi^2 = 0.22$, $P = 0.89$			$\chi^2 = 0.095$, $P = 0.76$	

Note: *n* – number of individuals.

genotype and allele frequency distributions in control samples of Russians and Tatars was similar to those reported previously for German [19] and Americans from the New York area [20]. However, it differs from those reported for individuals from Canada [16] and Italy [19].

The serotonin receptor 2A gene (*HTR2A*) (13q14-q21) is often considered as one of the major unipolar depression candidate gene. The studies of polymorphic locus *A–1438G* of the *HTR2A* gene have shown that heterozygous genotype *A*/*G* (52%) was the most frequent in the group Russians control while homozygous genotype *G*/*G* (52%) was observed more frequently in the UD patient sample. However, the frequency of the *A*/*A* genotype in both groups was 37%. Moreover, the frequency of allele *G* was the highest in both examined Russian groups: 59% in the control group and 71% in the UD patients. The allele *A* frequencies were 41% and 29%, respectively. The analysis of the genotype ($\chi^2 = 7.64$, *d.f.* = 2, $P = 0.02$) and allele ($\chi^2 = 5.88$, *d.f.* = 1, $P = 0.02$) frequency distribution of *A–1438G* polymorphic locus in Russians revealed the presence of statistically significant differences in both examined groups. It was established that in the individuals of Russian ethnicity, genotype *G*/*G* (OR = 1.68, 95%CI 1.16-2.44) and allele *G* (OR = 143, 95% CI 1.06-1.92) of *HTR2A* gene might be considered as a risk factor. In ethnic Tatars, no significant differences were observed between the UD patients and control in the allele ($\chi^2 = 0.56$, *d.f.* = 1, $P = 0.46$) and genotype ($\chi^2 = 2.03$, *d.f.* = 2, $P = 0.36$) distribution at polymorphic locus *A–1438G* of the *HTR2A* gene.

According to the published data, there are population differences in allele frequencies of *A–1438G* polymorphism [21]. Thus, the allele *A* frequency exceeds the allele *G* frequency in ethnic Japanese. In contrast, in

Table 3. Genotype and allele frequency distribution at polymorphic loci of the serotonin transporter gene in UD patients and healthy individuals

Group		*n*	Genotypes (%)					Alleles (%)		
***SLC6A4* (*Stin2VNTR*)**			***12/12***	***12/10***	***12/9***	***10/10***	***10/9***	***12***	***10***	***9***
Tatars	Patients	99	44 (44)	31 (31)	5 (5)	16 (16)	4 (4)	123 (62)	67 (34)	8 (4)
	Control	170	65 (38)	83 (49)	2 (1)	17 (10)	2 (1)	213 (63)	122 (36)	3 (1)
			$\chi^2 = 12.89$, $P = 0.01$					$\chi^2 = 6.24$, $P = 0.04$		
Russians	Patients	75	27 (36)	27 (36)	2 (2)	18 (24)	1 (2)	83 (55)	65 (43)	3 (2)
	Control	158	52 (33)	82 (52)	3 (2)	16 (11)	2 (1)	193 (61)	113 (36)	10 (3)
			$\chi^2 = 8.245$, $P = 0.04$					$\chi^2 = 2.58$, $P = 0.28$		
***SLC6A4* (*5-HTTLPR*)**			***L/L***		***L/S***		***S/S***	***L***		***S***
Tatars	Patients	99	25 (25)		45 (46)		29 (29)	95 (48)		103 (52)
	Control	170	46 (27)		75 (44)		49 (29)	167 (49)		173 (51)
			$\chi^2 = 0.11$, $P = 0.95$					$\chi^2 = 0.06$, $P = 0.80$		
Russians	Patients	75	25 (34)		40 (53)		10 (13)	91 (61)		59 (39)
	Control	161	50 (31)		84 (52)		27 (17)	184 (57)		138 (43)
			$\chi^2 = 0.48$, $P = 0.79$					$\chi^2 = 0.522$, $P = 0.47$		

Note: *n* – number of individuals.

Caucasians allele *G* frequency exceeds the allele *A* frequency [21]. It has been reported also that polymorphic locus *A–1438G* of *HTR2A* gene is associated with various psychic disorders such as unipolar depression, obsessive-compulsive disorder, food behaviour disorder and schizophrenia [22]. In addition, the evidence has been reported that depressive patients have increased density of serotonin receptor 2A in prefrontal cortex (review [22]). Meyers at al. [22] have found that *HTR2A A–1438G* and *A783G* polymorphisms, assumed to be in strong linkage disequilibrium, could significantly modify promoter activity both in vivo and in vitro and thus affect the level of gene expression and serotonin receptor A2 density in the brain. Thus, haplotype *HTR2A*A*G* including loci *A783G* and *A–1438G*, respectively, was associated with increased gene expression. Choi et al. [23] have observed the association of *G/G* genotype of *HTR2A* gene with major depression in the Korean population.

Serotonin receptor 1B is highly important for regulation of serotonin neurotransmission since as well as with serotonin receptor 1A it serves both as an autoreceptor on serotonin containing neurons of raphe nuclei and as a heteroreceptor at other neurons lacking serotonin. It was suggested that polymorphic marker *G861C* of the *HTR1B* gene can affect the expression of the gene and accordingly the receptor density [24].

The prevalence of the *G/G* genotype was observed both in the UD patient group (52%) and in healthy donors (53%) while comparing the genotype and allele frequency of *G861C* polymorphism of *HTR1B* gene in Russians. The values of genotype *G/C* frequency in Russian sample were close both in the UD patients (41%) and healthy individuals (42%). These values were 7% and 5%, respectively, in case of genotype *C/C*. In the UD patients the allele frequencies were 73% for *G* and 26% for *C*. The *G/C* genotype was the most common in ethnic Tatar sample both in the UD patient (51%) and in control (45%). The rarest genotype was *C/C*, which was observed in 15% and 11% in both groups, respectively. The frequency of the homozygous genotype in the UD patient group was 34%, while in healthy individuals it was observed in 44% cases. However, no significant differences were found in the allele ($\chi^2 = 0.10$, *d.f.* = 1, $P = 0.76$) and genotype ($\chi^2 = 0.22$, *d.f.* = 2, $P = 0.89$) frequencies distribution between UD patients and healthy Russian individuals. In ethnic Tatar sample no significant differences were also observed between UD and controls in the allele ($\chi^2 = 1.86$, *d.f.* = 1, $P = 0.46$) and genotype ($\chi^2 = 2.75$, *d.f.* = 2, $P = 0.46$) frequency distribution in polymorphic locus *G861C* of the *HTR1B* gene.

Published findings involving association of polymorphic locus *G861C* of the *HTR1B* gene with unipolar depression are contradictory. Huang et al. (United States) [24] have revealed the association of the allele

C of the *HTR1B* gene with UD and with substance abuse. However, these results are controversial to earlier findings reported by Fehr et al. [25], demonstrating an association of allele *G* of the *HTR1B* gene with alcohol addiction but not with UD, anxiety, and narcolepsy. The C allele of the *HTR1B* gene was also associated with asocial behaviour in ethnic Finns with alcohol addiction [26] as well as with obsessive-compulsive disorder in Canadians [27]. Huang et al. [28] have demonstrated decreased serotonin receptor 1B density in the brain of suicide victims carrying allele *G* in polymorphic locus *G861C*. As the *G* to *C* substitution at 861 position is synonymous, this locus does not modify amino acid structure of the receptor 1B protein. Therefore, its association with the decreased level of receptor activity can be caused by indirect effect on transcription or linkage disequilibrium with some functionally significant polymorphism.

Two polymorphic markers in the serotonin transporter gene *SLC6A4* (17q11.2-12) were examined for the association with unipolar depression. It was shown that insertion-deletion polymorphism in the promoter region (*5-HTTLPR*), which is associated with the presence (allele *L*) or the absence (allele *S*) of 44 bp fragment can affect transcriptional and functional activity of the gene [29]. Variable-number tandem repeats (*VNTR*) comprising of 17 bp region are located in the second intron, and contain 9 (*Stin2.9*), 10 (*Stin2.10*) or 12 (*Stin2.12*) tandem repeats [30]. Studies of embryonic stem cells have shown that this polymorphic locus can also affect gene transcription, while its allelic variants differ in their enhancer activity [31].

Comparative analysis of the genotype frequencies distribution of the polymorphic locus *Stin2VNTR* of the *SLC6A4* gene in the healthy donors and the UD patients in the Tatar ethnic group have shown significant difference between them ($\chi^2 = 12.89$, *d.f.* = 4, $P = 0.01$). This difference was caused by the lower frequency of the *12/10* genotype in the UD patient group (31%) compared to control group (49%). The odds ratio (OR) showed that the *12/10* genotype has protective function (OR = 0.62, 95%CI 0.44-0.88). Both in the control and the UD patient groups, the *12/12* genotype was observed relatively frequently (in 44% of UD patients and in 38% of healthy individuals), while heterozygous genotypes carrying the *Stin2.9* allele were demonstrated with the lowest frequency (below 5%). Homozygous allele *Stin2.9* was not detected. The *Stin2.12* allele frequencies prevailed in both examined groups (62% in UD patients and 63% in healthy controls). The *Stin2.10* allele ranked second in frequency (34% in UD patients and 36%, in healthy controls). The rare *Stin2.9* allele was observed with a low frequency (below 4%) in both groups. Comparative analysis of the allele frequency distribution of *Stin2VNTR* polymorphic locus of the *SLC6A4* gene in the Tatar group has shown no significant differences between the UD patients and the healthy individuals.

In Russians, a significant genotype frequency distribution ($\chi^2 = 8.25$, *d.f.* = 4, $P = 0.04$) of *Stin2VNTR* polymorphism of *SLC6A4* gene between healthy individuals and UD patients was observed similar to that demonstrated in Tatars. In Russians, the marker of UD development risk was *10/10* genotype (OR = 1.84, 95%CI 1.26-2.72), while heterozygous *12/10* genotype (OR = 0.64, 95CI 10.43-0.95) was protective.

An association of rare *Stin2.9* allele with unipolar depression has been revealed previously [32, 33]. Similar distribution of allele and genotype frequencies was observed in the same polymorphic locus of the *SLC6A4* gene in ethnic Russians and Germans. It has been shown that the amount of serotonin transporter on thrombocytes increases in UD patients carrying *12/10* and *10/10* genotypes [34].

The studies of polymorphic locus *5-HTTLPR* of the *SLC6A4* gene in healthy controls and UD patients from Tatar population have shown that the heterozygous *L/S* genotype was most frequently observed in the both groups (44% and 46%, respectively). The *S/S* genotype was observed with equal frequency (29%) both in the UD patients and the healthy donors group. At the same time, the *L/L* genotype frequencies were 25% and 27%, respectively. In both groups, the *S* and *L* allele frequencies were similar: 52% and 48% in the UD patients versus 51% and 49% in control. The differences in the allele and genotype frequencies of *5-HTTLPR* polymorphic locus between the UD patients and healthy individuals in Tatars were not significant. While comparing genotype and allele frequency distribution in ethnic Russians no differences between the UD patients and the healthy individuals were detected. Only two of fourteen studies have shown significant association of UD with the *S* allele of polymorphic locus *5-HTTLPR* in the *SLC6A4* gene [35, 36].

These results show that for Russians, unipolar depression genetic risk markers are the *G/G* genotype (OR = 1.68) and the *G* allele (OR = 1.43) of the *HTR2A* gene and the *10/10* genotype (OR = 1.84) of the *SLC6A4* gene. Genetic markers of UD resistance in Russians and Tatars were the *12/10* genotype (OR = 0.64 and OR = 0.62, respectively) of *SLC6A4* gene. However, our results do not support the association of polymorphisms: *C–1019G* of serotonin receptor 1A gene (*HTR1A*), *G861C* of serotonin receptor 1B gene (*HTR1B*), and *5-HTTLPR* of serotonin transporter gene (*SLC6A4*) with unipolar depression in all the examined groups examined.

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