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Ethnic differences in the serotonin transporter polymorphism (5-HTTLPR) in several European populations

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ABSTRACT

The serotonin transporter (5-HTT) is a protein that has a major role in divergent psychiatric disorders, personality traits and behaviors, by regulating serotonergic synaptic function. Transcriptional activity of the 5-HTT gene (*5-HTT* or *SLC6A4*) is modulated by a polymorphic repetitive element (5-HTT gene-linked polymorphic region, 5-HTTLPR), which consists of a 44-base pairs insertion–deletion in the promoter region, creating a short (*S*) allele and a long (*L*) allele. Ethnic differences in the allele frequencies of the 5-HTTLPR exist between Caucasian and Asian populations. This study investigated ethnic differences in 5-HTTLPR in 1804 healthy Caucasian subjects from several European populations living in Croatia and the Russian Federation. The genotype and allele frequency of the 5-HTTLPR differed significantly ($P<0.001$) between male and female Croats, Russians, Tatars and Bashkirs, due to the lower frequency of the *S* allele (38% and 37%) and *S/S* genotype (14% and 15%) in Croat men and women compared to other studied groups. When male and female data were collapsed, Russians had marginally different allele and genotype distribution compared to Bashkirs and Tatars. Bashkirs and Tatars had similar allele and genotype frequency. The higher frequency of the *S/S* genotype was found in Tatars and Bashkirs compared to Croats and Russians. Gender related differences occurred only in the allele distribution within Bashkir population. These ethnic differences might be responsible for the inconsistent findings in the studies of the association between various psychiatric disorders, personality traits, behaviors and 5-HTTLPR across different ethnicities, and should be controlled to enable the generalization of results across various population groups.

1. Introduction

The serotonergic (5-hydroxytryptamine, 5-HT) system is known to modulate mood, emotions, sleep, appetite, and cognitive functions, and thus is implicated in the control of numerous behavioral and physiological functions (Schloss and Williams, 1998). The impairment of the central 5-HT neurotransmission has been associated with the etiology of various psychiatric disorders, addictions, and altered behaviors such as extreme violence, hostility, aggression, suicidal behavior, and impulsivity (Lucki, 1998; Stahl, 1998). The 5-HT transporter (5-HTT) regulates the entire 5-HT system and its receptors via modulation of extra-cellular fluid 5-HT concentration (Murphy et al., 2004). The 5-HTT is a protein which terminates the action of 5-HT after it is released from the nerve terminals into the synapse, and it regulates the magnitude and duration of 5-HT neurotransmission (Lesch et al., 1996; Schloss and Williams, 1998). The 5-HTT is the site of action of widely used reuptake inhibiting antidepressants such as selective serotonin reuptake inhibitors and traditional tricyclic antidepressants. A dysfunction of 5-HTT has been implicated in the etiology of psychiatric disorders such as mood disorders, obsessive–compulsive, and substance abuse disorders (Serretti et al., 2006).

A single gene (*5-HTT* or *SLC6A4*) encoding the human 5-HTT has been cloned and mapped on chromosome 17q11.1-q12 (Ramamoorthy et al., 1993). The 5-HTT is highly evolutionarily conserved, since *SLC6A4*

Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; 5-HTT, serotonin transporter, 5-HTTLPR, serotonin transporter gene-linked polymorphic region; *5-HTT* or *SLC6A4*, 5-HTT gene; *L*, long allele; NS, non-significant; PCR, polymerase chain reaction; *S*, short allele.

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homologues have been identified in over ten species, including non-human primates, rodents, worms (*C. elegans*), fly (*Drosophila*), and even in prokaryotes (Murphy et al., 2004). Transcriptional activity of the *SLC6A4* is modulated by a polymorphic repetitive element (5-HTT gene-linked polymorphic region, 5-HTTLPR) located upstream of the transcription start site (Lesch and Gutknecht, 2005). A deletion/insertion in the 5-HTTLPR creates a short (*S*) allele and a long (*L*) allele, i.e. 14- and 16-repeat alleles, which alter the promoter activity (Heils et al., 1996; Collier et al., 1996). The uptake of 5-HT is approximately two-fold higher in cells containing the homozygous *L/L* form of the *SLC6A4* than either the *L/S* or *S/S* forms (Lesch and Mossner, 1998), while the *S* allele is related to reduced transcriptional efficiency and therefore decreased 5-HT expression and uptake (Lesch et al., 1996). Volumetric neuroimaging studies have shown that the *S* allele is associated with reduced grey matter volume in the limbic system and disrupted amygdala–cingulate coupling (Heinz et al., 2005; Pezawas et al., 2005). Fourteen allelic variants (14-A, 14-B, 14-C, 14-D, 15, 16-A, 16-B, 16-C, 16-D, 16-E, 16-F, 19, 20 and 22) have been found in Japanese and Caucasian people (Nakamura et al., 2000). The 5-HTTLPR has been studied in several psychiatric disorders and personality traits, and many positive associations with *S* allele have been reported (Caspi et al., 2003; Feinn et al., 2005; Hoefgen et al., 2005; Lasky-Su et al., 2005), while other studies did not replicate these data (Mendes de Oliveira et al., 1998; Mendlewicz et al., 2004).

Differences in population genetic structure and substructure between cases and controls can lead to false positive or false negative association tests. Ancestry differences corresponding to ethnic groups may be important in determining disease risk factors and optimizing treatment (Tian et al., 2008). The allele frequencies between Caucasian and Asian populations are different, since *S* allele is found in 42% of Caucasians and in 79% of Asians (Gelernter et al., 1997; Kunugi et al., 1997; Ng et al., 2006; Serretti et al., 2006). In contrast to other Caucasian populations, population from Russia comprised of different ethnic groups, which could contribute to the different allele frequencies of the *SLC6A4*. Therefore, the hypothesis of this study was that ethnic differences in the polymorphism of the *SLC6A4* might be found among Caucasian subjects from several European populations. In this study, we analyzed the genotype and allele frequencies of the polymorphous locus of the *SLC6A4* gene (5-HTTLPR) in healthy individuals from four European populations living in Croatia and the Russian Federation.

2. Methods

2.1. Sample population and study design

A total sample of 1804 subjects was included in this multi-centric study. DNA samples were obtained from European healthy subjects (Croats, Russians, Tatars and Bashkirs). The Croatian sample comprised of 665 Caucasians of the South Slavic origin (461 men, 204 women), who were recruited from 2001 to 2006 from the University Hospital Dubrava, Zagreb, Croatia, and who filled in the questionnaire answering the questions about their medical history, smoking and drinking habits. The participants from the Russian Federation were randomly selected from the general population of the Volga–Urals region: 261 Bashkirs (151 men, 110 women), 380 Tatars (184 men, 196 women) and 498 Russians (238 men, 260 women). Bashkirs and Tatars are Caucasians of the Turkish descent, with a varying Mongoloid component, and Russian subjects are Caucasians of the East Slavic origin. They were collected from 1997 to 2001. All subject were without a personal or familial (first degree) history of neuropsychiatric disorders and suicidal behavior. All individuals were unrelated and belonged to the native ethnic group of the regions studied; they filled the questionnaire answering the questions about their national origin up to three degree relatives. Written informed consent was obtained from all participants, after explaining the aims and procedures of the study, under procedures approved by the Ethics committees of the Corresponding Centers.

2.2. DNA genotyping for the 5-HTTLPR polymorphous locus

Genomic DNA was extracted from peripheral blood using standard phenol–chloroform (Russian Federation) or salting out method (Croatia). Polymerase chain reaction (PCR) amplification of polymorphic loci in the *SLC6A4* gene was performed in total volume of 15 μL containing 150 ng DNA, 0.5 μM of each specific primer (Syntol, Moscow, Russia; Sigma Aldrich, USA), 0.200–0.250 mM of each dNTP (Helicon, Moscow, Russia; Invitrogen, USA), 0.5 units Taq polymerase (Silex, Moscow, Russia; Qiagen, Germany) and 1× PCR buffer (67 mM Tris–HCl, pH 8.8, 6.7 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 0.01% Tween-20) (Silex, Moscow, Russia; Qiagen, Germany). The primers used for the amplification of the 5-HTTLPR were 5′-GGCGTTGCCGCTCTGAATGC-3′ and 5′-GAGGGACTGAGCTGGACAACCAC-3′ (for Croatian samples) or 5′-CTTGTTGGGGATTCTCCCGCCTGGCGTT-3′ and 5′-TCGAGGCTGAGCGTC-TAGAGGGACTGAGCTGG-3 (as described by Heils et al., 1996) (for Russian Federation samples). Cycle conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s, 30 s annealing at 55 °C, extension at 72 °C for 1 min as well. A final extension was carried out at 72 °C for 5 min. PCR products were separated in 2% agarose gel (for Croatian samples), or 7% acrylamide gel (for Russian Federation samples), and visualized with ultraviolet light after ethidium bromide staining. All laboratory procedures were performed blind to subject status.

2.3. Statistical data analysis

The genotype frequency was assessed for Hardy–Weinberg equilibrium with a Chi-square (χ^2) test. Statistical analysis of the results (differences in genotype and allele frequencies between four groups) was performed using a χ^2 test. Bonferroni correction was used for multiple testing, using the total number of pair comparison of ethnic groups as correction factor and the level of significance was set to $\alpha = 0.0083$. In addition, power calculation (pc) of the power of performed tests was given for all comparisons, while the desired power should be pc ≥ 0.800. All statistical analyses were performed with SPSS version 12.0 and with SigmaStat 2.0 (Jandell Scientific Corp. San Raphael, California, USA).

3. Results

The sample of 1804 healthy individuals from 4 European populations was analyzed for the genotype and allele frequencies of the 5-HTTLPR (Tables 1 and 2). Genotype frequencies of the 5-HTTLPR were in accordance with Hardy–Weinberg equilibrium.

3.1. Frequency of the serotonin transporter gene variants (5-HTTLPR) in male and female Croats, Russians, Tatars and Bashkirs

The genotype and allele frequencies of the 5-HTTLPR among all four populations were significantly different ($P<0.001$) when men and women were analyzed separately (Table 1). There were significant differences in the genotype ($\chi^2=40.259$; $df=6$; pc=1.000; $P<0.001$) and allele ($\chi^2 = 37.952$; $df=3$; pc=1.000; $P<0.001$) frequencies in male Croats, Russians, Tatars and Bashkirs, and in the genotype ($\chi^2 = 17.752$; $df=6$; pc=0.911; $P=0.007$) and allele ($\chi^2= 14.607$; $df=3$; pc=0.919; $P=0.002$) frequencies in female Croats, Russians, Tatars and Bashkirs (Table 1). Since the power of performed tests exceeded 0.8, these differences are significant.

3.2. Gender related differences in the serotonin transporter gene variants (5-HTTLPR)

When Croats, Bashkirs, Tatars and Russians were analyzed separately according to the gender, no significant gender related differences in genotype frequencies of the 5-HTTLPR between Croats,

Table 1
Frequencies (percentages) of the serotonin transporter gene variants (5-HTTLPR) in male and female Croat, Russian, Tatar and Bashkir subjects

	Croats, *n* (%)		Russians, *n* (%)		Tatars, *n* (%)		Bashkirs, *n* (%)	
	Men	Women	Men	Women	Men	Women	Men	Women
	n=461	*n*=204	*n*=238	*n*=260	*n*=184	*n*=196	*n*=151	*n*=110
Genotypes								
L/L	173 (37.53)	81 (39.70)	82 (34.45)	74 (28.46)	54 (29.35)	52 (26.53)	32 (211.19)	37 (33.64)
L/S	224 (48.59)	92 (45.10)	107 (44.96)	136 (52.31)	82 (44.56)	88 (44.90)	70 (46.36)	51 (46.36)
S/S	64 (13.88)	31 (15.20)	49 (20.59)	50 (19.23)	48 (26.09)	56 (28.57)	49 (32.45)	22 (20.00)
χ^2	χ^2=0.709; pc=0.103; *P*=0.701		χ^2=2.915; pc=0.300; *P*=0.233		χ^2=0.486; pc=0.085; *P*=0.784		χ^2=7.354; pc=0.677; *P*=0.025 NS after Bonferroni correction	
Alleles								
L	570(61.82)	254 (62.25)	271 (56.93)	284 (54.62)	190 (51.63)	192 (48.98)	134 (44.37)	125 (56.81)
S	352 (38.18)	154 (37.75)	205 (43.07)	236 (45.38)	178 (48.37)	200 (51.02)	168 (56.93)	95 (43.18)
χ^2	χ^2= 0.0078; pc=0.048; *P*=0.929		χ^2=0.451; pc=0.096; *P*=0.502		χ^2=0.433; pc=0.094; *P*=0.511		**χ^2=7.398; pc=0.789; *P*=0.007** S after Bonferroni correction	

N is the number of subjects. S=significant. NS=not significant.

Russians, Tatars, or Bashkirs, or in allele frequencies of the 5-HTTLPR between Croats, Russians, and Tatars were found (Table 1). Bashkir men and women had significantly different allele frequency, with marginally significant power of performed test (pc=0.789), however this difference was present after Bonferroni correction. Since the power of performed tests for other comparisons was less than desired (values ranged from 0.048 to 0.300), we might have missed to detect significant gender differences when they actually existed (Table 1).

3.3. Ethnic differences in the serotonin transporter gene variants (5-HTTLPR) in male and female Croats, Russians, Tatars and Bashkirs

Male and female Croats, Bashkirs, Tatars and Russians had significantly different genotype and allele frequencies of the 5-HTTLPR, Table 1. Within male subjects, significant differences in genotype and allele frequencies of the 5-HTTLPR were found between Croats and Tatars (χ^2= 14.229; *df*=2; pc=0.945; *P*<0.001 and χ^2= 10.870; *df*=1; pc=0.928; *P*<0.001), and Croats and Bashkirs (χ^2= 30.458; *df*=2; pc=1.000; *P*<0.001 and χ^2= 27.643; *df*=1; pc=1.000; *P*<0.001), respectively. Russian men had significantly different genotype and allele frequencies compared to Bashkir men (χ^2= 10.744; *df*=2; pc=0.854; *P*=0.005 and χ^2= 11.185; *df*=1; pc=0.935; *P*<0.001), and similar genotype and allele frequencies compared to male Croats and Tatars. Tatar and Bashkir men had similar genotype and allele frequencies of the 5-HTTLPR. These non-significant differences in the allele and genotype frequencies might be explained by the less than desired power of performed tests (*pc*<0.800) and Bonferroni correction (Table 1). Within female subjects, the only significant differences were found in the genotype (χ^2= 13.441; *df*=2; pc=0.930; *P*<0.001) and allele (χ^2= 13.749; *df*=1; pc=0.974; *P*<0.001) frequencies between Croats and Tatars. Other populations did not differ significantly in the genotype and allele frequencies of the 5-HTTLPR, due to the lower power of performed tests (pc<0.800) and Bonferroni correction, Table 1.

To elucidate which genotype contributed to these ethnic differences, male and female ethnic groups were analyzed additionally according to the presence of homozygous *S/S* genotype versus the combined *L/L* and *L/S* genotypes. Significant (χ^2= 29.288; *df*=3; pc=0.999; *P*<0.001) differences in the frequency of *S/S* and *S/L+L/L* genotypes were found between male, but not female (χ^2= 11.557; *df*=3; pc=0.831; *P*=0.009, NS due to Bonferroni correction) Croats, Russians, Tatars and Bashkirs. Croat and Tatar men (χ^2= 12.813; *df*=1; pc=0.963; *P*<0.001) and women (χ^2= 9.736; *df*=1; pc=0.896; *P*<0.001), and Croat and Bashkir men (χ^2= 24.828; *df*=1; pc=1.000; *P*<0.001) differed significantly, while the other studied groups had similar frequency (due to the lower power of performed tests (pc<0.800) and Bonferroni correction) of S/S and *S/L+L/L* genotypes.

3.4. Frequency of the serotonin transporter gene variants (5-HTTLPR) in Croat, Russian, Tatar and Bashkir populations

The genotype (χ^2= 40.259; *df*=6; pc=1.000; *P*<0.001) and allele (χ^2= 37.925; *df*=3; pc=1.000; *P*<0.001) frequencies of the 5-HTTLPR among all four populations (when data for men and women were collapsed) were significantly different between Croats, Russians, Tatars and Bashkirs (Table 2). The largest difference was found in the frequency of the *S/S* genotype (14% in Croats, 20% in Russians, and 27% in Tatars and Bashkirs). Genotypes frequencies were significantly different between Croats and Bashkirs (χ^2= 24.929; *df*=2; pc=0.999; *P*<0.001), and Croats and Tatars (χ^2= 29.585; *df*=2; pc=1.000; *P*<0.001), while Russians did not differ significantly in the genotype frequency of the 5-HTTLPR with

Table 2
The data from the literature and present study on 5HTTLPR *LL*, *LS* and *SS* genotypes and *L* and *S* alleles

Country	Genotype, *n* (%)			Allele, *n* (%)	
	LL	LS	SS	L	S
Croatia (present study)	254 (38.2)	316 (47.5)	95 (14.3)	824 (62.0)	506 (38.0)
Russia: Russians (present study)	156 (31.3)	243 (48.8)	99 (19.9)	555 (55.7)	441 (44.3)
Russia: Tatars (present study)	106 (27.9)	170 (44.7)	104 (27.4)	382 (50.3)	378 (49.7)
Russia: Bashkirs (present study)	69 (26.4)	121 (46.4)	71 (27.2)	259 (49.6)	263 (50.4)
Germany (Lang et al., 2004)	85 (37.3)	102 (44.7)	41 (18.0)	272 (59.6)	184 (40.4)
Austria (Willeit et al., 2003)	51 (34.9)	65 (44.5)	30 (20.6)	167 (57.2)	125 (42.8)
United Kingdom (Surtees et al., 2006)	648 (33.4)	944 (48.7)	348 (17.9)	2240 (57.7)	1640 (42.3)
Hungary (Szekely et al., 2004)	53 (35.1)	69 (45.7)	29 (19.2)	175 (58.0)	127 (42.0)
Spain (Gutiérrez et al., 1998)	29 (34.9)	37 (44.6)	17 (20.5)	95 (57.2)	71 (42.8)
Italy (Nonnis et al., 2008)	21 (14.0)	86 (57.3)	43 (28.7)	128 (42.7)	172 (57.3)
PR China (Li et al., 2007)	13 (13.0)	32 (31.0)	58 (56.0)	58 (28.0)	148 (72.0)
Korea (Kim et al., 2000)	12 (4.8)	103 (40.9)	137 (54.4)	127 (25.1)	377 (74.9)
Japan (Katsuragi et al., 1999)	4 (4.0)	31 (30.7)	66 (65.3)	39 (19.3)	163 (80.7)

Croats and Tatars, due to the lack of power of performed tests (pc=0.789 and 0.640) and Bonferroni correction (P=0.01 and 0.033), respectively, Table 2. Bashkirs did not differ significantly in the genotype frequency of the 5-HTTLPR with Russians and Tatars (Table 2). *L* and *S* allele frequencies were significantly different between Croats and other populations: Russians (χ^2= 8.907; df=1; pc=0.865; P=0.003), Bashkirs (χ^2= 22.996; df=1; pc=0.999; P<0.001) and Tatars (χ^2= 26.611; df=1; pc=1.000; P<0.001), Table 2. As the power of performed tests exceeded 0.8, these differences are highly significant. Russians did not differ significantly in the allele frequency of the 5-HTTLPR with Tatars and Bashkirs, due to the lack of power of performed tests (pc=0.600 and 0.595) and Bonferroni correction (P=0.026 and 0.027), respectively, Table 2. Bashkirs and Tatars did not differ significantly in the allele frequency of the 5-HTTLPR (Table 2).

4. Discussion

4.1. Ethnic differences in the genotype and allele frequency of the 5-HTTLPR in male and female Croats, Russians, Tatars and Bashkirs

The results from the present study showed significant ethnic differences in the genotype and allele frequencies of the 5-HTTLPR in healthy individuals from four European populations living in Croatia and the Russian Federation. The largest differences were found between Croats and other studied groups, presumably because Croats are moderately homogenous Caucasian sample, while groups from Russian Federation were recruited from Volga–Ural region (situated at the edge between Europe and Asia), with ethnically, historically, and culturally heterogeneous population. Ethnic groups of this region belong to different language groups: the Slavic group (Russian) of the Indo-European language family, and the Turkish group (Bashkirs, Tatars) of the Altaian language family. Bashkirs and Tatars anthropologically belong to Caucasians with a varying Mongoloid component (Bermisheva et al., 2002). The contribution of the *S/S* genotype was responsible for the significant ethnic differences observed between male and female Croats and Tatars, and male Croats and Bashkirs, presumably due to the moderate Asian influence, while other studied populations had similar frequencies of the *S/S* genotype. Since the *S* allele results in reduced transcriptional activity of the *SLC6A4* gene (Heils et al., 1996; Lesch and Gutknecht, 2005), the Asian population is believed to have lower transcriptional activity of the *SLC6A4* gene. As *S* allele is associated with an increased risk of affective disorders and pathological behaviors (Lesch and Gutknecht, 2005), our results would suggest that the prevalence of affective disorders would be higher in Tatars and Bashkirs, than in Croats and Russians. However, a cross-national study on the life-time morbidity rate of affective disorders showed a lower morbidity rate in Asian populations compared to European populations (Weissman et al., 1996). Besides, Europeans generally scored higher in extraversion and novelty seeking than Asians (McCrae and Terracciano, 2005), and have higher scores on neuroticism compared to subjects from South and Southeast Asia (McCrae and Terracciano, 2005). Thus, if the *S* variant of the *SLC6A4* contributes to affective disorders, personality traits and pathological behaviors, it may require interactions with other factors such as ethnic, socio-cultural, geographical factors, gender, age, chronic alcohol use (Hariri et al., 2006; Li and He, 2007), and exposure to environmental stress (Caspi et al., 2003). The association between particular gene polymorphisms and mood disorders, behaviors or cognition is not simple, since the gene effects on the brain function are small and therefore the sample size must be large (Lesch and Gutknecht, 2005; Goldberg and Weinberger, 2004). Besides gene × gene interactions, or gene × environment interactions, other epigenetic factors (Mill and Petronis, 2007) might be important as main determinants of complex-inheritance disorders and personality traits.

Our results confirmed that ethnic differences in the 5-HTTLPR, previously found between Caucasian and Asian populations (Gelernter et al., 1997; Kunugi et al., 1997; Ng et al., 2006; Serretti et al., 2006), exist also in Caucasian (or mostly Caucasian) subjects from four European populations. The different distribution of the alleles and genotypes of the 5-HTTLPR from individuals living in Europe (Gutierrez et al., 1998; Lang et al., 2004; Szekely et al., 2004; Nonnis Marzano et al., 2008; Surtees et al., 2006; Willeit et al., 2003, present study), China (Li et al., 2007), Korea (Kim et al., 2000) and Japan (Katsuragi et al., 1999) is shown in Table 2. These ethnic differences might be responsible for the inconsistency (i.e. non-replication) of the associations of 5-HTTLPR with suicidal behavior (Li and He, 2007), anxiety, depression, and aggression (Lesch and Gutknecht, 2005), personality traits (Munafo et al., 2005), or response to antidepressant treatment (Serretti et al., 2006).

4.2. Gender related differences in the 5-HTTLPR

In agreement with literature data (Szekely et al., 2004), no significant gender related differences in genotype and allele frequencies of the *SLC6A4* were found between Croat, Russian and Tatar men and women. However, since the power of the performed tests was lower than desired, we might have failed to detect gender differences where they actually existed. On the contrary, the distribution of the allele, but not genotype, was significantly different within male and female Bashkirs. This difference might be explained by the sampling procedure, since Bashkirs were recruited from different regions of the Bashkortostan Republic (Ufa, Baymakskiy, Arkhangelskiy, Abzelilovskiy, Sterlibashevskiy, Burzyanskiy), and therefore a varying Mongoloid component, present in this huge area, might have contributed significantly to the gender related differences in the allele frequency of the *SLC6A4*. Namely, male and female Bashkirs might have been recruited from different regions and different subpopulations.

4.3. The limitations and advantages of the study

The limitation of the study was that additional SNP of the 5-HTTLPR (A to G substitution on the *L* variant) was not measured (Hu et al., 2005). The power of the performed tests was lower than 0.800 in some comparisons, indicating that we probably failed to detect the existing differences. To elucidate the possible effect of gender on the genotype and allele frequencies of the *SLC6A4*, larger groups are needed. The advantage of the study was that 5-HTTLPR was evaluated separately in Croat, Russian, Tatar and Bashkir men and women, and that a conservative Bonferroni correction was used for multiple testing, with a significance set to α=0.0083. Since the study included 1804 subjects, and significant differences were found with the desired power of the performed tests (more than 0.800), the study had a satisfactory effect size to detect ethnic differences in 5-HTTLPR. Therefore, the significant ethnic differences were detected between Croats and other populations, whereas Russians showed modest differences in the genotype and allele frequencies of the *SLC6A4* compared to other populations.

Prevalence of hereditary diseases in population depends on population genetic structure. Genes are the only consistent risk factors of susceptibility to major psychiatric disorders across populations. The results from this multi-centric study showed ethnic differences in the genotype and allele frequencies of the 5-HTTLPR among four European population of different ethnic affiliation, which might be responsible for the divergent findings regarding suicidal behavior and 5-HTTLPR in our populations (Gaysina et al., 2006; Hranilovic et al., 2003), suggesting that ethnicity must be controlled in the further studies of genetic basis of suicidal behavior. As there are other polymorphisms in the *SLC6A4* gene, and an A/G substitution on *L* allele, the further studies should elucidate these polymorphisms to allow the evaluation of the haplotype of the *SLC6A4* gene, to identify more homogenous participant groups, and to minimize false positive and false negative results in assessing genetic risk factors for particular trait or disease.

5. Conclusion

The present results showed significant ethnic differences in allele and genotype frequencies of the 5-HTTLPR between Croats and Russians, Bashkirs and Tatars. These ethnic differences might be responsible for the inconsistent findings in the studies of the association between various psychiatric disorders, treatment outcomes and 5-HTTLPR across different populations, and should be controlled to enable the generalization of results across various population groups.

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