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The Serotonin Transporter Gene: Polymorphism and Haplotype Analysis in Russian Suicide Attempters

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Key Words
Suicidal behavior · Serotonin transporter · Serotonin deficit

Abstract
The focus on gender-specific genes associated with female suicide is justified by the possible dimorphic nature of the serotonergic system and by the greater number of suicide attempts in females. We performed analysis of the promoter *(5-HTTLPR)* and intron 2 (*STin2* VNTR) polymorphisms and haplotypes of the serotonin transporter gene in Russian suicide attempters, separately in men and women. Our findings indicate the contribution of the *SLC6A4* gene to susceptibility for suicidal behavior in women, but not in men. The *L/L* genotype ($p = 0.013$, OR = 2.09) and *L10* haplotype ($p = 0.04$, OR = 1.77) were associated with suicide in Russian women only. Further investigations of this gene in different phenotypic groups are necessary.

Introduction

Suicidal behavior is believed to be a result of interaction between a lot of genetic and environmental factors. Gender-related differences in suicidal behavior are known: suicide attempts are more frequent in women than in men, but men are three- to fourfold more likely to complete suicide. The differences in environmental risk factors between men and women have been investigated; however, the role of genetic risk factors are still unclear. The current consensus is that genetic factors in males and females are probably of similar magnitude. Whether this heritability is due to the same genes is still to be addressed [1–4].

Suicide or serious suicide attempts are associated with a serotonin (5-HT) deficit that appears to be independent of psychiatric diagnosis [5]. The serotonin transporter (5-HTT) is believed to be primarily responsible for the termination of action of 5-HT after it is released from the nerve terminal into the synapse. It is located on the presynaptic neuron and takes up one 5-HT molecule concurrently with one Na^+ ion, decreasing extracellular fluid concentrations of 5-HT to levels where postsynaptic receptor activation ceases [6]. The human serotonin transporter protein is encoded by a single gene *(SLC6A4)*, mapped to chromosome 17q11.1–q12 [7]. Two common polymorphisms in the *SLC6A4* gene have been identified. A functional polymorphism *(5-HTTLPR)* in the 5′ regulatory region involves two major alleles – *S* (short) and *L* (long) alleles – that correspond to the presence of 14- or 16-repeat units of a 20- to 23-bp incomplete repeat. The *S* allele was found to reduce transcription efficiency for the *SLC6A4* gene, resulting in decreased gene expression and serotonin uptake in lymphoblast cell lines [8]. A second polymorphism, a 17-bp variable number of tandem repeats (*STin2* VNTR), located in intron 2, involves two major alleles *(STin2.10* and *STin2.12)* that correspond to 10-

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or 12-repeat units of the 17-bp VNTR. It has been suggested that the VNTR region may act as a transcriptional regulator of the *SLC6A4* gene [9]. Many studies have examined the association between the *5-HTTLPR* polymorphism and suicide, but have yielded inconsistent results [for review, see 10]. Conflicting results on the relationship between the *5-HTTLPR* polymorphism and vulnerability to suicidal behavior may be due to gender effects on 5-HT neurotransmission. Clear sex-specific differences in serotonergic system were demonstrated in a number of studies [11]. Both animal and human studies showed that females may have higher serotonergic activity [5]. Anatomical sexual dimorphism has been suggested [12] in the raphe nuclei, the area with higher density of serotonin transporter [7, 13]. Several studies have reported that SSRI efficacy is higher in females than in males [14]. Moreover, recent studies have reported that alteration of gene expression affects serotonin synthesis by sexual hormones in macaques [15]. These results suggest that the modulation of serotonergic neuron activity is gender dependent, which may indicate the involvement of sexual hormones in serotonergic neurotransmission. According to these findings, it seems valuable to accept gender as an important factor in the study of serotonergic function in the CNS. The aim of the present study is to assess two genetic variants of the *SLC6A4* gene for association with suicide attempts in Russian men and women.

Materials and Methods

Samples

We investigated a total sample of 394 subjects. Cases were 150 persons (52 men and 98 women) – hospitalized in the Clinical Republic Hospital, Ufa, Russia after suicide attempt. The mean age (± SD) was 33.80 ± 15.68 years for men and 33.42 ± 15.10 years for women. Systematic information on suicidal behavior was collected by interview with patients and their relatives by an experienced suicidologist. A suicide attempt was defined according to Mann as intentional self-harm that was not self-mutilatory in nature and required medical evaluation and treatment in an emergency or intensive unit. Suicide attempts were classified as violent – hanging (4.2%), jumping from a high place or under a vehicle (6.3%), cutting (8.5%), or nonviolent – drug overdose (81%). Forty-seven patients (31.3%) admitted to previous suicide attempts. The mean number of previous suicide attempts in the study population was 1.7 (range 1–8). The diagnosis of underlying psychiatric disorders was made according to the ICD-10 criteria. Some patients were diagnosed with more than one psychiatric disorder. The most common primary diagnoses in the sample were: personality disorders (29.3%), depressive disorders (17.3%), substance dependence (16%), schizophrenia spectrum disorders (14.7%). Other diagnoses were: bipolar affective disorder I (2%); posttraumatic stress disorder (1.33%).

The control group consisted of 244 volunteers – 132 men (mean age ± SD, 35.24 ± 10.58 years) and 112 women (mean age ± SD, 39.24 ± 12.20 years) without a personal or familial (first degree) history of neuropsychiatric disorders and suicidal behavior. All subjects that participated in this study came from the Russian population which belongs to a Slavic group of the Indo-European language family. The study was approved by the Biology Ethics Committee of Bashkortostan Republic and written informed consent was obtained from all subjects.

Genotyping

Genomic DNA was extracted from peripheral blood using a standard phenol-chlorophorm method. Polymerase chain reaction amplification of both polymorphic loci in the *SLC6A4* gene were performed in total volumes of 15 μl containing 50 ng DNA, 0.5 μM of each specific primer (Syntol, Moscow, Russia), 0.8 mmol dNTPs mix (Helicon, Moscow, Russia), 0.5 units Taq polymerase and 1.5 μl 10× 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). The primers and cycle conditions used for the amplification of both loci are described elsewhere [8, 9]. Polymerase chain reaction products were separated in 7% acrylamide gel and visualized with ultraviolet light after ethidium bromide staining. For technical reasons we did not have genotypic information for 6 individuals for either *5-HTTLPR* or *STin2* VNTR loci.

Statistical Analysis

Genotype and allele frequencies as well as Hardy-Weinberg equilibrium were calculated using Microsoft Excel macro PHARE version 2.1 (http://bioinformatics.org/macroshack/programs/PHARE/description.html). For association analysis of individual genotypes and alleles, Fisher's exact test was carried out using the RxC program (http://www.marksgeneticsoftware.net/). Odds ratios (OR) with 95% confidence intervals (CI) were calculated using Woolf's method. For pairwise linkage disequilibrium and haplotype analysis, the EH program (Rockefeller University, New York, USA, http://linkage.rockefeller.edu/ott/eh.htm) and the 2LD program (Institute of Psychiatry, King's College London, London, UK, http://www.iop.kcl.ac.uk/IoP/Departments/PsychMed/GEpiBSt/software.shtml) were used. Bonferroni correction was used for multiple testing, using the total number of loci as correction factor (the level of significance was set to $\alpha = 0.025$).

Results

The sample of 144 suicide attempters and 244 healthy control subjects was genotyped for the *5-HTTLPR* and *STin2* VNTR polymorphisms of the *SLC6A4* gene. Distributions of genotype frequencies of both loci were in accordance with Hardy-Weinberg equilibrium for both control subjects and suicide attempters (data not shown). Distribution of genotype and allele frequencies of the *5-HTTLPR* polymorphism in control subjects and suicide attempters is shown in table 1. A tendency for significant difference in the *5-HTTLPR* genotype distribution was

Table 1. Distribution of genotype and allele frequencies of the *5-HTTLPR* polymorphism in control subjects and suicide attempters

Genotypes and alleles	Frequencies (%)					
	men		women		total	
	control subjects (n = 132)	suicide attempters (n = 49)	control subjects (n = 112)	suicide attempters (n = 95)	control subjects (n = 244)	suicide attempters (n = 144)
L/L	37 (28)	15 (31)	28 (25)	39 (41)	65 (27)	54 (38)
L/S	66 (50)	25 (51)	53 (48)	40 (42)	119 (49)	65 (45)
S/S	29 (22)	9 (18)	31 (28)	16 (17)	60 (25)	25 (17)
χ^2 (p)[a]		0.31 (NS)		7.06 (**0.03**)		5.89 (0.053)
L	140 (53)	55 (56)	103 (49)	118 (62)	249 (51)	173 (60)
S	124 (47)	43 (44)	115 (51)	72 (38)	239 (49)	115 (40)
χ^2 (p)[a]		0.28 (NS)		9.03 (**0.002**)		5.97 (**0.014**)

Figures in bold indicate level of significance < 0.05.
[a] Compared to corresponding control group.

Table 2. Distribution of genotype and allele frequencies of the *STin2* VNTR in control subjects and suicide attempters

Genotypes and alleles	Frequencies (%)					
	men		women		total	
	control subjects (n = 132)	suicide attempters (n = 50)	control subjects (n = 112)	suicide attempters (n = 94)	control subjects (n = 244)	suicide attempters (n = 144)
9/10	2 (2)	1 (2)	3 (3)	0 (0)	5 (3)	1 (1)
9/12	1 (1)	1 (2)	2 (2)	3 (3)	3 (2)	4 (3)
10/10	24 (19)	6 (12)	19 (17)	18 (19)	43 (18)	24 (17)
10/12	54 (41)	24 (48)	44 (40)	42 (45)	98 (41)	66 (46)
12/12	51 (39)	18 (36)	44 (40)	31 (33)	95 (39)	49 (34)
χ^2 (p)[a]		1.89 (NS)		3.98 (NS)		3.60 (NS)
9	3 (1)	2 (2)	5 (2)	3 (2)	8 (1)	5 (2)
10	104 (39)	37 (37)	85 (38)	78 (41)	189 (39)	115 (40)
12	157 (60)	61 (61)	134 (60)	107 (57)	291 (60)	168 (58)
χ^2 (p)[a]		0.53 (NS)		0.69 (NS)		0.13 (NS)

[a] Compared to corresponding control group.

revealed between suicide attempters and controls in total samples (p = 0.053). When we analyzed the results of genotyping in male and female groups separately, the significant differences in the *5-HTTLPR* genotype frequencies were revealed between the suicide and control groups for women (p = 0.03, lost after Bonferroni correction), but not for men. The increase in the *L/L* genotype frequency was observed in women of the suicide group when compared to women of the control group ($\chi^2 = 6.05$, p = 0.013, OR = 2.09, 95% CI 1.11–3.95). Distributions of genotype and allele frequencies of the *STin2* VNTR polymorphism are presented in table 2. Frequencies of genotypes and alleles did not differ significantly between the control and suicide groups, either in the total sample, in men, or in women.

Maximum likelihood analysis of haplotype distribution demonstrated the presence of linkage disequilibrium between the two polymorphisms in control subjects (men $D' = 0.43$, women $D' = 0.33$), suicide attempters (men $D' = 0.20$; women $D' = 0.43$), and the overall sample (men $D' = 0.37$; women $D' = 0.38$). Analysis of distribution of the estimated haplotype frequencies (table 3) re-

Table 3. Distribution of estimated haplotype frequencies for the *SLC6A4* gene in control subjects and suicide attempters

Haplotypes	Frequencies (%)					
	men		women		total	
	control subjects (n = 132)	suicide attempters (n = 48)	control subjects (n = 112)	suicide attempters (n = 93)	control subjects (n = 244)	suicide attempters (n = 141)
L9	20 (8)	3 (3)	20 (9)	17 (9)	40 (8)	20 (8)
L10	42 (16)	17 (18)	26 (12)	35 (19)	68 (14)	52 (18)
L12	79 (30)	33 (35)	63 (27)	65 (35)	142 (29)	98 (34)
S9	6 (2)	4 (4)	1 (1)	1 (1)	7 (1)	5 (1)
S10	16 (6)	8 (8)	25 (11)	14 (8)	39 (8)	22 (7)
S12	101 (38)	31 (32)	89 (40)	54 (29)	190 (39)	85 (31)
χ^2 (p)[a]		2.44 (NS)		9.48 (0.093)		7.58 (NS)

[a] Compared to corresponding control group.

vealed no significant difference between suicide attempters and control subjects. Further analysis showed overrepresentation of haplotype *L10* among female suicide attempters compared to female controls ($\chi^2 = 4.17$, d.f. = 1, p = 0.04, OR = 1.77, 95% CI 1.01–3.17), but significance was lost after Bonferroni correction. Haplotype *S12* had lower frequency in the female suicide group than in female controls ($\chi^2 = 5.12$, p = 0.022, OR = 0.62, 95% CI = 0.41–0.98). It also had lower frequency in total suicide group than in total control group ($\chi^2 = 6.02$, p = 0.01, OR = 0.67, 95% CI = 0.49–0.93). The differences retained significance after the correction for multiple testing.

Discussion

This is the first association study of the serotonin transporter gene with suicidal behavior in a Russian (eastern European) population. In our female sample, a higher frequency of the *5HTTLPR L* allele was demonstrated for the suicide attempters compared to the normal controls. Over the past few years, numerous studies on the association between the *5-HTTLPR* polymorphism and suicidal behavior have been published, however with conflicting results [for review, see 1, 2, 10]. The meta-analysis based on results of 18 studies has been performed by Lin and Tsai [10] and provided evidence supporting the association of the of the *5-HTTLPR S* allele with suicidal behavior in the psychiatric population, and also with violent suicide. Perhaps, our study was unable to find an effect of the *S* allele, because the sample was composed of patients with suicidal behavior that was not violent (in 81% of cases) or lethal. Moreover, our suicide patients were recruited from general and not psychiatric population. As ICD-10 diagnostic information was available for a proportion of subjects, we performed the analysis independent of psychiatric diagnosis. Additionally, our study was conducted in Russian population, which has a higher frequency of the *S* allele than other Caucasian populations. Therefore, any effect of the *5-HTTLPR S* allele on suicidal behavior would require larger sample sizes than would be needed in other Caucasian populations. However, we should emphasize that the association of the *L* allele with suicide was true for Russian women only, but not for men. There is a recently published article by Mizuno et al. [16], where gender differences in association with *5-HTTLPR* and anxiety (a suicide-related trait) are shown: females with the l/s genotype showed higher anxiety than those with the s/s genotype in both state and trait anxiety. Controversially, males with the s/s genotype showed higher anxiety than those with the l/s genotype [16]. A biological explanation of the *L* allele overrepresentation in suicidal women may involve an interaction between the *5-HTTLPR* polymorphism and low gonad hormonal level. In this hypothesis, differences in either gene-environment interactions, in which the environment can modify the effect of a gene, or epistasis (gene-gene interactions) could be responsible. Baca-Garcia et al. [17] showed that the proportion of women-carriers of the *L* allele in the menses or menopause was significantly higher among suicide attempters than in the general population. Unfortunately, we cannot test this hypothesis because we do not have data on the hormonal status of our sample.

To our knowledge, there are only three reports on the association of the *STin2* VNTR polymorphism with sui-

cide [18–20]. In one of them, de Lara et al. [18] found a significant association of suicide completion with having at least one copy of the *STin2.10* allele. In our study we did not find significant differences in genotype and allele frequencies of the *STin2* VNTR polymorphism between control and suicide groups. However, we found that *L10* haplotype was associated with suicidal behavior in Russian females, although the association was not significant after Bonferroni correction. At the same time, the *S12* haplotype was shown to be associated significantly with a lower risk of suicide in the Russian female population. Therefore, our results are consistent with the report by Hranilovic et al. [19] of an association of haplotype *L10* with completed suicide in a Croatian population. Single-marker analyses in case-control studies are prone to generate conflicting results in common disease association studies. This may be due to the fact that common psychiatric disorders are predicted to be associated with common alleles [21]. The use of haplotypes as more specific risk markers than single alleles is a new design for case-control studies. Gene-based haplotype, i.e. a combination of alleles located within a gene unit, independent of genetic heritability, could potentially be the most precise markers possible for a given gene, as it would contain all the variations in the gene.

In conclusion, our findings indicate that the *SLC6A4* gene contributes to susceptibility for suicidal behavior in women, but not in men. Because of the limitations of our sample and the weak statistical significance of our findings, further investigations of this gene, gene-environment and gene-gene interactions are necessary. These results may help understand better the pathopsychology of the human brain in suicidal behavior and its prevention and treatment.

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References

1 Turecki G: Suicidal behavior: is there a genetic predisposition? Bipolar Disord 2001;3:335–349.
2 Bondy B, Buettner A, Zill P: Genetics of suicide. Mol Psychiatry 2006;11:336–351.
3 Baldessarini RJ, Hennen J: Genetics of suicide: an overview. Harv Rev Psychiatry 2004;12:1–13.
4 Hawton K: Sex and suicide. Gender differences in suicidal behaviour. Br J Psychol 2000;177:484–485.
5 Mann JJ, Brent DA, Arango V: The neurobiology and genetics of suicide and attempted suicide: a focus on the serotonergic system. Neuropsychopharmacology 2001;24:467–477.
6 Purselle DC, Nemeroff CB: Serotonin transporter: a potential substrate in the biology of suicide. Neuropsychopharmacology 2003;28:613–619.
7 Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, et al: Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. Proc Natl Acad Sci USA 1993;90:2542–2546.
8 Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P: Organization of the human serotonin transporter gene. J Neural Transm 1994;95:157–162.
9 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP: Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621–2624.
10 Lin PY, Tsai G: Association between serotonin transporter gene promoter polymorphism and suicide: results of a meta-analysis. Biol Psychiatry 2004;55:1023–1030.
11 Hrdina P: Sex-related differences: do they matter? J Psychiatry Neurosci 2000;25:319–320.
12 Cordero ME, Valenzuela CY, Torres R, Rodriguez A: Sexual dimorphism in number and proportion of neurons in the human median raphe nucleus. Brain Res Develop Brain Res 2000;124:43–52.
13 Frazer A, Gerhardt, GA, Daws LC: New views of biogenic amine transporter function: implications for neuropsychopharmacology. Int J Neuropsychopharmacology 1999;2:305–320.
14 Martenyi F, Dossenbach M, Mraz K, Metcalfe S: Gender difference in the efficacy of fluoxetine and maprotiline in depressed patients: a double-blind trial of antidepressants with serotonergic or norepinephrinergic reuptake inhibition profile. Eur Neuropsychopharmacol 2001;11:227–232.
15 Gundlah C, Lu NZ, Mirkes SJ, Bethea CL: Estrogen receptor beta (ERbeta) mRNA and protein in serotonin neurons of macaques. Mol Brain Res 2001;91:14–22.
16 Mizuno T, Aoki M, Shimada Y, Inoue M, Nakaya K, Takahashi T, Itoyama Y, Kanazawa M, Utsumi A, Endo Y, Nomura T, Hiratsuka M, Mizugaki M, Goto J, Hongo M, Fukudo S: Gender difference in association between polymorphism of serotonin transporter gene regulatory region and anxiety. J Psychosom Res 2006;60:91–97.
17 Baca-Garcia E, Vaquero C, Diaz-Sastre C, Ceverino A, Saiz-Ruiz J, Fernandez-Piquera J, de Leon J: A pilot study in a gene-hormone interaction in female suicide attempts. Eur Arch Psychiatry Clin Neurosci 2003;253:281–285.
18 Lopez de Lara C, Dumais A, Rouleau G, Lesage A, Dumont M, Chawky N, Alda M, Benkelfat C, Turecki G: STin2 variant and family history of suicide as significant predictors of suicide completion in major depression. Biol Psychiatry 2006;59:114–120.
19 Hranilovic D, Stefulj J, Furac I, Kubat M, Balija M, Jernej B: Serotonin transporter gene promoter (5-HTTLPR) and intron 2 (VNTR) polymorphisms in Croatian suicide victims. Biol Psychiatry 2003;54:884–889.
20 Yen FC, Hong CJ, Hou SJ, Wang JK, Tsai SJ: Association study of serotonin transporter gene VNTR polymorphism and mood disorders, onset age and suicide attempts in a Chinese sample. Neuropsychobiology 2003;48:5–9.
21 Cardon LR, Bell JI: Association study designs for complex diseases. Nat Rev Genet 2001;2:91–99.