Association of the *SLC6A4, SLC6A3, COMT* and *BDNF* Gene Polymorphisms with Suicidal Behavior: A Case Control Study

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Research article

Keywords: Gene polymorphism, suicidal behavior, depressive symptoms, anxiety

Posted Date: August 21st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-56679/v1

Abstract

Background

Suicide causes about 1 million deaths per year. Familial transmission of suicide, as well as the involvement of serotonin and dopamine systems in suicidal behavior, was confirmed by previous studies. We investigated an effect of 11 polymorphisms of 9 genes related to dopamine and serotonin transmission (*SLC6A4, HTR1A, HTR2A, HTR1B, SLC6A3, DRD4, DRD2, COMT,* and *BDNF)* on the risk of suicide.

Methods

The study was performed on 100 psychiatric clinic patients with repeated suicide attempts. Genomic DNA was obtained from venous blood. Genotyping was performed using locus-specific PCR. Statistical analysis was carried out using a Pearson Chi-squared test, ORs, with the corresponding 95% CIs and one-way ANOVA.

Results

Association of the *SLC6A3* 40–45 bp VNTR and *SLC6A4* rs25531 loci with suicidal behavior was tested for the first time. We have shown that a short allele of *SLC6A3* 40–45 bp VNTR locus might increase the risk of suicidal attempts (p = 0.009). Contrary to previous research, we have found that the Val-allele of *COMT* rs4680 is associated with suicide (p = 0.028). T-allele of *BDNF* rs6264 and A-allele of *SLC6A4* rs25531 were associated with suicidal behavior with p = 0.029 and p = 0.047, respectively. ANOVA showed the lack of association between these loci and severity of depressive symptoms, but revealed a possible effect of *SLC6A4* 40 bp VNTR and *HTR1B* loci on anxiety symptoms.

Conclusions

Our results suggest that allelic variants of *SLC6A3, SLC6A4, COMT* and *BDNF* genes might be considered as risk factors for repeated suicide attempts in patients with different mental disorders. Loci *SLC6A4* 40 bp VNTR and *HTR1B* rs6296 may affect anxiety symptoms.

1. Background

According to the World Health Organization, more than 800 000 people die from suicide each year [1]. Many family, twin, and adoption studies provide evidence for familial transmission of suicide and suicidal behavior [2–6]. The contribution of genetic risk factors was confirmed even after controlling for hereditary mental disorders [7]. The aggregation of suicide in families also cannot be fully explained by a similar environment [8]. Molecular studies have shown that suicide attempts could be associated with altered serotonin (HT) and dopamine (DA) transmission [9–12]. Given the above data, the search for an association between suicidal behavior and genes encoding important pathways of serotoninergic and

dopaminergic transmission (HT and DA transporters, receptors, ferments for HT and DA biosynthesis) is relevant.

The promoter region of the serotonin transporter gene (*SLC6A4/5HTT*) contains the variable number of tandem repeats (VNTR-polymorphism). The short (S) allele with 14 repeats is associated with lower expression activity than the long (L) allele with 16 repeats [13, 14]. Later, a single nucleotide polymorphism (SNP) A → G (rs25531) was detected within the sixth repeat of the S- and L-alleles. It was shown that the expression level of the L_G allele is lower than of the L_A allele. A meta-analysis of 39 studies of the *SLC6A4* VNTR-polymorphism demonstrated a significant association between suicidal behavior and the S-allele. The influence of the rs25531 polymorphism was not considered in this study [15].

Polymorphism C1019G (rs6295) exists in the promoter region of the *HTR1A* gene. It might enhance or decrease gene expression depending on the location on the presynaptic or postsynaptic membrane [16, 17]. *Post-mortem* study has discovered that the *HTR1B* gene polymorphism G861C (rs6296) affects gene expression activity and as a result changes the density of the receptors [18]. In the case of *HTR1A* gene polymorphism A1438G (rs6311), it was shown that the A-allele increases the promoter activity [19]. Association studies for these genes were also performed. Meta-analyses of these investigations detected a significant association between *HTR2A* (rs6311) and suicidal behavior [20] but failed to find any associations for *HTR1A* (rs6295) and *HTR1B* (rs6296) polymorphisms [21, 22].

A 40–45 bp VNTR-polymorphism exists in the 3'-UTR region of the transporter gene (*SLC6A3/DAT1*). Several studies have confirmed the effect of the length of this locus on gene expression, although contradictory results have been obtained [23–26]. It was shown that the 9R-allele may result in a high risk of depression and angry-impulsive personality traits [27, 28]. The dopamine receptor gene (*DRD4)* contains a 48 bp VNTR-polymorphism in exon 3 and 120 bp In/Del polymorphism in the promoter region. The 7-repeat variant of the *DRD4* VNTR-polymorphism was found to be associated with such personal traits as impulsivity, aggression, depression and novelty-seeking behavior [29, 30]. *DRD4* In/Del polymorphism might affect gene expression activity—the long allele is associated with lower expression level than the short allele [31]. The density of the DRD2 receptors in the striatum depends on the allelic variant of the rs1800497 (C/T) polymorphism. It was shown that T-allele carriers have a lower density of DRD2 receptors [32]. The *DRD2* rs1800497 polymorphism is associated with impulsivity and suggested to be related to suicidal attempts [33, 34]. The enzyme catechol-O-methyltransferase (COMT) plays an important role in dopamine metabolism. SNP rs4680 (G/A or Val/Met) in the *COMT* gene leads to reduced enzyme activity [35]. A meta-analysis of six studies has demonstrated that the *COMT* rs4680 polymorphism has a modestly significant association with suicidal behavior [22].

Another gene associated with mental disorders is the brain-derived neurotrophic factor (*BDNF*) gene [36]. BDNF and serotonin are shown to regulate each other positively: HT increases the *BDNF* expression and BDNF stimulates HT neurons' growth and survival [37]. The *BDNF* gene contains a common

polymorphism rs6264 which leads to the replacement of methionine with valine (Val66Met). Met-allele is associated with decreased protein activity [38] and possibly with suicide attempts [39].

This study aims to estimate the contribution of 11 polymorphisms in the genes *SLC6A4 (5HTT), HTR1A, HTR2A, HTR1B, SLC6A3 (DAT1), DRD4, DRD2, COMT* and *BDNF* to suicidal behavior and severity of symptoms of depression and anxiety in patients. As described above, all polymorphisms under study affect gene expression, protein product activity or were shown to be associated with personality traits.

2. Methods

2.1 Subjects

A sample of patients (N = 100) who had attempted suicide at least two times and were monitored by a psychotherapist was used for this study. A survey of patients and blood samples was carried out at the Moscow Research and Clinical Center for Neuropsychiatry of the Healthcare Department of Moscow. Depressive symptoms were evaluated with help of Hamilton's Depression Rating Scale (HAMD) and Beck's Depression Inventory (BDI). Spielberger's test was used for evaluation of the degree of situational and personal anxiety. Demographic and clinical characteristics of samples and results of medical examinations of patients are shown in Table 1. Detailed data of the patient's characteristics are shown in Additional file 1. The control group (N = 200) was a sample of the East Slavic population of the city of Moscow and the regions of Central Russia (99 men (49.5%) and 101 women (50.5%); mean age: 36 ± 8.2 years).

2.2 DNA isolation and genotyping

Genomic DNA was obtained from 250 μL of EDTA-anticoagulated venous blood using innuPREP Blood DNA Mini Kit (Analytik Jena AG, Germany), according to the manufacturer's recommendations.

The SNP and VNTR genotyping was carried out using locus-specific PCR as described previously [40–45].

2.3 Statistical analysis

Hardy–Weinberg equilibrium calculator software (https://wpcalc.com/en/equilibrium-hardy-weinberg/) was used to calculate the correspondence of the genotype distribution in the population sample to the Hardy–Weinberg equilibrium. Allele and genotype frequencies between cases and control groups were compared using a Pearson Chi-squared test. The strength of associations between allelic variants of studied polymorphic loci and suicidal behavior was estimated using odds ratios (ORs), with the corresponding 95% confidence intervals (95% CIs). One-way ANOVA was performed to determine the effect of studied polymorphisms on symptoms of depression, situational anxiety, and personal anxiety. The normal quantile–quantile (QQ) plot and Shapiro–Wilk test were used to test whether our data were

normally distributed. The analysis of frequencies of genotypes and alleles, Chi-squared test and ANOVA were conducted using Statistica (data analysis software system, version 7, http://www.statsoft.com). All tests were conducted at a level of significance $p < 0.05$.

3. Results

In the control samples, all polymorphisms were in the Hardy–Weinberg equilibrium. The results of the polymorphic loci genotyping in cases and control samples are shown in Table 2.

The strongest association was found for VNTR-polymorphism of the *SLC6A3* gene. Given the data suggesting the effect of length of this locus on gene expression and high activity of the 10R allele compared with the 9R allele [23–26], we classified cases and controls as carriers of the long (≥10) and short (<10) alleles. The short allele (S) was more common in cases than it was in the control group ($p=0.009$). SS and LS genotype frequencies were higher and LL genotype frequency was lower in cases than in controls ($p=0.019$).

Another gene of the dopaminergic system associated with suicide attempts was the *COMT* gene. In the group of cases, the G-allele (Val) frequency of *COMT* rs4680 was significantly higher than with the control group ($p = 0.028$). However, the genotype distributions of *COMT* rs4680 were not found to be significantly different between cases and controls, AA genotype frequency was decreased and GA and GG frequencies were increased in the group of cases compared with the control group (17%, 53%, 30%, respectively, in cases and 29.4%, 47.9%, 22.7%, respectively, in controls; $p = 0.064$).

We also found a statistically significant association between the T-allele of *BDNF* rs6264 and suicidal behavior. In the group of patients, the T-allele was more common than with controls ($p = 0.029$). The difference in genotype distribution was not statistically significant. However, CC genotype tends to be less common in the group of cases, while frequencies of CT and TT genotypes are higher in cases than with the control group (61%, 32%, 7%, respectively, in cases and 70.7%, 26.6%, 2.7%, respectively, in controls; $p = 0.076$).

We analysed two loci of the *SLC6A4* gene: rs25531 and VNTR 44 bp polymorphism. We found that the rs25531 polymorphism of *SLC6A4* showed some statistically significant associations. The A-allele frequency of rs25531 was higher in cases than it was in controls ($p=0.047$). VNTR-polymorphism of *SLC6A4* was not found to be associated with suicidal behavior.

Because of the complexity of *SLC6A4* organization, we additionally analysed the distribution frequencies of its allelic variants according to their functional characteristics. To verify whether alleles with low or high expression activity are associated with suicidal behavior, we grouped alleles and genotypes according to their expression levels: high (L_A) and low (S and L_G) alleles with high and low expression activity, respectively, and high/high (L_A/L_A), high/low (L_A/S_A, L_A/L_G) and low/low (S_A/S_A, S_A/L_G, L_G/L_G) genotypes.

We found that the distribution of *SLC6A4* gene genotypes and alleles showed that alleles with high expression tend to be increased in patients with suicidal behavior compared with the control group, but this association was not statistically significant (p = 0.098).

The alleles of the dopamine receptor DRD4 exon 3 were grouped into long (≥7) and short (<7) allelic variants, associations between this locus and suicide were not found. No significant associations between suicidal behavior and *DRD2* rs1800497, *DRD4* In/Del, *5HTR1A* rs6295, *5HTR2A* rs6311 and *5HTR1B* rs6296 alleles and genotypes were discovered. All results of genotyping of cases and controls are shown in Additional files 1 and 2, respectively.

To determine the effect of studied polymorphisms on the patient's clinical characteristics we conducted the one-way ANOVA. This analysis showed the lack of association between loci which were significant in the Chi-squared test and severity of the symptoms of depression, situational anxiety, and personal anxiety in patients with suicidal behavior. However, we observed a significant effect of *SLC6A4* VNTR 44 bp on the situational anxiety and *HTR1B* loci on the personal anxiety (p = 0,0358 and p = 0,0132, respectively). The most significant results of one-way ANOVA are shown in Table 3 and results for all loci are shown in Additional file 3.

4. Discussion

Genes related to neurotransmission and neurotrophic function have been widely studied in various mood and behavior disorders. To confirm possible associations studied previously and find out new associations, we investigated *SLC6A3* (40—45 bp. VNTR), *DRD2* (rs1800497), *DRD4* (In/Del 120 bp. and 48 bp. VNTR), *COMT* (rs4680), *SLC6A4* (VNTR 44 bp. and rs25531), *HTR1A* (rs6295), *HTR2A* (rs6311), *HTR1B* (rs6296) and *BDNF* (rs6264) polymorphic loci in patients with suicidal behavior. The study was carried out with a sample of patients who had attempted suicide at least two times. We have supposed that the repeated suicidal attempts are not the result of impulsivity or mood disorders only but show the genetic predisposition to suicidal behavior. We have found statistically significant associations between suicidal behavior and *SLC6A3* (40 bp. VNTR), *COMT* (rs4680), *SLC6A4* (rs25531), *BDNF* (rs6264) loci.

Although the association between *SLC6A3* 40 bp. VNTR-polymorphism and mood disorders, as well as personality traits, was confirmed [27, 28], we do not know about research that studied an effect of this locus on suicidal tendencies. We discovered a strong association between short (≤ 9 repeats) allele and suicidal behavior (p = 0.009). Carriers of at least one S-allele (SS and LS genotypes) have a significantly higher risk of suicide (p = 0.019). Given the data of low expression activity of the 9R-allele [26], we can suppose that suicidal behavior may be associated with dopamine transporter deficiency and consequently with increased dopamine signalling.

Our research may complement the results of a meta-analysis that demonstrated that the *COMT* rs4680 polymorphism is associated with suicidal behavior [22]. The meta-analysis was performed on six studies and showed that Met (A) allele with low activity is associated with suicidal attempts. However, the authors draw attention to the dependence of the results on the inclusion of all six studies. When five of

the six studies were individually excluded from the analysis, the relationship between *COMT* Met-allele and suicide was no longer significant. According to our results, suicidal behavior is associated with the *COMT* G-allele (Val) (56,5% in cases vs. 46,6% in controls, p=0,028). Taking these data into account, we can conclude that the COMT rs4680 polymorphism possibly does not affect suicidal behavior.

We also confirmed the results of a meta-analysis that showed the risk of suicide in carriers of the Met-allele of *BDNF* rs6264 [39]. The Met-allele was more common in the cases than in the control group (23% vs. 16%, p = 0,029).

We did not confirm the results of the meta-analysis that showed that the S-allele of the 44 bp. *SLC6A4* VNTR-polymorphism is a risk factor for suicide [15]. As far as we know, the relationship between *SLC6A4* rs25531 and suicidal attempts was not verified before. We have found that the rare G-allele was even rarer in cases (5% vs. 9.9%, p = 0.047). We suppose this locus may contribute to predisposition to suicidal behavior together with other loci, but its contribution is small. A bigger sample is required to verify this association. We also tried to establish whether the effects of 44 bp VNTR and rs25531 polymorphisms on *SLC6A4* expression are associated with suicide. We classified alleles and genotypes according to their functional activity and did not find statistically significant associations (Table 2).

The effect of dopamine receptor genes polymorphism on suicidal behavior is not well understood. Although some studies have shown an association between *DRD2* rs1800497 or haplotypes with this SNP and suicidal behavior [33, 34] no differences between allelic and genotype frequencies of this locus in our samples of patients and controls were found. Just like our research, previous studies failed to find an association between *DRD4* 48 bp VNTR-polymorphism and suicidal attempts [29, 46]. We also did not find *DRD4* In/Del 120 bp. to be associated with suicide. In our knowledge, this is the first study aimed to establish if the *DRD4* in/del 120 bp. polymorphism related to suicidal behavior.

Our data on the lack of association between *HTR1A* (rs6295) and *HTR1B* (rs6296) polymorphisms and suicide are consistent with previous meta-analyses [21, 22]. Contrary to the results of a meta-analysis showing a link between *HTR2A* (rs6311) and suicidal behavior [20], we did not find statistically significant differences.

It was previously shown that all of these genes are associated with major depression. We tried to verify if these loci affect severity of depressive symptoms. ANOVA showed the lack of association between these loci and depression symptoms, as well as with anxiety symptoms, but revealed an effect of *SLC6A4* 40 bp VNTR and *HTR1B* loci on situational and personal anxiety, respectively.

5. Conclusions

In this study, we show that polymorphism of the *SLC6A4, SLC6A3, COMT* and *BDNF* genes might be associated with the risk of suicidal behavior. For the first time, the effect of the dopamine transporter gene (*SLC6A3)* 40–45 bp VNTR-polymorphism on suicidal behavior was examined and we have found that a short allele may increase the risk of suicidal attempts. We also confirmed the results of previous

studies of the *BDNF* rs6264 locus, the T-allele of which is associated with suicide. In contrast to the meta-analysis of studies of the rs4680 *COMT* gene polymorphism, which shows that the G-allele (Val) is associated with suicide, we obtained data on the possible association of suicidal behavior with the Met-allele. The association of *SLC6A4* rs25531 polymorphism with suicide was first studied, but because the G-allele is rare in the population, this result requires verification on a large sample.

We showed that *SLC6A4* 40 bp VNTR and *HTR1B* loci might be related to severity of anxiety symptoms.

Further studies are required to verify if these polymorphic loci are associated with suicidal behavior only or if they might increase the risk of severe mental illness leading to suicide attempts.

Abbreviations

OR — odds ratio

CI — confidence interval

VNTR — variable number tandem repeat

SNP — single nucleotide polymorphism

HAMD — Hamilton's Depression Rating Scale

BDI — Beck's Depression Inventory

Declarations

Ethics approval and consent to participate

All blood samples were collected with the informed consent of the investigated persons after participant's personal statement signature. The Ethics Committee of the Institute of Molecular Genetics (Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov sq. 2, Moscow, Russia) approved the study (protocol 03\19, 19 February 2019).

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no conflict of interest.

Funding

The present study was supported by the Russian Foundation for Basic Research (projects no. 19-04-00383, no. 17-29-02203-ofi-m, no. 19-015-00380). The funding body played no role in the study design, the collection, analysis and interpretation of the data or the preparation and approval of the manuscript.

Authors' contributions

ER performed genotyping, statistical analysis, data interpretation, and wrote the original draft. MS performed DNA isolation and genotyping. PS contributed to conceptualization and edited the article. AG collected blood samples and patients data. AR contributed to conceptualization and edited the article. DS made contributions to the methodology and genotyping. VV led the investigation and made contributions to the funding acquisition, methodology, and design of the study. All authors have read and approved the manuscript.

Acknowledgements

The work was conducted on the base of the Center for Collective Use, Institute of Gene Biology, Russian Academy of Sciences (GK02.451.11.7060).

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Tables

Table 1. Demographic and psychiatric characteristics of patients

n	total	100
	female	80
	male	20
Age, years	min	18
	max	77
	mean	31.54
BDI	min	8
	max	53
	mean	28.85
HAMD	min	7
	max	35
	mean	21.19
Situational anxiety	min	25
	max	80
	mean	55.52
Personal anxiety	min	25
	max	80
	mean	56.44

Table 2. Genotype and allele frequency of *SLC6A4, HTR1A, HTR2A, HTR1B, SLC6A3, DRD4, DRD2, COMT* and *BDNF* genes in patients with suicidal behavior

Genotype/Allele	Total. n (%)		χ 2 (p-value)	ORs
	Control	Suicide		
SLC6A3 40 bp VNTR*				
LL	113 (69.3)	52 (52)	7.96 (0.019)++	0.48
LS	46 (28.2)	44 (44)		1.998
SS	4 (2.5)	4 (4)		0.6
L	272 (83.4)	148 (74)	6.86 (0.009)++	0.565
S	54 (16.6)	52 (26)		1.77
COMT rs4680				
AA (Met/Met)	48 (29.4)	17 (17)	5.51 (0.064)+	0.49
GA (Val/Met)	78 (47.9)	53 (53)		1.23
GG (Val/Val)	37 (22.7)	30 (30)		1.46
A (Met)	174 (53.4)	87 (43.5)	4.83 (0.028)++	0.67
G (Val)	152 (46.6)	113 (56.5)		1.49
BDNF rs6264				
CC (Val/Val)	183 (70.7)	61 (61)	5.14 (0.076)+	0.65
CT (Val/Met)	69 (26.6)	32 (32)		1.3
TT (Met/Met)	7 (2.7)	7 (7)		2.71
C (Val)	435 (84)	154 (77)	4.77 (0.029)++	0.64
T (Met)	83 (16)	46 (23)		1.56
SLC6A4 rs25531				
AA	129 (82.2)	90 (90)	3.92 (0.141)	1.95
AG	25 (15.9)	10 (10)		0.59
GG	3 (1.9)	0		0.22
A	283 (90.1)	190 (95)	3.95 (0.047)++	2.08
G	31 (9.9)	10 (5)		0.48
SLC6A4 VNTR 44 bp				
LL	68 (43.3)	41 (41)	4.18 (0.12)	0.91
LS	59 (37.6)	48 (48)		1.53

SS	30 (19.1)	11 (11)		0.52
L	195 (62.1)	130 (65)	0.44 (0.507)	1.13
S	119 (37.9)	70 (35)		0.88
SLC6A4 VNTR 44 bp + rs25531 **				
high/high	49 (31.2)	35 (35)	4.45 (0.108)	1.19
high/low	67 (42.7)	50 (50)		1.34
low/low	41 (26.1)	15 (15)		0.5
high	165 (52.5)	120 (60)	2.75 (0.098)+	1.35
low	149 (47.5)	80 (40)		0.74
DRD2 rs1800497				
CC	95 (58.3)	48 (48)	2.85 (0.241)	0.66
CT	63 (38.6)	47 (47)		1.41
TT	5 (3.1)	5 (5)		1.66
C	253 (77.6)	143 (71.5)	2.48 (0.115)	0.72
T	73 (22.4)	57 (28.5)		1.38
DRD4 120 bp In/Del				
LL	116 (71.2)	75 (75)	0.82 (0.664)	1.22
LS	41 (25.1)	23 (23)		0.89
SS	6 (3.7)	2 (2)		0.53
L	273 (83.7)	173 (86.5)	0.73 (0.393)	1.24
S	53 (16.3)	27 (13.5)		0.8
DRD4 48 bp VNTR ***				
SS	114 (71.7)	67 (67)	1.77 (0.414)	0.8
LS	41 (25.8)	32 (32)		1.35
LL	4 (2.5)	1 (1)		0.39
S	269 (84.6)	166 (83)	0.23 (0.631)	0.89
L	49 (15.4)	34 (17)		1.12
HTR1A rs6295				
CC	42 (25.8)	23 (23)	2.14 (0.343)	0.86

CG	91 (55.8)	51 (51)		0.82
GG	30 (18.4)	26 (26)		1.56
C	175 (53.7)	97 (48.5)	1.33 (0.248)	0.81
G	151 (46.3)	103 (51.5)		1.23
HTR2A rs6311				
GG	63 (38.6)	43 (43)	2.61 (0.271)	1.2
AG	72 (44.2)	47 (47)		1.12
AA	28 (17.2)	10 (10)		0.54
G	198 (60.7)	133 (66.5)	1.77 (0.184)	1.28
A	128 (39.3)	67 (33.5)		0.78
HTR1B rs6296				
GG	81 (49.7)	57 (57)	2.31 (0.315)	1.34
CG	69 (42.3)	39 (39)		0.87
CC	13 (8)	4 (4)		0.48
G	231 (70.9)	153 (76.5)	2 (0.157)	1.34
C	95 (29.1)	47 (23.5)		0.75

The most significant results marked + $p < 0.1$, ++ $p < 0.05$

*—genotypes classified as LL, LS and SS (L≥10, <10)

**—genotypes classified as LL, LS and SS (L≥7, S<7)

*** — genotypes classified according to their expressional activity

Table 3. One-way ANOVA. Effects of gene polymorphisms on the depression and anxiety symptoms

Source of variability	SS	DF	F	P
Depression symptoms, Beck scale				
SLC6A3 40 bp VNTR*	455,9	2	2,0572	0,0824
Error	7 353,0	83		
Depression symptoms, Hamilton scale				
HTR1A	108,1	2	2,0277	0,1381
Error	2 211,6	83		
Situational anxiety				
DRD4 48 bp VNTR**	741,7	2	2,2842	0,1082
Error	13 475,7	83		
SLC6A4 44 bp VNTR + rs25531***	757,3	2	2,3348	0,1032
Error	13 460,2	83		
SLC6A4 44 bp VNTR	1 096,3	2	3,4675	**0,0358**
Error	13 121,1	83		
Personal anxiety				
COMT	422,0	2	2,1451	0,1236
Error	8 064,9	82		
SLC6A4 44 bp VNTR + rs25531***	454,7	2	2,3208	0,1046
Error	8 032,2	82		
SLC6A4 44 bp VNTR	441,7	2	2,2508	0,1118
HTR1B	850,7	2	4,5678	**0,0132**
Error	7 636,2	82		

Statistically significant results are in bold

DF – degrees of freedom, SS – sum of squares, F – F-test, P – probability

*—genotypes classified as LL, LS and SS (L≥10, <10)

**—genotypes classified as LL, LS and SS (L≥7, S<7)

*** — genotypes classified as high/high, high/low and low/low according to expressional activity

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