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Suicide ideators and attempters with schizophrenia –
the role of *5-HTTLPR, rs25531*, and *5-HTT VNTR Intron 2* variants

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Abstract

Aim. To examine the role of *5-HTTLPR*, *rs25531* and *5-HTT VNTR* Intron 2 variants in subjects with psychotic disorders manifesting suicide ideation and behaviour.

Methods. The study included 519 subsequently hospitalized subjects who were genotyped for *5-HTTLPR, rs25531* and *5-HTT VNTR In2* variants. Clinical assessments included structured psychiatric interview, sociodemographic characteristics, suicide ideation and behaviour (SIBQ), severity of psychopathology (PANSS) and depression (CDSS).

Results. Three subgroups were identified: suicide attempters (N=161), suicide ideators (N=174) and subjects who never reported suicide ideation or behaviour (comparative group, N=184). Major findings: 1) Suicide attempters scored highest on the CDSS, while no differences between the three clinical subgroups were detected in the PANSS scores; 2) Suicide attempters were more frequently the carriers of L_A allele, while subjects in the comparative group were more frequently the carriers of low expression *5-HTTLPR/5-HTT rs25531* haplotype SL_G,; 3) No difference was found between the three clinical groups in the *5-HTT VNTR In2* variants; 4) Subjects with *5-HTTLPR/5-HTT rs25531* intermediate expression haplotype (L_AL_G, SL_A) scored higher on the PANSS general psychopathology subscale; 5) There was no association between suicide attempt or ideation and *5-HTTLPR/In2* or *5-HTTLPR/rs25531/In2* haplotype distribution.

Conclusion. The suicide ideators, attempters and controls did not differ significantly in *5-HTTLPR* or *5-HTT VNTR In 2* variants, but *5-HTTLPR/5-HTT rs25531* haplotype might be a useful genetic marker in distinguishing these three clinical groups.

Key words: suicide ideation, suicide attempt, schizophrenia, serotonin transporter, polymorphism, *5-HTTLPR*, *rs25531*, haplotype

1. Introduction

Suicide ideation affects majority of people with schizophrenia, a large proportion of whom eventually attempt and complete suicide (De Hert et al., 2001). Today suicide is the leading cause of premature death in schizophrenia with approximately 5% completing suicide. (Inskip et al., 1998, Palmer et al., 2005).

Findings from the epidemiological genetic studies suggested a genetic origin to substantial familial susceptibility both to schizophrenia and suicide behavior (Shih et al., 2004; Brent and Mann, 2005). Disturbances in serotonin (5-hydroxitryptamine, 5-HT) transmission are the most frequently reported neurobiological substrates of suicide and the gene coding for the serotonin transporter (5-HTT) (GenBank accession no. X70697) has been a well-explored candidate for genetic studies on suicide. The *5-HTT* promoter harbors a functional 43 bp insertion/deletion polymorphism (*5-HTTLPR*), which yields a short and a long allele. The *S* allele is associated with a nearly 50% reduction of basal 5-HTT activity in vitro (Heils et al., 1996) and reduced in vivo 5-HTT binding in Caucasians (Praschak-Rieder et al., 2007). Other polymorphisms have been found in the proximity of the Ins/Del locus such as rs 25531, rs 25532, rs 2020933. The rs 25531, a polymorphism nearest to the *5-HTTLPR*, results in an A to G substitution and has been shown to modulate the effect of *5-HTTLPR* on transcription efficacy. The rs 25531 polymorphism is located immediately outside of the *5-HTTLPR* segment, so they can be considered as two independent polymorphisms (Bonvicini et al.,2010.). The G allele of rs25531 is in phase with the *5-HTTLPR* long allele and mitigates transcriptional efficacy more than does the *5-HTTLPR* short allele. Therefore, the modulation of the *5-HTTLPR* by rs25531 results in haplotypes with high (L_A) or low (L_G, S_A or S_G) transcriptional efficacy (Hu et al., 2006; Martin et al., 2007). A variable number tandem repeats (VNTR) polymorphism within intron 2 (*5-HTT VNTRin2*) contains 9, 10 ("*s*", short allele) or 12 copies ("*l*", long allele) of the 16 or 17-base pair repeats (Ogilvie and Harmer,1997). Although intron parts of a gene are not transcribed, both common repeats „*s"* and „*l*" alleles are purported to increase its transcription, with „*l"* allele having stronger enhancer-like properties than „*s*" allele (MacKenzie and Quinn, 1999).

A systematic review of twelve studies with biallelic *S/L* genotyping demonstrated a higher frequency of the low activity *S* allele in mood disorders and with suicidal behavior (Anguelova et al., 2003). Only one study examined the role of *5-HTTLPR/ rs25531* haplotype and *5-HTT VNTR* Intron 2 variants in suicide attempters with schizophrenia (De Luca et al., 2006). De Luca and colleagues genotyped 290 Caucasian subjects with schizophrenia and among these patients, 92 had a history of suicide attempt. No association with history of suicide attempt was found in the *5-HTTLPR* haplotype, but they found significant association with the intron 2 VNTR polymorphism, as well as association between suicide attempt and haplotype distribution.

The aim of this study is to examine the role of *5-HTTLPR, rs25531*, and *5-HTT VNTR Intron 2* variants in subjects with psychotic disorders who are manifesting suicide ideation and attempts, or none of these phenomena.

2. Patients and Methods

The study included 519 subsequently hospitalized patients who met the following criteria: (a) an ICD-10 diagnosis of psychotic disorder (ICD-10, 1990) and (b) age ≥18 years. They were recruited over the three years period (from 2007. to 2010.) from the Department of Psychiatry, University Hospital Center Zagreb. The study protocol was approved by the institutional ethical committee and all participants gave written informed consents.

2.1. Patients

Clinical assessments included structured psychiatric interview, collection of sociodemographic characteristics, severity of psychopathology (using the Positive and Negative Syndrome Scale, PANSS) (Kay et al., 1988), suicide ideation and behaviour (using „Suicide ideation and behaviour questionnaire“, SIBQ) (Marušič et al, 2007) and depression using the "Calgary Depression Scale for Schizophrenia" (CDSS; Addington, 1990). The latter consists of 14 questions, divided into two sections. The first section includes ten questions in yes/no format assessing suicidality as a dimension that arises from passive to active suicide thoughts, and suicide behaviour and attempt. The 10th question is intended for suicide attempters and assesses desire for attempting suicide with a 5 points Likert scale (from 1- "I never wanted to die" to 5 - "I was determined to die"). The last four questions are related to the family history of attempted and completed suicides.

Three subgroups were identified:
- suicide attempters (group 1), 161 subjects who were admitted after attempting suicide;
- suicide ideators (group 2), 174 subjects who were admitted for having serious and active suicide ideation; and
- comparative group (group 3), 184 subjects who were hospitalized due to worsening of symptoms that was not accompanied by suicide ideation or behaviour at the time of admission or ever before

Being aware that patients tend to suppress and distort this information, special attention was given to the assessment of suicide ideation and behaviour. Data gathered from the clinical interview and SIBQ were double-checked with the medical charts and family members. In 11 cases SIBQ results indicated that subjects attempted suicide, but interview revealed that they took large amount of anxiolytics to calm down and fall asleep, but not to kill themselves (N=6), or it was not possible to confirm suicide attempt (N=5). These subjects were excluded and analyses were performed on 519 subjects. All ratings were performed at the time of inclusion.

2.2. Genotyping

The DNA was extracted from whole blood with EDTA as described by Miller et al. (1998.). The *5-HTTLPR* genotyping consisted of two steps – first the "*S*" and "*L*" alleles were identified, and then the „*L*" allele was disentangled into "L_A" and "L_G". For "*S*" and "*L*" allele discrimination, we used a method described by Rauch et al. (2002) with our modification (Bozina et al. 2007). For the discrimination of the "L_A" and "L_G" structure, specific fluorogenic probes were used in real time polymerase chain reactions method based on a procedure

described by Hu et al. (2006) with our modification: the 25 μl reaction volume contained 15 ng of genomic DNA, 12.5 μl of Taqman master Mix (Applied Biosystems), and 5μl of Assay Mix that contained 2.5 μM of each primer, 3 μM of L_A probe, 2μM of L_G probe and 1 μl of 4% DMSO. Amplification was performed in a 7500 Real Time PCR System in 96 –well plates under the following PCR conditions: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 96°C for 15 sec and 62.5°C for 1min 30 sec. In order to acquire reliable controls and to check some questionable results, we performed genotype sequencing (3130 x 1 Genetic Analyzer, Applied Biosystems), also based on a previous study (Hu et al., 2006). Furthermore, in order to double check the real time PCR method, every tenth sample was genotyped according to the published PCR-RFLP methods (Lonsdorf et al., 2009). *5-HTT VNTR* Intron 2 analysis was performed according to a method described previously (Ito et al., 2002).

2.3. Data analysis

The statistical analysis was performed using SPSS for Windows version 15 (SPSS Inc., Chicago, IL, USA). Study subjects were described using means and standard deviations for interval measures and frequencies and percentages for categorical variables. For testing the normality of data distribution, Kolmogorov–Smirnov goodness of fit test was used (with $p>0.05$ considered as a non-significant departure from normality). Chi-square tests were used to compare the groups for different categorical variables, including allele and genotype frequencies. Analyses of variance were used for comparison of normally distributed interval variables and Kruskal-Wallis H test and Mann-Whitney U test were used in case of non-normal distributionsand. Statistical hypotheses were tested at alpha error rate set to 0.05, except in three post-hoc paired comparisons between groups where alpha error rate for each contrast was lowered to 0.017 according to the Bonferroni adjustment. The equivalence of the genotype distribution to the Hardy-Weinberg equilibrium was tested using the online software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). In addition, haplotype based analysis of the associations of genetic polymorphic variants of the SERT gene and PANSS scores and the suicide behaviour-groups were tested using program UNPHASED ver. 3.0.10 (Dudbridge, 2003) and Arlequin ver. 3.01 (Excoffier et al., 2006), respectively.

The power analysis was performed with the use of G* Power3 (release 3.1.3 for Windows XP, 2011) (Faul et al., 2007, 2009). This study had a power of 0.353 to detect a small effect size (0.10, alpha=0.017), 0.999 to detect a medium effect size (0.30, alpha=0.017) and 1.0 to detect a large effect size (0.50, alpha=0.017) in the biallelic genotype distribution and the expression-groups genotype distribution (N=519); and a power of 0.256 to detect a small effect size (0.10, alpha=0.017), 0.999 to detect a medium effect size (0.30, alpha=0.017) and 1.0 to detect a large effect size (0.50, alpha=0.017) in the *5-HTTLPR/rs25531* haplotypes distribution (N=519). For the allele frequency analysis (N=1038) the study had a power of 0.797 to detect a small effect size (0.10, alpha=0.017), 1.0 to detect a medium effect size (0.30, alpha=0.017), and 1.0 to detect a large effect size (0.50, alpha=0.017). Effect size conventions were determined according to the method of Buchner et al. (1997).

3. Results

3.1. Differences in general characteristics

The descriptive statistics for the entire sample (column *All*) and for each of the three clinical subgroups are shown in Table 1. The Kruskal-Wallis *H* test showed that subjects differed statistically significantly with regard to age. The post-hoc Mann-Whitney *U* test showed that suicide ideators were significantly younger than the comparative group ($p = 0.001$), whereas attempters did not differ in age from the comparative group nor from the ideators. The percentage of women differed between the groups, being the highest in the comparative group (adjusted standardized residual for group 1 was –3.1, for group 2 it was –1.8, and for group 3 it was 4.7). The comparative group also had the highest percentage of subjects with university degree (adjusted standardized residual for group 1 was –2.1, for group 2 it was –0.2, and for group 3 it was 2.3). The three groups did not differ significantly in terms of marital status, parenthood, type of psychotic disorder or family history. The percentage of subjects who were retired due to mental illness (*n*=40, i.e. 24.8% of group 1, adjusted standardized residual = 3.6) was statistically significantly higher among suicide attempters than among the ideators (*n*=25, 14.4%, adjusted standardized residual = –0.8) or the comparative group (*n*=19, 10.3%, adjusted standardized residual = –2.7), χ^2=13.98, df=2, p=0.001.

3.2 Symptomatic profile

The psychopathology was assessed using PANSS and results for the entire sample show that the mean positive subscale score was 26.67 (SD=7.11), negative subscale score 22.68 (SD=6.91), general psychopathology subscale score 52.78 (SD=8.82) and total PANSS score was 102.14 (SD=16.5). ANOVAs revealed no statistically significant difference among the three clinical subgroups when these scores were compared (for positive subscale F=2.93, df_1=2, df_2=512, p=0.054; for negative subscale F=0.29, df_1=2, df_2=512, p=0.747; for general psychopathology F=1.67, df_1=2, df_2=511, p=0.190). The suicide attempters scored highest on the Calgary Depression Scale for Schizophrenia (CDSS) – mean score was 10.4 (SD=6.5), while scores in the suicide ideation group and the comparative group were 8.7 (SD=6.4) and 4.3 (SD=5.1), respectively (Kruskal-Wallis χ^2=82.57, df=2, p<0.001).

3.3. Serotonin transporter

3.3.1. The *5-HTTLPR* biallelic structure

Looking at the biallelic structure of the *5-HTTLPR* polymorphism, the genotype frequencies in the entire sample (see the first column of Table 2) did not differ significantly from the Hardy-Weinberg equilibrium, χ^2=1.49, df=2, p=0.220. There was also no significant difference in the frequencies among the three subclinical groups (see the second, third and the fourth columns of Table 2 for the frequencies). The *LL* genotype was more frequent among suicide attempters, but the difference did not reach statistical significance (χ^2=4.953, df=2, p=0.084).

3.3.2. The *5-HTTLPR/rs25531* haplotype

The triallelic structure of the *5-HTTLPR* polymorphism indicated five haplotypes:, L_A/L_A, L_A/L_G, S/L_A S/L_G, S/S (see Table 2 for frequencies). Out of all "*L*" alleles, 6% were "L_G" alleles which would be considered as "*L*" alleles in the biallelic structure, but their function is much closer to the "*S*" alelles. The "L_A" allele was significantly more frequent among suicide attempters than in the other two groups (χ^2=6.487, df=2, p=0.039). Data are given in Table 2.

3.3.3. The *5-HTTLPR* expression groups

The next step was to group the *5-HTTLPR/rs25531* haplotypes according to their expression into "high expression" (L_A/L_A), "intermediate expression" (L_A/L_G and S/L_A) and "low expression" (S/L_G and S/S) genotypes. The frequencies were: 139 (0.27), 276 (0.53) and 104 (0.20), respectively. The three clinical groups differed with respect to the frequency of "low expression" genotypes which was more frequent in the comparative group (0.24) than in the other two groups (0.14 in group 1, 0.22 in group 2, χ^2=6.58, df=4, p=0.143), but it was not statistically significant.

3.3.4. The *5-HTT VNTR* Intron 2 variants

The *5-HTTVNTR-In2* frequencies for the entire sample (see the first column in Table 3) did not differ from the Hardy-Weinberg equilibrium (χ^2=1.31, df=2 p=0.253). There was no difference between the three clinical groups in the genotype frequencies or with regard to allele frequencies. Only four subjects were genotyped with 9 copies (all with suicide ideation). They were merged with subjects with 10 copies (9+10) in the further analysis. Data are given in Table 3.

3.3.5. Haplotype analysis - *5-HTTLPR/In2* and *5-HTTLPR/rs25531/In2*

The haplotype analysis of the 5-*HTTLPR/In2* and *5-HTTLPR/rs25531/In2* showed no significant differences between the three clinical groups. Data shown in Table 4.

3.4. Association between the examined genetic variants and subjects' psychopathology

We found statistically significant difference between the *5-HTTLPR* expression groups and PANSS general psychopathology subscale (Table 5). Subjects with the *5-HTTLPR* intermediate expression haplotypes (L_A/L_G, S/L_A) scored higher than both high-expression and low-expression group (F=3.89, df_1=2, df_2=513, p=0.021). Other comparisons did not reach the level of statistical significance. Furthermore, poor attention (PANSS item G11) was associated with both 5-*HTTLPR/In2* and *5-HTTLPR/rs25531/In2* haplotypes (χ^2=10.86, df=3, p=0.0125; χ^2=12.68, df=5, p=0.0265). The combination of "*S*" and "*s*" alleles was associated with lower scores on poor attention in 5-*HTTLPR/In2* analysis (AddVal -0.159, CI -0.385 - 0.067, specific test χ^2=4.059, p=0.043) and higher in *5-HTTLPR/rs25531/In2* analysis (AddVal 0.4047, CI -0.053 - 0.824, specific test χ^2=3.896, p=0.048). However, those associations did not remain statistically significant after Bonferroni corrections. The stereotyped thinking (N7 item) was associated with 5-*HTTLPR/In2* haplotype only with the borderline significance (χ^2=7.439, df=3,

p=0.057). The combination of "*S*" and "*s*" alleles was associated with higher scores on stereotyped thinking (AddVal 0.2571, CI 0.078 - 0.4364; specific test χ^2=6.567, p=0.0103).

4. Discussion

Our findings indicate that suicide ideators and attempters with psychotic disorders can be distinguished from the comparative group of subjects with psychotic disorders who do not express suicide ideation, nor have ever attempted suicide, on both clinical and biological level.

The three clinical groups did not differ significantly in *5-HTTLPR* – biallelic variant, however, looking at the *5-HTTLPR/rs25531* haplotype offers an entirely different perspective on the matter. Suicide attempters were more frequently carriers of „L_A" allele, while subjects in the comparative group were more frequently carriers of lower expression haplotypes (*S/*L_G, *S/S*). Only two studies reported higher frequencies of „*L*" allele among subjects who completed suicide. Du and colleagues analyzed a functional polymorphism in the 5' regulatory region of the *5-HT* transporter gene from postmortem brain samples of 24 depressed suicide victims and 31 control subjects and found significantly higher frequencies of „*L*" allele among depressed subjects (Du et al., 1999). This finding appears to be in accordance with the monoamine hypothesis of lower availability of serotonin as a key feature of depression. In the second study, 95 suicides and 120 healthy controls were compared and the first were characterized with higher frequency of „*L*" allele, but the difference did not reach the level of statistical significance (Fitch et al., 2000). However, as previously mentioned, most of the studies in the field detected higher frequency of „*S*" allele which was associated with anxiety (Lesch et al., 1996) and aggression (Sander et al., 1998). Results from our population (subjects of Croatian origin) suggest modest association between „*L*" and „*s*" allele in suicide cases (of unknown psychiatric history) in comparison to healthy population (Hranilovic et al., 2000). Altogether, there is still a small number of studies with *5-HTTLPR/rs25531* haplotype. One of the first in suicidology, published by Zalsman and colleagues, explored 191 subjects with mood disorder and 125 healthy volunteers, and reported that lower expressing transporter alleles, independently predicted greater depression severity and predicted greater severity of major depression with moderate to severe life events compared with the higher expressing L_A allele, but no associations with suicidal behavior was found (Zalsman et al., 2006). Similarly, we also showed that lower expressing alleles are not associated with sucide ideaion or attempts. As already mentioned, De Luca and colleagues found no association between the *5-HTTLPR/rs25531* haplotype and suicide attempts in schizophrenia (De Luca et al., 2006). However, they did find significant association with the intron 2 VNTR polymorphism. Our study is quite comparable in methodology to this one as we also used positive controls (and not healthy subjects), but results are different as we found no difference between the three clinical groups in the *5HT In2 VNTR* genotype and allele frequencies. **Interestingly, we found only four alleles with 9 copies within the intron 2 VNTR polymorphism which represents the frequency of 0.4%. In another study performed among the Croatian population, authors reported that this allele was present in 1% of the healthy controls, and 2% of suicide victims. This allele seems to be rare in other European Caucasian populations (healthy controls) as well – English 3%, French and german 1%, Scottish 1%, Spanish 2% (Cho et al., 2005).**

Suicide attempters in our study scored significantly higher on PANSS scale assessing general psychopathology. They also scored higher on depression. It could be argued that suicide behaviour in schizophrenia does not emerge from psychotic symptoms such as hallucinations and delusions, but rather from depressive mood (Cotton et al., 1985; Strosahl et al., 1992, Acosta et al., 2006), agitation (Hawton et al., 2005) and insight into illness (Kim et al., 2003). Depression and insight into illness together might lead to hopelessness which is an extremely dangerous suicide risk factor.

Suicide ideators were younger than other study participants. This is in accordance with previous findings that suicide risk changes with time and suicide ideation occurs mostly early in the course of illness (Palmer et al., 2005; Nordentoft et al., 2004). Suicide ideators and attempters were mostly men with lower level of education and retired due to illness. The two characteristics (male gender and education) are also well known suicide risk factors, and early retirement might be an indicator of poor social functioning and more significant psychopathological burden which might have a negative impact on quality of life and desire to continue living.

Study's main limitation is a cross-sectional design, and the strength, in our opinion, is in using positive controls, not healthy ones. From the point of someone who works in psychiatric clinical practice and deals exclusively with subjects with schizophrenia, it seems to be crucial (for both diagnostics and treatment) to find differences within this very population, not in comparison with healthy people. We believe that our results might add on attempts to integrate clinical and genetic data.

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Table 1 – Study sample

	All (N = 519)	Group 1 – SA (N = 161)	Group 2 – SI (N = 174)	Group 3 – Comparative (N = 184)	Test of differences among groups
Age M (SD)	33.7 (11.3)	33.3 (11.3)	31.7 (11.9)	35.9 (11.7)	Kruskal-Wallis χ^2=10.55, df=2, p=0.005
Age at illness onset M (SD)	28.2 (10.0)	28.0 (9.7)	27.6 (10.4)	29.1 (9.7)	Kruskal-Wallis χ^2=4.64, df=2, p=0.098
Type of psychosis N (%)					χ^2=8.92, df=10, p=0.540
paranoid schizophrenia	376 (72.4)	108 (67.1)	127 (73.0)	141 (76.6)	
disorganized schizophrenia	43 (8.3)	15 (9.3)	14 (8.0)	14 (7.6)	
residual schizophrenia	17 (3.3)	9 (5.6)	4 (2.3)	4 (2.2)	
other schizophrenia	14 (2.7)	3 (1.9)	7 (4.0)	4 (2.2)	
persistent delusional disorder	27 (5.2)	11 (6.8)	9 (5.2)	7 (3.8)	
schizoaffective disorder	42 (8.1)	15 (9.3)	13 (7.5)	14 (7.6)	
Family history N (%)					χ^2=5.55, df=4, p=0.235
psychosis	131 (25.2)	40 (25.3)	47 (27.6)	44 (24.9)	
other mental illness	141 (27.2)	49 (31.0)	52 (30.6)	40 (22.6)	
negative family history	233 (44.9)	69 (43.7)	71 (41.8)	93 (52.5)	
Women N (%)	278 (53.6)	71 (44.1)	84 (44.3)	123 (66.8)	χ^2=20.81, df=2, p<0.001
Education N (%)					χ^2=17.02, df=6, p=0.028[a]
no formal education	9 (1.7)	5 (3.1)	1 (0.6)	3 (1.6)	
primary school	54 (10.4)	22 (13.7)	20 (11.5)	12 (6.5)	
high school	328 (62.2)	106 (65.8)	105 (60.3)	117 (63.6)	
university student	53 (10.2)	13 (8)	23 (13.2)	17 (9.3)	
university degree	75 (14.4)	15 (9.3)	25 (14.4)	35 (19.02)	
Marital status N (%)					χ^2=4.51, df=4, p=0.607[b]
single	315 (60.7)	97 (60.2)	113 (64.9)	105 (57.1)	
married	162 (31.2)	49 (30.4)	53 (30.5)	60 (32.6)	
divorced	35 (6.7)	12 (7.5)	7 (4.02)	16 (8.7)	
widow	4 (0.7)	1 (0.6)	1 (0.5)	2 (1.08)	

Parenthood N (%)	192 (36.9)	60 (37.3)	57 (32.8)	75 (40.8)	χ^2=2.737, df=2, p=0.259
Employment status N (%)					χ^2=14.079, df=4, p=0.007
employed	171 (32.9)	43 (26.7)	60 (34.5)	68 (36.9)	
unemployed	251 (48.3)	73 (45.3)	85 (48.9)	93 (50.5)	
retired	94 (18.1)	43 (26.7)	29 (16.7)	22 (11.9)	

Note: SA stands for subjects who attempted suicide, SI stands for subjects who reported suicide ideation, Comparative stands for subjects without suicide attempt or ideation. [a]"No formal education" was merged with „primary school" due to theoretical frequencies less than 5. [b] Category „widow" was merged with „divorced" due to theoretical frequencies less than 5.

Table 2 - *5-HTTLPR/rs25531* allele, genotype and haplotype frequencies

SEROTONINE TRANSPORTER	All (N = 519)	Group 1 – SA (N = 161)	Group 2 – SI (N = 174)	Group 3 – Comparative (N = 184)	Test of differences among groups
5-HTTLPR					
Genotype					χ^2=5.012, df=4, p=0.286
L/S	250 (0.48)	82 (0.51)	82 (0.47)	86 (0.47)	
L/L	183 (0.35)	61 (0.38)	60 (0.35)	62 (0.34)	
S/S	86 (0.17)	18 (0.11)	32 (0.18)	36 (0.19)	
Alelle					χ^2=4.995, df=4, p= 0.082
L allele	616 (0.59)	204 (0.63)	202 (0.58)	210 (0.57)	
S allele	422 (0.41)	118 (0.37)	146 (0.42)	158 (0.43)	
5-HTTLPR/rs25531					
Haplotype					χ^2=7.534, df=8, p=0.480
S/L_A	232 (0.44)	78 (0.48)	76 (0.44)	78 (0.42)	
L_A/L_A	139 (0.27)	44 (0.27)	47 (0.27)	47 (0.26)	
L_A/L_G	44 (0.08)	17 (0.11)	13 (0.08)	14 (0.08)	
S/L_G	19 (0.04)	4 (0.02)	6 (0.03)	9 (0.05)	
S/S	85 (0.17)	18 (0.11)	32 (0.18)	36 (0.19)	
Allele					χ^2=6.487, df=2, p=0.039
L_A	554 (0.53)	183 (0.57)	183 (0.53)	186 (0.51)	
$S + L_G$	484 (0.47)	139 (0.43)	163 (0.47)	182 (0.49)	
***5-HTTLPR/rs25531* expression groups**					χ^2=6.58, df=4, p=0.143
high expression (L_A/L_A)	139 (0.27)	44 (0.27)	47 (0.27)	47 (0.26)	
intermediate expression (L_A/L_G, S/L_A)	276 (0.53)	95 (0.59)	89 (0.51)	92 (0.50)	
low expression (S/L_G, S/S)	104 (0.20)	22 (0.14)	38 (0.22)	45 (0.24)	

Table 3 - *5-HTTVNTR-In2* allele and genotype frequencies

SEROTONINE TRANSPORTER	All (N = 519)	Group 1 – SA (N = 161)	Group 2 – SI (N = 174)	Group 3 – Comparative (N = 184)	Results of statistical tests for comparison of the three groups
5-HTT Intron2 VNTR					
Allele					χ^2=2.694, df=2, p=0.260
12	648 (0.63)	193 (0.60)	229 (0.66)	226 (0.61)	
9+10 *	390 (0.37)	129 (0.40)	119 (0.34)	142 (0.39)	
Genotype					χ^2=5.037, df=4, p=0.284
12/9+10	232 (0.45)	67 (0.42)	75 (0.43)	90 (0.49)	
12/12	208 (0.40)	63 (0.39)	77 (0.44)	68 (0.37)	
9+10/9+10	79 (0.15)	31 (0.19)	22 (0.13)	26 (0.14)	

Note: SA stands for subjects who attempted suicide, SI for subjects who reported suicide ideation, Comparative stands for subjects without suicide attempt or ideation.

* Since only 4 alleles with 9 copies were genotyped (all in subjects with suicide ideation), they were grouped with alleles with 10 copies for the further analysis (9+10)

Table 4 - Haplotype analysis, *5-HTTLPR/In2* and *5-HTTLPR/rs25531/In2*

	Haplotype Frequencies		
Haplotype	Group 1 – SA (N = 161)	Group 2 – SI (N = 174)	Group 3 – Comparative (N = 184)
5-HTTLPR/In2*			
L, 12	0.194	0.193	0.187
L, 9+10	0.239	0.245	0.248
S, 12	0.292	0.31	0.243
S, 9+10	0.275	0.252	0.322
5-HTTLPR/rs25531/In2*			
$L_{A,}$ *12*	0.246	0.217	0.188
$L_{G,}$ *12*	0.038	0.028	0.025
S, 12	0.204	0.257	0.233
$L_{A,}$ *9+10*	0.319	0.314	0.319
$L_{G,}$ *9+10*	0.029	0.027	0.039
S, 9+10	0.164	0.157	0.203

Note: * Results of statistical tests for comparison of the three groups in 5-*HTTLPR/In2* and *5-HTTLPR/rs25531/In2* analysis p=n.s.

Table 5 – Means (and standard deviations in parentheses) for subjects' psychopathology and depression in different 5-HTTLPR/rs25531 expression groups

Scale	High expression (L_A/L_A) (N=139)	Intermediate expression (L_A/L_G, S/L_A) (N=276)	Low expression (S/L_G, S/S) (N=104)	Test of differences among groups
PANSS – Positive symptoms	26.14 (7.52)	26.95 (6.98)	26.68 (7.10)	$F=0.59$, $df_1=2$, $df_2=512$, $p=0.557$
PANSS – Negative symptoms	23.02 (6.81)	23.04 (6.81)	21.60 (7.22)	$F=1.59$, $df_1=2$, $df_2=512$, $p=0.204$
PANSS – General psychopathology	51.61 (9.31)	53.75 (8.50)	51.54 (8.88)	$F=3.89$, $df_1=2$, $df_2=511$, $p=0.021$
PANSS – Total score	100.77 (17.62)	103.74 (15.90)	99.83 (16.30)	$F=2.78$, $df_1=2$, $df_2=511$, $p=0.063$
CDSS	8.38 (6.73)	7.56 (6.46)	7.12 (6.46)	Kruskal-Wallis $\chi^2=2.38$, $df=2$, $p=0.304$

Note. PANSS stands for "Positive and Negative Symptoms Scale". CDS stands for "Calgary Depression Scale for Schizophrenia".