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Emotional Distress in Parents of Psychotic Patients is Modified by Serotonin Transporter Gene (5-HTTLPR) - Brain-Derived Neurotrophic Factor Gene Interactions

Vera Golimbet, Margarita Alfimova, Galina Korovaitseva, Lilia I. Abramova, and Vasily G. Kaled
Russian Academy of Medical Sciences (Russia)

Caregiving of a family member with psychotic disorder is considered among the most significant stressors and relatives of a sufferer experienced psychological and physical burden that may be the cause of neurotic states. There is growing evidence that sensitivity of individuals to depressogenic effects of stressful factor is moderated by genetic variants of serotonin transporter (SERT) and brain-derived neurotrophic factors (BDNF). We examined the association of the 5-HTTLPR SERT and Val66Met BDNF polymorphisms with signs of depression and anxiety measured with the Minnesota Multiphasic Personality Inventory (MMPI) in 235 unaffected parents of patients with major psychosis and 102 age-matched controls. A significant effect of the SERT-BDNF interaction on Depression and Psychasthenia scales was found in the group of parents, but not in the control group. Carriers of the Val/Val x SS variant scored higher as compared to other allelic combinations. The results obtained revealed that the SERT-BDNF interactions might moderate the level of anxiety and depression caused by caregiving status in parents of psychotic patients.

Keywords: distress, gene polymorphism, relatives, psychosis, serotonin, BDNF.

Cuidar a un miembro de la familia con un trastorno psicótico es considerado como uno de los estresores más significativos y los familiares de una persona que sufre dicho trastorno experimentan una carga psicológica y física que puede ser la causa de estados neuróticos. Hay cada vez más evidencia de que la sensibilidad de los individuos hacia los efectos depresógenos de los factores estresantes es moderada por variantes genéticas del transportador de la serotonina (SERT) y de factores neurotróficos derivados del cerebro (BDNF). Examinamos la asociación de los polimorfismos 5-HTTLPR SERT y Val66Met BDNF con los signos de la depresión y la ansiedad medidos con el Minnesota Multiphasic Personality Inventory (MMPI) en 235 familiares sanos de pacientes con psicosis mayor y 102 controles apareados en edad. Se encontró un efecto significativo de la interacción SERT-BDNF en las escalas de Depresión y Psicastenia en el grupo de familiares pero no en el grupo control. Los portadores de la variante Val/Val x SS puntuaban más alto en comparación con otras combinaciones alélicas. Los resultados obtenidos pusieron de manifiesto que las interacciones SERT-BDNF podrían moderar el nivel de ansiedad y depresión causado por el estatus de cuidador de los padres de pacientes psicóticos.

Palabras clave: malestar, polimorfismo genético, familiares, psicosis, serotonina, BDNF.
There is a vast body of evidence showing that psychosocial stressors people face during their lifetime exert a significant pathogenic influence on their psychological health. However, individuals vary substantially in their vulnerability to stressors. The causes of such variation are thought to relate, in part, to genetic factors. For the last years, evidence has been accumulating for a modifying role of specific molecular-genetic variants in emotional distress. The most promising candidate appears to be the serotonin transporter (SERT) gene. The SERT codes for a serotonin transporter, which selectively removes serotonin out of the synaptic cleft thus controlling the level of this neurotransmitter.

The transcriptional activity of the SERT is modulated by a repetitive element, SERT linked polymorphic region (5-HTTLPR), in the promoter, represented by the variants assigned to the short (S) and the long (L) alleles, which differentially regulate SERT expression, with the L/L genotype yielding in vitro higher levels of serotonin transporter function than the L/S or S/S genotypes. The 5-HTTLPR polymorphism has been reported for association with some personality traits, e.g. Neuroticism and Agreeableness, carriers of the S allele demonstrating more negative emotions, relating to depression and anxiety (Greenberg et al. 2000; Lesch et al. 1996; Munafo et al. 2003) A potential role of the S allele in the development of negative emotional state under chronic stressful condition in humans is supported by the findings of higher frequencies of the S allele in patients with depression (Lesch, 2001; Lotrich & Pollock, 2004) and posttraumatic stress disorder (Lee et al. 2005) as well as in suicidal attempters (Li & He. 2007) though some studies reported negative results (Caspi et al. 2003; Golimbet, Alfimova, Shcherbatykh, & Rogaev (2003; Mendlewicz et al. 2004). The findings of the last few years have demonstrated that the relation of the 5-HTTLPR polymorphism to the development of depression seemed more complicated, namely it modified an individual response to stressful life events that resulted in increasing of anxiety-related traits or development of diagnosable depression. Caspi et al. (2003) have revealed that, under stressful life events, the carriers of the S allele were more vulnerable to depression than those with the LL genotype. This finding has been confirmed in many other studies that examined the effect of different psychosocial stressors on the vulnerability of individuals to depression (Alfimova et al. 2007; Brummet et al. 2008; Cervilla et al. 2007; Grabe et al. 2005; Kaufman et al. 2004; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Sjoberg et al. 2005; Taylor et al. 2006; Wilhelm et al. 2006) However some studies did not find the interaction effect between the 5-HTTLPR polymorphism and stressful life events (Gillespie, Whitfield, Williams, Heath, & Martin 2005; Scheid et al. 2007).

Further research in this field has demonstrated a more complex gene-environmental effect on the vulnerability to depression. It has been shown that the 5-HTTLPR gene can moderate the development of depressive symptoms under the environmental stress in interaction with the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene (Kaufman et al. 2006; Kim et al. 2007). BDNF promotes the development and function of serotonergic neurons and mood-related effects. As it has been shown earlier, this neurotrophin modulates serotonin transporter function in vitro (Mossner et al. 2000). Changes in BDNF expression cause anxiety-like behaviors in serotonin transporter knockout mice (Ren-Patterson et al., 2005). The BDNF gene bears a frequent single nucleotide polymorphism at nucleotide 196 G/A, located at the 5’proBDNF-sequence, which produces an amino acid substitution (valine/methionine) at codon 66 (Val66Met). A Met allele makes the gene activity less sensitive to stimuli by inducing BDNF secretion than the Val/Val genotype (Chen et al. 2004). The Val66Met polymorphism has been tested for association with some psychiatric conditions and psychological traits. The data regarding which allele is related to depression are controversial. In several studies, a Val allele was associated with mood disorders (Lohoff et al. 2005; Neves-Pereira et al 2002; Ribeiro et al. 2007; Sklar et al 2002; Strauss et al. 2005), higher levels of depressive symptoms (Duncan, Hutchison, Carey, & Craighead, 2008) and higher anxiety-related personality traits (Lang et al. 2005; Sen et al. 2003) in European populations. In Asian samples, the higher frequency of a Met allele was reported in patients with depression compared to controls (Hwang et al. 2006; Kim et al. 2008). However some studies reported no association between the Val66Met polymorphism and depression-related phenotypes (Surtees et al. 2007). A meta-analysis of 8 association studies also revealed no effect of any variant on the development of mood disorders (Gratacos et al. 2007). It should be mentioned that both European and Asian samples were included in the analysis.

Recently, it has been shown that the Val66Met and 5-HTTLPR polymorphisms exert an interaction effect on the vulnerability to depression scores (Kaufman et al. 2006; Kim et al. 2007; Wichers et al. 2008; Aguilera et al. 2009). Carriers of a Met allele and SS genotype had the highest risk of depression in the presence of stressful life events. At the same time, a recent neuroimaging study has demonstrated a protective role of the Met allele against 5-HTTLPR S allele-induced effects on a brain circuitry on the neural systems level (Pezawas et al. 2008).

Severe disease of a family member is considered among the most significant stressors and relatives of sufferer experienced psychological and physical burden that may be the cause of neurotic states. A number of studies revealed that parenting a son or daughter with schizophrenia frequently results in considerable emotional distress, with loss, fear and self-blame for the disease (Angermeyer, Liebelt, & Matschinger, 2001; Brown & Wiste, 1998; Ferriter & Huband, 2003; Salleh et al., 1994). According to our data mild depressive and anxiety symptoms may be found in about 16% of relatives of schizophrenia (Alfimova et al. 2007) It has been noticed that comparing to family members of patients with physical diseases relatives of psychotic patients have
the same level of so-called objective burden relating to the patient’s symptoms, behavior and socio-demographic characteristics, as well as the changes in household routine, family or social relations, work, physical health etc, but higher subjective burden, i.e. burden relating to mental health and distress among family members (Magliao et al. 2005). Recently, Brummett et al. (2008) reported that the effect of the 5-HTTLPR polymorphism on symptoms of depression was moderated by caregiving status in the group of relatives of patients with Alzheimer’s disease.

To extend this finding, we examined the association of the 5-HTTLPR and Val66Met BDNF polymorphisms with signs of depression and anxiety in unaffected parents of patients with major psychosis who were caregivers of their children. We hypothesized that 5-HTTLPR and Val66Met polymorphisms might moderate emotional distress in parents of psychotic patients, but not in comparison subjects without the burden of disease of a family member, separately or in interaction with each other and that under stressful conditions carriers of the SS 5-HTTLPR genotype and the Val/Val BDNF genotype would be more vulnerable to distress than their counterparts with other genotypes. The latter prediction is based on the fact that most studies reported the protective effect of the Met allele for anxiety- and depressive-related genotypes. Moreover, Pezawas et al. (2008) argue for the protective role of this allele in the risk architecture of depression through its biologic interaction with the 5-HTTLPR because the Met allele is less sensitive to stimuli that induce BDNF secretion than are Val/Val genotypes. The authors suggest that in the brain this allele may lessen the developmental impact of the 5-HTTLPR S allele on amygdala–rAC circuitry.

To elucidate gene x environment interactions, we tested the following groups which were at different risk of developing signs of anxiety and depression:
(a) high-genetic-risk x high-environmental-risk group (relatives of psychotic patients with the SS and Val/Val genotypes);
(b) low-genetic-risk x high environmental risk (relatives of psychotic patients with other 5-HTTLPR and BDNF genotypes);
(c) high-genetic-risk x low-environmental-risk group (controls with the SS and Val/Val genotypes) and
(d) low-genetic-risk x low- environmental-risk group (controls with other 5-HTTLPR and BDNF genotypes).

Methods

Participants

A sample has been selected from parents of patients admitted to clinical departments of Mental Health Research Center, Russian Academy of Medical Sciences. The parents who agreed to participate in the study were asked to meet with a psychiatrist (Dr. Abramova and Dr. Kaleda) and to complete the Minnesota Multiphasic Personality Inventory (MMPI). Subjects with a current or past episodes of psychotic (schizophrenia, schizoaffective disorder, schizophreniform disorder or atypical psychosis) or affective disorder (bipolar disorder, major depression, cyclothymia) and those who have T-scores more than 70 on scales L or K or more than 80 on scale F were not included in the study (see explanations in section Assessment Instruments). A final sample consisted of 235 parents of patients with psychosis, 109 men, 126 women, aged from 38 to 66 years, mean age 51.0±7.8 years. Patients, 73 men, 48 women, mean age 23.7±6.5 years, mean illness duration 3.3±3.4 years, were diagnosed with DSM-IV categories of schizophrenia (73 patients), schizoaffective psychosis (34) or affective disorder with psychotic symptoms (14). In most of patients (56), illness duration was less than one year. Patients were not included in the present study. Clinical data on the patient sample were presented only as the additional description of the parent sample.

A control group was recruited from the community by word of mouth. A volunteer who agreed to participate in the study was asked if he or she had ever visited a psychiatrist. Those who answered “no” were suggested to complete the MMPI and a stressful-life events scale (SLE). Individuals whose test was invalid (T-scores more than 70 on scales L or K or more than 80 on scale F) and who answered “yes” on the SLE item “Chronic disease of a first-degree relative” were excluded. A final control sample consisted of 102 genetically unrelated subjects, 28 male, 74 female, aged from 37 to 73 years, mean age 47.4±8.6 years. The groups of parents and controls were matched by age but not by sex. The percentage of females in the latter group was higher (53,6% vs 72,5%). All participants were ethnic Russians from Moscow and the surrounding region. After being informed about the goals of the investigation, each subject gave a written consent for participation in the study. The study was approved by the Ethics Committee of Mental Health Research Center.

Assessment instruments

All subjects were suggested to complete the Minnesota Multiphasic Personality Inventory (MMPI). An MMPI version used in the study included 377 items. The MMPI measures various psychopathologies, including emotional states of depression and anxiety, and has been widely administered in studies measuring vulnerability to stress in clinical and non-clinical populations (Alessi, White, Ray, & Stewart, 2001; Franklin, Repasky, Thompson, Shelton, & Uddo, 2002; Scheibe Bagby, Miller, & Dorian, 2001; Stilley, Miller, & Tarter, 1997). The MMPI has a number of true/false items, which are scored for 10 basic clinical scales (Hypochondriasis (Hs), Depression (D), Hysteria (Hy), Psychopathic deviate (Pd), Masculinity-Femininity...
(Mf), Paranoia (Pa), Psychasthenia (Pt), Schizophrenia (Sc), Hypomania (Ma) and Social introversion (Si)) and 3 validity scales (L, F, K)). The L Scale (lie scale) was developed to detect attempts by patients to present themselves in a favorable light. The F Scale is used to detect attempts at “faking good” or “faking bad.” This scale asks questions designed to determine if test-takers are contradicting themselves in their responses. The K Scale or “defensiveness scale” is a more effective and less obvious way of detecting attempts to present oneself in the best possible way. Cut-off point for normal variability on clinical scales is estimated as 70 T-scores. MMPI-profiles with L or K > 70 and/or F>80 are considered to be invalid. Vulnerability to stress emerges in elevation on the classical neurotic triad - scales Hypochondriasis (Hs), Depression (D), Hysteria (Hy) - and moderate elevation on Psychasthenia (Pt) scale. It should be noted that MMPI is commonly regarded as a personality inventory.

To be sure that we measure distress caused by psychological burden of having a schizophrenic child rather than personality traits, we developed a questionnaire to assess the current balance between the capacity to experience happiness/pleasure vs negative emotions which are the most frequent replies to the disease of a loved one: grief/depression, fear, embarrassment, resentment, anger and guilt. Responses were on a Likert-type scale from one to four asking respondents to assess how accurate the word described them during the child’s illness. In a subgroup of 49 relatives the composite measure of this questionnaire correlated with MMPI Depression and Psychasthenia ($p=.04$ and $p=.02$, respectively).

**Molecular-genetic methods**

DNA extraction, PCR performance and electrophoresis were carried out as described elsewhere for the 5-HTTLPR polymorphism (Lesch et al. 1996) and for the BDNF Val66Met polymorphism (Neves-Pereira et al. 2002). Genotype distribution for both polymorphisms was in accordance to the Hardy-Weinberg equilibrium in both groups. The frequency of 5-HTTLPR alleles was 54.5% for an allele L and 45.5% for an allele S and that of BDNF alleles was 81.7% for a Val allele and 18.3% for a Met allele in the control group. The frequency distribution in the group of parents was as follows: 69.6% for an allele L and 30.4% for an allele S; 79.5% for a Val allele and 20.5% for a Met allele. These values were similar to previously reported frequencies for Caucasians: 57% for the L allele and 43% for the S allele (Lesch and Mossner, 1998) and 78-84% for the Val allele and 16-22% for the Met allele (Gratacos et al. 2007). The genotype frequencies were as follows: LL – 42.1%; LS - 40.2%; SS - 17.8%; Val/Val – 68.6%; Val/Met – 29.4% and Met/Met – 2.0% in the control group and LL – 37.9%; LS - 40.4%; SS - 21.7%; Val/Val – 69.4%; Val/Met – 28.9% and Met/Met – 1.7% in the group of parents. No differences between parents and controls were found in the distribution of 5-HTTLPR alleles ($\chi^2=0.2; df=1; p=.78$) and genotypes ($\chi^2=1.0; df=2; p=.59$) and Val66Met alleles ($\chi^2=0.4; df=1; p=.53$) and genotypes ($\chi^2=0.8; df=2; p=.67$).

In further analysis, we used combinations (diplotypes) Met/Val x LL, Met/Val x LS, Met/Val x SS, Val/Val x LL, Val/Val x LS and Val/Val x SS. Combinations of 5-HTTLPR genotypes with the Met/Met genotype have been discarded from the analysis because of the very low frequency of the Met/Met genotype.

**Data analysis**

Multivariate analysis of covariance (MANCOVA) was employed to test associations between 5-HTTLPR and BDNF and personality factors. In the MANCOVA model, 9 personality factors (Hs, D, Hy, Pt, Sc, MA and Si) which were correlated served as one composite response variable; genotypes were independent variables and sex and age were used as covariates to exclude their potential confounding effects on associations. This approach was used to adjust for multiple testing. MANCOVA was followed by post-hoc Fisher (LSD) tests in order to specify risk genotypes for every personality factor. All procedures were performed using statistical software “Statistics for Windows 6.0”. Power calculation was conducted using the G-Power analysis program. The power of the study was estimated as 80%.

**Results**

At the first step of the study, we compared mean MMPI profiles for parents of psychotic patients and controls. The data are presented on Fig.1. Scores for both profiles were within normal limits (i.e. T-score< 70). MANCOVA with group status (parents or controls), sex and age as predictors of scores on MMPI scales revealed that group status contributed to the composite response variable ($F=3.3; df=9, p<.01$). Marginal effects of sex and age on the dependent variable ($F=1.85; df=9, p=.06$ and $F=1.81; df=9, p=.07$, respectively) were observed. Post hoc tests revealed effects of group status for scales Pt ($p<.01$) and Ma ($p<.01$), controls scoring higher than parents. No between-group differences on scales that Hs, D, Hy and Pt were observed.

At the second stage of the analysis, we studied the effect of SERT and BDNF genotypes on MMPI scores in parents and controls. MMPI scores by single genotypes and their combinations are shown in Tables 1-4. There were no significant differences between controls or parents with different SERT or BDNF genotypes. However a significant interaction effect of 5-HTTLPR and Val66Met polymorphisms on the composite response variable ($F=1.6, df=18, p=.05$) was observed in the group of parents. No
interaction effect of both polymorphisms was found in the control group ($F=1.0$, $df=18$, $p=.39$). Univariate AVCOVs for each personality factor showed the effect of BDNF x 5-HTTLPR interactions for scales D ($F=3.1$, $df=2$, $p=.05$) and Pt ($F=3.3$, $df=2$, $p=.04$). The marginal effects were found for scales Hs ($F=2.9$, $df=2$, $p=.05$) and Si ($F=2.7$, $df=2$, $p=.07$). No effect was seen for scales Hy ($F=0.7$, $p=.47$); Pd ($F=1.6$, $p=.21$), Pa ($F=1.1$, $p=.35$), Sc ($F=1.8$, $p=.16$) and Ma ($F=0.1$, $df=2$, $p=.90$). Post hoc Fisher (LSD) test revealed that carriers of the Val/Val x SS variant had the

![Figure 1. Average MMPI profiles for parents of psychotic patients and controls. Axis X – MMPI scales, axis Y – T-scores. No differences were found between parents and controls on MMPI scales.](image)

Table 1

Scores on MMPI Scales (mean and standard deviation) in controls for 5-HTTLPR and Val66Met genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MMPI scales</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hs</td>
</tr>
<tr>
<td>LL (5-HTTLPR) n=43</td>
<td>55.9</td>
</tr>
<tr>
<td>LS (5-HTTLPR) n=41</td>
<td>55.7</td>
</tr>
<tr>
<td>SS (5-HTTLPR) n=18</td>
<td>52.8</td>
</tr>
<tr>
<td>Val/Val n=70</td>
<td>55.6</td>
</tr>
<tr>
<td>Val/Met n=30</td>
<td>54.8</td>
</tr>
<tr>
<td>Met/Met n=2</td>
<td>48.0</td>
</tr>
</tbody>
</table>
highest scores for these scales (Table 4) as compared to other allelic combinations.

We also compared scores on MMPI scales for the groups with high/low genetic and environmental risk as specified in the Introduction. MANCOVA revealed the significant effect of group status on MMPI scores ($F = 1.7$, $df = 30, p = .01$). Post hoc Fisher (LSD) test showed that the group with high-genetic-risk x high-environmental-risk, which comprised the relatives of psychotic patients with the SS and Val/Val genotypes, had significantly higher scores on scales Hy ($p = .03$), D ($p = .03$) and Pt ($p = .03$). The MMPI profiles for groups with different combinations of genetic and environmental risks are presented on Fig.2.

### Table 2
Scores on MMPI Scales (mean and standard deviation) in parents for 5-HTTLPR and Val66Met genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MMPI scales</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hs</td>
</tr>
<tr>
<td>LL (5-HTTLPR)</td>
<td>59.1, 56.1, 57.0, 54.6, 51.3, 51.7, 54.5, 52.6, 53.4, 51.6</td>
</tr>
<tr>
<td>n=89</td>
<td>12.2, 12.6, 10.1, 9.4, 9.8, 10.7, 11.0, 10.8, 10.6, 10.5</td>
</tr>
<tr>
<td>LS (5-HTTLPR)</td>
<td>56.8, 56.2, 55.0, 54.2, 51.7, 50.7, 53.7, 51.9, 53.4, 52.0</td>
</tr>
<tr>
<td>n=107</td>
<td>11.0, 12.3, 10.8, 10.7, 11.0, 10.9, 10.9, 12.3, 9.5, 11.0</td>
</tr>
<tr>
<td>SS (5-HTTLPR)</td>
<td>61.8, 58.6, 57.3, 54.1, 52.6, 53.5, 56.9, 55.5, 53.0, 53.3</td>
</tr>
<tr>
<td>n=39</td>
<td>15.7, 12.6, 11.4, 11.3, 12.4, 14.6, 13.6, 16.2, 8.5, 10.5</td>
</tr>
<tr>
<td>Val/Val</td>
<td>59.3, 56.0, 57.0, 54.0, 53.7, 52.3, 54.9, 53.7, 54.6, 51.7</td>
</tr>
<tr>
<td>n=163</td>
<td>14.5, 12.8, 11.6, 10.5, 10.0, 11.5, 11.2, 11.4, 9.6, 11.4</td>
</tr>
<tr>
<td>Val/Met</td>
<td>57.5, 56.1, 54.1, 52.5, 46.9, 50.5, 53.8, 51.4, 53.1, 52.5</td>
</tr>
<tr>
<td>n=68</td>
<td>9.7, 12.3, 9.9, 9.2, 11.2, 10.9, 12.4, 12.2, 9.1, 9.7</td>
</tr>
<tr>
<td>Met/Met</td>
<td>51.4, 49.8, 56.8, 54.8, 53.4, 53.2, 52.6, 59.4, 49.8, 47.8</td>
</tr>
<tr>
<td>n=5</td>
<td>8.6, 8.0, 11.8, 10.3, 12.3, 23.0, 9.7, 22.5, 10.3, 7.6</td>
</tr>
</tbody>
</table>

The combinations of 5-HTTLPR genotypes with the Met/Met genotype have been discarded from the analysis because of the very low frequency of the Met/Met genotype.

### Table 3
Scores on MMPI Scales (mean and standard deviation) in Controls with Different Val66Met × 5-HTTLPR Diplotypes

<table>
<thead>
<tr>
<th>Val66Met × 5-HTTLPR diplotype</th>
<th>MMPI scales</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hs</td>
</tr>
<tr>
<td>Met/Val × LL</td>
<td>53.9, 58.8, 52.6, 59.1, 53.2, 61.0, 54.0, 56.5, 57.9, 55.0</td>
</tr>
<tr>
<td>n=15</td>
<td>16.9, 9.5, 9.9, 10.0, 8.8, 12.6, 8.9, 15.8, 18.2, 9.2</td>
</tr>
<tr>
<td>Met/Val × LS</td>
<td>53.7, 55.8, 52.5, 55.7, 48.0, 52.6, 53.7, 50.6, 52.6, 55.5</td>
</tr>
<tr>
<td>n=13</td>
<td>9.5, 7.5, 10.1, 7.8, 8.2, 11.8, 11.4, 17.6, 10.6, 11.1</td>
</tr>
<tr>
<td>Met/Val × SS</td>
<td>56.2, 56.5, 51.7, 50.7, 44.0, 54.5, 56.2, 50.8, 56.0, 52.8</td>
</tr>
<tr>
<td>n=8</td>
<td>16.5, 7.5, 16.1, 14.9, 14.2, 8.8, 14.0, 10.9, 9.6, 7.9</td>
</tr>
<tr>
<td>Val/Val × LL</td>
<td>57.0, 59.9, 52.2, 51.4, 48.3, 57.2, 59.3, 54.6, 58.1, 51.6</td>
</tr>
<tr>
<td>n=25</td>
<td>14.5, 10.9, 12.3, 10.2, 12.7, 8.2, 12.7, 10.8, 10.2, 9.3</td>
</tr>
<tr>
<td>Val/Val × LS</td>
<td>55.7, 51.6, 57.1, 54.9, 48.6, 56.4, 56.9, 53.4, 55.9, 52.4</td>
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<tr>
<td>n=28</td>
<td>14.1, 10.3, 11.7, 11.9, 11.3, 14.0, 12.1, 17.7, 11.6, 11.9</td>
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<tr>
<td>Val/Val × SS</td>
<td>52.4, 54.4, 50.3, 49.5, 55.4, 55.8, 59.7, 59.7, 51.6, 56.1</td>
</tr>
<tr>
<td>n=13</td>
<td>5.6, 8.8, 7.5, 7.5, 7.7, 22.1, 8.9, 19.2, 18.6, 11.7</td>
</tr>
</tbody>
</table>
Table 4
Scores on MMPI Scales (mean and standard deviation) in Parents of Psychotic Patients with Different Val66Met × 5-HTTLPR Diplotypes

<table>
<thead>
<tr>
<th>Val66Met × 5-HTTLPR diplotype</th>
<th>MMPI scales</th>
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<tbody>
<tr>
<td></td>
<td>Hs</td>
<td>D</td>
<td>Hy</td>
<td>Pd</td>
<td>Mf</td>
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<td>Pt</td>
<td>Sc</td>
<td>Ma</td>
<td>Si</td>
<td></td>
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<tr>
<td>Met/Val × LL</td>
<td>59,8</td>
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<tr>
<td>n=27</td>
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The combinations of 5-HTTLPR genotypes with the Met/Met genotype have been discarded from the analysis because of the very low frequency of the Met/Met genotype.

* scores for the Val/Val × SS diplotype were significantly higher comparing to the Val/Val × LS (p<.01), Val/Met × LS (p=.04) and Val /Met × SS (p=.04) on scale Hs.

** scores for the Val/Val × SS diplotype were significantly higher comparing to the Val/Val × LS (p<.01) and Val /Met × SS (p=.03) on scale D. A trend towards the level of significance (p=.08) was found for the Val/Val × LL and Val/Met × LL diplotypes.

# scores for the Val/Val × SS diplotype were significantly higher comparing to the Val/Val × LS (p=.003), Val/Met × LL (p=.04) and Val/Met × SS (p=.02) on scale Pt. A trend towards the level of significance (p=.06) was found for the Val/Val × LL and Val/Met × LS diplotypes.

Figure 2. MMPI profiles of groups with different levels of genetic and environmental risks for the development of emotional distress under the stressful life event (a mental disease of a child).
High-genetic-risk x high-environmental-risk group – parents of psychotic patients with the SS and Val/Val genotypes.
Low-genetic-risk x high-environmental-risk group – parents of psychotic patients with other 5-HTTLPR and BDNF genotypes).
High-genetic-risk x low-environmental-risk group – controls with the SS and Val/Val genotypes Low-genetic-risk x low-environmental-risk group - controls with other 5-HTTLPR and BDNF genotypes.
Axis X – MMPI scales, axis Y – T-scores.
Discussion

In accordance to our hypothesis, we have tested 4 groups of subjects at different risk of developing symptoms of anxiety and depression with the following gene x environmental combinations: (a) high-genetic-risk x high-environmental-risk group (relatives of psychotic patients with the SS x Val/Val diplotype); (b) low-genetic-risk x high environmental risk (relatives of psychotic patients with other 5-HTTLPR and BDNF genotypes); (c) high-genetic-risk x low-environmental-risk group (controls with the SS x Val/Val diplotype) and (d) low-genetic-risk x low-environmental-risk group (controls with other 5-HTTLPR and BDNF genotypes). As a result, we have revealed that the first group has significantly higher scores on scales Hy, D and Pt. Scale D reflects anxiety and depression symptoms and scale Pt taps obsessive-compulsive features, as well as abnormal fears, self-criticism, difficulties in concentration, and guilt feelings. Therefore, this finding has confirmed our hypothesis that response to chronic stress related to the burden of having the affected child can be mediated by SERT (5-HTTLPR) and BDNF (Val66Met) gene interactions. In our study, parents with the Val/Val x SS genotype combination have the highest levels of depressive and anxiety signs, i.e., are more vulnerable to emotional distress.

The results of the study are in line with previous reports that SERT x BDNF interactions convey vulnerability to depression (Aguilera et al. 2009; Kaufman et al. 2006; Kim et al. 2007; Wichers et al. 2008). However these studies revealed the highest level of depression symptoms for individuals with the SS 5-HTTLPR and the Met BDNF allele that contrasted to our results. The discrepancy may be explained by the differences in a nature of stressful factor (current or past stressors), racial characteristics of the sample and outcome measures. For instance, studies of Kaufman et al (2006) and Wichers et al. (2008) considered childhood adversities, while we studied a current stressor (a psychiatric disease of a family member). The study of Kim et al. (2007) investigated the effect of current stressful life events but the distribution of SERT x BDNF genotypes in the Asian population of Koreans significantly differed from those in the European population of Russians. It should be noted also that the association of the Val/Val genotype with anxiety-related personality traits and mood disorders was reported in many studies (Lang et al. 2005; Lohoff et al. 2005; Neves-Pereira et al. 2002; Sen et al. 2003; Sklar et al. 2002; Strauss et al. 2005) conducted in Caucasians. Moreover, our finding is supported by the data of Pezawas et al. (2008) who reported the decrease of structural covariance between amygdala and anterior cingulate in the brain of carriers with the variants containing a S allele and the Val/Val genotype that might reflect abnormal “wiring” conferring a risk of depression.

Also our study extends the results of an earlier publication (Brummett et al. 2008) which demonstrated that the caregiving experience might serve as a mediator in the path between the 5-HTTLPR genotype and signs of depression and anxiety. We show that the SS variant of this polymorphic marker may modify the development of emotional distress in parents of a patient with psychotic disorder.

It should be mentioned that our study has a limitation concerning the specifics of the group used for G x E study. It is well known that relatives of schizophrenic patients can share some features predisposing to psychosis. Moreover, a number of MMPI scales have been implicated as vulnerability indicators for schizophrenia in family studies (Moldin, Gottesman, & Erlenmeyer-Kimling, 1987). Thus, the possibility left that results of our study would just reveal the association between genetic polymorphism and some intermediate phenotypes emerging in relatives. To minimize this possibility, we included in the study only psychiatrically-well parents of psychotic patients who had been examined by the psychiatrist. Mean scores for all MMPI scales in this group were within normal limits. The average MMPI profile of parents did not feature elevations on scales which were thought to be characteristic of high-risk individuals for psychosis, e.g., scales Pd and Sc. In our study, the genetic variant predicts elevations on scales D and Pt, i.e. the scales related to anxiety and depressive symptoms. Vulnerability to stress usually emerges in elevations on the classical neurotic triad (scales Hs, D, Hy) and moderate elevation on scale Pt (Beresin, Miroshnikov, & Rojanez, 1976). Elevations on all these scales or some of them have been observed in many studies which used the MMPI as an instrument for measuring response to stress in clinical and non-clinical populations (Alessi et al. 2001; Franklin et al. 2002; Scheibe et al. 2001; Stilley et al. 1997). Interestingly, the recent study of MMPI profiles in parents of children with schizophrenia and attention deficit hyperactivity disorder (ADHD) reported elevations on neurotic triad scales in both groups, while the elevation on scale Sc was seen only in patients of schizophrenics (Subotnik et al. 2005). The authors argue that higher scores on scale Sc indicate the genetic predisposition to schizophrenia and elevations on neurotic triad scales reflect distress caused by psychological burden of having a child with a chronic behavioral disorder.

Also, the results should be treated with caution, because we do not use a multiple testing correction. Therefore, the possibility of Type I error could not be excluded.

It has been noticed that, comparing to family members of patients with physical diseases, relatives of psychotic patients have the same level of so-called objective burden, which concerns the patient’s symptoms, behavior and socio-demographic characteristics, as well as the changes in household routine, family or social relations, work, physical health etc, but higher subjective burden, i.e. burden relating to mental health and distress among family members (Magliano et al. 2005). An importance of developing instruments allowing to distinguish between “objective” burden...
and “subjective” burden was articulated earlier (Reine, Lancon, Simeoni, Duplan, & Auquier, 2003). A molecular-genetic variant likely plays a modifying role between objective and subjective burden and may be taken into account in elaboration of psychosocial programs aimed at helping informal caregivers to cope with the stressful situation.

In conclusion, the study reveals that SERT - BDNF gene interactions contribute to emotional distress experienced by parents of psychotic patients. This finding can be considered as an additional evidence for the modifying role of genetic factors in individual sensitivity to stressful life events.

References


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