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ORIGINAL RESEARCH ARTICLE

Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden

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Previous studies have yielded conflicting results as to the putative role of the functional polymorphism of the promoter region of the serotonin transporter gene (SLC6A4) in the etiology of anxiety-related traits and depressive disorders. Recently, a significant gene–environment interaction was found between life stressors, the short allele of the SLC6A4 polymorphism and depression. The aim of the present study was to investigate if such a gene–environment interaction could be replicated within a different population with a different risk structure. A total of 1005 subjects from a general population sample (Study of Health in Pomerania) were genotyped. Mental and physical distress were assessed on 38 items of the modified complaint scale (BL-38). The interaction between the SLC6A4 genotype, social stressors and chronic diseases with regard to the BL-38 score was evaluated by ANOVA. There was no independent association of genotype with mental and physical distress. However, significant interactions between genotype, unemployment and chronic diseases ($F = 6.6$; $df = 3, 671$; $P < 0.001$) were found in females but not in males. The genotype explained 2% of the total variance of the BL-38 score and 9.1% of the explained variance. The results partly confirm previous findings of a significant gene–environment interaction of the short allele, indicating a higher mental vulnerability to social stressors and chronic diseases. The relevance of this finding is sustained by the fact that the sample characteristics and the risk structure were highly different from previous studies.
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The SLC6A4 polymorphism of the promoter region of the coding gene of the serotonin transporter consists of a 44-bp insertion/deletion that is characterized by a short (s) allele with lower basal and induced transcriptional activity and a long (l) allele with a higher transcriptional activity of the serotonin transporter gene.[1–5] Given the implication of the serotonergic system in the regulation of mood, anxiety responses, aggressive behaviour, pain perception and autonomic functions like sleep, appetite and sexuality behavioural effects of this functional polymorphism appear to be possible. Initial studies have found an association with the s-allele in neuroticism (increased levels of depression, hostility and anxiety)[6] and harm avoidance.[7] Two following studies replicated[8,9] these findings, others did not.[10–12] Association studies with depressive disorders also yielded conflicting results.[13] However, Neumeister *et al*[14] demonstrated a higher susceptibility to depressive reactions during tryptophan depletion in women carrying the s/s-genotype. Recently, Caspi *et al*[15] found strong evidence for a gene–environment interaction in a birth cohort sample of age 26 in New Zealand. The number of life stressors was significantly associated with higher ratings of depression in subjects with the s/s than the l/l genotype, whereas the s/l genotype was intermediate. Since types and frequencies of life stressors may differ among populations, the assumed gene–environment interaction may be difficult to replicate in different samples, thereby questioning the validity of gene–environment interaction of the 5-HTT polymorphism. Furthermore, due to the numerous implications of the serotonergic system, a much broader range of mental and physical well being could be influenced by this polymorphism.

The aim of the present study was to verify the gene–environment interaction of the s-polymorphism of the promoter of the 5-HTT gene with life stressors in a

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different population with different sociodemographic characteristics and a different risk structure for mental stressors than in the study population of Caspi *et al*.[15]

Material and methods

Sample

The Study of Health in Pomerania (SHIP) is a cross-sectional study of the population in Western Pomerania, the north-east coastal region of Germany.[16] As being part of the former German Democratic Republic, our general population sample had to face major social and economic upheavals during the last 13 years. Many jobs had been lost, the rate of unemployment was about 20% during the study period, social networks had been disrupted in many cases and, due to the higher age of our sample, physical illnesses are supposed to play an important role in the perceived mental and physical distress of the sample. From the total population of 212 157 people living in the study area, a random sample was drawn. Data were collected between October 1997 and May 2001. The SHIP population comprised 4310 participants (68.8% of eligible subjects) aged 20–79 years.

In order to perform univariate analysis of variance (ANOVA), the left-skewed distribution of BL-38 scores (modified complaint scale from von Zerssen[17]) in the total SHIP population had to be adjusted to an acceptable normal distribution: A sample of 505 subjects with moderate and high BL-38 scores (≥ 58; mean−standard deviation/2) was matched for age, sex, marital status and education, with 505 subjects from the total population. In all, 1005 subjects were genotyped for the SLC6A4 polymorphism. All information was present in 976 subjects. Due to this sampling procedure, the selected subjects were preferentially female (69.3%; $\chi^2 = 177$; $P < 0.001$), had higher mean scores in mental and physical distress (BL-38 scores) (75.8; SD 19.7 *vs* 62.7; SD 13.3; $T = 24.2$; df = 4284; $P < 0.001$), higher mean frequencies of chronic diseases (1.01; SD 1.04 *vs* 0.72; SD 0.71; $T = 8.7$; df = 4308; $P < 0.001$) and a higher age (51.6; SD 15.4 *vs* 49.9; SD 16.7; $T = 3$; df = 4308; $P = 0.003$) than the nongenotyped volunteers of the SHIP population.

All subjects were interviewed concerning sociodemographic characteristics, education, employment status, health and health behaviour. Chronic diseases were considered when present within the last year prior to the interview. The following diseases were evaluated: myocardical infarction, stroke, diabetes, deep vein thrombosis, kidney disease, chronic bronchitis, arthritis and degenerative diseases of the joints and spine, osteoporosis, peptic ulcer, pancreatitis, gastrointestinal bleedings, migraine, thyroid disease, cancer, multiple sclerosis and Parkinson's disease.

The diagnosis of deep vein thrombosis ($N = 11$) was excluded from the sum score of chronic diseases because the presence of thrombosis was near-significantly associated with the s-allele ($\chi^2 = 3.6$; df = 1; $P = 0.06$). None of the other somatic diagnoses were associated with any genotype of the 5-HTT promoter ($P > 0.1$). All the remaining items were summarized to a score reflecting the overall chronic disease burden.

Assessment of mental and physical distress

The modified version of von Zerssen's complaints scale[17–19] was used to assess psychological and somatic symptoms by self-report on 38 items. The presence of each symptom was rated on a four-point scale (absent = 1, mild = 2, moderate = 3, severe = 4). The following items were assessed: (1) back- and lower back pain; (2) joint pain; (3) weakness; (4) abdominal feeling of fullness; (5) faintness; (6) heartburn; (7) irritability; (8) nervousness; (9) heavy legs; (10) sleeplessness; (11) dizziness; (12) fatigue; (13) headache; (14) deafness; (15) sudden difficulty in breathing; (16) feelings of suffocation; (17) tachycardia or palpitations; (18) anxiety; (19) stomach ache; (20) loss of energy; (21) poor concentration; (22) inner tension; (23) sensitivity to weather; (24) depression; (25) numbness of hands or feet; (26) globus sensation; (27) breathlessness; (28) difficulty in swallowing; (29) chest pain; (30) nausea; (31) rumination; (32) nervous legs; (33) hypersensitivity against warmth; (34) hypersensitivity against cold; (35) excessive need of sleep; (36) tremors; (37) neck and shoulder pain; (38) loss of weight. The Cronbach's alpha (0.93) indicated a very high internal consistency of the BL-38 scale.

Social support was assessed using the five items of the 'tangible support' subscale, which were rated on a five-point scale (1 = never; 2 = seldom; 3 = sometimes; 4 = often; 5 = always).[20,21] The five questions covered the following domains: Do you have someone you can talk to about personal problems, someone who can drive you to your doctor we you are sick, someone who prepares your meal or helps you along with activities of daily living when you can't do it by yourself and someone who borrows you some money when you are in need?

Genotyping

For genotyping, DNA was isolated from leukocytes by standard phenol/chloroform extraction. The known 5-HT transporter variants (s/s, s/l, l/l) located in the 5′ region 1 kb upstream of the exon 1 (accession no: X76753) were determined by PCR. DNA (0.1 μg) was added to a final volume of 25 μl consisting of 1.5 mmol/l $MgCl_2$, 1 U expanded high-fidelity polymerase and buffer (Roche), 0.125 mmol/l dNTPs (Biozyme), 0.5 pmol forward primer 5′-ATG TCC CTA CTG CAG CCT CC and 0.5 pmol reverse primer 5′-AGT CCG CGC GGG ATT C. PCR conditions were 35 cycles of 30 s at 94°C, 50 s at 62°C and 60 s at 72°C. A 440- and/or 396-bp fragment was generated, respectively, and genotypes were distinguished by size on a 2% agarose gel using a Kodak digital imager.

Statistical analyses

Comparisons between groups of allele frequencies and other categorical variables were made using χ^2

tests. Univariate analyses of variance were calculated with the mental and physical distress score (BL-38) as dependent variable and the genotype, the status of employment (unemployed *vs* employed) and the number of chronic diseases (0, 1, 2, 3, 4 or more) during the last year as independent variables. Covariates were age and social support. Another covariate covering the educational status (eight levels with increasing quality of education) was omitted from the analyses because of its insignificance (females: $F=0.03$; $df=1, 673$; $P=0.96$; males: $F=1.4$; $df=1, 298$; $P=0.24$). None of the gene–environment interactions were influenced by the variable education. The analyses were calculated separately for males and females. In order to increase the statistical power, a combined analysis of genotypes (s/s and s/l together *vs* l/l) according to the hypothesis was performed.

Results

The percentages of the SLC6A4 genotypes were s/s = 15.4%, s/l = 48.5%, l/l = 36.1% in females and s/s = 17.9%, s/l = 43.7%, l/l = 38.4% in males ($\chi^2=2.3$; $df=2$; $P=0.32$). The distribution of the genotypes was in Hardy–Weinberg equilibrium ($\chi^2=0.14$, $P=0.93$). Table 1 shows the distribution of the independent variables in females and males in the study sample.

In females, low social support ($F=11.8$; $df=1, 673$; $P=0.001$) and chronic diseases ($F=10.1$; $df=4$; 670; $P<0.001$) were associated with high mental and physical distress. Unemployment ($F=2.9$; $df=1, 673$; $P=0.09$) and the 5-HTT genotype (s/s and s/l *vs* l/l) ($F=0.7$; $df=1, 673$; $P=0.39$) were not independently associated with mental and physical distress in females. Significant effects of interaction between the genotype and unemployment ($F=4$; $df=1, 673$; $P=0.047$) (Figure 1), the genotype and chronic diseases ($F=4.6$; $df=4, 670$; $P=0.001$) (Figure 2) and between the genotype, unemployment and chronic diseases ($F=6.6$; $df=3$; 671; $P<0.001$) emerged. The equation including the genotype explained 22.8% of the total variance of the BL-38 score (adjusted $R^2=0.228$). When omitting the genotype from the equation, the explained variance decreased to 20.9%.

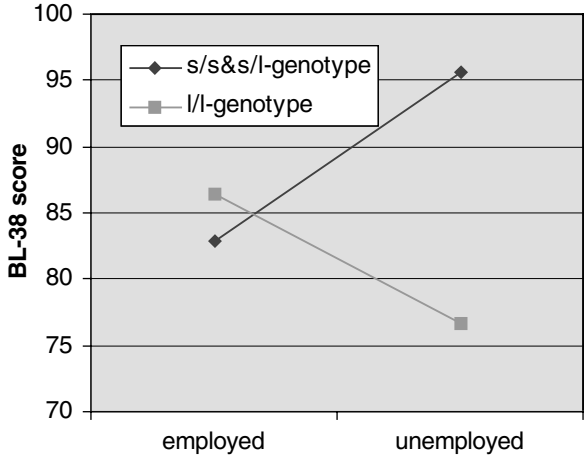

Figure 1 Interaction between the genotype (s/s and s/l *vs* l/l) and employment status with regard to perceived mental and physical distress (BL-38 score) in females ($n=674$). Values are means adjusted for age and social support by ANOVA.

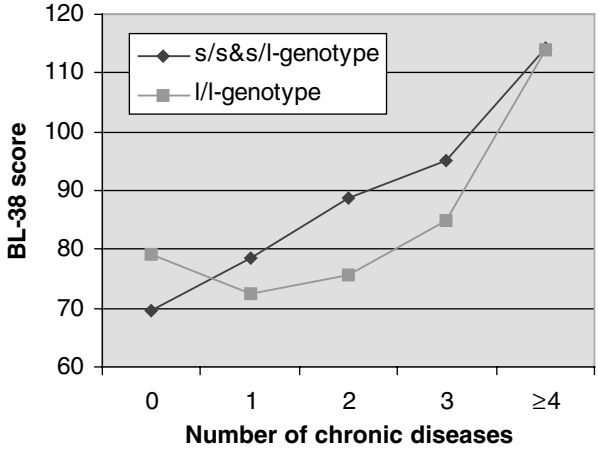

Figure 2 Interaction between the genotype (s/s and s/l *vs* l/l) and the number of chronic diseases with regard to perceived mental and physical distress (BL-38 score) in females ($n=674$). Values are means adjusted for age and social support by ANOVA.

Table 1 Description of the study sample ($n=976$)

	Females (n = *674*)	*Males* (n = *302*)
Employed	584 (86.6%)	274 (90.7%)
Unemployed	90 (13.4%)	28 (9.3%)
Number of chronic diseases		
0	247 (36.6%)	138 (45.7%)
1	228 (33.8%)	106 (35.1%)
2	130 (19.3%)	32 (10.6%)
3	50 (7.4%)	20 (6.6%)
≥4	19 (2.8%)	6 (2%)
l/l genotype	243 (36.1%)	116 (38.4%)
s/l genotype	327 (48.5%)	132 (43.7%)
s/s genotype	104 (15.4%)	54 (17.9%)

In males, only the variable 'chronic diseases' were significantly associated with impaired mental and physical well being ($F=6.8$; $df=1, 298$; $P<0.001$). Unemployment ($F=2.9$; $df=1, 298$; $P=0.09$), age ($F=3.8$; $df=1, 298$; $P=0.05$), social support ($F=0.05$; $df=1, 298$; $P=0.82$) and the genotype ($F=0.01$; $df=1, 298$; $P=0.9$) did not attain statistical significance. None of the interactions between the genotype and the variables unemployment and chronic diseases were significant ($P>0.3$). The model explained 18.1% of the total variance of the BL-38 score.

Discussion

The s-allele of the 5-HTT promoter region showed an interaction with life stressors in females but not in males in our sample. There was no independent association of the genotype with mental and physical health. These results provided partly support to the study of Caspi *et al*,[15] who investigated 847 subjects from a birth cohort study at the age of 26 in New Zealand and found a significant interaction between the s-allele, life-stressors and depression.

The risk factors for mental and physical distress were *a priori* chosen from the data set of the Study of Health in Pomerania[16] and represent relevant stressors especially in a population with a mean age of 52 years (SD 15 years). It is important to note that our general population sample had been investigated after it had experienced an 8–11-year period of a hitherto unknown social and economic decline that commenced with German reunification and is still ongoing. As a consequence of this socioeconomic upheaval, a great deal of jobs vanished whereas, at the same time, the previously tightly woven social networks have gradually dissolved. Additionally, the study region has also undergone dramatic demographic changes with older—and consequently also much sicker—people making up for an ever greater proportion of a steadily diminishing whole population. It may therefore come to no surprise that chronic disease was highly associated and unemployment was modestly associated with mental and physical distress in males and females, whereas social support only emerged as a strong predictor variable in females, pointing to gender-specific risk factors for mental and physical distress.

The self-rating questionnaire captures a broad range of mental and physical distress corresponding to a general health concept. Our results underscore the circumstance that the gene–environment interaction modulates not only symptoms restricted to depression but also a much broader range of mental and physical symptoms. However, this is not very surprising given the fact that the serotonin system is supposed to modulate many functional areas like mood, anxiety, sleep, appetite, sexual and somatosensory (eg pain) functions. Moreover, the very high internal consistency in which all 38 items of the questionnaire are statistically highly intercorrelated also emphasizes the entity of the assessed symptoms as a broad dimension of perceived distress.

The genotype variable explained 2% of the variance of the BL-38 sum score in females. This means that, besides other known and unknown environmental, biological and psychological factors that contribute to the development of mental and physical symptoms of distress, the s/l-polymorphism of the 5-HTT increases (s-allele) or relatively decreases (l-allele) the overall load of symptoms to some degree. Given that only 22.8% of the total variance of the BL-38 score could be explained by all independent variables in the equation, the 2% explained variance due to the genotype seems relatively high.

Some further issues and limitations of the study need attention:

1. The selection of psychosocial and other risk factors and the method of assessment represent always an explorative approach to a complex system and might differ among populations.
2. In our sample, medical risk factors were strongly associated with impaired mental and physical health, and did significantly interact with the s-allele in females. However, the selection of the diseases was *a priori* given by the design of SHIP. Results might have been different if diseases were assessed with an alternate spectrum of diagnoses or with other groups of diagnoses, for example, assessing joint related diseases not on one but on two items like arthritis of the major joints and chronic degenerative disease of the spine.
3. The interaction between the s-allele was found in females but not in males. A different psychosocial risk structure in males may contribute to the finding. Thus, a gene–environment interaction might emerge when assessing different risk factors in males. Moreover, a gender-specific genetic vulnerability also could contribute to this finding.[22] The male sample was smaller than the female sample; thus, a lack of statistical power may have contributed to the negative finding.
4. Our sample comprised subjects on a wide spectrum of BL-38 scores, also including relatively more subjects with high BL-38 scores than in the primary SHIP sample ($n = 4310$). This led to a much higher standard deviation and higher means of the BL-38 score in the genotyped sample. Though this sampling procedure was deliberately chosen, it might have influenced the statistical results.

Analyses including psychosocial stressors, physical stressors, biological factors and genotypes may help to approach multidimensional interactions as might occur in complex diseases, and may help to quantify the effects of various risk factors. Further analyses on gene–environment interaction of the functional polymorphism of the serotonin transporter gene are warranted and should, due to the relevance of serotonin for platelet aggregation,[23,24] be extended to atherosclerosis.[25,26]

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