Serotonin Transporter Gene Promoter Polymorphism and Somatoform Symptoms

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Introduction: Symptoms of somatoform and affective disorders are thought to be connected to serotonergic neurotransmission because serotonin is known to regulate the functions relevant in these disorders, such as pain and mood. Previous studies have reported associations of these disorders with a functional polymorphism in the promoter region of the serotonin transporter gene, a limiting factor of the serotonergic neuronal system, as its alleles have been associated with differences in levels of synthesized transporter and therefore differences in reuptake efficiency.

Method: Ninety-one patients with at least 2 unexplained physical symptoms were clinically evaluated and genotyped for the triallelic genotypes of the serotonin transporter gene polymorphism; patients were recruited from 2001 until 2004. *DSM-IV* diagnoses were assessed using the International Checklists for *ICD-10* and *DSM-IV*. Somatic complaints were quantified with an interview version of the Screening for Somatoform Symptoms, persistent symptoms in the last 2 years (SOMS-2) and the SOMS-7 (current symptoms in the last 7 days). Depressive symptoms were quantified with the Beck Depression Inventory (BDI).

Results: Subjects with higher-expressing allele variants of the serotonin transporter gene (L′L′ and L′S′) had significantly more somatic symptoms in the last 2 years (trait) than those with lower-expressing variants (S′S′) ($P < .01$). No differences could be found in regard to short-term somatic symptoms (ie, in the last 7 days). Neither depressive symptoms nor a comorbid diagnosis of major depression was associated with allelic variants.

Conclusion: Somatoform symptoms may be associated with a functional polymorphism in the promoter region of the serotonin transporter gene.

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The term *somatoform disorders* refers to a group of psychiatric disorders with somatic symptoms not caused by a well-known medical condition. These bodily symptoms tend to persist and tend to occur together with symptoms of other organ systems ("multisomatoform disorders").[1] The pathophysiology of these syndromes is not fully understood, but neurotransmitters are considered to be relevant for symptom development and maintenance.[2]

Serotonin (5-hydroxytryptamine [5-HT]) is a prominent neurobiologic marker and is known to regulate functions such as pain, sleep, and mood,[3–5] which are related to somatoform disorders as well as affective disorders. In depression, possible actions of 5-HT might be primarily central, modulating cognition and affect regulation,[6,7] whereas pathways of contribution for serotonin to somatoform symptoms could be 2-fold: through modulation of pain perception via direct cerebral action, or through peripheral pathways, eg, via contribution to muscle metabolism or action on different receptor subtypes in pain.[5,8]

One limiting factor for the 5-HT neuronal system is the 5-HT transporter gene (*SLC6A4*). The 5-HT transporter regulates the action of 5-HT by transporting it from synaptic spaces into presynaptic neurons, thus terminating its action. *SLC6A4* encodes this transporter. A polymorphism within the promoter region (5-HT transporter gene–linked polymorphic region, *5-HTTLPR*) has been reported, and its allelic variants are associated with differences in the regulations of gene expression and in the efficiency of 5-HT reuptake.[3,9] The *5-HTTLPR* was thought to be biallelic, with a less efficient S ("short") and an L ("long") variant, but recent findings suggest that the L allele can be subtyped into L_A ("long-A") and L_G ("long-G"), the latter of which is thought to be similar to the S allele in terms of reuptake efficiency.[10–13]

Studies on "somatoform-associated disorders"[2] such as chronic fatigue syndrome or temporomandibular disorder have found higher rates of high-expressing genotypes compared to control groups.[3,4] In affective disorders, there may be associations between the high-expressing allele (L_A or "L" in a biallelic model) and higher response rates to antidepressant treatment.[11,14] Other studies did not find an association between *5-HTTLPR* and depression.[9] These inconsistencies could result from differences in study design.

We hypothesized a positive association between *5-HTTLPR* and the number of unexplained somatic symptoms because the serotonergic system plays a major role in

pain perception and pain thresholds. We examined patients with at least 2 unexplained symptoms and measured the frequency of the triallelic form of *5-HTTLPR* from blood samples. We also controlled for the possible influence of depression.

METHOD

Sample and Psychometric Instruments

Ninety-one subjects (mean [SD] age = 50.89 [12.65] years; 39.6% men, 60.4% women) with a history of at least 2 unexplained physical symptoms were included in this study (see Table 1); patients were recruited from 2001 until 2004. Participants were a subsample of 2 prior studies and were recruited from primary care practices. The procedures were fully explained to the participants. Additionally, they were asked whether they agreed to let their blood be drawn and tested for genetic variants. All participants gave their informed consent to the procedures. Study protocol was approved by the Ethics committee of the German Society of Psychology (DGPs). Detailed inclusion criteria are described elsewhere.[15,16] Of 289 subjects, 104 agreed to have their blood tested for *5-HTTLPR*, and 13 were excluded because the correct alleles could not be determined. Eighty-six (94.51%) of the participants were diagnosed with at least 1 *DSM-IV* somatoform disorder—ie, somatization disorder, undifferentiated somatization disorder, pain disorder, conversion disorder, or hypochondriasis (mean [SD] number of disorders = 1 [0.58]). Participants were mainly of German (ie, Caucasian) origin (n = 89, 97.8%), with 2 (2.2%) reporting other origins.

Subjects were evaluated by laboratory analyses, medical history, interviews, and self-rating scales. We used the International Diagnostic Checklists for *ICD-10* and *DSM-IV*[17] to assess psychiatric morbidity, and the interview version of the Screening for Somatoform Symptoms, persistent symptoms in the last 2 years (SOMS-2)[18] was used to assess somatoform complaints during the last 2 years. This interview is equivalent to the symptom list used in the *DSM-IV* criteria for somatization disorder. The questionnaires assessed current somatic symptoms using the SOMS-7 (persistent symptoms in the last 7 days, reflecting the "state" version of the SOMS-2),[18] and depressive symptoms were quantified with the Beck Depression Inventory (BDI).[19]

Genotyping

Genomic DNA was isolated from whole blood according to standard procedures. The *5-HTTLPR* polymorphism was determined in its triallelic form, in which the A/G SNP rs25531 leads to a further differentiation of the L allele into L_A and L_G.[10] The alleles were genotyped by polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphisms (RFLP) analysis using the following primers: forward primer: 5-CTC CCT GTA CCC CTC CTA GG-3′; reverse primer: 5′-TGC AAG GAG AAT GCT GGA G-3′. PCR was performed with 50 ng DNA in a total volume of 15 µL containing 1.5 µL PCR buffer, 0.6 µM each primer, 3 µL Q-solution (Qiagen, Hilden, Germany), and 0.15 µL (0.75 units) Taq polymerase (Qiagen, Hilden, Germany). After denaturation at 95°C for 15 minutes, 35 cycles of PCR were performed with the following conditions: 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. 15 µL PCR product was digested with 1 µL *Msp*I and 2 µL Puffer Y-Tango (Fermentas, St. Leon-Rot, Germany) in a total volume of 20 µL at 37°C overnight. The samples were run on a 3% agarose gel and visualized by ethidium bromide staining. For the L_A-allele bands of 245 bp and 38 kb and for the L_G-allele bands of 162 kb, 83 kb, and 38 kb were obtained, whereas the S allele was visible by an uncut band of 211 bp.

Statistical Analyses

SPSS software (version 15.0 for Windows; SPSS Inc., Chicago, Illinois) was used for statistical analyses. Missing values for the self-report scales were imputed using an expectation-maximization algorithm. The triallelic genotypes were reclassified into a biallelic model by their levels of expression. L_G and S, which are associated with lower levels of expression than L_A, were reclassified as S′, and L_A was reclassified as L′.[13] Group differences were described using Kruskal-Wallis rank analysis (or Mann-Whitney test/χ^2 test where appropriate). Associations were assessed using the Cramer V; since *P* values calculated in this statistical procedure are actually approximations of *P* values, designations of these values are shown within quotation marks ("*P*").

RESULTS

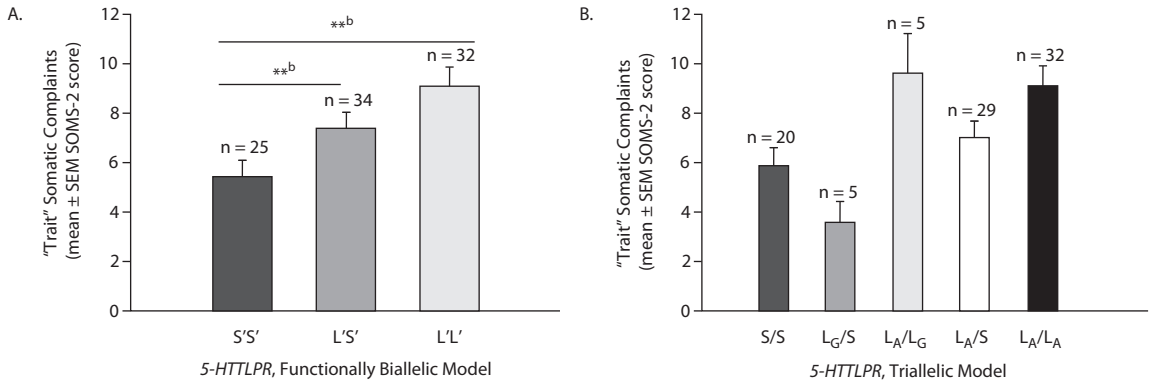
Genotype distribution was in Hardy-Weinberg equilibrium in the triallelic ($\chi^2 = 6.25$, $df = 4$, $P > .05$) and the biallelic model ($\chi^2 = 5.89$, $df = 2$, $P > .05$). *5-HTTLPR* biallelic genotype groups did not differ in terms of age, sex, and comorbid major depressive disorder (Table 1). For the biallelic model, there was a significant group difference for somatic complaints during the 2 years prior (SOMS-2) ($H = 12.11$, $df = 2$, $P < .01$). Participants with the L′L′ (mean [SD] number of symptoms = 9.13 [4.44]) and the L′S′ (mean [SD] number of symptoms = 7.41 [3.7]) variants reported significantly more persistent somatic complaints than those with the S′S′ variant (mean [SD] number of symptoms = 5.4 [3.24]) ($U = 282.5$, $P < .05$, and $U = 193.5$, $P < .01$, respectively). There was no significant group difference for current depressive symptoms (BDI) ($H = 3.71$, $df = 2$, $P > .10$) or somatic complaints during the previous 7 days (SOMS-7) ($H = 0.53$, $df = 2$, $P > .10$). Figure 1 shows the "trait" somatic complaints for the 2 allelic models; for the means and standard deviations (SOMS-2, SOMS-7, BDI) for the biallelic model, see Table 1. For the triallelic model, the groups were too small to test for differences, but the Cramer V revealed a significant association between genotype and "trait" somatic

Table 1. Demographic Features of the Study Sample (N = 91)[a]

	Biallelic Genotype Reclassification[b]				
	S′S′ (n = 25)	L′S′ (n = 34)	L′L′ (n = 32)	Statistic	*P*
Age, y	52 (12.21)	52.09 (13.63)	48.63 (12.28)	$\chi^2 = 2.05$	.359
Gender, n (%)					
Male	12 (48)	10 (29.4)	14 (43.8)	$\chi^2 = 4.16$	.125
Female	13 (52)	24 (70.6)	18 (56.3)		
Comorbid major depressive disorder, n (%)					
Yes	4 (16)	5 (14.7)	7 (21.9)	$\chi^2 = 0.88$	.646
No	21 (84)	29 (85.3)	25 (78.1)		
SOMS-2 score[c]	5.4 (3.24)	7.41 (3.70)	9.13 (4.44)	$H = 12.11$	< .01
SOMS-7 score	34.83 (40.74)	33.84 (64.24)	31.26 (48.07)	$H = 0.53$	> .10
BDI score	12.93 (10.85)	13.23 (9.48)	10.48 (8.35)	$H = 3.71$	> .10

[a]Values shown as mean (SD) unless otherwise noted; df = 2 for all statistical comparisons.
[b]Reclassification of the triallelic genotypes on the basis of expression levels. L_G and S, having lower levels of expression than L_A, were reclassified as S′, and L_A was reclassified as L′.
[c]Specific between-genotype comparisons are shown in Figure 1.
Abbreviations: BDI = Beck Depression Inventory; SOMS-2 = Screening for Somatoform Symptoms, persistent symptoms in the last 2 years; SOMS-7 = SOMS, persistent symptoms in the last 7 days.

Figure 1. "Trait" Somatic Complaints in the Previous 2 Years for (A) the Functionally Biallelic Model[a] and (B) the Triallelic Model



[a]Biallelic genotype = reclassification on the basis of levels of expression; S′S′ = S/S, L_G/S, L_G/L_G; L′S′ = L_A/S, L_A/L_G; L′L′ = L_A/L_A.
[b]L′L′ and L′S′ reported more symptoms than S′S′ (**$P < .01$).
Abbreviation: SOMS-2 = Screening for Somatoform symptoms, last 2 years.

complaints (Cramer's V = 0.28, *"P"* < .05); 40.6% of the participants with the L_AL_A genotype reported a high number of somatic complaints in the last 2 years (compared to 10% of those with the SS genotype). There were no significant associations for "state" somatic complaints or current depressive symptoms (Cramer's V = 0.16 and 0.21, *"P"* > .05).

Further analysis on a possible association between depression and *5-HTTLPR* also failed to show significant results (binary logistic regression with diagnosis of major depression as dependent variable and SOMS-2 scores and biallelic genotype as covariates; data not shown).

DISCUSSION

We expected the allelic variation in *5-HTTLPR* to be positively associated with the number of unexplained physical symptoms in patients with somatoform disorders. Accordingly, we found that persistent somatic symptoms are related to higher-expressing alleles of *SLC6A4*, which are thought to facilitate higher reuptake rates of serotonin. Similar findings have been shown for chronic fatigue syndrome and temporomandibular disorders.[3,4] It is possible that higher reuptake rates cause lowered 5-HT concentrations in the extracellular space, thus creating a relative hypofunction of the serotonergic system that in turn might cause or amplify somatic symptoms.[3,4] However, the causality of the connection between *5-HTTLPR* allelic variants and somatoform symptoms remains to be clarified as there are also findings contrary to our results. For other somatoform-associated disorders such as fibromyalgia, links to lower-expressing allele (S) were reported.[20] Our results suggest that serotonergic pathways are associated with a lasting tendency to experience physical symptoms ("trait") but not with the perception of acute symptoms ("state"). Considering the reduced tryptophan concentration found in patients with somatization disorders[8] and the efficacy of serotonergic

medication on pain symptoms in somatoform disorders,[21] there is evidence for the influence of serotonergic pathways in the development and maintenance of somatoform disorders.

The results of previous studies on mood disorders have been inconsistent, sometimes revealing associations with *5-HTTLPR* alleles that were not confirmed in other studies.[9] We found no significant associations between allelic genotypes and depressive symptoms or comorbid major depression, possibly because affective disorders were not the predominant diagnoses for participants.

There are some limitations to the interpretation of our findings. External validity may be limited, as we did not include a symptom-free control group in our design. In addition, the participants of this study were mainly of German origin. Previous studies have shown considerable variations in the distribution of *5-HTTLPR* alleles in different cultures[22]; therefore, our assumptions might be limited to Caucasian populations. Furthermore, we examined only a single candidate gene. In regard to the heterogeneity of symptoms in both somatoform and affective disorders, interactions of multiple genes as biologic bases for the psychiatric phenotypes are thought to be more likely.[9] Future research, therefore, should control for gene-gene interactions.

It might also be interesting to compare differences in allelic genotypes for patients with subsyndromes to further investigate the effects of serotonin in somatoform symptomatology. Due to the small sample size, it was not suitable to compare subgroups in our study, and the results might only be considered preliminary. However, our results confirm the association of somatoform symptoms with the serotonergic system, and to our knowledge this is one of the first reports on somatization and gene expression.

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