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ORIGINAL RESEARCH ARTICLE

A polymorphism (5-HTTLPR) in the serotonin transporter promoter gene is associated with DSM-IV depression subtypes in seasonal affective disorder

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Serotonergic mechanisms are thought to play an important role in the pathogenesis of seasonal affective disorder (SAD). The expression of the serotonin transporter (5-HTT) is regulated in part by an insertion/deletion polymorphism in the serotonin transporter gene promoter region (5-HTTLPR). The 5-HTTLPR short allele *(s)* has been associated with anxiety-related personality traits and depression, and one study observed an association between the 5-HTTLPR *s*-allele and SAD and the trait of seasonality. We genotyped 138 SAD patients and 146 healthy volunteers with low seasonality for 5-HTTLPR. No difference between patients and controls was found for genotype distribution and *s*-allele frequency. However, genotype distribution and allele frequencies were strongly associated with DSM-IV depression subtypes. Melancholic depression was associated with the 5-HTTLPR long *(l)* allele and atypical depression with the 5-HTTLPR *s*-allele (two-sided Fisher's exact test: genotype distribution: $P=0.0038$; allele frequencies: $P=0.007$). Our data are compatible with the hypothesis of a disease process that is not causally related to 5-HTTLPR, but involves 5-HT neurotransmission and 5-HTTLPR somewhere on its way to phenotypic disease expression.
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Although the impact of seasons on the incidence of mood disorders has been known since ancient times, only in the 1980s of our century seasonal affective disorder (SAD) has been described as a distinct nosologic entity.[1] Typically, patients with SAD, winter type, fulfill the diagnostic criteria for recurrent major depressive or bipolar disorder according to DSM-IV criteria and suffer from depressive episodes during fall and winter, alternating with remission or hypomania/mania in spring and summer. Common symptoms in SAD include depressed mood and the so-called atypical or reverse neurovegetative symptoms, such as hypersomnia, hyperphagia, fatigue, carbohydrate craving, and subsequent weight gain. The tendency to experience seasonal variations in mood, feeding behavior, energy, and social activity has been termed seasonality and can be measured using the global seasonality score (GSS). Although the etiology of SAD is still unclear, a substantial heritable component in seasonality has been shown in twin studies.[2,3] A solid body of literature suggests involvement of serotonin (5-HT) in the pathogenesis of SAD.[4] Serotonergic parameters, including central 5-HTT availability,[5] hypothalamic 5-HT concentrations, and peripheral serotonergic parameters[6] show seasonal fluctuations. A variety of research parameters, such as the tryptophan depletion paradigm,[7] hormonal challenge studies,[8] and *in vivo* imaging of central 5-HTT availability[9] have shown alterations in serotonergic parameters in depressed patients with SAD. Some studies also suggest that serotonergic alterations may be trait markers in SAD.[7] Genes involved in serotonergic neurotransmission are thus good candidates in the genetic research of SAD.

The 5-HTT is a member of the family of the Na^+/Cl^--dependent membrane transporters and controls the spread of the serotonergic signal in time and space by reuptake of 5-HT from the synaptic cleft immediately after its release. A polymorphism in the 5-HTT promoter gene region (5-HTTLPR)[10] with two common alleles, consisting of a 44-bp insertion (*l*-allele) or deletion (*s*-allele), has been shown to regulate 5-HTT expression *in vitro*. The presence of one or two

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copies of the *s*-allele led to a significant reduction in the amount of 5-HTT mRNA and 5-HTT expressed by human cell lines.[11] Besides anxiety-related personality traits,[11] affective disorder,[12] and violent suicide,[13] the 5-HTTLPR *s*-allele has been associated with SAD and seasonality.[14] In view of these findings, 138 unrelated patients (82.6% females, 17.4% males; mean age: 37.5 ± 11.3 years) from our outpatient unit for SAD and 146 unrelated healthy volunteers with low seasonality levels (85.6% females, 14.4% males; mean age: 28.2 ± 10.5 years) were studied and genotyped for 5-HTTLPR from autumn 1997 to spring 2000. Serotonin is known to play a key role in the regulation of food intake[15] and sleep.[16] Since feeding behavior and sleep are altered in opposite directions in melancholic and atypical depression, data were analyzed for differences in 5-HTTLPR genotype distribution between melancholic and atypical depression subtypes according to DSM-IV.

Genotype and allele distributions did not differ significantly between patients and controls (two-sided Fisher's exact test: genotype: $P=0.499$; allele distribution: $P=0.933$; see Table 1) and were within the Hardy–Weinberg equilibrium in both groups. In contrast to the Rosenthal *et al*[14] study, our data did not show an association between 5-HTTPLR and seasonality measured using the GSS in patients (one-way ANOVA: $F=0.221$, df=2, $P=0.802$; Kruskal–Wallis $\chi^2=0.371$, df=2, $P=0.831$). The effect of 5-HTTLPR on the GSS in healthy controls was not analyzed, since a score of 6 or less was requested for control subjects to take part in the study. Power calculations for a χ^2 test assuming the genotype frequencies published by Rosenthal *et al*[14] as true population frequencies showed a probability of 0.81 to observe a *P*-value of 0.01 (or less) and 0.93 for a *P*-value of 0.05 for a sample size of $n=140$ in each group (as in our study). The probability to observe a *P*-value of 0.58 or larger (as in our study sample when using χ^2 statistics) was <0.0015 when assuming the Rosenthal *et al* data as true population frequencies. Hence, the hypothesis of an association between 5-HTTLPR and SAD, as strong or stronger than the association found by Rosenthal *et al*, should be rejected for our sample. A limitation of our study is the age difference between SAD patients and healthy controls (two-tailed $t=7.17$; df=282; $P<0.001$). However, this shortcoming is considerably offset by the fact that the age of illness onset in patients did not differ from the mean age of the control subjects (age of SAD onset: 28.4 ± 11.9 years; age controls: 28.2 ± 10.5 years; two-tailed $t=0.159$; df=276; $P=0.874$). Stratification effects due to ethnical inhomogeneity of the study subjects or their ancestors may in part be responsible for the divergent findings of the Rosenthal *et al* study and the present one, as 5-HTTLPR genotypes frequencies have been shown to vary considerably across different ethnic groups. In the present study, substantial effort has been made to avoid ethnical stratification by including only Caucasian subjects with central European origin. Adding external validity to our data, a recently conducted meta-analysis in samples from various nationalities provides no evidence for an association between 5-HTTLPR and SAD.[17]

While no significant differences in 5-HTTLPR genotype distribution were found between bipolar and unipolar depression (two-sided Fisher's exact test: $P=0.581$), a marked difference in 5-HTTLPR genotype (two-sided Fisher's exact test: $P=0.0038$) and allelic distribution (two-sided Fisher's exact test: $P=0.007$) was found between patients with SAD suffering from the atypical depression subtype according to DSM-IV[18] and patients suffering from the melancholic subtype (see Table 2). Carriers of the *s*-allele were significantly more likely to suffer from atypical depression, while patients homozygous for the 5-HTTLPR *l*-allele were more likely to suffer from melancholic depression (odds ratio: 3.956, 95% CI 1.640–9.774). There was no difference in age (one-way ANOVA $F=0.697$, df=2, $P=0.50$) and gender distribution (two-sided Fisher's exact test: $P=0.792$) between the respective genotype groups. The association of 5-HTTLPR with DSM-IV depression subtypes is of interest insofar as larger scale twin studies provided substantial evidence for hereditability and familiality of depressive subtypes.[19] Melancholic depression has been shown to reliably identify a subset of severely depressed individuals with distinct clinical features.[20] Moreover, biological research paradigms allow to differentiate between melancholic and nonmelancholic depression,[21] and, interestingly, lower plasma tryptophan levels have been shown for melancholic depression.[21] Of course, future studies will have to prove whether the association between 5-HTTLPR and depression subtypes in SAD found in our study can be generalized to nonseasonal depression.

Table 1 5-HTTLPR[a] genotype distribution and allele frequencies in patients with seasonal affective disorder and healthy controls

	I/I	*I/s*	*s/s*[b]	*Frequency I*	*Frequency s*[c]
Patients with seasonal affective disorder ($n=138$)	44 (31.9%)	71 (51.4%)	23 (16.7%)	57.6 %	42.4%
Healthy controls ($n=146$)	51 (34.9%)	65 (44.5%)	30 (20.5%)	57.2%	42.8%

[a]5-HTTLPR: serotonin transporter gene linked polymorphic region.
[b]Genotype distribution: Fisher's exact test, two-sided: $P=0.499$.
[c]Allele distribution: Fisher's exact test, two-sided: $P=0.932$.

Table 2 5-HTTLPR[a] genotype distribution and allele frequencies: depression subtype according to DSM-IV[b] in patients with seasonal affective disorder

	I/I	I/s[c,d]	s/s	*Frequency* I	*Frequency* s[e]
Patients with melancholic depression (n=34)	19 (55.9%)	11 (32.4%)	4 (11.8%)	72.1%	27.9%
Patients with atypical depression (n=104)	25 (24.0%)	60 (57.7%)	19 (18.3%)	52.9%	47.1

[a]5-HTTLPR: Serotonin transporter gene linked polymorphic region.
[b]DSM-IV: *Diagnostic and Statistical Manual*, American Psychiatric Association, 4th edn.
[c]Genotype distribution, *I/I vs I/s vs s/s*: Fisher's exact test, two-sided: P=0.0038.
[d]Genotype distribution, *I/I vs I/s + s/s*: Fisher's exact test, two-sided: P=0.0012.
[e]Allele distribution: Fisher's exact test, two-sided: P=0.007.

The overall pattern of results derived by studies on 5-HTTLPR and its effects on brain 5-HTT availability,[22] 5-HT uptake in human platelets,[23] seasonal variations in blood 5-HT levels[6] and on the therapeutic response to serotonin reuptake inhibitors (SSRIs),[24] and to sleep deprivation[25] is most suggestive for a functional dominance of the *s*-allele. We therefore analyzed *I/s* heterozygous patients grouped with both, *s/s* and *I/I* homozygous patients (ie *I/I vs Is + ss* and *II + Is vs ss*). The analysis of *s*-carriers *vs I/I* homozygous patients revealed a strong effect on DSM-IV depression subtypes (*I/I vs Is + ss:* two-sided Fisher's exact test: P=0.0012), while this was not the case when all *I*-carrying patients were grouped together (*ss vs II + Is:* two-sided Fisher's exact test: P=0.44). Our data are so far in line with the original *in vitro* findings,[11] as they are compatible with a functional dominance of the 5-HTTLPR *s*-allele.

The presence of reverse vegetative symptoms in SAD patients with atypical depression is suggestive for a reduced central 5-HT neurotransmission,[15] at least in key areas involved in the regulation of feeding behavior and sleep. Our own group reported a reduction in hypothalamic 5-HTT availability in patients with SAD.[9] Although the data in this study were not evaluated separately for patients with melancholic and atypical depression, by far the greater part of the patients in this study suffered from atypical depression. However, results on *in vivo* effects of 5-HTTLPR are controversial,[22,26,27] and the complex interaction of pre- and postsynaptic elements renders simple quantitative conclusions on 5-HT neurotransmission impossible. However, high-resolution imaging techniques using selective 5-HTT ligands will give the opportunity to measure possible differences in 5-HTT availability between depression subtypes *in vivo*. These studies may furthermore help to answer the question, whether the association of 5-HTTLPR with depression subtypes, as found in the present study, is due to functional effects of this polymorphism itself, or whether linkage disequilibrium may be held responsible for the present finding.

Studies investigating the relationship between 5-HTTLPR and response to selective serotonin inhibitors,[24] therapeutic sleep deprivation,[25] and the combination of sleep deprivation and bright light therapy (BLT),[28] found the *I*-allele to be associated with better treatment outcome. The favorable response to BLT predicted by the presence of atypical depressive symptoms in SAD[29] raises the question, whether 5-HTTLPR may serve as a treatment predictor to bright light as well. As a nonpharmacological treatment modality, BLT offers ideal imaging possibilities to study the *in vivo* dynamics of 5-HTT regulation from the depressed state to symptom remission in patients with SAD. In conclusion, our data do not support a direct role of 5-HTTLPR in the etiology of SAD. However, they are compatible with a disease process that involves 5-HTTLPR, possibly via 5-HT uptake, somewhere on its way to the phenotypic disease expression. Our results furthermore suggest that studies beyond the unitary depression model could be a promising strategy in the future biological research of affective disorders.

Methods

Subjects

SAD was diagnosed according to Rosenthal and DSM-IV criteria for SAD. [1,18] All patients were untreated for at least 6 months prior to the diagnostic interview. Patients with psychiatric diagnoses other than SAD were not included in the study. Healthy volunteers were recruited by advertisement, before study inclusion, psychiatric disorders were excluded using the Structured Clinical Interview for DSM-IV axis I Disorders (SCID-DSM-IV). Control subjects with a history of substance abuse or a history of psychiatric illness were not allowed to enter the study. All patients and controls were given a German version of the Seasonal Pattern Assessment Questionnaire (SPAQ)[30] to assess the GSS. The SPAQ is a self-rating scale that measures seasonal variations in sleep, appetite, mood, energy, weight, and social behavior. The GSS has a maximum score of 24 subjects with a GSS score of more than 11 are susceptible for SAD caseness. We excluded all control subjects with a GSS score greater than 6. All subjects gave written informed consent; blood samples were handled anonymously. The Ethics Committee of the

University of Vienna approved the investigation of 5-HTTLPR genotypes in patients with SAD and healthy controls.

Genotyping

Genomic DNA was isolated from peripheral nuclear blood cells according to standard procedures. Polymerase chain reaction (PCR) amplification was performed using the primers described by Deckert *et al.*[31] PCR products were separated on a 3% agarose gel and visualized by ethidium bromide staining. All laboratory procedures were carried out blind to subject's status.

Statistical analysis

Association tests on contingency tables (genotype *vs* case/control, allele frequencies *vs* case/control, etc.) were calculated using Fisher's exact test for testing the null hypothesis of independence of rows and columns in a contingency table with fixed marginals against a two-sided alternative. The association between GSS and genotype was calculated using a one-way ANOVA and a Kruskal–Wallis rank-sum test. All tests were performed using the statistical software package R, version 1.2.1.[32]

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