Transmission disequilibrium of polymorphic variants in the tryptophan hydroxylase-2 gene in children and adolescents with obsessive–compulsive disorder

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Abstract

Dysfunction of the central serotonergic system has been implicated in the pathophysiology of obsessive–compulsive disorder (OCD). The genetic contribution to the development of OCD is particularly high in early-onset OCD. The aim of this study was to investigate the effect of polymorphic variants in the gene of the novel brain-specific tryptophan hydroxylase-2 (TPH2), the rate-limiting enzyme of serotonin (5-HT) synthesis in the brain, in OCD with disease onset in childhood and adolescence. We analysed two common single nucleotide polymorphisms (SNPs) of TPH2 in the putative transcriptional control region and in intron 2 of the TPH2 gene in a unique family-based sample of OCD patients with onset of the disease in childhood and adolescence comprising 71 complete, independent trios. The transmission disequilibrium test was used to determine transmission of alleles and haplotypes from parents to offspring. In this first study of TPH2 in OCD, analysis of the SNPs, rs4570625 and rs4565946, revealed a significant preferential transmission of haplotype G-C to children and adolescents with OCD. Moreover, a trend towards preferential transmission of the C allele of SNP rs4565946 to the patients was found. The genotype relative-risk estimate for homozygous C allele carriers of SNP rs4565946 was 2.58 (95% CI 0.98–6.82). In conclusion, the results link TPH2 variations to the pathogenesis of early-onset OCD and further support the aetiological relevance of 5-HT signalling in OCD.

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Key words: Early-onset OCD, family study, serotonin, TPH2.

Introduction

Obsessive–compulsive disorder (OCD) is a relatively common condition which usually begins in adolescence or early adulthood. OCD is characterized by intrusive, disturbing, repetitive thoughts (obsessions), and repetitive or ritualistic behaviours (compulsions) performed to neutralize or prevent discomfort or some dreaded event. These thoughts and behaviours cause marked distress and are time-consuming or significantly interfere with a patient’s normal functioning. Obsessions may include fears of contamination or illness, needs for symmetry, religious preoccupations, superstitions, and thoughts about sexual behaviours. Comorbidity of OCD with depression, anorexia nervosa, and Tourette’s syndrome is considerable.
(Abramowitz, 2004; Angst et al., 2005; Halmi et al., 2003; Pauls, 1992; Santangelo et al., 1994). Based on the results of family and twin studies, the involvement of a genetic factor in OCD is likely. In a large set of studies using operationalized criteria the risk to first-degree relatives ranged from 3% to 35% for narrowly defined OCD (Alsobrook et al., 1999; Pauls et al., 1995; Rasmussen, 1993). The twin data indicate a heritability of up to 68% (Andrews et al., 1990; Torgersen, 1983, 1990). In 60% of patients OCD develops before the age of 25 yr, and onset of disease can already occur in childhood (Flament et al., 1990). Familial loading is higher in early-onset OCD, indicating that genetic factors may be of special importance in OCD with early onset (Nestadt et al., 2000; Pauls et al., 1995). Although the pattern of inheritance in OCD does not appear to be straightforward, molecular studies may provide valuable clues as to the contribution of genetic factors in the disorder (Cavallini et al., 2000).

Serotonin (5-HT) influences many brain functions such as emotions, cognition, motor function, and pain as well as circadian and neuroendocrine functions including food intake, sleep, and reproductive activity. While there are multiple presynaptic and post-synaptic 5-HT receptor subtypes mediating these complex actions of 5-HT, a major factor in the availability of 5-HT is its rate of production. 5-HT is synthesized in a two-step process, with the effect of the enzyme tryptophan hydroxylase (TPH) representing the rate-limiting step. Until recently, TPH was thought to be a single enzyme, responsible for synthesizing 5-HT both in the serotonergic neurons of the midbrain raphe as well as in the periphery, where it influences smooth muscle tone of blood vessels and of the gastrointestinal tract, platelet activity, and immune responses (Essmann, 1978; Mössner and Lesch, 1998). Unexpectedly, however, mice lacking TPH continue to produce normal concentrations of 5-HT in serotonergic neurons of the brain (Walther et al., 2003). In contrast, 5-HT concentrations in peripheral tissues of these knockout mice were reduced to <4% of normal levels. This pointed to the existence of a second, brain-specific form of TPH, which was termed TPH2. Indeed, this novel TPH2 was identified exclusively in the brain, while the classical TPH isoform, now termed TPH1, was detected in the periphery, especially in the duodenum and blood, but not in the brain, apart from the pineal gland (Walther and Bader, 2003; Walther et al., 2003).

The discovery of differential expression of classical TPH1 synthesizing 5-HT in peripheral tissues, and the novel TPH2 synthesizing 5-HT in serotonergic neurons of the midbrain raphe, may shed new light on the pathophysiology of a variety of psychiatric disorders, particularly OCD. Dysregulation of the serotonergic system in OCD is suggested by the differential effectiveness of treatment with antidepressant drugs. Clomipramine and selective serotonin reuptake inhibitors (SSRIs) represent the most effective pharmacological treatment available for OCD, with these drugs modulating synaptic 5-HT levels by way of inhibiting the 5-HT transporter (Baumgarten and Grozdanovic, 1998; Fineberg and Gale, 2005; Zohar et al., 2000). However, efficacy of 5-HT selective antidepressants is not a proof for dysregulation of the 5-HT system in OCD. Conversely, OCD patients treated experimentally with meta-chlorophenylpiperazine (m-CPP), which binds to several 5-HT receptor subtypes, experience an exacerbation of their obsessive-compulsive symptoms, further suggesting the importance of the serotonergic system in the pathophysiology of OCD (Zohar et al., 1988).

It has, therefore, been hypothesized that synthesis of 5-HT influences the likelihood of developing OCD. However, several studies over the past years of genetic variants of the TPH1 gene yielded negative results. In a study of Frisch et al. (2000), no association of TPH1 polymorphisms with OCD was observed. Similarly, we did not find an effect of a TPH1 variant on disease risk in children and adolescents with OCD (Walitza et al., 2004). Since these findings need to be re-interpreted in view of TPH1 being responsible for 5-HT synthesis in peripheral organs, while TPH2 controls synthesis of 5-HT in the brain, family-based studies on the possible association of TPH2 variations in OCD are urgently required. In order to elucidate the role of brain 5-HT synthesis in the pathophysiology of OCD, we investigated transmission of TPH2 alleles and haplotypes in a unique sample of children and adolescents with OCD and their parents.

Method

Study sample

The patient sample consisted of 71 children and adolescents (41 males, 30 females) with childhood-onset OCD (mean age 13.29 yr, S.D. = 2.6; mean age at onset 11.73 years, S.D. = 2.9) and both of their biological parents. All index patients had received in-patient treatment for OCD at the Departments of Child and Adolescent Psychiatry of the Universities of Würzburg, Marburg, Freiburg or Technical University of Aachen. The patients and their biological parents were all of German origin. All patients (children and adolescents) agreed to participate in the study and all participants gave written informed consent (in case
of minors their parents gave written informed consent). The ethics committees of the Universities of Würzburg, Marburg, Aachen and Freiburg approved the study. Exclusion criteria for patients were: lifetime history of psychotic disorder, Tourette’s syndrome, autistic disorder, alcohol dependence and mental retardation (IQ <70). Patients were also excluded when the obsessive–compulsive symptoms occurred only as a part of another psychiatric disorder. For inclusion into the study, at first all patients had to fulfill the diagnostic criteria for OCD according to DSM-IV (APA, 2000). To assess the criteria, all patients were interviewed with the Children’s Yale–Brown Obsessive Compulsive Scale (C-YBOCS; Goodman et al., 1989). Second, to appraise comorbid disorders, we used ‘Diagnostisches Interview bei psychischen Störungen im Kindes- und Jugendalter’ (DIPS) – children and parents version (Unnewehr, 1995). Subjects with comorbid disorders were only included in the study if the obsessive–compulsive symptoms were most prominent. This meant: (1) OCD had to predate the onset of the comorbid disorders except attention deficit hyperactivity disorder (ADHD), (2) senior clinicians (C.W., S.W.) diagnosed the predominance of the OCD symptoms. Forty-eight patients (67.6%) had no comorbid diagnosis. Twenty-three of the children and adolescents with OCD had comorbid diagnoses; the most frequent ones were ADHD which was observed in six children and different anxiety disorders (n = 6). In the rest of the cases there were conduct disorders (n = 3), depressive disorders (one patient with single episode, one with recurrent episode and two patients with dysthymic disorders). Eating disorders occurred in two patients, dyslexia in one patient and one patient had complex motor tic disorders (but no Tourette’s syndrome).

**TPH2 polymorphisms and genotyping**

Screening of the human *TPH2* gene revealed two common single nucleotide polymorphisms (SNPs), which were found to represent common allelic variants of *TPH2* in the general population (Gutknecht et al., unpublished observations) and were chosen for association analyses. Both SNPs were listed in the SNP database of the National Centre of Biotechnology Information (NCBI) but had not been verified and tested for allele frequencies. SNP rs4570625 is located in the putative transcriptional control region of *TPH2*, at position –703 with respect to the transcription start site (+1). SNP rs4565946 is located in intron 2 of *TPH2*. Genotyping of the two SNPs was performed as described by Gutknecht et al. (unpublished observations). Briefly, for detection of SNP rs4570625, a 204-bp polymerase chain reaction (PCR) product containing the SNP was amplified by PCR using the following reaction mix: 20 ng of genomic DNA in 75 mM Tris–HCl (pH 9.0), 20 mM ammonium sulphate, 0.01% Tween-20, 1.5 mM magnesium chloride, 0.4 μM of each of the primers, forward (5’-TTT TAT GAA AGC CAT TAC ACA T) and reverse (5’-TTC CAC TCT TCC AGT TAT TTT A), 0.25 mM dNTP, and 1 U Taq polymerase. After an initial denaturation for 5 min at 95 °C, 36 cycles of denaturing at 95 °C for 45 s, annealing at 51.9 °C for 45 s and extension at 72 °C for 45 s were performed, followed by a final extension at 72 °C for 3 min. PCR products were digested with PstI at 37 °C for 16 h. PCR products were visualized on a 3% agarose gel containing ethidium bromide. The undigested PCR product carries the G variant, whereas the digested product with two fragments of 55 and 149 bp contains the T allele. Determination of the SNP rs4565946 was performed in a similar PCR procedure, with 0.24 μM each of the forward primer 5’-CAT CCA AGG CTG TGT CCA TA and reverse primer 5’TGT GTC ACG TTG GCC TTT TA yielding a 225-bp product. Annealing temperature was 56.3 °C. PCR products were digested with BpuI. The undigested PCR product carries the T variant, whereas the digested product with two fragments of 93 and 132 bp contains the C allele.

**Statistics**

Parental genotypes were checked for Hardy–Weinberg equilibrium. The standard single-allele transmission disequilibrium test (Spielman et al., 1993) and haplotype transmission disequilibrium test with phase-certain haplotypes of *TPH2* were carried out with GENEHUNTER 2 (Kruglyak et al., 1996). Phase-certain haplotypes are haplotypes which can be unambiguously reconstructed from genotype data. In the case of subjects who are heterozygous at both loci, haplotypes may not be unambiguously reconstructable. All tests were performed at a two-sided comparison-wise significance level of α=0.05. We report uncorrected p values. In addition, we did a Bonferroni correction for multiple testing. Finally, we estimated allele frequencies, genotype relative risks (Knapp et al., 1995; Scherag et al., 2002), as well as interval estimates in order to address the accuracy of the study.

**Results**

In the present study sample of 71 patients with childhood-onset OCD, 16 patients (22.5%) had obsessions only, 17 patients (24.0%) had compulsions only and 38 (53.5%) had both obsessions and compulsions.
The average score of overall severity of OCD symptoms assessed by C-YBOCS was 22.16 (S.D. = 8.30; median 23.0; range of C-YBOCS scores: 5–38). C-YBOCS scale scores suggested that the patients had a moderate severity of OCD symptoms. The data are comparable to other recorded C-YBOCS scores in studies of children and adolescents with OCD (Geller et al., 2001; Hanna et al., 1995).

The two SNPs located in the putative transcriptional control region (SNP rs4570625) and in intron 2 (rs4565946) of TPH2 were found to be in linkage disequilibrium ($D' = 0.615$, $p < 0.001$). Both SNPs were in Hardy–Weinberg equilibrium in the 142 parental genotypes ($p > 0.8$). The distance between SNPs rs4570625 and rs4565946 is 4846 bp. A graphic illustration of the location of the SNPs is given in Figure 1.

The genotype and allele frequencies of SNPs rs4570625 and rs4565946 are given in Table 1. When transmission was analysed by transmission disequilibrium test, no preferential transmission of either allele of SNP rs4570625 was observed (Table 2). The intronic SNP rs4565946, on the other hand, showed a trend towards preferential transmission of allele C in patients with early-onset OCD (44 transmitted, 28 untransmitted; $p = 0.059$). In addition, this tendency was further supported by the haplotype analysis which indicated a preferential transmission of the G-C haplotype to patients ($p = 0.035$) (Table 3). Correspondingly, the G-T haplotype was transmitted to the patients less frequently ($p = 0.022$).

Finally, as our investigation only comprises 71 trios, we calculated estimates and 95% confidence intervals (CI) for SNP rs4565946. The estimated allele frequency of the C allele was 0.48 (95% CI 0.40–0.56), whereas the genotype relative-risk estimates for heterozygous carriers compared to homozygous T carriers was 1.78 (95% CI 0.84–3.77); for homozygous C carriers it was 2.58 (95% CI 0.98–6.82). In sum, this reflects a possible contribution of rs4565946 to the total risk of early-onset OCD. For SNP rs4570625 no valid calculations were possible as asymptotic theory did not hold.

### Table 1. Genotype and allele frequencies of TPH2 SNPs rs4570625 and rs4565946 in 71 patients with early-onset OCD

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genotypes</th>
<th>Alleles</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>G/G</td>
<td>T/T</td>
</tr>
<tr>
<td>rs4570625</td>
<td>53.5%</td>
<td>42.3%</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>C/T</td>
</tr>
<tr>
<td>rs4565946</td>
<td>33.8%</td>
<td>50.7%</td>
</tr>
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</table>

### Table 2. Transmission disequilibrium test of polymorphic variants in TPH2 of 71 patients with early-onset OCD and their biological parents

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele</th>
<th>Transmitted</th>
<th>Not transmitted</th>
<th>$\chi^2$</th>
<th>$p$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4570625</td>
<td>G</td>
<td>23</td>
<td>28</td>
<td>0.49</td>
<td>0.484</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>28</td>
<td>23</td>
<td>0.49</td>
<td>0.484</td>
</tr>
<tr>
<td>rs4565946</td>
<td>C</td>
<td>44</td>
<td>28</td>
<td>3.56</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>28</td>
<td>44</td>
<td>3.56</td>
<td>0.059</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genotype</th>
<th>Relative risk</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4565946</td>
<td>C/T</td>
<td>1.78 (compared to T/T)</td>
<td>0.84–3.77</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>2.58 (compared to T/T)</td>
<td>0.98–6.82</td>
</tr>
</tbody>
</table>

* Two-sided uncorrected asymptotic $p$ values.
† For SNP rs4570625 no valid calculations of genotype relative risks were possible as asymptotic theory did not hold.

The average score of overall severity of OCD symptoms assessed by C-YBOCS was 22.16 (S.D. = 8.30; median 23.0; range of C-YBOCS scores: 5–38). C-YBOCS scale scores suggested that the patients had a moderate severity of OCD symptoms. The data are comparable to other recorded C-YBOCS scores in studies of children and adolescents with OCD (Geller et al., 2001; Hanna et al., 1995).

### Table 3. TDT for phase-certain haplotypes of TPH2 SNPs rs4570625 and rs4565946

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Transmitted</th>
<th>Not transmitted</th>
<th>$\chi^2$</th>
<th>$p$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4570625-G</td>
<td>29</td>
<td>15</td>
<td>4.45</td>
<td>0.035</td>
</tr>
<tr>
<td>rs4565946-C</td>
<td>36</td>
<td>23</td>
<td>5.25</td>
<td>0.022</td>
</tr>
<tr>
<td>rs4570625-G</td>
<td>19</td>
<td>36</td>
<td>5.25</td>
<td>0.022</td>
</tr>
<tr>
<td>rs4565946-T</td>
<td>21</td>
<td>18</td>
<td>0.23</td>
<td>0.631</td>
</tr>
<tr>
<td>rs4570625-T</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>rs4565946-T</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Two-sided uncorrected asymptotic $p$ values.

![Figure 1](chart.png) Location of SNPs rs4570625 and rs4565946. The TATA box, transcription start site (+1), start codon (ATG), and the first three of 11 exons of TPH2 are shown.
Discussion

In the present study we investigated polymorphic variants of TPH2, which codes for the rate-limiting 5-HT synthesizing enzyme in the brain, in a sample of 71 patients with onset of OCD in childhood and adolescence, and their biological parents. Analogous to the impact of other candidate genes of the serotonergic pathway on disease risk, a small to moderate effect of polymorphic variants of TPH2 was anticipated. Since disease risk is frequently modulated by variants in regions which control expression or stability of gene transcripts, common polymorphic variants in the putative transcriptional control region and in intron 2 of TPH2 were selected for analysis in OCD (Figure 1).

In the present study we observed a trend towards preferential transmission of the C allele of rs4565946 as well as a significant preferential transmission of the SNPs rs4570625 and rs4565946 haplotype G-C to children and adolescents with OCD. Taken together with the genotype relative-risk estimate of 2.58 (95% CI 0.98–6.82) for homozygous C carriers of rs4565946, our findings suggest a pathogenetic role of TPH2 in the development of early-onset OCD. However, after a global Bonferroni correction none of the comparisons remained significant at a global $\alpha = 0.05$.

In contrast to the previous negative findings of TPH1 with OCD (Frisch et al., 2000), and negative findings in our earlier study (Walitza et al., 2004) of one SNP each in three serotonergic genes TPH1, 5-HTT and 5-HT1B receptor in early onset OCD (including 64 patients of the present sample), it may, therefore, be the synthesis of 5-HT in the brain via TPH2 which exerts its influence on OCD risk. Our finding suggests TPH2 as a plausible susceptibility gene for early-onset OCD.

In summary, this is the first study to assess whether polymorphic variants of TPH2 are associated with OCD. The results link TPH2 variations to the pathogenesis of early-onset OCD and further support the aetiological relevance of 5-HT signalling in OCD.

Acknowledgements

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Statement of Interest

None.

References


