Tryptophan hydroxylase-2 gene variation influences personality traits and disorders related to emotional dysregulation

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Abstract
Variation in the tryptophan hydroxylase-2 gene (TPH2) coding for the rate-limiting enzyme of serotonin (5-HT) synthesis in the brain modulates responses of limbic circuits to emotional stimuli and has been linked to a spectrum of clinical populations characterized by emotional dysregulation. Here, we tested a set of common single nucleotide polymorphisms (SNPs) in and downstream of the transcriptional control region of TPH2 for association with personality traits and with risk for personality disorders in two cohorts comprising of 336 healthy individuals and 420 patients with personality disorders. Personality dimensions were assessed by the Tridimensional Personality Questionnaire (TPQ) and the revised NEO Personality Inventory (NEO-PI-R). Personality disorders were diagnosed with the Structured Clinical Interview of DSM-IV and were allocated to clusters A, B, and C. Individual SNP and haplotype analyses revealed significant differences in genotype frequencies between controls and cluster B as well as cluster C patients, respectively. In both patient groups, we observed overrepresentation of T allele carriers of a functional polymorphism in the upstream regulatory region of TPH2 (SNP G-703T, rs4570625) which was previously shown to bias responsiveness of the amygdala, a structure critically involved in emotionality. Furthermore, significant effects of TPH2 variants on anxiety-related traits defined primarily by the TPQ Harm Avoidance were found in healthy individuals. The results link potentially functional TPH2 variants to personality traits related to emotional instability as well as to cluster B and cluster C personality disorders. These findings implicate alterations of 5-HT synthesis in emotion regulation and confirm TPH2 as a susceptibility and/or modifier gene of affective spectrum disorders.

Received 27 February 2006; Reviewed 3 April 2006; Revised 9 October 2006; Accepted 24 October 2006

Key words: Anxiety, emotion regulation, gene variation, personality disorders, personality traits, serotonin, tryptophan hydroxylase 2.

Introduction
While traits of emotionality are essential, stable, and continuously distributed dimensions of normal human personality, pathological manifestations of emotion regulation are ubiquitous in a wide spectrum of psychiatric conditions. Variance in personality traits, including those related to emotional dysregulation, such as anxiety, depression, and aggression, is thought to be generated by a complex interaction of environmental and experiential factors with a number of gene products involving distinct neural circuits such as the brain serotonin (5-HT) system. Although twin studies have indicated that individual variation in measures of anxiety-related personality traits is moderately heritable, only few of the potentially relevant genes have actually been identified (Lander and...
Examples for those implicated in anxiety-related traits and emotional dysregulation are the genes for the 5-HT transporter, 5-HT1A receptor, and monoamine oxidase A (Deckert et al., 1999; Jacob et al., 2005; Lesch, 2004; Lesch and Gutknecht, 2004; Meyer-Lindenberg et al., 2006; Schinka et al., 2004; Sen et al., 2004; Strobel et al., 2003).

Tryptophan hydroxylase-2 (TPH2), the rate-limiting enzyme of 5-HT synthesis in the brain tightly regulates 5-HT neurotransmission (Walther et al., 2003). The human TPH2 gene spans approximately 100 kb, consists of 11 exons, and is located on chromosome 12q21.1. Variation in TPH2 has been linked to a spectrum of clinical populations characterized by emotional dysregulation. Single nucleotide polymorphism (SNP) and haplotype analyses of the TPH2 locus spanning 28 kb between exons 5 and 7 revealed evidence for association of TPH2 variants with depression (Zill et al., 2004). Examination of SNPs further downstream and distributed over a region of 24 kb between exons 7 and 9 in a sample of patients with bipolar I and II disorders identified an affective disorder-associated haplotype in TPH2 (Harvey et al., 2004). Zhou et al. (2005) reported a haplotype-based association with major depression and anxiety disorders in a white US population and with major depression in African Americans. The frequency of the same haplotype, which was predictive of lower 5-hydroxyindoleacetic acid (5-HIAA) concentrations in cerebrospinal fluid, was also increased in suicide attempters in African Americans and a Finnish population. Zhang and co-workers (2004) reported an association between an extremely rare Arg441His substitution in TPH2, resulting in a loss of function when expressed in a cell model, and depression (see also Blakely, 2005).

Although higher TPH2 mRNA concentrations were found in the dorsolateral prefrontal cortex (Brodmann Area 46) of post-mortem brain from 23 individual with completed suicide compared to controls, this difference was not significant (De Luca et al., 2006). Investigation of the same region from samples of bipolar and schizophrenic patients revealed higher concentrations of TPH2 mRNA in the bipolar group in comparison with controls (De Luca et al., 2005). Measurement of TPH2 mRNA in the brainstem of depressed patients who had committed suicide, demonstrated greater expression in the dorsal and median raphe nuclei compared to matched non-psychiatric controls (Bach-Mizrachi et al., 2005). These findings have to be interpreted on the basis that TPH2 is transcribed in the somatodendritic segment of 5-HT neurons and a likely variable fraction of TPH2 mRNA is transported to terminal fields.

We previously reported transmission disequilibrium of variants in the transcriptional control region of TPH2 in children and adolescents with attention deficit hyperactivity disorder (ADHD) and showed preferential transmission of a haplotype linked to the transcriptional control region of TPH2 in early onset obsessive–compulsive disorder (OCD), suggesting a biological link between these disorders possibly based on common emotional instability (Mossner et al., 2006; Walitza et al., 2005). Finally, functional magnetic resonance imaging (fMRI) was employed to provide evidence that acute tryptophan depletion, which results in a transient reduction of brain 5-HT, as well as one of the potentially functional variants in the upstream regulatory region of TPH2 (SNP G-703T, rs4570625) biases the responsiveness of the amygdala, a structure critically involved in the modulation emotional behaviours (Brown et al., 2005; Canli et al., 2005).

In the present study, we evaluated the role of TPH2 variants and haplotypes in the modulation of individual differences in personality traits by a population genetic study of two cohorts, healthy volunteers and patients with personality disorders. We predicted that the TPH2 variation would be associated with personality traits related to anxiety and depression. Our hypothesis was based on several lines of evidence: anxiety-related personality traits are modulated by central 5-HT function, tryptophan depletion-elicited transient reduction of brain 5-HT moderates the reactivity of neural circuits controlling cognition and emotionality, and alterations in 5-HT signalling are associated with anxiety and affective spectrum disorders, whereas 5-HT uptake inhibitors are an effective treatment for these conditions.

Methods and materials

Healthy volunteers

For investigation of association between TPH2 polymorphisms and personality/behavioural traits as well as for case-control comparison, 336 healthy volunteers of German descent (247 females, mean age $\pm$ S.D. = 22.4 $\pm$ 5.3 yr) were recruited at the Dresden University of Technology (Strobel et al., 2004). All participants completed the German versions of the revised NEO Personality Inventory (NEO-PI-R; Ostendorf and Angleitner, 2003) and the Tridimensional Personality Questionnaire (TPQ; Weyers et al., 1995), based on the concept that there may be a genetic and
psychopathological continuum from normal personality to personality disorders and that it may be less difficult to identify genes for psychopathology by searching for genes influencing personality traits. The NEO-PI-R is based on the five-factor model of personality (Costa and McCrae, 1992). It consists of 241 items and assesses individual differences in 30 personality facets, which are subsequently aggregated and reduced to the five personality domains Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness. The TPQ is based on Cloninger’s model of personality (Cloninger, 1987; Cloninger et al., 1991). Its 100 items assess individual differences along the temperament dimensions Novelty Seeking, Harm Avoidance, and Reward Dependence. Each of the three temperament scales comprises four subscales, the Reward Dependence subscale Persistence being considered as a separate fourth temperament factor. The German versions of the NEO-PI-R, and the TPQ, respectively, have acceptable psychometric properties (Ostendorf and Angleitner, 2003; Weyers et al., 1995).

**Patient cohort**

Inclusion criteria were personality disorders according to DSM-IV criteria and age between 18 and 60 yr. The Structured Clinical Interview of DSM-IV (SCID), using the corresponding criteria of personality disorders, has previously been demonstrated to be highly sensitive to personality and behavioural changes. In the present study, personality disorders were therefore diagnosed with the SCID-II and were allocated to clusters A, B, and C operationalized as follows: Cluster A (odd-ecentric) comprises paranoid, schizoid, and schizotypal personality disorders. Cluster B (dramatic-emotional) encompasses antisocial, borderline, histrionic, and narcissistic personality disorders. Cluster C (anxious-fearful) includes avoidant, dependent, and obsessive-compulsive personality disorders, and a category called personality disorders not otherwise specified.

Exclusion criteria were medical conditions that have significantly changed the previous level of functioning, and lifetime diagnosis of schizophrenia or other psychotic disorders. The assessment of the personality disorders, including all the psychometric testing was performed by a single experienced psychiatrist (C.J.). Following screening of a large cohort of patients with personality disorders at the Department of Psychiatry and Psychotherapy, University of Würzburg, 420 patients were selected based on availability of complete TPH2 genotype data and presence of an exclusive cluster B (n = 316; 183 females, mean age ± s.d. = 35.3 ± 13.0 yr) or cluster C diagnosis (n = 104; 65 females, mean age ± s.d. = 38.5 ± 12.7 yr). Patients with an exclusive cluster A diagnosis were rare and therefore not included. The preselection avoided confounding effects of comorbidity for other personality disorders. Seventy-three percent of the cohort (n = 307) were diagnosed with an Axis I disorder according to DSM-IV (SCID-I) and ICD-10 criteria. Eighty patients had an Axis I diagnosis of affective disorders. The predominant entities were depressive episode (F32, n = 30), recurrent depressive disorder (F33, n = 28), and bipolar affective disorder (F31, n = 18). A neurotic, stress-related, or somatoform disorder was diagnosed in 166 patients. Among most prevalent diagnostic subgroups were adjustment disorder (F43.2, n = 99) and phobic anxiety disorder (F41, n = 18).

The study was carried out in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the University of Würzburg. Written informed consent was obtained from all individuals after the procedures and goals of the study had been fully explained. All of the participants were of German ethnicity which renders ethnic admixture unlikely (Reif et al., 2006).

**Genotyping**

DNA was extracted either from EDTA blood using the QiAamp Blood kit (Qiagen, Hilden, Germany) or from buccal swabs using the BuccalAmp system (Epigenic Technologies, Madison, WI, USA). Four SNPs found to represent common allelic variants of TPH2 in the general population were chosen for association analyses. All SNPs were listed in the SNP database (dbSNP) of the National Center of Biotechnology Information (NCBI) but had neither been verified nor tested for allele frequencies. Their corresponding reference number in dbSNP, their position in the gene relative to the transcription start site, and details of the genotyping procedure are given in Table 1. Genotype-based allele frequencies were determined on 116 chromosomes of a sample of unrelated subjects and were found to be 22.4% for the T allele of SNP rs4570625, 5% for the A allele of SNP rs11178997, 4% for the G allele of SNP rs4341581 and 31% for the T allele of SNP rs4565946, respectively.

**Statistical analyses**

Pairwise linkage disequilibrium between the four SNPs was assessed using 2LD (Zhao, 2004).
Association analyses were performed using the WHAP program (http://www.genome.wi.mit.edu/~shaun/whap/) developed by S. Purcell and P. Sham (Institute of Psychiatry, London, UK). WHAP uses a regression-based approach and calculates likelihood ratio tests to perform single-marker and haplotype association tests for dichotomous as well as for quantitative traits. By randomly reassigning the trait values a large number of times (10,000 in the present study), WHAP provides permutation-based p values which reflect the proportion of replicates that produce values of likelihood ratio statistics at least as large as those observed in the actual data. For single-marker analyses, the program provides both local (i.e. marker-specific) tests for allelic association and global marker-trait association tests where the global p values reflect the proportion of times the sum of likelihood ratio statistics for the single-marker associations in the permuted datasets are at least as large as the sum of likelihood ratio statistics observed in the actual data. For haplotype analyses, WHAP estimates haplotype frequencies using an expectation maximization algorithm and performs tests of global haplotype-trait association. It also permits tests of haplotype-specific associations by comparing each of a specific haplotype vs. all other haplotypes. Haplotypes with estimated frequencies of <1% were not considered for analysis. Tests for single-marker and haplotype association between the TPH2 and personality traits in the control sample were based on age- and gender-residualized and z-standardized personality scores to account for possible influences of these demographic variables. The residualization was performed by multiple regression. In the case that hypotheses were tested by more than one test (e.g. the hypothesis of an association between TPH2 markers and personality disorder was tested by two separate tests for cluster B and cluster C), a Bonferroni correction was applied to the global p value. The Bonferroni-correction factors are given in the table notes.

Results

Linkage disequilibrium and Hardy–Weinberg equilibrium

Table 2 depicts pairwise linkage disequilibrium (LD) between the four SNPs (physical marker order: rs4570625–0.23 kb–rs11178997–3.0 kb–rs4341581–1.6 kb–rs4565946) which was determined using those participants for which genotype information for all four SNPs was available (n = 756). Hardy–Weinberg equilibrium (HWE) was determined separately for the controls, cluster B, and cluster C personality disorder. Slight deviations from HWE were observed only for the rs11178997 genotype in cluster B patients (χ² = 1.89, p = 0.169) and for the rs4565946 genotype in cluster C patients (χ² = 2.71, p = 0.100; all other p ≥ 0.364).

Table 1. Location of TPH2 variants and genotyping procedure

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>Position</th>
<th>Alleles</th>
<th>Primers</th>
<th>Restriction enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4570625</td>
<td>−703, transcrip</td>
<td>G/T</td>
<td>F: ttcctagatttcctacgagag</td>
<td>ApoI</td>
</tr>
<tr>
<td></td>
<td>tional control</td>
<td></td>
<td>R: aagcctttctctcttctctcct</td>
<td></td>
</tr>
<tr>
<td>rs11178997</td>
<td>−473, transcrip</td>
<td>T/A</td>
<td>P*: tctgctaacctatttggcatacct</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tional control</td>
<td></td>
<td>R: gatactcctgataaagagaaaat</td>
<td></td>
</tr>
<tr>
<td>rs4341581</td>
<td>+2533, intron 1</td>
<td>T/G</td>
<td>F: agggtaaagagggcttctctctcttct</td>
<td>HpyCH4V</td>
</tr>
<tr>
<td>rs4565946</td>
<td>+4144, intron 2</td>
<td>C/T</td>
<td>R: gtaagctttgttaataaaagcttctctct</td>
<td>Bsu10I</td>
</tr>
</tbody>
</table>

Table 2. Linkage disequilibrium between the investigated TPH2 SNPs

<table>
<thead>
<tr>
<th>SNP 1</th>
<th>SNP 2</th>
<th>D’</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4570625</td>
<td>rs11178997</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rs4570625</td>
<td>rs4341581</td>
<td>0.81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rs4570625</td>
<td>rs4565946</td>
<td>0.83</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rs11178997</td>
<td>rs4341581</td>
<td>0.93</td>
<td>0.11</td>
</tr>
<tr>
<td>rs11178997</td>
<td>rs4565946</td>
<td>0.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rs4341581</td>
<td>rs4565946</td>
<td>0.89</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Modifying primer (a/t); @ allele-specific primer.
Table 3. Single-marker association of TPH2 with cluster B and cluster C personality disorder

<table>
<thead>
<tr>
<th>TPH2 SNP</th>
<th>Allele</th>
<th>Cluster B</th>
<th></th>
<th>Cluster C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p</td>
<td></td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>rs4570625</td>
<td>G</td>
<td>0.81</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0.19</td>
<td><strong>0.41</strong></td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>rs11178997</td>
<td>T</td>
<td>0.95</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.05</td>
<td>0.43</td>
<td>0.070</td>
<td></td>
</tr>
<tr>
<td>rs4341581</td>
<td>T</td>
<td>0.97</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.03</td>
<td><strong>-0.20</strong></td>
<td>0.611</td>
<td></td>
</tr>
<tr>
<td>rs4565946</td>
<td>C</td>
<td>0.52</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0.48</td>
<td><strong>-0.12</strong></td>
<td>0.292</td>
<td></td>
</tr>
<tr>
<td>Global</td>
<td>p</td>
<td><strong>0.038</strong></td>
<td></td>
<td><strong>0.001</strong></td>
<td></td>
</tr>
</tbody>
</table>

%, Frequency of TPH2 alleles; b, logistic regression coefficient (constrained to equal zero for frequent alleles); bold values: p < 0.05; a uncorrected significance of local permutation tests; b Bonferroni-corrected significance of global permutation tests, adjusted according to the number of personality disorder clusters examined (corrected p = empirical p × 2).

Single-marker association tests in personality disorder

Table 3 presents allele frequencies of all SNPs in the total sample and results of permutation tests for global and local (i.e. marker-specific) association of the TPH2 variants with cluster B and cluster C personality disorder. In both patient groups, a significant global association was observed (cluster B: pcorr = 0.038; cluster C: pcorr = 0.001; p values corrected for multiple testing, see notes to Table 3). Marker-specific tests showed that the global association with cluster B personality disorder was mainly due to a significant positive regression weight for the rs4570625 T allele (b = 0.41, p = 0.005), indicating higher frequency of the T allele in cluster B patients compared to controls. Similarly, the rs4570625 T allele was positively associated with cluster C personality disorder (b = 0.67, p = 0.001). Moreover, the rs11178997 A allele was positively associated with cluster C personality disorder (b = 0.43, p = 0.002), indicating a higher frequency of the A allele in cluster C patients compared to controls. These marker-specific effects remained significant even when considering only those patients without Axis I diagnoses in order to exclude confounding effects of comorbidity with other major psychiatric disorders (all p < 0.05).

In subsequent analyses, we explored whether the associations observed at the cluster level were due to associations of rs4570625 and rs11178997 with specific personality disorders, respectively. Because most of the patients were diagnosed with several subtypes within a personality disorder cluster, patients were characterized based on their main diagnosis in SCID-II (in terms of the number of fulfilled criteria per personality disorder) resulting in non-overlapping groups of patients. Tests were restricted to groups consisting of at least 30 patients. Analyses of specific personality disorders were therefore performed only for histrionic and narcissistic personality disorder (cluster B) and for avoidant and OC personality disorder (cluster C) (Table 4).

Within cluster B, the global association of rs4570625 and rs11178997 with histrionic personality disorder just missed the Bonferroni-corrected level of significance (pcorr = 0.054), whereas the global association with narcissistic personality disorder was not significant. Despite the lack of significance for histrionic personality disorder, we observed a highly significant single-marker association between rs4570625 and histrionic personality disorder (b = 0.41, p = 0.008). Within cluster C, there was no significant global association with avoidant personality disorder (pcorr = 1.00), but a significant global association with OC personality disorder (pcorr = 0.010). This global association was attributable to positive associations with both rs4570625 and rs11178997 (rs4570625: b = 0.75, p = 0.008; rs11178997: b = 1.07, p = 0.018).
reflecting a higher frequency of the rs4570625 T allele and the rs11178997 A allele in patients with OC personality disorder.

**Haplotype association tests in personality disorder**

Table 5 summarizes the results of the haplotype analyses. Two haplotype analyses were performed to test for global TPH2 haplotype association with cluster B and cluster C personality disorder, respectively. Both analyses yielded highly significant results (cluster B: \( p = 0.0004 \); cluster C: \( p = 0.0004 \)). Tests for specific TPH2 rs4570625-rs11178997-rs4341581-rs4565946 haplotypes revealed significant positive regression weights for the T-T-T-C haplotype (cluster B: \( b = 0.48, p = 0.004 \); cluster C: \( b = 0.47, p = 0.042 \)) as well as for the T-A-T-C haplotype (cluster B: \( b = 0.69, p = 0.012 \); cluster C: \( b = 1.07, p = 0.001 \)). Furthermore, a significant negative regression weight was observed for the rare T-T-T-T haplotype for cluster B only (\( b = -1.71, p = 0.0002 \)). Altogether, haplotype associations with cluster B and cluster C personality disorders largely reflected the results of the single-marker association with rs4570625.

**Association tests in personality disorder patients with comorbid Axis I disorders**

Subsequent analyses examined whether the single-marker association results for rs4570625 and
rs11178997 were attributable to possible influences of comorbidity with Axis I disorders. The highest frequencies of comorbidity of cluster B or C personality disorders with groups of Axis I diagnoses were observed for affective disorders (F3x, \(n = 80\)) and anxiety-related disorders comprising neurotic, stress-related, or somatoform disorders (F4x, \(n = 166\)).

Table 6 presents the results of the respective association tests. Highly significant global associations of rs4570625 and rs11178997 were observed for both F3x disorders (\(p_{corr} = 0.002\)) and F4x disorders (\(p_{corr} = 0.009\)). Marker-specific tests revealed highly significant positive regression weights for both markers, indicating that the rs4570625 T allele and the rs11178997 A allele were more frequent in the respective patient groups.

Subsequent analyses for specific F3x and F4x disorders were performed only for groups with \(n \geq 15\), i.e. bipolar affective disorder (F31, \(n = 18\)), depressive episode (F32, \(n = 30\)), recurrent depressive disorder (F33, \(n = 28\)), phobic anxiety disorder (F41, \(n = 18\); two subjects with an F41.x diagnosis other than F41.0 were excluded from the analyses) and for adjustment disorder (F43.2, \(n = 99\); two subjects with an F43.x diagnosis other than F43.2 were not considered). For F43.2, significant associations emerged at both the global level (\(p_{corr} = 0.002\)) and the local level (rs4570625: \(b = 0.63, p = 0.002\); rs11178997: \(b = 1.06, P < 0.001\)). For the other subgroups, no global association was observed at the Bonferroni-adjusted level of significance (all \(p \geq 0.126\)). Therefore, subsequent marker-specific tests were not performed.

In order to exclude the possibility that personality disorder–SNP associations are based on Axis I disorders, patients without Fx diagnosis were also compared with F3x and F4x groups as well as with controls. Whereas results for rs4570625 and rs1117897 remained unchanged when comparing controls with personality disorder patients without Fx diagnosis (Table 6), no significant effects were observed when comparing patients with and without Axis I comorbidity in this cohort (global tests, all \(p_{corr} \geq 0.804\)). However, the comparatively low statistical power due to the small sample sizes do not entirely rule out an effect of personality disorders on the risk to subsequently develop an Axis I disorder.

**Association between TPH2 polymorphisms and personality traits**

At the global level, the TPH2 markers were significantly associated with TPQ Harm Avoidance (\(p = 0.013\), Table 7), a significant marker-specific association was observed for rs4341581 (\(b = 0.70, p = 0.001\)), indicating higher Harm Avoidance scores in carriers of the rs4341581 G allele. Furthermore, haplotype analyses revealed a significant global haplotype association with Harm Avoidance (\(p = 0.028\), Table 8). On the haplotype-specific level, this global haplotype association was mainly accounted for by higher Harm Avoidance in carriers of the rs4570625-rs11178997-rs4341581-rs4565946 G-T-G-C haplotype (\(b = 0.69, p = 0.002\)).

Given the overlap between TPQ Harm Avoidance and NEO Neuroticism in the current sample (\(r = 0.66, p > 0.001\)), we conducted further analyses to examine whether potential associations between TPH2 variants and Harm Avoidance are also evident for
Neuroticism. The global association missed the Bonferroni-adjusted level of significance ($p_{corr} = 0.092$), but there was a significant marker-specific association with rs11178997 ($b = 0.44$, $p = 0.025$), reflecting higher Neuroticism scores in carriers of the rs11178997 A allele. Haplotype association with Neuroticism also did not reach significance (global $p = 0.173$, Table 8). Despite this lack of a global haplotype association with Neuroticism, the G-T-G-C haplotype which was associated with Harm Avoidance was also significantly associated with Neuroticism ($b = 0.43$, $p = 0.048$). Furthermore, the T-A-T-C haplotype, which was strongly associated with cluster B and cluster C personality disorder (also see Table 5), was significantly and positively associated with Neuroticism in healthy controls.

For the remaining TPQ and NEO personality traits, no significant global association with $TPH2$ variants emerged (all $p_{corr} > 0.402$, adjustment based on seven traits analysed). Therefore, single-marker
tests were not done. Likewise, no significant haplotype association was observed (all \( p_{corr} \geq 1.00 \) for these traits.

**Discussion**

The present results link potentially functional TPH2 variants, particularly SNP rs4570625, to personality traits related to emotional instability as well as to cluster B and cluster C personality disorders. While rs4570625 affected both patients with personality disorders and healthy volunteers, distinct associations were observed between rs11178997 and patients as well as between rs4341581 and healthy individuals, respectively. Haplotype analyses largely reflected the findings of single-marker analyses. The association between rs4570625 and personality disorders remained significant when Axis I disorders were excluded, although an incremental effect was noted in patients with a comorbid F3x disorder cannot be ruled out.

Because certain temperamental traits and personality dimensions are considered to represent risk factors for the development of psychiatric disorders, we examined whether genetic variation in TPH2 is associated with individual differences in personality dimensions in healthy volunteers using the TPQ and NEO-PI-R. Our analyses focused on anxiety- and depression-related traits, namely on TPQ Harm Avoidance and NEO Neuroticism. Both traits show considerable conceptual and empirical overlap. However, in contrast to Costa and McCrae (1992) who do not provide a biopsychological explanation for individual differences in NEO Neuroticism, Cloninger and associates (Cloninger, 1987; Cloninger et al., 1991) postulate a primarily serotonergic modulation of individual differences in TPQ Harm Avoidance. Based on this view and on additional evidence from association studies with other gene variants of the 5-HTT polymorphisms are associated with individual differences in Harm Avoidance. Our results are supported by a report of Reuter and co-workers (2006) also demonstrating a significant association between rs4570625 and TPQ Harm Avoidance (\( \chi^2 = 10.06, \text{d.f.} = 2, p = 0.007 \)) in an independent cohort of healthy volunteers. Taken together, these findings implicate alterations of 5-HT synthesis in emotion regulation and confirm TPH2 as a susceptibility and/or modifier gene of affective spectrum disorders.

5-HT is critically involved in the modulation of cognition and affect (Clarke et al., 2004; Murphy et al., 2002; Riedel et al., 2002). Common variation within the transcriptional control region of serotonergic genes, such as the 5-HT transporter (5-HTT) and the 5-HT\(_{1A}\) receptor (HTR1A), are associated with anxiety- and depression-related traits (Lesch et al., 1996; Strobel et al., 2003), and were shown to influence brain activation in response to emotional stimuli across a number of different paradigms (Canli et al., 2005; Hariri et al., 2005; Heinz et al., 2005; Pezawas et al., 2005). Unlike 5-HTT and HTR1A, much less is known about the impact of variants in the transcriptional control region of TPH2, which codes for the rate-limiting enzyme of 5-HT synthesis specifically in the brain.

While single-marker and haplotype analyses of variants within the structural gene provided initial evidence for a link between TPH2 and affective disorders (Harvey et al., 2004; Zill et al., 2004), recent studies further underscored the impact of TPH2 variation and altered TPH2 brain expression on disorders featuring emotional dysregulation including depression, bipolar disorder, anxiety disorders, and suicide (Bach-Mizrachi et al., 2005; De Luca et al., 2005a; Preisig et al., 2005; Zhou et al., 2005). Likewise, other disorders commonly sharing a biological relationship based on emotional instability, such as ADHD and OCD, have also been linked to TPH2 variation (Mossner et al., 2006; Walitzka et al., 2005). Subsequently, functionality and thus the intrinsic relevance of variants in the upstream regulatory region of TPH2, particularly of SNP rs4570625 or a variation in LD with it, was confirmed by fMRI with an affective face-matching paradigm that revealed an allele-dependent responsiveness of the amygdala, which modulates emotional behaviours (Brown et al., 2005; Canli et al., 2005). Moreover, using a passive emotional picture perception task, measurement of event-related potentials (ERPs) during early steps of visual processing revealed an additive effect of both the TPH2 SNP rs4570625 and the low-expression short variant of 5-HTT on the sensory encoding of affective stimuli (Herrmann et al., 2006). A potential impact of TPH2 variation on amygdala activation is also supported by the work of Cools and co-workers (2005) examining the effects of acute tryptophan depletion, which results in a transient reduction of cerebral 5-HT, on brain activation to fearful faces. They found that reduction in 5-HT signalling increases amygdala responses to fearful faces suggesting that variation in threat sensitivity interacts with 5-HT function to influence the processing of emotional stimuli. The increase in amygdala response is likely to be a consequence a compensatory decrease of 5-HT transporter function and concomitant increase of synaptic 5-HT
availability (Milak et al., 2005). The findings that both the rs4570625 T variant, which may result in low functional expression of TPH2, and impaired 5-HT function increase amygdala responsiveness to emotional facial expressions indicate the presence of, and further underscore the physiological significance of, regulatory TPH2 variation in cognitive and affective brain processes.

Although SNP rs4570625 affects amygdala response to emotional stimuli by having the potential to modify expression of TPH2, the transcriptional control region and regulatory elements of TPH2 expression have not yet been studied in detail. Elucidation of a functional effect of regulatory SNPs will require further detailed biochemical and allele-specific expression studies. Indirect evidence that the potentially functional impact of the two SNPs, either individually or in concert, results in lower enzyme activity is provided by a study in mouse strains exhibiting allelic variation in TPH2 function. For example, mice of the BALBc strain carrying a loss-of-function Pro447Arg mutation in Tph2 show altered emotionality and behavioural deficits including impaired maternal behaviour (Zhang et al., 2004; V. Carola, K. P. Lesch, C. Gross, unpublished observations).

Taken together, imaging approaches and results from genetic modification of 5-HT signalling in mice indicate that anxiety-related behaviour and increased stress reactivity probably represents a consequence of increased terminal 5-HT availability. This mechanism is also consistent with recent theoretical models of anxiety that are primarily based upon pharmacologically derived data. On the other hand, the cumulative reduction in serotonergic impulse flow to septo-hippocampal and other limbic and cortical areas involved in the control of anxiety is believed to explain the anxiolytic effects of ligands with selective affinity for the 5-HT1A receptor in some animal models of anxiety-related behaviour. While increased 5-HT signalling and activation of other serotonergic receptor subtypes that have been shown to mediate anxiety may contribute to anxiety-related behaviour, multiple downstream neurotransmitter pathways or neurocircuits, including dopaminergic, noradrenergic, GABAergic, glutamatergic, and peptidergic transmission, as suggested by overexpression or targeted inactivation of critical genes within these systems (Lesch et al., 2003), have been implicated in the processing of this complex behavioural trait. Since avoidance induced by conflict and fear is only one dimension of anxiety-related responses, other components including autonomic systems activation, responsiveness to stress, 5-HT dynamics, and neuronal excitability in limbic circuitries appear to be involved in anxiety.

Cluster B and cluster C personality disorders are genetically complex and are like other disorders of cognitive and emotional dysregulation considered to be related to altered serotonergic neurotransmission. While conditions of low 5-HT elicited by tryptophan depletion are associated with cognitive impairment, the prefrontal cortex, which plays a crucial role in working memory, cognition, and control behaviours, displays high densities of inhibitory 5-HT1A receptors and excitatory 5-HT2A receptors which are targeted by an extensive network of serotonergic projections from the raphe nuclei (Martin-Ruiz et al., 2001; Puig et al., 2003, 2005). Although pyramidal neurons of the prefrontal cortex co-express both receptors, their NMDA receptor-mediated ionic and synaptic currents are inhibited by physiological concentrations of 5-HT (Yuen et al., 2005). In addition, 5-HT inhibits pyramidal neurons indirectly through activation of 5-HT1A and 5-HT4 receptors located on GABAergic interneurons. Finally, dorsomedial prefrontal cortex involvement is supported by clinical observations of executive-function deficits, including failure to regulate emotions, difficulty to cope with stress, and inability to restrain impulsive or aggressive actions (Amat et al., 2005; Evers et al., 2005; Heinzel et al., 2005). Since TPH2 is the rate-limiting enzyme in the biosynthesis of 5-HT in the brain, genetic variation in TPH2 activity is likely to represent a critical factor in the pathogenesis of syndromal dimensions characteristic for cluster B and cluster C personality disorders.

In conclusion, allelic variation in TPH2 function is likely to play a critical role in the development and modulation of individual differences in anxiety-related personality traits. Whether TPH2 variation contributes to the general tendency for individuals who score higher on anxiety factors in personality questionnaires to be at higher risk for psychopathological sequelae of stressful life events is currently the objective of further study. It likewise remains to be investigated whether therapeutic responses to serotonergic agents are influenced by TPH2 variation.

Acknowledgements

We express our gratitude to all probands for their participation. The excellent technical assistance of N. Steigerwald is greatly appreciated. The work was supported by the Deutsche Forschungsgemeinschaft (SFB 581; GRK 1156, KFO 125/1), the Fritz Thyssen Stiftung, and the European Commission (NEWMOOD LSHM-CT-2003–503474).
Statement of Interest

None.

References


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