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No association between a TPH2 promoter polymorphism and mood disorders or monoamine turnover[☆]

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Abstract

Background—Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in serotonin synthesis. TPH2 is a recently discovered isoform that is expressed predominantly in serotonin neurons. Associations are reported of TPH2 polymorphisms with MDD, bipolar disorder and suicidal behavior. This study examines a single nucleotide polymorphism in the putative promoter region of the TPH2 gene.

Methods—One hundred nine bipolar, 324 major depressive disorder, and 130 healthy volunteers were genotyped for the rs4131347 (–C8347G) promoter SNP. Association was assessed with diagnosis, suicide attempt status, severity of psychopathology and cerebrospinal fluid monoamine metabolite levels of 5-HIAA, HVA, and MHPG. General linear models and logistic regression tested the effect of genotype* childhood abuse interactions on psychopathology severity and suicide attempt.

Results—There was no association between genotype and either mood disorder, suicide attempt status, psychopathology severity or CSF monoamine metabolite levels.

Conclusions—No association was detected between the rs4131347 (–C8347G) SNP in the promoter region of the TPH2 gene and mood disorders, suicidal behavior or monoamine function.

Keywords

TPH2; Single nucleotide polymorphism; Bipolar disorder; Major depressive disorder; Suicide attempt

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Conflict of Interest No conflict declared.

1. Background

Serotonergic dysfunction has been implicated in the etiology of major depressive disorder (MDD), bipolar disorder, schizophrenia, substance use disorders, aggression and suicidal behavior. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in serotonin synthesis. There are two TPH isoforms; TPH1, expressed in peripheral tissues; and the more recently discovered TPH2, expressed predominantly in the brain (Walther et al., 2003). TPH2 mRNA has been detected in multiple brain regions including frontal cortex, thalamus, hippocampus, hypothalamus and amygdala (Zill et al., 2004b) perhaps originating with gene expression in brainstem serotonin raphe neurons (Zill et al., 2007; Bach-Mizrachi et al., 2006).

After some, but not all, initial studies reported TPH1 single nucleotide polymorphism (SNP) associations with suicidal behavior (Bellivier et al., 2004), mood disorder (Tsai et al., 1999), and aggression (Rujescu et al., 2002), attention turned to TPH2. Some haplotype studies suggest association of the TPH2 gene to major depression (Zill et al., 2004a; Van Den Bogaert et al., 2006; Zhou et al., 2005), suicide (Zill et al., 2004c), suicide attempt (Zhou et al., 2005; de Lara et al., 2007), and bipolar disorder (Lopez et al., 2007; Harvey et al., 2004; Van Den Bogaert et al., 2006), and others disagree (De Luca et al., 2004, 2006, 2005b).

SNP studies have reported associations between TPH2 genotype and MDD and bipolar disorder in a genetically isolated Swedish sample (Van Den Bogaert et al., 2006), and with MDD and suicidal behavior in Chinese (Ke et al., 2006) and German Caucasian (Zill et al., 2004a) samples. These associations have yet to be replicated, and studies of over 20 other TPH2 SNPs have not detected associations with psychiatric disorders or suicidal behavior (Van Den Bogaert et al., 2006; Zill et al., 2004a, 2004c; Lopez et al., 2007; De Luca et al., 2005b, 2004; Harvey et al., 2004).

Two SNPs in the putative promoter region of the TPH2 gene, rs4131347 (–C8396G) and rs11178997 (–473A/T) are of potential greater interest because they may affect gene expression, which has been shown to be increased in the brainstem of depressed suicides (Bach-Mizrachi et al., 2006). They were not associated with suicide in a postmortem sample of schizophrenic and bipolar cases (De Luca et al., 2005a), but the sample was too small to consider an effect of diagnosis. Therefore, in this study we examined rs4131347 (–C8396G) SNP (hereafter referred to as (–C8347G) in accordance with GenBank notation) in a much larger mood disorder sample, for possible association with mood disorder and suicide attempt, and explored putative genotype environmental interaction effects on severity of psychopathology ratings in mood disorders. We also carried out exploratory analysis of CSF levels of the monoamine metabolites for serotonin (5-HIAA), dopamine (HVA), and norepinephrine (MHPG) with respect to genotype.

2. Methods

DNA samples were obtained from 433 unrelated psychiatric patients and 130 healthy volunteers who gave written consent as required by the Institutional Review Board. Volunteers had responded to advertisements. Patients had been referred for evaluation of major depressive episodes or other major psychiatric disorders. Patients and healthy volunteers were assessed by psychiatrists or clinical psychologists and diagnosed according to DSM-IV criteria for Axis I and Axis II diagnoses using the SCID-I, SCID-II or SCID-NP (First et al., 2002; First et al., 1996). Ratings of depression severity, hopelessness, impulsivity, aggression, hostility and suicidal ideation were conducted using the Hamilton Depression Rating Scale (17 Item), Beck Depression Inventory, Beck Hopelessness Scale, Barratt Impulsivity Scale, Brown–Goodwin Lifetime History of Aggression scale, Buss–Durkee Hostility Scale and the Scale for Suicidal Ideation as previously described (Oquendo et al., 2004).

With the exception of African Americans, ethnic groups did not differ significantly in genotype frequency. Therefore we omitted African American subjects and combined all other ethnic groups for subsequent analyses. The remaining sample was predominantly Caucasian $N=529$ (94%), with $N=26$ (4.6%) Asians, and $N=8$ (1.4%) identifying as belonging to more than one ethnic group. Patient diagnoses were: $N=109$ (21%) bipolar disorder and $N=324$ (63%) major depressive disorder. $N=187$ (43%) of patients reported at least one prior suicide attempt.

2.1. DNA extraction and genotyping

DNA samples were isolated as previously described from buffy coat fraction and from buccal mucosa cheek swabs (Huang et al., 2003) (BuccalAmp DNA Extraction Kit, Epicentre, Madison, WI, USA). The TPH2 polymorphism (GenBank dbSNP: rs4131347; -C8347G) was typed by PCR and *Sma* I restriction enzyme. Briefly, the oligonucleotide primer set design used sense MTPH8437-2F: 5'-TTGGTCCAGGGTTCATCAT-3' and antisense MTPH8437-2R: 5'-TTGTGGCCAAGAACTTTGG-3'. PCR was carried out in a 20 μ l volume, containing 100 ng DNA, 40 ng of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.15 mM MgCl₂, 5% DMSO, 0.001% gelatin, 200 μ M of each dNTP, and 0.8 U of JumpStart REDTaq DNA Polymerase (Sigma, MO, USA). Samples were processed in a Robocycler (Stratagene, CA, USA). Thirty temperature cycles were carried out, consisting of 30 s at 95 °C, 30 s at 55 °C, and 40 s at 72 °C, followed by a final extension step of 72 °C for 4 min. The PCR fragments were digested with *Sma* I restriction enzyme (NE Biolab, MA, USA) and the digested PCR products were separated on a 1.5% agarose gel.

2.2. CSF monoamine metabolite analysis

Lumbar puncture was performed in a subsample of mood disorder patients and controls ($N=143$) and cerebrospinal fluid 5-HIAA, HVA, and MHPG were assayed by high-performance liquid chromatography (HPLC) with electrochemical detection as described previously (Placidi et al., 2001).

2.3. Statistics

Genotype and allelic distributions in diagnostic groups and controls, and suicide attempters and non-attempters, were compared groups using the Chi² contingency table test. One-way analysis of variance was used to compare psychopathology rating scales, and CSF 5-HIAA, HVA, and MHPG levels by genotype. General linear models (GLMs) and logistic regression were used in exploratory analyses to test for possible effects of interactions between genotype and history of childhood abuse on clinical measures of severity of psychopathology including, hopelessness, self-rated and clinician-rated depression, aggression, hostility and impulsivity, and presence or absence of suicide attempt and suicidal ideation. The models were run for all psychiatric patients combined and then for MDD and bipolar disorder patients separately. In general linear models, clinical measure was the dependent variable and independent variables were childhood abuse (yes/no), genotype, and an interaction term of childhood abuse* genotype. In logistic regression models, presence or absence of suicide attempt and suicidal ideation was the dependent variable, and independent variable as described above.

3. Results

3.1. Genotype effects

The genotype distributions in the healthy volunteer and patient groups were in Hardy-Weinberg equilibrium as assessed by the χ^2 test ($\chi^2=.09$, $df=1$, $p=0.76$; $\chi^2=0.09$, $df=1$, $p=0.764$, respectively). There were no differences between any diagnostic group and healthy volunteers in genotype (any Axis I diagnosis: $\chi^2=.531$, $df=2$, $p=.839$; bipolar: $\chi^2=.1894$, $df=2$, $p=.388$; MDD: $\chi^2=.759$, $df=2$, $p=.684$) or allelic frequency (Table 1).

There were no differences in genotype or allelic frequency between suicide attempters and non-attempters (psychiatric patients only: $\chi^2=1.072$, $df=2$, $p=.585$). There were no differences by genotype on severity of psychopathology for depression, suicidal ideation, hopelessness, aggression, hostility or impulsivity, or incidence of reported childhood physical or sexual abuse, within major diagnostic groups (MDD, BP), or in all mood disorder patients combined (data available on request). There was no association of genotype or allele with CSF 5-HIAA, HVA, or MHPG levels (Table 2).

3.2. Genotype and history of childhood abuse interaction

There were no significant interaction effects on any clinical measure in the GLMs or logistic regression models in the combined mood disorder group (MDD and bipolar) or the bipolar disorder group. In the MDD group, the presence of the CG genotype and a positive history of childhood abuse were associated with higher Beck Depression Inventory scores (model: $F=3.385$, $p=.005$; CG* abuse: $b=8.426$, $SE=3.04$, $t=2.765$, $p=.006$), greater likelihood of suicidal ideation (model $\chi^2=59.9$, $df=6$, $p<.001$; CG* abuse B 1.24, $SE=.523$, $Wald=5.345$, $df=1$, $p=.021$), and higher lifetime impulsivity scores (model: $f=6.776$, $p=.001$; CG* abuse $b=9.865$, $SE=4.193$, $t=2.352$, $p=.019$). The CC genotype and a positive history of abuse were associated with lower impulsivity scores (CC* abuse $B=-23.01$, $SE=9.81$, $t=-2.497$, $p=.02$).

4. Discussion

We found no association of the TPH2 promoter SNP rs4131347 (–C8347G) with MDD, bipolar disorder, suicide attempt or ideation, or with severity of current depression, hopelessness, lifetime aggression, hostility or impulsivity. We also found no association of genotype with CSF levels of 5-HIAA, HVA, or MHPG. The only other study to examine this SNP also found no association with suicide in a small postmortem sample of bipolar disorder and schizophrenic cases (De Luca et al., 2006). No effect of this polymorphism on transcription factor-binding sites or TPH2 mRNA expression has also been reported (De Luca et al., 2006; De Luca et al., 2005a). Both our group and De Luca and colleagues have found higher TPH2 gene expression in depressed suicides and bipolar disorder, respectively (Bach-Mizrachi et al., 2006; De Luca et al., 2005a). This altered gene expression could be due to a structural gene variant, or due to effects of transcription factors, or compensation for a variant with impaired catalytic activity, however there is no evidence, to date, that the promoter polymorphism we report on in this study has functionality in gene expression, or is associated with altered CSF monoamine levels.

Given the multidimensionality of mood disorders, genetic variation may affect only certain characteristics or symptom clusters of a disorder. In exploratory analyses, we found no association between genotype and severity of clinical characteristic including current depression, suicidal ideation, hopelessness, lifetime aggression, hostility or impulsiveness, either within mood disorder subgroups (MDD or bipolar) or in the combined mood disorder group. When we evaluated a possible interaction between reported childhood adversity (physical and/or sexual abuse before the age of 15), genotype, and severity of psychopathology, effects were observed on suicidal ideation, self-rated depression, and impulsivity. However, these effects occurred primarily in the heterozygote CG genotype group, while the CC and GG genotypes had comparable severity. There is no plausible functional explanation why heterozygotes should be more severely affected compared with either homozygote group, which do not differ from each other. Given the lack of functionality found for this SNP by us and by (De Luca et al. 2006; 2005a), these findings may indicate a linkage disequilibrium with some as yet unidentified functional SNPs, or they may be spurious.

4.1. Limitations

The CC genotype was relatively rare and thus this study may have been underpowered for identifying effects associated with that genotype when the mood disorder group was divided into smaller subsamples in the GLMs. Complex disorders such as MDD and bipolar disorders most likely involve multiple genes and epigenetic phenomena and thus association studies of single SNPs will only provide, at best, a partial insight into the genetic basis of such disorders.

5. Conclusion

The –C8347G polymorphism in the TPH2 gene is not associated with MDD and bipolar disorders or with suicide attempt or CSF levels of 5-HIAA, HVA, or MHPG in psychiatric patients.

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Table 1

Genotype distributions, allele frequencies for TPH2 gene SNP rs4131347 (–C8347G) in healthy volunteers and psychiatric subjects with a history of bipolar disorder or MDD

	Genotype distribution (%)				Allele frequency (%)			
	N	GG	CG	CC	P	G	C	P
Healthy volunteers	130	68 (52)	53 (41)	9 (7)		189 (73)	71 (27)	
All mood disorder patients	433	239 (55)	167 (39)	27 (6)	.839	645 (74)	221 (26)	.564
Bipolar disorder	109	62 (57)	36 (33)	11 (10)	.388	160 (73)	58 (27)	.863
Major depression	324	177 (55)	131 (40)	16 (5)	.684	485 (75)	163 (25)	.502
Suicide attempt (yes) ^a	187	103 (55)	70 (37)	14 (8)	.626	276 (74)	98 (26)	.687

^a *p* values are for comparisons of suicide attempters to non-attempters in mood disorder patients only.

Table 2

TPH2 gene SNP rs4131347 (–C8347G) genotype and CSF 5-HIAA, HVA, and MHPG levels (pmol/ml) in mood disorder and controls groups combined

	GG	CG	CC	
	(N=8)	(N=52)	(N=83)	
	(Mean±SD)	(Mean±SD)	(Mean±SD)	
CSF 5-HIAA	93.95±30.9	100.9±27.6	98.71±23.8	$F=.927, df=2, p=.398$
CSF HVA	204.3±80	202.8±77.3	228.6±55.2	$F=.391, df=2, p=.677$
CSF MHPG	46.04±20.4	47.52±20	42.25±17.5	$F=.256, df=2, p=.769$