

NEURONAL TRYPTOPHAN HYDROXYLASE (TPH2) IN SUICIDAL BEHAVIOUR

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Abstract

Serotonin (5-hydroxytryptamine, 5-HT) is a signalling molecule involved in many brain functions as well as in a variety of psychiatric conditions, including suicidal behaviour. Tryptophan hydroxylase (TPH), the rate-limiting enzyme of 5-HT biosynthesis, plays a critical role in 5-HT metabolism and thus in the regulation of 5-HT neurotransmission. There are two isoforms of TPH, so-called peripheral (TPH1) and neuronal (TPH2), encoded by the two distinct genes. TPH1 is abundant in the peripheral tissues, while TPH2 was detected exclusively within the nervous system. Since its discovery in 2003, TPH2 gene has attracted great interest as a potential factor contributing to diathesis of suicidal behaviour. However, initial positive findings indicating an association of TPH2 genetic variants with suicide have not been replicated consistently. Here we provide an overview of published studies to date, that have evaluated the contribution of TPH2 gene to the etiopathogenesis of suicidal behaviour.

Keywords

Serotonin • Tryptophan hydroxylase • Gene • Polymorphism • Suicide

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1. Introduction

Suicide is a major mental health problem and increasingly a serious public health issue. According to World Health Organization (WHO), over one million of people worldwide annually die from suicide (<http://www.who.int/topics/suicide/en>). Among people aged 15-34 years, suicide appears to be one of the three leading causes of mortality in many countries. WHO estimates that one suicide attempt occurs approximately every three seconds, while one completed suicide occurs approximately every minute. Yet, suicide is regarded as a preventable cause of death. Therefore, strategies for reducing suicide rates, which would rely on the identification of individuals at highest risk, have become an important international health goal [1]. The causes of suicide are heterogeneous and include complex gene-environment interactions. A number of broad factors have been identified which interact to place an individual at risk; including socio-cultural influences, psychiatric conditions, neurobiology,

genetics, and stressful events [2]. Neurobiological correlates of suicide have been repeatedly linked to deviations in brain monoamines, particularly serotonin (5-hydroxytryptamine, 5-HT) [3]. In addition, epidemiological data from family, twin and adoption studies have provided evidence for the role of genes in the etiology of suicidal behaviour [4]. During the last decade, considerable effort has been devoted towards identifying genetic risk factors for suicidal behaviour. Variations in genes related to the metabolism and functioning of 5-HT have been the most widely investigated as potential candidates [5]. Our attempts in this regard have been also focused on the genes encoding 5-HT synaptic proteins, such as serotonin transporter [6,7] and serotonin receptors [8,9]. So far, only promoter polymorphism of 5-HT transporter gene has emerged as being implicated in the susceptibility to suicidal behaviour [5].

Tryptophan hydroxylase (TPH, EC 1.14.16.4) is the rate-limiting enzyme in the biosynthesis of 5-HT and plays a vital role in the regulation of 5-HT neurotransmission. For many years it

was believed that TPH was encoded by a single gene, today referred to as TPH1. TPH1 gene has been extensively investigated for its association with suicidal behaviour [10,11]. It was not until recently that a generation of mice lacking this gene led to the discovery of a second TPH isoform, encoded by a distinct gene [12]. This newly identified isoform, termed neuronal TPH or TPH2, was detected specifically within the nervous system, while the previously known isoform, called thereafter peripheral isoform or TPH1, was found to be abundant in peripheral tissues such as pineal gland and the gut. Recent genetic deletion [13-15] and expression [16] studies convincingly point to TPH2 as the exclusive isoenzyme responsible for the production of 5-HT in the brain. Consequently, variants in TPH2 gene have become regarded as prime candidates for the susceptibility to serotonin-related psychiatric disorders, including suicide. The present paper provides overview of studies that have so far evaluated the role of TPH2 gene in the susceptibility to suicidal behaviour.

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2. Sequence variation in TPH2 gene

TPH2 gene (NCBI GeneID: 121278) covers a region of about 93.5 kilobases on human chromosome 12q21.1. Polymorphisms and mutations of TPH2, a highly polymorphic gene, have been of great interest for their potential involvement in the regulation of 5-HT signalling, as well as in the etiopathogenesis of disorders related to 5-HT dysfunction. So far, 700 TPH2 sequence variants have been recorded in dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). The majority of them represent common single nucleotide polymorphisms (SNPs) located mainly in non-coding regions of the gene. Coding sequence variants appear to be rare, as is the case for most human genes. Three nonsynonymous mutations of TPH2 gene have been implicated in the etiopathogenesis of different psychiatric disorders, although statistical validation of these results remains controversial due to extremely low frequency of the mutant alleles [17-19]. Effects of these and several other coding mutations on the activity, stability and solubility of the TPH2 enzyme have been comprehensively studied [19-21]. Conversely, very little is known about the functional consequences of common variants in TPH2, despite a great number of studies investigating their association with different psychiatric disorders. Most of the available functional data relates to rs4570625, a common SNP located in the promoter region of the gene. Neuroimaging studies have provided evidence for the impact of rs4570625 on the intermediate phenotypes such as reactivity of amygdala [22-25] and amygdalar and hippocampal volume [26]. The respective SNP and a nearby SNP rs11178997 were reported to affect TPH2 expression in different *in vitro* systems, though data were not unequivocal [27-29]. On the other hand, in post-mortem sections of human pons, rs4570625 only weakly correlated with TPH2 mRNA levels, while strong correlation was found between TPH2 mRNA levels and SNPs located in exon 7 (rs7305115) and introns 5 (rs2171363), 6 (rs4760815), 7 (rs6582078) and 8 (rs9325202) [30]. Similarly, in human prefrontal cortex, TPH2 mRNA expression was found to be

correlated with intronic SNP rs10748185 [31], but not with promoter SNPs rs11178997 and rs41313475 [32]. Taken together, correlation between genetic variation of TPH2 and expression levels of this gene should be further evaluated in different cellular systems as well as in native brain tissues. Besides genomic variation, alternative splicing and RNA editing of TPH2 transcripts should be also considered as a substrate of TPH2 phenotypic diversity [33].

3. TPH2 expression in the brain of suicide subjects

A number of post-mortem studies have examined expression of TPH2 in the brain of suicide victims. The majority of them have found increased levels of TPH2 protein [34-36] and mRNA [31,37,38] in suicide patients as compared to control subjects. Severe suicidality has been consistently associated with low brain or cerebrospinal fluid (CSF) concentrations of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) [4]. Furthermore, subjects with highly lethal suicide attempts were shown to have diminished brain 5-HT synthesis [39]. These findings seemingly contradict results on the increased brain expression of TPH2 in suicide. It was speculated that over-expression of TPH2 in suicide could reflect a homeostatic response to deficient serotonin levels [38]. However, it may also be a consequence of acute or early life stressful events [40-42]. It should be noted that several studies reported no significant changes of TPH2 expression in brains of suicide victims [43-45]. It was recently hypothesised, based on contemporary research, that association between 5-HT and certain psychiatric conditions might arise as a consequence of events taking place during neural development [46]. Namely, altered 5-HT synthesis during brain development period could potentially shape a lifelong risk for suicidal behaviour. Therefore, environmental and genetic factors that influence TPH2 function either in adulthood or during brain development period might be of interest as potential substrates in the etiology of suicide behaviour.

4. Association between variants in TPH2 gene and suicidal behaviour

Since its discovery, TPH2 gene has attracted great interest as a candidate for the genetic background of suicidal behaviour [12]. Results of population-based studies investigating association of TPH2 single loci and/or haplotypes with suicidal behaviour, in different populations and within different diagnostic categories, are summarized in Table 1 and Table 2. Out of nine studies, three reported positive results for the association of TPH2 variants with suicide completion (Table 1), while four out of eight reported positive results for the association of TPH2 variants with suicide attempt (Table 2). In addition, two studies, one yielding negative [47] and another positive [48] results, have applied family-based approach to address association of TPH2 polymorphisms with suicidal attempt in bipolar disorder patients.

Haplotype analyses appear to be the most robust tools for genetic dissection of complex disorders. Initial TPH2 haplotype study on German population reported differences between suicide victims and control subjects in the distribution of three haplotypes comprising of 10 intronic SNPs (after Bonferroni correction, the results remained significant only for the haplotype that was present only in control subjects with the frequency of 3%) [49]. In addition, single marker analysis demonstrated differences between the groups in the distribution of one SNP in intron 5 (rs1386494) [49]. A follow-up study examined associations between 15 TPH2 SNPs and suicide attempt in three different populations [50]. Positive results in haplotype and single locus analyses were demonstrated in two out of three populations; however, single SNPs showing significant associations varied between the populations [50, Table 2]. Haplotype study of 12 TPH2 SNPs on French Canadian population provided some evidence for the involvement of TPH2 5'-upstream variants in suicide completion in major depression [51]. However, positive results on the association of suicide with rs1386494 [49], could not be replicated on this population [51]. Haplotype analyses performed on USA Caucasians suggested that TPH2 gene might play a role in major depression, but not in suicide [52]. Furthermore, haplotypes derived

Table 1. Population-based studies on the association of common variants in TPH2 gene with suicide completion.

CASE subjects		CONTROL subjects		Origin	Investigated SNPs (ID and/or location)	Results	Year of publication (reference)
Diagnosis	N	Diagnosis	N				
Not available	263	Healthy subjects	266	German Caucasian	10 intronic SNPs (introns 5 and 6)	Single marker association with rs1386494 (intron 5); Association with 3 haplotypes encompassing 10 SNPs	2004 [49]
SCH or BD	22	SCH or BD without SC	47	Undefined	rs11178997, rs4131347 (promoter)	No single marker or haplotype association	2006 [45]
MDD	114	MDD without SC	145	French Canadian	rs4448731, rs6582071, rs7963803 (5'-UTR); 9 intronic SNPs (introns 1, 4, 5, 6, 7 and 8)	Single marker association with rs4448731, rs6582071 (5'-UTR), rs4641527 (intron 1) and rs1386497 (intron 8); Association with haplotype encompassing rs4448731, rs6582071, rs7963803 (5'-UTR), rs4641527 (intron 1) and rs2129575 (intron 4)	2007 [51]
72 MDD and 4 no MDD	76	68 healthy subjects, 12 no MDD disorders	80	USA Caucasian	6 intronic SNPs (intron 5)	No single marker or haplotype association	2008 [52]
Not available	288	Healthy subjects	327	Estonian	14 tagging SNPs (9 in introns, 1 in 3'-UTR, 4 in adjacent regions)	No single marker or haplotype association	2009 [56]
Not available	234	Healthy subjects	260	Japanese	15 tagging SNPs (4 in promoter, 8 in introns, 2 in exons, one in 3'-UTR)	No single marker or haplotype association	2009 [55]
Not available	369	Healthy subjects	373	German Caucasian	rs4290270 (exon 9)	Association with AA genotype	2010 [33]
Various	154	Undefined	289	West European Caucasian	rs10748185 (intron 2)	No association	2010 [31]
Not available	281	Healthy subjects	286	Croatian	rs4570625 (promoter)	No association	2010 [58]

BD - bipolar disorder; ID - identification number in NCBI Single Nucleotide Polymorphism database (dbSNP); MDD - major depression disorder; SC - suicide completion; SCH - schizophrenia; SNP - single nucleotide polymorphism; UTR - untranslated region.

from 20 and 8 TPH2 SNPs demonstrated no significant association with suicide attempt in either alcohol dependence [53] or bipolar disorder [54] patients, respectively; single locus associations were also not detected in these two studies [53,54]. Finally, two recent haplotype analyses based on 14 and 15 tagging SNPs, and performed on fairly large samples of suicide victims of Japanese [55] and Estonian [56] origin, respectively, strongly suggested that common SNPs in the TPH2 gene are unlikely to be susceptibility factors for suicide completion.

Several studies have focused on the single SNPs in the TPH2 upstream region that could participate in the regulation of TPH2 expression. Of particular interest was rs4570625 due to the related functional data [22-29]. A positive result was reported for the association of this SNP with suicide attempt in Korean population [57]. Two other studies [50,53], however, found no

evidence for the effect of rs4570625 on suicide attempt in three different populations (Table 1). Results on suicide victims, obtained recently by the group of Mouri et al. [55] and our group [58], demonstrated no effect of rs4570625 on suicide commitment in Japanese and Croatian population, respectively. The association observed in the Korean population could possibly be related to depression or depression associated with a suicide attempt, since no differences in the genotype distributions were found between the groups of depressive patients with and without history of suicide attempt (Table 1) or the groups of non-suicidal depressive patients and healthy controls [57]. Similarly, as demonstrated by a family-based approach, genetic variation of TPH2 did not appear to be an independent risk factor for suicidal behaviour in bipolar disorder patients, though it was associated with both bipolar disorder and suicide attempt

[48]. Four other SNPs in TPH2 promoter region have been analysed. Among them, rs11178997 [48,50,56,59,60] and rs4131347 [60,61] consistently yielded negative results for the association with suicide attempt or completion. The same SNPs have not demonstrated correlation with TPH2 mRNA levels in human prefrontal cortex [32], although rs11178997 was reported to modulate transcriptional activity *in vitro* [27-29]. SNP rs6582071 was associated with suicide completion in one [51], but not in two other studies [55,56], while rs4448731 was associated with suicide completion [51], but not with suicide attempt [54]. No data on the functional consequences of these two SNPs exist. Three additional SNPs with inferred functional significance were evaluated for the association with suicide: intronic SNP rs10748185 and common exonic SNPs rs7305115 and rs4290270. SNPs rs10748185

Table 2. Population-based studies on the association of common variants in TPH2 gene with suicide attempt.

CASE subjects		CONTROL subjects		Origin	Investigated SNPs (ID and/or location)	Results	Year of publication (reference)
Diagnosis	N	Diagnosis	N				
Various	150	Healthy volunteers	196	Finnish Caucasian	rs4570625, rs11178997 (promoter); rs7305115 (exon 7); rs4290270 (exon 9); NTPH93329 (3'-UTR); rs6582070, rs1872834 (intergenic region); 8 intronic SNPs (introns 2, 4, 5, 8)	Single marker association with rs1352251 and rs1473473 (intron 8); Association with haplotype encompassing C_8872342, C_15836061 (intron 5), rs7305115 (exon 7), C_8872308, rs1352251 and rs1473473 (intron 8)	2005 [50]
	104		315	African Americans		Single marker association with C_245410 (introns 2), C_15836061 (intron 5) and C_8376042 (intron 8); Haplotype association: the same as in Finnish Caucasians	
	123		62	Southwestern American Indians		No single marker or haplotype association	
SCH	83	SCH without SA	170	Undefined (recruited in Toronto area)	rs11178997 (promoter); rs10784941 (intron 1)	No single marker or haplotype association	2005 [59]
MDD	102	MDD without SA	123	Han Chinese	rs7305115 (exon 7)	Association with G allele	2006 [62]
Alcohol dependence	102	Alcohol dependence without SA	251	German Caucasian	rs4570625 (promoter); 19 intronic SNPs (introns 1, 4, 5, 6, 7, 8 and 9)	No single marker or haplotype association	2007 [53]
		Healthy volunteers	305			No single marker or haplotype association	
BD or MDD	187	BD or MDD without SA	246	94% USA Caucasian, 6% other	rs4131347 (promoter)	No association	2008 [61]
		Healthy subjects	130			No association	
MDD	181	MDD without SA	143	Korean	rs4570625 (promoter)	No association	2009 [57]
		Healthy subjects	176			Association with GG genotype and G allele	
BD	140	BD	148	undefined (recruited from Southeast and South of Brazil)	rs4448731 (5'-UTR); 7 intronic SNPs (introns 2, 4, 5, 7, 8)	No single marker or haplotype association	2010 [54]
MDD	215	MDD without SA	215	Han Chinese	rs7305115 (exon 7)	Association with G allele	2010 [63]

BD - bipolar disorder; ID - identification number in NCBI Single Nucleotide (dbSNP) or Celera Discovery System (CDS) database; MDD - major depression disorder; SA - suicide attempt; SCH - schizophrenia, SNP - single nucleotide polymorphism; UTR - untranslated region.

and rs7305115 were reported to correlate with TPH2 expression in human prefrontal cortex [31] and pons [30], respectively, while rs4290270 was demonstrated to govern TPH2 mRNA splicing and editing in human amygdala [33]. Two studies on Han Chinese population have reported association of rs7305115 with suicide attempt in major depressive patients [62,63]. A positive association was also observed

between rs4290270 and suicide completion in German Caucasians [33], while rs10748185 was not associated with suicide completion in West European Caucasian population, though statistical power of the this study was insufficient to detect small size effect [31]. Therefore, it might be beneficial to re-evaluate association results on these presumably functional SNPs in different populations.

As mentioned earlier, a number of studies have found increased expression of TPH2 in the brains of suicide subjects and it was speculated that this over-expression could be a compensatory response to the presence of a mutant enzyme with impaired catalytic activity. One such mutation, resulting with decreased enzyme activity, stability and solubility [20], was identified in 9 unrelated, severely depressed

patients, 6 of whom had a history of suicidal behaviour [17]. Screening of our group of subjects affected with the most extreme form of suicidal behaviour showed, however, no presence of the mutated allele (unpublished data); identical findings were obtained also on the two smaller samples of suicidal subjects [51,52]. Future studies could address potential involvement of the other two functional TPH2 mutations that were found to be associated with attention deficit/hyperactivity disorder [18] and bipolar affective disorder [19]. In order to dissect the potential role of yet unknown TPH2 mutations, it might be worthy to undertake re-sequencing of the coding region of the TPH2 gene in the selected group of subjects with the most severe form of suicidal behaviour.

5. Concluding remarks

Studies of haplotypes derived from a number of tagging SNPs strongly suggest that common variants of the TPH2 gene are unlikely

to have major role in the susceptibility to suicidal behaviour. Positive findings, although present in the literature, were limited to individual studies and could not be replicated in independent populations. The fact that different associating SNPs were detected in positive studies might reflect insufficient statistical power for reliable detection of markers with a very small effect size. A number of individuals per group mainly ranged between 100 and 300, and only exceptionally above that [33] (Table 1 and 2). Sample sizes on the order of several thousand cases and controls or trios have been recommended for studies in psychiatric genetics [64]. Therefore, any future studies on the association of TPH2 gene variants with suicide should be based on a larger number of subjects. Furthermore, association may become apparent only in combination with variants of one or more additional genes that functionally interact with TPH2 (epistasis) or when environmental factors (such as history of stressful life events) are considered. Stronger associations may

be observed with heritable endophenotypes of suicidal behaviour compared to the suicidal behaviour per se. Therefore, we recommend identification of TPH2 variants associated with intermediate phenotypes of suicidal behaviour, such as trait aggression/impulsivity, early-onset major depression, neurocognitive function, and cortisol social stress response [65]. Finally, further studies on the functional consequences of particular TPH2 gene variants, which could be associated with suicidal behaviour, should be encouraged.

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