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Functional promoter polymorphism of the neuronal isoform of tryptophan hydroxylase (*Tph2*) in suicide

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Abstract

The association between suicide and G-703T polymorphism of the tryptophan hydroxylase 2 (*Tph2*), the rate limiting enzyme in the biosynthesis of the neurotransmitter serotonin, was studied in a sample of 291 suicide victims and 280 healthy subjects of Croatian origin. No significant differences were found between the groups. Obtained results do not support involvement of the investigated polymorphism in the susceptibility to suicide completion.

Keywords: suicide; *Tph2*; association study

1. **Introduction**

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of 5HT. There are two isoforms of TPH, so-called peripheral (*Tph1*) and neuronal (*Tph2*), the later being expressed exclusively within the nervous system (Walther et al., 2003). A single nucleotide polymorphism (SNP) G-703T (rs4570625 in dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/), located in the promoter region of *Tph2* gene, was of great interest for the potential involvement in the regulation of 5HT signaling. Respective SNP was shown to have significant effect on the *Tph2* gene expression in different cellular systems (Lin et al., 2007; Chen et al., 2008), although data were not unequivocal (Scheuch et al., 2007). Furthermore, it influenced *in vivo* reactivity of limbic system, i.e. amygdalae (Brown et al., 2005; Canli et al., 2005), as well as prefrontal and parietal cortices (Reuter et al., 2008). Molecular genetic studies revealed association of G-703T polymorphism with individual differences in emotion-related (Gutknecht et al., 2007; Reuter et al., 2007a) and executive (Reuter et al., 2007b; Osinsky et al. 2009) functions, as well as with certain neuropsychiatric conditions, including suicidal attempt (Yoon et al., 2009). The present study, based on a relatively large sample of suicide victims belonging to Croatian population, re-evaluated potential involvement of G-703T polymorphism in the etiopathogenesis of suicidal behavior.

2. Materials and methods

Data on our cohorts of suicide victims and control subjects, as well as the procedures for genotyping and statistical analyses, are given in the expanded Materials and Methods section in the online data supplement.

3. Results

The genotype frequencies of G-703T polymorphism accorded well with Hardy-Weinberg equilibrium in both control (χ^2=1.637, *d.f.*=2, *P=0.4411*) and victim (χ^2=0.060, *d.f.*=2, *P=0.9702*) group. There were no significant differences between the groups in either genotype (χ^2=1.545, *d.f.*=2; *P=0.4620*) or allele (FET, *P=0.7159*) frequencies (Table 1).

4. Discussion

The present study focuses research on the involvement of *Tph2* gene in suicidal behavior to ethnically homogenous population of Croatian origin. Study was based on the relatively large

sample (N=291) of subjects who have completed suicide, mainly (97%) by violent means. The statistical power of our sample to detect differences associated with an odds ratio (OR) of 2.0 and 1.5, at the level of significance of 0.05, amounted to 94.4% and 49.9%, respectively. No differences were found between the control subjects and suicide victims in the distribution of either genotype or allele frequencies of G-703T polymorphism (Table 1), suggesting that the respective polymorphism has no influence on suicide completion in our population. Even when only violent suicide victims (N=282) were taken into consideration, no association was found (data not shown). The very recent study on suicide victims of Japanese origin (Mouri et al., 2009), as well as the two previous studies on suicide attempters (Zhou et al., 2005; Zill et al., 2007) also found no evidence for the effect of G-703T polymorphism on suicidal behavior. Yoon et al. (2009), on the other hand, reported excess of GG homozygotes among suicidal depressed patients as compared to healthy controls. Since no differences between depressive patients with and without history of suicide attempt or between the groups of non-suicidal depressive patients and healthy controls were found, it seems plausible that the observed association was not related to suicidal attempt itself (as concluded by the authors), but possibly to depression or depression associated with suicidal attempt.

Frequency of -703T allele of G-703T polymorphism in our control sample, being 20.4 %, fits well in the range of 18%-23%, reported for other Caucasian populations (Zhou et al., 2005; Reuter et al., 2007a; Zill et al., 2007). It should be noted that significantly higher frequencies of -703T allele were found in non-Caucasian populations such as African American (39%) (Zhou et al., 2005), American Indian (48%) (Zhou et al., 2005), Korean (57%) (Yoon et al., 2009) and Japanese (45%) (Mouri et al., 2009).

Complex disorders such as suicide are likely to involve multiple genes along with epigenetic influences and the main limitation of our study is that it considers effect of a single genetic factor. Another limitation, the lack of records for suicide victims on the psychiatric co-morbidity, should also be mentioned. Nevertheless, our results, as well as findings on other populations (Zhou et al., 2005; Zill et al., 2007; Mouri et al., 2009), even ethnically very distant ones, clearly speak against the major role of functional variant G-703T of the *Tph2* gene in the susceptibility to suicide. Serotonergic dysfunction reportedly plays a major role in the etiopathogenesis of suicidal behaviour (for review see Mann, 2003). Our earlier research has also given some support to this concept (Hranilovic et al., 2003; Jernej et al., 2004; Stefulj et al., 2006). Therefore, association studies on genes encoding synaptic proteins of this transmitter, including recently discovered *Tph2*, should be encouraged, with negative reports being as important as positive ones in obtaining realistic picture of true genetic influences.

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Table 1. Allele and genotype counts and frequencies of the G-703T polymorphism of tryptophan hydroxylase 2 (*Tph2*) promoter in Croatian control population and suicide victims.

	P [a]	**Control subject (N=280)**	**Suicide victims (N=291)**
Genotypes	*0.4620*		
GG		183 (65.4 %)	181 (62.2 %)
GT		80 (28.6 %)	96 (33.0 %)
TT		17 (6.1 %)	14 (4.8 %)
Alleles	*0.7159*		
G		446 (79.6 %)	458 (78.7 %)
T		114 (20.4 %)	124 (21.3 %)

[a] *P* values for differences in genotype and allele distributions between the control and victim group were calculated by Chi-square and Fisher's Exact Test, respectively.

Supplement

Functional promoter polymorphism of the neuronal isoform of tryptophan hydroxylase (*Tph2*) in suicide

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This material supplements but does not replace the content of the peer-reviewed paper published in *Psychiatry Research*.

Materials and Methods

Subjects

Blood samples of suicide victims (N=291; 80% males, 20% females; mean age 53 ± 20 years) were obtained during autopsy at the Department of Forensic Medicine, University of Zagreb. Victims committed suicide mainly by violent means, i.e. hanging (56.4%), penetrating lesions (30.0%), jumping from height or under a train (8.7%), CO and other poisoning (3.1%), and drowning (1.7%). The control group (N=280; 79% males, 21% females; mean age 48 ± 17 years) consisted of blood donors without personal or family history of neuropsychiatric disorders, including suicidal behavior. Individuals from both groups were of the Croatian origin and were recruited from the same geographical area (larger area of the city of Zagreb) Informed written consent was obtained from all control subjects as well as from victim's relatives. The study was approved by the Ethical Committee of the Medical faculty, University of Zagreb.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard phenol / chlorophorm / isoamyl alcohol procedure. Genotyping of G-703T polymorphism was based on the restriction fragment length polymorphism (RFLP) analysis following amplification of the target sequence by polymerase chain reaction (PCR) throughout 40 cycles (95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec). PCR was carried out in a total volume of 15 μl containing 100 ng of DNA, 1.5 mM $MgCl_2$, 50 μM dNTP, 0.5 U of Taq DNA polymerase (Applied Biosystems) and 0.5 μM

primers. Sequences of the primers (5'-TTTCCATGATTTCCAGTAGAGAG-3' and 5'-AAGCTTTTTCTGACTTGACAAAT-3') were taken from Canli et al. (2005). Aliquots (5 µl) of PCR products were digested overnight with 5U of Xap (Fermentas) in a total volume of 20 µl, and fractionated on 3% agarose gels stained with ethidium bromide. The uncut fragment of 309 bp corresponded to T allele, while G allele gave fragments of 285 bp and 24 bp, in accordance with the reported sequence of the human *Tph2* gene.

Statistical analysis

Statistical analyses were performed using GraphPad InStat (version 3.05) software. Differences in genotype distribution between case and control groups as well as presence of Hardy-Weinberg equilibrium were tested by two sided chi-square (χ^2) test for independence. Allele frequencies were compared using two-sided Fisher's Exact Test (FET). The statistical power of the study was calculated by on-line available software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). The level of significance was in all analyses set at $\alpha = 0.05$.

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