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Association study of the TPH2 Gene with Major Depressive Disorder in the Han Chinese Population

Jing Du^{a,*}
Zhaofeng Zhang^a
Weidong Li^b
Lin He^b
Jianhua Xu^a
Yongyong Shi^{b,*}

^a Key Lab. of Reproduction Regulation of NPFPC, SIPPR, IRD, Fudan University, Shanghai

^b Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Shanghai Jiao Tong University, Shanghai

CHINA

ABSTRACT – Background and Objectives: Tryptophan hydroxylase 2 (TPH2) catalyzes the rate-limiting step in serotonin biosynthesis in the nervous system. Several variants of human TPH2 have been reported to be associated with a spectrum of neuropsychiatric disorders such as unipolar major depression, bipolar disorder and suicidality etc. Recent studies suggested that two variants (T212 and A375) in the exon 7 and exon 9 were associated with major depressive disorder (MDD).

Methods: To replicate these findings, two polymorphisms located in exons 7 and 9 of TPH2 (rs7305115 and rs4290270, respectively) were analysed by DNA sequence in the case–control sample study in 191 MDD and 191 healthy volunteers. Statistical analyses were carried out using the program SPSS. The comparison of allele and genotype frequencies of each polymorphism between case and control groups was carried out on the online software SHEsis. All subjects were unrelated southern Han Chinese.

Results: No difference was observed on the allelic or genotypic distribution of TPH2 gene polymorphisms between the groups. However, the two-marker haplotype covering components T212 (rs7305115) A and A375 (rs4290270) T were observed to have a significantly protective effect MDD in female (corrected $p = 0.0032$; OR = 0.241[95% CI = 0.099-0.587]).

Conclusions: The results suggest that TPH2 might be associated with a lower risk of female MDD. However, confirmatory studies in independent samples are needed.

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List of abbreviations

MDD: major depressive disorder.
 5-HT: Serotonin (5-hydroxytryptamine)
 ADHD: attention-deficit/hyperactivity disorder
 TCAs: tricyclic antidepressants
 SSRIs: selective serotonin reuptake inhibitors
 MAOIs: monoamine oxidase inhibitors
 TPH: Tryptophan hydroxylase
 TPH1: Tryptophan hydroxylase 1
 TPH2: Tryptophan hydroxylase 2
 5-HTT: serotonin transporter
 5-HTR: serotonin receptor
 MAOA: monoamine oxidase A
 SNPs: single nucleotide polymorphisms
 ESE: exonic splicing enhancer
 OR: odds ratio
 SR proteins: Ser/Arg-rich proteins
 SD: standard deviation

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a monoaminergic central neurotransmitter that is widely distributed in the human central nervous system and in certain peripheral tissues. 5-HT has been shown to influence a variety of peripheral and brain physiological functions, including sleep-wake cycle, mood, appetite, aggression, neuroendocrine regulation, neurogenesis and haemostasis¹⁻³. Dysregulation of brain serotonergic homeostasis has been implicated in many neuropsychiatric disorders, including major depressive disorder (MDD), attention-deficit/hyperactivity disorder (ADHD), autism, aggression and suicidal behaviour, bipolar disorder and anxiety disorder¹. Most antidepressant drugs,

including many tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs), have their effect by increasing levels of extracellular 5-HT by inhibiting its reuptake or metabolism⁴⁻⁶. Serotonin homeostasis is modulated mainly by its receptors, transporter, and enzymes of its biosynthetic pathway. Genes encoding proteins involved in the serotonergic system, including tryptophan hydroxylase (TPH), serotonin transporter (5-HTT), serotonin receptor (5-HTR) and monoamine oxidase A (MAOA), are major genes in association studies of affective disorders⁷⁻⁹.

TPH is the rate-limiting enzyme in the serotonin biosynthetic pathway and plays an important role in the regulation of serotonin function¹⁰. Up to now, two different TPH enzymes are expressed by two distinct genes: TPH1 and TPH2 genes. TPH1 has been thought as the sole rate-limiting enzyme for brain serotonin synthesis¹¹ studied for several decades. Recently researchers found that *TPH1* expresses predominantly in the periphery¹² while tryptophan hydroxylase-2 (*TPH2*) is exclusively expressed in neuronal cell types and is the predominant isoform of serotonin in the brain¹³. *TPH2* then has been identified as a neuronal-specific isoform which controls brain serotonin synthesis^{14,15}. Currently, more than 430 single nucleotide polymorphisms (SNPs) in *TPH2* have been identified in cohorts of various neuropsychiatric disorders or in general population. A number of them are coding mutations with unknown functions¹⁵⁻¹⁸.

MDD is complex, polygenic disorders with genetic, environmental and biochemical influences as potential contributing factors, and affect tens of millions of people with enormous social and economic impact. Given the widespread and disabling nature of the illness, MDD represents a rising public health

concern. It is estimated that MDD will become the second leading cause of disability globally by the year 2020¹⁹. The overall contribution of genetic factors in the origin of these diseases is approximately 40%. The genetic pathogenesis of MDD remains unclear. The identification of the role of *TPH2* in brain serotonin synthesis has opened a new area to explore the molecular and genetic mechanisms of serotonin-related neuropsychiatric disorders. Therefore, functional characterization of *TPH2* may ultimately provide important insights into the pathophysiology of these disorders. Two polymorphisms located in exons 7 and 9 of *TPH2* (rs7305115 and rs4290270, respectively) were shown to be associated with MDD²⁰. However, there have been no consistent findings concerning the relationship between two polymorphisms and MDD as well as therapeutic response in

Asian population²¹. Following these inconsistent results, in this study we investigate the association between these two genetic variants of the *TPH2* gene and MDD in a Chinese Han population.

Materials and methods

Samples

The sample set consisted of 190 unrelated MDD cases (131 males and 59 females), and 190 normal controls (99 males and 91 females) recruited from the Chinese Han population. The mean age of MDD cases was 33.7 years (\pm 11.8) and the mean age of the controls was 32.4 years (\pm 11.0) (Table 1). All subjects were born in Shanghai. All patients

Table 1
Sociodemographic characteristics of the sample population

Study sample	Sample Size	Mean Age (SD)	Mean Age of Onset (SD)	Male/Female	Female %
MDD	190	33.7 (11.8)	32.1 (11.6)	131/59	31.1
Control	190	32.4 (11.0)		99/91	47.9

were interviewed by two independent psychiatrists and were diagnosed strictly according to DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition). All subjects gave an informed consent, the details of which were reviewed and approved by the local ethical committee. Controls were randomly selected from the Shanghai general population. The study complied with the guidelines of our local Medical Ethical Committee and all participants recruited in this study provided written informed consents.

Genotyping

Genomic DNA was extracted from venous blood with the QIAamp DNA Blood Kit (QIAGEN, Hilden, Germany) according to the manufactures' instructions. Genotyping was performed without knowledge of the clinical status of the subjects. The sequences of the PCR primers and cycling conditions are given in Table 2. PCR was carried out in a 15 μ l reaction mixture containing 10 ng of DNA, 10 pmol of each primer, 2.5 mM MgCl₂, 0.2 mM dNTP and 0.25 U Taq DNA polymerase

Table 2
Primers used for the PCR

Markers	Primer	Sequence	Fragment size	Condition (35 cycle)
rs7305115 (Pro312Pro)	F	5'- atcagaagcaccaaacat-3'	491bp	30 s 94°C
	R	5'- cggaccagatgaggagt-3'		30 s 55°C 60 s 72°C
rs4290270 (Ala375Ala)	F	5'- ttcaggaagcgtaagact-3'	349bp	30 s 94°C
	R	5'- aggtgccaaatcctctat-3'		30 s 55°C 60 s 72°C

(Sigma, St. Louis, MO, USA). All reactions had an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 94°C for 30s denaturation, 55°C for 30s annealing and 72°C for 1 min, and finally at 72°C for 10 min on a Gene Amp PCR system 9700 (Applied Biosystems, Foster City, CA). Preparation of DNA for sequencing included incubation of PCR products with 0.1 U of shrimp alkaline phosphatase (Roche, Basel, Switzerland) and 0.5 U of exonuclease I (New England Biolabs Inc., Beverly, MA) at 37°C for 45 min, followed by heat inactivation at 85°C for 20 min. The PCR products were sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems, Foster city, CA). The sequences were analysed in an ABI PRISM model 3100 DNA Sequencer (PE Applied Biosystems, Perkin-Elmer) to determine the genotypes of variation at the same position on both forward and reverse sequences. Any differences were resolved by re-genotyping samples (with an overall error rate < 0.05%).

Prediction of ESE site disruption:

Change in ESE motifs due to single base substitutions were calculated by use of the ESEfinder3.0 program (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese finder.cgi?process = home>)¹³.

Statistics

Statistical analyses were carried out using the program SPSS (version 19.0). The odds ratio (OR) and their 95% confidence intervals were estimated for the effects of alleles. Haplotype distribution was estimated using the program UNPHASE²². The comparison of allele and genotype frequencies of each polymorphism between case and control groups was carried out on the online software SHEsis (<http://202.120.7.14/analysis/myAnalysis.php>)²³. In all of the analyses, $p < 0.05$ was considered statistically significant, after Bonferroni correction. Power analysis of our sample was performed using the G*Power 3 programme²⁴.

Results

To examine the association between MDD and the polymorphisms in the TPH2 gene, we detected genotype and allele frequencies of two SNPs, T212 (rs7305115) A and A375 (rs4290270) T in the 191 MDD patients and the 191 healthy controls. The distribution of the two polymorphisms was in Hardy–Weinberg equilibrium (Table 3). The data for genotypes and allele frequencies are shown in Table 3. As shown in Table 2, no significant

Table 3
Individual marker analysis using SHIEsis

Exon	The number of Genotypes and Genotype frequency (freq.)	Hardy-Weinberg equilibrium test			P value	The number of alleles and allele frequency (freq.)		P value	OR (95%CI)
		chi2	df	P value		G	A		
Exon 7									
(rs7305115)	GG 56 (0.299) GA 84 (0.449) AA 47 (0.251)	1.852	1	0.17	0.94	196 (0.524)	178 (0.476)	0.71	0.95 [0.71~1.26]
Pro312Pro)	case 54 (0.284) control 86 (0.453)	1.691	1	0.19		194 (0.511)	186 (0.489)		
Exong 9									
(rs4290270)	TT 42 (0.221) TA 96 (0.505) AA 52 (0.274)	0.034	1	0.85	0.85	180 (0.474)	200 (0.526)	0.99	0.99 [0.75~1.32]
Ala375Ala	case 39 (0.206) control 101 (0.534)	0.973	1	0.32		179 (0.474)	199 (0.526)		

differences in allele or genotype frequencies of the two polymorphisms between the case groups and the control group were observed.

Haplotypes with probabilities greater than 1% accounted for the majority of haplotype diversity. The A-T Haplotype which was more frequent in female controls, was observed to be significantly associated with protect MDD in female (OR = 0.241, $p = 0.0008$, corrected $p = 0.0048$) (Table 4). We adjusted the p value using the Bonferroni correction so as to control type 1 error.

ESEfinder program was used to identify potential binding sites splicing factors in exon 7 of *TPH2* gene. We found some changes of putative ESE motifs, SF2/ASF, SC35 and SRp40, in *TPH2* predicted by ESEfinder (Figure 1).

In this study, power analysis showed that the statistical power of our sample to detect a significant association ($p < 0.05$) was 99.8% in genotypic comparisons for MDD when a large effect size ($w = 0.8$) was presumed. This indicates that the sample size in our study was sufficient to achieve a relatively low risk of a type II error.

Discussion

Psychiatric disorders such as MDD place a large burden on society and health service. Every year, almost one million people die from suicide (http://www.who.int/mental_health/prevention/suicide/suicideprevent/en/). Numerous genetic studies in search for the genes involved in mental disorders have been performed.

Components of the serotonin system are being studied as risk factors in MDD, obsessive-compulsive disorder and autism¹ and also play an important role in the clinical effecti-

Table 4
The haplotype analysis of two markers using UNPHASE

Haplotype		Case (freq)	Control (freq)	P value	Corrected P value	OR (95%CI)
Total	rs7305115 rs4290270					
1	A A	137 (0.365)	118 (0.311)	0.116	0.116	1.275 [0.942~1.726]
2	A T	41 (0.111)	67 (0.179)	0.008	0.048	0.573 [0.378~0.869]
3	G A	60 (0.162)	81 (0.216)	0.059	0.059	0.701 [0.485~1.014]
4	G T	136 (0.362)	112 (0.295)	0.05	0.05	1.359 [1.001~1.844]
Global P				0.004	0.016	
Male	rs7305115 rs4290270					
1	A A	88 (0.342)	68 (0.348)	0.889	0.889	0.972 [0.657~1.439]
2	A T	35 (0.135)	32 (0.167)	0.338	0.338	0.776 [0.462~1.305]
3	G A	39 (0.150)	32 (0.167)	0.626	0.626	0.881 [0.530~1.465]
4	G T	96 (0.373)	62 (0.317)	0.22	0.22	1.280 [0.8631~1.897]
Global P				0.577	0.577	
Female	rs7305115 rs4290270					
1	A A	49 (0.421)	50 (0.272)	0.007	0.028	1.941 [1.188~3.172]
2	A T	6 (0.054)	35 (0.190)	0.0008	0.0032	0.241 [0.099~0.587]
3	G A	21 (0.183)	49 (0.266)	0.098	0.098	0.618 [0.348~1.096]
4	G T	40 (0.343)	50 (0.272)	0.192	0.192	1.396 [0.845~2.307]
Global P				0.0005	0.002	

veness of antipsychotic drugs²⁵. Alterations in the 5-HT system have also been related to specific symptoms and treatment of MDD. Recently, the associations between TPH2 variants, methylation and MDD had been identified in multiple populations²⁶⁻²⁸. However, the contribution of the TPH2 gene to MDD is controversial and the relationship between TPH2 gene and MDD remains elusive.

In the present study, the frequencies of all the haplotypes were larger than 3%. Initially, we conducted a total association study without consideration of gender. To explore whether a gender difference existed in the association between MDD and TPH2, we conducted a comparison within different gender

groups. The haplotypes at the rs7305115 and the rs4290270 was significantly associated with female MDD, where a gene-sex interaction was observed. The haplotypes A/T, which were more frequent in female control subjects (19.0%) than in patients (5.4%), might be significant protective effect against MDD in female (corrected $p = 0.0032$). Conversely, the haplotype A/A was more frequent in patients (42.1%) than in control subjects (27.2%), suggesting that haplotype G/G is a risk haplotype for female MDD (corrected $p = 0.028$). All these results suggest that at least one susceptibility locus for MDD lies within, or very close to, the region spanning TPH2 genes in Chinese subjects.

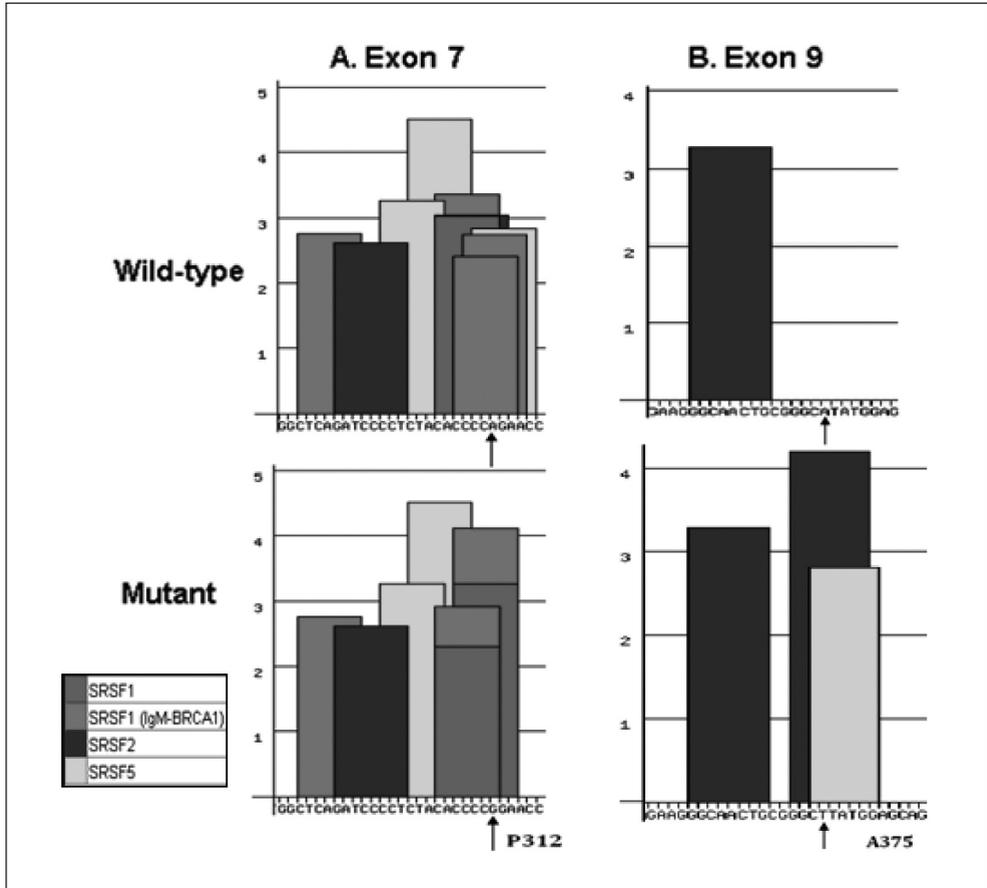


Figure 1. The two TPH2 exonic mutations were predicted by ESEfinder to disrupt ESE sites of one or more of three different SR proteins (SRSF1 (red), SRSF1(IgM-BRCA1)(pink), SRSF2 (blue), SRSF5 (green)).

Predicted ESE sites within wild-type TPH2 exons 7 (A) and 9 (B) are shown in the top panels.

Coloured bars represent individual ESE sites with the matrix score (higher score represents a stronger prediction) plotted on the y-axis and the position of the ESE within rs7305115(P312) and rs4290270(A375) represented on the x-axis. Matrix score changes induced by each mutation are represented in the lower panels.

If matrix scores for a particular SR protein fell below threshold levels as predicted by ESEFinder3.0

(SRSF1 = 1.956, SRSF1(IgM-BRCA1) = 2.383, SRSF2 = 2.67, SRSF5 = 2.676),

the coloured bar representing an ESE site was lost.

Although our evidence could be a chance finding, it fits well with the evidence of gender differences in the risk of MDD which shows that females are more often affected than males. Women are twice as likely to suffer from depression as men. Women with MDD experience an earlier age of onset, a greater variety of symptoms and an increased

number of episodes of depression compared to men²⁹. Many studies showed that female hormones (e.g. estrogen) treatment have antidepressant effects^{30,31}. Ovarian hormones can interact with serotonergic function to influence affect. Estrogen has been shown to increase the density of 5HT2A receptors in brain regions associated with mood³² and can

facilitate serotonergic transmission by enhancing serotonin synthesis and/or decreasing serotonin reuptake thereby alleviating depressive symptoms³³.

None of the individual SNPs showed positive association whereas haplotype analysis gave a significant association with MDD. This may be explained by the fact that haplotype analysis has a higher power than individual genetic markers in association analysis, since haplotype analysis takes into account the correlation between the individual markers³⁴.

The two SNPs investigated are synonymous. rs7305115 has been found to influence gene expression in post-mortem human pons in which the A allele was associated with higher levels of expression³⁵. rs4290270 may also have functional effects on gene expression³²⁻³³. This may be due to exon skipping or alterations in mRNA stability. There is increasing evidence that many human disease genes harbour exonic mutations that affect pre-mRNA splicing³⁶⁻³⁹. Bioinformatics analysis revealed that the rs7305115 variant changes the overlapping ESE motifs for SRSF2 (SC35) and SRSF5 (SRp40) to motifs for SRSF1 (SF2/ASF) and SRSF1 (IgM-BRCA1), while overlapping SC35 and SRp40 motifs are formed by the rs4290270 variant.

Our results raise the possibility that TPH2 gene variants might be involved in the development of MDD. However, several issues should be noted in the present study. The major limitations of the present study were the relatively small sample size which is liable to result in a stratification bias. Protective haplotype could vary according to ethnic differences. Thus, further studies using a larger number of subjects in different ethnic groups should be performed to determine whether the TPH2 gene haplotype may be truly involved in the development of MDD. Also, the choice of SNPs was based on previous research and focused only on the exons

of the TPH2 gene. Accordingly it is necessary that further research provide more complete coverage of the TPH2 gene and investigate other variants that may have an effect on the expression of the gene.

Further work with large sample size or from different populations and corresponding functional analysis is still required to fully elucidate the exact role of TPH2 in the pathogenesis of MDD and other psychiatric disorders.

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Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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* Corresponding author:

Jing Du and Yongyong Shi

E-mail address: dujing42@126.com (Jing Du)

shiyongyong3@gmail.com (Yongyong Shi)