Progress in Neuro-Psychopharmacology & Biological Psychiatry 56 (2015) 97–100

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Association study of *TPH2* polymorphisms and bipolar disorder in the Han Chinese population

Shiqing Chen [a], Xiaoye Huang [a], Tao Yu [a], Xin Li [a], Yanfei Cao [a], Xingwang Li [a], Fei Xu [a], Fengping Yang [a], Forrest Fabian Jesse [a], Mingqing Xu [a], Weidong Li [a], Lin He [a,b,c,*], Guang He [a,**]

[a] *Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, China*

[b] *Institutes of Biomedical Sciences, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China*

[c] *Institute for Nutritional Sciences, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China*

ARTICLE INFO

Article history:
Received 4 April 2014
Received in revised form 26 July 2014
Accepted 16 August 2014
Available online 21 August 2014

Keywords:
Association
Bipolar disorder
Case–control study
Chinese Han population
TPH2

ABSTRACT

Objective: Bipolar disorder (BPD) is a serious and common mental disorder with high heritability. The serotonergic system is known to be implicated in the etiology of the disorder. Tryptophan hydroxylase isoform-2 (*TPH2*), which controls the synthesis of serotonin in the brain, has been suggested as a candidate gene for BDP. The aim of this study was to examine the association between the polymorphisms in *TPH2* and BPD.
Methods: We conducted a case–control study by genotyping six SNPs (rs10784941, rs1386494, rs2171363, rs4760816, rs1386486, and rs1872824) in 506 bipolar patients and 507 controls of Chinese Han origin.
Results: rs10784941 was not in the Hardy–Weinberg equilibrium and therefore excluded from further analysis. rs1386486 and rs1872824 showed statistically significant differences between cases and controls in genotype frequencies (rs1386486: $p = 0.043351$; rs1872824: $p = 0.016563$), but no association in allele frequencies. Strong LD was found among rs1386494, rs2171363 and rs4760816, but no positive association with BPD was found for haplotypes.
Conclusion: Our results indicate that in the Han Chinese population *TPH2* may be a potential susceptibility gene for bipolar disorder. Further studies are needed to validate this association.

1. Introduction

Bipolar disorder (BPD) is a common and severe mood disorder characterized by mania or hypomania and depression (Craddock and Sklar, 2013). The disability caused by BPD and the high incidence of relapse result in huge personal and social burdens. Recent estimation of the lifetime prevalence of bipolar disorder was made at 2.4% (Merikangas et al., 2011). BPD has a strong genetic basis with high heritability (Althoff et al., 2005; Smoller and Finn, 2003), and many candidate regions and genes have been reported (Barnett and Smoller, 2009; Serretti and Mandelli, 2008). Several association and functional studies have found that the serotonergic system is involved in the pathogenesis of BPD, and that tryptophan hydroxylase isoform-2 (TPH2) plays a key role in the serotonergic system.

Serotonin (5-HT) is an important neurotransmitter implicated in various physiological processes, such as cell proliferation, mobility, differentiation, organ development, neuronal migration and synaptogenesis (Gaspar et al., 2003). It has been suggested that 5-HT is involved in certain pathological functions in the central nervous system (Lucki, 1998; Meltzer et al., 1998). Previous studies have shown that the expression of 5-HT is lower in the brains of BPD patients (Sobczak et al., 2002). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the synthesis of serotonin (5HT), and has two isoforms, TPH1 and TPH2. *TPH1* is mainly expressed in the peripheral nervous system, whereas *TPH2*, located on chromosome 12q21, is preferentially expressed in the brain to control 5-HT synthesis in the central nervous system (Walther et al., 2003; Zhang et al., 2004; Zill et al., 2004). Some studies have indicated that *TPH2* is associated with BPD (Campos et al., 2011; Cichon et al., 2008; De Luca et al., 2005; Harvey et al., 2007; Lin et al., 2007; Roche and McKeon, 2009; Van Den Bogaert et al., 2006; Xiang et al.,2014), although the opposite results have also been reported (Campos et al., 2010; Choi et al., 2010).

This study was designed to examine the association between *TPH2* polymorphisms and BPD. We identified six single nucleotide

Abbreviations: BPD, bipolar disorder; DR, dorsal raphe nucleus; DSM-IV, Diagnostic and Statistical Manual for Mental Disorders, 4th Edition; LD, linkage disequilibrium; PD, panic disorder; SNP, single nucleotide polymorphism; TPH, tryptophan hydroxylase; TPH2, tryptophan hydroxylase isoform-2.

* Correspondence to: L. He, Institutes of Biomedical Sciences, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China. Tel./fax: +86 21 62822491.
** Correspondence to: G. He, Bio-X Institutes, Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, China. Tel./fax: +86 21 62822491.
E-mail addresses: helinhelin3@gmail.com (L. He), heguang@sjtu.edu.cn (G. He).

http://dx.doi.org/10.1016/j.pnpbp.2014.08.008
0278-5846/

Table 1
Demographic characteristics of patients and control subjects.

Phenotype	Participants, no.	Sex, M/F, no.	Age, mean ± SD	Onset age, mean ± SD	Bipolar I disorder	Bipolar II disorder	Cyclothymic disorder
BPD	506	284/222	37.85 ± 11.4	26.7 ± 10.6	419	83	4
Control	507	287/220	36.39 ± 8.7				

polymorphisms (SNPs) (rs10784941, rs1386494, rs2171363, rs4760816, rs1386486, and rs1872824) in 506 bipolar patients and 507 healthy controls of Han Chinese origin.

2. Materials and methods

2.1. Subjects

For the case–control study, we recruited 506 unrelated bipolar disorder patients (284 males and 222 females, age: 37.8 ± 11.4, onset age: 26.7 ± 10.6) and 507 controls (287 males and 220 females, age: 36.4 ± 8.7). Demographic characteristics of the sample are summarized in Table 1. All the patients were diagnosed according to the DSM-IV criteria. The final diagnosis on every patient was made by two independent psychiatrists on the basis of interview data and hospital case notes. None of the controls exhibited symptoms of any psychiatric problem and all were in good health. The procedure was fully explained to the participants all of whom signed an informed consent. All participants came from Anhui and were of Chinese Han origin. The study protocol was reviewed and approved by the Ethics Committee of the Human Genetics Center in Shanghai. DNA of each participant was obtained via phenol-chloroform method from peripheral blood samples.

2.2. Genotyping

Six SNPs (rs10784941, rs1386494, rs2171363, rs4760816, rs1386486, and rs1872824) were genotyped using TaqMan® technology. All the markers are located in the intron region. The SNPs were genotyped with ABI 7900 DNA detection system (Applied Biosystems, Foster City, California) using TaqMan® probes, designed by Applied Biosystems. The standard 5 μl PCR reaction of procedure was carried out using TaqMan® Universal PCR Master Mix reagent kits according to the supplier's guidelines.

2.3. Statistical analysis

SHEsis (http://analysis.bio-x.cn/myAnalysis.php) was used to analyze Hardy–Weinberg equilibrium, allelic and genotypic distributions and pairwise linkage disequilibrium (LD) (Shi and He, 2005). Linkage disequilibrium of all pairs of SNPs was estimated with D′ according to the standardized measurement. Haplotype distribution was initially performed on Haploview 4.0RC1 (Barrett et al., 2005) and further analysis was carried out on SHEsis. For all analyses, p values were two tailed and the significance level was set at $p < 0.05$.

3. Results

Among the control subjects, the observed genotype distributions were in Hardy–Weinberg equilibrium for all the SNPs except rs10784941, which was excluded from further analysis. The allele and genotype frequencies of the five SNPs are listed in Table 2. Rs1386486 and rs1872824 showed statistically marginal differences between cases and controls in genotype frequencies (rs1386486: $p = 0.043351$; rs1872824: $p = 0.016563$), but no significant differences in allele distribution. For the other genetic variants, no significant associations were observed.

We calculated D′ and r^2 for all possible combinations of the SNPs (shown in Table 3 and Fig. 1). Strong LD was found among rs1386494, rs2171363 and rs4760816. In addition, haplotype analysis showed that haplotype rs1386494–rs2171363–rs4760816 was in a block defined by the confidence interval algorithm in Haploview 4.0RC1 and we therefore continued the haplotype analysis of these three SNPs using SHEsis (shown in Table 4). Those haplotypes with an estimated frequency of less than 3% in both case and control groups were excluded from analysis. However, no significant association between this block and BPD was observed.

4. Discussion

For the purposes of the study, we hypothesized that *TPH2* polymorphisms might be associated with BPD and we analyzed six SNPs (rs10784941, rs1386494, rs2171363, rs4760816, rs1386486, and rs1872824) in 506 bipolar patients and 507 controls of Chinese Han origin. rs1386486 and rs1872824 showed significant differences in genotype frequencies (rs1386486: $p = 0.043351$; rs1872824: $p = 0.016563$). However, given the slight significance, larger samples are probably needed to consolidate our results.

Serotonin exists in the entire brain, regulating multiple physiological activities, one of which is higher nervous activity. The serotonin system is characterized by the fact that a relatively small amount of 5-HT

Table 2
Allele and genotype distribution in bipolar patients and controls.

SNP ID		Allele frequency		p value [a]	Permutated p value	Genotype frequency			p value	H–W p value
		A	G			AA	AG	GG		
rs1386494	Case	943 (0.943)	57 (0.057)	0.277428	0.6982	444 (0.888)	55 (0.110)	1 (0.002)	0.440348	0.603372
	Control	920 (0.931)	68 (0.069)			429 (0.868)	62 (0.126)	3 (0.006)		0.643185
		C	T			CC	CT	TT		
rs2171363	Case	523 (0.530)	463 (0.470)	0.827183	0.9995	142 (0.288)	239 (0.485)	112 (0.227)	0.212909	0.551496
	Control	515 (0.526)	465 (0.474)			125 (0.255)	265 (0.541)	100 (0.204)		0.061565
		A	G			AA	AG	GG		
rs4760816	Case	526 (0.533)	460 (0.467)	0.357523	0.8082	143 (0.290)	240 (0.487)	110 (0.223)	0.438200	0.625436
	Control	520 (0.554)	418 (0.446)			140 (0.299)	240 (0.512)	89 (0.190)		0.439436
		C	T			CC	CT	TT		
rs1386486	Case	466 (0.502)	462 (0.498)	0.201646	0.5609	128 (0.276)	210 (0.453)	126 (0.272)	**0.04335**	0.041142
	Control	483 (0.532)	425 (0.468)			123 (0.271)	237 (0.522)	94 (0.207)		0.303095
		A	G			AA	AG	GG		
rs1872824	Case	390 (0.401)	582 (0.599)	0.053764	0.1891	85 (0.175)	220 (0.453)	181 (0.372)	**0.01656**	0.201857
	Control	419 (0.445)	523 (0.555)			84 (0.178)	251 (0.533)	136 (0.289)		0.086564

[a] Pearson's p value, significant p (<0.05) values are in boldface.