Neurosci Bull February 1, 2014, 30(1): 33–42. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1380-5

·Original Article·

Genes in the serotonin pathway are associated with bipolar affective disorder in a Han Chinese population

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ABSTRACT

Serotonin plays an important role in mood regulation, but the involvement of serotonin pathway genes in the development of bipolar I disorder (BP-I), a mood disorder, is not clear. We selected 21 single-nucleotide polymorphisms (SNPs) within the *HTR2A* gene, 8 within the *SLC6A4* gene and 23 within the *TPH2* gene for genotyping using the GoldenGate genotyping assay. A total of 375 patients with BP-I and 475 normal controls were recruited. Two out of 21 SNPs (rs1475196 and rs9567747) in the *HTR2A* gene and 1/23 SNPs (rs17110566) in the *TPH2* gene were significantly associated with BP-I, both genotype-wise and allele-wise. Furthermore, a specific haplotype in the *HTR2A* gene showed a significant association with BP-I. Our results indicate that the *HTR2A* and *TPH2* genes in the serotonin pathway play important roles in susceptibility to BP-I.

Keywords: bipolar affective disorder; serotonin pathway; *TPH2*; *HTR2A*; *SLC6A4*

INTRODUCTION

People with bipolar affective disorder (BPD) experience severe, disruptive mood swings. At some points in their life, patients with BPD may experience abnormally elevated mood, energy, and activity to a degree that interferes with the functions of ordinary life. At other times, they may endure the opposite state with abnormally low mood, energy, and activity[1]. The lifetime prevalence of BPD is 0.3% to 1.5% with a similar ratio in men and women[2]. Despite the high prevalence together with psychosocial impairment and a high risk of suicide in patients with BPD, the pathogenesis of the disorder is still largely unknown. Family, twin, and adoption studies consistently found that BPD is highly heritable, estimated at up to 85%[3-5]. Moreover, there are substantial interactions between genetic and environmental factors[5].

The monoamine hypothesis, which mainly concerns the three classic neurotransmitters serotonin, noradrenalin, and dopamine, includes the concept that disruption of serotonergic function is involved in the pathogenesis of many psychiatric disorders, such as BPD, major depression disorder, and schizophrenia[6, 7]. Serotonin (5-hydroxytryptamine, 5-HT) influences a broad range of behavioral functions, including the control of mood, the sleep-wake cycle, appetite, sexual behavior and cognition[8-10]. Serotonin homeostasis is mainly modulated at pertinent targets such as receptors, transporter, and enzymes in its biosynthetic pathway. Tryptophan hydroxylase 2 (*TPH2*),

5-HT receptor 2A (*HTR2A*), and solute carrier family 6, member 4 (*SLC6A4*) in serotonin pathway are among the key regulatory genes. Studies demonstrated that the *TPH2* gene is predominantly expressed in the brainstem, especially in neurons of the raphe nuclei, and the encoded protein catalyzes the first and rate-limiting step in the biosynthesis of serotonin[11]. Recent studies have provided some evidence for the involvement of the *TPH2* gene in BPD and major depression disorder[12-17]. The *HTR2A* gene encodes one of the neurotransmitter receptors for serotonin, and may alter the levels of serotonin metabolites. Increased levels of 5-HT2 receptors have been associated with mood disorders and sleep disturbances[18-21]. Postmortem studies on people with depression have shown changes in 5-HT2A receptors in different brain regions, which implicate it in mood regulation[22-24]. In addition, a genome-wide linkage study of a large multigenerational BPD pedigree identified a susceptibility locus on chromosome 13q where the *HTR2A* gene is located[25], with subsequent replication in 13 Australian BPD pedigrees[26]. Large association studies[27-29], meta-analysis[30], and convergent functional genomics also support the involvement of *HTR2A* in BPD[31]. The human 5-HT transporter (5-HTT) is encoded by the serotonin transporter gene (*SLC6A4*) on 17q11.2-12[32], and is responsible for terminating the action of 5-HT in the synaptic cleft[33]. Low levels of platelet and brain 5-HTTs have been reported to be associated with depression and suicide[34-36]. However, previous studies of the possible involvement of *SLC6A4* in BPD have produced conflicting results[37-41].

In the current study, we investigated in a Han Chinese population the involvement of *TPH2*, *HTR2A* and *SLC6A4* in serotonin pathway in the development of BPD, using a case-control association design. Only patients with bipolar I disorder (BP-I) who had more than one manic episode were included in order to reduce the phenotypic heterogeneity due to various clinical manifestations.

PARTICIPANTS AND METHODS

Samples

Patients in the BP-I group: 375 in-patients or out-patients at the Guangzhou Brain Hospital were included in the study. All patients were interviewed individually by trained psychiatrists using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) Axis I Disorders[42]. Patients were excluded if they had any other current Axis I diagnosis other than BPD, or neurological disease, or significant physical illness. Patients with history of consciousness disturbance after traumatic brain injury were also excluded.

A total of 475 community volunteers were recruited as normal controls using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Non Patient Edition to screen for a lifetime absence of psychiatric illness. Participants with significant physical illness, pregnancy, or any psychiatric disorder were excluded.

All participants were Han Chinese. This study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Boards of Guangzhou Brain Hospital and West China Hospital, Sichuan University. Written informed consent was given by all subjects.

Genotyping

Peripheral blood (5 mL) was collected from all participants, and genomic DNA was extracted according to the standard phenol-chloroform procedure[43]. A total of 52 single-nucleotide polymorphisms (SNPs) (21 SNPs in *HTR2A*, 8 in *SLC6A4*, and 23 in *TPH2*) were selected based on SNP tagging of Han Chinese in Beijing through the HapMap database (http://hapmap.ncbi.nlm.nih.gov). The coverage of tagging SNPs, calculated by Tagger Server (http://www.broadinstitute.org/mpg/tagger/), on average captured 70% of the information in the targeted region with max r^2 ≥0.8. The distributions of these SNPs are shown in Figs. 1, 2 and 3. A total of 250 ng DNA was genotyped by the GoldenGate genotyping assay following the manufacturer's instructions (BeadStation 500, Illumina Inc., San Diego, CA).

Statistical Analysis

Student's *t*-test and the χ^2 test were used for continuous variables and categorical variables as appropriate. Analysis was performed using the SPSS 12.0 statistical software package[44]. Hardy-Weinberg equilibrium and intermarker linkage disequilibrium (LD) as expressed by r^2 and D' values were calculated using Plink 1.07 (http://pngu.mgh.harvard.edu/purcell/plink). Categorical association tests between cases and controls were analyzed using the χ^2

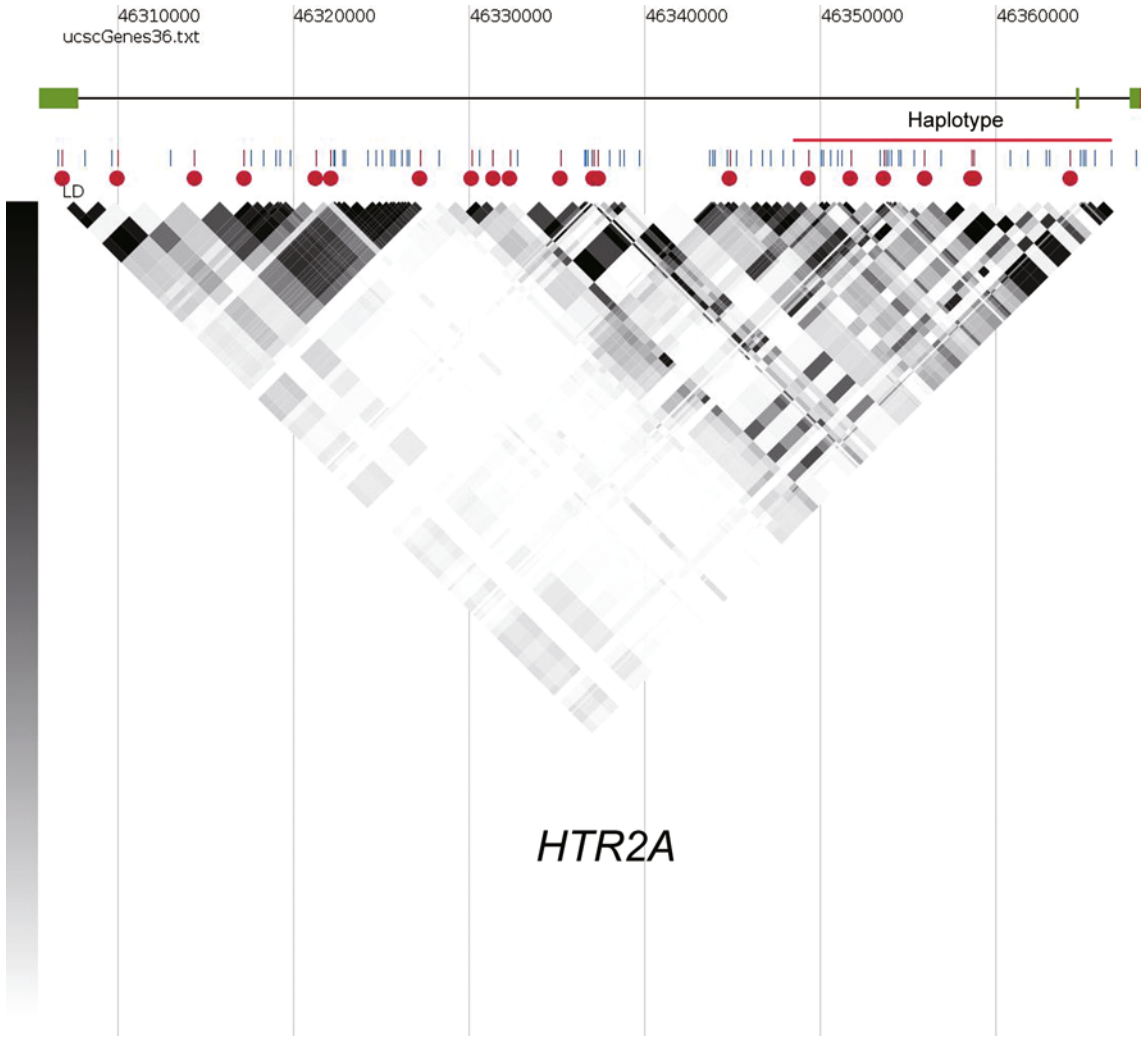

Fig. 1. Genomeview from Goldsurfer2 showing the selection of 21 tagSNPs (red dots) in relation to the coding region of *HTR2A* (blue lines). Linkage disequilibrium (LD) is measured in r^2.

test or Fisher's exact test, as appropriate. Multiple marker haplotypes were analyzed by Plink 1.07 with a moving-window strategy combining 2 to 8 neighboring SNPs. For each combination of loci within one gene, we obtained both global *P* values that assessed the significance of association for all the haplotypes of the loci, and *P* values that assessed the significance of association for specific haplotypes. For the present analysis, the minimum haplotype frequency was set at 0.05 (i.e. haplotypes that occurred with a frequency of <5% in controls were excluded). Bonferroni correction was used to reduce false-positives due to multiple tests[45].

RESULTS

Demographic Characteristics

Sex and age distribution did not differ significantly between patients with BP-I and normal controls, but there was a significant difference in years of education. The age at onset in patients with BP-I was 24.3 ± 8.9 years (mean ± SD) (Table 1).

Hardy-Weinberg Equilibrium Test

The genotypic frequencies of 52 SNPs within the *TPH2*, *HTR2A*, and *SLC6A4* genes were in Hardy-Weinberg disequilibrium in the control group (*P* >0.05 for all SNPs).

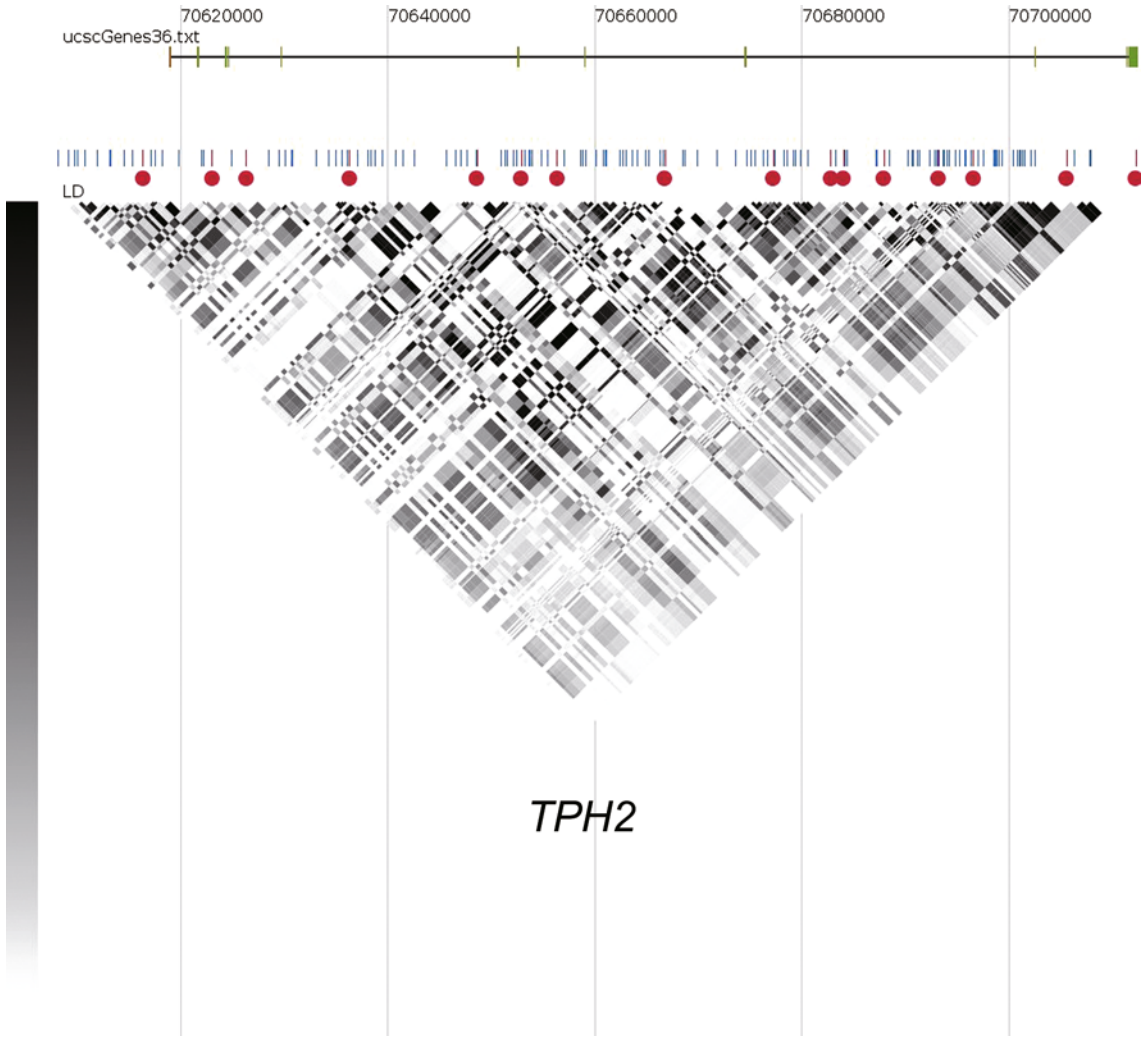

Fig. 2. Genomeview from Goldsurfer2 showing the selection of 15 tagSNPs (red dots) in relation to the coding region of *TPH2* (blue lines). Linkage disequilibrium (LD) is measured in r^2.

Comparison of Patients with BP-I and Normal Controls

Effects of individual SNPs Analysis for effects of individual SNPs revealed that 2/21 SNPs (rs1475196 and rs9567747) in the *HTR2A* gene and 1/23 SNPs (rs17110566) in the *TPH2* gene showed a statistically significant association with BP-I both genotype-wise and allele-wise (Table 2). No individual SNPs in the *SLC6A4* gene were significantly associated with BP-I.

Effects of multi-marker haplotypes The aim of multiple-marker analysis is to find haplotypes with significant associations that can be used to map true susceptibility in the region. Consequently, only those haplotypes with a global *P* value of <0.05, and at least one specific haplotype with a corrected *P* value of <0.05 are shown (Table 3). We found that haplotypes with multiple-markers (SNPs rs1928038 - rs1475196 - rs9562689 - rs9567747 - rs1328684 - rs1328684) showed significant association with BPD-I, with a global *P* value of 0.003, and three specific haplotypes (CAAGAA, GAGGAA, and CCGAAA) with corrected *P* values of <0.05.

DISCUSSION

In the present study, we found that 2 out of 21 SNPs (rs1475196 and rs9567747) in the *HTR2A* gene and 1 out of 23 SNPs (rs17110566) in the *TPH2* gene were significantly

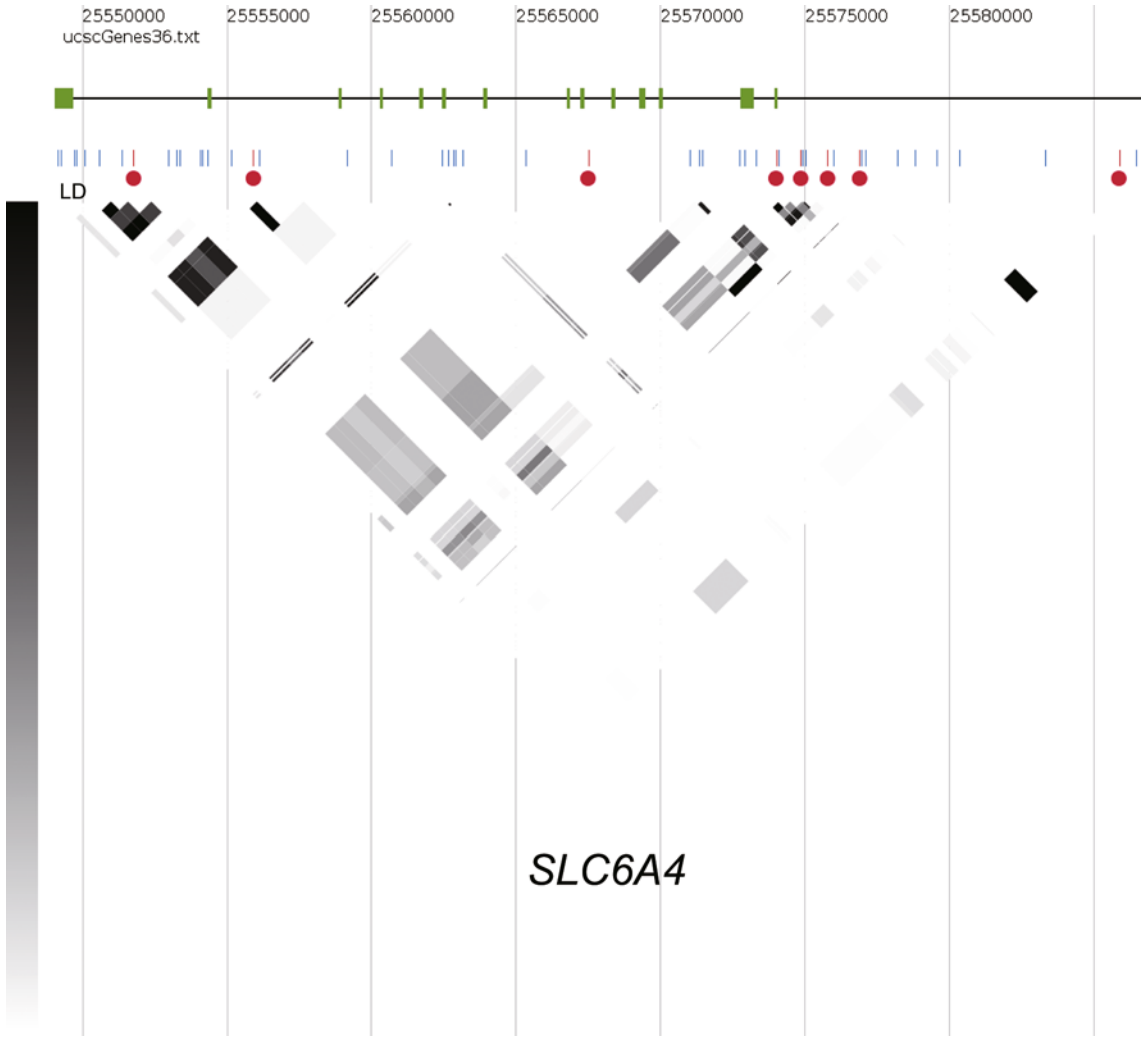

Fig. 3. Genomeview from Goldsurfer2 showing the selection of 8 tagSNPs (red dots) in relation to the coding region of *SLC6A4* (blue lines). Linkage disequilibrium (LD) is measured in r^2.

associated with BP-I both genotype-wise and allele-wise. Furthermore, a specific haplotype within the *HTR2A* gene also showed significant association with the disease by multi-marker haplotype analysis. The combined evidence from the current study supports the hypothesis that genes in the serotonin pathway, especially the *HTR2A* and *TPH2* genes, play important roles in susceptibility to BP-I.

The *TPH2* gene is located on chromosome 12q21.1, spanning ~93 kb and containing 12 exons[11, 46]. This gene encodes a member of the pterin-dependent aromatic-acid hydroxylase family and plays an important role in the regulation of serotonin function[46]. Many previous studies suggested that this chromosome region might be associated with BPD. For example, significant linkage between BPD and chromosome 12q23-24 has been reported in a number of independent linkage analyses[47-49]. Cichon *et al.* found a significant association between rs17110563 in the TPH2 gene, which encodes a Pro206Ser substitution, and BPD[50]. Zhang *et al.* reported that a rare loss-of-function mutation (G1463A) in the *TPH2* gene may represent an important risk factor for unipolar major depression[14]. Lopez *et al.* found that the haplotype G-T-A (rs1386494 - rs1007023 - rs9325202), located in the *HTR2A* gene, contributes to the risk of both BPD and suicide attempts in families with BPD[17]. Another report indicated that the C2755A polymorphism, which leads

Table 1. Demographic characteristics of patients with BPD-I and healthy controls

	Case (n = 375)	Control (n = 475)	χ^2/t	P
Sex (male:female)	169:206	236:239	1.73	0.189
Age (years)	31.58 ± 11.46	32.97 ± 12.89	1.63	0.103
Years of education	11.63 ± 3.63	10.65 ± 3.47	4.12	< 0.01
Age at onset (years)	24.32 ± 8.88			

Table 2. SNPs in HTR2A and TPH2 genes associated with BP-I

Gene	SNP	Genotype*	MAF		χ^2	OR	P	Bonf P
			Cases	Controls				
HTR2A	rs1475196	C/A	0.04	0.09	14.31	0.44	1.55E-04	0.007
HTR2A	rs9567747	G/A	0.34	0.259	16.26	1.55	5.53E-05	0.002
TPH2	rs17110566	A/G	0.10	0.17	13.79	0.58	2.04E-04	0.009

*Minor alleles are underlined. MAF, minor allele frequency; Bonfp, Bonferroni P-value; OR, odds ratio.

Table 3. Multi-marker* haplotype analysis within the *HTR2A* gene in cases and controls

Haplotype	Frequency in cases	Frequency in controls	χ^2 (1 df)	P
CAGAGG	0.06	0.08	1.16	0.29
CAAGAA	0.26	0.21	5.08	0.03
GAGGAA	0.09	0.06	5.74	0.02
CCGAAA	0.04	0.07	4.71	0.03
CAGAAA	0.56	0.60	2.69	0.10

*SNPs: rs1928038 - rs1475196 - rs9562689 - rs9567747 - rs1328684 - rs1328684. Global χ^2 = 15.89; df = 4; P = 0.003.

to an S41Y substitution, and two SNPs in the promoter region of the *TPH2* gene, T−703G (rs4570625) and T−473A (rs11178997), might be involved in susceptibility to BPD. The latter two promoter polymorphisms are predicted to affect POU3F2 binding and modulate *TPH2* gene expression[51]. In the current study, the associated SNP (rs17110566) in an intron region of the *TPH2* gene is unlikely to affect the coding sequence of *TPH2*, but it may be involved in BP-I by LD or by influencing *TPH2* mRNA expression. Taking into account the possible strong LD between rs17110566 and nearby variants, the causal variants may be harbored in the haplotype block we identified. As Cichon *et al.* suggested, further studies should be focused on detecting low-frequency and high-frequency penetrance variants in order to identify the full spectrum of susceptibility variants present in the haplotype blocks in the *TPH2* gene[50].

Studies on the *HTR2A* gene have also produced controversial results. Some previous studies suggested that this gene is associated with BPD[52-55], but others did not support these findings[56-58]. A recent Australian cohort study selected eight SNPs across the *HTR2A* gene and found that two common variants (rs2224721 and rs1923886) and a haplotype block (CCGCA) are associated with BPD[55]. In our study, we did not find significant association between these two common variants (rs2224721 and rs1923886) near the 5'UTR and BP-I. However, two other common variants (rs1475196 and rs9567747) near the UTR of the *HTR2A* gene [located close to functional polymorphisms of rs6313 (102T/C) and rs6314 (His452Tyr or C1354T) in the 3' end] showed a positive association with BP-I. The inconsistency between Australian cohort study and ours may be due to genetic heterogeneity in the different populations. Alternatively, false-positives may have been produced in either or both studies due to phenotypic heterogeneity. In order to reduce this heterogeneity,

we included only patients with BP-I who had more than one manic episode. It should also be noted that there is an overlapping region in the *HTR2A* gene between the haplotype block in our study and the Australia cohort study, so it is possible that this overlapping region harbors variants that have severe biological effects.

Many studies have explored a possible association between the *SLC6A4* gene and BPD but with inconsistent findings. Significant association has been found between *5-HTTLPR* polymorphism and BPD[38, 41, 59, 60], although conflicting results have also been reported[61, 62]. A study of BP-I with the *SLC6A4* gene in a Chinese population from Taiwan by Sun *et al.* indicated no significant association of any single polymorphism, including *5-HTTLPR.* However, an *SLC6A4* haplotype, which spans at least a 30-kb interval around the *SLC6A4* gene, may play a significant role in the etiology of BPD[39]. Our findings did not show any statistical significance for either individual SNPs or haplotype analysis, providing no evidence for the *SLC6A4* gene as a susceptibility gene of BPD-I.

Although many studies have shown associations between genes in the serotonin pathway and BPD, they mainly focused on several functional polymorphisms within these genes[14, 39, 50, 53]. There are two advantages in current study. First, we selected tagger SNPs throughout the candidate gene to investigate the association. Furthermore, only patients with BP-I were included in order to reduce phenotypic heterogeneity.

Previous studies showed that patients with BPD have better academic performance than controls[63-65]. This is partly in line with our finding that individuals with BP-I had a higher education level, probably due to their better academic ability. It has been suggested that genetic factors contribute to better academic performance as well as susceptibility to illness in patients with BPD[63, 66-68]. However, other studies have reported lower premorbid IQ scores or poorer school performance in such patients[64, 69-71]. It is worth exploring whether education level contributes to the susceptibility to BPD as an environmental factor; this would need a longitudinal study with sophisticated design.

Considering the small effect size of susceptibility genes for complex genetic disorders such as BPD, the sample sizes of 375 patients with BP-I and 475 controls in this study were still relatively small. Type II errors, through the lack of an observed association between the analyzed polymorphism and BP-I, cannot be ruled out. Ethnic differences should also be considered in explaining the results, as most previous studies were performed in other populations such as Caucasians[55]. Further analysis with a larger and independent sample is warranted. Another limitation of this study is that we performed the association analysis between genes in the serotonin pathway and BP-I only. In the future, it will also be important to explore the contribution of genes in the serotonin pathway (e.g. *TPH2* and *HTR2A*) in the pathology of other affective disorders such as major depression and BP-II with the same analysis strategy, considering previous positive findings. Nevertheless, the findings reported in this preliminary investigation are encouraging, because of the high relevance of the serotonin pathway to mood disorders. Information from individual variants and haplotypes may therefore facilitate the search for the causative risk alleles in these genes by cutting-edge technologies such as second-generation sequencing.

ACKNOWLEDGEMENTS

This work was partly funded by the National Natural Science Foundation of China (81261120415, 91232711, and 81130024), the National Basic Research Program of China (973 Program 2007 CB512301), and the Medical Scientific Research Foundation of Guangdong Province (A2010487) and Guangzhou City (2012A010011).

Received date: 2013-01-06; Accepted date: 2013-02-10

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