Polymorphisms in the human serotonin receptor 1B (HTR1B) gene are associated with schizophrenia: a case control study

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Abstract

Background: Schizophrenia is associated with multiple neurotransmitter disorders, including serotonin (5-hydroxytryptamine, 5-HT). The neuromodulatory action of serotonin on brain function largely depends on the action of specific subtypes of serotonin receptors. The serotonin receptor 1B (HTR1B) gene has been proposed to play putative roles in the development of multiple emotional and psychiatric disorders.

Methods: To study the relationship of HTR1B polymorphisms and schizophrenia, gene information was drawn from a cohort of 310 schizophrenic patients (152 men and 158 women) and 313 healthy controls (153 men and 160 women) of northern Han Chinese descent. The \( \chi^2 \) test was used to compare allele and genotype distributions between case and control groups. The haplotype and linkage equilibrium were also assessed in two group comparisons.

Results: We detected 14 SNPs. Male patients were observed to have higher frequencies of the A-allele and AA+AG genotype at rs1778258 than female patients (\( p = 0.012 \) and \( p = 0.015 \), respectively). Both the A-allele and AA+AG genotype were associated with schizophrenia risk (OR = 1.986 and OR = 2.061, respectively), although the statistical significance of the genotype was lost after Bonferroni correction. Linkage analysis showed that rs17273700, rs11568817, rs9361234 and rs58138557 polymorphisms exhibit strong linkage disequilibrium (LD). In addition, schizophrenic patients show stronger linkage between 11,568,817 and rs130058 than healthy controls.

Conclusions: HTR1B polymorphisms are associated with schizophrenia in the northern Han Chinese population, which provides an etiological reference for schizophrenia.

Keywords: Schizophrenia, Single-nucleotide polymorphisms (SNPs), Serotonin receptor 1B (HTR1B)
psychotherapeutic drugs. The HTRIB gene is located on chromosome 6 at position 77,460,848–77,464,022 (GR Ch38.p7). It has been suggested that schizophrenic patients show increased HTRIB mRNA levels in the hippocampus. Simultaneous upregulation of HTRIB and downregulation of HTR2A could decrease GABAergic activity, which leads to an increased glutamatergic efferent in the hippocampus [7]. In previous studies, there were inconsistent results on the genetic association between HTRIB gene variations and schizophrenia. For example, rs2143823 is considered to be related to schizophrenia in Croatians [10]. However, there was no correlation between G861C and schizophrenia in Portuguese, German and Brazilian patients [11–13]. The associations between C129T, T371G, T655C, C705T, G861C, A1099G, G1120A and schizophrenia were also negative in mixed populations in the United States [14]. In addition, the HTRIB haplotype may be implicated in the gender discrepancy of schizophrenia in Spanish populations [15].

As the function of the coding regions has been studied extensively, SNPs located in regulatory regions were taken in to consideration. Prior studies addressing the association between schizophrenia and HTRIB polymorphisms in the Chinese Han population include only the 5′-untranslated (5′UTR) and coding regions, with little data concerning the 5′-promoter and 3′-regulatory regions. Therefore, we chose to investigate these regions to improve etiologic knowledge of this disease. Expanding sequences were investigated in our study, including a 2285 bp 5′-promoter region and a 1277 bp 3′-untranslated region, in the northern Han Chinese population to further explore the relationship between HTRIB and schizophrenia.

**Materials**

**Subjects**

Our sample comprised 310 schizophrenic patients (152 men and 158 women) and 313 genetically unrelated, healthy volunteers (153 men and 160 women) comprising a combined 623 individuals. All participants enrolled were of northern Han Chinese descent. Blood samples from schizophrenic patients were provided by the Third People’s Hospital of Liaoning Province; those from healthy controls were supplied by the China Medical University’s Forensic Evidence Department. Inclusion criteria included a diagnosis of schizophrenia by trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). Exclusion criteria included the presence of other psychiatric disorders. The results of patient questionnaires confirming no history nor present evidence of any psychiatric disorder and no history of mental disease for at least three prior generations were used to select healthy controls. All subjects provided written informed consent prior to enrollment in this study.

**SNP selection**

The following factors were considered for SNP selection: (1) as the function of the HTRIB coding region has been studied extensively, SNPs located within HTRIB regulatory regions were selected; (2) based on prior reports, we found that current knowledge of the association between schizophrenia and HTRIB polymorphisms in the Chinese Han population include the 5′-untranslated region (UTR), while the promoter and 3′ regulatory regions have not been well studied and; (3) according to the HTRIB polymorphism distribution, we selected SNPs with favorable polymorphisms (MAF ≥ 0.1). Considering that the regulatory effects of sequences that are distal to the coding region may be limited, we selected a 2285 bp 5′-promoter region and a 1277 bp 3′-untranslated region for SNP investigation.

**DNA isolation and genotyping**

Genomic DNA was extracted by the phenol-chloroform method from whole blood [16]. A 2285 bp fragment in the 5′-promoter region and a 1277 bp fragment in the 3′-untranslated region were included in our analysis. Standard PCR was performed in a total reaction volume of 20 μl (adjusted with sterilized, deionized water) containing 2 μl genomic DNA as template (approximately 50 ng), 10 μl of 2 × GC Buffer, 0.2 μl (1 U) of Taq polymerase (Takara LA Taq, Dalian, China), 2 μl (3.75 nmol) of dNTPs, 1.5 μl (7.5 pmol) each of sense and antisense

| SNP                  | Chr. pos. | Base change | MAF | REGION |
|----------------------|-----------|-------------|-----|--------|
| rs1410535            | 77,465,335| –1932 T > C | 0.375 | 3′UTR |
| rs1778258            | 77,464,492| –1089 G > A | 0.113 | 5′UTR |
| rs1228114            | 77,464,263| –860 T > C | 0.121 | 3′UTR |
| rs11568817           | 77,463,665| –262 T > G | 0.116 | 5′UTR |
| rs130058             | 77,463,564| –161 A > T | 0.077 | 3′UTR |
| rs6297               | 77,462,224| *7 A > G   | 0.112 | 3′UTR |
| rs3827804            | 77,462,215| *16 G > A  | 0.011 | 3′UTR |
| rs140792648          | 77,462,065| *165_166 Ins AG | 0.029 | 3′UTR |
| rs9361234            | 77,461,965| *266 C > T | 0.113 | 3′UTR |
| rs183156887          | 77,461,962| *269 C > A | 0.0064 | 3′UTR |
| rs76194807           | 77,461,898| *333 G > T | 0.113 | 3′UTR |
| rs58138557           | 77,461,771| *459_460 Del GG | 0.115 | 3′UTR |
| rs13212041           | 77,461,407| *824 A > G | 0.256 | 3′UTR |

1 The A of the ATG start codon is designated as position 0
2 An asterisk (*) indicates alterations in the coding region, which is 1173 bp in length
### Table 2 Allelic distributions of the 14 SNPs and their associations with schizophrenia

| SNP     | Case (n=310) | Control (n=313) | p Value<sup>2</sup> | Codominant (AA/aa,Aa/aa,AA/AA,aA,aA) | Recessive | Dominant | Overdominant |
|---------|--------------|-----------------|----------------------|--------------------------------------|------------|----------|-------------|
| rs4140535 |              |                 |                      |                                      |            |          |             |
| T       | 394          | 0.635           | 0.025                | 0.725                                | 0.806      | 1.000    | 0.729       | 0.909       | 0.684       | 0.764       |
| CC      | 44           | 0.142           | 0.64                | 0.126                                | 0.147      |          |             |             |             |             |
| CT      | 138          | 0.445           | 0.25                | 0.055                                | 0.057      |          |             |             |             |             |
| TT      | 128          | 0.413           |                      |                                      | 0.396      |          |             |             |             |             |
| rs1778258 |              |                 |                      |                                      |            |          |             |
| G       | 556          | 0.897           | 0.585                | 1.000                                | 1.000      | 1.000    | 0.541       | 1.000       | 0.552       | 0.542       |
| AA      | 4            | 0.013           | 0.012                | 0.013                                | 0.016      |          |             |             |             |             |
| AG      | 56           | 0.181           | 0.049                | 0.014                                | 0.016      |          |             |             |             |             |
| GG      | 250          | 0.806           | 0.042                | 0.185                                | 0.162      |          |             |             |             |             |
| rs1727300 |              |                 |                      |                                      |            |          |             |
| T       | 554          | 0.894           | 0.423                | 0.772                                | 0.377      | 0.185    | 0.576       | 0.279       | 0.184       |
| CC      | 7            | 0.023           | 0.001                | 0.016                                | 0.006      |          |             |             |             |             |
| CT      | 52           | 0.168           | 0.049                | 0.014                                | 0.016      |          |             |             |             |             |
| TT      | 251          | 0.810           | 0.374                | 0.247                                | 0.465      | 0.276    | 0.663       | 0.356       |
| rs1228814 |              |                 |                      |                                      |            |          |             |
| C       | 517          | 0.834           | 1.000                | 0.374                                | 0.247      | 0.465    | 0.276       | 0.663       | 0.356       |
| AA      | 13           | 0.042           | 0.002                | 0.006                                | 0.002      |          |             |             |             |             |
| AC      | 77           | 0.248           | 0.185                | 0.071                                | 0.071      |          |             |             |             |             |
| CC      | 220          | 0.710           | 0.049                | 0.014                                | 0.014      |          |             |             |             |             |
| rs1156817 |              |                 |                      |                                      |            |          |             |
| T       | 559          | 0.902           | 0.315                | 1.000                                | 1.000      | 0.254    | 1.000       | 0.271       | 0.255       |
| GG      | 5            | 0.016           | 0.001                | 0.016                                | 0.016      |          |             |             |             |             |
| GT      | 51           | 0.165           | 0.049                | 0.014                                | 0.014      |          |             |             |             |             |
| TT      | 254          | 0.819           | 0.374                | 0.247                                | 0.465      | 0.276    | 0.663       | 0.356       |
| rs130058 |              |                 |                      |                                      |            |          |             |
| T       | 49           | 0.079           | 0.916                | 0.686                                | 0.816      | 0.434    | 1.000       | 0.449       | 0.816       |
| AA      | 265          | 0.855           | 0.079                | 0.816                                | 0.816      |          |             |             |             |             |
| AT      | 41           | 0.132           | 0.049                | 0.014                                | 0.014      |          |             |             |             |             |
| TT      | 4            | 0.013           | 0.006                | 0.006                                | 0.006      |          |             |             |             |             |
Table 2 Allelic distributions of the 14 SNPs and their associations with schizophrenia (Continued)

| SNP          | Allele<sup>1</sup> | Genotype | Case (n=310) No. | Frequency | Control (n=313) No. | Frequency | p Value<sup>2</sup> | Codominant (AA/aa, Aa/aa, AA/AA) | Recessive | Dominant | Overdominant |
|--------------|--------------------|----------|-----------------|-----------|---------------------|-----------|---------------------|-----------------------------------|------------|-----------|--------------|
| rs6297       |                    |          |                 |           |                     |           | 0.049               | 0.599 0.786 0.565 0.788 0.460 0.816 |            |           |              |
| A            | 541                | 0.873    | 536             | 0.865     |                     |           |                     |                                    |            |           |              |
| AA           | 237                | 0.765    | 231             | 0.738     |                     |           |                     |                                    |            |           |              |
| AG           | 67                 | 0.216    | 74              | 0.236     |                     |           |                     |                                    |            |           |              |
| GG           | 6                  | 0.019    | 8               | 0.026     |                     |           |                     |                                    |            |           |              |
| rs3827804    |                    |          |                 |           |                     |           | 0.802               | 0.800 0.800 0.800 0.800 |            |           |              |
| A            | 8                  | 0.013    | 7               | 0.011     |                     |           |                     |                                    |            |           |              |
| AG           | 8                  | 0.026    | 7               | 0.022     |                     |           |                     |                                    |            |           |              |
| GG           | 302                | 0.974    | 306             | 0.978     |                     |           |                     |                                    |            |           |              |
| AA           | 0                  | 0.000    | 0               | 0.000     |                     |           |                     |                                    |            |           |              |
| rs140792648  |                    |          |                 |           |                     |           | 0.862               | 0.860 0.860 0.860 0.860 |            |           |              |
| —            | 604                | 0.974    | 608             | 0.971     |                     |           |                     |                                    |            |           |              |
| —/—          | 294                | 0.948    | 295             | 0.942     |                     |           |                     |                                    |            |           |              |
| —/AG         | 16                 | 0.052    | 18              | 0.058     |                     |           |                     |                                    |            |           |              |
| AG/AG        | 0                  | 0.000    | 0               | 0.000     |                     |           |                     |                                    |            |           |              |
| rs9361234    |                    |          |                 |           |                     |           | 0.048               | 1.000 1.000 0.348 1.000 0.364 0.349 |            |           |              |
| C            | 559                | 0.902    | 555             | 0.887     |                     |           |                     |                                    |            |           |              |
| CC           | 254                | 0.819    | 247             | 0.789     |                     |           |                     |                                    |            |           |              |
| CT           | 51                 | 0.165    | 61              | 0.195     |                     |           |                     |                                    |            |           |              |
| TT           | 5                  | 0.016    | 5               | 0.016     |                     |           |                     |                                    |            |           |              |
| rs183156887  |                    |          |                 |           |                     |           | 1.000               | 1.000 1.000 1.000 1.000 1.000 |            |           |              |
| A            | 4                  | 0.006    | 4               | 0.006     |                     |           |                     |                                    |            |           |              |
| AC           | 4                  | 0.013    | 4               | 0.013     |                     |           |                     |                                    |            |           |              |
| CC           | 306                | 0.987    | 309             | 0.987     |                     |           |                     |                                    |            |           |              |
| AA           | 0                  | 0.000    | 0               | 0.000     |                     |           |                     |                                    |            |           |              |
| rs76194807   |                    |          |                 |           |                     |           | 0.048               | 0.725 0.242 0.472 0.340 0.725 0.242 |            |           |              |
| T            | 79                 | 0.127    | 71              | 0.113     |                     |           |                     |                                    |            |           |              |
| GG           | 234                | 0.755    | 247             | 0.789     |                     |           |                     |                                    |            |           |              |
| GT           | 73                 | 0.235    | 61              | 0.195     |                     |           |                     |                                    |            |           |              |
| TT           | 3                  | 0.010    | 5               | 0.016     |                     |           |                     |                                    |            |           |              |
Table 2 Allelic distributions of the 14 SNPs and their associations with schizophrenia (Continued)

| SNP     | Allele<sup>1</sup> | Genotype | Case (n=310) | Control (n=313) | p Value<sup>2</sup> | Codominant (AA/aa,Aa/aa,AA/Aa)<sup>3</sup> | Recessive | Dominant | Overdominant |
|---------|--------------------|----------|--------------|-----------------|---------------------|-------------------------------------------|-----------|-----------|-------------|
| rs58138557 |                    |          |              |                 |                     |                                           |           |           |             |
|         |                    | GG       | 558          | 0.900           | 554                 | 0.900                       | 0.016     | 0.016     | 0.016       |
|         |                    |         | 5            | 0.016           | 5                   | 0.016                       |           |           |             |
|         |                    |         | 52           | 0.168           | 62                  | 0.198                       |           |           |             |
|         |                    | GG/GG    | 253          | 0.816           | 246                 | 0.786                       |           |           |             |
|         |                    |          |              |                 |                     |                                           |           |           |             |
| rs13212041 |                    | G        | 160          | 0.258           | 160                 | 0.256                       |           |           |             |
|         |                    | AA       | 171          | 0.552           | 179                 | 0.572                       |           |           |             |
|         |                    | AG       | 118          | 0.381           | 108                 | 0.345                       |           |           |             |
|         |                    | GG       | 21           | 0.068           | 26                  | 0.083                       |           |           |             |

<sup>1</sup>The allele for each SNP indicated is that with the higher patient frequency.

<sup>2</sup>The significance levels of chi-squared tests for codominant, dominant, recessive and overdominant model were adjusted using the Bonferroni correction (significance level p = 0.0036).

<sup>3</sup>The SNP allele that is observed at a higher frequency in patients is designated A; the other allele is designated a.
primers. The primer sequences of the 5′-end fragment were 5′-TGGGTGTTGTGCTTTATGCTTT-3′ (sense), 5′-GGAGCAGAGGATAAGTTGCCATG-3′ (antisense), and the primer sequences of the 3′-end fragment were 5′-CCCTCTCTCTATCTCTCCCAAAGT3′ (sense), 5′-ACCCTATCCCTAATTGTTGTAAG-3′ (antisense; Taihe Biotechnology Co., Beijing, China). PCR conditions for the 5′-end fragment were: 94 °C for 1 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min; then final extension at 72 °C for 7 min. PCR conditions for the 3′-end fragment were: 94 °C for 1 min; by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min; and extension at 72°C for 7 min. The Sanger double-chain termination method (Taihe Biotechnology Co., Beijing, China) was employed for DNA sequencing.

Statistical analysis

Allele and genotype frequencies were calculated by direct counting. Haplotype blocks were determined by the confidence interval method in Haplovew. Using this method, six 5′-promoter region SNPs were included in a haplotype block, while the eight 3′-promoter region SNPs were not included in a haplotype block. Thus, we performed analysis for haplotypes formed by the 6 SNPs in the block (namely, the 5′ block) and the remaining eight SNPs (designated the 3′ block) [17].

In order to test Hardy-Weinberg equilibrium (HWE) and construct haplotype blocks, linkage equilibrium (LD) analysis (D′ and r2) were performed using Haplovew version 4.2 software (Broad Institute, Cambridge, MA, USA) [18]. The χ2 test was used to estimate correlations between the variance of polymorphism frequency distribution and schizophrenia. Statistical significance was defined as p < 0.05 (two-tailed). Statistical analyses were performed using SPSS Software19.0 (IBM, Armonk, NY, USA). A Bonferroni correction was applied for multiple comparisons to control for type I error, and the p-value was divided by the total number of loci or haplotypes [17].

Results

We identified 14 SNPs in the present study (Table 1). The genotype distribution was in accordance with Hardy-Weinberg equilibrium in the control group. A summary of allele and genotype frequencies is presented in Table 2. We found that male patients were observed to have a significantly higher A-allele frequency at rs1778258 than female patients (p = 0.012). The frequency of A-allele carriers (AA+AG genotype) among male patients was also significantly higher than among female patients (p = 0.015), although statistical significance was lost after Bonferroni correction. The presence of both the A-allele and AA+AG genotype increased schizophrenia risk (OR = 1.986 and OR = 2.061, respectively; Table 3).

| Group | rs1778258 | Male | Female | p value | OR | 95% CI |
|-------|-----------|------|--------|---------|----|--------|
| case  | A         | 41/304 | 23/316 | 0.012   | 1.986 | 1.161–3.398 |
| AA + AG | 38/152 | 22/158 | 0.015 | 2.061 | 1.152–3.684 |
| control | A       | 33/306 | 38/320 | 0.706 | 0.897 | 0.547–1.472 |
| AA + AG | 38/153 | 22/160 | 0.891 | 0.945 | 0.550–1.622 |

We found no significant associations between any other SNPs and schizophrenia.

Via linkage disequilibrium (LD) analysis, rs11568817 was found to be in strong linkage disequilibrium with rs17273700, rs9361234 and rs58138557 in northern Han Chinese patients (r2 > 0.8) [18]. We also found that, as compared to the healthy people, the linkage between rs11568817 and rs130058 in schizophrenic patients was more intense. Furthermore, LD analysis showed that the six 5′-promoter region SNPs are included in a haplotype using the confidence interval method, while the eight 3′-promoter region SNPs were not included in a haplotype block (Fig. 1). We next compared the frequencies of haplotypes formed by the six SNPs in block or the remaining eight SNPs between the patients and healthy people, and found that the haplotype frequency distributions for the 5′-promoter region C-G-C-A-G-A were significantly different among individuals in the case and control groups. (p = 0.044); however, statistical significance was lost after Bonferroni correction. No statistical differences between groups were observed for other haplotypes (Tables 4 and 5).

Discussion

In the present study, we investigated HTRIB polymorphisms in 623 individuals of northern Han Chinese descent, including 310 schizophrenic patients and 313 healthy controls. We ultimately detected 14 SNPs. According to previously reported observations, no evidence regarding a relationship between HTRIB and schizophrenia was found in any allele or major haplotype for T-261G, -182INS/DEL-181, A-161 T, C129T and G861C in Han Chinese patients [19]. Our study showed consistent results regarding rs11568817 (T-261G) and rs130058 (A-161 T). When patients were grouped by gender, male patients were observed to have a significantly higher frequency of A-allele at rs1778258 than female patients (p = 0.012). Based on multiple comparisons of genotype effects, we inferred that the most probable mode of inheritance of rs1778258 is the dominant model [20]. The frequency of the AA + AG genotype in male patients was also significantly higher than in female patients (p = 0.015), although statistical significance was lost after Bonferroni correction. Both the A-allele and AA+AG
genotype were associated with increased risk of schizophrenia (OR = 1.986 and OR = 2.061, respectively). Thus, our study provides evidence that rs1778258 involved with gender variations in schizophrenia.

It is understood that sex differences exist in brain function as well as the vulnerability, incidence, manifestation, and treatment of numerous psychiatric diseases, which are determined by inherent biological differences between males and females. For instance, males show a higher propensity for Parkinson's disease, autism, attention deficit hyperactivity disorder (ADHD) and addiction. Females also show higher susceptibility to Alzheimer's disease and anxiety/depression [21]. Most studies have found the onset age of schizophrenia to be earlier in men than in women, although there is no sex difference in overall incidence of schizophrenia [22]. Men with schizophrenia also present with more cognitive disturbances and greater reductions in temporal lobe volume than women with schizophrenia [23]. Moreover, evidence also supports sex differences in serotonin neurotransmission and psychiatric disorders caused by malfunctions in the serotonin system. Such differences are not only due to hormonal regulation, but are also attributable genetic effects [21, 24]. It was previously reported that higher whole blood 5-HT levels in women than in men are influenced by multiple genes on chromosomes 2, 6, and 17 [25]. In addition, the effects of sex hormones on serotonin regulation have also been reported. It has been shown that estradiol plays a protective role against the cognitive, positive, and negative symptom domains of schizophrenia [26]. Our results indicated that rs1778258 is associated with gender in schizophrenia. Since rs1778258 is located in the promoter region, it may have an influence on gene expression. Alternatively, there could be a functional site closely linked to rs1778258, and its variation thus could modify gene expression [27], which could have an influence on the serotonin system.

Previous genetic studies on schizophrenia have suggested that there is a gender-specific association between select dopamine genes and schizophrenia. Gender-specific associations between genotype and schizophrenia were also observed in GABAergic-mediated regulation of anterior cingulate cortex function. Male schizophrenic patients expressed significantly lower levels of GABA-Aa5, GABA-Ab1, and GABA-Ae, while the expression of GABA-Ab1 and GAD67 was significantly higher in female patients, as compared to sex-matched controls [28]. Since
it is known that these neurotransmitter systems are closely related [29], these differences may contribute to the gender-specific relationship between HTR1B polymorphisms and schizophrenia. Although sex differences are essential to study to fully understand the etiology of schizophrenia, unfortunately, few studies have been made regarding gender-specific associations between HTR1B and schizophrenia. The only association found to date was described in a Spanish population. The AAAC haplotype comprising rs6297, rs130058, rs1213366, and rs1213371 is significantly more frequent in female Spanish patients [15], which corroborates our finding that HTR1B polymorphisms and schizophrenia. Although sex differences are present greater anger and hostility [27]. Although three known functional sites (rs11568817, rs130058, rs13212041) were found in our study, we discovered no direct associations between HTR1B and schizophrenia by haplotype. We hypothesize that the onset of schizophrenia could be affected by multiple functional sites, but their effects are counteracted [30]. Moreover, schizophrenia is associated with multiple neurotransmitters that have been noted to have physical and functional interactions [32]. Even these sites lead to changes in the serotonin system, but do not necessarily cause schizophrenia.  

Our study showed that rs9361234, rs11568817 and rs17273700 are in strong linkage disequilibrium in the northern Han Chinese population. The linkage states of these SNPs are similar to those identified in Han Chinese populations in Beijing and southern China (1000 Genomes Project). We observed that rs58138557 was intensely linked to rs9361234, rs11568817 and rs17273700 (Fig. 1). In addition, schizophrenic patients show stronger linkage between 11,568,817 and rs130058 than healthy controls (r² = 0.78 and r² = 0.62, respectively; Fig. 1). Linkage disequilibrium is related to population. For example, the linkage between rs11568817 and rs130058 in Han Chinese is weaker in northern China than in southern China based on the 1000 Genomes Project. This difference is also found in European and African-American populations [30]. Moreover, schizophrenia-associated genes vary among distinct ethnic populations [33]. Therefore, linkage disequilibrium of HTR1B could be associated with schizophrenia in northern Han Chinese peoples. This study presents information on the linkage state of HTR1B in schizophrenic patients of northern Han Chinese descent, and thereby provided references for the etiology of schizophrenia in different populations.  

This study is limited by sample size and methods and, although we found that rs1778258 is related to gender in schizophrenia, unfortunately, the mechanism underlying this association was not addressed. This will require further exploration in the future. Because of the limitation of the candidate gene approach, caution must be taken in regards to the interpretation of the association we have observed. Since schizophrenia is a complex disease

| Table 4 | The relationship between 5'-region haplotype and schizophrenia |
|---------|---------------------------------------------------------------|
| Haplotype | Case(n = 310) | Control (n = 313) | p value | OR | 95% CI |
| S' rs1410535 | rs1778258 | rs17273700 | rs1228814 | rs11568817 | rs130058 |
| 1 | T | G | T | C | T | A | 391.840 | 0.632 | 389.998 | 0.623 | 0.770 | 1.040 | 0.827–1.309 |
| 2 | C | A | T | C | T | A | 62.620 | 0.101 | 70.112 | 0.112 | 0.583 | 0.898 | 0.627–1.288 |
| 3 | C | G | T | C | T | A | 58.900 | 0.095 | 58.844 | 0.094 | 1.000 | 0.011 | 0.692–1.477 |
| 4 | C | G | C | A | G | T | 48.980 | 0.079 | 48.202 | 0.077 | 0.916 | 1.033 | 0.683–1.564 |
| 5 | C | G | T | A | T | A | 39.060 | 0.063 | 30.048 | 0.048 | 0.266 | 0.011 | 0.692–1.477 |
| 6 | C | G | C | A | G | A | 11.780 | 0.019 | 25.040 | 0.040 | 0.044 | 0.474 | 0.236–0.953 |

Superscript 1Significance level p = 0.01
| Haplotype | Case (n = 310) | Control (n = 313) |
|-----------|---------------|------------------|
|           | N  | frequency | N  | frequency |
| 1 A G A C C G T A | 298 | 0.481 | 295 | 0.472 |
| 2 A G A C C G T G | 78 | 0.127 | 68 | 0.109 |
| 3 G G A C C G T G | 78 | 0.126 | 88 | 0.142 |
| 4 A G A C C T T A | 76 | 0.123 | 68 | 0.110 |
| 5 A G A T C G A A | 58 | 0.095 | 68 | 0.109 |
| 6 A G T C C G T A | 16 | 0.026 | 16 | 0.026 |
| 7 A A A C C G T A | 8  | 0.013 | 6  | 0.011 |

Significance level = 0.0083
that is influenced by both genetic and environmental factors, both biological and psychological events experienced by individuals could have an impact on its onset. Thus, any associations cannot be fully explained by simply reducing them to a two-dimensional relationship between genetic variance and disease. We suggest that genetic background and behavioral events of enrolled patients should be taken into consideration [34]. Furthermore we will attempt to implement analysis of accurately defined phenotypes involving scale scores and treatment response, as well as the convergent analysis of genetic, serum and brain imaging markers.

Conclusion
In this study, the HTR1B gene was found to be related to gender in schizophrenia, as the rs1778258 A-allele caused increased risk of schizophrenia in male patients. Linkage between rs11568817 and rs130058 is more intense in schizophrenic patients. Combining all results of this study, we assert that HTR1B has a putative relationship with schizophrenia in the northern Han Chinese population, which provides a reference schizophrenia etiology.

Abbreviations
S-HT: 5-hydroxytryptamine; ADHD: Attention deficit hyperactivity disorder; DSM: Diagnostic Criteria of American Diagnostic and Statistical Manual of Mental Disorders; GWAS: Genome-wide association study; HTR1B: Serotonin receptor 1B; LD: Linkage disequilibrium; SNP: Single-nucleotide polymorphism; UTR: Untranslated region

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Availability of data and materials
The datasets used in this study are available from the corresponding author upon reasonable request.

Authors’ contributions
BW conceived and designed this study. XX conducted the study, performed statistical analyses, and prepared the manuscript. JY revised the manuscript. MD, JX1, JX2, HP played important roles in interpreting the results and statistical analyses, and prepared the manuscript. JY revised the manuscript. BW conceived and designed this study. XX conducted the study, performed relevant assistance in conducting study. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of China Medical University. All subjects and/or patient guarantors provided written informed consent for participation in this study. This study was designed and performed according to the most recent version of the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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