Association between serotonin - related gene polymorphisms and early effects of sertraline in Chinese Han patients with panic disorder

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Abstract
Objective The aim of the present study was to examine the association of serotonin-related gene polymorphisms with PD risk. Then, we analyzed the correlation between these gene polymorphisms and response to sertraline drug.

Methods 230 patients with PD and 231 healthy controls were enrolled in the study. Panic Disorder Severity Scale (PDSS) were administered to all subjects, and all patients in the study were also assessed after 4 weeks of treatment. The SLC6A4 (rs140701, rs3813034, 5-HTTLPR and STin2), HTRA1 rs6295, HTR2A rs6313 and COMT rs4680 genes were genotyped and assessed for allele.

Results The allelic model showed that the SLC6A4 rs140701 variant was significantly associated with increased risk of PD (OR = 0.624, 95% CI 0.450-0.864, p <0.05), and significant results was found in the dominant model (OR = 0.546; 95% CI, 0.371-0.804, p <0.05). There was a significant difference in allele and genotype frequency between responders and nonresponders in the 5-HTTLPR polymorphism (OR = 0.205, 95% CI 0.128-0.328; OR = 0.249, 95% CI 0.155-0.401, both p <0.001), and indicated the PD patients with S-allele had a poorer response to sertraline than L-allele carriers.

Conclusions The present study suggest that the SLC6A4 rs140701 variant may be associated with susceptibility to PD, and 5-HTTLPR polymorphism may be a predictor of response to sertralines in the treatment of PD.

Background
Panic disorder is a common anxiety disorder characterized by sudden and unexpected panic attacks and accompany with obviously anticipatory anxiety. The estimated lifetime prevalence of PD is 3.4 to 4.7% [1, 2]. It typically occurs in young adults, and women are more likely to be affected than men[3]. However, The etiology of PD is complex and unclear. Family and twin studies have shown that genetic factors explain approximately 48% of the variance in the PD[4], and indicated genetic factors may play an important role in PD. To date, although genetic studies reported several susceptibility genes with PD, especially the serotonin-ergic system. such as serotonin transporter gene (SLC6A4), 5-HT1A receptor (5-HTR1A), 5-HT2A receptor (5HTR2A), and catechol-O-methyltransferase (COMT) genes were involved in PD [5-15]. However, few of them were replicated.
and the pathogenesis of PD remains to be clarified. It is important to note that previous studies have been conducted in other ethnic groups, there are few studies in the literature that have examined the relationship between serotonin-related gene polymorphisms and PD in Chinese population. With the advancement of pharmacogenetic technologies, genetic variation is known to contribute to individual response to antidepressants. Plenty of evidence suggest that genetic factor play an important role in the clinical effects of antidepressants. A number of candidate genes were involved in the antidepressant response and remission. For example, in one meta-analysis suggested that BDNF rs6265 (Val66Met) heterozygous genotype was associated with better selective serotonin-reuptake inhibitors (SSRIs) response compared to the homozygous genotypes, particularly in Asians[16]. However, to our knowledge, many previous studies have been conducted in major depressive disorder[17–20]. Few studies in the literature that have examined the relationship between serotonin-related gene polymorphisms and the response to antidepressant treatment in PD. In clinical practice, SSRIs and serotonin has been used widely to treat PD[21]. For example, the sertraline is significantly superior to placebo for PD patients, and the incidence of adverse events was not different between sertraline and placebo[22–24]. Hence, in this study, The main aim of this study was to investigate the association of candidate genes from both serotonergic pathways including regulatory and coding variants of the SLC6A4, 5-HTR1A, 5-HTR2A and COMT genes. Our secondary objective was to examine the association between candidate genes and early response to sertraline in PD.

Methods
Participants
233 patients with PD were recruited from inpatient and outpatient populations in the Department of Psychosomatic, Sichuan Provincial People’s Hospital between May 2015 and December 2018. Diagnosis of PD was conducted according to the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) (SCID)[25]. Patients with neurological diseases, and/or past or current episodes of major depression disorder, generalized anxiety disorder, manic disorder, bipolar disorder, schizophrenia or other psychiatric disorders were excluded. In addition, 231 healthy controls were recruited from the Center of Health Examination, Sichuan
Provincial People’s Hospital. The SCID was also administered by a trained clinical psychiatrist to exclude lifetime or current diagnosis of PD, major depression, generalized anxiety disorder and so on. All subjects in this study were Han Chinese in China, and all subjects were free of acute or chronic somatic disorders. All patients were free of antidepressants or other psychotropic medications intake within 2 weeks before their examination. Demographic data and clinical presentations were obtained from medical records or qualified interviews. A 3-mL EDTA-anticoagulated peripheral blood sample was collected from every individual.

Measures
Panic Disorder Severity Scale (PDSS)
The Panic Disorder Severity Scale comprises 7 items, and participants are instructed to rate each item from 0–4 based on the severity of each symptom, with possible responses ranging from “none” to “extremely severe” [26]. The scale was translated into Chinese by Xiong HF [27], and the Panic Disorder Severity Scale-Chinese Version has good internal consistency (Cronbach’s alpha) with the overall score (0.83).

Treatment
All patients received treatment for a 4-week period with sertraline (100–200 mg qd), other psychotropic medications were not permitted during the study except for benzodiazepine, which was prescribed occasionally for insomnia with a minimal dosage at bedtime. PD severity was assessed using the PDSS at baseline and after 4 weeks of treatment. The response of PD was defined as a reduction of the pre-treatment PDSS score of at least 40%[28].

SNP Genotyping
SNP genotyping was performed using an improved multiplex ligation detection reaction (iMLDR) technique which developed by Genesky Biotechnologies Inc. (Shanghai, China). The primer information of the reaction mixtures is described in Tables S1 and S2. The multiplex polymerase chain reaction (PCR) reaction volume (20 µl) included 1 x GC-I buffer (Takara), 3.0 mM Mg 2+, 0.3 mM dNTP, 1 U HotStar Taq Polymerase (Qiagen Inc., Hilden, Germany), 1 µl genomic DNA (5–10 ng/µl), and 1 µl Multiplex-PCR primermix. In addition, The 5-HTT of PCR reaction volume (10 µl) included 10 x buffer I (Qiagen Inc), Q Solution (Qiagen Inc.), 0.2 mM Mg 2+, 1 U HotStarTaq polymerase (Qiagen Inc.), 1 µl
genomic DNA, and 1 µl PCR primermix. The cycling program for PCR was 95 °C for 2 min, followed by 11 Cycles of 94 °C for 20 s, 65 °C for 40 s and 72 °C for 1.5 min, and each cycle decreased 0.5 °C. The third step, 24 cycles of 94 °C for 20 s, 59 °C for 30 s, finally, 72 °C for 2 min and 4 °C and a hold at 4 °C. In addition, the 5-HTT of cycling program for PCR was 95 °C for 2 min, 35 cycles of amplification consisted of 94 °C for 20 s and 72 °C for 1.5 min, and final extension at 68 °C for 60 min and a hold at 4 °C. The PCR product was purified with 5 U SAP and 2 U Exonuclease I at 37 °C for 1 h and then 15 min of 75 °C inactivation. The ligation reaction contained 1 µl of 10 × ligation buffer, 0.25 µl Tag DNA ligase, 5′ ligation primer (1 µM) 0.4 µl, 3′ ligation primer (2 µM) 0.4 µl, 2 µl purified multiplex PCR product, and 6 µl ddH 2 O mixture. The ligation cycling program was 38 cycles× (94 °C for 1.5 min and 56 °C for 4 min), and a hold at 4 °C. Sequencing was conducted on 0.5 µl ligation product, 0.5 µl Liz500 Size Standard, and 9 µl Hi-Di mixture (ABI3730XL). The raw data were analyzed by Gene Mapper v4.1 software (AppliedBiosystems, Foster City, CA USA). All primers, probes and labeling oligos were designed by and ordered from Genesky Biotechnologies Inc.

Statistical analysis
The data were analyzed using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). Student’s t-tests were used for inter group comparisons of continuous variables, with Pearson’s chi-square tests utilized for categorical variables. P values for the Hardy-Weinberg equilibrium (HWE) were tested by Pearson’s chi-square test, and P > 0.05 indicated no significant deviation in allele and genotype frequencies among subjects. Associations between single nucleotide polymorphism (SNPs) and disease status were determined based on the distributions of allelic frequencies and genetic models (additive, dominant, and recessive model), and odds ratios (ORs) and 95% confidence intervals (CIs) were performed in unconditional logistic regression analysis using PLINK v1.07, 20 adjusting for age, gender, educational level and resident location. To protect from Type I error, a Bonferroni correction was conducted. For all analyses, statistical tests were two-tailed and an alpha level of 0.05 was used to define statistical significance.

Results
Demographic data and clinical manifestations
A total of 233 patients with PD (92 men, 141 women) and 231 controls (98 men, 133 women) were
selected. The average age of the study sample was (35.65 ± 9.77) years, and the mean age of the
control group was (36.96 ± 7.82) years. 53.6 percent of the patients (n = 125) were residing in urban
locations, and 46.4 percent of the patients (n = 108) were residing in rural locations. No statistically
significant differences were noted between cases and controls in terms of sex, age, education level
and resident location(p > 0.05). In addition, the mean total duration of panic disorder was (2.49 ±
1.45) years. The PDSS score was 9.51 ± 3.81 before medication and 9.51 ± 3.81 after 4 weeks of
medication, the score was significantly reduced after medication (p < 0.05). According to the
definition of remission, the response rate was 42.1%(n = 98). The demographic details of the sample
are given in Table 1.

| Variable | PD (n = 233) | Controls(n = 231) | t/χ 2-value | p-value |
|----------|--------------|------------------|-------------|---------|
| Sex, n (%) |               |                  |             |         |
| Female   | 141(60.5)    | 133(57.6)        | 0.414       | 0.520   |
| Male     | 92(39.5)     | 98(42.4)         |             |         |
| Age      | 35.65 ± 9.77 | 36.96 ± 7.82     | 1.594       | 0.112   |
| Educational level, n (%) |          |                  |             |         |
| <Junior high school | 49(21.0) | 42(18.2) | 3.836       | 0.147   |
| High school | 95(40.8) | 80(34.6) |            |         |
| College and above | 89(38.2) | 109(47.2) |            |         |
| Resident location, n (%) |          |                  |             |         |
| Urban    | 125(53.6)    | 129(55.8)        | 0.266       | 0.635   |
| Rural    | 108(46.4)    | 102(44.2)        |             |         |
| Total duration of PD, years | 2.49 ± 1.45 |                |             |         |
| PDSS baseline | 15.47 ± 3.82 |                |             |         |
| PDSS 4-week | 9.51 ± 3.81 |                |             |         |

Association Of Serotonin-related Gene Polymorphisms With Pd Risk

All selected SNPs fulfilled the HWE in both cases and controls(p > 0.05). The linkage-disequilibrium
evaluated in PD group and controls for variants rs140701 and rs3813034 of SLC6A4 are shown in
Fig. 1 (r² > 0.9). The allele distributions of SLC6A4 (rs140701 and rs3813034) were significantly
different between PD cases and the controls(OR = 0.624, 95% CI 0.450–0.864, p = 0.004; OR = 0.705,
95% CI 0.509–0.975, p = 0.034; respectively), and only SLC6A4 rs140701 remained significant after
adjusting for Bonferroni’s multiple testing(p = 0.028). After adjustment for age, gender, educational
level and resident location, the dominant model of SLC6A4 rs140701 showed the relative risk of PD
for genotype CC + CT was lower than for genotype TT (OR = 0.546; 95% CI, 0.371–0.804, p = 0.004),
and after Bonferroni correction for multiple comparisons, the significance remained (p = 0.028). In addition, the dominant model of rs3813034 showed the relative risk of PD for genotype CA + AA was lower than for genotype CC (OR = 0.636; 95% CI, 0.432–0.935, p = 0.025). For serotonin-transporter-linked polymorphic region (5-HTTLPR) polymorphism, the relative risk of PD for genotype LL + LS was lower than for genotype SS (OR = 0.632; 95% CI, 0.436–0.916; p = 0.021). The additive model of rs140701 and rs3813034 showed similar association with PD(OR = 0.638, 95% CI 0.463–0.879, p = 0.009; OR = 0.721, 95% CI 0.526–0.988, p = 0.048; respectively). However, these correlations were no longer significant after adjusting for Bonferroni’s multiple testing (p > 0.05). Also, there were no significant associations of other SNPs with PD in allelic or other models (p > 0.05). (Table 2).
### Table 2

Association of serotonin-related gene polymorphisms with PD risk in Chinese Han population.

| Gene   | SNP      | Alleles and Genotypes (n, %) | PD (n = 233) | Controls (n = 231) | Model      | OR (95% CI) | p-value | p-value\textsuperscript{corr} |
|--------|----------|-----------------------------|--------------|-------------------|------------|-------------|---------|-----------------------------|
| SLC6A4 | rs140701 | C 76(16.3)                  | 110(23.8)    | 352(76.2)         | Allele\textsuperscript{a} | 0.624(0.45 0.86) | 0.004   | 0.028                      |
|        |          | T 390(83.7)                 | 13(5.6)      |                   | Additive\textsuperscript{b} | 0.638(0.46 0.87) | 0.009   | 0.063                      |
|        |          | CC 10(4.3)                  | 352(76.2)    |                   | Dominant\textsuperscript{b} | 0.546(0.37 1.08) | 0.004   | 0.028                      |
|        |          | CT 56(24.0)                 | 84(36.4)     |                   | Recessive\textsuperscript{b} | 0.752(0.32 1.75) | 0.438   | 1                          |
|        |          | TT 167(71.7)                | 134(58.0)    |                   |            |             |         |                             |
|        | rs3813034| C 386(82.8)                 | 357(77.3)    |                   | Allele\textsuperscript{a} | 0.705(0.50 0.97) | 0.034   | 0.238                      |
|        |          | A 80(17.2)                  | 105(22.7)    |                   |            |             |         |                             |
|        |          | CC 164(70.4)                | 139(60.2)    |                   | Additive\textsuperscript{b} | 0.721(0.52 0.98) | 0.048   | 0.336                      |
|        |          | CA 58(24.9)                 | 79(34.2)     |                   | Dominant\textsuperscript{b} | 0.636(0.43 0.93) | 0.025   | 0.175                      |
|        |          | AA 11(4.7)                  | 13(5.6)      |                   | Recessive\textsuperscript{b} | 0.831(0.36 1.19) | 0.662   | 1                          |
|        |          |                           |             |                   |            |             |         |                             |
|        | rs140701 | L 107(23.0)                 | 130(28.1)    |                   | Allele\textsuperscript{a} | 0.761(0.56 0.96) | 0.071   | 0.497                      |
|        |          | S 359(77.0)                 | 332(71.9)    |                   |            |             |         |                             |
| 5-HTTLPR|          | LL 22(4.9)                  | 20(8.7)      |                   | Additive\textsuperscript{b} | 0.785(0.59 0.99) | 0.010   | 0.700                      |
|        |          | LS 63(27.0)                 | 90(39.0)     |                   | Dominant\textsuperscript{b} | 0.632(0.43 0.91) | 0.021   | 0.147                      |
|        |          | SS 148(63.5)                | 121(52.3)    |                   | Recessive\textsuperscript{b} | 1.100(0.58 2.07) | 0.842   | 1                          |
| STin2  |          | 10 44(9.4)                  | 43(9.3)      |                   | Allele\textsuperscript{a} | 1.016(0.65 1.58) | 0.944   | 1                          |
|        |          | 12 422(90.6)                | 419(90.7)    |                   |            |             |         |                             |
|        |          | 10/10 5(2.1)                | 2(0.9)       |                   | Additive\textsuperscript{b} | 1.015(0.66 1.55) | 0.956   | 1                          |
|        |          | 12/10 34(14.6)              | 39(16.9)     |                   | Dominant\textsuperscript{b} | 0.932(0.57 1.50) | 0.711   | 1                          |
|        |          | 12/12 194(83.3)             | 190(82.2)    |                   | Recessive\textsuperscript{b} | 2.511(0.48 13.08) | 0.393   | 1                          |
| HTRA1  | rs6295   | G 359(77.0)                 | 358(77.5)    |                   | Allele\textsuperscript{a} | 1.026(0.75 1.39) | 0.870   | 1                          |
|        |          | C 107(23.0)                 | 104(22.5)    |                   |            |             |         |                             |
|        |          | GG 138(59.2)                | 138(59.7)    |                   | Additive\textsuperscript{b} | 1.026(0.75 1.39) | 0.554   | 1                          |
|        |          | GC 83(35.6)                 | 82(35.5)     |                   | Dominant\textsuperscript{b} | 1.022(0.70 1.48) | 0.560   | 1                          |
|        |          | CC 12(5.2)                  | 11(4.8)      |                   | Recessive\textsuperscript{b} | 1.086(0.46 2.51) | 0.772   | 1                          |
| HTR2A  | rs6313   | G 163(35.0)                 | 179(38.7)    |                   | Allele\textsuperscript{a} | 0.868(0.65 1.11) | 0.234   | 1                          |
|        |          | A 303(65.0)                 | 283(61.3)    |                   |            |             |         |                             |
|        |          | AA 106(45.5)                | 86(37.2)     |                   | Additive\textsuperscript{b} | 0.859(0.66 1.11) | 0.252   | 1                          |
|        |          | GA 91(39.1)                 | 111(48.1)    |                   | Dominant\textsuperscript{b} | 0.711(0.49 1.03) | 0.061   | 0.427                      |
|        |          | GG 36(15.4)                 | 34(14.7)     |                   | Recessive\textsuperscript{b} | 1.059(0.63 1.76) | 0.749   | 1                          |
| COMT   | rs4680   | A 143(30.7)                 | 118(25.5)    |                   | Allele\textsuperscript{a} | 1.291(0.96 1.72) | 0.021   | 0.028                      |
|        |          | G 323(69.3)                 | 344(74.5)    |                   |            |             |         |                             |
|        |          | AA 24(10.3)                 | 15(6.5)      |                   | Additive\textsuperscript{b} | 1.284(0.65 1.70) | 0.118   | 0.826                      |
|        |          | GA 95(40.8)                 | 88(38.1)     |                   | Dominant\textsuperscript{b} | 1.297(0.90 1.86) | 0.175   | 1                          |
|        |          | GG 114(48.9)                | 128(55.4)    |                   | Recessive\textsuperscript{b} | 1.654(0.84 3.24) | 0.236   | 1                          |

\textsuperscript{a}A" represent wild type and "a" represent mutant type: allele, a vs. A; additive, aa vs. Aa vs. AA; dominant, aa + Aa vs. AA, recessive, aa vs. Aa + AA. \textsuperscript{b}Chi-square test, \textsuperscript{corr} Logistic regression analyses by adjustment for age, gender, educational level and resident location. \textsuperscript{corr} adjusted by boferroni multiple comparison correction.

Association between serotonin related gene polymorphisms and early response to sertralines in the treatment of PD.
Finally, we investigated whether variations of gene could predict response to sertraline in Han Chinese population with PD. There was a significant difference in allele and genotype frequency between responders and nonresponders in the 5-HTTLPR polymorphism (OR = 0.205, 95% CI 0.128–0.328; OR = 0.249, 95% CI 0.155–0.401, all \( p^\text{corr} = 0.000 \); respectively), and the dominant and recessive model of 5-HTTLPR showed significant association with therapeutic response, indicated the PD patients with S-allele had a poorer response to sertraline than L-allele carriers. However, Genotype and allele frequencies of the other gene polymorphisms were not significantly different between responders and nonresponders (\( p > 0.05 \)). (Table 3).
### Table 3
Genotype and allele frequencies of serotonin-related gene polymorphisms between responder and nonresponder

| Gene | SNP     | Alleles and Genotypes (n, %) | Responder (n = 98) | Nonresponder (n = 135) | Model | OR (95% CI) | p-value | p-value\(corr\) |
|------|---------|------------------------------|-------------------|-----------------------|-------|-------------|---------|---------------|
| SLC6A4 | rs140701 | C 37(18.9) T 159(81.1) | 39(14.4) 231(85.6) | Allele\(^a\) | 0.726(0.44 3-1.188) | 0.201 | 1 |
|       |         | C 5(5.1) T 5(3.7)    | 5(3.7)           | Additive\(^b\)       | 0.638(0.47 0-1.198) | 0.159 | 1 |
|       |         | C 27(27.6) T 29(21.5) | 29(21.5)         | Dominant\(^b\)       | 0.694(0.39 1-1.232) | 0.160 | 1 |
|       |         | C 66(67.3) T 101(74.8) | 101(74.8)        | Recessive\(^b\)      | 0.715(0.20 1-2.542) | 0.446 | 1 |
|       | rs3813034 | C 155(79.1) A 41(20.9) | C 231(85.6)      | Allele\(^a\)         | 0.638(0.39 4-1.035) | 0.067 | 0.469 |
|       |         | C 63(64.3) A 101(74.8) | 101(74.8)        | Additive\(^b\)       | 0.669(0.42 3-1.060) | 0.113 | 0.791 |
|       |         | C 29(29.6) A 29(21.5)  | 29(21.5)         | Dominant\(^b\)       | 0.606(0.34 4-1.069) | 0.128 | 0.896 |
|       |         | C 6(6.1) A 5(3.7)   | 5(3.7)           | Recessive\(^b\)      | 0.590(0.17 5-1.991) | 0.332 | 1 |
| 5-HTTLPR |          | L 76(38.8) S 120(61.2) | L 239(85.6)      | Allele\(^a\)         | 0.205(0.12 8-0.328) | 0.000 | 0.000 |
| STin2 |          | L 16(16.3) S 6(4.4)  | 6(4.4)           | Additive\(^b\)       | 0.249(0.15 5-0.401) | 0.000 | 0.000 |
|       |          | L 44(44.9) S 19(14.1) | 19(14.1)         | Dominant\(^b\)       | 0.144(0.07 9-0.261) | 0.000 | 0.000 |
|       |          | L 38(38.8) S 110(81.5) | 110(81.5)        | Recessive\(^b\)      | 0.238(0.09 0-0.634) | 0.004 | 0.029 |
| HTRA1 | rs6295  | G 151(77.0) C 45(23.0) | G 208(77.0)      | Allele\(^a\)         | 1.000(0.64 6-1.549) | 0.999 | 1 |
|       |         | G 59(60.2) C 79(58.5) | 79(58.5)         | Additive\(^b\)       | 1.000(0.64 5-1.551) | 0.985 | 1 |
|       |         | G 33(33.7) C 50(37.0) | 50(37.0)         | Dominant\(^b\)       | 1.072(0.62 1-1.822) | 0.903 | 1 |
|       |         | G 6(6.1) C 6(4.4)  | 6(4.4)           | Recessive\(^b\)      | 0.713(0.22 3-2.281) | 0.826 | 1 |
| HTR2A | rs6313  | G 62(31.6) A 134(68.4) | G 101(37.4)      | Allele\(^a\)         | 1.292(0.87 5-1.906) | 0.197 | 1 |
|       |         | G 48(49.0) A 58(43.0) | 169(62.6)        | Additive\(^b\)       | 1.252(0.86 9-1.805) | 0.199 | 1 |
|       |         | G 38(38.8) A 53(39.3) | 53(39.3)         | Dominant\(^b\)       | 1.274(0.75 6-2.149) | 0.233 | 1 |
|       |         | G 12(12.2) A 24(17.8) | 24(17.8)         | Recessive\(^b\)      | 1.550(0.73 3-3.274) | 0.355 | 1 |
| COMT  | rs4680  | A 66(33.7) G 130(66.3) | A 77(28.5)       | Allele\(^a\)         | 0.786(0.52 8-1.169) | 0.234 | 1 |
|       |         | A 11(11.2) G 13(9.6)  | 13(9.6)          | Additive\(^b\)       | 0.793(0.53 7-1.172) | 0.294 | 1 |
|       |         | A 44(44.9) G 51(37.8) | 51(37.8)         | Dominant\(^b\)       | 0.705(0.41 8-1.189) | 0.174 | 1 |
|       |         | A 43(43.9) G 71(52.6) | 71(52.6)         | Recessive\(^b\)      | 0.843(0.36 1-1.969) | 0.954 | 1 |

\(^a\) A represent wild type and "a" represent mutant type; allele, a vs. A; additive, aa vs. Aa vs. AA; dominant, aa + Aa vs. AA; recessive, aa vs. Aa + AA. \(^b\) Chi-square test, \(^c\) Logistic regression analyses by adjustment for age, gender, educational level and resident location. \(p_{corr}\): adjusted by boferroni multiple comparison correction.

Discussion
In this present study, a significant relationship was found between the SLC6A4 rs140701 polymorphism and PD. The patients with PD had significantly higher frequencies of the TT genotype of rs140701, and this is consistent with the results of the previous study reported that only rs140701 polymorphism of SLC6A4 provided evidence of association with PD from 163 sample of African American[7]. SLC6A4 is involved in the transport of serotonin from synaptic spaces to presynaptic neurons, and maintaining the pool of available serotonin for subsequent release[29]. A plenty of evidence points to the involvement of the serotonin system in the neurobiology and pharmacotherapy of PD. Clinical studies demonstrated that SSRIs increasing the synaptic availability of 5-HT and are effective in the treatment of PD[30]. So specific SLC6A4 variants may have an influence on its function. Previous studies consistently found serotonin transporter (5-HTT) knockout mice show increased anxiety-like behaviour. For example, animal experiment found homozygous 5-HTT knockout mice were more anxious [31, 32]. 5-HTT overexpressing mice displayed reduced anxiety-like behaviour, whilst 5-HTT knockout mice showed increased anxiety-like behaviour[33]. These findings show that variation in 5-HTT gene produces robust changes in anxiety. In addition, imaging study shown the 5-HTTLPR genotype may alter resting brain function in emotion-related regions, including the amygdala and ventromedial prefrontal cortex[34]. Domschke found PD patients carrying the homozygous of the 5-HT1A -1019G risk allele or patients carrying the short risk allele of the 5-HTTLPR showed higher amygdala activation in response to happy faces[35]. Imaging data also revealed activations in areas associated with the fear circuit including amygdala, insula, and hippocampal areas[36, 37]. these alterations may suggest a important role of the 5-HTT gene in brain function that may be associated with the genetic susceptibility for PD. The above findings may contribute to our understanding of the mechanism linking SLC6A4 variants to PD.

In addition, there was no significant associations of SLC6A4 rs3813034, 5-HTTLPR and STin2 polymorphisms with PD, and inconsistent with findings from other studies. For example, previous research found the rs3813034 of SLC6A4 is a putative risk factor for PD and other behavioral disorders that involve dysregulation of serotonergic neurotransmission[38]. Also, we found no significant statistical differences in the genotype distributions or allele frequencies of the SNPs
between PD and control groups in 5-HTR1A rs6295, 5-HTR2A rs6313 and COMT rs4680. For example, in a study in which 119 PD patients and 119 healthy controls from Japanese population were included, no significant differences were found in the allele frequencies or genotype distributions of the COMT rs4680, 5-HTTLPR polymorphisms or the 5-HT1A (rs6295) between PD patients and controls[15]. A significant relationship was found between the COMTVal158Met polymorphism and PD[9, 13]. In other meta analysis, the COMT gene val158met polymorphism (rs4680) has been found to be associated with PD in European ancestry, but not Asian ancestry samples[39]. Previous studies also found pure PD was associated with HTR2A 102T-C (rs6313) polymorphism[40], but not in other study[13]. The above findings of genetic association studies suggest that certain 5-HT-related genes may contribute to the susceptibility to PD. however, these results are rather limited and inconsistent. These mixed results of studies might be partly attributable to different samples, sex, races, agoraphobia co-morbidity or severity of PD. For example, Previous study showed that the the HTR2A rs6311 polymorphism was associated with the severity of PD[41]. In addition, these conflicting findings indicate that the contribution of genetic factors to PD may involve a complex network of mutations. In the present study, we only found significant correlation between 5-HTTLPR polymorphism and treatment response to sertraline for 4-weeks in patients with PD. The results of our study indicated the PD patients with S-allele has been linked to poorer response to sertraline during the early stage of treatment, and suggest that 5-HTTLPR could be a predictor of response to sertralines treatment. 5-HTTLPR is a 44 base pair insertion–deletion polymorphism which can exist as a long (L) variant of a 16 repeat sequence or a short (S) variant of 14 repeats[42]. Previous researches indicated the L allele is associated with higher levels of transcription and concentrations of 5-HTT mRNA compared to the S allele, and the short (S) allele of the 5-HTTLPR polymorphism results in less efficient transcription[43-45]. In addition, SSRIs are effective in the treatment of PD, and SLC6A4 is the primary target. These findings may contribute to our understanding of the association between 5-HTTLPR polymorphism and antidepressant response. Consistent study found the 5-HTTLPR low-expression genotypes showed a more favorable response to exposure-based behavior therapy in PD with agoraphobia[46]. Similar findings were found in subjects with depressive disorder or premature
ejaculation treated with sertraline [47, 48]. However, previous report found no association of the 5-HTTLPR polymorphism with treatment response was observed in 102 patients with PD receiving sertraline or paroxetine[49]. Also, there was no significant associations between the 5-HTTLPR polymorphism and sertraline responses in major depression patients[50]. On the one hand, other polymorphisms of SLC6A4 may have a combined effect with 5-HTTLPR. In addition, variation in genes involved in the pharmacokinetics of SSRIs. Drugs metabolized by cytochrome P450 (CYP450) system and related genes of CYP450 enzymes may contribute to pharmacokinetics. For example, one study found the CYP2C19 genetic polymorphism is associated with Escitalopram treatment response in Chinese patients with PD[51]. On the other hand, functional variants in SLC6A4 may be related to epigenetic mechanisms and affect gene expression. For instance, epigenetic modifications of SLC6A4 gene is showing promising results as biomarkers for prediction of antidepressant response [52]. These association need to be further studied in PD with large sample size. However, we failed to find the relationship between others gene polymorphisms and treatment response to sertraline. which is inconsistent with previous studies that found the 5-HT1A receptor – 1019C/G polymorphism was strongly associated with response to treatment in PD receiving sertraline or paroxetinethe[49, 53]. In another study suggest that the genetic variant of the COMT enzyme may be related to treatment response to paroxetine in PD[54]. Inconsistent results may be related to small sample size, short follow-up periods, definition of response, antidepressant choice and ethnic differences. For instance, in one meta-analysis suggest that in Europeans 5-HTTLPR polymorphism may be a predictor of antidepressant response and remission, while it does not appear to play a major role in East Asians[55]. Considering these factors, these gene polymorphisms should be replicated in further study.

Conclusions
The present study suggest that the SLC6A4 rs140701 variant may be associated with susceptibility to PD, and 5-HTTLPR polymorphism may be a predictor of response to sertralines in the treatment of PD. However, the results of our study should be considered in light of the following limitations: (1) The sample size was limited and only from Sichuan provinces of western China, which may not completely
represent the Chinese ethnicity. (2) The etiology of PD is complex and comprises environmental and genetic factors, a potential gene-environment interaction or epigenetics study should be investigated. (3) We did not measure the relationship between plasma sertralines concentration and clinical response.

**Abbreviations**

BDNF: Brain-Derived Neurotrophic Factor; COMT: Catechol-O-Methyltransferase; CI:s: Confidence Intervals; CYP450: Cytochrome P450; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders fourth edition; HWE: Hardy-Weinberg Equilibrium; L: Long; ORs: Odds Ratios; PD: Panic disorder; PDSS: Panic Disorder Severity Scale; PCR: Polymerase Chain Reaction; SCID: Structured Clinical Interview for DSM-IV; SNPs: Single Nucleotide Polymorphism; S: Short; SLC6A4: Serotonin Transporter Gene; SSRIs: Selective Serotonin- Reuptake Inhibitors; STin2: intron 2; 5-HTR1A: 1A receptor; 5-HTR2A: 5-Hydroxytryptamine 2A receptor; 5-HTT: Serotonin Transporter; 5-HTTLPR: Serotonin-Transporter-Linked Polymorphic Region.

**Declarations**

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**Authors’ contributions**

ZLZ was critically involved in the study design and wrote the manuscript. YLH, JYW and WJM were involved in subject recruitment. BZ guided this research and supervised the entire project. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study was approved by the Sichuan Provincial People’s Hospital ethics committee, reference number: (2016) Ethics Review (29). All individuals provided written informed consent prior to the initiation of study procedures.

Consent for publication
Not applicable.

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

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Figures
Figure 1

Linkage disequilibrium in SLC6A4 gene polymorphisms (rs3813034 and rs140701).

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