Haplotype analysis confirms association of the serotonin transporter (5-HTT) gene with schizophrenia in the Han Chinese population

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\textbf{Abstract}

Serotonin transmission has long been suspected as being involved in the pathogenesis of schizophrenia. 5-HTT is a promising candidate gene for schizophrenia due to its critical role in regulating serotonin transmission and role in the mechanism of the atypical antipsychotic drugs. A common polymorphism STin2 VNTR in the 5-HTT gene has been extensively investigated in the genetic association studies, but the results are conflicting. Meanwhile, the SNPs of the 5-HTT gene have been much less explored. We therefore conducted a case-control study of the association between STin2 VNTR and three tagging SNPs in 5-HTT and schizophrenia in the Han Chinese population based on a cohort of 329 schizophrenic patients and 288 control subjects. No association was found in the single locus, but haplotype-based analyses revealed significant association between two haplotypes with schizophrenia even after Bonferroni correction ($P = 0.00000538$ and 0.011).

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Schizophrenia (OMIM #181500) is a chronic, debilitating psychotic disorder, affecting 0.5–1.0% of the population worldwide with devastating consequences for affected individuals and their families [28]. The genetic contribution to the etiology of schizophrenia has been shown to be upward of 80%, however the pathogenesis is complex and the specific factors that give rise to the disorder remain elusive [22,33].

Serotonin (5-HT) transmission has long been suspected of being involved in the pathogenesis of schizophrenia [1,15,16]. Postmortem brain tissue analysis and cerebrospinal fluid studies have identified a deficit of 5-HT function in the cortex of patients with schizophrenia [11,14,34]. 5-HT plays an important role in many physiological processes which are disturbed in schizophrenics, including cognition, mood, perception and attention [11]. Furthermore, it has extensive interaction with other neurotransmitter systems especially the glutamate system [2]. In the molecular level, many second-generation antipsychotic drugs such as clozapine, olanzapine, quetiapine are 5-HT\textsubscript{2} antagonists, whereas 5-HT receptor agonists are psychotogenic [1,28] In addition to acting as a neurotransmitter in mature brain, 5-HT has a role in neural development [32].

Serotonin transporter (5-HTT, SLC6A4) is a promising candidate gene for schizophrenia. 5-HTT is the major regulator of serotonin synthesis in the synaptic cleft, terminating the action of serotonin by uptaking serotonin from brain synapses into the presynaptic neuron [24,25]. The 5-HTT gene is mapped to chromosome 17q11.1–q12, within the 17p11–q25 region which has been reported to be linked with schizophrenia [3,38]. Dysregulation of 5-HTT has been reported in various complex behavioral traits and psychiatric disorders [14,23]. Besides, abnormal expression of 5-HTT has been reported in the brains of schizophrenic patients [12]. And postmortem studies have shown decreased 5-HTT affinity in the hippocampus in schizophrenics [6,21]. Clinically, 5-HTT is the primary target of SSRIs which are used as a first-line treatment for a number of psychiatric illnesses [11].

A lot of genetic association analyses between two common polymorphisms of 5-HTT have been carried out in different populations, 5-HTTLPR is a 44-bp insertion/deletion polymorphism in the 5′ regulatory region, involving two major alleles, namely ‘S’ (short) and ‘L’ (long). A lot of genetic association analyses between two common polymorphisms of 5-HTT have been carried out in different populations, 5-HTTLPR is a 44-bp insertion/deletion polymorphism in the 5′ regulatory region, involving two major alleles, namely ‘S’ (short) and ‘L’ (long).
diagnosed according to the criteria of DSM-IV (American Psychiatric Association). The diagnosis was checked and verified by two independent senior psychiatrists who reviewed the psychiatric case records. Written informed consent was obtained from either the participants or participants' relatives, after the procedure had been fully explained.

Besides STin2, we included SNPs from the dbSNP database (www.ncbi.nlm.nih.gov/SNP) and international HapMap project website (www.hapmap.org). Tagging SNPs were selected by Haplovier version 2.05 software [4]. Three intronic and non-functional SNPs, rs2054847, rs140700 and rs2020942 were expected to belong to the STin2-containing haplotype block [8]. Moreover, tagger SNP analysis by Haplovier showed they were among the tagger SNPs of this block. Thus these three SNPs were chosen for the present study. The positions of the chosen markers were shown in Fig. 1.

Genomic DNA was prepared from venous blood using the standard phenol/chloroform method. For genotyping of STin2 VNTR, primers were: forward: 5′-TGGTCTCTACCAGTCGGCG-3′; reverse: 5′- TGATGCATGTAGTCGAGCTGGCTTTCGGAG-3′; PCR were conducted in a final volume of 5 µl consisting of 10 ng DNA, 0.1 µl 10 µM primers, and 1 U Hotstart Taq polymerase (Qiagen). MegaBACE 1000 capillary electrophoresis (Amersham Biosciences, Piscataway, NJ) were used to discern the genotype of 10- and 12-repeat of the 17-bp VNTR.

SNP genotyping was performed using ligase detection reaction. Fluorescence-labeled primer was employed to detect the genotype of the (GT)₄ repeat using MegaBACE 1000 capillary electrophoresis. CLUMP 2.2 was used to compare the allelic, genotypic frequencies and haplotype distribution between cases and controls [30]. Bonferroni correction was used for multiple testing. Analysis of Hardy–Weinberg equilibrium and linkage disequilibrium were performed on SHEsis [31]. The G’POWER program was used for power calculations [7].

All genotype frequencies of the three SNPs and STin2 VNTR were in Hardy–Weinberg equilibrium (P > 0.05, Table 1). We observed no significant deviation in allelic or genotypic frequencies of any single one for the four markers examined (Table 1). For the allele frequen-

![Fig. 1. Diagram of the genomic structure of 5-HTT gene and the positions of the markers studied in the present work.](Image)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
<th>Number (frequency)</th>
<th>χ² (P-value)</th>
<th>Odds ratio (%95 CI)</th>
<th>Distancea</th>
<th>HWEb</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2054847</td>
<td>C/T</td>
<td>121 (0.188)</td>
<td>0.01 (0.94)</td>
<td>0.99 (0.74–1.132)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>10 (0.015)</td>
<td>101 (0.314)</td>
<td>211 (0.655)</td>
<td>0.01 (1.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>9 (0.032)</td>
<td>389 (0.316)</td>
<td>184 (0.652)</td>
<td>0.62 (0.66)</td>
<td></td>
</tr>
<tr>
<td>rs140700</td>
<td>G/A</td>
<td>584 (0.945)</td>
<td>1.82 (0.18)</td>
<td>0.68 (0.39–1.02)</td>
<td>11.456</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>34 (0.055)</td>
<td>277 (0.896)</td>
<td>30 (0.097)</td>
<td>1.74 (0.42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>20 (0.038)</td>
<td>244 (0.924)</td>
<td>18 (0.068)</td>
<td>0.24 (0.24)</td>
<td></td>
</tr>
<tr>
<td>rs2020942</td>
<td>G/A</td>
<td>121 (0.188)</td>
<td>0.01 (0.94)</td>
<td>0.99 (0.74–1.132)</td>
<td>14.956</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>10 (0.015)</td>
<td>101 (0.314)</td>
<td>184 (0.652)</td>
<td>0.01 (1.00)</td>
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<td>389 (0.316)</td>
<td>184 (0.652)</td>
<td>0.62 (0.66)</td>
<td></td>
</tr>
<tr>
<td>STin2</td>
<td>10/10</td>
<td>6 (0.025)</td>
<td>43 (0.179)</td>
<td>91 (0.793)</td>
<td>4.56 (0.10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/12</td>
<td>12/12</td>
<td>16 (0.10)</td>
<td>12/12</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/0</td>
<td>10 (0.015)</td>
<td>101 (0.314)</td>
<td>211 (0.655)</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

- **Table 1**: Allelic and genotypic frequencies of four markers among schizophrenic patients and normal controls.

  a Chi-square values or empirical P-values obtained using CLUMP(10,000 simulation).

  b Distance from rs2020847.

  c Test for departure from Hardy–Weinberg expectation in case and control groups using SHEsis.

On the other hand, the associations between single nucleotide polymorphisms (SNPs) of 5-HTH and schizophrenia have been much less explored compared with STin2 VNTR and 5-HTTLPR.

In the present study, we attempt to evaluate the role of 5-HTH in the pathogenesis of schizophrenia using a case-control approach involving 329 patients and 288 healthy subjects drawn from the Han Chinese population. STin2 VNTR and three SNPs of 5-HTT gene (rs2054847, rs140700, and rs2020942) were investigated.

329 unrelated schizophrenic patients (203 males, 126 females, mean age 49.54 ± 11.48) and 288 unrelated healthy subjects (159 males, 129 females, mean age 37.02 ± 8.16) were recruited in this study. All the subjects were from Shanghai and were Han Chinese in origin. All of the controls were interviewed to exclude any history of psychiatric disorder. Subjects with schizophrenia were strictly diagnosed according to the criteria of DSM-IV (American Psychiatric Association). The diagnosis was checked and verified by two independent senior psychiatrists who reviewed the psychiatric case records. Written informed consent was obtained from either the participants or participants' relatives, after the procedure had been fully explained.

Beside STin2, we included SNPs from the dbSNP database (www.ncbi.nlm.nih.gov/SNP) and international HapMap project website (www.hapmap.org). Tagging SNPs were selected by Haplovier version 2.05 software [4]. Three intronic and non-functional SNPs, rs2054847, rs140700 and rs2020942 were expected to belong to the STin2-containing haplotype block [8]. Moreover, tagger SNP analysis by Haplovier showed they were among the tagger SNPs of this block. Thus these three SNPs were chosen for the present study. The positions of the chosen markers were shown in Fig. 1.

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All genotype frequencies of the three SNPs and STin2 VNTR were in Hardy–Weinberg equilibrium (P > 0.05, Table 1). We observed no significant deviation in allelic or genotypic frequencies of any single one for the four markers examined (Table 1). For the allele frequen-
cies, the \( P \)-value was 0.94 and the odds ratio (OR) was 0.99 for both rs2020942 and rs2054847; the \( P \)-value was 0.18 and the OR was 0.68 for rs140700; the \( P \)-value was 0.07 and the OR was 1.48 for STin2 VNTR. As to the genotype frequencies, the \( P \)-value of both rs2020942 and rs2054847 was 1.00, the \( P \)-value for rs140700 was 0.42, and the \( P \)-value for STin2 VNTR was 0.10. The power of both rs2054847 and rs140700 was 78.9%, power of rs140700 was 30.8%, and power of STin2 VNTR was 66.0% at the level of OR = 1.5.

Table 2 shows the pair-wise linkage disequilibrium (LD) among each pair of markers. The three SNPs are in strong LD (\( D' > 0.7 \)), and in particular rs2020942 and rs2054847 were in complete LD (\( D' = 1.0, R^2 = 1.0 \)), which is inconsistent to the genotype data in HapMap database. Whereas LD between SNPs and microsatellites was much weaker (\( D' = 0.571 \) and 0.29).

A three-marker haplotype analysis was conducted with rs2054847, rs140700 and STin2 VNTR, since rs2020942 was in total LD with rs2054847. Table 3 showed the estimated haplotype frequencies in both groups, with Bonferroni corrected \( P \)-values. Globally, \( P = 4.67 \times 10^{-6} \) after Bonferroni correction, indicating a significant association. The haplotype A-G-10 (#4 in Table 3) displayed a strong association with schizophrenia (Bonferroni corrected \( P = 5.38 \times 10^{-3} \)), carried by about 8.4% of the schizophrenic patients versus 1.8% of controls. In addition, The 5\# haplotype A-G-12 is also significantly associated with schizophrenia (Bonferroni corrected \( P = 0.011 \)), carried by about 69.9% of patients versus 79.8% of controls.

No other significant associations were observed.

In this study, we carried out a case-control study of the association between four markers in 5-HTT and schizophrenia based on subjects drawn from the Han Chinese population. While no single locus associations was observed, we found that the frequencies of two haplotypes were significantly different between the patients and control subjects.

Our results support 5-HTT as a risk factor for schizophrenia in the Han Chinese population. This is consistent with some but not all previous reports. A study on a Japanese cohort investigating four SNPs of 5-HTT as well as 5-HTTLPR and 5-HTTVNTR, found no association with schizophrenia [31]. However, a meta-analysis found highly significant association between STin2 VNTR and schizophrenia from 12 population-based association studies [8]. What is more, a recent association study by Zabol et al. on Caucasians revealed a significant association between a haplotype consisting of STin2 VNTR and schizophrenia [37]. Interestingly, we repeated the result of Zabol et al. that the STin2-containing haplotype was associated with schizophrenia, suggesting that the 5-HTT region contributing genetic risk to schizophrenia lay in the haplotype block containing STin2. This also strengthened the data of the meta-analysis [8].

In this study, we did not detect statistically significant association between STin2 VNTR and schizophrenia (\( P = 0.07 \)) as did the meta-analysis, and this suggests that some allele combinations may constitute a risk genetic background, rather than any individual allele. Considering the \( P \)-value was <0.1% and the power was 66.0% at OR = 1.5, however, it could not be concluded that the lack of significant association is a negative result. It should be noted that a trend of increase in STin2 allele 12 frequencies was observed in schizophrenic patients [8], consistent with the result of the meta-analysis. This is also reminiscent of the studies of Liu et al. and Tsai et al. which reported marginal association of STin2 VNTR with schizophrenia in Chinese population, with an odds ratio of approximately 2.0 [17,35]. The odds ratio of STin2 VNTR in our study is 1.48, and this discrepancy may be also due to difference in sample size. Thus, a larger sample size may be required for a conclusive result on association between STin2 VNTR and schizophrenia. Finally, while the meta-analysis reported increased STin2 allele 12 frequencies in schizophrenic patients, our study found two haplotypes were associated with schizophrenia, the one containing allele 12 (\( P = 0.011 \)) and the one containing STin2 allele 10 (\( P = 5.38 \times 10^{-3} \)). Since haplotype analysis has taken into consideration the frequencies of single alleles, the difference between single allele and haplotype together with different sample size may result in this discrepancy.

rs2054847 and rs2020942 turned out to be in total LD, which was inconsistent with the LD data in the HapMap database, suggesting they belonged to one big LD block which may span across 3’ region of 5-HTT gene including STin2 VNTR. But the LD between these two SNPs with STin2 VNTR was not as high as expected, although rs2020942 is localized near to STin2 polymorphism (Fig. 1) and rs2054847 was predicted to be in the same LD block with STin2 polymorphism [8].

One recent neurochemical hypothesis on the etiology of schizophrenia has implicated a primary cortical hypothalamic-eyeball that enhances subcortical hyperdopaminergia and cortical hypodopaminergia [15]. In our view, 5-HT may contribute significantly to the circuit alteration due to its extensive interaction with other neurotransmitter systems especially the glutamate system. The physiological basis for this view is the role of 5-HT in the extensive biological processes which are relevant to the positive schizophrenic symptoms (such as hallucination), the negative symptoms (such as diminished emotional expression), and the cognitive symptoms (such as impaired long-term memories). Importantly, 5-HT is also involved in the early brain development [32]. The 5-HT neuronal network is among the earliest to develop in embryogenesis, where 5-HT may influence neurogenesis, neuronal apoptosis, and synaptic plasticity [32]. The involvement of 5-HT system components in the therapeutic action of antipsychotic drugs supports a role of 5-HT system in pathogenesis of schizophrenia [1,20,28]. Consistently, other components of 5-HT system in addition to 5-HT were also reported to be in association with schizophrenia [36]. The critical role of 5-HTT in regulating 5-HT...
transmission makes it a promising candidate gene for schizophrenia. And our results have added a support to the role of 5-HT transmission in the pathogenesis of schizophrenia.

In conclusion, here we conducted a case-control association study between genetic polymorphisms in 5-HTT and schizophrenia in the Han Chinese population. We demonstrated association between two STin2-containing haplotype combinations of the 5-HTT gene and schizophrenia. Therefore, we provide further evidence for a role of 5-HTT in pathogenesis of schizophrenia.

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References


