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# Association of *HTR2A* Polymorphisms with Risperidone Efficacy in Chinese Han Schizophrenia Patients

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**ABSTRACT:**

Association of *HTR2A* polymorphisms with risperidone efficacy in Chinese Han schizophrenia patients

**Objectives:** The 5-HT2A receptor is one of the most important serotonin receptors, serving as a major target for risperidone. Rs6311 and rs6313 polymorphisms in the *HTR2A* gene have been reported to be associated with response to risperidone treatment; however, many previous studies have yielded conflicting results. To confirm the importance of these polymorphisms in risperidone treatment and provide more evidence for the routine use of these pharmacogenetic biomarkers in clinical practice, we carried out an association analysis of rs6311 and rs6313 polymorphisms with risperidone efficacy in Chinese Han patients with schizophrenia.

**Methods:** Two independent cohorts of unrelated subjects were recruited from Henan and Shanghai (95 and 113 subjects, respectively). After 4- or 8-week risperidone monotherapy, the rs6311 and rs6313 polymorphisms of these subjects were genotyped with direct DNA sequencing. Clinical improvement was measured by the reduction of the PANSS scores (including the total and subscale scores). UNIANOVA and case-control study was used to evaluate the effects of rs6311 and rs6313 polymorphisms on the therapeutic efficacy of risperidone.

**Results:** As in previous studies, the rs6311G allele was found in complete linkage disequilibrium with the rs6313C allele. However, neither the rs6311 nor the rs6313 polymorphism significantly influenced clinical improvement during risperidone treatment. Neither the allele nor the genotype frequencies were significantly different between responder and non-responder subgroups.

**Conclusions:** No significant association between *HTR2A* polymorphisms (rs6313 and rs6311) and risperidone efficacy was found in the present study. The relative large sample size and two independent cohorts of subjects in the present study enhance the reliability of our findings.

**Keywords:** *HTR2A*, risperidone efficacy, pharmacogenetics, schizophrenia, Chinese Han

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## INTRODUCTION

Risperidone, a benzisoxazole derivative, is increasingly used as an atypical antipsychotic drug for the treatment of many psychotic disorders, such as schizophrenia, manic episodes associated with bipolar disorder, persistent aggression in conduct disorder, autistic disorder and persistent aggression in patients with Alzheimer's dementia[1]. Despite risperidone's proven safety and efficacy, considerable interindividual differences in drug response have been observed[2].

The interindividual variability of drug response

can be partially attributed to genetic factors involved in the pharmacokinetics and pharmacodynamics of risperidone. As the most relevant genes involved in risperidone metabolism, *CYP2D6* and *ABCB1* have been reported to be useful determinants of risperidone's plasma concentrations[3]. Genetic polymorphisms of these genes have been associated with risperidone-induced adverse effects[4,5]. Serotonin receptors and dopamine receptors are the main binding targets of risperidone. Investigations into the pharmacogenetics of risperidone response have found significant associations with some polymorphisms of dopamine receptors and serotonin receptors[1].

The affinity of risperidone for the 5-HT2A receptor, one of the most important members of the serotonin receptor family[6], is much stronger than that for serotonin receptors 6 and 7 as well as dopamine D2 receptors. Associations of some polymorphisms in the *HTR2A* gene with risperidone treatment response have been reported. Among these polymorphic markers, the 102T/C (rs6313) polymorphism has been most extensively studied. Lane et al. first reported that the *HTR2A* 102-C/C genotype might predict superior risperidone response (particularly for negative symptoms rather than positive symptoms) in Chinese Han patients with acutely exacerbated schizophrenia[6]. More recently, similar association analyses of the *HTR2A* 102T/C polymorphism with risperidone treatment response have been performed in Japanese and Korean populations of schizophrenia patients, as well as a Caucasian population of Alzheimer's disease patients[7-9]. However, the replication study on the association of risperidone treatment response with the *HTR2A* 102T/C polymorphism, in 73 Japanese schizophrenia patients, yielded results conflicting with the findings by Lane et al.[9].

Studies concerning the rs6311 (-1438 G/A) polymorphism in the *HTR2A* gene are less extensive than those concerning the 102T/C polymorphic marker, but also have yielded inconsistent results. Yamanouchi et al. reported that after adjustment for the effects of patient-related variables, the *HTR2A* diplotype did not significantly influence clinical performance[9], while Herken et al. reported that patients who were homozygous for the A/A genotype were found to have a better response to risperidone treatment than patients who had G/A or G/G genotypes[10].

In addition, the sample sizes of these studies were not very large, ranging from 45 to 100. In order to confirm the importance of these polymorphisms in risperidone treatment and provide more evidence for the routine use of these pharmacogenetic biomarkers in clinical practice, independent analysis and replication studies with larger sample sizes are needed. For this purpose, an association analysis of rs6311 and rs6313 polymorphisms in the *HTR2A* gene with risperidone treatment response in two independent cohorts of Chinese Han schizophrenic patients (n=208 in total) was carried out in the present study.

## METHODS

### Subjects

Two cohorts of unrelated Chinese Han in-patients with schizophrenia were enrolled in the present study. One hundred and thirteen patients (35 men and 78 women, aged 18-60 years) were recruited from Shanghai Mental Health Center, and 95 subjects (34 men and 61 women, aged 16-55 years) were recruited from Henan Provincial Mental Health Center. All patients recruited met the following criteria: (1) they satisfied the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for schizophrenia; (2) they had no physical complications or other substance abuse; (3) they had no history suggesting resistance to antipsychotic treatment; (4) they hadn't receive any medication for 4 weeks and (5) they hadn't previously received second generation antipsychotics. The study protocol was drawn up according to the principles of the Helsinki Accord and was reviewed and approved by the Shanghai

Ethical Committee of Human Genetic Resources. A statement of informed consent was obtained from all subjects after full explanation of the procedure.

**Clinical Assessment**

For the subjects recruited from Shanghai, the dosage of risperidone was 2mg/day initially and then gradually increased to 4mg/day within the first week, which was maintained until the end of week 2. After that, the dosage was adjusted according to individual tolerance. For the patients recruited from Henan, risperidone was given in a dosage of 1mg/day initially. Then the dosage was gradually increased to 6mg/day within the first week, after which the dosage was adjusted between 1mg/day - 6mg/day according to individual tolerance until the end of week 4. For all the participants, medication compliance was closely monitored and confirmed by nursing staff, and no other medication was given except biperidene for extrapyramidal side effects, flunitrazepam for insomnia and sennosides for constipation during the study period.

Clinical effect was assessed on the Positive and Negative Syndrome Scale (PANSS), including the positive, negative, and general psychopathology subscales. For patients recruited from Shanghai, clinical assessments were conducted on the day of admission, as well as at the end of week 4 and week 8, while clinical assessments of patients recruited from Henan were performed on the day of admission and at the end of week 4. In each cohort, all PANSS ratings were conducted independently by two qualified psychiatrists, who were blind to the genotype of the patients. The inter-rater reliability between the two psychiatrists was good. Risperidone treatment efficacy was measured in terms of the reduction in PANSS scores.

**Genotyping**

Genomic DNA was isolated from venous blood leukocytes using the phenol/chloroform method[11]. All patients' SNPs were genotyped by direct sequencing, using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) on an ABI PRISM 3100 DNA sequencer. The targeted DNA was amplified using a polymerase chain reaction (PCR), according to methods described previously[12]. The determination of genotypes was performed by researchers who were blind to the clinical outcome of the antipsychotic treatment.

**Statistical Analysis**

Statistical analysis was carried out as described previously[12] with minor modifications. Gender differences of demographic and clinical variables were examined firstly to affirm the homogeneity of the gender groups in our samples with the Student's t-test. Genotype frequencies were examined to test for Hardy-Weinberg equilibrium using Genepop 4.2.1 based on the Markov chain algorithm. Reduction of the total and subscale scores of the PANSS was used as a measure of clinical improvement of risperidone treatment. To evaluate the effects of rs6311 and rs6313 polymorphisms, a comparison of the mean clinical improvement among genotypic subgroups was performed using univariate analysis of variance (UNIANOVA) with the baseline PANSS scores as covariates to control for confounding factors. SPSS for Windows, version 11.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The above methodology was adopted only when the variables were in normal distribution and exhibited homoscedasticity between subgroups. The Kolmogorov-Smirnov test and Levene's test were used to check the normal distribution and the homoscedasticity of variances, respectively. Otherwise, the Kruskal-Wallis (KW) test was used for subgroup comparisons.

To substantiate the results, allele and genotype frequencies of each polymorphism were compared between responder and non-responder groups using the $\chi^2$ test on the online software SHEsis (http://analysis.bio-x.cn)[13]. Clinical responders were defined as patients with a larger

reduction in PANSS scores than the average level of all subjects. All tests were two-tailed and statistical significance was assumed at $p<0.05$.

## RESULTS

One hundred and eleven patients recruited from Shanghai completed the 4-week assessment and were eligible for further analysis; 97 of them completed the 8-week assessment. Eighty-seven subjects recruited from Henan completed the 4-week assessment and were eligible for further analysis. Gender differences of demographic and clinical characteristics of these patients are shown in Table 1. There was no significant difference between the male and female groups in the baseline PANSS scores, including the total scores and the subscale scores. This confirmed that there was no systematic difference of potentially clinically relevant outcomes between males and females.

Genotype frequencies of rs6311 and rs6313 are shown in Table 2. Neither exhibits any significant deviation from Hardy-Weinberg equilibrium. The rs6311G allele was in complete linkage disequilibrium with the rs6313C allele. UNIANOVA was used to examine the effects of rs6311 and rs6313 polymorphisms on risperidone treatment response, by comparing the mean clinical response improvement among genotypic subgroups. Baseline PANSS scores, including the total and the subscale scores, were selected as covariates. The results were summarized in Table 3. Neither the rs6311 nor the rs6313 polymorphism significantly influenced the clinical improvement of risperidone treatment, with or without adjustment for baseline score.

To confirm the results of the UNIANOVA, we also carried out a case-control study between responder and non-responder subgroups. Responders were defined as patients who exhibited larger reduction of PANSS scores than the average level. These results are shown in Table 4. Neither the allele nor the genotype frequencies of rs6311 and rs6313 were significantly different between responder and non-responder subgroups, in samples recruited from Henan or Shanghai. The results of the case-control study were in line with those of the UNIANOVA.

**Table 1: Gender differences of demographic and clinical characteristics of all subjects**

| | Patients remaining at week 4 | | | | | | | | Patients remaining at week 8 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample source | Henan | | | | Shanghai | | | | Shanghai | | | |
| Features | Total | Male | Female | p-value | Total | Male | Female | p-value | Total | Male | Female | p-value |
| Number | 87 | 32 | 55 | | 111 | 34 | 77 | | 97 | 30 | 67 | |
| Baseline score of | | | | | | | | | | | | |
| PANSS positive score | 22.2±4.7 | 21.8±5.1 | 22.5±5.2 | 0.565 | 21.3±7.4 | 21.0±8.2 | 21.5±6.1 | 0.739 | 21.6±6.9 | 21.3±8.8 | 21.7±6.5 | 0.797 |
| PANSS negative score | 24.0±6.5 | 25.3±7.9 | 23.2±5.9 | 0.158 | 17.4±8.4 | 18.2±8.2 | 17.0±9.7 | 0.525 | 17.9±8.8 | 18.3±8.2 | 17.8±9.8 | 0.780 |
| PANSS general score | 40.5±8.4 | 40.9±7.4 | 40.2±9.6 | 0.701 | 34.7±8.4 | 34.8±9.9 | 34.7±7.9 | 0.982 | 35.0±8.8 | 34.7±10.4 | 35.2±8.2 | 0.797 |
| PANSS total score | 86.7±16.8 | 88.0±14.1 | 85.9±17.8 | 0.543 | 72.9±17.9 | 73.5±19.8 | 72.7±16.7 | 0.834 | 74.0±18.7 | 74.0±19.7 | 73.9±18.0 | 0.982 |

*Data expressed as mean ± SD, †t test, degree of freedom is 1, ‡PANSS: Positive and Negative Syndrome Scale

**Table 2: Genotype frequencies of rs6311 and rs6313 in the HTR2A gene**

| | rs6311 | | | rs6313 | | |
|---|---|---|---|---|---|---|
| | Genotype | Frequency (%) | p-value for HWE | Genotype | Frequency (%) | p-value for HWE |
| Henan | GG | 18 (20.7) | 0.395 | CC | 18 (20.7) | 0.395 |
| | GA | 48 (55.2) | | CT | 48 (55.2) | |
| | AA | 21 (24.1) | | TT | 21 (24.1) | |
| Shanghai at week 4 | GG | 22 (19.8) | 0.112 | CC | 22 (19.8) | 0.112 |
| | GA | 45 (40.6) | | CT | 45 (40.6) | |
| | AA | 44 (39.6) | | TT | 44 (39.6) | |
| Shanghai at week 8 | GG | 17 (17.5) | 0.124 | CC | 17 (17.5) | 0.124 |
| | GA | 38 (39.2) | | CT | 38 (39.2) | |
| | AA | 42 (43.3) | | TT | 42 (43.3) | |

*HWE: Hardy–Weinberg equilibrium

**Table 3: UNIANOVA analysis of PANSS score reduction among genotypic subgroups**

| | Genotype (rs6311-rs6313) | Reduction of PANSS scores at week 4 | | | | | Reduction of PANSS scores at week 8 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | n | Positive | Negative | General | Total | n | Positive | Negative | General | Total |
| **Henan** | AA-TT | 21 | 4.81±4.61 | 4.76±4.68 | 7.10±8.33 | 17.00±16.08 | - | - | - | - | - |
| | GA-CT | 48 | 6.15±5.19 | 5.65±6.21 | 8.42±8.76 | 20.21±17.97 | - | - | - | - | - |
| | GG-CC | 18 | 6.61±4.45 | 4.44±6.63 | 8.89±8.04 | 19.94±15.05 | - | - | - | - | - |
| | p-value: unadjusted | | 0.534[§] | 0.559[§] | 0.790[§] | 0.809[§] | | - | - | - | - |
| | p-value: adjusted | | | | | | | - | - | - | - |
| **Shanghai** | AA-TT | 44 | 9.07±5.86 | 5.09±4.96 | 10.41±7.44 | 24.98±15.47 | 42 | 12.07±7.83 | 7.50±6.22 | 14.43±9.51 | 34.21±20.51 |
| | GA-CT | 45 | 9.44±6.55 | 5.07±4.72 | 10.27±6.53 | 24.73±13.62 | 38 | 12.00±6.95 | 6.95±5.20 | 13.00±8.30 | 31.63±15.30 |
| | GG-CC | 22 | 7.41±3.92 | 6.41±13.68 | 9.18±5.51 | 19.72±10.96 | 17 | 10.41±4.46 | 10.65±15.88 | 13.12±8.67 | 31.00±15.56 |
| | p-value: unadjusted | | 0.615[§] | 0.438[§] | 0.211 | 0.231 | | 0.064 | 0.913[§] | 0.541 | 0.297 |
| | p-value: adjusted | | | | 0.546 | 0.119 | | 0.723 | | 0.908 | 0.916 |

*Data are expressed as mean±SD, [†]PANSS: Positive and Negative Syndrome Scale, [‡]UNIANOVA: univariate analysis of variance, [§]The Kruskal-Wallis test is used because variables failed to pass the normal distribution test or the homoscedasticity test.

**Table 4: Case-control study of rs6311 and rs6313 polymorphisms in HTR2A gene between responder and non-responder groups: frequencies and significance levels**

| | Frequency of | | Reduction of PANSS scores at week 4 | | | | Reduction of PANSS scores at week 8 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Positive | Negative | General | Total | Positive | Negative | General | Total |
| **Henan** | Responder (n) | | 42 | 34 | 36 | 42 | | | | |
| | Non-responder (n) | | 45 | 53 | 51 | 45 | | | | |
| | Alleles (rs6311-rs6313) | A-T (%) | 50.0/53.3 | 52.9/50.9 | 51.4/52.0 | 51.2/52.2 | - | - | - | - |
| | | G-C (%) | 50.0/46.7 | 47.1/49.1 | 48.6/48.0 | 48.8/47.8 | - | - | - | - |
| | | p-value | 0.660 | 0.797 | 0.940 | 0.892 | - | - | - | - |
| | Genotypes (rs6311-rs6313) | AA-TT (%) | 21.4/26.7 | 23.5/24.5 | 25.0/23.5 | 23.8/24.4 | - | - | - | - |
| | | GA-CT (%) | 57.1/53.3 | 58.8/52.8 | 52.8/56.9 | 54.8/55.6 | - | - | - | - |
| | | GG-CC (%) | 21.4/20.0 | 17.6/22.6 | 22.2/19.6 | 21.4/20.0 | - | - | - | - |
| | | p-value | 0.850 | 0.822 | 0.927 | 0.986 | - | - | - | - |
| **Shanghai** | Responder (n) | | 51 | 44 | 47 | 51 | 44 | 47 | 42 | 41 |
| | Non-responder (n) | | 60 | 67 | 64 | 60 | 53 | 50 | 55 | 56 |
| | Alleles (rs6311-rs6313) | A-T (%) | 61.8/58.3 | 63.6/57.5 | 64.9/56.2 | 64.7/55.8 | 65.9/60.4 | 64.9/61.0 | 64.3/61.8 | 65.9/60.7 |
| | | G-C (%) | 38.2/41.7 | 36.4/42.5 | 35.1/43.8 | 35.3/44.2 | 34.1/39.6 | 35.1/39.0 | 35.7/38.2 | 34.1/39.3 |
| | | p-value | 0.603 | 0.359 | 0.194 | 0.179 | 0.427 | 0.575 | 0.724 | 0.464 |
| | Genotypes (rs6311-rs6313) | AA-TT (%) | 39.2/40.0 | 40.9/38.8 | 44.7/35.9 | 45.1/35.0 | 45.5/41.5 | 44.7/42.0 | 45.2/41.8 | 43.9/42.9 |
| | | GA-CT (%) | 45.1/36.7 | 45.5/37.3 | 40.4/40.6 | 39.2/41.7 | 40.9/37.7 | 40.4/38.0 | 38.1/40.0 | 43.9/35.7 |
| | | GG-CC (%) | 15.7/23.3 | 13.6/23.9 | 14.9/23.4 | 15.7/23.3 | 13.6/20.8 | 14.9/20.0 | 16.7/18.2 | 12.2/21.4 |
| | | p-value | 0.522 | 0.393 | 0.467 | 0.458 | 0.656 | 0.804 | 0.943 | 0.458 |

*Data before and after the slash represent frequency data (%) in the responder group and the non-responder group, respectively.
[†]$\chi^2$ test, degree of freedom is 1 for allele analysis and 2 for genotype analysis.

## DISCUSSION

Affinity and binding studies have implied that the 5-HT2A receptor is one of the major targets for atypical antipsychotics[14]. Consequently, genetic polymorphisms in the *HTR2A* gene might have some influence on atypical antipsychotic treatment response and partially explain inter-individual variations. Among the genetic variations existing within the *HTR2A* gene, rs6311 (-1438A/G) and rs6313 (102T/C) polymorphisms are of particular interest. The rs6311 SNP lies just upstream of two alternative promoters for the *HTR2A* gene, and promoter activity was significantly greater in the presence of A allele relative to the G allele[15]. The rs6313 polymorphism is a silent variant in exon 1, which has no influence on the amino acid sequence of the 5-HT2A receptor. However, the 102C variant was found to be associated with lower 5-HT2A receptor expression levels in the temporal cortex of both normal individuals and schizophrenia patients[16].

Although there have been a substantial number

of studies carried out, the association of rs6311 and rs6313 polymorphisms with atypical antipsychotic treatment response remains controversial. Lane et al. reported that after controlling for non-genetic confounders, in Chinese Han patients with acutely exacerbated schizophrenia, those with the C/C genotype of rs6313 had lower total scores, negative subscale scores, as well as general psychopathology scores on the Positive and Negative Syndrome Scale than patients with the T/C genotype, and that patients with the T/C and T/T genotypes had comparable total and subscale scores[6]. A replication study in a naturalistic setting with Korean acutely exacerbated schizophrenic patients treated with risperidone monotherapy yielded similar results, reporting that patients with the 102-T/T genotype showed less clinical improvement than did those with 102-T/C or 102-C/C genotypes, while patients with the C/C genotype exhibited no significant difference in clinical response from patients with the T/T genotype. However, in another replication study carried out in Japanese patients with schizophrenia, the 102T/C polymorphism, which was in complete linkage disequilibrium with the -1438G/A mutation, was found to have no association with clinical response to risperidone treatment. In addition, it has been shown that the 102-C/C genotype is more frequent among clozapine non-responders than responders[17,18]. Herken et al. suggested that in Turkish schizophrenic patients, the 102-TT genotype, which was in complete linkage disequilibrium with the 1438-AA genotype, was associated with better response to risperidone treatment[10]. Correia et al. reported that autistic patients homozygous for the *HTR2A*-1438G allele showed a poor response to risperidone[19].

In the present study, we carried out an association study of the rs6311 and rs6313 polymorphisms with risperidone treatment response in two independent cohorts of unrelated Chinese Han schizophrenic patients. As in previous studies, rs6311 and rs6313 polymorphisms were in complete linkage disequilibrium[9,20]. Allele frequencies were similar to those in the earlier studies enrolling Han Chinese[6,21], suggesting that our data was not biased. By UNIANOVA with baseline PANSS scores as confounding factors and the case-control study, which defined the responders as patients who exhibited larger reduction of PANSS scores than the average level, no significant association of rs6311 and rs6313 polymorphisms with response to risperidone was found. Our results are in line with the report of Yamanouchi et al.[9], conflicting with other previous studies regarding the association of *HTR2A* gene polymorphisms with risperidone treatment response.

The discrepancy may be explained by the following reasons. The first one is the type of illness. In some previous studies[6,8,19], association was found in patients with acutely exacerbated schizophrenia or autism, while in this study patients with chronic or acutely exacerbated schizophrenia were both included. Secondly, the study methodology and the ethnicity of the subjects vary. Thirdly, some previous studies[17,18] were concerned with the influence of *HTR2A* polymorphisms on clozapine treatment response, differing from the present study. Clozapine is distinguished from risperidone in its pharmacological profile[22]. In addition, different non-genetic factors were taken into consideration as confounding factors in different studies. Lane et al. reported that baseline PANSS scores and number of previous hospitalizations significantly affected the risperidone treatment response, while we only took the baseline PANSS scores into consideration. Last but not the least, the sample size of previous studies was small. Although we recruited a total number of 208 subjects, they were separated into 2 groups (113 and 95 subjects, respectively) by the sample source and analyzed independently. Thus, the limited sample size increases the risk of type II error.

Some strengths and limitations of this study should be acknowledged. Firstly, we recruited two independent cohorts of schizophrenic patients from Henan and Shanghai, respectively. These two cohorts of subjects were analyzed independently and could serve as a replication study for each

other. So stratification was reduced to the lowest extent, and reliability was enhanced. Secondly, to our knowledge, the sample size in this study is the largest among studies concerning association between the *HTR2A* gene and risperidone efficacy, although we might have had chance of false negative findings. Finally, many genetic and non-genetic factors can contribute to risperidone efficacy. Simultaneous examination of multiple genes, multiple polymorphisms as well as multiple non-genetic factors may be a more robust strategy; we only took limited factors into consideration.

## CONCLUSIONS

In summary, we conducted an association analysis of rs6311 and rs6313 polymorphisms in the *HTR2A* gene with risperidone efficacy in two independent cohorts of Chinese Han patients with schizophrenia. No significant association was found. Our findings may contribute to uncovering the real roles of rs6311 and rs6313 in risperidone treatment. However, due to the limitation of sample size and the limited factors taken into consideration, results of present study should be treated with caution. Independent analysis and replication studies with larger sample sizes and evaluating the effects of more comprehensive factors are still needed.

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