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Effects of the Dopamine D_3 Receptor (*DRD3*) Gene Polymorphisms on Risperidone Response: A Pharmacogenetic Study

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Previous observations of the anatomical distribution and pharmacological profile of the dopamine D_3 receptor (DRD3) have indicated its potential role in antipsychotic drug action. Risperidone, an effective first-line atypical antipsychotic agent, exhibits a relatively high affinity for this receptor. Recent studies have reported an association of the Ser9Gly polymorphism in the *DRD3* gene with therapeutic response to risperidone, but the results were inconsistent. We therefore postulated that the Ser9Gly polymorphism might be in linkage disequilibrium with an undetected variant that exerts a direct influence on risperidone efficacy. The present study genotyped eight single nucleotide polymorphisms (SNPs) distributed throughout the *DRD3* gene and examined five of these for association with treatment outcome, following an 8-week period of risperidone monotherapy in 130 schizophrenic patients from mainland China. Clinical symptoms were assessed before and after the treatment period, using the Brief Psychiatry Rating Scale (BPRS). The confounding effects of non-genetic factors were estimated and the baseline symptom score was included as a covariate for adjustment. Neither was any association observed between the five polymorphisms and improvement in total BPRS scores nor was any combined effect of these variants detected in the haplotype analysis. The current results indicate that genetic variations within the *DRD3* gene may not contribute significantly to interindividual differences in the therapeutic efficacy of risperidone.
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INTRODUCTION

In the last three decades, the dopamine hypothesis of schizophrenia has been the dominant force in driving the development of antipsychotic drugs. Given the tight correlation between the clinical potency and the D_2-binding affinity of the conventional neuroleptics (Creese *et al*, 1976; Seeman *et al*, 1976), the dopamine D_2 receptor is assumed to serve as a principal target for antipsychotic action. *In vivo* imaging studies have further demonstrated the importance of dopamine D_2 receptor occupancy in predicting antipsychotic response and side effects (Remington and Kapur, 1999). The advent of novel antipsychotics and the identification of additional dopamine receptor subtypes, however, have allowed for new insights into the wider diversity of pharmacological actions of antipsychotic drugs.

The dopamine D_3 receptor (DRD3) was cloned and characterized as a D_2-like receptor in 1990 (Sokoloff *et al*, 1990). Further observations on the preferential expression of this receptor in limbic and basal ganglia regions associated with cognitive, emotional, and motor functions (Murray *et al*, 1994; Suzuki *et al*, 1998), as well as other neurochemical and anatomical investigations (Schmauss *et al*, 1993; Gurevich *et al*, 1997), implicated a role for the D_3 receptor in the etiology of schizophrenia. It is postulated that the D_3 receptor plays an important role in the regulation of neurotransmission by inhibiting spontaneous secretion of the neurotransmitter (Kuzhikandathil and Oxford, 1999, 2000). Pharmacological studies have shown that many antipsychotic drugs also exhibit a high affinity for the D_3 receptor (Schwartz *et al*, 2000); thus, raising the question of the role this protein may play as an important target for antipsychotic drugs.

Compared with conventional neuroleptics, atypical antipsychotics are characterized by a low rate of extrapyramidal

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side effects (EPS) and a broader therapeutic profile, including efficacy in treating both positive and negative symptoms of schizophrenia. Risperidone, a benzisoxazole derivative with atypical antipsychotic activity, is widely used as a first-line drug for schizophrenia treatment. Higher antagonistic activity of risperidone at 5-HT_{2A} receptors than at D_2 receptors is assumed to well contribute to its atypical property but may not be sufficient by itself (Meltzer, 1999; Kapur and Remington, 2001). Competition experiments *vs* [^{125}I]iodosulpride showed that risperidone also displayed a high affinity for D_3 receptors ($K_i = 5.4$ nM) (Sokoloff *et al*, 1992). Recent studies have implicated a beneficial role for D_3 receptor antagonism in ameliorating negative symptoms of schizophrenia (Joyce and Millan, 2005). For example, administration of selective D_3 receptor antagonists enhances social interaction in isolation-reared rodents (Reavill *et al*, 2000; Brocco *et al*, 2004). Despite the potential role of D_3 receptor antagonism in the treatment of schizophrenia, several studies have showed that D_3 agonists rather than D_3 antagonists could produce antipsychotic effects (Witkin *et al*, 1998; Fink-Jensen, 2000). Consequently, the contribution of D_3 receptor antagonism to the antipsychotic activity of risperidone remains to be examined.

Among the single nucleotide polymorphisms (SNPs) identified in the *DRD3* gene, the Ser9Gly polymorphism has been the most extensively investigated. This polymorphic site in the first exon of the *DRD3* gene results in a serine to glycine substitution in the N-terminal extracellular domain of the receptor protein (Lannfelt *et al*, 1992). A series of reports including a recent meta-analysis have suggested an association in favor of the Gly-9 *vs* the Ser-9 variant with an increased risk of developing antipsychotic-induced tardive dyskinesia (Steen *et al*, 1997; Lerer *et al*, 2002; Bakker *et al*, 2006). Prior studies on the association between medication response and the Ser9Gly polymorphism were primarily a by-product of the research on the role of this polymorphism in schizophrenia. Two studies investigating a monotherapeutic strategy detected an excess of Gly-9 allele in responders to clozapine treatment (Shaikh *et al*, 1996; Scharfetter *et al*, 1999). However, a third report failed to replicate these results (Malhotra *et al*, 1998).

Recently, several studies have suggested an association between the D_3 receptor Ser9Gly polymorphism and antipsychotic response to risperidone, but the results were inconsistent (Szekeres *et al*, 2004; Lane *et al*, 2005; Reynolds *et al*, 2005). To further evaluate the importance of the DRD3 in the therapeutic efficacy of risperidone, we performed an association study on polymorphisms from different regions of the *DRD3* gene, in a relatively large sample of Chinese schizophrenic patients following risperidone treatment.

MATERIALS AND METHODS

Subjects

One hundred and forty-five Chinese Han schizophrenia patients participated in the study on admission, all of who were recruited from the Shanghai Mental Health Center. Diagnosis was confirmed by two independent psychiatrists conducting of clinical interviews based on the criteria of DSM-IV. Any subject suffering from a severe neurological or physical disease was excluded from the study. All patients were receiving atypical antipsychotic drug treatment for the first time. The study was conducted in compliance with all principles of the Helsinki Accord. A standard informed consent in the protocol, which was reviewed and approved by the Shanghai Ethical Committee of Human Genetic Resources, was obtained from the participants after the procedure had been fully explained.

Clinical Protocols

Before treatment with risperidone, all patients were subjected to at least a 4-week washout period. Risperidone was given at an initial dose of 2 mg/day, and dosage was gradually increased to 4 mg/day within the first week. The drug dose was maintained until day 14, and then adjusted according to individual tolerance. Medication compliance was closely monitored and confirmed by nursing staff. During the study period, no other drugs were given except flunitrazepam for acute insomnia and biperiden for moderate EPS. The Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1962) was used to assess clinical status at the beginning and after 8 weeks of treatment. Medication efficacy was evaluated in terms of a percent change in BPRS total scores. Clinical response was defined as an improvement from baseline of 40% or more in BPRS total score.

Genotyping

High-molecular-weight genomic DNA was prepared from venous blood using standard phenol chloroform extraction. Six SNPs including rs324028, rs717668, rs324026, rs6280, rs3773678, rs2134655 were selected from dbSNP (http://www.ncbi.nlm.nih.gov), spanning around 36.4 kb in the *DRD3* gene. SNP rs324028, SNP rs717668 in the 5′ noncoding region and SNP rs324026 in the 5′ promoter region of the *DRD3* gene are located 3741, 1074, and 203 bp upstream from the start codon, respectively. SNP rs6280 is in the coding sequence of the *DRD3* gene, resulting in an amino acid substitution of serine for glycine at position 9 (Ser9Gly). SNP rs3773678 and SNP rs2134655 are located in the introns. Other polymorphisms (Ala38Thr, $-7685G > C$) in the *DRD3* gene (Ishiguro *et al*, 2000; Staddon *et al*, 2005) were excluded from further tests for association with drug response because they were present at very low frequency in our sample.

All SNPs were genotyped by direct DNA sequencing, using a BigDye Terminator Cycle Sequencing Kit and an ABI PRISM 3100 DNA sequencer (PE Applied Biosystems, Perkin-Elmer). PCR amplification was performed on a Gene Amp PCR system 9700 (PE Applied Biosystems, Foster City, CA) in a volume of 10 μl containing 10 ng genomic DNA, 1 U *Taq* polymerase, 0.5 μM of each primer, 10 × PCR buffer, 1.5 mM $MgCl_2$, and 0.2 mM of each dNTP. PCR conditions consisted of an initial denaturation at 95°C for 3 min, 35 cycles of 30 s at 94°C, 30 s at annealing temperature, and 1 min at 72°C, followed by a terminal extension at 72°C for 7 min. Primer sequences and annealing temperatures are listed in Table 1. All genotypes were called blind to the clinical outcome of antipsychotic treatment.

Statistical Analysis

Deviations from Hardy–Weinberg equilibrium for each polymorphism were assessed using exact tests based on a Markov chain algorithm using GENEPOP 3.4 (Rousset and Raymond, 1995). Linkage disequilibrium (LD) of each pair of the six polymorphisms was measured by estimating standardized D' values on software 2LD (Zapata *et al*, 2001).

SPSS for Windows (version 10.0) was used for further statistical analyses. A comparison of the mean values of demographic and clinical variables between genotypic subgroups was made using ANOVA followed by Tukey's HSD test. These variables were also examined for gender differences using the Student's *t*-test or the nonparametric Mann–Whitney *U*-test. To determine the appropriate method, each variable was checked for normal distribution using the Kolmogorov–Smirnov test and for homogeneity of variances using the Levene's test. The influences of potential confounding factors (age, age of onset, gender, drug concentrations, and baseline BPRS scores) on symptom improvement were assessed in a stepwise regression procedure. Variables significantly correlated with the percentage change in total BPRS scores were selected as covariates for the control of confounding effects. In the analysis of genetic effects on therapeutic efficacy, the general linear model was used with or without adjustment for covariates. The factor-by-covariate interaction was tested to check the homogeneity of within-group regression slopes. Pairwise comparisons between genotypic subgroups were carried out using UNIANOVA. To confirm the results, allele and genotype frequencies of each polymorphism were compared between responder and non-responder groups using the online software SHEsis (http://202.120.7.14/analysis/myAnalysis.php). This software was also used to estimate the significance of differences in haplotype distributions between the two groups. Power analysis of our sample was performed using the G*Power program (Erdfelder *et al*, 1996). All tests were two-tailed and statistical significance was assumed at $p<0.05$.

RESULTS

Clinical Profiles

One hundred and thirty patients were available for assessment at the end of the treatment. Of them, 67 had no prior antipsychotic exposure and the remainder had prior exposure to conventional neuroleptics. Fifteen patients dropped out of the study owing to concurrent somatic illness ($n=3$), poor response ($n=6$), or noncompliance ($n=6$). Clinical profiles of the patients eligible for analysis are shown in Table 2. The patients were divided into different groups on the basis of gender. There were no

Table 1 Primer Sequences and Annealing Temperatures Used for PCR Amplifications

Markers	Primer	Sequence (5′–3′)	PCR product (bp)	Annealing temperature (°C)
rs324028	Forward	CTG-ATG-GGG-ACC-TGA-CAA-CT	467	59
	Reverse[a]	TCA-CGG-TTT-AAC-CCA-CCT-TC		
rs717668	Forward[a]	CAG-TAG-AGG-GTG-CCT-TAG-TCC	369	57
	Reverse	GTC-TCG-GTG-TTT-GTG-TCT-CC		
rs6280	Forward[a]	GCT-CTA-TCT-CCA-ACT-CTC-ACA	463	53
	Reverse	AAG-TCT-ACT-CAC-CTC-CAG-GTA		
rs3773678	Forward	GCC-ATG-GTG-GGA-GTA-TTG-AC	447	59
	Reverse[a]	TTG-GGA-GGC-TAC-AAC-AAC-CT		
rs2134655	Forward[a]	GAC-GGA-AAA-GGA-TCC-TCA-CT	375	57
	Reverse	AAA-CCT-GTG-GGA-CAT-CTG-AG		

[a]The primers were used for DNA sequencing.

Table 2 Baseline Demographics and Clinical Status of all Schizophrenic Patients

Patients	Total ($n=130$)	Male ($n=45$)	Female ($n=85$)	Significance
Age (years)	36.3±11.2	35.3±11.8	36.8±10.9	NS ($p=0.49$)
Age of onset (years)	30.3±10.3	28.8±9.7	31.2±10.6	NS ($p=0.21$)
Duration of illness (years)	6.0±8.6	6.3±9.1	5.8±8.4	NS ($p=0.22$)
Plasma risperidone levels (ng/ml)	7.0±5.6	6.7±6.1	7.1±5.3	NS ($p=0.63$)
Plasma 9-hydroxyrisperidone levels (ng/ml)	22.6±13.1	22.4±16.0	22.8±11.3	NS ($p=0.34$)
Total score of BPRS				
Initial	42.4±12.4	45.3±15.0	40.9±10.5	NS ($p=0.09$)
8 weeks	24.0±6.6	25.7±9.0	23.1±4.8	NS ($p=0.15$)

Data expressed as mean±SD.
NS, not significant.

significant differences between gender groups in age, age of onset, duration of illness, steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone, or in total BPRS scores at baseline and at the end of risperidone monotherapy. This indicates that our sample data were not significantly biased with respect to gender for these clinical parameters. In respect of treatment efficacy, the patients were classified as treatment responders ($n=72$) and non-responders ($n=58$), based on the percentage improvement in total BPRS scores before and after the 8-week medication. No significant differences in demographic characteristics or plasma levels of risperidone and 9-hydroxyrisperidone were observed between the two groups ($p>0.05$) (data not shown).

Effects of Individual Polymorphism on Clinical Improvement

All polymorphisms were successfully genotyped in the 130 schizophrenic patients. Genotype frequencies of these polymorphisms showed no significant deviations from Hardy–Weinberg equilibrium (data not shown). Two SNPs (rs324028 and rs324026) were found to be in complete LD ($D'=1$). This indicated that only one of these two SNPs could provide useful genetic information. Consequently, SNP rs324028 but not rs324026 was included in our association study. The genotypic distributions of five SNPs in the sample are shown in Table 3. There were no significant differences in the clinical parameters including age, age of onset, duration of illness, or drug concentrations between genotypes of analyzed polymorphisms ($p>0.05$).

The mean BPRS total score was reduced from 42.4 ± 12.4 to 24.0 ± 6.6 during the treatment period, with an improvement of 40.6% in general symptoms. The values of percentage improvement in total BPRS scores were normally distributed ($p=0.53$); therefore, the data were suitable for multiple regression analysis. The confounding effects of non-genetic factors on symptom improvement were then assessed by a stepwise regression analysis. No significant correlations were found between independent variables, such as age, age of onset, gender, or drug concentrations and the improvement of total BPRS scores, although the severity of illness (ie baseline BPRS scores) exerted a significant influence ($p<0.001$) on treatment efficacy (data not shown). Consequently, baseline BPRS score was identified as a relevant variable for treatment outcome and was included as a covariate in the analysis of association between *DRD3* genotype and symptom improvement.

The mean values of the covariate were not significantly different between genotypic subgroups of each polymorphism (one-way ANOVA $p>0.05$). Table 3 illustrates the effects of *DRD3* polymorphisms on the improvement in total BPRS scores, before and after adjusting for the covariate effects. No significant associations were found between the genotypes of the five polymorphisms and general symptom improvement. Pairwise comparisons between genotypic subgroups also failed to detect any significant differences.

Given the possible influence of the Ser9Gly mutation on the binding capacity of the D_3 receptor, we compared patients with the Gly9Gly genotype with those with the Ser9Ser or Ser9Gly genotype. The Gly9Gly genotype had no significant influence on clinical improvement ($p=0.418$). Moreover, there were no significant differences in allele or genotype distribution in any of the five polymorphisms between responder and non-responder groups.

LD between SNPs and Haplotype Analysis

Pairwise LD between the five markers was estimated (expressed in term of D'). As shown in Table 4, strong LD

Table 3 Genotype Frequencies of SNPs and Association Analyses with and without Adjustment for the Covariate

			BPRS			
Markers	**Genotype**	**Genotype frequency (%)**	**Baseline**	**Percentage change**	**Unadjusted effect**	**Adjusted effect**
	AA	67 (51.5)	41.8 ± 11.8	41.4 ± 15.3	NS ($p=0.631$)	NS ($p=0.213$)
rs324028	AG	46 (35.4)	43.7 ± 13.2	39.0 ± 19.9		
	GG	17 (13.1)	37.9 ± 11.5	43.7 ± 17.8		
	CC	66 (50.8)	42.2 ± 11.7	41.2 ± 15.4	NS ($p=0.585$)	NS ($p=0.672$)
rs717668	CT	46 (35.4)	43.1 ± 13.5	41.0 ± 17.9		
	TT	18 (13.8)	40.6 ± 12.1	36.6 ± 20.7		
	AA	64 (49.2)	42.2 ± 12.0	41.3 ± 15.5	NS ($p=0.813$)	NS ($p=0.407$)
rs6280	AG	49 (37.7)	43.4 ± 13.3	39.3 ± 20.0		
	GG	17 (13.1)	40.2 ± 11.3	41.5 ± 16.4		
	CC	89 (68.5)	41.7 ± 12.3	39.8 ± 17.8	NS ($p=0.668$)	NS ($p=0.525$)
rs3773678	CT	37 (28.5)	44.3 ± 13.0	42.0 ± 16.7		
	TT	4 (3.1)	39.0 ± 4.2	45.9 ± 11.5		
	GG	76 (58.5)	43.7 ± 13.5	42.0 ± 18.8	NS ($p=0.503$)	NS ($p=0.948$)
rs2134655	GA	46 (35.4)	39.9 ± 9.4	38.2 ± 15.2		
	AA	8 (6.2)	44.1 ± 15.8	41.1 ± 13.3		

NS, not significant.

was observed among the three SNPs near the 5′ end of the *DRD3* gene. The SNP rs3773678 located in the downstream intron was in much weaker LD with these markers ($D' \approx 0.20$), but this was not the case for another intron, SNP rs2134655.

Four SNPs (rs324028, rs717668, rs6280, rs2134655) with significant LD ($D' > 0.75$) were included in the haplotype analysis. Out of 16 possible combinations of alleles, only five haplotypes with a probability >3% represented the majority of the haplotype diversity (96%). The frequencies of these common haplotypes were compared between responder and non-responder groups. As shown in Table 5, there were no significant differences in haplotype distributions between the two groups (global $\chi^2 = 2.603$, $p = 0.626$).

DISCUSSION

The DRD3 has been suggested as a potential drug target for antipsychotic drugs (Schwartz *et al*, 2000). Recent studies have focused on the Ser9Gly polymorphism of the *DRD3* gene, but the results were inconclusive. Although it has been suggested that the Ser9Gly variant could induce a shift in intracellular signal transduction pathways, the molecular mechanisms and the clinical relevance of the switch remain unclear (Hellstrand *et al*, 2004). There might be other functional variants within the *DRD3* gene that affect transcriptional regulation, mRNA stability or splicing efficiency. In our study, eight polymorphisms spanning the *DRD3* gene were genotyped and five were investigated for their possible association with risperidone response in 130 unrelated schizophrenic patients from mainland China. However, no significant correlation was found between any variations and general symptom improvement, nor was a trend toward an excess of Ser9 variant observed in patients with better response. Furthermore, we did not detect significant discrepancy of allele, genotype or haplotype frequencies between responder and non-responder groups.

Our results indicate that the effect of variations in the *DRD3* gene on therapeutic efficacy of risperidone might be weak or absent. However, this does not rule out a potential role for the D_3 receptor in the action of risperidone. Pharmacological studies have suggested that D_3 receptor antagonism may contribute to the efficacy of atypical antipsychotics in relieving negative symptoms of schizophrenia (Joyce and Millan, 2005). The overall pharmacological effects of risperidone, however, may be mediated by multireceptor antagonism. In addition to its preferentially high affinity for 5-HT_{2A} and D_2 receptors, risperidone also displays relatively high affinity for histamine H_1 and alpha-adrenergic receptors (Leysen *et al*, 1994; Megens *et al*, 1994). Genetic variations of these receptors may produce confounding effects on assessing the predictive role of *DRD3* polymorphisms for risperidone response.

The current study attempted to identify genetic contributors to the clinical variability in risperidone response. However, true factors underlying interindividual variation in medication response may also include demographic, clinical, and environmental variables, which probably confound most pharmacogenetic association studies. The design of this study has some advantages in minimizing the impacts of potential confounding factors. First, patients involved in the current study were receiving atypical

Table 4 Pairwise LD Statistics for the Five Markers

	Distance[a] (bp)	rs324028	rs717668	rs6280	rs3773678	rs2134655
rs324028	0	—				
rs717668	2668	**0.84**[b]	—			
rs6280	3766	**0.98**	**0.83**	—		
rs3773678	24503	0.20	0.18	0.21	—	
rs2134655	36380	**0.76**	0.69	**0.84**	**0.82**	—

[a]Distance from rs324028.
[b]The standardized D' values >0.7 are boldfaced.

Table 5 Estimated Haplotype Frequencies of Four Polymorphisms in Responders ($n = 72$) and Non-responders ($n = 58$)

					Haplotype distribution (%)				
Haplotype[a]	rs324028	rs717668	rs6280	rs2134655	Responder	Non-responder	X^2	*p*-value	Odds ratio (95% CI)
1	A	C	A	A	21.0	23.6	0.247	0.619	0.860 (0.474–1.561)
2	A	C	A	G	45.3	38.8	1.077	0.299	1.306 (0.788–2.165)
3	A	T	A	G	2.2	3.6	0.441	0.507	0.605 (0.135–2.709)
4	G	C	G	G	4.4	1.8	1.269	0.260	2.435 (0.493–12.019)
5	G	T	G	G	24.5	27.1	0.223	0.637	0.872 (0.495–1.538)
Global							2.603	0.626	

[a]Haplotypes with a frequency <3% in both responders and non-responders were omitted from analysis.

antipsychotics for the first time. Prior drug treatment may produce confounding effects on clinical response to subsequent antipsychotic medications. It has been found that long-term effects of atypical antipsychotics on neurotransmitter receptors are somewhat different from those of conventional neuroleptics (White and Wang, 1983; Damask *et al*, 1996; Tarazi *et al*, 2002). In particular, long-term treatment with atypical antipsychotics but not with conventional neuroleptics significantly alters the abundance of 5-HT receptor subtypes in forebrain regions (Tarazi *et al*, 2002). Accordingly, the nature of our sample may reduce to some degree the effects of medical history on risperidone response. Second, the effects of other potential confounding factors (such as age, age of onset, gender, plasma drug concentrations, initial severity of illness) were estimated by regression analysis. We found that initial symptom severity of patients was closely related with their treatment outcome and this variable was controlled as a covariate for the primary variable of interest. Third, our patients were scheduled to receive a target dose of 4 mg/day of risperidone rather than a standard dose of 6 mg/day. A low risk of EPS was observed among these subjects, as was suggested by several other studies (Nyberg *et al*, 1999; Williams, 2001). Consequently, we were able to maintain a uniform dosage schedule for most patients. This may be useful for reducing individual differences with regard to drug concentration and the possible effect of this variable on medication response.

Power analysis revealed that the statistical power of our sample to detect a significant association ($p < 0.05$) was about 90% in allele or genotype comparisons and over 80% for haplotype analysis when a medium effect size ($w = 0.3$) was presumed. This indicates that the sample size in our study was sufficient to achieve a considerably low risk of a type II error.

In summary, we conducted a detailed association study of the *DRD3* gene with risperidone response, but did not detect any significant effect of *DRD3* polymorphisms. Our results indicate that variants in either coding or regulatory regions of the gene are not likely to greatly affect the therapeutic efficacy of risperidone for the mainland Chinese population. The assumed predictive role of markers within the *DRD3* gene for risperidone treatment outcome should be further evaluated. It may be helpful to investigate a set of markers from different genes that might exert a combined or synergistic effect on drug action, as risperidone has also been found to interact with dopamine D_2, D_4, serotonin $5\text{-}HT_{2A}$, histamine H_1, adrenergic α_1, and α_2 receptors. In addition, appropriate control of confounding factors would be useful for further pharmacogenetic studies to focus on the effect of genetic variation on antipsychotic response.

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