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Association Studies of *DRD2* and *COMT* Gene Polymorphisms with Risperidone-induced Amenorrhea in Female Schizophrenia Patients

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Abstract

Object: To study the association between *dopamine D2 receptor* (*DRD2*) and *catechol-O-methyltransferase* (*COMT*) gene polymorphisms and the risperidone-induced amenorrhea resulted from hyperprolactinemia in female schizophrenia patients.

Patients and methods: According to International Diagnostic and Classification of Diseases tenth edition (ICD-10) criteria, 45 Chinese female schizophrenic patients (25 patients with amenorrhea, and 20 patients with eumenorrhea) were recruited by trained psychiatrists in this study. Sanger sequencing was utilized to determine the *DRD2* and *COMT* genotypes from peripheral venous blood samples.

Results: There were no significant differences between amenorrhea patients and eumenorrhea patients in age, disease courses and risperidone dosages (P>0.05). Also, no significant differences were observed in rs6277, rs1079598 and rs4680 polymorphisms between the two groups.

Conclusion: These results suggest that *DRD2* rs6277, rs1079598 and *COMT* rs4680 gene polymorphisms show no significant correlation with risperidone-induced amenorrhea in Chinese female schizophrenia patients.

Keywords: Schizophrenia; Amenorrhea; *DRD2*; *COMT*

Introduction

Schizophrenia is a serious mental disorder that affects social function of patients. Clinical characteristics of schizophrenia are early onset age and prolonged disease course. With the extensive application of antipsychotics, there are significant improvements in the remission rate of psychotic symptoms such as impaired social function in schizophrenic patients. However, in recent years, disruption of endocrine system was observed in the patients especially for women with long-term use of antipsychotic drugs. It has been reported that antipsychotic drugs could lead to delayed and reduced menstruation or even life-long amenorrhea by blocking the dopamine receptors [1]. This adverse drug reaction has a broad impact on medication compliance and life quality in women with schizophrenia. Thus, this concern has become one of the main problems in the treatment of female psychiatric patients for many years. Therefore, more correlation studies should focus on the disrupted endocrine system and antipsychotic drugs.

Risperidone is a new atypical antipsychotic drug which has good effects on both positive and negative symptoms of schizophrenia. And fewer side effects such as extrapyramidal motor symptoms (EPS) and excessive sedation were observed after usage of risperidone. Therefore, the risperidone is one of the most widely used antipsychotic drugs in the treatment of schizophrenia [2]. Side effects of risperidone such as hyperprolactinemia which leads to amenorrhea, decreased libido, weight gain and infertility have also been reported [3-5]. However Not all risperidone-induced hyperprolactinemia would result in amenorrhea in female. The agnogenic adverse reaction of risperidone varies in patients, which indicates that genetic differences are a key factor. The mechanism of risperidone-induced amenorrhea needs to be further studied.

Dopamine dysfunction has been considered to be an important reason for the pathogenesis of schizophrenia, and the treatment of schizophrenia with risperidone mainly by antagonizing dopamine receptors [6,7]. Recent studies have shown that the gene polymorphisms in *dopamine D2 receptor gene* (*DRD2*) and *catechol-O-methyltransferase gene (COMT)* are associated with schizophrenia, antipsychotic treatment efficacy, and adverse reactions. For instance, polymorphism of *DRD2* rs6277 was found to be a susceptible factor of schizophrenia, and patients with *DRD2* rs6277 and rs1079598 variants have high risk for antipsychotic-induced weight gain [8,9]. Besides, *COMT* rs4680(Val>Met) polymorphism showed associated with schizophrenia, and the Met allele was also associated with the improvement of negative symptoms after risperidone treatment [10,11]. Furthermore, the *DRD2* rs6277 variants have been associated with clozapine-induced hyperprolactinemia [12]. It has been reported that the prolactin level in patients carrying *COMT* rs4280 Met allele is higher than that in patients without this allele after treated with risperidone [13]. However, the relationship between genetic factors and amenorrhea caused by risperidone-induced hyperprolactinemia has not been reported. In this study, in order to explore the genetic mechanism of risperidone-induced amenorrhea in female schizophrenia patients,

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we studied the schizophrenia susceptible *DRD2* rs6277, rs1079598 gene polymorphism and risperidone-induced hyperprolactinemia related *COMT* rs4680 gene polymorphism.

Materials and Methods

Clinical data

Clinical data of hospitalized patients with schizophrenia was collected at the Demobilized Soldiers Corning Hospital in Liaoning Province in February 2015.

Inclusion criteria were 1) meeting the diagnostic criteria for schizophrenia in ICD-10; 2) female patients; 3) age 18-40 years old; 4) treatment for at least 6 months with single medication of risperidone; 5) hyperprolactinemia (hyperprolactinemia defined as: prolactin>566 uIU/ml) occurred after the use of risperidone.

Exclusion criteria were 1) associated with metabolic diseases, endocrine diseases or connective tissue diseases; 2) taking medicine which may affect women menstrual; 3) history of ovarian resection; 4) suffering from other mental or neurological diseases; 5) eating disorders, alcohol abuse or dependence; 6) pregnant or lactating women. Women with schizophrenia whose menstrual interrupted after taking risperidone for at least 6 months were classified as amenorrhea group. Women with schizophrenia who have normal menstruation after taking risperidone for at least 6 months were classified as menstrual normal group. A total of 45 patients were included, 25 patients with amenorrhea and 20 patients with eumenorrhea.

The protocol and informed consent were approved by the Ethics Committee of the Peking University Institute of Mental Health in accordance with the guiding principles of the Guideline for Good Clinical Practice of the International Conference on Harmonization (ICH-GCP). Prior to the enrolment, written informed consent was obtained from the subjects or their legal guardians.

Clinical evaluation

Questionnaires about patient's general information and disease related situation including age, sex, date of birth, education level, ethnicity, marital status, age of onset, course of disease, past medical history and medication history, as well as the current medication list were conducted by all patients.

Laboratory evaluation

Blood collection: The venous blood was collected from the subjects at 6-7 AM after 8-12 hours fasting by nurses. The blood samples were kept in 5 mL coagulation tubes produced by a domestic mountain medical company.

Blood sample testing: (1) Prolactin: UniCel DxI800 Access automatic micro particle chemiluminescence instrument manufactured by Beckman Coulter Inc. was used for detection, and chemiluminescence immunoassay was used for determination. PRL detection range is 5.3~4240 μIU/mL, the normal range is 58-566 μIU/mL.

(2) *DRD2*, *COMT* genotype determination: The PCR primers were designed by Primers 3.0 software online according to the gene sequence of *DRD2* rs6277, rs1079598 and *COMT* rs4680 gene locus, upstream primer of rs4677: AGTCTTCAGAGGGGGAAAGG, downstream primer of rs4677: GGAATGGGACCTTTCACAGA, upstream primer of rs1079598: AGGCTAAGTCCTCCTTCTAC, downstream primer of rs1079598: TCAGGGAAGGCTTTCTAGAGG, upstream primer of rs4680: ACCAGGGAGGTGAAATACCC, downstream primer of rs4680: GATGACAAGGCCCCACTCT. PCR reacted in a 20 μL system, which include 1.0 μL of template DNA, 2.0 μL of 10×NH_4^+ buffer, 0.5 μL of dNTP, 0.2 μL of EXTaq enzyme and 0.8 μL of each pair of primers. The PCR conditions were as follows: pre-denaturation at 95°C for 3 min; denaturation at 95°C for 30 s, annealing at 58°C for 45 s, extension at 72°C for 1 min, a total of 35 cycles; extension at 72°C for 10 min. Finally, sequencing of the PCR products was carried out using the ABI 3730XL automatic sequencer.

Statistical methods

The independent samples t-test was used to compare the general demographic data, and the $\chi 2$ test was used to determine whether the genotype distribution accorded with Hardy-Weinberg equilibrium. The difference of genotype and allele distribution was analyzed by $\chi 2$ test and Fisher's exact test. All data were analysed using SPSS 19.0, $P<0.05$ for the difference was statistically significant.

Results and Discussion

The basic characteristics of the two groups of subjects

The mean age of the amenorrhea patients was (32.6 ± 6.0) years, the course of disease (10.7 ± 3.4) years, the risperidone dosage (5.4 ± 2.2) mg; the mean age of the normal menstrual patients was (35.6 ± 4.7) years, the course of disease (9.4 ± 3.9) years, risperidone dosage (4.9 ± 2.5) mg. There were no significant differences in age, course of disease and dose of risperidone between the two groups ($P>0.05$), showed in Table 1.

There was no significant difference between the two groups in the prolactin level ($P>0.05$), and the prolactin level is (1741.7 ± 1073.1) uIU/mL in menorrhea patients and (1485.2 ± 772.8) μIU/mL in normal menstrual patients.

Hardy-Weinberg equilibrium test

There was no significant difference in frequency distribution of *DRD2* rs6277, rs1079598 and *COMT* rs4680 gene polymorphism genotype ($P>0.05$), which accorded with Hardy-Weinberg equilibrium law.

Association analysis of *DRD2* rs6277, rs1079598 and *COMT* rs4680 polymorphisms with risperidone-induced hyperprolactinemia resulted in amenorrhea

In the amenorrhea group and normal menstruation group, results of *DRD2* rs6277, rs1079598 and *COMT* rs4680 gene polymorphism

Variable	Amenorrhoea	Normal menstruation	P
Age (years old)	32.6 ± 6.0	35.6 ± 4.7	>0.05
Course of disease (years)	10.7 ± 3.4	9.4 ± 3.9	>0.05
Risperidone dosage (mg)	5.4 ± 2.2	4.9 ± 2.5	>0.05
Prolactin level (uIU/mL)	1741.7	1485.2 ± 772.8	>0.05

Table 1: Demographics and clinical data for the studied subjects.

SNP	Group	Number	Allele frequency		P	Genotype frequency			P
			C	T		CC	CT	TT	
rs6277	Amenorrhoea	25	49	1	>0.05	24	1	0	>0.05
	Normal menstruation	20	36	4		17	12	1	

Table 2: Allele and genotype distribution of rs6277.

SNP	Group	Number	Allele frequency		P	Genotype frequency			P
			G	A		GG	GA	AA	
rs4680	Amenorrhoea	25	39	11	>0.05	15	9	1	>0.05
	Normal menstruation	20	27	13		10	7	3	

Table 3: Allele and genotype distribution of rs4680.

SNP	Group	Number	Allele frequency		P	Genotype frequency			P
			T	C		CC	CT	TT	
rs1079598	Amenorrhoea	25	29	21	>0.05	9	11	5	>0.05
	Normal menstruation	20	24	16		8	8	4	

Table 4: Allele and genotype distribution of rs1079598.

were showed in Tables 2-4. The results showed that there were no significant differences in genotype frequency and allele frequency between the two groups (P>0.05).

Discussion

Dopamine is the one of the most important regulator of Prolactin (PRL), and in vivo and in vitro studies showed that dopamine is a potent inhibitor for prolactin. In the hypothalamic arcuate nucleus, dopamine is released from tuberoinfundibular dopamine (TIDA) neurons to the anterior pituitary, which inhibits the secretion of prolactin continuously. Risperidone is a strong antagonist of DRD2, which increases the level of prolactin by antagonizing DRD2 on the anterior pituitary prolactin cells to influence the prolactin level significantly. The incidence of hyperprolactinemia caused by risperidone is reaching to as high as 72-100%. Hyperprolactinemia could lead to reduction of estrogen by inhibiting the secretion of gonadotropin (GnRH), resulting in amenorrhea in female [14].

Risperidone is widely used in the treatment of schizophrenia, and the main adverse reaction of this drug is hyperprolactinemia, but not all risperidone-induced hyperprolactinemia in female patients will cause amenorrhea. In this study, the patients with hyperprolactinemia caused by taking risperidone were recruited, and we found that a part of the patients had amenorrhea, while others were normal, and the levels of prolactin were not significantly different between the two groups. This result indicates that this individual difference may be due to genetic differences among patients. In previous studies, individual differences of adverse effects such as efficacy and weight gain in risperidone therapy have been reported, but the genetic mechanism of amenorrhea caused by risperidone-induced hyperprolactinemia in female schizophrenia patients has not been reported [9,15].

Catechol-O-methyltransferase (COMT) is a widely existed enzyme in the human body, which can catalyze the degradation of catecholamine's including dopamine, norepinephrine, and epinephrine [16]. rs4680 is a well-studied SNP in the COMT gene. The 158th codon of COMT is mutated from valine (Val) to methionine (Met). This reduced the activity of COMT enzyme, and leading to slow down the degradation of dopamine, which may be responsible for central nervous disease [17]. Gao et al. studied 83 cases of Chinese patients with schizophrenia, and found that the prolactin levels of schizophrenia patients with Met allele were significantly higher than Val/Val patients after 8 weeks immunotherapy with risperidone [13]. Another study has shown that the cognitive function of schizophrenia patients with homozygous Met allele was significantly better than that with other alleles after receiving risperidone therapy [18].

Risperidone functions as a blocking agent for D2 receptors, and the side effects such as risperidone-induced hyperprolactinemia and risperidone efficacy has been confirmed to have association with the polymorphism of the gene. It has been reported that female schizophrenia patients with TaqIA gene polymorphism had a higher level of prolactin secretion after taking risperidone [19]. The rs6277 locus changes the stability and translation level of *DRD2* mRNA and alters the expression of DRD2 in dopamine channel [20]. Rs6277 had association with plasma prolactin increasing and schizophrenia in the study of different populations, which is also one of susceptible locus in Chinese Han population schizophrenia patients [21-23]. A DRD2 haplotype (-141delC and TaqIA) tended to correlate with better clinical performance of risperidone in Japanese schizophrenia patients [24]. Moreover, patients with *DRD2*-141C Ins/Del polymorphism had a better treatment with risperidone. Prolactin secretion of patients with A-241G polymorphism was significantly higher than that of non-carriers [19].

In this study, a total of 45 Chinese female schizophrenic patients were recruited, which were divided into amenorrhea group and normal menstrual group. It has shown that there was no significant association between *DRD2* rs6277, rs1079598 and *COMT* rs4680 gene polymorphism and amenorrhea caused by risperidone-induced hyperprolactinemia. This may suggest that amenorrhea caused by risperidone-induced hyperprolactinemia in female schizophrenia patients is due to multiple gene interaction or cumulative effect [25]. In addition, this adverse event may also be associated with more complex metabolic pathways.

Conclusion

An important limitation of this study is the number of patients; therefore, the result can't explain the whole situation of this region, China and Asian people. However, the present findings generate important hypotheses in a sample of Chinese schizophrenia patients that may lay the foundation for future study in other populations. In the follow-up research, we will further increase the number of cases. Furthermore, we will also expand the relevant gene list by searching for possible regulatory pathways through metabolic pathways and

regulatory network analysis between risperidone target gene set and the amenorrhea-related gene set, and re-sequencing the target genes using high-throughput sequencing. To carry out a stratified study on schizophrenia patients with high prolactin caused by risperidone treatment, study the relevance between gene polymorphism and normal menstruation and amenorrhea phenotype and hormone levels and other indicators by association analysis. We hope to improve the safety and tolerance of risperidone in the treatment of schizophrenia, and to achieve the precise medical treatment of schizophrenia by studying the genetic polymorphism of patients with amenorrhea induced by risperidone.

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Disclosure

The authors report no conflicts of interest in this work.

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