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Association of catechol-O-methyltransferase gene polymorphisms with schizophrenia and negative symptoms in a Chinese population

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Abstract

The gene encoding Catechol O-methyltransferase (COMT), a dopamine catabolic enzyme, has been associated inconsistently with schizophrenia in spite of consistent evidence for dopaminergic dysfunction in the prefrontal cortex (PFC) of schizophrenia. Since one contribution to this inconsistency might be genetic heterogeneity, this study investigated whether the COMT gene was associated with the development and symptoms of schizophrenia in relatively genetically homogeneous Chinese schizophrenic patients. We analyzed two polymorphisms (rs740603 and rs4818) of the COMT gene in a case-control study of 604 Han Chinese (284 patients and 320 controls). The patients' psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS). We found no significant differences in the rs740603 and rs4818 genotype and allele distributions between the patient and control groups. Quantitative trait analysis by the UNPHASED program showed that the rs740603 and rs740603(G)-rs4818(G) haplotypes were associated with negative symptoms in the schizophrenic patients, particularly among female patients. Thus, the COMT gene polymorphisms may not contribute to the susceptibility to schizophrenia, but may contribute to the negative symptoms of schizophrenia among Han Chinese.

Keywords

Case-control; Association; Schizophrenia; COMT; Negative symptom

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Introduction

The COMT gene has been studied widely among schizophrenics, using both case-control and family designs [Sagud *et al.*, 2010]. Moreover, the COMT gene lies on chromosome 22q11.1–q11.2, a region which is significantly associated with schizophrenia in several linkage studies [Pulver *et al.*, 1994; Owen, 2005]. The COMT Val158/108Met (rs4680) polymorphism is the gene variant studied most widely in psychiatry, because of its functional relevance [Okochi *et al.*, 2009]. However, current data other variants of the COMT gene could also be relevant [Shield *et al.*, 2004; Molero *et al.*, 2007]. Several other SNPs that were tested for an association with schizophrenia have shown conflicting results [Chen *et al.*, 2004; Handoko *et al.*, 2005]. A recent study analyzing 14 polymorphisms in a case-control study of 876 Han Chinese found that none of these polymorphisms contributed to the risk of developing schizophrenia or its subtypes, as assessed using allele/genotype and haplotype analyses [Chen *et al.*, 2011].

The role of the COMT Val/158/Met polymorphism in the modulation of cognitive performance and presumed prefrontal cortical function was first studied over 10 years ago [Egan *et al.*, 2001]. Since that study several lines of evidence point to dysfunction of COMT in the prefrontal cortex as involved in psychotic symptoms and in the response to antipsychotic treatment [Molero *et al.*, 2007; Wang *et al.*, 2010]. Since clinical phenotypes rather than the whole spectrum of schizophrenia may be more closely related to certain susceptibility genes, a quantitative trait test may lead to better understanding of the genotype-phenotype relationships in schizophrenic patients [Tao *et al.*, 2006; Wang *et al.*, 2010]. In the present study, therefore, we performed a case-control association study to assess whether a COMT haplotype and a specific genotype of the COMT gene influenced the clinical symptoms of schizophrenia as assessed using a quantitative trait test in Chinese patients with schizophrenia. The two SNPs selected for our investigation were rs 4818, which is located in exon 4 at 64 bp 3' to Val158Met, and rs740603 within intron 1, which defines a synonymous A-G change that is about 6.1 kb 5' to Val158Met. These two SNPs were chosen on the basis of the SZgene database (<http://www.schizophreniaforum.org/res/sczgene/geneoverview.asp?geneid=420>), the NCBI SNP database (www.ncbi.nlm.nih.gov/projects/SNP/) and a review of the literature, in order to have a minimum allele frequency (MAF) of 0.15. We also chose the synonymous COMT gene polymorphism rs4818 C/G, because recent evidence suggests that it accounts for greater variation in COMT activity than the functional Val158Met polymorphism [Nackley *et al.*, 2006]. The COMT rs740603 was identified as another potentially functional variant, based on its positive selection in unrelated European-American and African-American populations [Ittiwut *et al.*, 2011].

Methods

Subjects

Two hundred and eight-four schizophrenia patients (male/female=203/81) were recruited from Beijing Hui-Long-Guan hospital. Two independent experienced psychiatrists diagnosed each patient on the basis of the Structured Clinical Interview for DSM-IV (SCID). All schizophrenic patients were chronic, with at least 5 years of illness, were Han

Chinese, and were between 25 and 75 years old (mean 48.3 ± 10.1 years). All patients had been receiving stable doses of oral antipsychotic drugs for at least 3 months before entry into the study. Antipsychotic drug treatment of the patients consisted mainly of monotherapy with clozapine (n=119), risperidone (n=83), perphenazine (n=20), sulpiride (n=19), chlorpromazine (n=17), haloperidol (n=12), with 14 patients on other agents. Each patient's mean daily dose of antipsychotics, including both the first- and the second-generation antipsychotics was converted to daily mean chlorpromazine milligram equivalents using standard guidelines [Woods, 2003; American Psychiatric Association., 2004; American Psychiatric Association., 2006]. The mean antipsychotic dose (in chlorpromazine equivalents) was 484 ± 506 mg/day. The patients had been on their respective medications for 44.2 ± 50.6 months at the time of the investigation.

Three hundred and twenty healthy Han Chinese controls (male/female=140/180) without a current or past psychiatric history were selected from the local Beijing area. They were from 25–73 years old (mean 47.6 ± 12.4 years). A psychiatrist conducted psychiatric interviews to rule out psychiatric disorders among controls.

All subjects gave their written informed consent to participate in the study. The protocol was approved by the Institutional Review Board of the Beijing Hui-Long-Guan hospital.

Genotyping

We extracted genomic DNA for the polymerase chain reaction (PCR) from the whole blood sample using standard protocols.

According to the International SNP Consortium (<http://www.ncbi.nlm.nih.gov=SNP>) and Haploview software (<http://www.hapmap.org>), we selected 2 tag SNPs (rs740603 and rs4818) in the COMT gene. These two SNPs were determined using a restriction fragment length polymorphism (RFLP) assay after polymerase chain reaction amplification and digested with Mlu I or Bcl I, according to previously reported methods [Beuten *et al.*, 2006; Roussos *et al.*, 2008]. Genotyping was duplicated and carried out blind to the clinical status.

Clinical assessment

Four psychiatrists, who had previously completed a training session in rating the positive and negative syndrome scale (PANSS), assessed the patient's psychopathology using this scale. After training, they maintained an inter-rater correlation coefficient greater than 0.8 for the PANSS total score.

Statistical analysis

Deviation from Hardy–Weinberg equilibrium was calculated by Chi-square tests. The differences of genotypic and allelic frequency between cases and controls were analyzed using SPSS 12.0. Bonferroni corrections were applied to each test to adjust for multiple testing. Other statistical analyses were done using UNPHASED 3.010, including linkage disequilibrium, haplotype analysis and quantitative trait analysis. Quantitative trait analysis indicates the association of gene polymorphisms and severity of symptoms. The power (power defined as the chance that true differences will actually be detected) of the sample

was calculated with Quanto Software [Gauderman, 2002], with known risk allele frequencies and a schizophrenia population prevalence of 0.01, and we examined log additive, recessive and dominant models.

Results

Table 1 shows that the patients and control subjects significantly differed in sex ($p < 0.001$) and smoking ($p < 0.001$). SNP rs740603 was genotyped in 284 patients and 320 controls, and SNP rs4818 in 277 patients and 290 controls. The χ^2 goodness-of-fit test showed that the genotypic distributions of the two SNPs rs740603 and rs4818 in both patient and control groups were consistent with Hardy–Weinberg equilibrium (all $P > 0.05$). Analysis showed modest linkage disequilibrium between the two markers in both patients ($D' = 0.77$, $r^2 = 0.47$) and controls ($D' = 0.72$, $r^2 = 0.39$).

Table 2 shows no significant differences in the rs740603 and rs4818 genotype and allele distributions between the patient and control groups (both $p > 0.05$; Table 2). In males we found a significant association between the rs4818 genotype and schizophrenia at the genotypic ($\chi^2 = 8.035$, $df = 2$, $p = 0.018$), but not at the allelic level ($\chi^2 = 0.87$, $df = 1$, $p = 0.35$). Furthermore, we found no significant overall difference in the estimated haplotype frequencies between cases and controls ($\chi^2 = 1.891$, $df = 3$, $p = 0.59$) or in any individual haplotype between cases and controls (all $p > 0.05$).

Table 3 shows a significant association between negative symptoms and rs740603 ($n = 284$, $p = 0.025$, adjusted $p = 0.032$; effect size = 0.22), but not for rs4818 ($n = 277$, $p = 0.054$, adjusted $p = 0.066$; effect = 0.18). However, these results did not pass Bonferroni test. The rs740603 association with negative symptoms was stronger in females ($\chi^2 = 8.782$, $p = 0.003$, adjusted $p = 0.007$; Bonferroni correction $p < 0.05$; effect size = 0.41), but not significant in males ($p > 0.05$).

As shown in Table 4, further significant associations were identified between the rs740603-rs4818 haplotypes and negative symptoms ($p = 0.045$). This association remained statistically significant after 10,000 permutations (adjusted $p = 0.039$). Furthermore, negative symptoms were less severe in patients carrying the rs740603(G)-rs4818(G) haplotype than those patients with three other haplotypes ($p = 0.01$). We showed a significant association between the rs740603-rs4818 haplotypes and negative symptoms in females ($p = 0.021$; adjusted $p = 0.037$). Furthermore, negative symptoms were less severe in female patients carrying the rs740603(G)-rs4818(G) haplotype ($p = 0.009$). We showed no associations in male patients (data not shown). In addition, there were no significant differences in terms of dosage, type (typical vs atypical antipsychotics) or duration of antipsychotic treatment in patients carrying the rs740603(G)-rs4818(G) haplotype than in those patients with three other haplotypes (all $p > 0.05$) in the combined patient groups or when the male and female patients were examined separately (all $p > 0.05$). Similarly, there were no significant differences in number of years of illness among the patients carrying the rs740603(G)-rs4818(G) haplotype than those patients with three other haplotypes ($p > 0.05$) in the combined patient groups or when the male and female patients were examined separately (both $p > 0.05$).

This total sample had 0.77–0.84 power to detect dominant, recessive and log additive polymorphic inheritance in schizophrenia with an odds ratio (OR) of 2 or greater ($\alpha=0.05$, two tailed test).

Discussion

Two major findings evolved from the present study. (1) The COMT gene rs740603 and rs4818 polymorphisms did not seem to strongly contribute to the susceptibility to schizophrenia. (2) The COMT gene may be associated with negative symptoms in Han Chinese schizophrenic patients, especially females. Our finding of no association between the COMT rs740603 and rs4818 polymorphisms and schizophrenia is consistent with most previous studies. For example, recent meta-analyses found no evidence for a relationship between the COMT gene and schizophrenia [Fan *et al.*, 2005; Munafo *et al.*, 2005; Barnett *et al.*, 2007]. Moreover, the COMT gene has generally not been associated with schizophrenia in several previous studies in the Han Chinese population including a recent study of 14 SNPs including two SNPs in our present study [Tsai *et al.*, 2004; Yu *et al.*, 2007; Chen *et al.*, 2011].

A number of modifier genes may affect clinical features of schizophrenia without altering susceptibility to the illness [Fanous and Kendler, 2005], and our quantitative trait analysis showed that rs740603 was associated with negative symptoms, and the patients carrying allele A at rs740603 showed more negative symptoms. The rs4818 polymorphism alone showed only a trend ($p=0.054$) association with negative symptoms, but the rs740603-rs4818 haplotypes were associated with negative symptoms, particularly in females. Patients carrying rs740603(G)-rs4818(G) scored lower than those with other haplotypes, which is consistent with the quantitative trait analysis for a single SNP. Our results suggest that the COMT gene may contribute to the severity of negative symptoms in Chinese Han schizophrenia.

Several authors have also found significant differences in negative symptoms in relation to the COMT genotype. For example, Pelayo-Teran *et al.* [2008] found that patients with a Val158 homozygote genotype had a higher severity of negative symptoms at the onset the illness. Furthermore, they reported that the Val158 homozygote patients exhibited higher negative symptom severity scores after 6 weeks of treatment [Pelayo-Teran *et al.*, 2011]. Also, Stefanis *et al.* [2004] found an association between the Val158 allele and negative schizotypy in a large non-psychotic sample. Another study also reported a higher severity of psychotic symptoms and a poorer improvement in negative symptoms to neuroleptic treatment in chronic schizophrenic patients with the Val/Val polymorphism [Molero *et al.*, 2007]. Interestingly, a recent study showed an association of the rs4633-rs4680 haplotype of the COMT gene with negative symptoms in Han Chinese schizophrenics [Wang *et al.*, 2010]. Taken together, COMT genetic variations seem to be involved in the negative symptoms of schizophrenia.

However, several authors have not found COMT genotypes related to any significant symptom differences [Inada *et al.*, 2003; Koen *et al.*, 2004; Tsai *et al.*, 2004; Strous *et al.*, 2006]. A recent study of 14 SNPs including the two SNPs in our present study found no

association with the PANSS scores among schizophrenia patients in a Han population from Taiwan [Chen *et al.*, 2011]. Such a discrepancy may be attributable to several reasons. First, population stratification could lead to a resetting of population gene frequencies. The sample from Taiwan may not be representative of the entire Han Chinese population, because of a possible population stratification bias [Yeh *et al.*, 2010; Chen *et al.*, 2011]. Second, the COMT genotypes can be regarded as trait-dependent features, while the assessment of illness intensity by PANSS is state-dependent. Therefore, a comparison of genotypes with the intensity of illness at any given time could be misleading. Our study's chronic schizophrenic inpatients had comparatively higher levels of negative symptoms compared to the outpatients, who were studied in Taiwan [Chen *et al.*, 2011]. Hence, the differences in severity of clinical symptoms might be responsible for the discrepancy. Third, inconsistency can arise from differences in clinical subtypes of schizophrenia, in stages of disease progression (acute vs. chronic or active phase vs. remission), in illness courses, and in exposure to different types, dosages and durations of antipsychotic medications. The possible effects of antipsychotic treatment and illness duration on negative symptoms in our patients clearly distinguish the two studies. All patients had been receiving stable doses of oral antipsychotics for at least three months before entry into the study, and this medication could contribute to higher levels of negative symptoms [Kapur, 2004]. However, we found no association of the dose, type (atypical vs typical antipsychotics) and duration of treatment with the haplotype rs740603(G)-rs4818(G) that was associated with negative symptoms. All our patients also were chronic, with at least 5 years of illness, but we found no significant difference in the number of years of illness among patients with the haplotype associated with negative symptoms.

This study has several limitations. First, we only genotyped two polymorphisms, while the total COMT gene variants in this 35 kb gene includes at least 50 polymorphisms. Second, although we found the rs4818 genotype association with schizophrenia in males ($p=0.018$), but not females, suggesting a sexual dimorphism between COMT variants and the genetic predisposition to schizophrenia [Shifman *et al.*, 2002; Sazci *et al.*, 2004; Pelayo-Teran *et al.*, 2008; Hoenicka *et al.*, 2010], the sample size is too weak to clarify this question. The positive result was based on 81 female with small numbers per genotype group. It is highly possible that we do see only false-positive results. Third, our sample size was comparatively small, and this could lead to false positive or negative results due to the lack of statistical power and selection of samples. It would be useful to extend the sample to about 800 subjects for a power of 0.80, with the relative risk of 2. This could help to clarify a possible association of the COMT rs740603/rs4818 polymorphisms and schizophrenia. Fourth, there were many samples that were not included in the genotype analysis of rs4818, and this might lead to bias in the statistical analysis due to the imbalance in the number of two SNPs in our current study. Fifth, population stratification or the unmatched sex of our samples could be confounders. However, the Chinese in Beijing are ethnically relatively homogenous, and our examination of ethnic markers showed no differences between the schizophrenics and controls. A replication study with genomic controls or a family-based population study would help to address this limitation. Sixth, a misclassification of genotypes is possible in spite of our quality controls, but such misclassification would typically bias the results towards no effect. Seventh, other polymorphisms could be

functionally associated with schizophrenia or with the psychopathological symptoms of schizophrenia. Examining other functional variants of COMT is warranted.

In summary, our case-control association study did not provide support for COMT variants being associated with schizophrenia in a Chinese population. However, the COMT gene may contribute to the negative symptoms of schizophrenia in females. This finding remains preliminary due to limited sample size and our low statistical power and requires replication in larger samples in different ethnicities.

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Table 1

Demographics of patients and normal control subjects

	Schizophrenia (n=284)	Control subjects (n=320)
Sex, M/F	203/81	140/180
Age (years)	48.3±10.1	47.6±12.4
Education (years)	10.1±8.1	10.4±8.4
Body Mass Index (kg/m ²)	24.1±4.5	24.6±4.8
Smokers	57.0%	45.8%
Duration of illness (years)	26.2±9.7	NA
Age of onset (years)	23.9±6.5	NA
Hospitalization numbers	3.6±2.7	NA
Duration of antipsychotic Treatment (years)	5.1±4.6	NA
PANSS		NA
Positive subscale	10.7±5.2	
Negative subscale	17.5±7.2	
General subscale	21.9±6.6	
Total	50.2±15.7	

Note: Patients and control subjects significantly differed in sex ($p<0.001$) and smoking ($p<0.001$).

Table 2
Comparison of allele and genotype frequencies of two COMT polymorphisms between schizophrenic cases and controls

Group(n)	Genotypic association(%)				Allelic association(%)					
	G/G	A/G	A/A	χ^2	P	G	A	χ^2	P	
rs740603										
All	Case(284)	56(19.7)	133(46.8)	95(33.5)	0.236	0.888	245(43.1)	323(56.9)	0.011	0.915
	Control(320)	67(20.9)	144(45.0)	109(34.1)			278(43.4)	362(56.6)		
Male	Case(203)	35(17.2)	101(49.8)	67(33.0)	2.556	0.279	171(42.1)	235(57.9)	0.037	0.847
	Control(140)	31(22.1)	58(41.4)	51(36.4)			120(42.9)	160(57.1)		
Female	Case(81)	21(25.9)	32(39.5)	28(34.6)	1.837	0.399	74(45.7)	88(54.3)	0.145	0.703
	Control(180)	36(20.0)	86(47.8)	58(32.2)			158(43.9)	202(56.1)		
rs4818										
All	Case(277)	37(13.4)	132(47.7)	108(39.0)	3.692	0.158	206(37.2)	348(62.8)	0.586	0.444
	Control(290)	44(15.2)	115(39.7)	131(45.2)			203(35.0)	377(65.0)		
Male	Case(197)	22(11.2)	92(46.7)	83(42.1)	8.035	0.018	136(34.5)	258(65.5)	0.867	0.352
	Control(129)	20(15.5)	40(31.0)	69(53.5)			80(31.0)	178(69.0)		
Female	Case(80)	15(18.8)	40(50.0)	25(31.2)	1.399	0.497	70(43.8)	90(56.2)	1.372	0.241
	Control(161)	24(14.9)	75(46.6)	62(38.5)			123(38.2)	199(61.8)		

Table 3

The quantitative trait test for association of 2 SNPs with psychiatric symptoms

	Mean	Variance	χ^2	P
rs740603 (n=284)				
Positive subscore	G=10.48, A=10.73	47.98	0.191	0.662
Negative subscore	G=16.29, A=18.43	125.9	5.037	0.025
General psychopathology	G=21.28, A=22.38	141.2	1.181	0.277
Total score	G=47.69, A=51.54	762.9	2.692	0.101
rs4818 (n=277)				
Positive subscore	G=10.78, C=10.50	47.50	0.220	0.639
Negative subscore	G=16.32, C=18.22	125.7	3.689	0.054
General psychopathology	G=21.70, C=22.06	142.9	0.116	0.733
Total score	G=48.37, C=50.77	766.3	0.967	0.325

Table 4

The quantitative trait test for association of haplotype with negative symptoms

Haplotype	Mean	Variance	χ^2	P
rs740603-rs4818	17.41	123.8	8.039	0.045
GG	15.80	123.8	6.594	0.010
GC	17.85	123.8	0.000	0.987
AG	20.95	123.8	2.025	0.152
AC	18.41	123.8	3.340	0.068