GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 16, Number 9, 2012

Pp. 1138–1141
DOI: 10.1089/gtmb.2012.0061

No Association of Catechol-O-Methyltransferase Polymorphisms with Schizophrenia in the Han Chinese Population

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Aims: Genetics play a major role in the etiology of schizophrenia (SZ). Catechol-O-methyltransferase (COMT) is one of the promising candidate genes for SZ. A nonsynonymous single-nucleotide polymorphism (SNP), rs4680, causing a Valine (Val) to Methionine (Met) substitution, has been widely studied in relation to psychiatric phenotypes, including SZ, but with conflicting results. We conducted a two-stage study to examine the association of COMT polymorphisms with SZ in the Han Chinese population. *Results:* Association analysis of nine SNPs in 768 patients and 1348 controls failed to detect any positive markers or haplotypes. Then, we tested rs4680 in a validation sample of 963 patients and 992 controls, and no significant association was observed, but the cases significantly deviated from Hardy–Weinberg equilibrium ($p = 5.7e\text{-}4$). There was no association of rs4680 with SZ in the combined sample ($n = 4071$, $p = 0.110$, odds ratio = 1.08). *Conclusions:* Our results do not support the association of COMT with SZ in the Han Chinese population.

Introduction

Schizophrenia (SZ) is a complex genetic disease with a heritability of ~80%–85% (Cardno and Gottesman, 2000). Dopamine (DA) signaling is strongly implicated in the pathophysiology of SZ, as evidenced by the fact that antipsychotic drugs target mainly on the DA receptors. Catechol-O-methyltransferase (COMT) catalyzes the degradation of the catecholamine neurotransmitters, including DA, norepinephrine, and epinephrine, thereby influencing the DA concentration in the dorsolateral prefrontal cortex (PFC). COMT maps at chromosome 22q11.2, a region that has been implicated in linkage areas of SZ (Lewis *et al.*, 2003) and whose deletion causes the velo-cardio-facial syndrome. Thus, COMT is regarded as one of most promising candidate genes for SZ. It contains a missense polymorphism Val158Met (rs4680), resulting in a G to A substitution that changes Valine (Val) into Methionine (Met) at codon 108/158 in exon 4. rs4680 significantly affects the protein abundance and enzyme activity, with a higher COMT activity in individuals with Val homozygotes compared with those with Met homozygotes (Chen *et al.*, 2004). This single-nucleotide polymorphism (SNP) has been widely studied for its connection with SZ susceptibility, but with conflicting results. Meta-analyses do not support the association with SZ (Fan *et al.*, 2005; Munafo *et al.*, 2005; Okochi *et al.*, 2009). Several previous studies in the Han Chinese population failed to find a positive association with SZ (Fan *et al.*, 2005; Yu *et al.*, 2007; Kang *et al.*, 2010; Chen *et al.*, 2011); but these studies chiefly focused on only one SNP and the sample sizes involved were relatively small.

In this work, we aimed to evaluate the association of COMT with SZ in a two-stage study. In the discovery stage, we derived relevant data from our genome-wide association study (GWAS) data (Yue *et al.*, 2011), involving nine SNPs in 768 SZ cases and 1348 healthy controls. In the validation stage, we tested the genetic distribution of rs4680 in 963 cases and 996 controls.

Materials and Methods

Subjects

All participants were unrelated Han Chinese recruited from the North of China. The initial GWAS sample consisted of 768 unrelated subjects with SZ (360 males and 408 females, aged 33.5 ± 8.7 years) and 1348 control subjects (658 males and 690 females, aged 31.1 ± 13.2 years). For validation, an independent sample consisting of 1030 cases (503 males and 527 females, aged 33.44 ± 11.60 years) and 1032 controls (483 males and 549 females, aged 41.00 ± 14.00 years) was recruited from northern China. The consensus diagnoses were made by at least two experienced psychiatrists according to the

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Diagnosis and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria for SZ. None of the patients had severe medical complications. Healthy controls were recruited from communities with a simple nonstructured interview performed by psychiatrists, who excluded individuals with history of mental health and neurological diseases. The control subjects live in the same area with patients, and were group matched to patients by gender, age, and ethnicity.

The study was approved by the Medical Research Ethics Committee of the Institute of Mental Health, Peking University. All participants enrolled in the study signed written informed consent.

Genotyping

Peripheral blood samples were collected from all subjects. Genomic DNA was extracted using the Qiagen QIAamp DNA Mini Kit. The genotyping of the GWAS was performed on Illumina HumanHap610-Quad BeadChips, as previously described (Yue *et al.*, 2011). In the validation stage, the genotypes of rs4680 were determined using the Sequenom MassARRAY system (Sequenom iPLEX). Nine hundred sixty-three of 1030 cases and 992 of 1032 controls were successfully genotyped, the calling rate being 94.8%. The primer pair for the polymorphism comprised the forward primer ACGTTG GATGACCCAGCGGATGGTGGATTT, and the reverse primer ACGTTGGATGTTTTCCAGGTCTGACAACGG.

Statistical analysis

Allelic and genotypic association tests were analyzed using PLINK 1.07 (Purcell *et al.*, 2007). Haplotype analyses were performed using UNPHASED 3.1 (Dudbridge, 2008). The overdominance test compared heterozygosity versus homozygosity state at the rs4680 polymorphism using Pearson's χ^2 test in R.

Results

Genotypic distributions of the 9 SNPs in the discovery sample did not deviate from Hardy–Weinberg equilibrium (HWE; $p > 0.001$). rs4680 significantly deviated from HWE in the patient group of the validation sample ($p = 5.7e\text{-}4$). Allele and genotype frequencies of the 9 SNPs did not significantly differ between patients and controls in the 768 SZ cases and 1348 controls (Table 1), nor did the haplotypes (data not shown). There was no association of rs4680 with SZ in the validation sample, nor in the combined sample ($n = 4071$, $p = 0.11$, odds ratio [OR] = 1.08; Table 1). Overdominance test (GG + AA vs. GA) in the combined sample showed nominal significance ($\chi^2 = 3.86$, $p = 0.049$), with GA being overrepresented in the cases.

Discussion

DA signaling is strongly implicated in the etiology of SZ. Because of its essential role in DA metabolism, COMT has been postulated to be involved in the development of SZ. The functional polymorphism, rs4680, leads to remarkable alteration of the enzyme efficiency. This functional relevance has stirred a big research interest for this marker in relation to cognitive function and psychiatric conditions. Although some studies supported an association of Val or Met allele with SZ, more studies failed to find any association (Fan *et al.*, 2005; Munafo *et al.*, 2005; Okochi *et al.*, 2009). Cumulative evidence suggests that there is an inverted-U-shape relationship between DA activity in PFC and working memory performance

Table 1. Allelic and Genotypic Distributions of Three Single-Nucleotide Polymorphisms in Patients and Controls

St	*SNP*	*1/2*	*gr*	n	*1 (MAF)*	p (d = *1)*	*1/1*	*1/2*	*2/2*	p (d = *2)*	*HWE*	*OR (95% CI)*
1	rs4646312	C/T	SZ	768	548 (35.7)	0.585	95 (12.4)	358 (46.6)	315 (41)	0.465	0.695	1.04 (0.91–1.18)
			ctl	1346	938 (34.8)		174 (12.9)	590 (43.8)	582 (43.2)		0.208	
1	rs165656	C/G	SZ	766	412 (26.9)	0.218	54 (7)	304 (39.7)	408 (53.3)	0.455	0.854	1.09 (0.95–1.26)
			ctl	1345	677 (25.2)		81 (6)	515 (38.3)	749 (55.7)		0.612	
1	rs165722	T/C	SZ	766	412 (26.9)	0.233	54 (7)	304 (39.7)	408 (53.3)	0.472	0.854	1.09 (0.95–1.26)
			ctl	1348	680 (25.2)		81 (6)	518 (38.4)	749 (55.6)		0.517	
1	rs2239393	G/A	SZ	768	552 (35.9)	0.546	96 (12.5)	360 (46.9)	312 (40.6)	0.419	0.639	1.04 (0.91–1.19)
			ctl	1348	944 (35.0)		176 (13.1)	592 (43.9)	580 (43)		0.209	
1	rs4680	A/G	SZ	768	428 (27.9)	0.091	58 (7.6)	312 (40.6)	398 (51.8)	0.196	0.858	1.13 (0.98–1.3)
			ctl	1348	687 (25.5)		79 (5.9)	529 (39.2)	740 (54.9)		0.251	
1	rs4646316	T/C	SZ	768	530 (34.5)	0.709	90 (11.7)	350 (45.6)	328 (42.7)	0.461	0.873	1.03 (0.90–1.17)
			ctl	1348	915 (33.9)		169 (12.5)	577 (42.8)	602 (44.7)		0.101	
1	rs165774	A/G	SZ	768	228 (14.8)	0.077	18 (2.3)	192 (25)	558 (72.7)	0.214	0.775	1.18 (0.98–1.41)
			ctl	1348	348 (12.9)		24 (1.8)	300 (22.3)	1024 (76)		0.716	
1	rs174699	C/T	SZ	768	606 (39.5)	0.999	120 (15.6)	366 (47.7)	282 (36.7)	0.983	0.940	1 (0.88–1.14)
			ctl	1346	1062 (39.5)		213 (15.8)	636 (47.3)	497 (36.9)		0.690	
1	rs165599	G/A	SZ	768	756 (49.2)	0.943	189 (24.6)	378 (49.2)	201 (26.2)	0.975	0.665	1 (0.88–1.13)
			ctl	1348	1330 (49.3)		330 (24.5)	670 (49.7)	348 (25.8)		0.828	
2	rs4680	A/G	SZ	963	540 (28.0)	0.745	54 (5.6)	432 (44.9)	477 (49.5)	0.117	5.7e-4	0.98 (0.85–1.12)
			ctl	992	547 (27.6)		71 (7.2)	405 (40.8)	516 (52)		0.525	
1&2	rs4680	A/G	SZ	1731	968 (28.0)	0.110	112 (6.5)	744 (43)	875 (50.5)	0.127	0.006	1.08 (0.98–1.20)
			Ctl	2340	1234 (26.4)		150 (6.4)	934 (39.9)	1256 (53.7)		0.184	

st, stage; ctl, control; MAF, minor allele frequency; SZ, schizophrenia; SNP, single-nucleotide polymorphism; OR, odds ratio; HWE, Hardy–Weinberg equilibrium; CI, confidence interval.

(Goldman-Rakic *et al.*, 2000), and that Val homozygotes may favor cognitive tasks demanding flexibility at the expense of lower stability, while Met homozygotes may perform better on tasks demanding more stability than flexibility (Durstewitz and Seamans, 2008). Therefore, the alleles of rs4680 might play a modulatory role rather than simply being "good" or "bad" in the onset of SZ (Tunbridge *et al.*, 2006), and the precise effect of COMT activity on PFC function will be dependent on precisely where on the inverted-U curve the individual in question lies in any given environmental or genetic context.

Recently, Costas *et al.* (2011) proposed an overdominant model through their meta-analysis of Val158Met and SZ. In this model, they suggest that both too high and too low levels of DA signaling may be risk factors (i.e., heterozygotes perform better than homozygotes), with heterozygotes at rs6280 conferring a weak protective effect (OR = 0.947). This result was in agreement with a Spanish study (Hoenicka *et al.*, 2010). This model is intriguing, considering the inverted-U-shaped relationship of DA signaling and brain function, but has yet to be validated.

In this work, we extended rs6280 to nine common SNPs, spanning from intron 1 to the 3'UTR of COMT. Our analysis showed that there was no association of COMT polymorphisms or haplotypes with SZ in the Han Chinese population. In contrast to findings of Costas *et al.* (2011), we detected a nominally significant overrepresentation of rs6280 heterozygotes in the combined patient group compared with controls ($p = 0.049$). Therefore, our data do not support the overdominant model of COMT rs6280 concerning SZ.

Of note is a strong deviation from HWE in the SZ cases in the validation sample ($p = 5.7e\text{-}4$), and a nominal deviation in the combined sample ($p = 0.006$). Although testing of HWE is commonly used for quality control in large-scale genotyping, there is little consensus regarding how to deal with the departure from HWE in the cases. Underlying reasons include genetic drift, population subdivision, genotyping error, and natural selection (Lachance, 2009). However, we genotyped another 22 SNPs in the same validation sample simultaneously and there were no severe departures from HWE ($p > 0.001$) for these markers. These facts mitigate the likelihood of genetic drift, or population subdivision. Considering the calling rate of 94.8% we cannot exclude the possibility of genotyping errors. Otherwise, the lack of HWE for rs6280 in the cases might be selection-related.

There were several limitations to our study. Assuming a disease allele frequency of 0.25, and a type I error of 0.05, our sample has an 80% power to detect a variant with an OR larger than 1.22 (Dupont and Plummer, 1998). Although we tested nine SNPs within COMT, the selection of candidate SNPs was not unbiased but dependent on the design of the commercial array. The effect of rs4680 probably depends on the genetic background and environmental factors; therefore, testing rs4680 without accommodating other epistatic factors would result in reduced power and limited reproducibility in this work. Further studies taking into account the genetic context would be more revealing.

In conclusion, our study does not support the association of rs6280 with SZ in the Han Chinese population. The deviation from HWE of rs4680 in SZ cases calls for further investigation.

Acknowledgments

The authors thank all participants for their cooperation in our study. This work was supported by research grants from the National High-Tech Research and Development Program of China (2009AA022702), the National Natural Science Foundation of China (81000578, 81071087, and 81071088), the National Basic Research Program of China (2011CB707805), and the International Science and Technology Cooperation Program of China (2010DFB30820).

Author Disclosure Statement

The authors declare that they have no competing interests.

References

Cardno AG, Gottesman, II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12–17.

Chen CY, Lu RB, Yeh YW, *et al.* (2011) Association study of catechol-O-methyltransferase gene polymorphisms with schizophrenia and psychopathological symptoms in Han Chinese. Genes Brain behav 10:316–324.

Chen J, Lipska BK, Halim N, *et al.* (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821.

Costas J, Sanjuan J, Ramos-Rios R, *et al.* (2011) Heterozygosity at catechol-O-methyltransferase Val158Met and schizophrenia: new data and meta-analysis. J Psychiatr Res 45:7–14.

Dudbridge F (2008) Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum Hered 66:87–98.

Dupont WD, Plummer WD (1998) Power and sample size calculations for studies involving linear regression. Control Clin Trials 19:589–601.

Durstewitz D, Seamans JK (2008) The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. Biol Psychiatry 64:739–749.

Fan JB, Zhang CS, Gu NF, *et al.* (2005) Catechol-O-methyltransferase gene Val/Met functional polymorphism and risk of schizophrenia: a large-scale association study plus meta-analysis. Biol Psychiatry 57:139–144.

Goldman-Rakic PS, Muly Er, Williams GV (2000) D(1) receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 31:295–301.

Hoenicka J, Garrido E, Martinez I, *et al.* (2010) Gender-specific COMT Val158Met polymorphism association in Spanish schizophrenic patients. Am J Med Genet B Neuropsychiatr Genet 153B:79–85.

Kang C, Xu X, Liu H, *et al.* (2010) Association study of catechol-O-methyltransferase (COMT) gene Val158Met polymorphism with auditory P300 in Chinese Han patients with schizophrenia. Psychiatry Res 180:153–155.

Lachance J (2009) Detecting selection-induced departures from Hardy-Weinberg proportions. Genet Sel Evol 41:15.

Lewis CM, Levinson DF, Wise LH, *et al.* (2003) Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. Am J Hum Genet 73:34–48.

Munafo MR, Bowes L, Clark TG, *et al.* (2005) Lack of association of the COMT (Val158/108 Met) gene and schizophrenia: a meta-analysis of case-control studies. Mol Psychiatry 10: 765–770.

Okochi T, Ikeda M, Kishi T, *et al.* (2009) Meta-analysis of association between genetic variants in COMT and schizophrenia: an update. Schizophr Res 110:140–148.

Purcell S, Neale B, Todd-Brown K, *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575.

Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry 60:141–151.

Yu R, Zhang XN, Huang XX, *et al.* (2007) Association analysis of COMT polymorphisms and schizophrenia in a Chinese Han population: a case-control study. Am J Med Genet B Neuropsychiatr Genet 144:570–573.

Yue WH, Wang HF, Sun LD, *et al.* (2011) Genome-wide association study identifies a susceptibility locus for schizophrenia in Han Chinese at 11p11.2. Nat Genet 43:1228–1231.

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