Genes, Brain and Behavior
Official publication of the International Behavioural and Neural Genetics Society

Genes, Brain and Behavior (2009) **8:** 228–237

Genetic variants in COMT and neurocognitive impairment in families of patients with schizophrenia

S.-Y. Liao[†,‡,1], S.-H. Lin[†,§,1], C.-M. Liu[¶], M. H. Hsieh[¶], T. J. Hwang[¶], S. K. Liu[¶,], S.-C. Guo[††], H.-G. Hwu[‡,§,¶] and W. J. Chen[*,†,§,¶]**

[†]*Genetic Epidemiology Core Laboratory, Division of Genomic Medicine Research Center for Medical Excellence,* [‡]*School of Medicine, College of Medicine,* [§]*Institute of Epidemiology, College of Public Health and* [¶]*Department of Psychiatry, College of Medicine and National Taiwan University Hospital, National Taiwan University,* [**]*Department of Psychiatry, Far Eastern Memorial Hospital, Taipei, and* [††]*Ju Shan Mental Hospital, Taoyuan, Taiwan*

**Corresponding author: Dr W. J. Chen, Institute of Epidemiology, College of Public Health, National Taiwan University, 17 Xuzhou Road, Taipei 100, Taiwan. E-mail: wjchen@ntu.edu.tw*

This study examined the relations of genetic variants in catechol-*O*-methyltransferase (*COMT*) gene, including rs737865 in intron 1, rs4680 in exon 4 (Val158Met) and downstream rs165599, to schizophrenia and its related neurocognitive functions in families of patients with schizophrenia. Totally, 680 individuals from 166 simplex (166 affected members and 354 nonpsychotic first-degree relatives) and 46 multiplex families (85 affected members and 75 nonpsychotic first-degree relatives) were interviewed using Diagnostic Interview for Genetic Studies, administered Wisconsin Card Sorting Test (WCST) and Continuous Performance Test (CPT), and drawn for venous blood. Both categorical (dichotomizing families on affected members' neurocognitive performance) and quantitative approaches toward the WCST and CPT performance scores were employed using the family-based association test and the variance components framework, respectively. Both false discovery rate and permutations were used to adjust for multiple testing. The genotypes of rs4680 were associated with both the WCST and CPT performance scores in these families, but not with schizophrenia *per se* in either whole sample or subgroup analyses. Meanwhile, the other two single nucleotide polymorphisms were differentially associated with the two tasks. For WCST indexes, regardless of subgroup analyses or quantitative approach, only rs737865 exhibited moderate associations. For CPT indexes, rs737865 exhibited association for the subgroup with deficit on CPT reaction time, whereas rs165599 exhibited association for the subgroup with deficit on CPT d′ as well as quantitative undegraded d′. Our results indicate that the genetic variants in COMT might be involved in modulation of neurocognitive functions and hence conferring increased risk to schizophrenia.

Keywords: COMT, continuous performance test, family-based association study, gene, quantitative trait, schizophrenia, Wisconsin Card Sorting Test

Received 28 July 2008, revised 3 November 2008, accepted for publication 7 November 2008

Introduction

The catechol-*O*-methyltransferase (*COMT*) gene has been one of the most studied schizophrenia susceptibility genes on the basis of several lines of evidence. One line is from the biochemical functions of COMT, emphasizing that the enzyme is involved in the catabolism of monoamines, especially dopamine (Karoum *et al.* 1994), and its dysfunction has been the core of the dopamine hypothesis of schizophrenia (Carlsson 1978). Another line of evidence is from gene mapping efforts. Microdeletion of 22q11, where the *COMT* gene is located, was found to be associated with schizophrenia (Karayiorgou *et al.* 1995; Murphy *et al.* 1999; Usiskin *et al.* 1999). Several linkage and association studies further suggested 22q11 region as a probable schizophrenia susceptibility locus (Karayiorgou *et al.* 1998; Palmatier *et al.* 1999).

A functional polymorphism of COMT in exon 4, a G/A nucleotide transition denoted as rs4680, generates a valine-to-methionine (Val/Met) substitution and lowers enzyme's activity (Lachman *et al.* 1996). The Val allele was associated with the cognitive symptoms in schizophrenia (Egan *et al.* 2001). In a case–control study of Ashkenazi Jews, the COMT Val/Met polymorphism along with two other polymorphic sites, rs737865 in intron 1 and downstream rs165599 near 3′ untranslated region, constituted a G-G-G haplotype and exhibited a strong association with schizophrenia (Shifman *et al.* 2002).

However, subsequent studies have been inconsistent regarding the association of COMT genetic variants with schizophrenia (Fan *et al.* 2005; Munafo *et al.* 2005; Tsai *et al.* 2004; Williams *et al.* 2005). The frequencies of the G-G-G haplotype varied substantially across different ethnic groups, indicating that the role played by the COMT variants in relation to schizophrenia might differ in different populations (Palmatier *et al.* 2004). Another issue is the genetic heterogeneity of schizophrenia. When studying a complex disorder, targeting biological traits found in both affected subjects and their

[1] These authors contributed equally to this study.

doi: 10.1111/j.1601-183X.2008.00467.x

unaffected relatives, so called endophenotypes (Gottesman & Gould 2003), may improve the power to detect susceptibility genes (Freedman *et al.* 1997; Kremen *et al.* 1994). There is empirical evidence supporting deficits in neurocognitive function assessed by the Wisconsin Card Sorting Test (WCST) (Robinson *et al.* 1980) and the Continuous Performance Test (CPT) (Rosvold *et al.* 1956) to be vulnerability markers to schizophrenia. Although the functional rs4680 of the *COMT* gene has been intensively investigated for its relations to the performance on the WCST (Barnett *et al.* 2007; Bruder *et al.* 2005; Egan *et al.* 2001; Minzenberg *et al.* 2006; Rybakowski *et al.* 2006; Szoke *et al.* 2006) as well as the CPT (Eisenberg *et al.* 1999; Stefanis *et al.* 2005), the results remained inconsistent and the other single nucleotide polymorphisms (SNPs) in the gene have been rarely examined in this respect. Furthermore, none of these studies used the method of family-based association test (FBAT) (Lake *et al.* 2000), which is immune from confounding by population stratification.

The aims of this study were (1) to examine the relations of three COMT genetic variants, including rs737865 in intron 1, rs4680 in exon 4 (Val158Met) and downstream rs165599, to schizophrenia in families of patients with schizophrenia by means of FBAT; (2) to examine the relations of these genetic variants to schizophrenia in subgroups stratified by affected members' deficit on the WCST or the CPT; and (3) to test the relationship between the SNP genotypes and the quantitative traits of WCST and CPT performance scores under the variance components framework.

Methods

Subjects

Study subjects included both patients with schizophrenia and their first-degree relatives from the Study on Etiological Factors of Schizophrenia, in which affected members from both simplex (i.e. without affected siblings) and multiplex families (i.e. at least two affected siblings) were recruited from National Taiwan University Hospital and Ju-Shan Psychiatric Hospital (a private hospital in Tao-Yuan County, Taiwan) from 2002 to 2005. The exclusion criteria for patients to be recruited were: severe neurological abnormality, prominent substance use problems, mental retardation and aboriginal ancestry. Then, the first-degree relatives of patients with schizophrenia were recruited. There were 166 simplex and 46 multiplex families of patients with schizophrenia recruited for this study, with a total of 680 individuals. This study was approved by the institutional review boards of the participating hospitals. Written informed consent was obtained from all subjects after complete description.

Measurements

All participants were interviewed by well-trained assistants using the Diagnostic Interview for Genetic Studies (DIGS), which was designed specifically for family-genetic studies of schizophrenia and bipolar disorder with good interrater reliabilities (Nurnberger *et al.* 1994). The Chinese version of the DIGS was translated by two psychiatrists and one psychiatric epidemiologist, and its reliability was good in Taiwanese population (Chen *et al.* 1998b). The Chinese version of the Family Interview for Genetic Studies (FIGS) (NIMH Genetics Initiative 1992) was used to collect relevant information on relatives who were not interviewed for the study. On the basis of information assembled in the DIGS, the FIGS and clinical information from medical records, best estimate lifetime psychiatric diagnoses were determined using the criteria specified by the Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) (American Psychiatric Association 1994).

In addition, each participant was administered two neurocognitive tests, the WCST and the CPT, and drawn for venous blood that was used for DNA extraction.

Neurocognitive assessment

Wisconsin Card Sorting Test

We used a computerized version of WCST (Lin *et al.* 2000) to measure the abstract function of cognition. The subjects were instructed to match a 'response' card to one of the four 'stimulus' cards on the basis of three dimensions (color, form or number) by pressing one of the 1–4 number keys on the computer keyboard. The subjects were required to determine which one was correct and not informed of the correct sorting principles, nor were they told when the principle shifted during the test. Four performance indices as described in the WCST manual (Heaton *et al.* 1993) were used for subsequent analyses: (1) Perseverative Errors, the number of errors that were perseverative, reflecting the tendency toward perseveration; (2) Categories Achieved, the number of times that 10 consecutive correct responses were made, reflecting overall success; (3) Conceptual Level Response, proportion of consecutive correct responses occurring in runs of three or more, reflecting insight into the correct sorting principles; and (4) failure to maintain set, the number of times subject makes between 5 and 9 correct responses in a row, reflecting efficiency of sorting. Standardized *z*-scores with adjustment for age, sex and educational level were derived for individual WCST indices against a group of 392 healthy controls (Lin, S.-H., Liu, C.-M., Liu, S.-K., Hwang, T.J., Hsieh, M.H., Hwu, H.-G., and Chen, W.J. unpublished data).

Continuous performance test

A CPT machine from Sunrise System, v. 2.20 (Pembroke, MA, USA), was used to assess sustained attention. The procedure has been described in detail elsewhere (Chen *et al.* 1998a). Each subject undertook two CPT sessions: the undegraded task and the degraded task. During the undegraded session, subjects responded to the target stimulus, a '9' preceded by '1'. During the degraded session, a pattern of snow was used to toggle background and foreground dots so that the image was not distinct. Sensitivity d', estimated from the hit rate (probability of response to target trials) and false-alarm rate (probability of response to nontarget trials), reflects an individual's ability to discriminate target stimuli from nontarget ones. In addition, the reaction time (i.e., the mean time to respond correctly) for each session was also used for the analyses. The adjusted *z* scores of the CPT indexes were derived by means of standardizing the raw scores with adjustments for sex, age and education against a community sample of 345 individuals (Chen *et al.* 1998a).

Among 680 participants, 566 (224 being affected members) completed the WCST and 577 individuals (227 of them were affected members) completed the undegraded CPT and 565 (225 being affected members) completed the degraded CPT.

SNP genotyping

Three SNPs (rs737865, rs4680 and rs165599) on the COMT were chosen for genotyping, and their positions on the gene are displayed in Fig. 1. The genotypings were performed using the method of matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS). The procedure has been described in detail elsewhere (Liu *et al.* 2006). In brief, we used SpectroDESIGNER v2.0.0.17 software (Sequenom, San Diego, CA, USA) to design primers and probes flanking the SNPs. A DNA fragment (100–300 bp) encompassing the SNP site was amplified by the polymerase chain reaction (PCR) (GeneAmp 9700 thermocycler; Applied Biosystems, Foster City, CA, USA). After PCR amplification, primer extension was performed by adding the probe. Different extension products were differentiated by mass through MALDI–TOF as

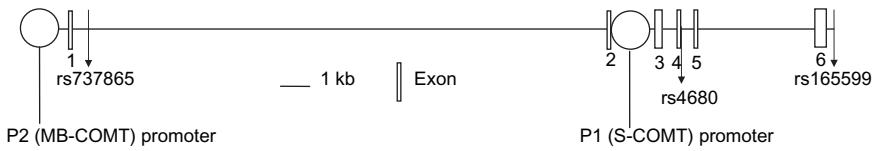

Figure 1: Positions of the three SNPs in the COMT gene. The location of each SNP is indicated by arrow.

follows. The reaction mixture was plotted on the SpectroCHIP. A nitrogen laser with nanosecond-wide pulses interrogated the samples on the SpectroCHIP in the high-vacuum environment of the TOF MS after introducing to the SpectroREADER. Acquired spectra were interpreted and corrected for their genotypes automatically after transferring to the MassARRAY Server (Sequenom, San Diego, CA).

Statistical analysis

The three SNP markers were checked for Mendelian inheritance using UNKNOWN V5.23 (Terwilliger & Ott 1994) and PEDCHECK V1.1 (O'Connell & Weeks 1998). We used Procedure ALLELE in SAS/GENETICS release 8.2 to test for Hardy–Weinberg equilibrium. The software HAPLOVIEW (Barrett *et al.* 2005) was used to calculate D' and r^2 of linkage disequilibrium.

Qualitative approach

The outcome was first treated as qualitative using schizophrenia as affection and subjected to both single locus and haplotype association analyses using software FBAT version 1.7.2 (Horvath *et al.* 2001). The null hypothesis assumes no association under the presence of linkage and was advised to be used when testing for association in an area of known linkage (Lake *et al.* 2000). Three genetic models (additive, dominant and recessive models) are allowed in the FBAT program. We compared the results in different models and reported the model that had the most significant results. In addition to the whole sample analyses, subgroup analyses were also conducted separately for WCST- or CPT-based deficit and nondeficit families, with a deficit family defined as having at least one deficit affected member in the family. As there has been no well-established threshold for a deficit on a particular neurocognitive test, two methods were explored in this study. First, we used a series of different thresholds, starting with median and then median minus 0.5 *z* score, and median minus 1 *z* score. Second, the thresholds for some indexes were selected on the basis of greatest magnitude of recurrence risk ratios among unaffected relatives obtained from our previous family studies. For the WCST, an adjusted *z* score of <1.0 for Perseverative Errors and that less than -1.0 for Categories Achieved were chosen (Lin *et al.*, unpublished data), whereas for the CPT d′, an adjusted *z* score of less than -2.5 was chosen (Chen *et al.* 2004a). These cutoff points have been applied in previous fine mapping studies (Liu *et al.* 2006, 2008). Plus or minus 0.5 *z* score around these thresholds were also explored in this study. These two methods were then compared on the basis of FBAT test statistics and the threshold with the most significant results was reported. Some families were excluded from the subgroup analyses because of the lacking of affected members' tests score.

The issue of multiple testing was dealt with in two ways. First, we adopted the method of false discovery rate (Benjamini & Hochberg 1995) to account for the number of SNPs and performance indexes within a subgroup for a particular neurocognitive test. Second, to assess the magnitude of false positivity in our subgroup-specific association tests, we calculated an empirical *P* value using a permutation approach. We divided the total number of families into deficit and nondeficit subgroups in 10 000 permutations, and then the frequency of a FBAT test statistic greater than the greatest one obtained in real data from the subgroup-specific association tests for each marker in the 10 000 permutations was counted for each subgroup.

Quantitative approach

Next, the WCST or CPT performance scores were treated as quantitative traits and their relations with the COMT SNPs were evaluated using software QTDT V2.4.6 (Abecasis *et al.* 2000a,b). Under the variance components framework, in which the variance is decomposed into environmental, polygenic and additive components, the model allows tests for population stratification, within-family association and total association. If the coefficient of population stratification is retained in the model, the within-family association test is suggested to indicate the magnitude of marker-trait association and its significance level can be estimated by means of an empirical *P* value on the basis of Monte-Carlo simulations (e.g. 1000 times for this study). When there is no evidence of population stratification, the total association test is used instead, which is more powerful than the within-family association test in detecting the marker-trait association (van den Oord 2002).

Results

Qualitative analyses for whole sample

The number of affected members and their relatives for the whole sample as well as their distribution in the performance scores on the WCST and CPT are shown in Table 1. When the phenotype was defined as fulfilling the DSM-IV criteria of schizophrenia and subjected to family-based association analysis for each SNP, there were no significant associations with any SNP markers for the whole sample, simplex families or multiplex families (Table S1).

When haplotype analyses were conducted for the three SNPs, a block between rs4680 and rs165599 was found ($D' = 0.64$). Similar to the results of single-locus analyses, none of the haplotypes was associated with schizophrenia for the whole sample, simplex families or multiplex families (data not shown).

Qualitative analyses for subgroups

When a range of cutoff points, either around median or some preselected thresholds, were tried for subgroup analyses, different thresholds were selected for individual indexes on the basis of the most significant association in FBAT test statistics (Tables S2 and S3). For WCST indexes, the threshold was either >0.0 (Perseverative Errors) or less than -0.5 adjusted *z* score (Categories Achieved, Conceptual Level Responses and Failure to Maintain Set). Meanwhile, for CPT indexes, the threshold was less than -2.5 (undegraded d′), less than -2.0 (degraded d′), or $>$median (both undegraded and degraded reaction time). Their demographic features and distribution in the performance scores on the WCST and CPT are shown in Table 1.

Table 1: Descriptive data of all subjects and subgroups by means of deficit* status on the WCST and the CPT

Variable	Total ($N = 680$)	Deficit in PE (>0) ($N = 397$)	Deficit in CA (<−0.5) ($N = 383$)	Deficit in CLR (<−0.5) ($N = 352$)	Deficit in FMS (<−0.5) ($N = 293$)	Deficit in Ud′ (<−2.5) ($N = 156$)	Deficit in Dd′ (<−2.0) ($N = 299$)	Deficit in URT (>Med) ($N = 336$)	Deficit in DRT (>Med) ($N = 320$)
Affected siblings, *n* (%)	251 (36.9)	150 (37.8)	142 (37.1)	133 (37.8)	110 (37.5)	57 (36.5)	110 (36.8)	133 (39.6)	129 (40.3)
Multiplex†	85 (33.9)	60 (40.0)	53 (37.3)	51 (38.3)	42 (38.2)	24 (42.9)	40 (36.4)	62 (46.6)	64 (49.6)
Male gender†	152 (60.6)	91 (60.7)	90 (63.4)	80 (60.2)	66 (60.0)	34 (59.6)	60 (54.5)	80 (60.2)	75 (58.1)
Age, mean (SD)	32.2 (8.8)	31.0 (8.3)	32.0 (8.7)	31.6 (8.6)	32.2 (8.8)	31.2 (7.8)	31.9 (8.7)	33.4 (8.2)	32.7 (8.3)
Unaffected first-degree relatives, *n* (%)	429 (63.1)	247 (62.2)	241 (62.9)	219 (62.2)	183 (62.5)	99 (63.5)	189 (63.2)	203 (60.4)	191 (59.7)
WCST, mean (SD)									
Perseverative Errors (PE)	0.40 (1.35)	0.76 (1.47)	0.72 (1.48)	0.80 (1.53)	0.72 (1.68)	0.96 (1.68)	0.66 (1.51)	0.52 (1.40)	0.40 (1.39)
Categories Achieved (CA)	−0.14 (1.04)	−0.36 (1.03)	−0.45 (1.06)	−0.49 (1.05)	−0.29 (1.12)	−0.50 (1.04)	−0.35 (1.09)	−0.24 (1.03)	−0.16 (1.04)
Conceptual Level Response (CLR)	−0.28 (1.19)	−0.54 (1.19)	−0.64 (1.23)	−0.71 (1.24)	−0.56 (1.33)	−0.77 (1.28)	−0.57 (1.27)	−0.39 (1.22)	−0.29 (1.19)
Failure to Maintain Set (FMS)	−0.24 (0.96)	−0.27 (0.97)	−0.28 (0.98)	−0.34 (0.93)	−0.53 (0.93)	−0.41 (1.02)	−0.36 (0.98)	−0.25 (1.02)	−0.20 (1.03)
CPT, mean (SD)									
Undegraded d′ (Ud′)	−0.70 (1.53)	−0.84 (1.56)	−0.99 (1.66)	−0.99 (1.69)	−1.01 (1.70)	−1.81 (2.12)	−1.22 (1.79)	−1.04 (1.63)	−0.72 (1.46)
Degraded d′ (Dd′)	−1.11 (1.60)	−1.32 (1.65)	−1.38 (1.69)	−1.41 (1.69)	−1.37 (1.66)	−1.86 (1.76)	−1.77 (1.71)	−1.35 (1.60)	−1.08 (1.46)
Undegraded reaction time (URT)	0.15 (1.11)	0.20 (1.16)	0.23 (1.21)	0.21 (1.23)	0.32 (1.25)	0.39 (1.53)	0.27 (1.30)	0.52 (1.16)	0.31 (1.11)
Degraded reaction time (DRT)	−0.34 (1.53)	−0.33 (1.69)	−0.45 (1.76)	−0.46 (1.77)	−0.44 (1.77)	−0.81 (2.08)	−0.53 (1.89)	−0.26 (1.70)	0.22 (1.23)

*A deficit family was defined as having at least one affected sibling with an adjusted *z* score worse than the threshold for a particular neuropsychological test. Med, median; median of URT, 0.34, median of DRT, 0.08.

†The proportion among affected siblings, not among the total sample. For each multiplex family, there were at least two siblings affected with schizophrenia, although some did not participate in the study.

Table 2: Single-locus association between the COMT genetic polymorphisms and schizophrenia using FBAT program under dominant model, stratified by family's WCST-deficit status

		Deficit family					Non-deficit family				
Test/SNP	Allele	Frequency	*N**	*Z*†	*P* value	FDR‡	Frequency	*N**	*Z*†	*P* value	FDR‡
WCST Perseverative Errors											
rs737865	C	0.290	35	—	—		0.274	29	—	—	
	T	0.710	18	2.674	**0.0075**	0.0900	0.726	12	−0.324	0.7456	
rs4680	A	0.292	48	—	—		0.245	27	−0.552	0.5807	
	G	0.708	22	−1.272	0.2035		0.755	9	—	—	
rs165599	A	0.497	45	—	—		0.489	28	—	—	
	G	0.503	41	−1.070	0.2847		0.511	26	0.965	0.3346	
WCST Categories Achieved											
rs737865	C	0.292	36	—	—		0.270	28	—	—	
	T	0.708	20	2.359	**0.0183**	0.0732	0.730	10	0.289	0.7728	
rs4680	A	0.288	50	—	—		0.254	25	0.594	0.5525	
	G	0.712	20	−0.636	0.5245		0.746	11	—	—	
rs165599	A	0.492	45	1.511	0.1307		0.497	28	−1.156	0.2479	
	G	0.508	37	—	—		0.503	30	—	—	
WCST Conceptual Level Response											
rs737865	C	0.286	35	—	—		0.281	29	—	—	
	T	0.714	17	2.414	**0.0158**	0.0948	0.719	13	0.587	0.5569	
rs4680	A	0.306	46	—	—		0.234	29	−0.535	0.5930	
	G	0.694	21	−0.823	0.4102		0.766	10	—	—	
rs165599	A	0.487	41	1.496	0.1347		0.503	32	−0.971	0.3317	
	G	0.513	33	—	—		0.497	34	—	—	
WCST Failure to Maintain Set											
rs737865	C	0.295	29	—	—		0.274	35	−0.898	0.3692	
	T	0.705	11	2.197	**0.0280**	0.0840	0.726	19	—	—	
rs4680	A	0.316	35	—	—		0.238	40	0.673	0.5011	
	G	0.684	18	−0.636	0.5245		0.762	13	—	—	
rs165599	A	0.486	34	1.272	0.2032		0.501	39	—	—	
	G	0.514	25	—	—		0.499	42	−0.709	0.4786	

Values in bold: $P < 0.05$.

*The number of the informative family.

†The test statistic of FBAT. For a dominant model, only the allele with the greater magnitude of Z was shown; whereas for an additive model, the Z was equivalent for both alleles.

‡False discovery rate, calculated on the basis of 12 tests for each subgroup (3 SNPs × 4 indexes) only for those FBAT test statistics with $P < 0.05$.

When families were subgrouped for the family-based association analysis, only those with deficits either on the WCST or CPT exhibited associations with the COMT genetic variants on the basis of FBAT test statistics. For subgrouping by WCST indexes (Table 2), the T allele of rs737865 was associated with schizophrenia for deficit families on all of the four indexes of WCST, with the most significant one for Perseverative Errors ($P = 0.0075$). However, after adjustment using false discovery rate, the association becomes nonsignificant ($P = 0.09$). For subgrouping by CPT indexes (Table 3), the A allele of rs165599 was associated with schizophrenia for deficit families on both the undegraded and degraded d′, and the T allele of rs737865 was associated with schizophrenia for deficit families on both the undegraded and degraded reaction time. After adjustment using false discovery rate, all the associations remained significant except that of rs165599 with degraded d′.

The robustness of the subgroup-specific association analysis was then evaluated by means of 10 000 permutations for the most significant finding for the WCST and CPT indexes, respectively. For the association of rs737865 with schizophrenia for families with deficit on Perseverative Errors, families were randomly assigned into deficit (120 families) and nondeficit (73 families) subgroups, respectively. Out of 10 000 permutations, the FBAT test statistics was greater than the greatest one obtained in real data (i.e. $Z = 2.674$, $P = 0.0075$) for 259 times, that is an empirical P value of 0.0259. Similarly, for the association of rs165599 with schizophrenia for families with deficit on undegraded d′, families

Table 3: Single-locus association between the COMT genetic polymorphisms and schizophrenia using FBAT program, stratified by family's CPT-deficit status

		Deficit family					Nondeficit family				
Test/SNP	Allele	Frequency	*N**	$Z^{\dagger}$	*P* value	FDR‡	Frequency	*N**	$Z^{\dagger}$	*P* value	FDR‡
Undegraded CPT d′ (Additive Model)											
rs737865	C	0.301	14	—	—		0.283	50	—	—	
	T	0.699	14	0.106	0.9156		0.717	50	0.513	0.6082	
rs4680	A	0.311	21	0.700	0.4838		0.259	56	—	—	
	G	0.689	21	—	—		0.741	56	0.065	0.9485	
rs165599	A	0.478	24	3.051	**0.0023**	**0.0276**	0.501	64	—	—	
	G	0.522	24	—	—		0.499	64	0.906	0.3650	
Degraded CPT d′ (Dominant Model)											
rs737865	C	0.270	25	—	—		0.299	40	—	—	
	T	0.730	10	1.279	0.2008		0.701	20	1.393	0.1635	
rs4680	A	0.298	40	—	—		0.244	34	—	—	
	G	0.702	17	−0.973	0.3304		0.756	13	0.385	0.7004	
rs165599	A	0.463	35	2.212	**0.0269**	0.0807	0.522	37	−1.491	0.1359	
	G	0.537	23	—	—		0.478	45	—	—	
Undegraded CPT Reaction Time (Dominant Model)											
rs737865	C	0.276	32	—	—		0.301	31	0.962	0.3359	
	T	0.724	15	2.835	**0.0046**	**0.0278**	0.699	15	—	—	
rs4680	A	0.281	39	−0.146	0.8840		0.263	36	—	—	
	G	0.719	17	—	—		0.737	13	−0.965	0.3346	
rs165599	A	0.473	43	0.996	0.3194		0.519	29	—	—	
	G	0.527	36	—	—		0.481	33	−0.602	0.5474	
Degraded CPT Reaction Time (Dominant Model)											
rs737865	C	0.263	32	—	—		0.307	33	−0.180	0.8575	
	T	0.737	13	2.538	**0.0111**	**0.0444**	0.693	17	—	—	
rs4680	A	0.263	41	−0.844	0.3985		0.277	33	—	—	
	G	0.737	14	—	—		0.723	16	−0.896	0.3705	
rs165599	A	0.481	40	0.630	0.5286		0.506	32	0.368	0.7127	
	G	0.519	32	—	—		0.494	36	—	—	

Values in bold: $P < 0.05$.

*The number of the informative family.

†The test statistic of FBAT. For a dominant model, only the allele with the greater magnitude of *Z* was shown, whereas for an additive model, the *Z* was equivalent for both alleles.

‡False discovery rate, calculated on the basis of 12 tests for each subgroup (3 SNPs × 4 indexes) only for those FBAT test statistics with a $P < 0.05$.

were randomly assigned into deficit (45 families) and nondeficit (150 families) subgroups. Compared with the corresponding FBAT test statistics ($Z = 3.051$, $P = 0.0023$), only eight times of permutations were greater than this, that is an empirical *P* value of 0.0008.

Software QTDT analyses

When the WCST and CPT performance scores were treated as quantitative traits and subjected to the variance component analysis using the software QTDT by pooling all the participants together, the results with significant association with any one of the three SNPs are displayed in Table 4. As there was evidence for population stratification in the analysis of rs737865 with the Categories Achieved and in that of rs165599 with the undegraded CPT d′, within-family association tests were reported. The results suggested a moderate association for rs737865 with WCST Categories Achieved and a borderline association for rs165599 with undegraded CPT d′. For the remaining analyses that did not indicate existence of population stratification, the results of total association rather than within-family association were reported. There were significant associations for rs4680 with three indexes of WCST (Perseverative Errors, Categories Achieved and Conceptual Level Response) and two indexes of CPT (both the undegraded and degraded d′). As rs4680 is a functional polymorphism, the direction of the association was further evaluated by means of linear regression analyses using the mixed-effect model to account for the within-family correlation. The results indicated that more copies of the Val allele were associated with better performance on the WCST

Table 4: Results* of tests of population stratification, within-family association, and total association for individual SNPs and neurocognitive functions in all subjects (affected members and relatives) by means of software QTDT (the figures shown here are *P* values)

SNP and neurocognitive task	Population stratification	Within-family association†	Total association
rs737865			
WCST (Categories Achieved)	0.0258	0.0170	
rs4680			
WCST (Perseverative Errors)			0.0070
WCST (Categories Achieved)			0.0188
WCST (Conceptual Level Response)			0.0038
CPT (Undegraded d′)			0.0040
CPT (Degraded d′)			0.0158
rs165599			
CPT (Undegraded d′)	0.0414	0.0410	

*Only the results of tests with $P < 0.05$ are shown here.

†Empirical *P* value from 1000 permutation tests.

(i.e. greater Categories Achieved and Conceptual Level Response and less Perseverative Errors) or CPT (i.e. greater d′).

Discussion

This study examined the relations of three COMT SNPs to schizophrenia and its related deficits in neurocognitive functions in families of patients with schizophrenia in Taiwan. Unlike previous studies in Caucasian populations, we did not find any of the SNPs to be associated with schizophrenia in the whole sample. However, when families were categorized as deficit on either the WCST or the CPT, some SNPs did exhibit associations with schizophrenia. Furthermore, when these neurocognitive functions were treated as quantitative trait loci, they also exhibited association with some of the COMT genetic variants.

Our results highlight the utility of identifying homogeneous subgroups or using schizophrenia-related neurocognitive functions in examining the association of genetic variants to schizophrenia. This study used two different neurocognitive tests, each having its own theoretical and empirical basis for its relations to schizophrenia susceptibility. Executive functions, particularly those of frontal lobe (Alvarez & Emory 2006), as measured by the WCST were found to be impaired in patients with schizophrenia (Goldberg *et al.* 1987; Koren *et al.* 1998) as well as their first-degree relatives (Wolf *et al.* 2002). Meanwhile, sustained attention deficits as measured on the CPT have been shown to be present not only in patients with schizophrenia but also in subjects with schizotypal personality disorder and in nonpsychotic relatives of patients with schizophrenia (Chen & Faraone 2000; Cornblatt & Keilp 1994). The recurrence risk ratio for CPT performance among parents or siblings was higher than that of schizophrenia alone (Chen *et al.* 2004a, 1998b). Sustained attention deficit in nonpsychotic first-degree relatives of patients with schizophrenia was worse in multiplex than in simplex families (Tsuang *et al.* 2006).

A challenging issue is how to apply these neurocognitive tasks to the association analysis on the relations between COMT and schizophrenia. In our categorical approach, we adopted two methods to adjust for multiple testing. The false discovery rate analyses indicate that at least three associations (rs165599 with schizophrenia for families with deficit on undegraded CPT d′, and rs737865 with schizophrenia for families with deficit on both undegraded and degraded CPT reaction time) remained significant. Even for the WCST indexes, all the false discovery rates ranged between 0.073 and 0.095. It should be pointed out, however, that the correction is likely to be too conservative because the SNPs and neurocognitive tasks were chosen based on *a priori* knowledge rather than randomly. Meanwhile, the permutations revealed that our choice of threshold for a particular performance index had an acceptable false-positivity rate. For the quantitative approach, we also employed permutations to derive empirical *P* values. Taken together, the robustness of our results is supported by these safeguarding evaluations.

Among the three SNPs of the *COMT* gene investigated in this study, the functional meaning of rs4680 is the most well known (Lachman *et al.* 1996). The genotypes of rs4680 were associated with both the WCST and the CPT performance traits in the families with schizophrenia, but not with schizophrenia *per se* in either whole sample or subgroup analyses. As our sample included nonpsychotic relatives of patients with schizophrenia, our results extend the findings from a recent meta-analysis of 12 studies on the relations between COMT rs4680 and the WCST performance in both patients with schizophrenia and healthy controls, which concluded that a small but significant relation between rs4680 and executive function was limited to healthy individuals (Barnett *et al.* 2007).

Intriguingly, our results indicated that the Val allele was associated with better rather than worse WCST or CPT performance, which is in opposite direction to that found in Caucasian populations. The paradoxical findings might be explained by an inverted 'U'-shape relationship between dopamine levels and prefrontal cortex function (Cools & Robbins 2004; Mattay *et al.* 2003). This model indicates that an optimal functioning occurs within a narrow range of dopamine level, and both excessive and insufficient dopamine levels impair working memory performance. Furthermore, the COMT enzyme may modulate the balance of tonic and phasic dopamine function in different ways depending on area-specific neurochemical environment (Bilder *et al.* 2004). As individuals may have different tonic dopamine functions, the effect of Val/Met polymorphism on neurocognitive task is not straightforward (Williams *et al.* 2007). The inconsistent results might be because of different tonic dopamine functions among participants across studies, which remained unaccounted for in all the association analyses.

It is interesting to note that the other two SNPs examined in this study were differentially associated with the two neurocognitive tasks. For WCST indexes, regardless of

subgroup analyses or quantitative approach, only rs737865 exhibited moderate associations. For CPT indexes, rs737865 exhibited association for the subgroup with deficit on CPT reaction time, whereas rs165599 exhibited association for the subgroup with deficit on CPT d′ as well as quantitative undegraded d′. The rs737865 in intron 1 may be linked to *Hin*dIII SNP, which is within the P2 promoter. Hence, the rs737865 may affect the transcription of membrane-bound form of COMT (Palmatier *et al.* 2004), which is highly expressed in the brain. Meanwhile, rs165599 was found to be just downstream to the polyadenylation signal at exon 6 (Tenhunen *et al.* 1994), indicating that rs165599 might be related to the polyadenylation process that could influence the efficiency of mRNA translation (Colgan & Manley 1997). Whether the functional roles played by these two SNPs are related to modulation of dopamine function and hence individual neurocognitive performance warrants future investigation.

Our choice of the three SNPs was based on previous findings that the haplotype constituted by these SNPs exhibited strong association with schizophrenia, such as G-G-G (rs737865–rs4680–rs165599) in Ashkenazi Jews (Shifman *et al.* 2002), A-A-G in Caucasians (Handoko *et al.* 2005), and A-G-A in the Irish (Chen *et al.* 2004b). Surprisingly, the coefficient *D*′s among the three SNPs in this study were relatively low, with only two of them forming a loose block (rs4680 and rs165599). This may also explain the lack of association of the haplotypes with schizophrenia in this study, which is consistent with a case–control study in Chinese Han population (Yu *et al.* 2007) and highlights the ethnic differences in the allelic compositions in *COMT* gene (Palmatier *et al.* 2004). Whether there are other loci in tight linkage disequilibrium with the three SNPs that are involved with schizophrenia-related cognitive function warrants more investigation.

Our results should be interpreted with its limitations in mind. First, the current diagnosis of schizophrenia relies on psychiatric interview, which might not be free from individual subjectivity and likely to involve clinical heterogeneity. Second, the sample size of this study limits our power in detecting the association for some subgroup analysis. Third, despite our efforts in using the state-of-art methods in judging the statistical significance of the results (e.g. false discovery rate analysis and permutation-based empirical *P* value), the associations found in this study need future replication in independent samples.

In summary, this study examined the relations of three COMT genetic variants, including rs737865 in intron 1, rs4680 in exon 4 (Val158Met) and downstream rs165599, to schizophrenia and its related neurocognitive functions in families of patients with schizophrenia. Our results indicate that the genetic variants in COMT might be involved in modulation of neurocognitive functions and hence conferring increased risk to schizophrenia, and acknowledge the SNP genotyping work done by the National Genotyping Center (NGC), NSC, Taiwan.

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Acknowledgements

This work was supported by the National Health Research Institutes, Taiwan (NHRI-90-8825PP, NHRI-EX91,92-9113PP, NHRI-CN-MG-9006S, and NHRI-EX93-9113PP), National Science Council, Taiwan (NSC-91-3112-B-002-011, NSC-92-3112-B-002-019, NSC-93-3112-B-002-012, and NSC95-3114-P002-005-Y), and NTU grant 97HP0023. The authors thank Po-Chang Hsiao of the National Taiwan University Center for Genomic Medicine for helping prepare the database.

Supporting Information

The following supporting information are available for this article.

Table S1: Single-locus association between the COMT genetic polymorphisms and schizophrenia using FBAT program

Table S2: Single-locus association between the COMT genetic polymorphisms and schizophrenia using FBAT program under dominant model, stratified by family's WCST-deficit status with a range of cutoff points (only alleles and tests with a $P < 0.05$ are shown here)

Table S3: Single-locus association between the COMT genetic polymorphisms and schizophrenia using FBAT program under additive/dominant model[a], stratified by family's CPT-deficit status with a range of cutoff points (only alleles and tests with $P < 0.05$ are shown here)

Additional Supporting Information may be found in the online version of this article.

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