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ORIGINAL RESEARCH ARTICLE

Separate and interacting effects within the catechol-*O*-methyltransferase (*COMT*) are associated with schizophrenia

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Several lines of evidence have implicated the catechol-*O*-methyltransferase (*COMT*) gene as a candidate for schizophrenia (SZ) susceptibility, not only because it encodes a key dopamine catabolic enzyme but also because it maps to the velocardiofacial syndrome region of chromosome 22q11 which has long been associated with SZ predisposition. The interest in COMT as a candidate SZ risk factor has led to numerous case–control and family-based studies, with the majority placing emphasis on examining a functional Val/Met polymorphism within this enzyme. Unfortunately, these studies have continually produced conflicting results. To assess the genetic contribution of other *COMT* variants to SZ susceptibility, we investigated three single-nucleotide polymorphisms (SNPs) (rs737865, rs4633, rs165599) in addition to the Val/Met variant (rs4680) in a highly selected sample of Australian Caucasian families containing 107 patients with SZ. The Val/Met and rs4633 variants showed nominally significant associations with SZ ($P<0.05$), although neither of the individual SNPs remained significant after adjusting for multiple testing (most significant $P=0.1174$). However, haplotype analyses showed strong evidence of an association; the most significant being the three-marker haplotype rs737865-rs4680-rs165599 (global $P=0.0022$), which spans more than 26 kb. Importantly, conditional analyses indicated the presence of two separate and interacting effects within this haplotype, irrespective of gender. In addition, our results indicate the Val/Met polymorphism is not disease-causing and is simply in strong linkage disequilibrium with a causative effect, which interacts with another as yet unidentified variant ~20 kb away. These results may help explain the inconsistent results reported on the Val/Met polymorphism and have important implications for future investigations into the role of COMT in SZ susceptibility.
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Schizophrenia (SCZD (MIM 181500)) is a severe, debilitating disorder characterised by delusional beliefs, hallucinations, disordered speech, and deficits in emotional and social behaviour with an average lifetime morbid risk of 1%. Several lines of evidence have implicated the catechol-*O*-methyltransferase gene (COMT (MIM 116790)) as a candidate for schizophrenia (SZ), not only due to it encoding a key dopamine catabolic enzyme but also because it maps to the velocardiofacial syndrome (VCFS (MIM 192430)) region of chromosome 22 which has been long-associated with SZ. Over 20% of patients with VCFS also have SZ or schizoaffective (SA) disorder;[1,2] moreover, the 1.5–3 Mb microdeletions associated with VCFS have been found in 0.6–2% of adult SZ[3] patients and in 6% of cases with onset below the age of 16 years.[4] The *COMT* region on chromosome band 22q11.2 was in the eighth ranked bin of the genome scan meta-analysis of SZ[5] and gave a $P<0.00009$ in another meta-analysis.[6]

COMT has a common functional substitution of Valine for Methionine at position 158/108 (codon 158 of the membrane-bound form, MB-COMT; codon 108 of the soluble form, S-COMT) with the Met allele being more thermolabile even at physiological temperature.[7] The Val variant has a higher enzymatic activity leading to more efficient degradation of dopamine, and lower than normal prefrontal

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dopamine levels. The Met allele has 25–50% of the enzyme activity of the Val allele[8,9] and is correlated with superior performance on prefrontal executive cognition and working memory tasks.[10–12]

Li *et al*[13] were the first to demonstrate excess transmission of the Val allele among family trios with SZ and since then many other studies have followed, including an extension of the original work.[14] A meta-analysis carried out by Glatt *et al*[15] evaluated a collection of 14 case–control and five family-based studies between 1996 and 2002. Overall, the case–control studies showed no indication of an association between either of the alleles and SZ, but the family-based studies found modest evidence implicating the Val allele in SZ risk. They concluded that the family-based studies might be more accurate and suggested that the Val allele may be a small but reliable risk factor for SZ in populations with European ancestry, but that its role in Asian populations remained unclear. A study in a large Ashkenazi sample (720 SZ patients, 2970 controls) reported only modest support for the Val/Met polymorphism ($P = 0.024$), but a highly significant association for two other polymorphisms (in intron 1 and 3′ UTR), and for a core haplotype of three markers ($P = 9.5 \times 10^{-8}$).[16]

Materials and methods

In the present study, we have attempted to perform an independent replication in an Australian sample of 50 Caucasian affected sib-pair (ASP) families. This study received approval from the relevant institutional ethics committees. Families were recruited opportunistically, a form of ascertainment that is effective and economic, since it avoids excessive time and resources being spent on pedigrees that prove to be ineligible. *Inclusion criteria.* Only pedigrees with Caucasian ancestry were included, since ethnicity may form the basis for genetic (locus) heterogeneity at certain susceptibility loci. An ASP family had to include a proband with DSM-IV[17] diagnosis of SZ plus at least one other sibling affected with either SZ or SA disorder—since molecular genetic studies combine schizophrenia and schizoaffective disorder into a 'core' phenotype;[18,19] blood samples were obtained from the affected siblings and available parents; if one/no parent was available, up to three unaffected siblings were asked to participate. Affected subjects were defined as those with DSM-IV SZ or SA based on the Diagnostic Interview for Genetic Studies (DIGS),[20] medical records and Family Interview for Genetic Studies (FIGS)[21] sources of information. Unaffected subjects were defined as those for whom blood samples were obtained for genetic phase information and genotyping error checking. *Exclusion criteria.* Subjects who were unable to give informed consent, subjects whose psychosis was judged secondary to substance use or a known neurological disorder such as epilepsy, based on the consensus diagnostic procedure, and subjects with severe mental retardation were excluded. All available diagnostic information for each case was reviewed independently by two psychiatrists, who then met to assign a consensus Best Estimate Final Diagnosis. This study has received approval from the ethics committee. The average age of affected individuals was 34.7 (range = 18–63, SD = 9.91).

Four single-nucleotide polymorphisms (SNPs) were utilized (Table 1) extending from intron 1 to the 3′UTR. Full sequence and other linked information can be found through the Electronic-Database by using the NCBI identification numbers for SNPs. Genotyping was performed via a primer extension reaction and MALDI-TOF mass spectrometry (MassARRAY, Sequenom Inc., San Diego, CA, USA) as previously described.[22,23] To estimate error rates due to genotyping technical causes in our laboratory, an SNP has been genotyped twice in another study on 3268 DNAs independently at different times. Of the 6536 genotypes, there were only seven unresolved errors (not attributable to Mendelian, DNA or other nontechnical causes), which is an error frequency of 0.11%. Indeed, utilising the PEDMANAGER programme (MP Reeve and MJ Daly, personal communication), only one Mendelian-inheritance inconsistency was observed in our data. Specifically, a family with one genotyped parent (2/2) and three genotyped affected siblings (2/2, 1/2, 1/1) was inconsistent at locus rs165599. This family's rs165599 genotypes were not included in subsequent analyses. Assuming only one of the four genotypes in this family is erroneous, results in an observed error rate of only 1/756 (0.13%), consistent with the estimate obtained from our study of 3268 DNAs.

Deviations from Hardy–Weinberg equilibrium (HWE) were assessed using the HWSIM programme[24]

Table 1 Matrix of pair-wise LD correlations with $|\Delta|$ given below the diagonal and the four associated eigenvalues are given along the diagonal (bold). D'-values are also given above the diagonal

Locus	*Distance from rs737865 (bp)*	*rs737865*	*rs4633*	*rs4680*[a]	*rs165599*
rs737865	0	**2.4848**	0.769	0.768	0.215
rs4633	20114	0.4629	**0.8450**	1.000	0.464
rs4680[a]	21150	0.4552	0.9845	**0.6549**	0.444
rs165599	26660	0.1703	0.3562	0.3351	**0.0153**

[a]Some studies refer to this SNP as rs165688.[16,35,37,39]

(Kidd Lab Website). Since some of the analyses contained small numbers of observations in some cells, *P*-values for all analyses were estimated empirically through the use of Monte–Carlo simulations (10 000 iterations in each case) based on observed allele frequencies. Significance levels were estimated as the proportion of times the simulated distribution reached or exceeded the observed deviation from HWE. Intermarker linkage disequilibrium (LD) between the four SNPs ($|\Delta|$ = Pearson correlation; D' = Lewontin's standardized LD coefficient) was calculated using the LDMAX programme (Abecasis Website). Nonindependence between the SNPs was examined using the SNPSpD world wide web interface.[25]

The data were analysed for association using the TDTPHASE programme.[26] This programme can perform both multilocus Haplotype-based Haplotype Relative Risk (HHRR) analysis on nuclear families with unphased genotype data and multilocus Transmission Disequilibrium Tests (TDT) with phased genotype data, and has the advantage of being able to conduct either unconditioned or conditioned analyses on multiple loci. When the phase is unknown, an unconditional logistic regression is performed on the full likelihood of parents and offspring. This approach classes transmitted (T) haplotypes as cases and nontransmitted (NT) haplotypes as controls and is equivalent to the HHRR approach of Terwilliger and Ott;[27] and is strictly a test of association (as opposed to the TDT, which is a test of both linkage *and* association). Maximum-likelihood estimations of case/control haplotype frequencies in parents are obtained using the expectation-maximization (EM) algorithm (note: we *did not* estimate missing parental genotypes by looping over allele values that are consistent with pedigree data (*-missing* option)). Since the EM algorithm does not accurately estimate haplotype frequencies $<1\%$,[28] such haplotypes were excluded (*-zero 0.01* option). Conditional analyses test for the equality of odds ratios (ORs) for haplotypes identical at conditioning loci.[29] Homozygous parent tests (HPT) use only the parents that are homozygous at the conditioning loci. To maximise use of our data, we analysed all affected offspring in each family using the HHRR approach. Therefore, to investigate the effect of nonindependence of the transmissions—due to our use of multiple patients from the same family—we permuted *P*-values (10 000 replicates) using the same permuted transmission status for siblings (*-robustperm* option), which gives a valid test in the presence of linkage. For aesthetic reasons, we chose not to present 95% confidence intervals (CIs) for our permuted *P*-values; however, we note that due to the large number of replicates (10 000) they will be very small. For example, using the recommended[30,31] approach of Wilson[32] for calculating accurate CIs; permuted *P*-values of 0.05, 0.01, 0.001 and 0.0001 have corresponding 95% CIs of 0.046–0.055, 0.0082–0.0122, 0.0005–0.0018 and 0.000005–0.00057, respectively.

To further investigate potential bias generated by intrafamilial correlation, we analysed the data using the TDT of the TRANSMIT programme[33] with the robust variance (*-ro*) option to take account of the correlations. ORs were calculated using the T/NT counts estimated by the TDTPHASE EM algorithm and thus are not true ORs, although we would expect them to be very close to true ORs. The conditional allelic model assumes that the mode of inheritance is multiplicative, although it was recently observed to be valid under any genetic model (F Dudbridge, personal communication). Nonetheless, we also stratified our data according to genotype at the conditioning loci, thus providing a conditional test which is insensitive to the underlying genetic model.

Results

Of the 50 Australian Caucasian families, 46 contained two affected siblings and four contained three affected siblings. Also, three of the 50 families had an even stronger family history of SZ, with one ASP having an affected aunt and two ASP families having an affected parent. At a maximally informative locus, the 104 affected siblings would provide data for 208 allelic transmissions/nontransmissions. However, because not all families had both parents available for genotyping (5/46 and 1/4 with zero; 21/46 and 2/4 with one (of these five had one, three had two, and one had three additional unaffected siblings typed); 20/46 and 1/4 with both parents available), and because the EM algorithm could not resolve all phase ambiguities, the number of estimated allele transmissions/nontransmissions ranged from 105 (rs165599) to 120 (rs737865). None of the SNP genotypes deviated significantly from HWE in both the parents and children ($P>0.05$). Using the approach of Nyholt,[25] the four SNPs produced an effective number of independent SNPs (M_{eff}) of 3.17, representative of significant intermarker LD (see Table 1). Indeed, except for rs737865-rs165599, all pair-wise SNP combinations are in significant LD ($P<0.05$). Moreover, rs4633 and rs4680 are in almost complete LD ($|\Delta| = r = 0.9845$).

Individual HHRR analysis of SNPs rs4633 and rs4680 reached nominal significance ($P=0.04$; Table 2). However, the robust permutation analysis adjusting for the four loci tested revealed a nonsignificant corrected *P*-value of 0.1174. On the other hand, haplotype analyses clearly showed evidence of an association (Table 3a), the most significant being the three-marker haplotype rs737865-rs4680-rs165599 ($P=0.00056$). Significant association remained after robust permutations for the rs737865-rs4633 ($P=0.0035$), rs737865-rs4680 ($P=0.0035$), rs737865-rs165599 ($P=0.0544$), rs737865-rs4633-rs165599 ($P=0.0025$), rs737865-rs4680-rs165599 ($P=0.0022$) and rs737865-rs4633-rs4680-rs165599 ($P=0.0023$) haplotypes. The robust permutation procedure was also used to estimate the significance of the best result, correcting for multiple testing, for tests of

Table 2 Results for allelic HHRR analysis

			Transmitted		*Nontransmitted*			
SNP	*Allele*	*Polymorphism*[a]	*Count*	*Frequency*	*Count*	*Frequency*	P_{global}	*OR*[b]
rs737865	1	A	93	0.7750	96	0.8000	0.6359	1
	2	G	27	0.2250	24	0.2000		1.16
rs4633	1	C	43	0.4019	58	0.5421	0.0396	1
	2	T	64	0.5981	49	0.4579		1.76
rs4680	1	G/Val	44	0.4112	59	0.5514	0.0398	1
	2	A/Met	63	0.5888	48	0.4486		1.76
rs165599	1	A	78	0.7429	67	0.6381	0.1000	1.64
	2	G	27	0.2571	38	0.3619		1

[a]Based on coding (forward) strand.
[b]OR = odds ratio compared to the reference haplotype which has OR = 1.

individual haplotypes. The most aberrant haplotypes for loci rs737865-rs4633 (1–1), rs737865-rs4680 (1–1), rs737865-rs165599 (1–2), rs737865-rs4633-rs165599 (1–1–2), rs737865-rs4680-rs165599 (1–1–2) and rs737865-rs4633-rs4680-rs165599 (1–1–1–2) all remained significant, producing corrected HHRR permuted *P*-values of 0.0061, 0.0040, 0.0463, 0.0012, 0.0012 and 0.0012, respectively.

Individual TDT analysis for SNPs rs737865, rs4633, rs4680 and rs165599 by the TRANSMIT programme[33] found that the global results were very similar both with ($\chi^2_1 = 5.66 \times 10^{-6}$; $P = 0.9981$, $\chi^2_1 = 2.82$; $P = 0.0930$, $\chi^2_1 = 2.94$; $P = 0.0866$, $\chi^2_1 = 1.52$; $P = 0.2180$) and without the robust estimator ($\chi^2_1 = 9.40 \times 10^{-6}$; $P = 0.9976$, $\chi^2_1 = 3.52$; $P = 0.0605$, $\chi^2_1 = 3.76$; $P = 0.0526$, $\chi^2_1 = 1.99$; $P = 0.1583$). Analogously, TRANSMIT TDT analysis for the two-SNP, three-SNP, and four-SNP haplotypes found that the global results were very similar both with and without the robust estimator. Finally, the same pattern of results obtained from the HHRR analyses were obtained from TDTPHASE phase certain haplotype (TDT) analysis (Table 3b). These results, together with the robust permutation *P*-values indicate that the effect of transmission nonindependence was negligible and our analyses represent a reliable test of association.

As shown in Table 3, haplotypes containing either rs737865 and rs4633, or rs737865 and rs4680 were the most significant (for complete results of haplotypic HHRR analysis, see Supplementary Table 1 (http://www.qcmhr.uq.edu.au/bryan/supplementary%20table%201.pdf)). To investigate whether separate effects existed, we performed analyses conditional on the SNPs initially showing strongest individual significance (eg Kilding *et al*[34]). HHRR association tests conditioning on alleles at rs4633 and rs4680 (Table 4) indicated a significant and separate effect at rs737865 (*Full Model* $P_{\text{global}} = 0.0124$ and 0.0147, respectively). Furthermore, this finding remained significant after robust permutation for rs737865 conditioned on rs4633 or rs4680, both producing *Full Model* global HHRR *P*-values of 0.0060. This indicates that the separate effects observed for rs737865 and rs4633/rs4680 were not due to a correlation between transmissions. These results were supported by homozygous parent HHRR analyses (Table 5), which indicate that the effect of rs4633/rs4680 is moderated by the presence of allele 1 (A) at rs737865 (robust permuted $P_{\text{global}} = 0.0001$). The *Full Model* and homozygous parent HHRR results did not remain significant between rs737865 and rs165599 after permutation ($P_{\text{global}} = 0.0640$, $P_{\text{global}} = 0.0637$). Interestingly, subsequent analyses comparing the *Full Model* to one not allowing interaction between rs737865 and rs4633 or rs4680 provided significant evidence ($P = 0.0264$, $P = 0.0284$, respectively) for interaction between these effects. The same results were observed when conditioning was performed on genotypes (data not shown).

Given Shifman *et al*'s[16] recently reported sex-specific genetic effects for *COMT* polymorphisms (ie, rs737865 associated in both sexes; rs4680 associated in males; rs165599 associated in females) and to further examine the individual effects of rs737865 and rs4633/rs4680, we separately performed HHRR tests on the female and male SZ patients. Our data also found sex-specific associations; however, they were not in the same direction as reported by Shifman *et al*.[16] Specifically, rs737865 was primarily associated in female subjects, while rs4633, rs4680 and rs165599 were primarily associated in male subjects. Importantly, the 1-1-1-2 haplotype was significantly *under*transmitted and clearly drives the haplotype association in both male (1 T *vs* 13 NT) and female subjects (0 T *vs* 5 NT) (Table 6). We also note sex-specific haplotype analysis found a different haplotype significantly *over*transmitted in male (1-2-2-1) compared to female subjects (2-1-1-2), even though the latter haplotype was only observed a total of six

Table 3 Results for (a) haplotypic HHRR analysis, (b) haplotypic TDT analysis

		Transmitted		*Nontransmitted*				
SNPs	*Haplotype*	*Count*	*Frequency*	*Count*	*Frequency*	P_{global}	$P_{individual}$*	*OR*[a]
(a)								
rs737865-rs4680-rs165599	1-1-1	11	0.1183	14	0.1505	0.00056[b]	0.5186	0.71
	1-1-2	1	0.0107	18	0.1935		0.0000068[c]	0.05
	1-2-1	49	0.5269	34	0.3656		0.0266	1.31
	1-2-2	9	0.0967	7	0.0752		0.6005	1.17
	2-1-1	11	0.1183	10	0.1075		0.8167	1
	2-1-2	11	0.1183	7	0.0752		0.3194	1.43
	2-2-1	1	0.0107	3	0.0322		0.3013	0.30
rs737865-rs4633-rs4680-rs165599	1-1-1-1	10	0.1075	13	0.1398	0.0012[d]	0.5035	0.70
	1-1-1-2	1	0.0107	18	0.1935		0.0000068[c]	0.05
	1-2-1-1	1	0.0107	1	0.0107		1	0.91
	1-2-2-1	49	0.5269	34	0.3656		0.0266	1.31
	1-2-2-2	9	0.0967	7	0.0752		0.6005	1.17
	2-1-1-1	11	0.1183	10	0.1075		0.8167	1
	2-1-1-2	11	0.1183	7	0.0752		0.3194	1.43
	2-2-2-1	1	0.0107	3	0.0322		0.3013	0.30

		Transmitted		*Nontransmitted*				
SNPs	*Haplotype*	*Count*	*Frequency*	*Count*	*Frequency*	P_{global}	$P_{individual}$*	*RR*[e]
(b)								
rs737865-rs4680-rs165599	1-1-1	11	0.1719	13	0.2031	0.0010[f]	0.7235	2.05
	1-1-2	1	0.0156	18	0.2812		0.000017[g]	0.11
	1-2-1	28	0.4375	13	0.2031		0.04346	3.12
	1-2-2	6	0.0938	4	0.0625		0.5619	1.99
	2-1-1	7	0.1094	6	0.0938		0.7814	1
	2-1-2	10	0.1562	7	0.1094		0.5111	3.01
	2-2-1	1	0.0156	3	0.0469		0.3063	0.82
rs737865-rs4633-rs4680-rs165599	1-1-1-1	10	0.1562	12	0.1875	0.0021[h]	0.6946	2.52
	1-1-1-2	1	0.0156	18	0.2812		0.000017[g]	0.11
	1-2-1-1	1	0.0156	1	0.0156		1	1.27
	1-2-2-1	28	0.4375	13	0.2031		0.0435	3.57
	1-2-2-2	6	0.0938	4	0.0625		0.5619	2.2
	2-1-1-1	7	0.1094	6	0.0938		0.7814	1
	2-1-1-2	10	0.1562	7	0.1094		0.5111	3.20
	2-2-2-1	1	0.0156	3	0.0469		0.3063	0.92

*$P_{\text{individual}}$ = significance of individual haplotypes obtained by grouping all others together.
[a]OR = odds ratio compared to the reference haplotype which has OR = 1.
[b]Permuted $P_{\text{individual}} = 0.0022$.
[c]Permuted $P_{\text{individual}} = 0.0012$.
[d]Permuted $P_{\text{individual}} = 0.0023$.
[e]RR = relative risk compared to the reference haplotype which has RR = 1.
[f]Permuted $P_{\text{individual}} = 0.0023$.
[g]Permuted $P_{\text{individual}} < 0.0001$.
[h]Permuted $P_{\text{individual}} = 0.0034$.

times in female subjects (robust permuted $P_{\text{individual}} = 0.0294$).

To further investigate sex-specific effects, we separately examined the transmission of alleles from fathers and mothers. Interestingly, these results suggest rs737865 associated alleles in female subjects were transmitted from their fathers, while male rs4633/rs4680 and rs165599 associated alleles were transmitted from their mothers. However, analysis of haplotypes indicated no preferential paternal/maternal transmission, producing a similar pattern of results to the

Table 4 Results for conditional HHRR analysis

		Full Model[a]			*No Interaction Model*[b]			*Test for interaction*[c]		
Conditioned on	*SNP*	χ^2	*df*	P_{global}	χ^2	*df*	P_{global}	$\Delta\chi^2$	Δdf	$P_{interaction}$
rs4633										
Alleles	rs737865	8.7787	2	0.0124[d]	3.8474	1	0.0498	4.9313	1	0.0264
	rs4680	0	0	1	0	1	1	0	−1	—
	rs165599	1.4606	2	0.4818	0.9112	1	0.3398	0.5494	1	0.4586
Genotypes	rs737865	4.9760	3	0.1736	3.5048	1	0.0612	1.4712	2	0.4792
	rs4680	0.0059	1	0.9389	0.0059	1	0.9389	0	0	—
	rs165599	5.1735	3	0.1595	1.3529	1	0.2448	3.8206	2	0.1480
rs4680										
Alleles	rs737865	8.4427	2	0.0147[d]	3.6416	1	0.0564	4.8011	1	0.0284
	rs165599	1.5199	2	0.4677	0.9677	1	0.3253	0.5522	1	0.4574
Genotypes	rs737865	4.6457	3	0.1997	3.3253	1	0.0682	1.3204	2	0.5168
	rs165599	4.8559	3	0.1827	1.4118	1	0.2348	3.4441	2	0.1787
rs165599										
Alleles	rs737865	5.6821	2	0.0584	0.7076	1	0.4002	4.9745	1	0.0257
Genotypes	rs737865	13.4694	3	0.0037	0.9426	1	0.3316	12.5268	2	0.0019

[a]The *Full Model* is a test of equality of ORs for haplotypes identical at conditioning loci.
[b]The *No Interaction Model* is the 'main effects' model, which assumes that the haplotype risk depends only on the constituent allelic risks. All interaction terms are included within the conditioning and test loci, but there are no interactions between conditioning and test loci.
[c]Comparing the likelihoods between the *Full* and *No Interaction Model* provides a test for interactions between test and conditioning loci.
[d]Permuted *Full Model* global HHRR *P*-value = 0.0060.

Table 5 Results for homozygous parent HHRR analysis

		Transmitted		*Nontransmitted*			
SNPs	*Haplotype*	*Count*	*Frequency*	*Count*	*Frequency*	P_{global}	*OR*
rs737865-rs4633	1/1-1	2	0.0833	14	0.5833	0.000124[a]	1
	1/1-2	22	0.9167	10	0.4167		15.4
rs737865-rs4680	1/1-1	2	0.0833	14	0.5833	0.000124[a]	1
	1/1-2	22	0.9167	10	0.4167		15.4
rs737865-rs165599	1/1-1	21	0.8750	13	0.5417	0.0093	5.92
	1/1-2	3	0.1250	11	0.4583		1

[a]Permuted $P_{\text{global}} = 0.0001$.

sex-specific analyses performed without regard to parental origin.

Finally, sex-specific HHRR analyses conditional on the SNPs initially showing strongest individual significance suggested that the separate effects were more prominent in the female compared to male subjects. Sex-specific homozygous parent HHRR analyses indicated that the moderating effect of allele 1 (A) at rs737865 on rs4633/rs4680 (and rs165599) is present in both sexes, although due to the reduced sample size the female results were not significant via permutation (most significant HPT male $P_{\text{global}} = 0.0301$, female $P_{\text{global}} = 0.1242$). Nonetheless, the general trends in allele transmission *vs* nontransmission are consistent across sex, suggesting multiple and interacting *COMT* effects exist in both male and female subjects with SZ.

Discussion

Conditional HHRR tests indicated the presence of two separate effects, one effect at or in strong LD with

Table 6 Results for sex-specific haplotypic HHRR analysis

		Transmitted		*Nontransmitted*			
SNPs	*Haplotype*	*Count*	*Frequency*	*Count*	*Frequency*	P_{global}	$P_{individual}^{*}$
Male							
rs737865-rs4633-rs4680-rs165599	1-1-1-1	8	0.1270	8	0.1270	0.0108	1
	1-1-1-2	1	0.0158	13	0.2063		0.00024
	1-2-1-1	1	0.0158	0	0.0000		0.2377
	1-2-2-1	37	0.5873	22	0.3492		0.0071
	1-2-2-2	3	0.0476	4	0.0635		0.6969
	2-1-1-1	7	0.1111	7	0.1111		1
	2-1-1-2	5	0.0793	7	0.1111		0.5430
	2-2-2-1	1	0.0159	2	0.0318		0.5553
Female							
rs737865-rs4633-rs4680-rs165599	1-1-1-1	2	0.0667	5	0.1667	0.0046	0.2210
	1-1-1-2	0	0.0000	5	0.1667		0.0065
	1-2-1-1	0	0.0000	1	0.0333		0.2362
	1-2-2-1	12	0.4000	12	0.4000		1
	1-2-2-2	6	0.2000	3	0.1000		0.2741
	2-1-1-1	4	0.1333	3	0.1000		0.6871
	2-1-1-2	6	0.2000	0	0.0000		0.0027

$^{*}P_{\text{individual}}$ = significance of individual haplotypes obtained by grouping all others together.

rs737865, another at or in strong LD with rs4633/rs4680. Owing to the high LD between rs4633 and rs4680, we were unable to separate these SNPs. On the other hand, conditional analyses suggest any transmission distortion observed for rs165599 is due to it being in LD with the effect at or near rs4633/rs4680. Given the well-documented difficulties in replicating the association between the Val/Met (rs4680) polymorphism and SZ,[15] and the recently published family-based association study using a large sample of the Irish Study of High-Density SZ Families (ISHDSF) with only modest association,[35] it seems unlikely that this polymorphism is disease-causing. The finding of excess transmission of the Met allele in some studies, and of the Val allele in others, suggests that this polymorphism is simply in LD with a causative effect. That is, these contradictory results are due to the sampling of different haplotypic backgrounds (given the Val and Met alleles are typically equifrequent, it is not surprising that the Val allele is on the common haplotype in some populations, but on the rare haplotype in others), and/or by random chance, the studies have ascertained individuals with or without the modifying effect at or near rs737865.

Meanwhile, the effect of genotype on Wisconsin Card Sorting Test performance was found to be of similar effect size in control subjects and patients with SZ,[10] indicating that the effect of the Val/Met polymorphism on prefrontal execution cognition was *not* necessarily an SZ-related phenomenon, but rather a generalized human characteristic. Hence, it is more likely that a mutation other than and in strong LD with Val/Met is a causative allele.

In most assayed tissues, the S-COMT predominates, but in the brain MB-COMT is more prevalent.[36] However, according to Bray *et al*,[37] in human brain the Val transcripts are expressed at a slightly lower level than those encoding the Met allele. Thus, individuals homozygous for the Val alleles might not have as high COMT activities in their brains as in their blood or liver, because of the lower level of Val transcripts in the brain. The level of expression might be regulated by another SNP, perhaps rs737865, located closer to the promoter for MB-*COMT*. Palmatier *et al*[38] have also suggested recently that the P2 promoter of MB-COMT as the region of relevance for SZ. Bray *et al*[37] also argued that rs737865 might not have a peripheral effect on S-COMT, since this soluble form of COMT is transcribed by use of a separate promoter located 3' of this SNP.

A combined effect of multiple susceptibility genes is currently accepted as a paradigm in complex diseases. Xu *et al*[39] genotyped 85 SNPs in 23 genes (including seven SNPs of *COMT*) in a case/control sample and they suggested that *COMT* and *ALDH3* may be the most common combination in the dopamine metabolism pathway involved in predisposition to paranoid SZ. This again highlights the possibility that *COMT* is interacting with or capturing another allele as a SZ risk factor.

We are aware of three family-based association studies examining the role of *COMT* in SZ with Caucasian/European descent subjects. The earliest one was carried out by Kunugi *et al*,[40] and utilized a combination of seven families from Wales and 13 from England (as well as two from Japan); the result was not statistically significant. The second is Egan

et al's[10] that employed both case/control and family-based methods in a single sample; therefore, the groups are not entirely independent. The third is the recently published sample of the ISHDSF with only modest association for the Val/Met of rs4680, and no association signals for rs737865 and rs165599.[35]

The family-based study by Egan *et al*[10]—which also closely matches our study in terms of strict phenotyping, ethnicity and sample size—utilised 104 family trios and found a genotypic relative risk of 1.471 for the Val allele (75 transmissions *vs* 51 nontransmissions). Using the Genetic Power Calculator[41] and assuming a disease prevalence of 0.01 and risk allele frequency of 0.5, our sample has a power of 0.83 and 0.52 to replicate the Egan *et al*[10] finding at a nominal significance level of 0.05 under an additive and multiplicative model, respectively. Furthermore, under these additive and multiplicative models, our 54 independent ASPs (46 families with two affected siblings, four families with three affected siblings (ie, sibship of size S as being equivalent to $(S-1)$ independent ASPs)[42]) are equivalent to 106 and 110 trios, respectively.[43] Hence, our sample is comparable in power to other *COMT* association studies in Caucasian schizophrenia samples.

We also note that because our SZ cases are highly selected in terms of family history, compared to a standard case–control or family-trio association study (ie, with cases unselected for family history), our sample will have considerably more power to detect gene associations. Indeed, Antoniou and Easton[44] recently showed that for additive, multiplicative and dominant models, such selected cases provide approximately twice the power to detect association compared to unselected cases. In other words, the power to detect association increases when affected relatives are ascertained, since this will increase the probability that affected individuals have a multifactorial disease for (the same) genetic reason(s). Therefore, we consider our sample to be equivalent to approximately 200 trios unselected for family history.

In conclusion, utilizing four SNPs, we found strong evidence supporting the presence of two separate and interacting effects within the 27.22 kb *COMT* gene associated with SZ susceptibility. Together with the results from previous research,[16,35,37] this study does not support a causative role of the Val/Met polymorphism in SZ susceptibility. However, our results confirm the genetic contribution of other *COMT* variants to SZ and indicate the presence of more than one functional polymorphism. Therefore, to further investigate the involvement of *COMT* variants on SZ (ie, distinguish between functional and nonfunctional variants), it will be necessary to vigorously evaluate—utilising statistical approaches similar to those presented here—all of the polymorphic markers in the gene and its nearby regulatory elements for association with SZ, followed by functional molecular and cellular studies.

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Electronic-Database Information

URLs for data presented herein are as follows:

Abecasis Website, http://www.sph.umich.edu/csg/abecasis/GOLD/download/index.html (for GOLD, accessed January 30, 2003)

Genetic Power Calculator, http://statgen.iop.kcl.ac.uk/gpc/

Kidd Lab Website, http://krunch.med.yale.edu/hwsim/ (for HWSIM)

National Center for Biotechnology Information, Single Nucleotide Polymorphism Database, http://www.ncbi.nlm.nih.gov/SNP/ (for reference identification numbers for SNPs)

National Institute of Mental Health (1999) FIGS face sheet, http://zork.wustl.edu/nimh/figs/FIGS.pdf

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for SCZD, *COMT*, and VCFS)

MIT Genome Centre, http://www.broad.mit.edu/ftp/distribution/software/pedmanager/ (for PEDMANAGER)

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Supplementary Information accompanies the paper on Molecular Psychiatry website (http://www.nature.com/mp)