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European Neuropsychopharmacology 12 (2002) 299–303

EUROPEAN NEURO-PSYCHOPHARMACOLOGY

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Functional catechol-*O*-methyltransferase gene polymorphism and susceptibility to schizophrenia

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Received 21 August 2001; received in revised form 15 March 2002; accepted 15 March 2002

Abstract

Genetic polymorphism of catechol-*O*-methyltransferase (COMT), involved in the degradation of catecholamine neurotransmitters, has been investigated as a candidate for modifier of susceptibility to development of schizophrenia. To address this issue further, we carried out a study in Korean schizophrenic patients and controls. The study population consisted of 103 Korean inpatients diagnosed as schizophrenic and their 103 age and sex matched controls. The patients were divided into two groups on the basis of history of aggressive behavior, family history of schizophrenia and related disorders, and age at onset of the disease. The *COMT* genotypes were determined by a PCR based method. No statistically significant overall associations between the *COMT* genotypes and risk of schizophrenia were observed. However, subjects with at least one low activity associated *COMT-L* allele showed a tendency of elevated risk for schizophrenia (OR=1.7, 95% CI=0.9–3.1) compared with those homozygous for the high activity associated *COMT-H* alleles. Moreover, when cases were stratified by family history of schizophrenia, a significant combined effect was seen: the cases with concurrent family history of schizophrenia and the *COMT-L* allele containing genotypes had an almost 4-fold (OR=3.9, 95% CI=1.1–14.3) higher risk of schizophrenia compared to controls with the *COMT-HH* genotypes. Future studies with larger sample sizes are, however, needed to confirm this novel finding.

Keywords: Schizophrenia; Catechol-*O*-methyltransferase polymorphism; Family history; Violence; Age at onset

1. Introduction

Schizophrenia is a severe and common psychiatric disorder affecting ~1% of the world population. As a leading cause of psychiatric admissions, it is viewed as a major public health concern. However, our knowledge of the structural or functional pathology of schizophrenia is very limited. The only etiological factor with a reasonably firm foundation is inheritance, as evidenced by family, twin, and adoption studies that point to substantial inheritability (Gottesman and Shields, 1982; Gottesman, 1991; Kendler and Diehl, 1993). Since childhood onset of schizophrenia is known to be related to familial schizophrenia (McClellan, 2000), family history and childhood onset of schizophrenia are important steps in sub-grouping of this disorder.

The advent of molecular genetics was a turning point in schizophrenia research, enabling, for example, testing of candidate-gene polymorphisms with presumed functional relevance for the disease (Martin, 1987; Baron and Rainer, 1988; Owen and Craddock, 1996). One of the most interesting of these candidate-genes is that encoding for

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0924-977X/02/$ – see front matter
PII: S0924-977X(02)00030-5

catechol-*O*-methyltransferase (COMT), an enzyme catalyzing *O*-methylation of biologically active or toxic catechols. It is a major component of the metabolism of drugs and neurotransmitters such as *L*-dopa, noradrenaline, adrenaline, and dopamine (Price-Evans, 1993). The activity of COMT exhibits a trimodal distribution of activity levels (Weinshilboum, 1978), which is known to be caused by an amino acid substitution: a single G→A base pair change in exon 4 of the *COMT* gene results in an valine→methionine amino acid change at codon 158 of membrane bound COMT (MB-COMT) and codon 108 of soluble COMT (S-COMT). This change is associated with 3–4-fold lower enzymatic activity of COMT (Lachman et al., 1996). Approximately 25% of Caucasians are homozygous for the high activity allele (*COMT-H*), another 25% are homozygous for the low activity allele (*COMT-L*), and the remaining half exhibit heterozygous *COMT-HL* genotypes, associated with intermediate enzyme activity (Aksoy et al., 1993; Lachman et al., 1996). There are, however, marked racial differences in the genetically determined COMT activity. Based on a recent global survey (Palmatier et al., 1999), the frequency of homozygotes for the *COMT-L* allele is only ~6% in the East Asian populations.

Although the results of early studies on the activity of COMT in peripheral blood as a potential marker for psychiatric disorders like schizophrenia and depression were not too convincing (Dunner et al., 1977; Phillippu et al., 1981; Puzynski et al., 1983; Karege et al., 1987), interest was renewed by observations made in velo-cardio-facial syndrome (VCFS). VCFS is a congenital anomaly, known to be caused, in most cases, by a micro-deletion on chromosome 22q11 (Kelly et al., 1993), a region where *COMT* gene has also been mapped (Winqvist et al., 1992). Subsequently, 2% of schizophrenia patients have been shown to carry deletion in chromosome 22q11 region (Karayiorgou et al., 1995), and several linkage studies have suggested linkage between genetic markers at this locus and schizophrenia (Lasseter et al., 1995; Schwab et al., 1995).

Some recent studies reported an association between the *COMT-L* allele containing genotypes and schizophrenia (Ohmori et al., 1998) or bipolar affective disorder (Li et al., 1997). Moreover, several recent studies have reported a significant association between the *COMT* low activity genotypes and aggressive and violent behavior in schizophrenic patients (Strous et al., 1997a; Lachman et al., 1998; Kolter et al., 1999). However, in some studies no association between the *COMT* genotypes and schizophrenia were seen (Daniels et al., 1996; Chen et al., 1997; Strous et al., 1997b; Liou et al., 2001).

In this study, we addressed further the possible role of *COMT* gene polymorphism in predisposition to schizophrenia in a Korean study population. Factors like violence, family history, and age at onset were taken into account in the statistical evaluations.

2. Experimental procedures

2.1. Study subjects

Schizophrenic cases (63 males, age 42.4±8.3; 40 females, age 41.7±7.9) were recruited from two psychiatric hospitals located in the suburbs of Seoul, Korea. Two board certified psychiatrists directly interviewed patients. Diagnoses were assigned using standard diagnostic criteria (DSM-IV) and were based on the individual interview and medical records. Final diagnoses of schizophrenia were made upon agreement between the two psychiatrists. We included schizophrenia-related chronic psychoses, including schizotypal personality disorder, but excluded schizoaffective disorder. Altogether 103 schizophrenic cases were finally included in the study.

The controls were recruited among patients admitted to the department of surgery for non-cancer operations in Seoul National University Hospital and Korea Cancer Center Hospital. Subjects with cancer, systemic disease, neurological disease, and mental disorder other than schizophrenia were excluded from the study. Each patient was individually matched to one control according to sex and age. Consequently, the final study population consisted of 103 cases and their 103 age and sex matched referents.

All participants in the study gave written informed consent to an Institutional Review Board-approved protocol of Seoul National University Hospital.

Schizophrenic cases were categorized on the basis of history of aggressive behavior, family history of schizophrenia, and age at onset. Violence classification was based on review of all available hospital records, and was conducted essentially as described by Lachman et al. (1998). Violent subjects had histories of violent or threatening behavior towards another person at least twice, including hitting, kicking, slapping, biting, choking, throwing objects, and using weapons. Non-violent subjects had no known history of violence. Subjects showing intermediate levels of violence, such as a single assault, verbal threats, or gestures, were not included in the study.

Family history of schizophrenia-related disorders was elicited from all available family members of each patient. Although use of a standardized interview schedule (e.g. the Family Interview Scale) in the collection of family history could provide more reliable data for comparison studies, no such scale has been fully standardized in Korea preventing us from applying this procedure in the present study. Instead, two psychiatrists directly interviewed all available close family members and collected integrated family histories about all first-degree relatives of the patients. The questions used by the interviewers were largely compatible with the Family Interview Scale.

Age of onset of the disease was examined by dichotomizing the cases based on whether they were less than 15 years old, or at least 15 years old, when they experienced the first psychotic symptom.

2.2. Genotype determination

DNA was extracted from frozen blood with a Qiagen kit. In the *COMT* genotype analysis a 217 base pair fragment was first amplified using a forward oligonucleotide primer 5′-TCG TGG ACG CCG TGA TTC AGG-3′ and a reverse primer 5′-AGG TCT GAC AAC GGG TCA GGC-3′ (Bioneer, Seoul, south Korea) described by Yim et al. (2001). Subsequently, the PCR product was digested by *Nla* III restriction enzyme (New England Biolabs, Beverly, MA, USA) for 3 h at 37 °C. After electrophoresis with 100 V for 35 min on 3% Metahphor agarose gels (FMC, Maine, USA) containing 0.5 μg/ml ethidium bromide, the gels were photographed under UV light. Restriction fragments of 114, 83 and 20 bp revealed the *COMT-H* allele, whereas in the presence of *COMT-L* allele the 114-bp fragment was cut into 96- and 18-bp fragments.

2.3. Statistical analysis

For matched samples odds ratio (ORs) and 95% confidence intervals (95% CI) for the association between *COMT* genotypes and schizophrenia risk were estimated by conditional logistic regression. In all other analyses unconditional logistic regression analyses were used. Subjects with the *COMT-HH* genotype were designated as the referent category in these analyses.

3. Results

The demographic characteristics of the study subjects are presented in Table 1. The frequency of *COMT-LL* genotype (5.8%) in controls (Table 2) was similar to that previously reported in Asian populations (Chen et al., 1997; Ohmori et al., 1998; Palmatier et al., 1999). No statistically significant overall associations between the *COMT* genotypes and risk of schizophrenia were observed. However, subjects with at least one *COMT-L* allele showed a tendency of elevated risk for schizophrenia (OR=1.7, 95% CI=0.9–3.1) compared with those homozygous for the *COMT-H* allele. Moreover, when the cases were stratified by family history of schizophrenia (Table 3), there was a significant combined effect between family history of schizophrenia and the *COMT* genotypes: cases with a family history of schizophrenia and the *COMT-L* allele containing genotypes had an almost 4-fold (OR=3.9, 95% CI=1.1–14.3) higher risk of schizophrenia compared to controls with the *COMT-HH* genotype.

Since the family history was not available for the controls we also performed a case-only analysis to test the differences in genotype between schizophrenia cases with and without family history. In this analysis subjects with the *COMT-L* allele containing genotypes showed a tendency of elevated risk for familial schizophrenia cases (OR=2.76, 95% CI=0.78–10.23) (data not shown).

Table 1
Demographic characteristics of the study subjects

Demographic characteristics	Patients (n=103)		Controls (n=103)	
	n	%	n	%
Sex				
Male	63	61.2	63	61.2
Female	40	38.8	40	38.8
Age (years)				
Under 29	5	4.9	5	4.9
30–39	36	34.9	36	34.9
40–49	42	40.8	42	40.8
50–59	20	19.4	20	19.4
Mean age (S.D.)	42	(7.8)	42	(7.8)
History of violence				
No	56	54.4	–	–
Yes	47	45.6	–	–
Family history				
No	88	85.4	–	–
Yes	15	14.6	–	–
Age of onset				
≥15 years	96	93.2	–	–
<15 years	7	6.8	–	–

4. Discussion

Although no statistically significant overall associations between the *COMT* genotypes and risk of schizophrenia were observed, subjects with at least one *COMT-L* allele showed a tendency of elevated risk for schizophrenia compared with those homozygous for *COMT-H* allele. Contrasting the earlier observations (Strous et al., 1997b; Lachman et al., 1998; Kolter et al., 1999), no relationship was found between the *COMT* genotypes and either the childhood onset subtype or violence in schizophrenia (data not shown). On the other hand, the homozygous high activity *COMT* genotype, rather than the low activity allele containing genotypes, was recently associated with high levels of aggression rated by the Overt Aggression Scale in schizophrenia (Jones et al., 2001).

One reason for the above discrepancies may be different tools used for evaluation of violence. For instance, based on the report of Kay et al. (1988) suggesting that suicidal behavior may not be a hallmark of the aggressive patient, we excluded suicidal attempt behavior from aggressive behaviors.

The limitations in the present study design, such as the relatively small sample size restricting the statistical power to detect plausible effects and the potential misclassification of diseases and subtypes of the diseases due to our inability to use the standardized interview scale, must also be considered. In the latter case, however, if anything the non-differential misclassification would cause bias towards null.

Although genetic loading is seen more often in the

Table 2
Distribution of *COMT* genotypes in the study groups

Genotype	Patients (n=103)		Controls (n=103)		OR (95% CI)[a]
	n	%	*n*	%	
HH	56	54.4	68	66.0	1.0 (ref.)
HL	38	36.9	29	28.2	1.7 (0.9–3.2)
LL	9	8.7	6	5.8	1.8 (0.6–5.2)*
HL+LL	47	45.6	35	37.0	1.7 (0.9–3.1)

*P=0.08 by likelihood test for trend.
[a] OR, odds ratio; 95% CI, confidence interval. Adjusted odds ratios based on regression coefficients and its standard error from conditional logistic regression model.

Table 3
Distribution of *COMT* genotypes according to the family history of schizophrenia

Genotype	Controls (%)	Patients (%)			
		Family Hx (+)	OR (95% CI)	Family Hx (−)	OR (95% CI)
HH	68 (66.0)	5 (33.3)	1.0 (ref.)	51 (58.0)	1.0 (ref.)
HL	29 (28.2)	9 (60.0)	4.2 (1.2–16.1)	29 (33.0)	1.3 (0.7–2.6)
LL	6 (5.8)	1 (6.7)	2.3 (0.0–27.6)	8 (9.1)	1.8 (0.5–6.2)
HL+LL	35 (34.0)	10 (66.7)	3.9 (1.1–14.3)	37 (42.0)	1.4 (0.8–2.7)

relatives of people with childhood onset schizophrenia than of those with adult onset schizophrenia, this disorder is rarely diagnosed in children under 15 years of age. Dividing patients into those who experienced symptoms younger than or at least 15 years old might also be somewhat arbitrary.

A suggestive combined effects of the *COMT* genotype and family history was observed in this study. However, since family history was not available for the controls it remains to be confirmed in future studies whether the *COMT-L* alleles exert any significant additional effect over family history.

To conclude, despite the limitations of this study, it gives some support to suggestions that functional polymorphism of *COMT* gene modifies individual susceptibility to schizophrenia. As a novel finding, we found that this effect was especially strong among individuals with a family history of schizophrenia. Future studies with larger sample sizes are, however, needed to confirm this novel finding.

Acknowledgements

This work was supported by grant no. 2000-0245 Seoul National University Hospital Research Fund and by the Center for Functional Analysis of Human Genome, FG-2-3.

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