Genotype determining low catechol-*O*-methyltransferase activity as a risk factor for obsessive-compulsive disorder

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ABSTRACT In the present study, we address the role of the gene for catechol-*O*-methyltransferase (COMT), a key modulator of dopaminergic and noradrenergic neurotransmission, in the genetic predisposition to obsessive-compulsive disorder (OCD). We show that a common functional allele of this gene, which results in a 3- to 4-fold reduction in enzyme activity, is significantly associated in a recessive manner with susceptibility to OCD, particularly in males. This association is further supported by psychiatric evaluation of patients who carry microdeletions encompassing the *comt* gene. The mechanism underlying this sex-selective association remains to be defined and may include a sexual dimorphism in COMT activity, although close linkage with a nearby disease susceptibility locus cannot be excluded at this point.

Obsessive-compulsive disorder (OCD) is a common and severe psychiatric illness that affects 1–3% of the population (1, 2) and is characterized by anxiety-producing intrusive thoughts and performance of anxiety-reducing rituals. Very little is known about the pathogenesis of the disorder. Several studies suggest a genetic component in the etiology of OCD (3, 4). In addition, the selective response of the illness to treatment with serotonin reuptake inhibiting agents has led to the hypothesis that OCD may be associated with dysregulation of serotonergic neurotransmission (5, 6). Although serotonin reuptake inhibitors are clearly the first-line pharmacotherapy for OCD, complete relief of symptoms is rare during treatment with these medications, and 30–40% of patients remain clinically unchanged (7). Augmentation of serotonin reuptake inhibitor treatment with dopamine antagonists appears to be useful for a subset of OCD patients (8), thus implicating involvement of dopaminergic pathways as well in the illness.

It has been previously described that patients with 22q11 microdeletions manifest a number of psychiatric phenotypes, including schizophrenia and OCD (9, 10). A more recent follow-up study (11) on behavioral phenotypes observed in patients with the 22q11 deletion reported OC symptoms in the majority of these patients, thus providing even stronger evidence that the 22q11 locus harbors gene(s) predisposing to OCD. The gene for catechol-*O*-methyltransferase (COMT) (12, 13), which is involved in the inactivation of catecholamines including the neurotransmitter dopamine (14), maps to the 22q11 region (15), and is frequently deleted in patients with 22q11 microdeletions (9). *comt* therefore represents an attractive candidate gene for OCD.

MATERIALS AND METHODS

Diagnostic Criteria—OCD Case Sample. The subjects in this study are genetically unrelated individuals who were recruited from the National Institute of Mental Health Adult OCD Outpatient Clinic and from local private practitioners. All subjects met criteria from the *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition* (*DSM-III-R*) (16) for OCD. Subjects genotyped for this study were all caucasians. Since variation of the COMT activity among different races has been described (17), we were careful to exclude two Asian subjects from the study. Presence of tics was assessed during the clinical evaluation on 68 of the 73 OCD subjects. Eighteen (13 males and 5 females) were found to have comorbid tics. Appropriate informed consent was obtained from all patients. Forty milliliters of blood was drawn from all participating individuals and DNA was extracted by using the Blood and Cell Culture DNA midi-kit (Qiagen, Chatsworth, CA).

Control Sample. The control sample was composed of 148 ethnically matched and unrelated caucasians (75 males and 73 females), who were recruited from the same location as the case sample (Washington, DC, metropolitan area). Diagnoses were made from SADS-L (18) psychiatric interviews according to criteria from the *DSM-III-R*. All diagnoses were made by two raters who were blind to subject identities, and diagnostic differences were resolved in a consensus conference. All control subjects were adults passed the age considered to be at risk for most major psychiatric disorders. Appropriate informed consent was obtained.

Genotypings. For detection of the *Nla*III polymorphism in codon 158, the following primers were used: F, 5′-TCACCATCGAGATCAACCCC; and R, 5′-ACAACGGGTCAGGCATGCA. PCR was performed with the thermostable enzyme *Taq* polymerase (1.5 units per sample) (AmpliTaq, Perkin–Elmer/Cetus) and a programmable PCR apparatus (MJ Research, Cambridge, MA). Target sequences were amplified in a 10-μl reaction mixture containing 100 ng of genomic DNA in 75 mM KCl/10 mM Tris·HCl, pH 9.2/1.5 mM MgCl$_2$/5 pmol of each primer/100 mM of each dNTP (dATP, dCTP, dGTP, dTTP)/[^{32}P]dCTP. Amplification was as follows: 94°C × 3 min (1×), 94°C × 30 sec/64°C × 1 min/72°C × 1 min (35×), 72°C × 7 min (1×), 4°C × 5 min. The amplified product (5 μl) was digested with *Nla*III in a 10-μl reaction volume, according to the manufacturer's specifications. The digested product was diluted 1:1 with formamide-dye, denatured at 95°C × 5 min, and electrophoresed in a 6% 1 × TBE polyacrylamide gel, at 1,200 V × 2.5 h at room temperature.

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Gels were dried and signal was detected by overnight autoradiography without an intensifying screen.

RESULTS

Unlike simple mendelian disorders caused by highly penetrant but rare functional polymorphisms (mutations) in a single gene, the genetic component of complex psychiatric disorders is likely to be associated with low penetrance but common functional variations in a number of susceptibility genes. In humans, a common polymorphism is associated with a 3- to 4-fold variation in COMT enzyme activity. It has been recently shown that this variation in activity is due to a $G \rightarrow A$ transition at codon 158 of the *comt* gene that results in a valine (Val)-to-methionine (Met) substitution (19–22). The two alleles [Val[158] or High (H) activity, and Met[158] or Low (L) activity] and the three genotypes [Val[158]/Val[158] or High/High (H/H); Val[158]/Met[158] or High/Low (H/L); Met[158]/Met[158] or Low/Low (L/L)] can be identified with a PCR-based restriction fragment length polymorphism analysis using the restriction enzyme *Nla*III (22).

We tested the distribution of the *comt* genotypes (H/H, H/L, L/L) and alleles (H, L) in an OCD sample (Table 1), consisting of 73 caucasian subjects (42 males and 31 females), and a control group consisting of 148 ethnically matched caucasians (75 males and 73 females), recruited from the same location as the case sample (Washington, DC, metropolitan area), and screened using the SADS-L structured psychiatric diagnostic interview (18) to ensure there was no history of psychiatric illness. A χ^2 test of homogeneity in genotype distributions (Table 2) among cases and controls and males and females was highly significant ($\chi^2 = 27.91$, 6 df, $P = 0.0001$). Partitioning of χ^2 into three components with 2 df each provided the following empirical significance levels: $P = 0.0222$ for the Disease main effect, $P = 0.0745$ for the Sex main effect, and $P = 0.0005$ for the Disease-by-Sex interaction. Because of the high significance of the interaction term, Disease effects were investigated separately for males and females, resulting in $P = 0.0002$ for males and $P = 0.0658$ for females (Table 2). Clearly, association between *comt* and disease is highly significant in males, but not very pronounced (not formally significant) in females. Thus, we computed odds ratios (approximate relative risks) in males relative to one genotype over another. Results in Table 3 show that genotypes H/L and H/H do not differ significantly from each other in their effect as risk factors for disease, whereas the L/L genotype appears to be a strong risk factor. Thus, the Met[158] (low activity) allele appears to act in a recessive manner. Pooling genotypes H/L and H/H leads to an approximate relative risk of 5.91 for genotype L/L versus non-L/L (last line in Table 3).

Analogous results were obtained when analyses were carried out for alleles rather than genotypes (Tables 4 and 5). Again, association is essentially confined to males ($\chi^2 = 13.68$, $P = 0.0002$) and is highly significant. Confidence intervals for the approximate relative risks in Table 3 are somewhat wide, partly reflecting limits on our sample size. For genotype L/L as a risk factor (when contrasted to non-L/L genotypes), although the estimated relative risk is 5.91, the true value may range from 2.4 through 14.5 (95% confidence). On the other hand,

Table 1. Genotype distribution at *comt* gene by disease and sex

Genotypes	OCD sample		Control sample	
	Males	Females	Males	Females
L/L	20 (0.48)	3 (0.10)	10 (0.13)	16 (0.22)
L/H	17 (0.40)	19 (0.61)	44 (0.59)	29 (0.40)
H/H	5 (0.12)	9 (0.29)	21 (0.28)	28 (0.38)
	42	31	75	73

Table 2. Chi-square test of homogeneity in genotype distributions at *comt* gene

Source	χ^2	df	$2P$
Disease	7.61	2	0.0222
Sex	5.19	2	0.0745
D $\times$ S	15.11	2	0.0005
Total	27.91	6	0.0001

Males ($n = 117$), $\chi^2 = 17.19$, $2P = 0.0002$; females ($n = 104$), $\chi^2 = 5.44$, $2P = 0.0658$.

genotypic and allelic associations are quite consistent, which is not reflected in the confidence intervals. Thus, the width of these intervals provides a conservative picture of the real situation.

In addition, as noted above, dopamine antagonist augmentation of serotonin reuptake inhibitor treatment appears to be preferentially (but not exclusively) effective in OCD patients with comorbid chronic tic disorder (8), who are more frequently males (23). Our sample includes only 13 male patients with comorbid tics, but it is interesting to note a trend for an increased frequency of comorbid tic disorders among males with the L/L genotype than among males with non-L/L genotype in this sample [46% vs. 26%, odds ratio = 2.24 (0.56, 8.91)] (data not shown).

DISCUSSION

The results presented in this report along with the drug response data mentioned above suggest that dopaminergic and/or noradrenergic neurotransmission systems, and more specifically neurotransmitter inactivation by COMT, may be involved in the pathophysiology of OCD and could be targeted in the treatment of this disease. Studies using COMT inhibitors have provided some evidence to support an involvement of this enzyme in terminating the action of dopamine, as well as norepinephrine at central nervous system synapses (24–27). To our knowledge, however, no systematic studies have correlated the effect on neurotransmitter clearance with behavioral phenotypes. The availability of an animal model in which the *comt* gene is deleted by gene-targeting approaches (J.A.G. and M.K., unpublished data) will help address these issues. We should note, however, that close linkage with a nearby disease susceptibility locus cannot be excluded at this point.

The highly significant association between the Met[158] low activity (L) *comt* allele, the L/L genotype, and OCD in males, means that this result is unlikely to have arisen by chance alone. A nonrandom association between a genetic polymorphism and a phenotype can arise because of a direct relationship or close linkage between the gene and the disease phenotype, or because subpopulations that have different frequencies of the polymorphism also happen to differ in average susceptibility to the disease (population stratification). We note, however, that the sex-selectivity of the association observed in our sample makes it highly unlikely that it is the result of biases related to population-stratification effects. Of course, transmission of the *comt* alleles in OCD patients needs to be tested within families as well. In addition, it is important to emphasize that our control sample has been evaluated psychiatrically. The ob-

Table 3. Odds ratio (approximate relative risk) for disease with genotype as a risk factor (confidence interval computed as logit limits)

Genotype	Odds ratio	95% confidence interval
L/L vs. H/L	5.18	(2.02, 13.29)
H/L vs. H/H	1.62	(0.53, 5.00)
L/L vs. H/H	8.40	(2.44, 28.91)
L/L vs. (H/L and H/H)	5.91	(2.40, 14.53)

Table 4.   Allele distribution at *comt* gene by disease and sex

Alleles	OCD sample		Control sample	
	Males	Females	Males	Females
L	57 (0.68)	25 (0.40)	64 (0.43)	61 (0.42)
H	27 (0.32)	37 (0.60)	86 (0.57)	85 (0.58)

served frequencies of the *comt* alleles in this control sample were: high activity allele = 0.58, and low activity allele = 0.42, and they did not differ between males (0.57/0.43) and females (0.58/0.42). The observed frequencies are in very close agreement with frequencies previously reported for another North American caucasian control sample that had also been evaluated psychiatrically (28), as well as for a European sample of Spanish origin, where psychiatric illness had also been excluded (29). Another caucasian control sample of British origin that was *not* psychiatrically evaluated has been recently described (30) with a higher frequency of the low activity allele in both males and females (0.53). To our knowledge, however, frequencies as high as 0.68 for the low activity allele, such as the one observed in our male OCD sample, have not been reported even in earlier determinations of allele frequencies based on enzymatic assays (31). Indeed, we note that even after considering the allele frequencies of the British control sample, the male-specific association described here still remains significant ($P < 0.05$). The results presented in this report are further supported by psychiatric evaluation of patients with 22q11 microdeletions encompassing the *comt* gene (9–11). As mentioned in the Introduction, the most recent study on psychiatric symptoms and disorders present in patients with 22q11 microdeletions (11) reported a high frequency of obsessive-compulsive symptoms among patients with this deletion. Moreover, 2 of the 25 patients (13 males and 12 females) also met full criteria for obsessive-compulsive disorder. Although the numbers were small, it is of interest to note that both patients who met the full criteria for OCD were male and had the low activity *comt* allele on the intact chromosome (11, 28). The observation that 17.6% of psychiatrically evaluated healthy individuals are carriers of the L/L genotype suggests that inheritance of the disease is not simple but involves most likely a number of susceptibility genes and possibly environmental influences.

A biological basis for the observed sex-selective association remains to be defined. The following observations may be relevant. First, it has been reported that gonadal hormones can affect onset and severity of OCD. For example, there are reports of onset and exacerbation of OCD during pregnancy (32), when levels of gonadal steroids are markedly elevated, and reports of OCD symptom relief in women when gonadal steroids are suppressed (33). Second, comparisons of COMT activity and thermostability in hepatic tissue in humans indicated an epigenetically determined, significantly lower COMT activity in females (34). A limited number of studies in other species suggest that COMT activity can be reduced epigenetically by estrogens (35) and can be affected by the process of sexual differentiation of the brain (36, 37). Along these lines, it has been suggested (37) that estrogens could directly modulate neurotransmission and neuronal excitability through conversion into catecholestrogens, which could competitively

Table 5.   Chi-square test of homogeneity in allele distributions at *comt* gene

Source	χ^2	df	2P
Disease	7.84	1	0.0052
Sex	5.03	1	0.0250
D × S	5.89	1	0.0152
Total	18.76	3	0.0003

Males, $\chi^2 = 13.68$, $2P = 0.0002$; females, $\chi^2 = 0.01$, $2P = 0.9097$.

inhibit methylation of dopamine by COMT, resulting in an increase of dopamine concentration (38, 39).

It is conceivable that females have evolved mechanisms to compensate for their lower levels of COMT activity, and thus are relatively less vulnerable to develop OCD in association with the low activity COMT genotype (to a degree not detectable in our sample). Of course, other explanations are possible for the finding presented in this report. We note, for example, that estrogens are formed in the developing and adult male brain by the action of aromatase on testosterone and can mediate the effect of the male hormone (37). Aromatization is especially important in mediating the effects of testosterone surges in fetal life that are involved in the establishment of sex specificities in the central nervous system structure and function. A final testable possibility is that epigenetically challenged female homozygotes for the low activity COMT allele develop, in the context of a susceptible genotype, psychiatric disorders other than OCD.

The q11 band on chromosome 22 has been established as the smallest genomic interval (1.5 megabases) associated at present with a major psychiatric illness in a reproducible manner (9–11, 40, 41). This report extends our initial findings in this region by providing evidence that activity variation in one of the resident genes can explain aspects of the psychiatric phenotype associated with this genetic interval. It is of interest that previous studies on the *comt* allele distribution (22, 30, 42) failed to reveal a major effect of this gene on schizophrenia or bipolar disorder (29), suggesting diagnostic specificity of our finding and a complex contribution of the 22q11 region in the development of the psychiatric phenotype.

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