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Association Between the COMT Locus and Obsessive-Compulsive Disorder in Females But Not Males

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A polymorphism in the coding region of catechol-O-methyltransferase gene (COMT) was previously reported to be associated with obsessive-compulsive disorder (OCD), particularly in male probands. We attempted to replicate the previous finding using a family-based genetic design in haplotype relative risk (HRR) and transmission disequilibrium (TDT) analyses. Fifty-six OCD probands and their parents were genotyped for the COMT locus using established methods. Analysis of allele and genotype frequencies between the proband genotypes and the control (parental nontransmitted) genotypes failed to replicate the previous finding of gender divergence, gave no evidence of overall association, nor was linkage detected by TDT. However, further analysis of the COMT allele frequencies by proband gender gave evidence of a mildly significant association with the low-activity COMT allele in female probands ($P = 0.049$), but not in male probands. These findings indicate that COMT may be etiologically relevant to OCD in a gender-specific manner opposite to that shown in previous studies.

KEY WORDS: association study; haplotype relative risk; transmission disequilibrium; sexual dimorphism

INTRODUCTION

Obsessive-compulsive disorder (OCD) is characterized by recurring obsessions and/or compulsions [American Psychiatric Association, 1994] and has been estimated to affect nearly 5 million people in the United States [Karno et al., 1988] with a resultant economic impact of over $8 billion annually [DuPont et al., 1995; Hollander et al., 1998]. The etiology of this disorder remains largely unknown; converging lines of evidence from twin studies [Inouye, 1965; Carey and Gottesman, 1981; Torgersen, 1983; Rasmussen and Tsuang, 1986; Andrews et al., 1990], family genetic studies [Carey and Gottesman, 1981; Lenane et al., 1990; Bellodi et al., 1992; Black et al., 1992; Leonard et al., 1992; Nicolini et al., 1993; Pauls et al., 1995], and segregation analyses [Nicolini et al., 1991; Alsobrook et al., 1999; Cavalini et al., 1999] provide compelling evidence that OCD has a strong genetic component.

Recent efforts in the search for genes of etiologic importance in OCD have used the candidate gene approach [Catalano et al., 1994; Nicolini et al., 1996; Billett et al., 1997, 1998; Cruz et al., 1997; Cavalini et al., 1998], wherein current knowledge of disease pathology is used to make judicious a priori choices of genes for testing. Most of the studies to date have used an association paradigm with population-based controls, comparing allelic or genotypic frequencies between patients and an unrelated control sample. Such studies are of restricted utility because of the limitations inherent in case control designs. Molecular genetic studies that attempt to match index cases with population-based controls are highly sensitive to variation in population-specific allelic distributions. Frequently, improperly matched control samples result from limited phenotypic matching criteria such as gender and proband ethnicity; the case control design may be most reasonable when both samples are derived

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from the same genetically isolated population or are matched for ethnicity in multiple previous generations with a known genealogy. Positive findings from some studies that have used a case control paradigm have been fraught with difficulties [Gelernter et al., 1993].

A recent candidate gene analysis of OCD by Karayiorgou et al. [1997, 1999] reported positive findings for the COMT gene, which codes for the enzyme catechol-O-methyltransferase (EC 2.1.1.6), a component in the major degradative pathways of the neurotransmitters dopamine, epinephrine, and norepinephrine. The reported overall association of OCD and COMT was attributed to a gender-based association of the homozygous low-activity genotype of the COMT gene and risk for OCD in males. This is an intriguing finding given that there are very few reports indicating OCD exhibits gender-specific prevalence rates [Noshirvani et al., 1991]; indeed, family studies have failed to detect a gender-based differential relative risk among first-degree relatives of OCD probands. The present study was designed as a robust replication of the findings of Karayiorgou et al. [1997, 1999] by using a family-based genetic design to test for an association between COMT alleles with OCD. Our analyses used the haplotype relative risk (HRR) [Falk and Rubinstein, 1987; Terwilliger and Ott, 1992] and transmission disequilibrium test (TDT) methods [Ewens and Spielman, 1995]; by using nontransmitted parental alleles as a control sample, HRR analysis is not subject to the type I errors inherent in case control designs [Ewens and Spielman, 1995] due to hidden variations in population-specific allelic distributions.

MATERIALS AND METHODS

Sample Ascertainment

Probands were ascertained through OCD clinics at three sites: Israel; Paris, France; and New Haven, Connecticut. All probands met criteria for full OCD [American Psychiatric Association, 1987, 1994]. Informed consent was obtained from all probands and permission was obtained to contact parents; informed consent was then obtained from parents as well. In the case of probands less than 18 years of age, the proband's assent and parental consent were obtained. Diagnoses of OCD were made by direct clinical observation along with data obtained by a direct standardized interview (STOBS) [Spitzer et al., 1990; Pauls and Hurst, 1994]. The same interview was used at all three sites; it has previously been translated into Hebrew and French, then back-translated to confirm validity.

Genotyping

Blood samples or buccal swab samples were obtained from all probands and parents. DNA was isolated from blood essentially by the method of Lahiri and Nurnberger [1991]; DNA was isolated from buccal swabs using a Puregene kit (Gentra Systems, Minneapolis, MN). PCR and restriction digests were performed using primers and conditions essentially as given in Lachman et al. [1996] and modified by Palmatier et al. [1999]; one COMT primer was fluorescently tagged during oligosynthesis by a local university core facility, allowing detection of only the polymorphic *Nla*III restriction site. Following amplification and digestion, genotypes were determined by electrophoresis of samples in 4% Nu-Sieve 3:1 agarose (FMC BioProducts, Rockland, ME); detected allele sizes were 85 base pairs (the high-activity COMT allele) and 69 base pairs (the low-activity COMT allele). Genotypes were read directly from gel images.

Statistics

The SPSS statistical package (SPSS for Windows version 9.0) was used to store genotype information and perform calculations. HRR significance tests were by the Fisher exact; TDT significance tests were by the transmission disequilibrium chi-square [Ewens and Spielman, 1995]. The estimate of the sample's power is taken directly from the analytically derived power charts for an HRR sample of 50 families as in Terwilliger and Ott [1992]; our sample size of 56 probands and their parental controls is adequate to achieve $\geq 80\%$ power at $P = 0.05$ to detect a positive association.

RESULTS

We genotyped 56 probands with OCD (26 males and 30 females) and their 112 parents. The HRR method was used to construct a 2×2 contingency table (Table I) with the frequency counts of the high-activity and low-activity COMT alleles for probands and controls in each cell (haplotype-based HRR). This gave $P = 0.174$ (Fisher exact), indicating there was no significant difference in the distribution of alleles between the probands and controls. A comparison of genotypes between the probands and controls in a 2×3 table also indicated no significant difference (Pearson chi-square, $df = 2$, $P = 0.430$).

We then examined, in a similar fashion, the allele frequencies between male and female probands and their respective controls (Table I). Analysis of the male proband subset resulted in $P = 0.422$ (Fisher exact); analysis of the female proband subset resulted in $P = 0.048$ (Fisher exact), indicating that the COMT gene exhibits gender-divergent allele frequencies among OCD probands.

TABLE I. Haplotype-Based Haplotype Relative Risk Table of COMT Alleles

	High-activity allele, n (frequency)	Low-activity allele, n (frequency)
All probands	47 (0.21)	65 (0.29)
Controls	55 (0.25)	57 (0.25)
$P = 0.174$ (Fisher exact)		
Male probands	26 (0.25)	26 (0.25)
Controls	24 (0.23)	28 (0.27)
$P = 0.422$ (Fisher exact)		
Female probands	21 (0.21)	39 (0.29)
Controls	31 (0.25)	29 (0.25)
$P = 0.048$ (Fisher exact)		

The transmission disequilibrium test was used in an analogous manner to examine linkage to COMT in the overall sample and in the gender-specific subsets (Table II). The total sample had 78 informative transmissions, resulting in a chi-square$_{TD} = 0.82$, $P = 0.37$. Analysis of the male proband subset (40 informative transmissions) yielded a chi-square$_{TD} = 0.10$, $P = 0.75$, while analysis of the female proband subset (38 informative transmissions) yielded chi-square$_{TD} = 2.63$, $P = 0.105$.

DISCUSSION

The COMT genotypes of this sample of 56 OCD probands and their parents demonstrated overall allele frequencies similar to previous reports [Karayiorgou et al., 1997; Palmatier et al., 1999]. However, there was no evidence for an association between the COMT locus and OCD with the family sample taken as a whole. Interestingly, we found suggestive evidence of a differential allele distribution when comparing male and female subsets of the sample; the HRR gave a statistically significant association of the low-activity COMT allele in female but not male probands. For female probands, the TDT results showed a trend for linkage with the same low-activity allele that did not reach statistical significance, while for male probands there was no evidence for linkage. This is opposite to the findings of Karayiorgou et al. [1997], who demonstrated a significant association of the low-activity allele in male OCD probands. Thus, our main finding fails to replicate that earlier report.

While a larger sample size may be desirable, the present sample had $\geq 80\%$ power to detect a positive association of similar effect size as that of Karayiorgou et al. [1999]. It is possible for HRR analyses to exhibit type II error (rejecting an association when in fact one exists); this may be the case most often when there is an excess of homozygosity among parents, a condition that was not observed here. It was also possible that more information was available for female compared to male probands; however, this sample contained an equal number of families with male and female probands in the TDT analysis (26 each), with 38 informative transmissions for female probands and 40 informative transmissions for male probands. Thus, the sample did not appear to be inherently gender biased. It was also possible that, due to the limited sample size, heterogeneity among OCD subjects masked a true association; there is evidence that OCD has significant phenotypic subtypes [Alsobrook et al., 1999]. In that regard, one component of heterogeneity could be a differential age of onset distribution between the male and female probands. In our sample, the average age of OCD onset in males was 10.8 years, while that in females was 11.4 years; this is not a significant difference (t-test, df = 40, $P = 0.77$). Therefore, it is unlikely that age of onset heterogeneity contributes to the gender difference. Lastly, it remains possible that the conflicting findings from the small samples in this and the previous report represent false positives, with no true association.

There are several reports describing sexually dimorphic COMT activity in normal individuals [Boudikova et al., 1990], apparently due to differential hormonally regulated expression. Hippocampal [Ladosky et al., 1984] and hepatic COMT enzyme levels are reduced in females relative to males; hypothalamic levels, however, exhibit the opposite pattern with reduced levels in adult males [Ladosky et al., 1984]. Thus, women who inherit an allele or alleles for the reduced-activity isoform of COMT may have hippocampal enzyme activity levels that are even further reduced than males inheriting those same alleles. Those reduced activity levels may drop below a critical threshold, resulting in an increased susceptibility to OCD as was observed in our sample; a similar threshold model may apply to the hypothalamus. Both the hypothalamus and hippocampus communicate with the orbito-frontal cortex and associated frontal-striatal circuitry, regions that have been implicated in OCD [Fitzgerald et al., 1999]. The effect may not be specific for OCD, however. It is also possible that COMT acts in a more general manner, increasing overall susceptibility to psychiatric disorders in general, while other mechanisms (genetic or environmental) act in a disorder-specific manner. The low-activity COMT allele has been reported to be associated with OCD in two additional studies [Kinnear et al., 1999; Schindler et al., 1999], although with no observed gender divergence. COMT has also been shown to be associated with major depression and bipolar disorder. Given the multiple positive disease associations, COMT may represent a general susceptibility factor for psychiatric disorders, or a shared risk component across a smaller set of disorders.

While it appears that at least one locus may have an appreciable impact on the etiology of OCD, it is not a single-gene disease [Alsobrook et al., 1999]; there are very likely many molecular paths to a single observable

TABLE II. TDT for 78 Informative Transmissions of COMT Alleles

	High-activity allele, n (frequency)	Low-activity allele, n (frequency)
Total sample, number of transmissions Chi-square$_{TD} = 0.82$, $P = 0.365$	35 (0.45)	43 (0.55)
Male probands, number of transmissions Chi-square$_{TD} = 0.10$, $P = 0.752$	21 (0.53)	19 (0.47)
Female probands, number of transmissions Chi-square$_{TD} = 2.63$, $P = 0.105$	14 (0.37)	24 (0.63)

psychiatric outcome. The data presented here indicates that COMT may be a part of one of those paths.

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