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BRIEF REPORT

Association of the functional V158M catechol-*O*-methyl-transferase polymorphism with panic disorder in women

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Abstract

Panic disorder is an anxiety disorder with an estimated heritability of up to 48%. Pharmacological and genetic studies suggest that genes coding for proteins involved in the catecholaminergic system might be relevant for the pathogenesis of the disease. In the present study, we genotyped a single nucleotide polymorphism (472G/A = V158M) in the coding region of the catechol-*O*-methyl-transferase (COMT) gene in 115 patients with panic disorder and age- and sex-matched controls. Association analysis revealed a significant excess of the more active COMT allele (472G = V158) in patients with panic disorder ($p = 0.04$), particularly in female patients ($p = 0.01$), but not in male patients ($p = 1.0$). The assessment of a possible interaction of the COMT polymorphism with a previously reported functional 30-bp VNTR in the monoamine oxidase A promoter (MAOA–LPR) in female patients did not yield significant results. Our data support a role of the 472G/A (V158M) COMT polymorphism or a nearby locus in the pathogenesis of panic disorder in women.

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Key words: Association, COMT, gender, MAOA, panic disorder.

Introduction

Panic disorder is an anxiety disorder characterized by sudden and unexpected attacks of intense fear and anticipatory anxiety, often associated with agoraphobia, with a lifetime prevalence of 1–3%. Women are affected approximately twice as often as men (Weissman et al., 1997). Family and twin studies propose a strong genetic contribution to the pathogenesis of panic disorder with an estimated heritability of up to 48% (Hettema et al., 2001). Segregation studies, however, failed to identify a simple Mendelian pattern of inheritance (Vieland et al., 1996) and no major gene locus on the basis of genome-wide linkage studies has been published for panic disorder itself (Knowles et al., 1998; see, however, as part of wider, but at present still hypothetical syndromes: Gratacos et al., 2001; Hamilton et al., 2003). These findings have been interpreted as evidence for either genetic heterogeneity or complex inheritance with an interaction of environmental factors and multiple single genes.

The catechol-*O*-methyl-transferase (COMT) catalyses the inactivation of monoaminergic neurotransmitters by an extraneuronal transfer of a methyl group to catechol compounds. Significantly elevated erythrocyte COMT activity has been reported in patients with anxiety states (Shulman et al., 1978). In Parkinson's syndrome, which is successfully treated with COMT inhibitors, panic attacks have been observed to occur in up to 40% of patients (Richard et al., 1996). A single-nucleotide polymorphism (472G/A) in the COMT gene, mapped to chromosome 22q11.2 (Winqvist et al., 1991), causes an amino-acid change

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Table 1. Demographic and clinical data on patients and control subjects

Samples	*n*	Female	Male	Mean age (yr) (S.D.)
Patients (total)	115	74	41	37.8 (±11.0)
Without agoraphobia	32	16	16	38.8 (±11.2)
With agoraphobia	83	58	25	37.4 (±11.0)
Controls	115	74	41	41.5 (±10.6)

from valine to methionine at position 158 (V158M) of the membrane-bound enzyme and the valine allele (472G) has been reported to be associated with a 3- to 4-fold higher COMT activity compared to the methionine allele (472A) (Lachman et al., 1996). To date, three association studies of this V158M polymorphism with panic disorder have been performed with contradictory results of no association (Ohara et al., 1998), an association with the high activity valine allele in female patients (Hamilton et al., 2002) and an association with the low activity methionine allele (Woo et al., 2002). Additionally, a study by Rotondo et al. (2002) reported a significant association of the less active methionine allele of the V158M COMT polymorphism in patients with bipolar disorder without panic disorder, whereas there was no such effect in patients with bipolar disorder and concurrent panic disorder.

The monoamine oxidase A (MAOA) degrades monoamines by deamination. In patients with clinical anxiety, significantly elevated levels of platelet MAOA were reported (Mathew et al., 1980). Additionally, MAOA inhibitors are effectively used in the pharmacological treatment of panic disorder (Tyrer and Shawcross, 1988). A 30-bp VNTR (MAOA–LPR) present in 3, 3.5 (3a), 4, or 5 copies has been described in the promoter of the MAOA gene located on chromosome Xp11 (Sabol et al., 1998). The functionally more active longer alleles (3a, 4 and 5) were found to be significantly associated with panic disorder in the female subgroups of German and Italian patients (Deckert et al., 1999). Since MAOA and COMT both degrade noradrenaline and can mutually compensate for blockade in the respective other enzymatic pathway (Eisenhofer and Finberg, 1994), the more active MAOA and COMT polymorphisms described above could potentially show interactional effects in the pathogenesis of panic disorder.

In the present study, we looked for a possible association of the V158M COMT polymorphism with panic disorder and investigated a potential interaction with the previously reported MAOA–LPR in our case-control sample of 115 patients with panic disorder. Since family studies suggest differential underlying genetic patterns of transmission with regard to gender and the presence of agoraphobia (Maier et al., 1993), we also analysed subsamples differentiated for gender and the presence of agoraphobic avoidance.

Method

A sample of 115 unrelated Caucasian patients of German descent with panic disorder was investigated in this study (demographic and clinical data are shown in Table 1). In these patients, panic disorder was diagnosed by experienced psychiatrists on the basis of structured clinical interviews according to the criteria of DSM-III-R or DSM-IV [SADS-LA (Mannuzza et al., 1986) and CIDI (Robins et al., 1988; Wittchen et al., 1997)] and a review of medical records. Comorbid bipolar disorder was excluded in the patients under study. The patients diagnosed according to DSM-III-R criteria had already been included in a number of previous studies conducted by the corresponding author (Deckert et al., 1999; Steinlein et al., 1997). The control group consisted of 115 unrelated anonymous blood donors of German descent, who were matched according to gender and age (Table 1), but due to anonymity could not be controlled for the presence of psychiatric diseases. The study was approved by the respective local ethical committees and informed consent was obtained from all participating subjects.

Fragments containing the V158M COMT polymorphism were amplified with primers COMT-F (5′-TCACCATCGAGATCAACCCC) and COMT-R (5′-ACAACGGGTCAGGCATGCA). Standard PCR was carried out in a 25 μl vol containing 60 ng of genomic DNA, 10 pmol of each primer, 200 μM dNTPs, 1 U HotStarTaq™ DNA polymerase (Qiagen GmbH, Hilden, Germany), 50 mM KCl, 1.5 mM $MgCl_2$ and 10 mM Tris–HCl (pH 8.4). After an initial 15-min denaturation at 94 °C, 35 cycles were carried out consisting of 40 s at 94 °C, 40 s at the annealing temperature of 53 °C and 60 s at 72 °C, followed by a final extension time of 10 min at 72 °C in a Gene Amp PCR System 9700. Genotyping was performed by means of a restriction

Table 2. Allele and genotype frequencies of the 472G/A (V158M) COMT polymorphism in patients with panic disorder and controls

Samples diagnosis		COMT alleles G	COMT alleles A	χ^2 test[a]	COMT genotypes G/G	COMT genotypes G/A	COMT genotypes A/A	Armitage trend test[b]
Patients Panic total	Total ($n=115$)	117	113	$\chi^2=4.234$ $p=0.0396$*	28	61	26	$Z=-2.0920$ $p=0.0364$*
	Female ($n=74$)	80	68	$\chi^2=6.5705$ $p=0.0104$*	20	40	14	$Z=-2.6050$ $p=0.0092$*
	Male ($n=41$)	37	45	$\chi^2=0.0000$ $p=1.0000$	8	21	12	$Z=0.0000$ $p=1.0000$
Patients Panic without agoraphobia	Total ($n=32$)	32	32	$\chi^2=1.5429$ $p=0.2142$	7	18	7	$Z=-1.2675$ $p=0.2050$
	Female ($n=16$)	19	13	$\chi^2=4.3797$ $p=0.0364$*	4	11	1	$p=0.0386$*[c]
	Male ($n=16$)	13	19	$\chi^2=0.1890$ $p=0.6637$	3	7	6	$Z=0.4342$ $p=0.6641$
Patients Panic with agoraphobia	Total ($n=83$)	85	81	$\chi^2=3.8117$ $p=0.0509$*	21	43	19	$Z=-1.9707$ $p=0.0488$*
	Female ($n=58$)	61	55	$\chi^2=4.7144$ $p=0.0299$*	16	29	13	$Z=-2.1653$ $p=0.0304$*
	Male ($n=25$)	24	26	$\chi^2=0.1035$ $p=0.7477$	5	14	6	$Z=-0.3330$ $p=0.7391$
Controls	Total ($n=115$)	95	135		19	57	39	
	Female ($n=74$)	58	90		11	36	27	
	Male ($n=41$)	37	45		8	21	12	

[a] Compared to controls; d.f. = 1.
[b] Compared to controls; 2-sided.
[c] Fisher's exact test.
* Significant (p value $\leqslant 0.05$).

fragment length polymorphism (RFLP) assay with the restriction enzyme *Nla*III (2 U) as recommended by the manufacturer (New England Biolabs, Frankfurt, Germany) resulting in 65-, 18- and 13-bp bands for the A allele and 83- and 13-bp bands for the G allele respectively. A total of 8 μl of the digested product were mixed with 12 μl denaturing solution as described above and separated for 3 h on a 15% polyacrylamide gel (acrylamide:bisacrylamide = 49:1; Multigel-Long/Biometra, Göttingen, Germany) containing 1 × TBE at 20 V/cm. Bands were visualized by silver-staining. The MAOA–LPR was genotyped according to the published protocol (Deckert et al., 1999).

Differences in allele and genotype frequencies between cases and controls were analysed using χ^2 tests for alleles and Armitage trend tests or Fisher's exact tests for genotypes (Sasieni, 1997). Interaction of the two polymorphisms was explored by conditional logistic regression analysis, controlling for age differences in cases and controls. All statistics were calculated with the SAS statistical package (SAS/STAT, version 8.1, Cary, NC: SAS Institute Inc., 1999). Hardy–Weinberg equilibrium was examined by the program Finetti (Wienker TF, personal communication: April 2003).

Results

The genotype distribution of the V158M COMT polymorphism in the total control sample ($p=0.81$), in the total patient sample ($p=0.51$) and also in each of the male and female subsamples did not significantly differ from the expected numbers calculated on the basis of the observed allele frequencies according to Hardy–Weinberg equilibrium.

The allele and genotype frequencies for the V158M COMT polymorphism in patients and controls are shown in Table 2. Analysis of the allele and genotype distribution in the overall sample ($n=115$) revealed an association of the more active valine allele with panic disorder (alleles: χ^2 test, $p=0.0396$; genotypes: Armitage trend test, $p=0.0364$). In subsequent analyses differentiated for gender, female patients ($n=74$) showed an excess of the valine allele (alleles: χ^2 test, $p=0.0104$; genotypes: Armitage trend test, $p=0.0092$), whereas there was no such effect in the male subgroup of patients (alleles: χ^2 test, $p=1.0000$; genotypes: Armitage trend test, $p=1.0000$). Similarly, in female patients of both subgroups either with or without concurrent agoraphobia, we found an association of the valine allele [panic disorder with agoraphobia (alleles: χ^2 test, $p=0.0299$; genotypes: Armitage trend test, $p=0.0304$); panic disorder without agoraphobia (alleles: χ^2 test, $p=0.0364$; genotypes: Fisher's exact test, $p=0.0386$)]. In the total subgroup of male and female patients with panic disorder and concurrent agoraphobia, a trend towards an association of the valine allele was observed (alleles: χ^2 test, $p=0.0509$; genotypes: Armitage trend test, $p=0.0488$), which could not be found in the total subgroup of patients with pure panic disorder without agoraphobia (alleles: χ^2 test, $p=0.2142$; genotypes: Armitage trend test, $p=0.2050$). No significant associations could be identified in all other subgroups of patients.

Additionally, we performed a combined analysis of the distribution of the V158M COMT polymorphism and the MAOA–LPR in the female subgroup of patients ($n=74$). We found no evidence for an interaction of these two polymorphisms (COMT × MAOA: Wald $\chi^2=0.77$, $p=0.3814$).

Discussion

Our data of an association between the more active valine allele of the V158M COMT polymorphism and panic disorder, particularly in female patients, confirm the finding by Hamilton et al. (2002), who observed this association in women, but not in men in a family-based sample minimizing stratification effects. Both results suggest that there might be a gender-specific effect to the relevance of an increased catecholaminergic metabolism in the pathogenesis of panic disorder. However, the lack of an effect in our male subsample obviously could also be due to low power of the smaller male subsample.

In addition, we have observed that the more active allele of the COMT polymorphism might be preferably associated with panic disorder and agoraphobia compared to pure panic disorder without agoraphobia. This finding most certainly needs further assessment in larger, independent samples as it may just have been due to different sizes of the subsamples. Since MAOA inhibitors, however, have been reported to be efficient in the pharmacological treatment of panic disorder particularly with concurrent agoraphobia (Tyrer and Shawcross, 1988), it might be interesting to investigate, whether COMT inhibitors, increasing catecholamine levels like MAOA inhibitors, exhibit a similar differential effect.

Our results and those of Hamilton et al. (2002) are in line with clinical reports of increased COMT activity in anxious and depressed patients (Shulman et al., 1978) and a proposed clinical efficacy of COMT inhibitors for anxiety in Parkinson patients (Richard et al., 1996) as well as in depressed patients (Fava et al., 1999). Our results, however, are in contrast with the studies by Ohara et al. (1998) and Woo et al. (2002). The latter report of an association between the less active methionine allele and panic disorder is consistent with the observations in female COMT knock-out mice (Gogos et al., 1998). Both Asian studies, however, investigated considerably smaller samples ($n=29$ and $n=51$) than the American (70 multiplex families) and our study ($n=115$), which decreased the power of these studies and might have allowed for stratification effects. Moreover, it is not clear whether the control groups in these studies were adequately controlled for gender and age. Ethnic differences with regard to genetic background may also provide an explanation for the discrepant findings (Palmatier et al., 1999).

Our failure to find an interaction between the COMT and MAOA polymorphisms to increase the risk for panic disorder may have several causes. First, the size of the female subsample may have been too small to detect a small effect. Secondly, while both enzymes metabolize noradrenaline, MAOA additionally metabolizes serotonin and COMT dopamine. Therefore, under the assumption that in the pathogenesis of panic disorder the effects of MAOA might in fact be mediated by the serotonergic rather than the catecholaminergic system, MAOA and COMT would not necessarily have to interact with each other. Finally, as proposed by Hamilton et al. (2002) the V158M COMT polymorphism may not represent the clinically relevant polymorphism on chromosome 22q11.2, but reflect linkage disequilibrium with another nearby polymorphism. In different populations, this linkage disequilibrium might either affect the valine or the methionine allele. Such an interpretation would offer an alternative explanation for our failure to find an interaction between the more active valine allele of the

COMT polymorphism and the more active MAOA–LPR allele and also for the contradictory results in the association studies of the COMT polymorphism with panic disorder conducted so far. Fine mapping of 22q11.2 is required to test this hypothesis. Additionally, further studies might be conducted to probe the COMT polymorphism for association with more refined personality traits conferring vulnerability to anxiety rather than with the categorical psychiatric diagnosis of panic disorder (Henderson et al., 2000).

In conclusion, results from this study support the notion that the V158M COMT polymorphism, or a nearby locus on chromosome 22q11.2, contributes to the genetic susceptibility to panic disorder particularly in female patients with panic disorder. An interactional effect of the V158M COMT polymorphism and the MAOA–LPR in the pathogenesis of panic disorder is not supported by our study.

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Statement of Interest

None.

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