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BRIEF REPORT

Associations between serotonin-related gene polymorphisms and panic disorder

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Abstract

Studies suggest that vulnerability to panic attacks and panic disorder (PD) may be related to a deficient serotonin (5-HT) neurotransmission. In the present case-control study we investigated possible associations between PD phenotype and five candidate polymorphisms including 5-HT transporter (5-HTTLPR and VNTR), monoamine oxidase A (MAOA promoter region), tryptophan hydroxylase 1 (TPH1 218A/C) and 5-HT1B receptor (5-HT1BR 861G/C) genes. The study sample consisted of 158 patients with PD and 215 healthy control subjects. The analysis showed higher frequencies of LL genotype ($p=0.016$) and L allele variant ($p=0.007$) of 5-HTTLPR in the patients. No significant associations were observed between PD and other candidate gene polymorphisms. However, a higher frequency of longer allele genotypes of the MAOA promoter region was observed in female PD patients with agoraphobia than in female controls ($p=0.016$). These findings indicate that genetic variants conceivably related to lower 5-HT neurotransmission may be involved in the development of PD.

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Introduction

Panic disorder (PD) is a prevalent and disabling psychiatric condition characterized by recurrent panic attacks, anticipatory anxiety and the frequent development of agoraphobia. PD has high rates of co-occurrence with other psychiatric disorders, such as other anxiety and mood disorders (Weissman et al., 1997) indicating its complex neurobiological background. The data from twin and family studies suggest an involvement of genetic factors in the familial transmission of PD with the heritability estimate near 40% (Hettema et al., 2001); however the genetic factors responsible for the biological basis of PD have not yet been established.

Clinical and challenge studies point to the involvement of the serotonin (5-hydroxytryptamine, 5-HT) system in the neurobiology of PD. Treatment studies consistently demonstrate that medications increasing the synaptic availability of 5-HT, such as selective serotonin re-uptake inhibitors (SSRIs) and monoamine oxidase inhibitors, are effective in the treatment of PD (Bell and Nutt, 1998). Experimental studies show that lowering 5-HT levels by tryptophan depletion augments, whereas administration of the 5-HT precursor, 5-hydroxytryptophan (5-HTP), restrains panic response to panicogenic challenges in patients with PD (Schruers et al., 2000, 2002). These data support the hypothesis of a specific inhibitory influence of 5-HT in PD (Deakin and Graeff, 1991).

The availability of 5-HT in the brain depends on various factors including genetic regulation. Genes of particular interest are those coding for the key elements controlling 5-HT neurotransmission, such as 5-HT transporter (5-HTT), monoamine oxidase A (MAOA), tryptophan hydroxylase 1 (TPH1) and 5-HT1B receptor (5-HT1BR). Previous studies have indicated that certain variants in the promoter regions of 5-HTT and MAOA genes may influence 5-HT neurotransmission. For instance the long (L) allele variant of the 5-HTT linked polymorphic region (5-HTTLPR) was related to increased 5-HTT expression and 5-HT uptake in lymphoblasts (Lesch et al., 1996). Notably, the short (S) allele variant of 5-HTTLPR has been

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linked to anxiety-related traits in healthy subjects (Lesch et al., 1996) and there are ongoing efforts in defining the role of 5-HTTLPR in the risk for emotional traits and disorders. An upstream polymorphism of the MAOA gene has been shown to influence the activity and expression levels of MAOA. MAOA promoter region variants containing longer alleles displayed higher enzymic activity compared to shorter alleles (Deckert et al., 1999; Sabol et al., 1998). Clinically, a significant excess of functionally more active MAOA promoter alleles was observed in female, but not male patients with PD (Deckert et al., 1999). However, another family-based study did not find associations between PD and MAOA promoter polymorphism (Hamilton et al., 2000).

In the current study we aimed to detect possible associations between polymorphisms in the genes related to 5-HT neurotransmission and the PD phenotype. We hypothesized that the genetic variants conceivably reducing 5-HT neurotransmission, such as the long allele of 5-HTTLPR or longer alleles of the MAOA promoter region, could be associated with PD. In addition, we genotyped three other candidate polymorphisms, 5-HTT VNTR (Battersby et al., 1996), TPH1 218A/C (Nielsen et al., 1997) and 5-HT1BR 861G/C (Lappalainen et al., 1995), to explore their possible connection to PD.

Methods

Subjects

The study sample consisted of 158 patients (mean age 38.0 ± 12.9 yr, 80% females) recruited at the Clinic of Psychiatry of Tartu University Clinics and 215 healthy subjects recruited by newspaper advertisement in Tartu, Estonia. The Human Studies Ethics Committee of the University of Tartu approved the study protocol and all participants provided written informed consent. The diagnosis of PD according to DSM-IV criteria was verified using the Mini International Neuropsychiatric Interview (M.I.N.I. 5.0.0; Sheehan et al., 1998) and substantiated by psychiatric history and medical records. The interviews and diagnoses were made independently by two psychiatrists without knowledge of the genotypes of subjects. Concurrent agoraphobia was present in 87 (55%) of the patients. PD patients with history or current comorbidity with mood disorders or with other anxiety disorders were included in the study but no other psychiatric comorbidity was allowed. Among the patients 51% had comorbid major depression, 17% bipolar disorder and 3% other anxiety disorders, including social phobia and obsessive–compulsive disorder. The healthy subjects were matched to the patients by age and sex (mean age 39.8 ± 13.0 yr, 74% females). All subjects were interviewed using the M.I.N.I. and questioned about family psychiatric history. Only healthy subjects without personal or family history of psychiatric disorders among first-degree relatives were included in this study. The majority of the subjects were of Estonian ethnic background with a similar between-group distribution (98% among patients and 94% among controls). There were no significant differences in marital, occupational or smoking status between patients and controls.

DNA analysis

DNA was extracted from 5 ml of venous blood using a standard phenol–chloroform extraction. The polymorphisms were genotyped according to previously described protocols for 5-HT1BR 861G/C (Lappalainen et al., 1995), 5-HTT VNTR (Battersby et al., 1996), 5-HTTLPR (Lesch et al., 1996), TPH1 218A/C (Nielsen et al., 1997) and the MAOA promoter region (Sabol et al., 1998) respectively.

Data analysis

The genotype and allele frequencies between the patient and control groups were compared by χ^2 test using the software package STATISTICA 5.1 (StatSoft, Inc., Tulsa, OK, USA). Odds ratio (OR) values and 95% confidence intervals (CI) were calculated using STATA 6.0 (StataCorp, College Station, TX, USA). The association analyses were performed for all patients ($n = 158$), and separately for subgroups of those with agoraphobia ($n = 87$) and without agoraphobia ($n = 71$). The last two patient groups did not differ between each other by gender distribution, mean of age, per cent of ethnicity or comorbidity with other disorders. The results were considered as suggestively significant at the $p < 0.05$ level with a conservative estimation of significance after correction for multiple comparisons on four genes at the $p < 0.0125$ level.

Results

There were significant differences in the distribution of 5-HTTLPR genotypes and allele frequencies between patients and controls with the LL genotype and L allele variant being more frequent in patients (Table 1). The analysis in subgroups indicated that this difference was significant for both PD subgroups, except for the non-significant difference in genotypic frequency for PD patients with agoraphobia compared to controls (Table 1). The distributions of 5-HTTLPR genotypes

Table 1. Genotype and allele frequency distribution of 5-HTTLPR

		Genotype frequency (%)			Allele frequency (%)	
	n	SS	SL	LL	S	L
Total PD group	158	11 (6.9)	72 (45.6)	75 (47.5)	94 (29.7)	222 (70.3)
PD with AF	87	9 (10.3)	34 (39.1)	44 (50.6)	52 (29.9)	122 (70.1)
PD without AF	71	2 (2.8)	38 (53.5)	31 (43.7)	42 (29.6)	100 (70.4)
Controls	215	34 (15.8)	101 (47.0)	80 (37.2)	169 (39.3)	261 (60.7)
Statistics		$\chi^2=8.26$, df = 2, $p=0.016$[a]			$\chi^2=7.29$, df = 1, $p=0.007$[a]*	
		$\chi^2=4.86$, df = 2, $p=0.09$[b]			$\chi^2=4.73$, df = 1, $p=0.03$[b]**	
		$\chi^2=8.21$, df = 2, $p=0.017$[c]			$\chi^2=4.34$, df = 1, $p=0.04$[c]***	

PD, Panic disorder; AF, agoraphobia.
[a] Total PD group vs. controls; * OR 1.53, 95% CI 1.12–2.08.
[b] PD with AF vs. controls; ** OR 1.52, 95% CI 1.04–2.21.
[c] PD without AF vs. controls; *** OR 1.54, 95% CI 1.03–2.32.

Table 2. Genotype and allele frequency distribution of the MAOA promoter region polymorphism

		Allele frequency (%)				Genotype frequency (%)	
	n	1	2	3	4	All with 1 or 4	2–3/3–(3)
Total PD group	158	115 (40.5)	0 (0)	166 (58.5)	3 (1.0)	92 (58.2)	66 (41.8)
PD with AF	87	58 (37.4)	0 (0)	97 (62.6)	0 (0)	45 (51.7)	42 (48.3)
PD without AF	71	57 (44.2)	0 (0)	69 (53.5)	3 (2.3)	47 (66.2)	24 (33.8)
Controls	215	151 (40.3)	2 (0.5)	218 (58.1)	4 (1.1)	130 (60.5)	85 (39.5)
Statistics		$\chi^2=1.52$, df = 3, $p=0.68$[a]				$\chi^2=0.19$, df = 1, $p=0.66$[a]*	
		$\chi^2=3.07$, df = 3, $p=0.38$[b]				$\chi^2=1.94$, df = 1, $p=0.16$[b]**	
		$\chi^2=2.50$, df = 3, $p=0.48$[c]				$\chi^2=0.74$, df = 1, $p=0.39$[c]***	

PD, Panic disorder; AF, agoraphobia.
[a] Total PD group vs. controls; * OR 1.10, 95% CI 0.72–1.67.
[b] PD with AF vs. controls; ** OR 1.43, 95% CI 0.86–2.35.
[c] PD without AF vs. controls; *** OR 0.78, 95% CI, 0.45–1.37.

for the whole PD group, PD with agoraphobia group and controls were in agreement with Hardy–Weinberg equilibrium (HWE) ($p=0.26$, 0.53 and 0.82 respectively), but the frequency of this genotype significantly deviated from HWE in the PD without agoraphobia subgroup ($p=0.02$). Additionally, the comparison according to the functional classification of Lesch et al. (1996) of LL genotype vs. SS and SL genotypes indicated significant differences in genotypic frequencies between the total PD group and controls ($\chi^2=3.95$, df = 1, $p=0.047$; OR 1.53, 95% CI 1.01–2.31) as well as between the PD with agoraphobia group and controls ($\chi^2=4.57$, df = 1, $p=0.03$; OR 2.25, 95% CI 1.33–3.81).

The comparisons of MAOA promoter region variants between the groups were made according to functional classification of Sabol et al. (1998). There were no significant differences in genotype or allele frequencies of MAOA promoter region polymorphism between the patients and controls (Table 2). Separate analysis of this polymorphism in females did not show any significant difference in allele frequencies of MAOA promoter region polymorphisms between PD groups and controls (Table 3). However, a significantly higher frequency of functionally more active genotypes (3–3 or 2–3) of MAOA promoter region was observed in PD female patients with agoraphobia, but not in other female PD groups (Table 3).

Finally, no differences between patients and controls were found in allele or genotype distributions of 5-HTT VNTR, TPH1 218A/C or 5-HT1BR 861G/C polymorphisms for any of the studied groups

Table 3. Genotype and allele frequency distribution of MAOA promoter region polymorphism in females

		Allele frequency (%)				Genotype frequency (%)	
	n	1	2	3	4	All with 1 or 4	2–3/3–3
All PD females	126	98 (38.9)	0 (0)	151 (59.9)	3 (1.2)	75 (59.5)	51 (40.5)
PD with AF	68	46 (33.8)	0 (0)	90 (66.2)	0 (0)	33 (48.5)	35 (51.5)
PD without AF	58	52 (44.8)	0 (0)	61 (52.6)	3 (2.6)	42 (72.4)	16 (27.6)
Female controls	160	126 (39.4)	2 (0.6)	188 (58.8)	4 (1.2)	105 (65.6)	55 (34.4)
Statistics			$\chi^2=1.62$, df = 3, $p=0.66$[a]			$\chi^2=1.13$, df = 1, $p=0.29$[a]*	
			$\chi^2=4.19$, df = 3, $p=0.24$[b]			$\chi^2=5.84$, df = 1, $p=0.016$[b]**	
			$\chi^2=2.86$, df = 3, $p=0.41$[c]			$\chi^2=0.89$, df = 1, $p=0.34$[c]***	

PD, Panic disorder; AF, agoraphobia.

[a] All PD females vs. female controls; * OR 1.30, 95% CI, 0.80–2.10.

[b] PD females with AF vs. female controls; ** OR 2.02, 95% CI 1.14–3.59.

[c] PD females without AF vs. female controls; *** OR 0.73, 95% CI 0.38–1.41.

($p > 0.05$; data are available from the authors upon request). Also the distribution of the genotypes of these polymorphisms did not deviate from HWE in patients or controls. After correction for multiple comparisons on four genes, only the finding of a higher frequency of the L allele in 5-HTTLPR in patients remained statistically significant ($p < 0.0125$).

Discussion

5-HTT determines the magnitude and duration of postsynaptic receptor-mediated signalling, thus playing a pivotal role in the fine-tuning of 5-HT neurotransmission (Lesch and Mössner, 1998). A number of studies have attempted to find associations between 5-HTTLPR variants and psychiatric disorders. The results of both previous case-control and family-based association studies argued against a major role of 5-HTTLPR in PD (Deckert et al., 1997; Hamilton et al., 1999). However, Hamilton et al. (1999) have detected a more frequent occurrence of the 5-HTTLPR LL genotype in female PD probands compared to female controls. This finding is in line with our present results showing an association between the L allele as well as the LL genotype and PD. Thus, the 5-HTTLPR LL genotype that is related to a higher re-uptake of 5-HT may be a factor in the predisposition to PD. However, the history of or current comorbidity with mood disorders in our sample confounds the role of the 5-HTTLPR long allele genotype in PD. Considering that previous association studies in mood disorders have shown either lack of association with 5-HTTLPR variants (Minov et al., 2001; Rees et al., 1997) or positive association with short, but not long alleles of 5-HTTLPR (Hauser et al., 2003) it is not likely that our findings could be attributed to comorbidity with mood disorders. Interestingly, the presence of the long allele of 5-HTTLPR was found to be more frequent in excessively shy children (Arbelle et al., 2003) and in patients with obsessive–compulsive disorder (Bengel et al., 1999), implicating this gene variant in the development of pathological anxiety. Nevertheless, in healthy volunteers the short allele of 5-HTTLPR has been previously associated with anxiety-related personality traits (Lesch et al., 1996) and with a greater activation of the amygdala in response to fearful face stimuli (Hariri et al., 2002). Thus, lower activity of 5-HTT may be linked to anxiety proneness which is opposite to the anti-panic direction of this genotype in our results. Possibly, this discrepancy may be explained by different roles of the 5-HT system in the neuronal circuits of anxiety and panic attacks as proposed by Deakin and Graeff (1991). Further investigations and integrative approaches involving panic challenge and genetic studies are needed to explain the functional role of 5-HTT gene polymorphisms in different traits of fear and anxiety.

Similarly to previous findings of Deckert et al. (1999) we observed an excess of functionally more active MAOA promoter alleles in females with PD with agoraphobia than in female controls. However this association was not seen in the total group of PD females. Earlier, Inada et al. (2003) found a significant association with the 5-HT2A receptor gene 102T/C polymorphism in PD patients with agoraphobia, but not in the PD subgroup without agoraphobia. In a recent case-control study, Rothe et al. (2004) found a significant association between the 5-HT1A receptor gene 1019C/G polymorphism and PD with agoraphobia, but not in the total sample of PD

patients. These findings are in line with the data of Noyes et al. (1986) suggesting that PD with agoraphobia is a more severe variant of PD with a stronger genetic component.

In conclusion, we have detected an over-representation of the high-expressing variant of 5-HTTLPR in patients with PD and a higher frequency of transcriptionally more active MAOA promoter polymorphism in the subgroup of female PD patients with agoraphobia. These findings are in agreement with the hypothesis that genetic variants related to lower 5-HT neurotransmission are associated with PD. On the other hand, the non-functional serotonergic polymorphisms 5-HTT VNTR, TPH1 218A/C and 5-HT1BR 861G/C were not associated with PD, probably reflecting lack of influence of these polymorphisms on 5-HT neurotransmission. Interestingly, some recent studies have suggested that a newly identified TPH gene isoform 2 (TPH2), rather than TPH1, is preferentially expressed in the neuronal tissue and has functional polymorphisms involved in the regulation of the brain 5-HT synthesis (Zhang et al., 2004). These data indicate the importance of the inclusion of TPH2 polymorphisms in further genetic association studies in anxiety disorders.

It should be noted that after correction for multiple comparisons only results at significance level $p < 0.0125$ should be considered as significant. Therefore, the probability of false-positive findings on significance level $p < 0.05$ due to type I error could not be excluded. Another shortcoming of our study is a relative excess of female subjects. A reason of this disproportion seemed to be the exclusion of male PD patients with concurrent alcohol use disorders from our study. However, this also reflects higher rates of PD and probably a better response to recruitment in females. Other limitations of this study are relatively small sample size and significant comorbidity with mood disorders. Thus, additional larger studies are needed to validate and understand the association between 5-HTT and MAOA functional polymorphisms and PD.

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Statement of Interest

None.

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