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BRIEF REPORT

Association of a functional −1019C>G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia

Claudia Rothe[1], Lise Gutknecht[2], Christine Freitag[8], Ralf Tauber[3], Rainald Mössner[2], Petra Franke[4], Jürgen Fritze[5], Gerd Wagner[3], Gregor Peikert[3], Berit Wenda[3], Philipp Sand[2], Christian Jacob[2], Marcella Rietschel[4,6], Markus M. Nöthen[9], Henk Garritsen[7], Rolf Fimmers[8], Jürgen Deckert[1] and Klaus-Peter Lesch[2]

Departments of Psychiatry, University of [1] *Münster,* [2] *Würzburg,* [3] *Jena,* [4] *Bonn,* [5] *Frankfurt*
[6] *Central Institute of Mental Health, Mannheim, Germany*
[7] *Institute of Transfusion Medicine and Transplantation Immunology, University of Münster, Germany*
[8] *Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany*
[9] *Department of Medical Genetics, University of Antwerp, Belgium*

Abstract

Panic disorder is a common anxiety disorder which frequently co-occurs with agoraphobia. A functional promoter polymorphism in the serotonin receptor 1A (5-HT1A) gene has been found to be associated with major depression as well as anxiety- and depression-related personality traits. We investigated a possible association between this 5-HT1A gene promoter polymorphism and panic disorder by genotyping the −1019C>G single nucleotide polymorphism in 134 panic-disorder patients with and without agoraphobia and matched 134 controls. In our sample no significant evidence of allelic association in the combined panic-disorder group was found. However, our results show a significant association with the G allele in patients with panic disorder with agoraphobia ($p=0.03$, $n=101$). In conclusion, our findings do not support a major contribution of this polymorphism to the pathogenesis of panic disorder, but provide evidence for a possible role in the subgroup with agoraphobia.

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Key words: Agoraphobia, association, panic disorder, polymorphism, 5-HT1A gene promoter.

Introduction

Panic disorder is a severe mental disorder, which is defined by recurrent panic attacks and anticipatory anxiety. It is often accompanied by agoraphobia. The worldwide prevalence of panic disorder is 1–3% (Weissman et al., 1997). Psychosocial as well as genetic factors contribute to the aetiology of panic disorder.

Several preclinical and clinical studies implicate the serotonin receptor 1A (5-HT1A) in the pathogenesis of panic disorder. The 5-HT1A receptor is expressed in two distinct neuronal populations in the brain: as a presynaptic autoreceptor in the raphe nuclei, and as a post-synaptic heteroreceptor on neurons of the forebrain, mainly the hippocampus, septum and cortex (Kent et al., 2002). Activation of the presynaptic 5-HT1A receptor by selective agonists leads to a reduction in the firing rate of serotonergic neurons and causes the suppression of 5-HT synthesis, 5-HT turnover, and 5-HT release in serotonergic projection areas (Handley, 1995). 5-HT1A receptor responsivity is reduced in patients with panic disorder (Broocks et al., 2003; Lesch et al., 1992). 5-HT1A gene knockout mice display increased anxiety-like behaviour in various conflict tests (Heisler et al., 1998; Parks et al., 1998).

These observations suggest that allelic variations in the 5-HT1A receptor gene on chromosome 5q12.3 (Erdmann et al., 1995; Fargin et al., 1988; Kawanishi et al., 1998) could be predisposing factors for panic disorder. Functional relevance has recently been described for a −C1019G promotor polymorphism (Lemonde et al., 2003; Wu and Comings, 1999). It was not associated with major depression in a Spanish population (Arias et al., 2002), but has been found associated with major depression and suicidality in two

Address for correspondence: Dr K.-P. Lesch, Molecular and Clinical Psychiatry, Department of Psychiatry and Psychotherapy, University of Würzburg, Füchsleinstr. 15, 97080 Würzburg, Germany.
Tel.: +49-931-201 77600 *Fax*: +49-931-201 77620
E-mail: kplesch@mail.uni-wuerzburg.de

Table 1. Genotype and allele frequencies of the 5-HT1A promotor polymorphism in panic-disorder patients and controls

Sample	Genotypes			Armitage Trend test	Alleles		χ^2 test
	G/G	G/C	C/C		G	C	
Panic disorder							
Males ($n=49$)	16	23	10	$Z=-0.9083$, $p=0.3637$	55	43	$\chi^2=0.7375$, $p=0.3905$
Females ($n=84$)	26	36	22	$Z=-1.4420$, $p=0.1493$	88	80	$\chi^2=2.3108$, $p=0.1285$
Total ($n=133$)	42	59	32	$Z=-1.7025$, $p=0.0887$	143	123	$\chi^2=2.9963$, $p=0.0835$
Control							
Males ($n=49$)	9	31	9		49	49	
Females ($n=85$)	18	39	28		75	95	
Total ($n=134$)	27	70	37		124	144	
Panic disorder with agoraphobia							
Males ($n=33$)	13	16	4	$Z=-1.8805$, $p=0.0600$	42	24	$\chi^2=2.9692$, $p=0.0849$
Females ($n=68$)	21	30	17	$Z=-1.4659$, $p=0.1427$	72	64	$\chi^2=2.3566$, $p=0.1248$
Total ($n=101$)	34	46	21	$Z=-2.1689$, $p=0.0301$	114	88	$\chi^2=4.7633$, $p=0.0291$

Canadian samples (Lemonde et al., 2003). While another 5-HT1A receptor gene polymorphism (294 G/A) has been investigated in a Japanese panic-disorder sample with negative results (Inada et al., 2003), this functionally relevant promotor polymorphism has not yet been studied in panic disorder.

In the present study we therefore investigated whether the −1019C>G polymorphism may play a role in the pathogenesis of panic disorder by means of an association analysis in 134 German panic-disorder patients.

Methods

We investigated a sample of unrelated patients with panic disorder (85 female, 49 male) diagnosed by experienced psychiatrists according to DSM-IIIR/DSM-IV on the basis of structured interviews (SADS-LA, Mannuzza et al., 1986; CIDI, Robins et al., 1988; IDCL, Hiller, 1997) and medical records. Only patients with predominant panic disorder were included. The controls were unrelated, anonymous blood donors. All patients and controls were of German descent. They were matched for gender and age (average age of patients 37.3 ± 10.8 yr and of controls 41.0 ± 11.0 yr, mean ± standard error). The study design was approved by the local Ethics Committees. All participating subjects had given their informed written consent.

Genomic DNA was extracted from EDTA anticoagulated whole blood using QIAamp® DNA Mini kit (Qiagen GmbH, Hilden, Germany). A region of 163 bp (position −1158 to −996 from the translation start site ATG) containing the single nucleotide polymorphism at position −1019 was amplified by polymerase chain reaction (PCR) using the following reaction mix: 20 ng of genomic DNA in 75 mM Tris–HCl (pH 9), 20 mM ammonium sulphate, 0.01% Tween-20, 1.5 mM magnesium chloride, 0.4 μM of each of the primers, SNPR1A-nor (5′-GGC TGG ACT GTT AGA TGA TAA CG-3′) and SNPR1A-mod (5′-GGA AGA AGA CCG AGT GTG TCA T-3′), 0.4 mM dNTP, and 1 U *Taq* polymerase in a total reaction volume of 21 μl. The reverse primer (SNPR1A-mod) was modified (−1016A>T) to introduce a variable restriction site dependent on a C or a G in position −1019. After an initial denaturation step for 5 min at 95 °C, 35 cycles of denaturating at 95 °C for 30 s, annealing at 59.5 °C for 40 s and extension at 72 °C for 50 s were perfomed, followed by a final extension step at 72 °C for 5 min. Eight μl of the PCR product were digested with 1.5 U of *Bse*GI restriction enzyme in 33 mM Tris–acetate, 10 mM magnesium acetate, 66 mM potassium acetate and 0.1 mg/ml BSA at 55 °C overnight. The alleles were separated on a 5% agarose gel for 2 h and visualized under UV light in the presence of ethidium bromide. The undigested PCR product (163 bp) carries the C allele while the digested product with two fragments of 17 and 146 bp contains the G allele.

For statistical analysis, Armitage trend test for genotype frequency and χ^2 test for allele frequency (Sasieni, 1997) as well as conditional logistic regression were performed with a significance level set at $p=0.05$ using the SAS statistical package (SAS/STAT, 1999). Hardy–Weinberg equilibrium was tested by means of an online resource (Christensen, 2003). The statistic analyses were basically explorative and,

therefore, the results were not adjusted for multiple testing.

Results

The distribution of the genotypes of the C/G polymorphism did not differ significantly from those predicted by Hardy–Weinberg equilibrium in controls as well as patients (all cases with panic disorder, $p=0.21$; cases with panic disorder with agoraphobia, $p=0.46$; controls, $p=0.56$). We observed only a trend towards association with panic disorder in the total panic-disorder sample (genotype frequency, $p=0.0887$; allele frequency, $p=0.0835$; $n=134$). However, a significant association could be observed in the subgroup of panic-disorder patients with agoraphobia comparing the genotype distribution (Armitage trend test 2-sided: $Z=-2.1689$, $p=0.0301$, $n=101$). A similar difference was seen for the allele distribution with a significant excess of the G allele in patients with panic disorder and agoraphobia (χ^2 test: $\chi^2=4.763$, $p=0.029$, $n=101$). The results are summarized in Table 1.

Exploring the mode of inheritance by means of a conditional logistic regression analysis in patients with panic disorder with agoraphobia, we found an association for the recessive model. The genotype G/G was associated with a higher risk for the disorder ($p=0.019$, OR 2.148, 95% CI 1.159–4.188). In all other subgroups of patients no significant associations could be detected.

Discussion

In our study, an association between the -1019C>G 5-HT1A receptor gene polymorphism and panic disorder with agoraphobia is described for the first time. The results show a significant excess of the G/G genotype and the G allele of the -1019C>G 5-HT1A receptor gene polymorphism in the subgroup of patients with panic disorder with agoraphobia compared to controls.

The presence of the G variant prevents regulatory proteins (both transcriptional repressors and enhancers) from binding and thus contributes to altered 5-HT1A gene expression and 5-HT1A-mediated neurotransmission (Lemonde et al., 2003). It has been reported that the G allele is associated with major depression (Lemonde et al., 2003) as well as with anxiety- and depression-related personality traits such as the phobic trait harm avoidance in healthy volunteers (Strobel et al., 2003). Our observations are consistent with these findings as the association was only observed in the clinically more severely ill subgroup of patients with agoraphobia. Comorbidity between depression and agoraphobia is a clinically well-described phenomenon. It is considerably higher than between depression and other phobias, and is influenced by both common genetic and individual environmental factors (Kendler et al., 1993). While 27% of our patients had secondary major depression (DSM-IIR/DSM-IV), no association, however, could be observed for this subgroup and thus the observed association with panic disorder with agoraphobia could not attributed to this subgroup (results not shown).

Since our study used a case-control design and multiple statistical analyses, a false positive result in the agoraphobia subgroup as a consequence of population stratification or multiple testing cannot be excluded. The non-significant result in the overall sample, however, is as likely to be due to an inadequate sample size given the obvious small increase of risk due to the G allele. Further studies with independent, larger, and family-based designs are warranted in particular as the association with major depression is not consistently observed (Arias et al., 2002).

In summary, our findings suggest that the -1019 C>G 5-HT1A receptor gene polymorphism is not a major risk factor for panic disorder, but may contribute to the pathogenesis of agoraphobia in panic disorder.

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Statement of Interest

None.

References

Arias B, Arranz MJ, Gasto C, Catalan R, Pintor L, Gutierrez B, Kerwin RW, Fananas L (2002). Analysis of structural polymorphisms and C-1018G promoter variant of the 5-HT(1A) receptor gene as putative risk factors in major depression. *Molecular Psychiatry 7*, 930–932.

Broocks A, Meyer T, Opitz M, Bartmann U, Hillmer-Vogel U, George A, Pekrun G, Wedekind D, Ruther E, Bandelow B (2003). 5-HT(1A) responsivity in patients with panic disorder before and after treatment with aerobic exercise, clomipramine or placebo. *European Neuropsychopharmacology 13*, 153–164.

Christensen K (2003). Genetic calculation applets and other programs (http://kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/0.htm). Accessed May 2003.

Erdmann J, Shimron-Abarbanell D, Cichon S, Albus M, Maier W, Lichtermann D, Minges J, Reuner U, Franzek E, Ertl MA, Hebebrand J, Remschmidt H, Lehmkuhl G, Poustka F, Schmidt M, Fimmers R, Körner J, Rietschel M, Propping P, Nöthen MM (1995). Systematic screening for mutations in the promoter and the coding region of the 5-HT1A gene. *American Journal of Medical Genetics 60*, 393–399.

Fargin A, Raymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ (1988). The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. *Nature 335*, 358–360.

Handley SL (1995). 5-Hydroxytryptamine pathways in anxiety and its treatment. *Pharmacology & Therapeutics 66*, 103–148.

Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, Tecott LH (1998). Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. *Proceedings of the National Academy of Science USA 95*, 15049–15054.

Hiller W (1997). *International Diagnostic Checklists* (*DSM-IV*). Göttingen: Testzentrale.

Inada Y, Yoneda H, Koh J, Sakai J, Himei A, Kinoshita Y, Akabame K, Hiraoka Y, Sakai T (2003). Positive association between panic disorder and polymorphism of the serotonin 2A receptor gene. *Psychiatry Research 118*, 25–31.

Kawanishi Y, Harada S, Tachikawa H, Okubo T, Shiraishi H (1998). Novel mutations in the promoter and coding region of the human 5-HT1A receptor gene and association analysis in schizophrenia. *American Journal of Medical Genetics 81*, 434–439.

Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ (1993). Major depression and phobias: the genetic and environmental sources of comorbidity. *Psychological Medicine 23*, 361–371.

Kent JM, Mathew SJ, Gorman JM (2002). Molecular targets in the treatment of anxiety. *Biological Psychiatry 52*, 1008–1030.

Lemonde S, Turecki G, Bakish D, Lisheng D, Hrdina PD, Bown CD, Sequira A, Kushwaha N, Morris SJ, Basak A, Ou X-M, Albert PR (2003). Impaired trans-repression at a 5-HT1A receptor gene polymorphism associated with major depression and suicide. *Journal of Neuroscience 23*, 8788–8799.

Lesch KP, Wiesmann M, Hoh A, Muller T, Disselkamp-Tietze J, Osterheider M, Schulte HM (1992). 5-HT1A receptor-effector system responsivity in panic disorder. *Psychopharmacology 106*, 111–117.

Mannuzza S, Fyer AJ, Klein DF, Endicott J (1986). Schedule for Affective Disorders and Schizophrenia – Lifetime Version modified for the study of anxiety disorders (SADS-LA): rationale and conceptual development. *Journal of Psychiatric Research 20*, 317–325.

Parks CL, Robinson PS, Sibille E, Shenk T, Toth M (1998). Increased anxiety of mice lacking the serotonin1A receptor. *Proceedings of the National Academy of Sciences USA 95*, 10734–10739.

Robins LN, Wing J, Wittchen HU, Helzer JE, Babor TF, Burke J, Farmer A, Jablenski A, Pickens R, Regier DA, Sartorius N, Towle LH (1988). The Composite International Diagnostic Interview. An epidemiologic Instrument suitable for use in conjunction with different diagnostic systems and in different cultures. *Archives of General Psychiatry 45*, 1069–1077.

SAS/STAT (1999). SAS/STAT, Version 8.1. Heidelberg, Germany: SAS Institute GmbH.

Sasieni PD (1997). From genotypes to genes: doubling the sample size. *Biometrics 53*, 1253–1261.

Strobel A, Gutknecht L, Zheng Y, Reif A, Mössner R, Brocke B, Lesch KP (2003). Allelic variation of serotonin receptor 1A function is associated with anxiety- and depression-related traits. *Journal of Neural Transmission 110*, 1445–1553.

Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, Joyce PR, Karam EG, Lee CK, Lellouch J, Lepine JP, Newman SC, Oakley-Browne MA, Rubio-Stipec M, Wells JE, Wickramaratne PJ, Wittchen HU, Yeh EK (1997). The cross-national epidemiology of panic disorder. *Archives of General Psychiatry 54*, 305–309.

Wu S, Comings DE (1999). A common C-1018G polymorphism in the human 5-HT1A receptor gene. *Psychiatric Genetics 9*, 105–106.