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The symptomatic profile of panic disorder is shaped by the 5-HTTLPR polymorphism

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ABSTRACT

The short allele of a functional polymorphism (5-HTTLPR) in the serotonin transporter (5-HTT) promoter is associated with reduced serotonin transporter expression, lower in vivo 5-HTT binding, higher neuroticism and amygdala reactivity as well as facilitated fear conditioning and is a candidate variant for panic disorder. However, case–control studies have consistently failed to show an association between 5-HTTLPR and panic disorder. As psychiatric disorders are broadly defined phenotypes merging different symptoms to syndromes, they may not be very well suited for genetic association studies. An alternative approach is to measure symptoms along continuous symptom dimensions which may more appropriately reflect their biological underpinnings and may reveal subpopulations within clinical diagnostic groups. We recorded the symptomatic profile in 73 in panic disorder patients using observer-rated instruments (Panic Disorder Severity Scale, PDSS; Montgomery–Åsberg Depression Rating Scale, MADRS) and hypothesized more severe symptoms in patients carrying the 5-HTTLPR s-allele. We observed a strong association between bi- and triallelic 5-HTTLPR polymorphisms and the symptomatic profile. Carriers of the 5-HTTLPR s-allele suffered from most severe panic and depressive symptoms. Our data highlight the importance of defining appropriate phenotypes for psychiatric genetic studies and demonstrate that the 5-HTTLPR genotype may be related to the symptomatic profiles rather than to the vulnerability to develop panic disorder.

1. Introduction

Twin and family studies have accumulated evidence that panic disorder has a strong genetic underpinning with 30–40% of the variance in disease liability being explained by genetic factors (Hettema et al., 2001). Most studies investigating the genetics of panic disorder have focused on case–control comparisons of allele/genotype frequencies. Mostly these studied have used diagnostic categories as the dependent variable and not symptom severity that could represent a possibility of a more fine-grained measurement of illness expression. Candidate genes for panic disorder can be found in critical bottlenecks of neurotransmitter systems involved in anxiety and panic such as the serotonin system (Bell and Nutt, 1998; Maron and Shlik, 2006). Selective serotonin reuptake inhibitors (SSRIs) are effectively used to treat patients suffering from panic disorder (Baldwin et al., 2005). Thus the gene coding for the serotonin transporter (5-HTT) is a clear candidate for genetic studies on panic disorder. The *5-HTT* promoter harbors a functional 43 bp insertion/deletion polymorphism (5-HTTLPR), which yields a short and a long allele. The s-allele is associated with a nearly 50% reduction of basal 5-HTT activity in vitro (Heils et al., 1995), reduced in vivo 5-HTT binding in Caucasians (Praschak-Rieder et al., 2007; Reimold et al., 2007) and is associated with higher neuroticism (M. R. Munafo et al., 2009), higher amygdala reactivity (Munafo et al., 2008), and facilitated fear conditioning (Lonsdorf et al., 2009).

Several studies, including a recent metaanalysis (Blaya et al., 2007) have failed to find an association between the 5-HTTLPR genotype and panic disorder in case–control designs. Only one study (Kim et al., 2006) included symptom severity in their analysis, but did not find an association with the 5-HTTLPR genotype. However, this study investigated a Korean panic sample in which nearly all patients were 5-HTTLPR s-carriers. This lack of genotypic variance makes it hard to detect an association and leaves this question open to be tested in Caucasian samples.

The 5-HTTLPR is not the only functional variant of the 5-HTT gene. The G-allele of an A→G single nucleotide polymorphism (SNP) (db number rs25531), which is located in close proximity to 5-HTTLPR, has also been

Abbreviations: 5-HT, serotonin; 5-HTT, serotonin transporter; 5-HTTLPR, serotonin transporter linked polymorphic region; PDSS, Panic Disorder Severity Scale; MADRS, Montgomery–Åsberg Depression Rating Scale; SSRI, Selective Serotonin Reuptake Inhibitor; SNP, single nucleotide polymorphism.

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shown to reduce transcription rate, possibly due to creation of an AP2 transcription factor binding site (Kraft et al., 2005). The minor G-allele has a frequency of about 9–15% in Caucasian populations (Murphy et al., 2008) and is nearly always in phase with the 5-HTTLPR long allele, but only rarely observed on the 5-HTTLPR s-allele background (e.g. Wendland et al., 2006). It has become increasingly common to investigate the joint effects of 5-HTTLPR and rs25531 as the so called triallelic 5-HTTLPR, where individuals are classified based on putative 5-HTT expression levels. Individuals with the $long_A/long_A$ genotype are assumed to have the highest expression levels, while individuals carrying a $long_G$ allele have a reduced expression equivalent to the short allele (Hu et al., 2006). Only a single study (Strug et al., in press) to date has addressed the role of the triallelic 5-HTTLPR in panic disorder in a case/control study, but did not find an association with either bi-or triallelic 5-HTTLPR and the presence of panic disorder.

Because the 5-HTTLPR s-allele has been associated with both increased amygdala reactivity and facilitated fear conditioning, we hypothesized that it may also show an association with symptom severity in panic disorder. Fear conditioning has been proposed as a central mechanism in panic symptoms, particularly in phobic avoidance and anticipatory anxiety (Gorman et al., 2000).

In the current study we investigated the symptomatic profile as assessed by observer rated standardized questionnaires in a sample of 73 patients with panic disorder with/without agoraphobia in relationship to the bi- and triallelic 5-HTTLPR genotype.

2. Methods

2.1. Patient population

Patients were recruited from a randomized clinical equivalence trial of regular cognitive–behavioral group therapy (CBT) vs. internet-based CBT (ICBT) for panic disorder (Bergström et al., in preparation). Eighty-seven patients consented to participate in the study. Eleven patients were excluded because of excessive missing data and three patients were excluded because their pre-treatment PDSS mean item score was <1 indicating a questionable clinical significance of the panic disorder at admission. This left us with a final sample of 73 patients. There were 28 male (mean = 32.5 years, SD = 7.6, range 22–53) and 45 female (mean = 36.5 years, SD = 10.3, range 23–61). All except one patient, who was of South American origin, were Caucasians. The majority ($n = 41$) of the patients were referred from primary health care, 26 by self-referral and six by psychiatric outpatient care units. In the whole group, 31 patients were pharmacologically treated (SSRIs, other antidepressants, Benzodiazepine) at assignment to the study with a stable dosage for at least 2 months. Hence, symptoms were still present in this group in spite of medication. All patients provided written informed consent and the study was approved by the Ethics committee at the Karolinska Institutet and was carried out in accordance with the Declaration of Helsinki.

2.2. Diagnostic procedure

A psychiatrist or a resident in psychiatry under supervision by a psychiatrist performed an in-person structured clinical interview including the Mini-International Neuropsychiatric Interview (M.I.N.I. (Sheehan et al., 1998)). Inclusion criteria were: 1. Fulfilling DSM-IV criteria for panic disorder with/without agoraphobia (PD/A), 2. PD/A primary diagnosis, 3. Non-physiological etiology of panic symptoms, 4. age at least 18 years, 5. not suffering from severe depression or suicidal ideation, 6. having had a constant dosage for 2 months prior to inclusion if taking prescribed drugs for panic disorder, and 7. not undergoing other CBT or psychotherapy. The diagnostic procedures were performed blind to genotype and group assignment in the treatment trial.

2.3. Assessment instruments

The Panic Disorder Severity Scale (PDSS, (Shear et al., 1997)) is a 7-item observer-rated instrument which assessing panic symptom severity, rated on a 5-point Likert scale (0: no symptoms, 4: extremely severe symptoms). The Montgomery–Åsberg Depression Rating Scale (MADRS, (Montgomery and Åsberg, 1997)) is a 10-item observer-rated scale used to assess severity of depressive symptoms and is rated on a 7-point Likert scale (0: symptom is not present, 6 extremely severe symptoms). All ratings were performed at the time of inclusion of the study before the CBT treatment was started.

2.4. Genotyping

DNA was extracted from whole blood as described by Geijer et al. (Geijer et al., 1994). Genotyping for 5-HTTLPR was performed as described in detail earlier (Lonsdorf et al., 2009). Briefly, for the biallelic 5-HTTLPR two fragments, 336b (short) and 379 bp (long), were amplified by polymerase chain reaction (PCR), separated for 2 h at 180 V by gel-electrophoresis in TBE-buffer on a 2.5% Agarose gel containing ethidium bromide and visualized using ultraviolet light (UV). All genotypes were determined in duplicates. We used the modified nucleotide 7-deaza-dGTP in our initial protocol and could not obtain a satisfying digestion with either MSPI or HPAII enzymes to determine the triallelic 5-HTTLPR. This problem has been reported earlier (Grime et al., 1991) and therefore a different protocol was used to determine the rs25531 genotype.

For the triallelic 5-HTTLPR, PCR reactions were performed in a final volume of 20 µl containing 50 ng of genomic template, 0.2 mM each deoxynucleoside triphosphate (dNTP), 0.4 mM each primer, 0.05 U/µl Quiagen HotStar®Polymerase, 1 M Q-Solution and 1x Buffer. The primer (Thermo Scientific, Ulm, Germany) sequences were Forward: 5′-GGCGTTGCCGCTCTGAATGC-3′ and Reverse. 5′-GAGGGACTGAGCTGGACAACCAC-3′. Samples were amplified on a Biorad Tetrade (BIORAD, Hercules, CA, USA) with an initial denaturation step for 10 min at 94 °C followed by 32 cycles consisting of denaturation for 30 s at 95 °C, annealing for 30 s at 57 °C and elongation for 30 s at 72 °C and one final elongation step for 5 min at 72 °C. This yields a 486 bp (short) and a 529 bp (long) fragment. 8 µl of the PCR product separated for 2 h at 180 V on a 2.5% agarose gel containing GelRed® and visualized using UV light. 10 µl of the remaining PCR product were digested for 12 h at 37 °C with 0.1 µl MSP1 (New England Biolabs, Ipswich, MA, USA) and 1 µl buffer per sample. MSP1 recognizes and cuts a 5′-C/CGG-3′ sequence resulting in the following fragments: 340 bp, 127 bp and 62 bp for the L_A allele, 297 bp, 127 bp and 62 bp for the S_A allele, 174, 166, 127 and 62 bp for the L_G allele and 166, 131, 127 and 62 bp for the S_A allele. Fragments were run for 2 h at 180 V on 4% Agarose gels containing GelRed® and visualized via UV light. All biallelic 5-HTTLPR genotypes were thus determined using two different protocols that yielded identical results.

2.5. Data analysis

Statistical analysis were performed using SPSS for Windows version 15 (SPSS Inc., Chicago, IL, USA), and graphs were made using Origin®8 (OriginLab®, Northampton, MA, USA).

For the 5-HTTLPR analysis we used a step-wise approach: In the first step of the analyses, 5-HTTLPR (genotypes: l/l, s/l, s/s) and rs25531 (genotypes: AA and G-carriers (as only one patient with the G/G genotype was observed, G/G and G/A were combined) were treated as two independent biallelic loci. In a second step results based on the s-allele level (opposing s-carriers to l/l patients) are reported. In the third step, the 6 observed genotypes of the triallelic 5-HTTLPR (5-HTTLPR/rs25531) were grouped based on assumed expression (Kraft et al., 2005) into high (L_A/L_A), intermediate (L_A/L_G,