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A possible association between panic disorder and a polymorphism in the preproghrelin gene

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ABSTRACT

The aim of the study was to investigate whether polymorphisms in the preproghrelin gene are associated with anxiety disorders, such as panic disorder, in humans. Panic disorder is a severe anxiety disorder, characterized by sudden attacks of intense fear or anxiety in combination with somatic symptoms. The preproghrelin gene codes for two gut-derived circulating peptides that have been linked to anxiety-like behaviour in rodents: ghrelin (an orexigenic, pro-obesity hormone) and obestatin. In the present study, we genotyped three missense mutations in the preproghrelin gene in 215 patients suffering from panic disorder and in 451 controls. The A allele of the rs4684677 polymorphism was significantly associated with panic disorder, while there were no significant associations with the two other polymorphisms studied. We conclude that the rs4684677 (Gln90Leu) polymorphism in the preproghrelin gene may be associated with increased risk of panic disorder. It will be important to confirm these findings in additional panic disorder patient groups.

1. Introduction

Panic disorder is a severe anxiety disorder, characterized by sudden attacks of intense fear or anxiety, often in combination with physical symptoms such as heart palpitations and shortness of breath (American Psychiatric Association, 2000). Panic disorder has been shown to be hereditary (Hettema et al., 2001), but the genes responsible for the disease still remain to be elucidated. Several genes have been studied in relation to panic disorder. These include genes involved in serotonergic signalling, such as the serotonin transporter, several serotonergic receptors and genes involved in production and degradation of serotonin, but also other genes, such as brain-derived neurotrophic factor (BDNF), angiotensin and genes involved in hormonal systems, such as sex hormones and the hypothalamic-pituitary-adrenal (HPA) axis. These studies have yielded both positive and negative results. The most consistent finding is the association between panic disorder and a polymorphism in the catechol-*O*-methyltransferase (COMT) gene (Na et al., 2011).

The preproghrelin gene codes for two circulating peptides, ghrelin (Kojima et al., 1999) and obestatin (Zhang et al., 2005), that are produced predominantly by the stomach. Ghrelin is perhaps best known for its orexigenic (Wren et al., 2000) and adipogenic effects (Tschop et al., 2000). Interestingly, ghrelin has been shown to affect anxiety-like behaviour in rodents, although both anxiogenic (Asakawa et al., 2001; Carlini et al., 2002, 2004; Hansson et al., 2011; Kanehisa et al., 2006) and anxiolytic (Lutter et al., 2008) effects of ghrelin have been reported. Recently, we performed the first long-term study showing that chronic central infusion of ghrelin to rats increases anxiety-like behaviour (Hansson et al., 2011). We have also shown that gastrectomy, which substantially reduces circulating ghrelin levels, decreases anxiety- and depression-like behaviour in rats (Salome et al., 2011). Obestatin has also been implicated in anxiety-related behaviour but, in contrast to ghrelin, it decreases anxiety-like behaviour in rats (Carlini et al., 2007). Central infusion of antisense against GPR39 (previously thought to be a receptor for obestatin) to rats has also been shown to influence anxiety-like behaviour (Ishitobi et al., 2012). Inspired by the hypothesis

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emerging from these animal studies, that peptides derived from the preproghrelin gene may have a role in anxiety behaviour, we have begun to explore the clinical relevance of these findings. Here we sought to determine whether there is an association between panic disorder and polymorphisms in the preproghrelin gene in a Swedish population.

2. Materials and methods

2.1. Experimental design

The preproghrelin gene contains three known exonic polymorphisms, all of which are missense mutations. These are rs4684677, situated in exon 4, implying the amino acid exchange Gln90Leu; rs34911341, located in exon 3, implying the amino acid exchange Arg51Gln; and rs696217, also located in exon 3, and implying the amino acid exchange Leu72Met.

2.2. Subjects

The panic disorder group consisted of 215 patients (63 men and 152 women), meeting the DSM-IV criteria for panic disorder. Their mean age was 43 years (range 15–77 years). All of the patients had panic disorder as the main diagnosis and 83% also suffered from agoraphobia. Patients were screened with diagnostic interview schedules to exclude those with concurrent major depression, psychoses or substance use disorders. The controls were originally collected for a study of obesity, anthropometrics, and cardiovascular risk factors comprising 199 men born in 1944 (51 years old at the time of the assessment) and 252 women born in 1956 (40 years old at the time of the assessment) and living in Gothenburg, Sweden, who were drafted from a larger cohort recruited from the general population as previously described (Rosmond and Bjorntorp, 1998; Rosmond et al., 1998). Both patients and controls were Caucasians. These cohorts of patients and controls have been studied previously (Annerbrink et al., 2010; Ho et al., 2004; Olsson et al., 2004). The study was approved by the Research Ethics Committee at the University of Gothenburg. All participants provided written informed consent.

2.3. Genotyping

Venous blood was collected from each subject, and genomic DNA was isolated using the QIAamp DNA blood Mini Kit (Qiagen, Chatsworth, CA, USA). Initially, the polymorphisms were assessed for 175 patients and all controls using sequenom. For the polymorphism rs4684677, the remaining 40 panic disorder patients, as well as samples for which the sequenom analysis failed, were analysed using pyrosequencing. For the polymorphisms rs34911341 and rs696217, these samples were analysed using TaqMan SNP genotyping assays.

2.3.1. Sequenom analysis

Genotyping was performed at Sequenom Inc. in Hamburg, Germany, using the Sequenom iPLEX® Gold assay and MassARRAY® MALDI-TOF mass spectrometry platform in accordance with the manufacturer's instructions (Sequenom Inc., San Diego, CA). Primers for polymerase chain reaction (PCR) amplification and sequencing were designed using the Sequenom MassARRAY® System Designer software.

2.3.2. Pyrosequencing

The following primers were used for pyrosequencing (Ahmadian et al., 2006) (PSQ96 System; Biotage, Uppsala, Sweden) analysis of the rs4684677 polymorphism: forward: 5′-CATGTGGGGCTGCAAGGA-3′, reverse: 5′-TCCCTGCCCTGCCTCCTA-3′, and sequence: 5′-GCTGTGCTGCTGGTA-3′. The DNA was amplified using HotstarTaq Master mix kit (Qiagen), each reaction containing one unit HotStarTaq DNA polymerase, 1.5 mM magnesium chloride, 200 μM of each dNTP, 50 ng genomic DNA and 0.15 μM of each primer, in a total volume of 20 μl. The PCR comprised an initial denaturing step at 95 °C for 15 min, 45 cycles of 95 °C for 15 s, 58 °C for 15 s, and 72 °C for 15 s, followed by a final extension at 72 °C for 7 min. Twenty microlitres of the PCR product was used for the pyrosequencing. Fifteen picomoles of the sequencing primers was used to detect the polymorphism.

2.3.3. TaqMan SNP genotyping

The SNPs rs34911341 and rs696217 were analysed using Applied Biosystems pre-made reagents (C__25607739_20 and C___3151003_20, respectively, Applied Biosystems Inc., Foster City, CA, USA). The assays were performed under recommended conditions on an Applied Biosystems Prisma 7900 HT real-time PCR instrument, using standard reagents from Applied Biosystems.

2.4. Statistical analysis

Hardy–Weinberg equilibrium was checked in the control sample using Haploview. Association between polymorphisms and panic disorder was analysed with logistic regression using the count of the rare allele as numeric predictor. p-Values were corrected for multiple testing using permutation tests due to the linkage disequilibrium between the single nucleotide polymorphisms (SNPs).

3. Results

The genotype frequencies in the control sample were in Hardy–Weinberg equilibrium. Clinical data are summarized in Table 1. About 83% of the patients with panic disorder had agoraphobia as a second diagnosis (Table 1). The minor allele frequencies of the three SNPs rs4684677, rs34911341 and rs696217 were, respectively, 0.105, 0.012 and 0.105 in cases and 0.068, 0.004 and 0.099 in controls. The A allele of the rs4684677 polymorphism was significantly associated with panic disorder (odds ratio = 1.57, $p = 0.025$). No significant associations were found for either of the other two SNPs tested (rs34911341 and rs696217; Table 2).

4. Discussion

The present study supports an association between panic disorder and variations in the preproghrelin gene. We found that the A allele of the rs4684677 T > A polymorphism in the preproghrelin gene is significantly associated with increased risk of panic disorder (OR = 1.57). This polymorphism is a missense mutation located in exon 4 and is responsible for an amino acid exchange from Leu to Gln.

The SNP rs4684677 is situated in the part of the preproghrelin gene that codes for obestatin. It is possible therefore that the

Table 1
Clinical data.

	Number of cases	Age (S.D.)	Agoraphobia
Panic disorder	215	43.4 (12.4)	179
Male subsample	63	43.9 (11.4)	47
Female subsample	152	43.2 (13.0)	132
Controls	451	44.9 (5.5)	n.a.
Male subsample	199	51	n.a.
Female subsample	252	40	n.a.

Number of cases: number of subjects in each group, age (S.D.): mean age of each group (standard deviation is given in parentheses), agoraphobia: number of subjects suffering from agoraphobia as the second diagnosis. Numbers are given for cases and controls, and are also further divided into male and female subgroups.

Table 2
Association between panic disorder and SNPs in the preproghrelin gene.

SNP	Genotype	PD	Ctrl	OR	p	p_c	MAF
rs4684677	TT	174 (81%)	393 (87%)	1.57	0.025	0.05	0.068
	AT	37 (17%)	55 (12%)				
	AA	4 (2%)	3 (1%)				
rs34911341	CC	210 (98%)	447 (99%)	2.66	0.148	0.38	0.004
	CT	5 (2%)	4 (1%)				
	TT	0 (0%)	0 (0%)				
rs696217	GG	170 (79%)	367 (81%)	1.07	0.731	0.98	0.099
	GT	45 (21%)	79 (18%)				
	TT	0 (0%)	5 (1%)				

PD: panic disorder ($n = 215$), Ctrl: controls ($n = 451$), OR: odds ratio per minor allele, p: p-value using logistic regression, p_c: p-value corrected for multiple inference by permutation, MAF: minor allele frequency in controls.