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CRHR1 promoter hypomethylation: An epigenetic readout of panic disorder?

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Abstract
The corticotropin releasing hormone receptor 1 (CRHR1) is crucially involved in the hypothalamic-pituitary-adrenal axis and thus a major regulator of the stress response. *CRHR1* gene variation is associated with several mental disorders including anxiety disorders. Studies in rodents have demonstrated epigenetic regulation of *CRHR1* gene expression to moderate response to stressful environment. In the present study, we investigated *CRHR1* promoter methylation for the first time regarding its role in panic disorder applying a case-control approach (N=131 patients, N=131 controls). In an independent sample of healthy volunteers (N=255), *CRHR1* methylation was additionally analyzed for association with the Beck Anxiety Inventory (BAI) score as a dimensional panic-related intermediate phenotype. The functional relevance of altered *CRHR1* promoter methylation was investigated by means of luciferase-based reporter gene assays. In panic disorder patients, a significantly decreased *CRHR1* methylation was discerned ($p<0.001$). Accordingly, healthy controls with high BAI scores showed significantly decreased *CRHR1* methylation. Functional analyses revealed an increased gene expression in presence of unmethylated as compared to methylated pCpGl_CRHR1 reporter gene vectors. The present study identified a potential role of *CRHR1* hypomethylation – conferring increased *CRHR1* expression – in panic disorder and a related dimensional

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intermediate phenotype. This up-regulation of *CRHR1* gene expression driven by demethylation might constitute a link between the stress response and panic disorder risk.

1. Introduction

The human stress response as governed by the hypothalamic-pituitary-adrenal (HPA) axis is considered to play a crucial role in the pathophysiology of stress-related- and anxiety disorders. The corticotropin releasing factor (CRF) and its receptors are important components of the HPA axis and the stress response. By binding CRF, secreted from the hypothalamus upon stress signals, the corticotropin releasing hormone receptor 1 (CRHR1) triggers the release of the adrenocorticotropic hormone (ACTH), which in turn mediates cortisol excretion from the adrenal gland (Smith and Vale, 2006). *CRHR1* expression, however, is not limited to the pituitary gland, but found throughout the brain with strongest expression in the brainstem, the medial and basolateral amygdala and the cerebellum (Aguilera et al., 2004), and influences the noradrenergic stress response as relevant with respect to anxiety by corticotropic innervations of e.g. the locus coeruleus (Binder and Nemeroff, 2010; Valentino et al., 1983). Thus, the *CRHR1* gene constitutes a promising candidate gene for stress-related- and particularly anxiety disorders.

Along these lines, global *Crhr1* knockout mice have been reported to display decreased anxiety-like behavior along with blunted ACTH and cortisol levels (Timpl et al., 1998). Accordingly, conditional anterior forebrain (Wang et al., 2012) and limbic brain structure *Crhr1* knockout (Müller et al., 2003) as well as knockdown of *Crhr1* mRNA expression in the basolateral amygdala (Sztainberg et al., 2010) induced a significant decrease in anxiety levels. In a juvenile rhesus macaque model, anxious temperament (AT) – analogous to a human childhood anxiety-risk phenotype – and related brain metabolic activity were influenced by *CRHR1* variation (Rogers et al., 2013). Human fear acquisition deficits have been found to be driven by a single nucleotide polymorphism (SNP; rs878886) in the *CRHR1* gene located on chromosome 17 (Heitland et al., 2013, 2015), and healthy *CRHR1* rs17689918 risk allele carries scored higher on dimensional scales for anxiety sensitivity and agoraphobia (Weber et al., 2015). Finally, association of *CRHR1* polymorphisms (rs12944712, rs110402, rs12938031, rs4792887, rs242924) with stress- or anxiety-related phenotypes has been extended to a clinical context, i.e. post-traumatic stress disorder (Amstadter et al., 2011; Boscarino et al., 2012; White et al., 2013) and panic disorder (Ishitobi et al., 2012; Keck et al., 2008). In a recent multilevel approach, it was shown that carriers of the *CRHR1* rs17689918 risk allele displayed a significantly increased risk of panic disorder along with differential brain activation of the amygdalae and prefrontal cortices in a safety learning and differential conditioning task, respectively (Weber et al., 2015). Another study applying linkage and association analyses failed, however, to provide support for a role of *CRHR1* variation in panic disorder (Hodges et al., 2009).

Genetic effects might be modulated, strengthened or concealed by epigenetic mechanisms critically influencing gene regulation and mediating adaptation to environmental factors. Particularly, methylation of the cytosine pyrimidine ring in cytosine-guanine dinucleotides (CpG) has been shown to be of major functional significance by mainly silencing DNA transcription when occurring in the promoter region of a gene (Jaenisch and Bird, 2003; Suzuki and Bird, 2008). First studies with respect to anxiety disorders showed differential DNA methylation patterns in the monoamine oxidase A (*MAOA*) and glutamate decarboxylase 1 (*GAD1*) genes as well as in the oxytocin receptor (*OXTR*) gene to be associated with panic disorder (Domschke et al., 2013, 2012; Ziegler et al., 2016) and social anxiety disorder (Ziegler et al., 2015), respectively. To date, no data are available on the role of *CRHR1* methylation in regard to anxiety disorders despite evidence from a rodent study for *Crhr1* promoter demethylation induced by gestational hypoxia to be associated with anxiety-like behavior (Wang et al., 2013). Also, upon differential environmental stimulation, Sotnikov et al. observed bidirectional alterations of *Crhr1* gene expression in the amygdala of high anxious (HAB) and low anxious (LAB) mouse strains associated with a rescue of the respective extreme anxiety-phenotype to be regulated by dynamics in *Crhr1* promoter methylation (Sotnikov et al., 2014).

Given the converging body of evidence for a genetically driven and potentially epigenetically modified involvement of the corticotropin releasing hormone receptor 1 in anxiety as reviewed above, here we studied *CRHR1* promoter methylation for the first time regarding its role in panic disorder applying a case-control approach. In a large independent sample of healthy volunteers, *CRHR1* methylation was additionally analyzed for association with a dimensional anxiety phenotype as ascertained by the Beck Anxiety Inventory (BAI), which has been shown to have a particularly strong ability to assess acute panic-related symptomatology (Leyfer et al., 2006; Muntingh et al., 2011). Finally, the functional relevance of altered *CRHR1* promoter methylation was investigated by means of luciferase-based reporter gene assays. We hypothesized to discern decreased *CRHR1* methylation – conferring increased *CRHR1* expression – to be associated with panic disorder as well as with increased BAI scores.

2. Experimental procedures

2.1. Samples

The *panic disorder sample* – constituting the discovery sample – consisted of 131 German patients with panic disorder (f=85, m=46;