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Research paper

Effects of corticotropin-releasing hormone receptor 1 SNPs on major depressive disorder are influenced by sex and smoking status

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ABSTRACT

Background: The corticotropin-releasing hormone receptor 1 (*CRHR1*) gene has been repeatedly implicated in Major Depressive Disorder (MDD) in humans and animal models; however, the findings are not absolutely convergent. Since recent evidence from genome-wide association studies suggests that narrowing the phenotypic heterogeneity may be crucial in genetic studies of MDD, the aim of this study was to evaluate the effects of *CRHR1* polymorphisms on MDD while addressing the influence of sex and smoking status.

Methods: The association of the *CRHR1* SNPs rs12944712, rs110402, and rs878886 with MDD was evaluated in 629 Brazilian adults of European descent recruited from the general population [180 (28.6%) with lifetime MDD]. The sample was subdivided according to sex and smoking status

Results: Among nonsmokers, there were nominal associations between MDD and all tested SNPs (rs12944712, P=0.042; rs110402, P=0.031, and rs878886, P=0.040), regardless of sex. In addition, there were significant effects of rs110402 in women (P_{corr}=0.034) and rs878886 in men (P_{corr}=0.013). Among lifetime smokers, there were no significant associations between *CRHR1* SNPs and MDD

Limitations: The lack of a depression rating scale; scarcity of information on the functionality of the *CRHR1* SNPs; and relatively small sample sizes in some subgroups.

Conclusions: Our results strengthen the evidence for the role of *CRHR1* SNPs in MDD susceptibility and suggest that their effects may be modulated by sex and smoking status. These findings suggest the perspective that reducing phenotypic heterogeneity is warranted in genetic studies of MDD.

1. Introduction

Major Depressive Disorder (MDD) is characterized by depressed mood and decreased interest or pleasure in daily activities in addition to cognitive and physiological symptoms, leading to impaired function (American Psychiatric Association, 2013). The lifetime prevalence of MDD in the general population is approximately 15% (Kessler et al., 2003; Hasin et al., 2005), with a female: male ratio of 2:1 (Kessler, 2003).

MDD heritability has been estimated at 31–42% (Sullivan et al., 2000); however, the identification of specific genes involved in the etiology of MDD has remained a challenge. Most genome-wide association studies (GWAS) have not provided significant findings (Ripke et al., 2013). However, a recent study from the CONVERGE consortium observed two loci associated with MDD, one close to *SIRT1* and the other in an intron of the *LHPP* gene (CONVERGE consortium, 2015). These genes had previously been implicated in MDD using candidate gene approaches (Neff et al., 2009; Kishi et al., 2010; Kovanen et al., 2015). Additional genes are expected to play a role in such a complex disorder. One approach to identify such genes is to complement GWAS data with the most robust pathophysiological findings to design hypothesis-driven studies.

For the etiology of MDD, several lines of evidence point towards genes encoding components of the hypothalamic–pituitary–adrenal (HPA) axis (for review, see Wardenaar et al., 2011). There is evidence that the pathophysiology of MDD involves hyperactivity

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of the HPA axis (Holsboer, 2000; Wardenaar et al., 2011), which is activated in response to physical, pharmacological, and psychological stressors. The major activation pathway of the HPA axis involves the production of adrenocorticotropic hormone (ACTH) through the synergistic action of corticotropin-releasing hormone (CRH) signaling via CRH receptor 1 (CRH-R1) and arginine-vasopressin signaling via V1b receptor, resulting in cortisol release from the adrenal cortex (Bonfiglio et al., 2011).

CRH binding to CRH-R1 in the pituitary gland is considered to be the main regulator of the physiological response to stress, and this process has been associated with the pathophysiology of depressive and anxiety disorders (Binder and Nemeroff, 2010). Animal studies have demonstrated that elevated levels of CRH may induce anxiety-related behaviors, such as increased motor activity and startle response (Jones et al., 1998; Servatius et al., 2005). *Crhr1*-knockout mice present reduced stress-induced release of ACTH and corticosterone (equivalent to cortisol in humans) as well as less pronounced anxiety-related behavior than wild-type mice (Timpl et al., 1998). In addition, high basal levels of CRH are observed in the cerebrospinal fluid of patients with MDD (Banki et al., 1992), and this may lead to downregulation of CRH-R1. Postmortem human studies support this hypothesis, since it has been observed that CRH binding and CRH-R1 mRNA levels are decreased in the brain of depressed suicide victims (Merali et al., 2004). Therefore, it is likely that variations in the genes encoding components of this system are involved in the etiology of stress-related disorders, such as MDD.

The *CRHR1* gene, which encodes CRH-R1, is located on chromosome 17q21.31 region and contains 13 exons. Several studies have reported that *CRHR1* SNPs are associated with susceptibility to MDD and the severity of depressive symptoms (Liu et al., 2006; Papiol et al., 2007; Engineer et al., 2013; Ching-López et al., 2015), especially in the context of gene-environment interactions (Bradley et al., 2008; Grabe et al., 2010; Heim et al., 2009; Polanczyk et al., 2009; Kranzler et al., 2011; Laucht et al., 2013; Liu et al., 2013; Schatzberg et al., 2014; Starr et al., 2014). Nevertheless, mixed results have been reported, as studies have shown opposite effects of *CRHR1* alleles and haplotypes and/or failed to replicate previous findings (Stergiakouli et al., 2014). One argument often raised to explain the inconsistent results in association studies is clinical heterogeneity. The above mentioned CONVERGE study (CONVERGE consortium, 2015) highlighted the contribution of sex to phenotype as a potential reason for the heterogeneity in association studies, as their main approach was to reduce phenotypic heterogeneity to a sample of Chinese women with recurrent MDD. The higher prevalence of depression and anxiety-related disorders observed in women has been associated with sex-specific biological characteristics (Seney and Sibille, 2014). For example, women may have different stress reactivity through the HPA axis or perception of stressful events compared to men (Kudielka and Kirschbaum, 2005; Kishi et al., 2010). In addition, factors such as hormonal levels, oral contraceptive use, or menstrual cycle variations could influence genetic association findings. Interestingly, sex-specific associations involving polymorphisms in *CRHR1* have been suggested, with distinct effects on depression in men and women. For example, some significant associations were only observed in men but not in women (Heim et al., 2009; Wasserman et al., 2009). Notably, sex differences in the effects of *CRHR1* were also observed in the cortisol response (Heim et al., 2009).

Another major source of phenotypic heterogeneity in psychiatric conditions is the presence of other comorbidities. Although some studies have taken into account the clinical features sometimes related to depression, such as psychosis (Schatzberg et al., 2014) and suicidal behavior (Wasserman et al., 2009), most studies did not consider comorbidities often associated with MDD. In some cases, the investigated study sample was composed of patients with comorbid psychiatric disorders (Bradley et al., 2008; Wasserman et al., 2009), while in other studies, such conditions were exclusion criteria (Heim et al., 2009; Liu et al., 2013). Such clinical heterogeneity between and within samples may preclude the identification of robust and replicable genetic findings, since the presence of additional psychiatric disorders could precede the onset of MDD and confound the identification of etiological factors.

Nicotine dependence is one of the most common comorbidities associated with MDD, since the prevalence of MDD is substantially increased among smokers (Farrell et al., 2001; Scarinci et al., 2002). Although the exact biological mechanism underlying this relationship is unclear (Tsuang et al., 2012), there seems to be an overlap between the genetic factors associated with MDD and nicotine dependence. For example, HPA axis dysregulation and stress-related genes, including *CRHR1*, have also been linked to nicotine dependence (Richards et al., 2011; Tang et al., 2015). Therefore, nicotine dependence is a potential confounder in genetic association studies of stress-related disorders that should not be neglected.

Because MDD is a highly heterogeneous disorder, and studies have reported inconsistent findings for the effects of *CRHR1*, the aim of this study was to investigate the role of *CRHR1* SNPs on susceptibility to MDD in more homogeneous subgroups. For this purpose, the study sample was divided into subgroups according to factors that may contribute to the etiological heterogeneity of the disorder (i.e., sex and smoking status).

2. Methods

2.1. Study context and design

This is a cross-sectional study based on a population sample of adults assessed in the blood donation center at *Hospital de Clínicas de Porto Alegre* in Southern Brazil. A protocol assessing their sociodemographic characteristics and psychiatric profile, including data on mood, anxiety, and substance use disorders, was applied via a face-to-face interview of all subjects enrolled in this study (see Rovaris et al., 2013). Primarily, we performed gene-by-sex and gene-by-smoking interaction analyses of the total sample to support our hypothesis-driven approach to assess the influence of sex and smoking on the effects of *CRHR1* on MDD. Then, we performed association analyses subdividing the total sample in groups according to sex and smoking status to obtain more homogeneous subsamples and to clarify interpretation of the first-step analyses.

2.2. Ethics

This study was carried out in accordance with the Declaration of Helsinki. All subjects provided written informed consent using a form that was previously approved by the institutional review board (IRB) of the hospital (No. 00000921).

2.3. Subjects

The study sample included 629 Brazilian adults of European descent [180 (28.6%) with lifetime MDD]. Table 1 shows the characteristics of the sample according to sex, including the lifetime prevalence of smoking, and other comorbidities. The inclusion criteria were (a) being a native Brazilian of European descent and (b) aged 18 years or older. The exclusion criteria were: (a) evidence of a clinically significant neurological disease that might affect cognition (e.g., delirium, dementia, epilepsy, head trauma, and multiple sclerosis) and (b) current or past history of psychosis. Individuals diagnosed with lifetime bipolar disorder