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Nonsynonymous *HTR2C* polymorphism predicts cortisol response to psychosocial stress II: Evidence from two samples

Baldwin M. Way[a,*], Kirk Warren Brown[b], Jordan Quaglia[b], Nancy McCain[c], Shelley E. Taylor[d]

[a] Department of Psychology, The Ohio State University and Institute for Behavioral Medicine Research, The Ohio State University Wexner Medical Center, United States
[b] Department of Psychology, Virginia Commonwealth University, United States
[c] Department of Adult Health and Nursing Systems, Virginia Commonwealth University, United States
[d] Department of Psychology, University of California at Los Angeles, United States

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ABSTRACT

The 5-HT$_{2C}$ receptor is the primary serotonin receptor located in the corticotrophin releasing hormone (CRH) neurons of the hypothalamus. These neurons initiate the signaling cascade that culminates in cortisol release. Therefore, genetic variation in the 5-HT$_{2C}$ receptor gene (*HTR2C*) is a prime candidate for affecting cortisol reactivity to stress. Accordingly, we examined the association of a nonsynonymous polymorphism (Cys23Ser; rs6318) in *HTR2C* with stress reactivity in two Trier Social Stress Tests conducted at separate sites. In both Study 1 (N = 128) and Study 2 (N = 185), Cys23 homozygous females and hemizygous males had greater cortisol reactivity. There was no relation between this polymorphism and self-reported affective response (Studies 1 and 2) or cardiovascular reactivity (Study 2). Additionally, the short/short genotype of a polymorphism (5-HTTLPR) in the serotonin transporter gene was associated with greater cortisol reactivity in Study 1 as well as in Study 2 (previously reported). The Cys23Ser polymorphism and the 5-HTTLPR were independently associated with cortisol reactivity in both studies. These findings emphasize the important role of genetic variation in the serotonin system on regulating cortisol reactivity to social evaluative stress. Comparison of the present associations with those of prior studies underscores the likely importance of situational and psychological factors in determining the direction and magnitude of the association between genotype and phenotype.

1. Introduction

In daily life, people regularly encounter stressful events. When such events are uncontrollable, painful, or threatening, they can elicit activation of corticotrophin releasing hormone (CRH) neurons in the hypothalamus. This initiates a signaling cascade that culminates with the release of cortisol from the adrenal cortex. A key regulator of reactivity within this hypothalamic-pituitary-adrenal (HPA) axis is the serotonin (5-HT) system (Chaouloff et al., 1999). Serotonin neurons directly innervate CRH neurons in the hypothalamus (Liposits et al., 1987) as well as the limbic and paralimbic areas (Way et al., 2007) that project to the hypothalamus. Based on rodent data, the most prevalent serotonin receptor in CRH neurons is the 5-HT$_{2C}$ receptor (Heisler et al., 2007). Deletion of this receptor nearly eliminates the increased release of CRH from the hypothalamus following serotonin release. In humans, pharmacological challenge with an agonist acting on the 5-HT$_{2C}$ receptor leads to increases in cortisol release (Kahn et al., 1990; Seibyl et al., 1991). Therefore, the 5-HT$_{2C}$ receptor is a critical driver of HPA axis reactivity.

In the human 5-HT$_{2C}$ receptor gene (*HTR2C*), there is a polymorphism (rs6318) that leads to a substitution of a serine for a cysteine (Cys23Ser) in the coding region (Lappalainen et al., 1995). Although the precise mechanism by which this polymorphism affects cellular signaling has not been conclusively determined (Fentress et al., 2005; Okada et al., 2004; Walstab et al., 2011), accumulating evidence suggests that the Cys23Ser polymorphism impacts reactivity to psychological stress. In women homozygous for the Ser23 allele, high levels of exposure to stressful life events have been associated with higher depressive symptoms than in women carrying the Cys23 allele exposed to similar levels of life stressors (Brummett

* Corresponding author at: Department of Psychology, The Ohio State University, 1835 Neil Avenue, Columbus, OH, 43210, United States.
E-mail address: Way.37@osu.edu (B.M. Way).

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et al., 2014b). In a large sample of individuals with coronary artery disease, women homozygous for the Ser23 allele had higher risk of myocardial infarction and all-cause mortality than those carrying the Cys23 allele (Brummett et al., 2013). Males with the Ser23 allele (hemizygous) showed the same risk as women homozygous for the Ser23 allele. Thus, the Ser23 allele appears to be associated with multiple stress-related health outcomes.

Because the HPA axis shows dysregulation in certain subtypes of depression (Stetler and Miller, 2011) and in cardiovascular disease (Hamer et al., 2012; Pereg et al., 2011), cortisol may be a common contributor to these mental and physical health outcomes. In fact, the Ser23 allele was associated with greater cortisol release during an emotional recall task in a study of men (Brummett et al., 2012) and in a replication sample of men and women (Brummett et al., 2014a). This is consistent with findings showing that Ser23 carriers exhibit greater central dopamine release during a pain stressor (Mickey et al., 2012) known to increase cortisol levels (Peciña et al., 2013). In both the emotional recall task and the pain tasks, the Ser23 allele was associated with greater emotional reactivity. This suggests that the Ser23 allele is a risk factor for stress-related mental and physical health outcomes.

A more commonly used laboratory measure to assess cortisol reactivity is the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), which involves delivering a speech and performing mental arithmetic in front of an evaluative audience. Such social evaluative threat reliably impacts HPA axis activation and is a common experience in modern societies (Lehman et al., 2015; Smith et al., 2012). The goal of the present studies was to determine if the Cys23Ser polymorphism affects cortisol response in the TSST, an analogue to daily life social threat. To build confidence in the results, we tested this association with two study designs at separate sites. In Study 1, we examined the association of CysSer23 with TSST cortisol response in both working adults and college students in a correlational fashion. In Study 2, we sought to replicate the observed relation with an experimental TSST design in a mixed working adult and college student sample. Based on prior work (Brummett et al., 2014a, 2012), we anticipated that the Ser23 allele would be associated with greater cortisol reactivity.

In addition to examining the role of variation in the *HTR2C* gene in moderating stress reactivity, we also sought to replicate the effects of a polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene. A recent *meta*-analysis supports a role for the 5-HTTLPR short/short genotype in greater cortisol reactivity to stress (Miller et al., 2013), but there was heterogeneity in the results. For example, one study found that the long allele was associated with greater cortisol reactivity (Mueller et al., 2011). Therefore, to expand the knowledge base regarding the relationship between the 5-HTTLPR and stress reactivity, we examined this polymorphism's contribution to TSST-evoked cortisol response both independently and in the context of 5-HT_{2C} effects. To examine the specificity of these genetic associations to HPA axis functioning, we also examined their relations to cardiovascular and subjective negative emotional responses.

2. Methods: Study 1

2.1. Participants

Participants were 149 employees (n = 81; sample 1) and students (n = 68; sample 2) at Virginia Commonwealth University (VCU), a large public institution, and were recruited through poster and e-mail advertisements. Inclusion of two populations, college students and working adults, was designed to enhance the generalizability of the research findings across major sociodemographic groups. Inclusion criteria were age (> 18 years) and ability to read and write in English. Exclusion criteria were: 1) existing health conditions (e.g., autoimmune disorders) or health habits (e.g., regular cigarette, illicit drug, oral contraceptive use) that could affect stress responsiveness or increase health risks during the TSST; 2) health conditions (e.g., Cushing's disease, high blood pressure, psychiatric illness) or drug use (e.g., marijuana, tobacco) that could affect cortisol levels. Participants were asked to refrain from strenuous exercise, alcohol consumption, and smoking on the session day, and to refrain from consuming dairy products, caffeine, or eating < 1 h before the session (c. f., Gruenewald et al., 2004). Inclusion and exclusion criteria were checked at screening and upon entry into the TSST session. Of the initial participants, 5 were removed before analysis for procedural errors, and 11 for non-compliance in the self-report or TSST portion of the study. Three participants were removed for current psychiatric illness, and 1 was removed for English language comprehension difficulty. This left N = 129 participants for analysis (n = 62; sample 1; n = 67; sample 2).

Sample 1 (employees) was 71% female, with an average age of 38.34 years (SD = 11.47). Most were Caucasian (n = 42; 67.7%); the balance were Black or African American (n = 15; 24.2%); Asian (n = 4; 6.5%) and Hispanic or Latino(a) (n = 1; 1.6%). Sample 2 (students) was 61% female, with an average age of 21.09 years (SD = 3.43). This sample was more racially and ethnically diverse. Caucasians comprised 33.3% (n = 22), Black or African American participants comprised 30.3% (n = 20), and Asian individuals comprised 22.7% (n = 15) of the sample. The balance were Hispanic or Latino(a) (n = 3; 4.6%), mixed race (n = 1; 1.5%), another racial/ethnic group (n = 3; 4.6%), or undeclared (n = 2; 3.0%). Participants earned $100 for study completion. All procedures were approved by the Institutional Review Board at VCU.

2.2. Procedure

The testing session (2 h in duration) was conducted in a study-dedicated laboratory suite between 1 and 6 pm to control for diurnal cortisol variation. After an introduction to the study and receipt of written informed consent, baseline saliva for cortisol assay was collected, and blood for DNA extraction was drawn from the non-dominant arm by a trained phlebotomist via indwelling catheter with a butterfly needle and saline-lock access.[1] A local anesthetic cream was applied to the venipuncture site to minimize pain. Stress-relevant measures of current psychological state were also completed (see Section 2.3 below). All saliva and blood samples were processed immediately post-session and cryopreserved until analysis, after which any remaining samples were destroyed.

Following baseline data collection, participants began the TSST, which followed standard procedures (Kirschbaum et al., 1993). Participants spent 5 min mentally preparing a five-minute public speech before delivering it to a panel of two critical peer evaluators. For sample 1, the speech was to address: "Why you would be a good candidate for a job in which you would have to work effectively with university staff to come up with solutions to problems typically faced by employees in your department or unit." For sample 2, participants were asked to explain "Why you would be a good candidate for a job as an administrative assistant in the university Psychology Department." After this speech preparation period, two additional, brief self-report measures not discussed here (achievement goals and primary appraisal) were completed.

The experimenter waited in an adjoining control room during speech preparation, and subsequently, two public speech "evaluators" (trained confederates) entered the participant room and

[1] For other study purposes, two additional blood draws were made: 45 min and 85 min after TSST onset (data not reported here).