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# Corticosteroid receptor genes and childhood neglect influence susceptibility to crack/cocaine addiction and response to detoxification treatment

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ABSTRACT

The aim of this study was to analyze hypotheses-driven gene-environment and gene-gene interactions in smoked (crack) cocaine addiction by evaluating childhood neglect and polymorphisms in mineralocorticoid and glucocorticoid receptor genes (*NR3C2* and *NR3C1*, respectively). One hundred thirty-nine crack/cocaine-addicted women who completed 3 weeks of follow-up during early abstinence composed our sample. Childhood adversities were assessed using the Childhood Trauma Questionnaire (CTQ), and withdrawal symptoms were assessed using the Cocaine Selective Severity Assessment (CSSA) scale. Conditional logistic regression with counterfactuals and generalized estimating equation modeling were used to test gene-environment and gene-gene interactions. We found an interaction between the rs5522-Val allele and childhood physical neglect, which altered the risk of crack/cocaine addiction (Odds ratio = 4.0, P = 0.001). Moreover, a *NR3C2-NR3C1* interaction (P = 0.002) was found modulating the severity of crack/cocaine withdrawal symptoms. In the *post hoc* analysis, concomitant carriers of the *NR3C2* rs5522-Val and *NR3C1* rs6198-G alleles showed lower overall severity scores when compared to other genotype groups (P-values $\leq$ 0.035). This gene-environment interaction is consistent with epidemiological and human experimental findings demonstrating a strong relationship between early life stress and the hypothalamic-pituitary-adrenal (HPA) axis dysregulation in cocaine addiction. Additionally, this study extended in crack/cocaine addiction the findings previously reported for tobacco smoking involving an interaction between *NR3C2* and *NR3C1* genes.



## 1. Introduction

Smoked (crack) cocaine addiction is a growing health issue, especially in developing countries such as Brazil (Bastos, 2012). In the II Brazilian National Alcohol and Drugs Survey, the prevalences of lifetime crack and snorted cocaine use were estimated in 1.5% and 3.9%, respectively; while the dependence rate to this drug was reported to be 0.6% in the general population. Among adolescents, 0.8% had already smoked cocaine or its related forms (merla and oxi) at least once in their lifetime, being the prevalence of lifetime snorted cocaine use estimated in 3.6% (Abdalla et al., 2014; INPAD, 2014). Although crack users in Brazil are predominantly male (~78%), women are more vulnerable to complications related to drug use as homelessness and HIV infection, presenting also higher victimization rates than men in Brazilian samples (Bastos and Bertoni, 2014; Dias et al., 2011; Duailibi et al., 2008).

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Analyses of twin pairs have shown that the heritability of cocaine dependence is high in both genders (around 65%–79%) (Kendler et al., 2000; Kendler and Prescott, 1998). However, specific genetic factors are still largely unknown. Environmental effects also seem to be highly relevant, with childhood neglect and poor parent-child attachment being the main factors related to cocaine use during adulthood. Childhood neglect results in alterations of the hypothalamic-pituitary-adrenal (HPA) axis in humans (Gerra et al., 2009, 2010), and early life stress is related to epigenetic modifications of the glucocorticoid receptor gene in both rodents and humans (Anacker et al., 2014; Kundakovic et al., 2013). Furthermore, clinical and experimental findings are consistent with the connection between stress, cocaine use and the biology of substance use disorders. Cortisol increases the release of dopamine in mesocorticolimbic pathways (Oswald et al., 2005; Wand et al., 2007), and cocaine stimulates secretion of cortisol and corticosterone (Mello, 2010). There is also evidence that HPA axis dysregulation modulates craving and relapse in cocaine-abstinent addicts (Brady et al., 2009).

We have previously demonstrated an interaction effect between two functional SNPs (single nucleotide polymorphisms) from the mineralocorticoid (MR) and glucocorticoid receptor (GR) genes on tobacco smoking susceptibility (Rovaris et al., 2013b). Afterward, others have shown that SNPs located in the genes coding these receptors were also associated with cocaine addiction (Levran et al., 2014). MRs and GRs are transcription factors expressed in several brain areas (de Kloet et al., 2005) and modulate dopamine neurotransmission dynamics through cortisol availability (Sinha, 2008). These receptors must necessarily act together in coordinating all steps of stress response (Joels et al., 2008; Oitzl et al., 2010) and their binding to corticosteroids (cortisol in humans and corticosterone in rodents) results in dimerization and conformational changes that increase their interaction with DNA-responsive elements (Schoneveld et al., 2004). The dimerization process results in MR/MR, GR/GR and MR/GR complexes, which have distinct transactivation capacities (Liu et al., 1995; Savory et al., 2001; Trapp et al., 1994). Additionally, an 'MR/GR balance hypothesis' has been proposed (Joels et al., 2008; Oitzl et al., 2010). This theory suggests that an imbalance between MRs and GRs due to genetic and/or epigenetic variations would lead to an impaired stress response, increasing the susceptibility to stress-related disorders, including substance use disorders.

Within the MR coding gene (*NR3C2*), rs5522 is the most studied SNP (from dbSNP at www.ncbi.nlm.nih.gov). It comprises a A/G transition in the second exon, resulting in an isoleucine to valine change (Ile180Val). The *NR3C2* rs5522-Val allele has been associated *in vitro* with lowered efficiency of cortisol, but not aldosterone, as a ligand (DeRijk et al., 2006). Moreover, it was shown that such allele increases salivary and plasma cortisol responses to psychosocial stressor and dexamethasone suppression test (van Leeuwen et al., 2011). The *NR3C2* rs5522-Val allele was also linked to severity of attention-deficit/hyperactivity disorder (Kortmann et al., 2013), depressive symptoms in geriatric patients (Kuningas et al., 2007) and altered reward learning (Bogdan et al., 2010). Additionally, a genome wide association study (GWAS) has found a nominal association between *NR3C2* rs5522 SNP and severity of smoking behavior ($P = 1.52E{-}05$), with the Val allele increasing the number of smoked cigarettes per day (Berrettini et al., 2008).

In the GR coding gene (*NR3C1*), alternative splicing near the end of the primary transcript generates the two main receptor isoforms, termed GRα and GRβ, which differ in their length and molecular effect (Kino et al., 2009). The *NR3C1* rs6198 (A3669G) is a functional SNP that alters the rates of GRα/GRβ expression, where the G allele favors the expression of the GRβ isoform (Derijk et al., 2001). GRβ is unable to bind cortisol and acts as a dominant negative inhibitor of the classic MR and GR isoforms (Bamberger et al., 1997; Oakley and Cidlowski, 2013). As seen for the *NR3C2* rs5522 SNP, previous studies have associated the *NR3C1* rs6198-G allele with major depressive disorder (Szczepankiewicz et al., 2011) and altered cortisol response following a psychological stressor and dexamethasone suppression test (Kumsta et al., 2008). A protective effect of this allele on the clinical manifestation and course of bipolar disorder was also reported (Spijker et al., 2011, 2009).

Taking into account the links between the HPA axis, early life stress and cocaine addiction, as well as the evidence that *NR3C2* and *NR3C1* variants influence HPA axis reactivity, this study has three objectives. The first is to test main effects as well as interaction effects between *NR3C2* rs5522 and *NR3C1* rs6198 SNPs and childhood neglect in crack/cocaine addiction susceptibility. The second is to verify the potential role of theses SNPs on the severity of crack/cocaine withdrawal symptoms during detoxification treatment. Since it has been suggested that epistasis plays a role in the missing/phantom heritability of complex phenotypes (Zuk et al., 2012), the third goal of this study is to test if the interaction between *NR3C2* rs5522 and *NR3C1* rs6198 SNPs, previously associated to tobacco smoking susceptibility (Rovaris et al., 2013b), also affects the severity of crack/cocaine addiction.

## 2. Methods and materials

### 2.1. Design

This study had two designs to contemplate the objectives mentioned before: 1) it was a control free cross-sectional study; 2) It was a patient-only three-week longitudinal study (Fig. 1). The outcome evaluated over time was the response to a detoxification treatment measured by the severity of the withdrawal symptoms.

### 2.2. Sample

The sample comprised one hundred thirty-nine crack/cocaine-addicted women who were admitted to a three-week detoxification program in Southern Brazil. All participants were inpatient in a public psychiatric unit for female drug and alcohol disorders. These women were in a controlled abstinence situation, and had no access to alcohol, cigarettes or drugs. In addition, all patients had a

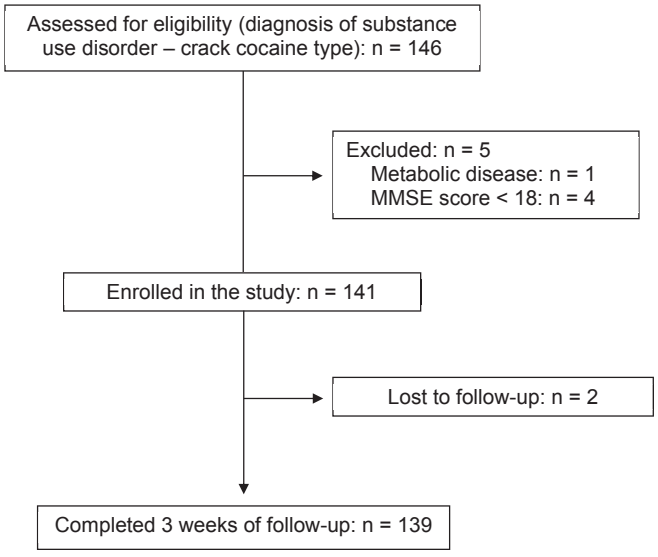


**Fig. 1.** Study flowchart. MMSE = Mini-Mental State Examination.