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Association between methylation of the glucocorticoid receptor gene, childhood maltreatment, and clinical severity in borderline personality disorder

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ABSTRACT

The hypothalamus-pituitary-adrenal axis (HPA) is essential in the regulation of stress responses. Increased methylation of the promoter region of the glucocorticoid receptor gene (*NR3C1*) has been described both in subjects with history of childhood trauma and in patients with Borderline Personality Disorder (BPD). However, no data on the possible association between a higher methylation of this gene and clinical severity is available. The aim of this study was to evaluate the association between *NR3C1* methylation status, the history of childhood trauma, and current clinical severity in subjects with BPD. A sample of 281 subjects with BPD (diagnosed by SCID-II and DIB-R semi-structured diagnostic interviews) was recruited. Clinical variables included previous hospitalizations, self-injurious behavior, and self-reported history of childhood trauma. DNA was extracted from peripheral blood. The results indicated a significant positive correlation between *NR3C1* methylation status and childhood maltreatment (specifically physical abuse). In addition, a positive correlation between methylation status and clinical severity (DIB-R total score and hospitalizations) was observed. These findings suggest that *NR3C1* methylation in subjects with BPD may be associated not only with childhood trauma but also with clinical severity, adding new evidence to the involvement of gene-environment interactions in this disorder.

1. Introduction

The hypothalamus-pituitary-adrenal axis (HPA) (Nemeroff, 1999) is among the most important biological systems involved in the regulation of response to stress. Activation of this axis leads to the secretion of glucocorticoids from the adrenal cortex via the release of corticotrophin-releasing factor (CRF) and adrenocorticotropin (ACTH). This system is regulated by the action of circulating glucocorticoids on glucocorticoid receptors in corticolimbic structures such as the hypothalamus or hippocampus. Early life stress has been associated with changes in HPA axis function in both preclinical and clinical studies (Heim and Nemeroff, 2001; Carpenter et al., 2007). This vulnerability of the HPA axis to early stress, together with its involvement in stress response, has prompted research into the role of this axis in Borderline Personality Disorder (BPD), especially because BPD is believed to be the result of an interaction between biologically-based temperamental vulnerabilities and stressful experiences in childhood (Linehan, 1993; Zanarini and Frankenburg, 1997; Beauchaine et al., 2009; Crowell et al., 2009). Most of the

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published evidence indicates that BPD is characterized by HPA axis hyperactivity and reduced feedback sensitivity, although some studies have shown contradictory results (for a review, see Wingenfeld et al., 2010).

The influence of environmental factors on biological processes might occur through epigenetic mechanisms such as DNA methylation, which inhibits transcription and reduces gene expression. Methylation of HPA axis genes may disrupt the proper functioning of the HPA axis. Epigenetic research on this axis has focused primarily on the glucocorticoid receptor (GR) since the methylation pattern of its promoter region has been linked to early stressful events in rats (Weaver et al., 2004). In humans, methylation of the promoter region of the GR gene (named *NR3C1*) is reported to be higher in children exposed to maternal stress during pregnancy (Oberlander et al., 2008; Radtke et al., 2011; Mulligan et al., 2012; Hompes et al., 2013), in victims of childhood trauma (McGowan et al., 2009; Labonte et al., 2012; Tyrka et al., 2012; Perroud et al., in press), in subjects with BPD (Dammann et al., 2011) and in subjects with BPD and history of childhood trauma (Perroud et al., 2011). While most of the aforementioned studies obtained DNA from peripheral blood, two (both from the same research group) obtained DNA from brain tissue samples (McGowan et al., 2009; Labonte et al., 2012). Those two studies found that suicide victims with a history of childhood trauma had higher levels of *NR3C1* methylation in the hippocampus compared to victims without such trauma and with control subjects.

To date, research on *NR3C1* methylation in BPD is scant. Dammann et al. (2011) compared 26 patients with BPD to 11 healthy controls to assess differences in the methylation pattern of *NR3C1* (and other neuropsychiatric genes). Those authors found that mean *NR3C1* methylation levels were significantly higher in BPD subjects than in the controls. Perroud et al. (2011) evaluated the association between childhood maltreatment and increased methylation of the *NR3C1* gene promoter in a sample of 101 subjects with BPD, 99 subjects with major depressive disorder (MDD), and 15 subjects with MDD and comorbid post-traumatic stress disorder (PTSD). Results of that study showed a positive correlation between sexual abuse, the severity of that abuse, the number of different types of maltreatment, and *NR3C1* methylation. In the BPD group, both sexual abuse and physical neglect, as well as the number of types of abuse and neglect, were associated with higher methylation levels. Moreover, BPD subjects without childhood maltreatment had higher *NR3C1* methylation levels than MDD subjects, suggesting that BPD could be, *per se*, linked to an increased methylation status.

In summary, data from these studies suggest that subjects with BPD who have suffered childhood trauma may have higher *NR3C1* methylation levels than those who have not. Moreover, taking into account that both specific kinds of childhood trauma (Martín-Blanco et al., 2014) and dysfunctions of the HPA axis have been linked to BPD severity (Carrasco et al., 2007), maybe the methylation status of *NR3C1* is also associated with the severity of this disorder. Therefore, the aim of the present study was to evaluate whether an association exists between *NR3C1* methylation status in a sample of subjects with BPD and the patients' history of childhood trauma and current clinical severity. Based on previous research, we hypothesized that both factors would be associated with higher methylation levels.

2. Materials and methods

2.1. Participants

A sample of 281 subjects with BPD was recruited from 3 hospitals with specific BPD units (Hospital de la Santa Creu i Sant Pau, Hospital Universitari de la Vall d'Hebron and Hospital de Igualada). Inclusion criteria consisted of: (a) diagnosis of BPD according to DSM-IV criteria, assessed through two semi-structured diagnostic interviews: the Spanish validated versions of both the Structured Clinical Interview for Diagnostic and statistical manual of mental disorders-IV (DSM-IV) Axis II Disorders (SCID-II) (Gómez-Beneyto et al., 1994) and the Revised Diagnostic Interview for Borderlines (DIB-R) (Barrachina et al., 2004); (b) no current episode of any Axis I disorder according to DSM-IV criteria, including substance dependence; (c) no severe physical conditions such as organic brain syndrome, neurological disease or mental deficiency; and (d) being Caucasian of European descent. Experienced psychiatrists carried out a clinical interview to collect sociodemographic and clinical variables including age, sex, marital status, educational level, employment status, current pharmacological treatment, past episodes of axis I disorders, previous hospitalizations, and self-injurious behaviour (self-mutilation and suicide attempts). The participants also completed the Childhood Trauma Questionnaire (CTQ-SF) (Bernstein et al., 2003), a self-reported questionnaire designed to assess traumatic experiences in childhood.

This study was approved by the Clinical Research Ethics Committee of the 3 collaborating hospitals and the study design followed the Declaration of Helsinki principles. All the participants gave informed consent to participate in the study and no compensation was given for participation.

2.2. Assessment instruments

- *Structured Clinical Interview for DSM-IV Axis II Personality Disorders* (SCID-II) (Gómez-Beneyto et al., 1994): SCID-II is a semi-structured interview to assess personality disorders according to DSM-IV criteria. The instrument has shown an adequate inter-rater reliability and a fine discrimination among the Axis II personality disorders. We used the validated Spanish version.
- *Revised Diagnostic Interview for Borderlines* (DIB-R) (Barrachina et al., 2004): DIB-R is a semi-structured interview to diagnose BPD within the last two years. This instrument has shown good psychometric properties. We used the validated Spanish version.
- *Childhood Trauma Questionnaire - Short Form* (CTQ-SF) (Bernstein et al., 2003): The CTQ is a questionnaire designed to retrospectively assess childhood abuse and neglect. The short version (CTQ-SF) was developed by means of exploratory and confirmatory analyses of the 70 original CTQ items, and its validity in clinical and non-referred populations as a screening tool for maltreatment histories in childhood has been tested. The 28 items on the CTQ-SF are rated on a 5-point, Likert-type scale ranging from Never True to Very Often True. This questionnaire is divided into five clinical subscales: sexual, physical, and emotional abuse, as well as physical and emotional neglect.

2.3. Procedure

Blood samples were systematically collected from the subjects upon admittance to the unit. Genomic DNA was extracted from peripheral leukocytes by using the salting out procedure (Autopure, Qiagen) (Miller et al., 1989). We used the same protocols of blood collection, DNA extraction and storage for all the samples in this study in order to avoid variability when measuring methylation (Wu et al., 2011). As in previous studies (Perroud et al., 2011), DNA bisulphite treatment and PCR amplification were performed by means of EpiTech Bisulfite kit and the PyroMark PCR kit (Qiagen) respectively, following the manufacturer recommendations. Finally, pyrosequencing was performed in a PyroMark Q24 (Qiagen).