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Contributions of the glucocorticoid receptor polymorphism (Bcl1) and childhood abuse to risk of bulimia nervosa

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ABSTRACT

This study evaluated the hypothesis that traumatic stress can increase risk of bulimia nervosa (BN) in individuals who are genetically disposed towards lower modulation of physiological stress reactions. We explored the extent to which childhood abuse (physical or sexual), variants of a main glucocorticoid receptor (GR) polymorphism (Bcl1), or their interaction, differentiated women with and without BN. Women seeking treatment for BN ($N=129$) and non-eating-disordered comparison women ($N=98$) provided blood samples for assays of the Bcl1 polymorphism, and completed structured interviews assessing eating symptoms, psychiatric symptoms and childhood abuse. Compared to normal-eaters, bulimic women were significantly more likely to carry the low-function Bcl1 C allele (CC or CG genotypes), to report a history of childhood abuse and, more importantly, to be positive for both factors. We interpret our findings as indicating that traumatic stress, when impacting individuals disposed to lower GR modulation, can be etiological for BN.

1. Introduction

Current etiological theories postulate that bulimia nervosa (BN) often implicates the activation of hereditary susceptibilities by environmental forces (e.g., Steiger, 2004; Bulik, 2005; Steiger and Bruce, 2007). In this study, we evaluated the hypothesis that traumatic stress can increase risk of BN in individuals who are genetically disposed towards lower inhibition of hypothalamic–pituitary–adrenal (HPA) axis stress responses.

Modal figures from clinical samples suggest that about 30% of adults with a bulimic syndrome report unwanted sexual experiences during childhood, whereas over half report physical maltreatment in childhood (see Wonderlich et al., 1997; Steiger and Bruce, 2008). Effects of childhood abuse have been thought to be enacted, in part, via stress-induced alterations in the functioning of the brain's main stress–response system, the HPA axis—exposure to traumatic stress having been linked to anomalous HPA-axis activity in eating- (Steiger et al., 2001; Díaz-Marsá et al., 2007) and non-eating-disordered populations (Stein et al., 1997; Yehuda et al., 2002). HPA-axis findings in BN are variable (see Brambilla and Monteleone, 2003), but a modal result seems to be "hypercortisolemia" (i.e., unusually high baseline cortisol levels)—which could reflect relatively low inhibition of cortisol release by HPA-axis glucocorticoid receptors (GRs).

The NR3C1 gene (located on chromosome 5q31) affects GR sensitivity and, in turn, strength of inhibitory feedback within the HPA axis (DeRijk et al., 2002). The most widely studied of polymorphisms associated with GR expression is the Bcl1 restriction fragment length polymorphism, a C/G single nucleotide polymorphism in intron B, 646 nucleotides downstream of exon 2. Suggesting that Bcl1 mediates inhibitory feedback within the HPA axis, Bcl1 high-function (G) allele carriers have been observed to display reduced cortisol responses following psychosocial stressors (Wüst et al., 2004; Kumsta et al., 2007) and greater suppression of cortisol after dexamethasone (van Rossum et al., 2003). Such findings lead to the conjecture that traumatic experiences might have more pronounced effects in individuals whose genetic propensities code for lesser modulatory feedback within the HPA axis. Consistent with the preceding, changes in methylation that would cause reduced hippocampal NR3C1 expression have been observed in suicide completers with a history of childhood abuse (McGowan et al., 2009). Likewise, corticotropin-releasing hormone receptor genes have been reported to modulate proneness to depression in previously traumatized adults (Bradley et al., 2008; Tyrka et al., 2009).

The Present Study: We tested the hypothesis that bulimic individuals would, more frequently than normal-eaters, carry the

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low-function allele of the Bcl1 polymorphism (associated with relatively low GR feedback) and report severe childhood stressors (i.e., sexual or physical abuse). As other syndromes that co-occur frequently with BN (notably major depressive disorder, some anxiety disorders and post-traumatic stress disorder) also implicate altered HPA-axis activity (Stein et al., 1997; Yehuda et al., 2002; Ströhle and Holsboer, 2003; Belmaker and Agam, 2008; DeRijk et al., 2008), we took steps to explore and control potential confounding effects of these other psychiatric entities.

2. Methods

2.1. Participants

This institutional ethics-board approved study was conducted with informed consent, between September 2001 and May, 2008. Eating-disordered women were recruited through a specialized Eating Disorder (ED) program for adults, and were 129 consenting women with a DSM-IV-TR (American Psychiatric Association, 2000) diagnosis of bulimia nervosa (BN). Of these women, 118 (91.5%) met criteria for BN-Purging (BN-P) subtype and 11 (8.5%) for BN-Nonpurging (BN-NP) subtype. Mean chronicity of illness was 8.16 (±6.13) years. According to data on 113 women for whom we had a reliable ED history, 50 (44.2%) had shown a prior history of anorexia nervosa or significant weight loss (an adult BMI below 17.5).

We also tested 98 normal-eater women, drawn from an age group comparable to that of our ED sample, and using public and school-based announcements so as to recruit comparable proportions of student and non-student participants to those found in our ED sample. To be eligible for the normal-eater group, participants had to be free of clinical ED symptoms according to the EDE, of an ED history according to semistructured interview, and to have BMI between 17.5 and 35. To avoid skewing the sample towards super-normalcy, we accepted 11 normal-eaters who emerged on structured interviews (see Measures section) as having had an Axis-I disorder within the past 12 months. Disorders detected included major depressive disorder ($n=3$), social phobia ($n=2$), specific phobia ($n=1$), post-traumatic stress disorder (PTSD: $n=2$), cannabis abuse ($n=1$), and cannabis dependence ($n=2$). We were unable to complete structured diagnostic interviews with one control participant, but a semistructured screening interview suggested that this individual had never suffered an Axis-I disorder.

Subjects were between the ages of 18 and 44 (mean = 25.17 ± 5.38 for bulimic subjects and 24.21 ± 5.72 for controls). Body Mass Index (BMI: kg/m^2) fell between 17.61 and 33.73 for bulimic participants (mean = 21.39 ± 3.89) and 18.02 and 33.02 for control subjects (mean = 22.10 ± 2.75). Clinical and control groups did not differ on either dimension. Within the eating-disordered group (information available on 120 of 129), 35 (29.2%) had a secondary-level education, 47 (39.2%) college-level, and 38 (31.7%) university level. Within the control group (information available on 97 of 98), 21 (21.6%) had a secondary-level education, 39 (40.2%) college, and 37 (38.1%) university level. Data on household income were available for 92 of the bulimic participants and for 72 of the normal-eater controls. Among the bulimic women, 23 (25%) reported a household income less than $10,000, 14 (15.2%) an income between $10,000 and $19,999, 13 (14.1%) an income between $20,000 and $29,999, 8 (8.7%) between $30,000 and $39,999, 12 (13.0%) between $40,000 and $49,999, and 22 (23.9%) an income above $50,000. Among the control women, 21 (23.3%) reported a household income less than $10,000, 14 (15.6%) an income between $10,000 and $19,999, 10 (11.1%) between $20,000 and $29,999, 5 (5.6%) between $30,000 and $39,999, 7 (7.8%) between $40,000 and $49,999, and 15 (16.7%) income above $50,000. Confirming the impression that clinical and control cases were very similar on these social-standing indices, chi-squared tests detected no significant differences on either variable. Limiting recruitment to unmedicated individuals was impractical (and undesirable on grounds of representativeness), and we therefore included 59 bulimic women (45.7% of the sample) and 1 normal-eater control who were using a psychoactive medication when tested.

The Quebec population from which this sample was drawn is skewed towards individuals of Caucasian, Western-European descent. Consequently, our bulimic sample included mainly Caucasians ($n=125$, or 96.9% of the sample), with rare Blacks ($n=2$, or 1.6% of the sample) and Asians ($n=2$, or 1.6% of the sample). Data on race were available for only 43 (43.9%) of the normal-eater participants. However, these data indicated racial composition of our control group—33 (76.7%) Caucasians, 5 (11.6%) Blacks and 5 (11.6%) Asians—to be quite similar to that of our bulimic group.

2.2. Measures

ED diagnoses and symptoms were assessed using the Eating Disorders Examination (EDE: Fairburn and Cooper, 1993), a semistructured interview assessing the presence and severity of core ED symptoms. The current "Gold Standard" for ED diagnosis, the EDE has established reliability and validity (Fairburn and Cooper, 1993).

Screening for comorbid (past 12 months) DSM-IV Axis-I disorders was accomplished using the Structured Clinical Interview for DSM-IV Axis-I disorders (SCID-I: First et al., 1996), a computer-guided, interview-based version of the Diagnostic Interview Schedule, Version IV (DIS4: Bucholz et al., 1991), and/or the Clinician-Administered Post-Traumatic Stress Disorder Scale (CAPS: Blake et al., 1995)—all "industry standard" measures, exhibiting excellent reliability, and convergent and discriminant validity. (Variations in interviews applied reflected shifts in study protocols occurring during the patient recruitment reported here.) Elsewhere, we have evaluated agreement between DIS4 and SCID-I diagnoses, and have obtained solid Kappas (and percent agreements) for the past 12-month presence of Axis-I disorders (Steiger et al., 2006).

Childhood abuse was assessed using the Childhood Trauma Interview (CTI: Fink et al., 1995), a roughly 30-minute structured interview addressing experiences of abuse occurring up to age 18. Interrater reliability for indices reflecting the nature, severity, frequency and duration of trauma are very good (Fink et al., 1995). As in earlier applications (e.g., Steiger et al., 2001), we utilized severity indices to isolate experiences of unambiguous physical or sexual maltreatment. Subjects were classified as having experienced childhood abuse when they received a score of 2 or greater on severity of sexual abuse or 3 or greater on severity of physical abuse. Examples of criterion-level sexual abuse were: fondling of genitals or breasts, or having to watch someone else being sexually abused. Examples of criterion instances of physical maltreatment were: being whipped on bare legs, slapped in the face, or pushed to the ground. We thought that lower ratings (reflecting concepts such as "being looked at in a sexualized way" or "pushed, but not pushed down") indicated abuse too ambiguously, and individuals reporting such instances were classified as "not abused". The "abused"/"not abused" distinction applied here therefore reflects relative levels of stress exposure, and not some absolute categorical phenomenon. We note that prior use of the same thresholds has led to interpretable effects in previous studies on clinical and neurobiological consequences of childhood abuse (see Steiger, 2004; Steiger and Bruce, 2007).

2.3. Genotyping

DNA samples, obtained from whole blood, were amplified by polymerase chain reaction (PCR) in a total volume of 20 µl, which contained 100 ng of genomic DNA, 200 µM of dNTPs, 10 pmol each of the forward and reverse primer, 1 U of Taq DNA Polymerase (Qiagen, Alameda, CA), 1× PCR buffer, and 1× Q solution (Qiagen). The forward primer (5′-AAA TTG AAG CTT AAC AAT TTT GGC-3′) and reverse primer (5′-GCA GTG AAC AGT GTA CCA GAC C-3′) were used to amplify a region encompassing Bcl1; C and G alleles were then resolved on a 2% agarose gel. The PCR protocol involved preheating the samples at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C (30 s), annealing at 59 °C (30 s), and extension at 72 °C (45 s), as well as a final hold of 7 min at 72 °C. The C and G alleles were subsequently studied by enzymatic digestion of 7 µl of the above mentioned PCR product using 5 U of Bcl1 and incubating overnight at 50 °C.

2.4. Statistical analysis

We explored bulimic versus nonbulimic differences as to genotype and abuse frequencies using chi-squared tests. To test for possible associations between Gene × Abuse interaction effects and diagnostic distinctions (e.g., bulimic versus nonbulimic), and to control for influences due to potential confounding variables, we applied hierarchical logistic regression analyses.

3. Results

Table 1 displays, for bulimic and normal-eater samples, the frequencies of a) Bcl1 genotypes and b) cases with or without at least one copy of the C allele. The table also shows rates, in both of our samples, of c) childhood sexual abuse, d) physical abuse, and e) either form of abuse. Hardy–Weinberg tests indicated genotype rates to be in equilibrium in our control sample [$\chi^2_{(1)}=2.63$, n.s.], but weakly in disequilibrium in our bulimic sample [$\chi^2_{(1)}=4.84$, $p<0.05$]. The latter

Table 1
Frequencies (and percentages) of cases in bulimic and normal-eater control groups displaying a) various genotypes or alleles and b) exposure to childhood abuse.

	Bulimic	Normal-eater
	n (%)	*n* (%)
a) Bcl 1 genotype		
CC	15 (11.6%)	4 (4.1%)
CG	74 (57.4%)	45 (45.9%)
GG	40 (31.0%)	49 (50.0%)
b) Bcl 1C allele (CC or CG genotype)		
C allele	89 (69.0%)	49 (50.0%)
No C allele	40 (31.0%)	49 (50.0%)
c) Sexual abuse	47 (36.4%)	7 (7.1%)
d)Physical abuse	73 (56.6%)	35 (35.7%)
e) Either form of abuse	89 (69.0%)	37 (37.8%)