Genetic and Environmental Predictors of Comorbid Symptoms and Treatment Response in Individuals with Bulimia-Spectrum Disorders

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Contribution of Authors

As first author on all three manuscripts, I participated in designing the studies and developed the research questions and hypotheses. I recruited participants, ran them through the studies, managed data collection and ran all statistical analyses. I wrote all three manuscripts and incorporated suggestions from co-authors.

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Abstract

Bulimia Nervosa (BN) is defined by eating disturbances, but frequently co-occurs with other psychiatric symptoms—including disturbances of mood, anxiety, substance-use and impulse control. This dissertation examined 1) how genetic factors and developmental experiences might be associated with patterns of psychiatric comorbidity in individuals with BN-spectrum disorders, and 2) how psychiatric comorbidity and genetic factors might impact treatment response in BN-spectrum individuals. The dissertation consists of three studies. Study 1 developed an empirically based classification of individuals with BN-spectrum disorders based on Axis-I comorbidity and compared resulting classes on variations in the serotonin transporter promoter polymorphism (5-HTTLPR) and exposure to childhood abuse. Results revealed two classes—a "high comorbidity" class and a "low comorbidity" class—which differed on genetic and developmental dimensions. The high comorbidity class displayed a greater likelihood of carrying 5-HTTLPR low function alleles and more sexual or physical abuse in childhood, implying that genetic variations and abuse may both influence comorbid psychopathological manifestations in BN. Study 2 examined possible associations between genetic and environmental factors and specific comorbid (Axis-I and Axis-II) disorders in individuals with BN-spectrum disorders. Findings revealed that 5-HTTLPR high-function alleles predicted the presence of comorbid Anxiety Disorders whereas childhood abuse predicted a history of Substance Abuse/Dependence, suggesting that genetic variations and

developmental history may help explain different comorbid symptom presentations observed among bulimic individuals. The third study in this dissertation explored the effects of psychiatric comorbidity and genetic factors on response to treatment in individuals with BN-spectrum disorders. Findings showed that Axis-I comorbidity had little effect on treatment outcome. In contrast, the presence of Borderline Personality Disorder predicted more severe impulsive symptoms pre- and post-treatment. The presence of Obsessive-Compulsive Personality Disorder predicted more severe depressive symptoms pre- and post-treatment, as well as poorer response of bulimic symptoms to treatment. With respect to genetic variables, homozygosity for 5-HTTLPR low-function variants predicted more severe purging behaviour, which persisted throughout treatment. Taken together, the present findings suggest that subgroups with different comorbid symptoms and disorders may display different environmental and genetic vulnerabilities and different responses to treatment for BN. Theoretical and clinical implications are discussed.

Résumé

La Boulimie Nerveuse (BN) est caractérisée par des perturbations alimentaires, mais est aussi fréquemment accompagnée par des symptômes psychiatriques – incluant des troubles de l'humeur, d'anxiété, d'abus de substances et de contrôle de l'impulsivité. Cette dissertation examine 1) comment les facteurs génétiques et les expériences durant l'enfance peuvent être associés à différents modèles de comorbidité psychiatrique chez les individus souffrant de troubles des conduites alimentaires de type boulimique et 2) comment la comorbidité des troubles psychiatriques et les facteurs génétiques peuvent affecter la réponse au traitement chez les individus souffrant de troubles des conduites alimentaires de type boulimique. Cette dissertation se compose de trois études. La première étude a développé une classification empirique des individus souffrant de troubles des conduites alimentaires de type boulimique basée sur la comorbidité des troubles présentés dans l'Axe I et a ensuite exploré les différences qui existent possiblement entre ces groupes par rapport au polymorphisme promoteur du gène du transporteur de la sérotonine (5-HTTLPR) et à l'exposition à l'abus dans l'enfance. Les résultats ont révélés deux groupes – un groupe possédant un haut niveau de comorbidité et un autre possédant un bas niveau de comorbidité – qui présentaient des dimensions génétique et de développement différentes. Les individus du groupe possédant un haut niveau de comorbidité avait plus de chances d'être porteurs d'allèles à faible fonction de 5-HTTLPR et d'avoir été exposés à des abus sexuels et physiques dans leur enfance.

Ces résultats signifient que les variations génétiques et l'abus dans l'enfance influencent possiblement la co-occurrence de psychopathologies chez les individus souffrant de troubles des conduites alimentaires de type boulimique. La deuxième étude a examiné les associations possibles entre les facteurs génétiques et environnementaux et certains désordres comorbides (Axe I et Axe II) chez les individus souffrant de troubles des conduites alimentaires de type boulimique. Les résultats démontrent que les allèles à haute fonction de 5-HTTLPR prédisent la co-occurrence de troubles d'anxiété, tandis que l'abus durant l'enfance prédit la présence d'abus ou de dépendance à certaines substances. Ces résultats suggèrent que les variations génétiques et l'histoire du développement peuvent aider à expliquer la présentation de différents symptômes parmi les individus souffrant de BN. La troisième étude rapportée dans cette dissertation a exploré les effets de la comorbidité psychiatrique et des facteurs génétiques sur la réponse au traitement des individus souffrant de troubles des conduites alimentaires de type boulimique. Les données de cette étude ont démontré que la co-occurence de troubles de l'Axe I avait peu d'effet sur les résultats du traitement. En revanche, la présence d'un trouble de personnalité limite prédisait le maintien de symptômes psychopathologiques généraux plus sévères après le traitement. De plus, la présence d'un trouble de personnalité obsessionnelle compulsive prédisait non seulement le maintien de symptômes psychopathologiques généraux plus sévères après le traitement, mais aussi le maintien des symptômes boulimiques. Concernant les variables génétiques, l'homozygosité des allèles à basse fonction de 5-HTTLPR prédisait des comportements purgatifs plus fréquents même après

le traitement. Ensemble, ces résultats suggèrent que les sous-groupes présentant des symptômes et des désordres comorbides peuvent variés dans leurs vulnérabilités environnementale et génétique et dans leurs différentes réponses aux traitements pour la BN. Les implications théoriques et cliniques sont discutées.

General Introduction

Although reports of bulimia as a symptom date back to the 1890's (Casper, 1983) bulimia as a syndrome seems not to have appeared in the literature until the late 1970's (Vandereycken, 1994). In an influential article in *Psychology Today*, Boskind-Lodahl and Sirlin (1977) described a neurosis they identified as *bulimarexia*: "The gorging-purging syndrome. Feeling rebuffed by men, America's affluent young women are devastating their bodies in the hope of perfection". In the ensuing years several papers appeared on the new bulimia syndrome, the most notable being Gerald Russell's now famous (1979) article describing 30 *bulimia nervosa* patients, who were characterized by uncontrollable urges to overeat and attempts to avoid weight gain by purging. Soon thereafter, bulimia nervosa (BN) was recognized as a new diagnosis in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III; American Psychiatric Association (APA), 1980). Throughout the 1970's, 80's and much of the 90's BN was regarded as an extreme manifestation of society's obsession with thinness and, as described in Boskind-Londahl and Sirlin's (1977) article, was often attributed to gender dynamics (Collier & Treasure, 2004). A shift in thinking came in the 1990's when accumulating evidence from twin and family studies uncovered an important genetic basis for eating disorders (Bulik, Sullivan, & Kendler, 1998; Bulik, Sullivan, Wade, & Kendler, 2000; Lilenfeld et al., 1998; Strober, Freeman, Lampert, Diamond, & Kaye, 2000). Since then, thinking has progressed to the current conceptualization of BN as a heterogeneous disorder with multiple causal factors, involving the interaction of environmental and

genetic processes (Collier & Treasure, 2004). In the last decade, much research has been directed at further understanding the genetic component of eating disorders (EDs), however, the specifics of which genes might contribute to EDs and how they may interact with environmental factors are still elusive and in need of further study.

Defining Characteristics

The core psychopathology in BN is the overevaluation of one's shape and weight. That is, individuals with BN judge their self-worth based largely, or even exclusively, on their shape, weight and the ability to control them (Fairburn & Harrison, 2003). Other symptoms of the disorder seem to be consequences of extreme attempts to control shape and weight. For example, efforts to restrict food intake often lead to episodes of uncontrolled eating, referred to as "binge eating". The revised version of the fourth edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV; APA, 2000) describes binge eating as eating an unusually large amount of food, in a discrete period of time, accompanied by a sense of loss of control. Episodes of binge eating are most often followed by compensatory behaviours aimed at preventing weight gain, such as purging (e.g., through self-induced vomiting or the use of laxatives or diuretics), fasting or excessive exercise. DSM-IV criteria for BN require that individuals engage in binge eating and compensatory behaviours at least twice a week (on average) for a period of 3 months. In addition, the criteria for anorexia nervosa cannot be met. The DSM-IV defines two subtypes of individuals with BN; those who compensate for overeating through purging (Purging Type) and those who do not

engage in purging, but compensate for overeating through other methods such as fasting and exercise (Nonpurging Type).

Relation to Other Eating Disorders

Anorexia Nervosa. Anorexia Nervosa (AN) and BN are united by the same core psychopathology; the overevaluation of shape and weight. As in BN, most other symptoms of AN stem from this core psychopathology (Fairburn & Harrison, 2003). To control shape and weight, individuals engage in extreme food restriction and avoidance of "fattening" foods in the same rigid way as do patients with BN—the major difference being that individuals with AN are "successful" in their attempts to lose weight, whereas individuals with BN usually maintain a normal weight due to intermittent episodes of overeating. A subgroup of individuals with AN do however also engage in regular binge eating and/or purging. The DSM-IV defines two subtypes of AN; a Restricting Type (AN-R) in which weight loss is accomplished through dieting fasting and/or exercise in the absence of binge eating or purging, and a Binge-Eating/Purging Type (AN-B/P), in which individuals maintain a low weight, but regularly engage in binge eating or purging (or both). The major difference between AN-B/P and BN is the relative balance of overeating and undereating, and the subsequent effect on body weight (Fariburn, Cooper, & Shafran, 2003). Studies comparing both AN subtypes to BN have found that the two groups who binge and purge (AN-B/P and BN) closely resemble each other and that AN-B/P can be distinguished from AN-R subjects on a range of demographic, clinical and psychometric variables (Dacosta & Halmi, 1992; Garner, Garfinkel, & O'Shaugnessy, 1985;Rosval et al., 2006). Moreover,

studies using taxometric methods suggest that AN-B/P is closer in nature to BN than it is to AN (Eddy et al., 2009; Gleaves, Lowe, Snow, Green, & Murphy-Eberenz, 2000). However, other studies show that AN-B/P and AN-R groups are similar and can be distinguished from BN on important variables such as longitudinal course, recovery, relapse and mortality rates (Eddy et al., 2002; Herzog et al., 1999).

Eating Disorder Not Otherwise Specified. The DSM-IV diagnosis of eating disorder not otherwise specified (EDNOS) is for EDs of clinical severity that fall outside the specified diagnoses of AN or BN. Many such cases are subthreshold forms of AN or BN. For example, an individual may meet all the criteria for AN except that they continue to have regular menses. Another example might be that all of criteria for BN are met except that the binge eating and compensatory behaviours occur at less than the requisite twice weekly. A special case of EDNOS that is under review to be in its own separate category of eating disorder in DSM-V is Binge Eating Disorder (BED). In BED recurrent episodes of binge eating occur at least twice weekly but in the absence of any compensatory behaviours (APA, 2000). EDNOS is the most common ED encountered in outpatient treatment settings (Fairburn et al., 2007). Research comparing BN to subthreshold BN syndromes diagnosed as EDNOS (EDNOS-BN) suggests that EDNOS-BN closely resembles BN in nature, duration and severity of associated psychopathology (Fairburn et al., 2007). For example, most research does not support a distinction between those individuals who engage in binge eating at threshold (2 or more times per week) versus subthreshold (less than 2 times per

week) frequency (Mond et al., 2006; for a review see Wilson, 1992). Similarly, Fairburn and Cooper (1984) found no clinical differences between individuals who vomited once or less per week and those who vomited at threshold levels (at least 2 times/week). In addition, research comparing individuals who engage in objective binge eating (in which an objectively large amount of food is consumed) to those who engage in subjective binge eating (in which the amount of food is not large, but is viewed by the subject as excessive) have found no differences between the two in terms of comorbid psychopathology, personality disorders and demographics (Niego, Pratt, & Agras, 1997; Pratt, Niego, & Agras, 1998; for a review see Wilson, 1992). More recent research utilizing taxometric analyses suggests that EDNOS cases closely resemble AN, BN or BED cases and may be better conceptualized as existing upon a continuum with DSM-IV EDs rather than as one distinct subgroup (Eddy et al., 2009; Mitchell et al., 2007).

Epidemiology

Reported prevalence of BN in young women in North America and Europe falls in the 1% to 2% range (Fairburn & Belglin, 1990; Garfinkel et al., 1995; Keski-Rahkonen et al., 2008; Whitehouse, Cooper, Vize, Hill, & Vogel, 1992). However, in subthreshold forms (e.g., in individuals who binge and purge at a frequency of once rather than twice a week), BN-spectrum EDs appear to be more prevalent, occurring in about 2.5-5% of young women (Garfinkel et al., 1995; Keski-Rahkonen et al., 2008; Whitehouse et al., 1992). Females are ten times more likely to develop BN than males (Garfinkel et al., 1995), with peak prevalence occurring in females in their late teens and early twenties (Hoek &

Van Hoeken, 2003; Keski-Rahkonen et al., 2008). Community studies suggest that only a minority of women with BN (about a third) are detected by the health care system (Hoek & Vann Hoeken, 2003; Keski-Rahkonen et al., 2008). Longitudinal outcome studies examining the natural course of BN suggest that the disorder is chronic, with about 50% of cases still meeting diagnostic criteria for the disorder after five years (Fairburn, Cooper, Doll, Norman, & O'Connor, 2000; Keski-Rahkonen et al., 2008).

A review of cross-historical and cross-cultural literature by Keel and Klump (2003) revealed that, as opposed to AN which can be found throughout history and in non-Western nations, BN is not found in earlier historical periods and occurs primarily in Western societies. Epidemiological data demonstrate a large and significant increase in BN over the latter half of the twentieth century (Keel & Klump, 2003), possibly due to increased media exposure to cultural ideals of thinness. Research also suggests that BN is more common in more industrialized environments, with individuals living in urbanised areas being 2.5 times more likely to develop BN than those in rural areas and individuals living in large cities (more than 100 000 inhabitants) being 5 times more likely to develop BN than those in rural communities (Van Son, Van Hoeken, Bartelds, Van Furth, & Hoek, 2006). Studies examining BN prevalence rates among different ethnic groups suggest that it is most commonly found in Caucasian women. Most studies show that rates of BN are lower in African American and Asian American women than in Caucasian women (Gross & Rossen, 1988; Johnson et al., 1984; Nevo, 1985). Conversely, Hispanic American women, although less concerned with

their weight in general, display a frequency of BN similar to that seen in their Caucasian counterparts (Crago, Shisslak, & Estes, 1996; Gross & Rossen, 1988; Johnson et al., 1984). In all ethnic minority groups, risk of BN is higher in females who are more identified with Western values (Crago et al., 1996), once again demonstrating the importance of cultural ideals in the development of BN. A common stereotype is that EDs are more prevalent in individuals of high socioeconomic status (SES). However, epidemiological studies suggest that this is not the case, with most studies showing no effect of SES on BN status and some even showing more BN in low SES environments (for a review see Gard & Freeman, 1996).

Comorbidity with Other Psychological Disorders and Traits

Although defined by disturbances in eating behaviour, BN frequently co-occurs with other DSM-IV Axis-I disorders, including mood, anxiety, and substance-use disorders, and Axis-II personality disorders. Moreover, individuals with BN often display specific character traits such as perfectionism, obsessive-compulsive features, impulsivity and affective instability.

Mood Disorders. Mood disorders (MDs) are the most common comorbid Axis-I disorder diagnosed in individuals with BN. Studies report lifetime prevalence rates of MDs in clinical samples of BN in the range of 70-90% (Brewerton et al., 1995; Bulik, Sullivan, Carter, & Joyce, 1996; Hudson, Pope, & Yurgelon-Todd, 1988). The most prominent mood disorder found in BN is major depressive disorder (MDD), with studies in clinical samples reporting prevalence rates of 60-80% (Brewerton et al., 1995; Godart et al., 2007; Herzog et al., 1999; Hudson et

al., 1988). In community samples, lifetime prevalence rates of MDD are lower than in clinical samples (falling in the 30-50% range), however the prevalence of MDD in community samples of BN is significantly higher than in individuals without an eating disorder in the population (Bushnell et al., 1994; Garfinkel et al., 1995; Hudson, Hiripi, Pope, & Kessler, 2007). Studies examining the familial coaggregation of MDs with EDs have produced mixed results, with some studies demonstrating significant coaggregation of EDs and MDs (e.g., Mangweth et al., 2003) and others reporting non-significant findings (e.g., Lilenfeld et al., 1998). Similarly, data from studies examining shared transmission of EDs and MDs using discordant monozygotic twin designs have produced mixed results, with some suggesting the presence of some shared genetic effect (e.g., Walters et al., 1992) and others not (e.g., Keel, Klump, Miller, McGue, & Iacono, 2005). Discrepant findings in family and twin studies may suggest both shared and independent transmission between EDs and MDs (Keel et al., 2005).

Anxiety Disorders. Lifetime prevalence rates of anxiety disorders (ADs) in individuals with BN are similar in both clinical and community samples, and generally range from 50-80% (Bulik et al. , 1996; Garfinkel et al., 1995; Godart et al., 2003; Hudson et al., 2007). Studies in community samples suggest that ADs are significantly more frequent in individuals with BN than in control subjects without an eating disorder (Garfinkel et al., 1995). The most common AD found in individuals with BN is social phobia, but generalized anxiety disorder, obsessive compulsive disorder, panic disorder and simple phobia also figure prominently in BN (for a review see Swinbourne & Touyz, 2007). Several

studies have shown that ADs generally precede the onset of BN (Bulik et al., 1996; Godart et al., 2003), leading researchers to speculate that early ADs may predispose individuals to BN. Twin studies supporting the existence of shared genetic transmission between BN and ADs (Keel et al., 2005; Kendler et al., 1995) further support such speculations and suggest that the relationship between BN and ADs may be due to common genetic factors.

Substance Abuse and Dependence. Studies in both clinical and community samples suggest that alcohol abuse or dependence occurs in roughly a third of individuals with BN (Garfinkel et al., 1995; Holderness, Brooks-Gunn, & Warren, 1994; Hudson et al., 2007; Lilenfeld et al., 1998). Drug abuse or dependence is somewhat less common in BN, with prevalence estimates ranging from 15-30% (Holderness et al., 1994; Hudson et al., 2007; Lilenfeld et al., 1998). Studies in both clinical and population based samples have found elevated rates of lifetime substance use disorders (SUDs) in women with BN compared to control women without an ED (Garfinkel et al., 1995; Lilenfeld et al., 1998). Despite the significant co-occurrence of BN with SUDs, twin studies consistently demonstrate no shared genetic factors between the two disorders (Keel et al., 2005; Kendler et al., 1995), suggesting independent transmission of BN and substance abuse.

Axis-II Disorders. BN frequently co-occurs with personality disorders (PDs), however reported prevalence rates of PDs in individuals with BN show marked inconsistencies across studies, with figures ranging from 27-93% (for a review see Vitousek & Manke, 1994). Inconsistencies may be due to various factors, including differences in methods of assessment, criteria utilized for diagnosis, and

recruitment methods across studies (Grilo, 2002). A meta-analysis of PDs and EDs including only studies that utilized DSM diagnostic criteria to diagnose PDs found that 44% of individuals with BN met criteria for Cluster C (Avoidant, Dependent or Obsessive-Compulsive) PDs (characterized by anxious, fearful behaviour) and 44% met criteria for Cluster B (Borderline, Histrionic, Narcissistic or Antisocial) PDs (characterized by dramatic, erratic behaviours) (Rosenvinge, Martinussen & Ostensen, 2000). A recent review paper examining 10 studies that included clinical BN samples and utilized clinical interview methods for diagnosis of PDs, found BPD and avoidant personality disorder (AVPD) to be the two most common personality disorders in BN, with estimated prevalence rates of 21% and 19% respectively (Cassin & Von Ransen, 2005). One common question in the study of PDs and EDs is whether the PD is pre-existing to the eating condition or a consequence of ED symptoms. ED symptoms such as malnutrition and purging can result in states that resemble features of PDs. One way to address potential confounds of ED state effects (like malnutrition) is to study recovered ED patients to see if PDs persist after symptom reduction. Zanarini et al. (1990) examined comorbid PDs in women with active BN and remitted BN and found no significant differences between the two groups with respect to PD prevalence, with 50% of active cases and 44.4% of remitted cases meeting criteria for a comorbid PD. Similarly, in a study of ED patients recovered from AN and BN (for at least 1 year) Matsunaga et al. (2000) demonstrated that PDs persist after recovery from EDs, however in contrast to the previous study, the rate of PDs in recovered patients (26%) was lower than expected in an active ED sample,

suggesting that recovery from an ED may have an attenuating influence on PD symptoms in some subjects.

Personality Traits. The relationship between personality traits and BN has been a source of interest for ED researchers over several decades. Research, mostly cross-sectional in nature, has delineated several personality traits as having potential importance for the aetiology of BN. More specifically, traits such as perfectionism, obsessionality, harm avoidance, impulsivity and affective instability have been associated with BN (Cassin & Von Ranson, 2005; Lilenfeld, Wonderlich, Riso, Crosby, & Mitchell, 2006; Steiger et al., 2009; Vitousek and Manke, 1994; Westen & Harnden-Fischer, 2001). As with the study of PDs a common problem in relating such traits to BN development is whether or not they are in fact traits that existed before the ED or whether they are influenced by states of the disorder, like malnutrition or binge eating and purging. In order to tease apart personality "trait" from ED "state" effects studies have been performed in remitted individuals with BN. For example, in a study comparing women recovered from BN (at least 1 year) to healthy control women on an array of personality traits (including perfectionism, obsessionality, impulsivity and affective instability) Kaye et al. (1998) found that perfectionism and obsessionality persist after recovery from BN. Likewise, in a study of women with active BN, remitted BN and no ED Von Ranson, Kaye, Weltzin, Rao, & Matsunaga (1999) found that obsessional traits persisted after recovery from BN and did not differ dramatically between active and remitted BN subjects. In addition, Lehoux, Steiger and Jabalpurlawa (2000) found that narcissistic traits

persisted after recovery from BN and did not differ significantly between active and recovered BN patients. Finally, Lilenfeld et al. (2000) examined personality traits in first-degree relatives (with no ED history) of individuals with BN and control subjects and found significantly more perfectionism, ineffectiveness and interpersonal distrust in relatives of BN subjects than those of control probands. Findings suggesting that personality disturbances run in families of individuals with BN and persist after recovery from BN suggest that such disturbances are trait-, rather than state-, related and may contribute to the pathogenesis of BN.

Etiology

BN is a heterogeneous disorder with multiple causal factors, involving the interaction of genetic processes and a range of environmental risk factors. In the last decade, much research has been directed at further understanding the etiology of BN, however there is still much to be known about the individual causal factors, in particular the genetic components and how they interact with environmental processes to contribute to BN.

Environmental Factors

Sociocultural Factors. Of the environmental variables thought to contribute to BN sociocultural influences are considered paramount. Rates of BN have risen over time and cross-culturally in conjunction with increased exposure to Western cultural ideals equating thinness with beauty, success, and power (Keel & Klump, 2003). In line with this, Stice, Schupak-Neuberg, Shaw, and Stein (1994) found a direct relationship between media exposure (arguably the strongest messenger of the thin ideal) and eating disorder symptoms in female college students.

Moreover, women with BN have been shown to hyper-internalize cultural thin ideals (Kendler et al., 1991). Stice's (1994, 2001) sociocultural theory for the development of BN posits that sociocultural pressures to be thin, internalization of the thin ideal and body dissatisfaction combine to foster dieting and negative mood, consequently increasing risk of BN symptoms.

Developmental Factors:

Family Functioning. Environmental factors within the family setting have also been thought to play a role in the development of BN. Studies have described families of individuals with Bulimia Nervosa (BN) as being 'chaotic', or as being characterized by high levels of conflict, low cohesiveness, or a lack of parental warmth and care (Schmidt, Humfress, & Treasure, 1997; Steiger, Van der Feen, Goldstein, & Leichner, 1989; Strober & Humphrey, 1987). Self-reports of family functioning by BN patients indicate their families to be typified by high levels of distress, more hostility and lesser support than do reports of normal controls (Humphrey, 1986; Latzer, Hochdorf, Bachar, and Canetti, 2002; Shissak, McKeon, and Crago, 1990). Although there are convergences between family interaction patterns and BN, recent thinking has moved away from the traditional notion that family interactions convey specific vulnerability to BN. Recent thinking conceptualizes family factors as playing more of a modulating role in the development of BN, enacted possibly through concurrent psychopathological traits (Steiger, Bruce, & Israel, 2003). In a review of the literature, Schmidt et al. (1997) reported that severity of family dysfunction was closely related to personality pathology in individuals with EDs, suggesting that family factors may

have an impact on personality traits, which in turn, influence risk of ED symptomatology.

Childhood Sexual and Physical Abuse. Traumatic childhood experiences have been frequently implicated as causal factors in the development of BN. Studies comparing individuals with BN to normal-eater control groups on measures of childhood sexual and physical abuse demonstrate a higher rate of abuse in BN women (Leonard, Steiger, & Kao, 2003; Rorty, Yager, & Rossotto, 1994; Steiger & Zanko, 1991; Welch & Fairburn, 1994). Data show that about 30% of adults with bulimia-spectrum disorders report a history of childhood sexual abuse, and 50% or more report a history of childhood physical abuse (Leonard et al., 2003; Fullerton, Wonderlich, and Gosnell, 1995; Rorty et al., 1994; Wonderlich, Brewerton, Jocic, Dansky, and Abbott, 1997). It is noteworthy that although such rates are elevated compared to control individuals, they are comparable to those found in other psychiatric patient groups (Steiger & Zanko, 1990; Welch & Fairburn, 1994)—suggesting that childhood abuse is not specific to BN, but rather, more strongly linked to generalized psychopathology. Consistent with this notion, in individuals with bulimia-spectrum disorders, childhood abuse has been more consistently associated with severity of general psychopathological symptoms than eating-specific ones (for a review see Schmidt, Humfress, & Treasure, 1997). For example, in individuals with BN a history of abuse has been associated with more self-destructiveness (Corstorphine, Waller, Lawson, & Ganis, 2007), submissiveness (Leonard et al., 2003), impulsivity (Myers et al., 2006), alcohol and substance abuse (Corstorphine et al., 2007; Fullerton et al.,

1995) and BPD (Steiger, Jabalpurwala, & Champagne, 1996). Taken together, research suggests that there is a strong, but non-specific association of childhood abuse with BN.

Genetic Factors

Until the 1990's research in the field of BN was mainly focused on environmental etiologic factors. In the past two decades, however, accumulating evidence from twin and family studies has uncovered an important genetic basis for BN. Family studies examining rates of EDs in first-degree biological relatives of individuals with and without a history of BN demonstrate that EDs aggregate in the families of individuals with BN. For example, Strober, Freeman, Lampert, Diamond, and Kaye (2000) found that the risk of BN was 4.4 times higher among relatives of BN probands than among relatives of comparison subjects. In addition, Lilenfeld et al. (1998) found that relatives of BN probands had a 12 times higher rate of EDNOS compared with relatives of control probands. Twin studies comparing concordance rates for BN among genetically identical (monozygotic) and nonidentical (dizygotic) twin pairs provide strong evidence that a significant proportion of the observed familial aggregation of BN is due to genetic factors (Bulik, Sullivan, Wade, & Kendler, 2000). Clinical- and community-based twin studies consistently show a 50-60% contribution of additive genetic effects to liability of BN (Bulik, Sullivan, & Kendler, 1998; Kendler et al., 1991; Wade, Neale, Lake, & Martin, 1999). Evidence of a strong genetic contribution to the risk of BN supports investigation into specific genetic factors that may underlie liability to the disorder. Studies investigating candidate

genes for BN have focused on genes coding for neurobiological agents that may regulate eating behaviour as well as other more general disturbances of mood, anxiety an impulse control. Serotonin (5-hydroxytryptamine: 5-HT) has been thought to be a promising agent for study since it has been implicated in the regulation of numerous biological, psychological and behavioural processes, including food intake and sleep patterns, and the regulation of mood, anxiety, aggression and behavioural impulses (Reif & Lesch, 2003). In the following sections evidence for a role of 5-HT in the etiology of BN will be reviewed and several genes involved in the regulation of 5-HT function will be introduced as possible candidate genes for BN.

5-HT System Functioning in BN. A large body of research has accumulated demonstrating altered 5-HT system functioning in BN. For example, individuals with active BN have been shown to display reduced platelet binding of 5-HT uptake inhibitors (Marazziti, Macchi, Rotondo, Placidi, & Cassano, 1988; Steiger, Young, et al.2001), diminished neuroendocrine responses to 5-HT precursors and agonists (Levitan, Kaplan, Joffe, Levitt, & Brown, 1997; Steiger, Gauvin, et al., 2001), reduced hypothalamic and thalamic 5-HT transporter availability (Tauscher et al., 2001), and increased 5-HT_{1A} activity throughout the cortex and raphe regions—suggesting increased presynaptic autoreceptor activity and decreased post-synaptic 5-HT availability (Tiihonen et al., 2004). The wish to clarify whether such reductions in 5-HT availability are a consequence of nutritional influences (e.g., dietary restriction of the amino acid tryptophan, used in 5-HT synthesis) or a pre-existing vulnerability factor for BN has led

researchers to examine 5-HT in individuals who have fully recovered from BN, to remove the possible confound of dietary factors. Studies in recovered individuals have revealed similar 5-HT abnormalities to those found in individuals with active EDs. For example, single photon emission computed tomography (SPECT) studies have shown reduced 5-HT_{2A} activity in the medial orbital frontal cortex of women who have recovered from BN (Kaye et al., 2001). Moreover, individuals recovered from BN and unaffected first-degree relatives of individuals with BN have both been shown to display reductions in platelet paroxetine binding similar to those found in individuals with active BN (Steiger et al., 2005; Steiger et al., 2006). Findings demonstrating presence of reduced 5-HT tone in the absence of active ED symptoms in at-risk individuals or in unaffected first-degree relatives suggest that 5-HT reductions may represent a pre-existing vulnerability factor or "endophenotype" for BN.

Candidate Genes. Before providing a review of the literature on candidate genes for BN a brief introduction to genetics will be provided. Genes are made from long molecules called deoxyribonucleic acid (DNA). DNA is made up of simple units called nucleotides—A,T,C, or G—that line up in a particular order. The order of these nucleotides carries genetic information. A single nucleotide polymorphism (SNP) is a variation in the DNA sequence occurring when a single nucleotide in the genome differs between one organism and another (e.g., AAG**C**CTA versus AAG**T**CTA). Each unique form of a gene polymorphism is called an allele (Goodsell, 1996). Allelic variations of gene polymorphisms are explored in candidate gene studies in an attempt to understand inter-individual

phenotypic differences, for example why one person might develop a certain trait or disorder and not another.

The Serotonin Transporter Promoter Polymorphism (5-HTTLPR) in BN. In keeping with findings linking 5-HT dysfunction to BN, evidence has suggested relevance, in the etiology of BN, of gene polymorphisms that code for 5-HT system functioning. The most extensively studied of the 5-HT gene polymorphisms has been the serotonin transporter promoter polymorphism (5-HTTLPR). 5-HTTLPR is a 44-base pair insertion deletion polymorphism in the 5′ flanking regulatory region of the serotonin transporter gene, originally thought to have "long" (L) and "short" (S) variants, which differentially modulate transcriptional activity (Lesch et al., 1996). Relative to the L allele, the 5-HTTLPR S allele has been associated with lesser transcription of 5-HT transporter protein (Heils et al., 1996; Lesch et al., 1996). Recent findings, however, suggest the existence of a low-frequency L-allele variant, L_G (an L allele with A→G SNP in its sequence), whose functioning may be comparable to that of the low-function, S allele (Hu et al., 2006; Zalsman et al., 2006). Such data imply that 5-HTTLPR may be triallelic, with S and L_G alleles regarded as "low-function" variants (S') and L_A regarded as a "high-function" allele (L'). Studies examining the traditional biallelic model of 5-HTTLPR in BN have found incongruent findings, with one study reporting an increased frequency of low function alleles in BN (DiBella, Catalano, Cavallini, Riboldi, & Bellodi, 2000), one reporting an increased frequency of the high function allele (Monteleone et

al., 2006) and two others reporting absence of association between 5-HTTLPR and BN (Lauzurica et al., 2003; Matsushita et al., 2004).

A possible explanation for inconsistent findings is that the biallelic model may be imprecise. Assuming that the triallelic model is viable, a traditional biallelic classification may potentially underestimate the presence of low-function variants and overestimate the presence of high-function variants by classifying L_G as an L (or high-function) allele. In the only study to examine triallelic 5-HTTLPR in EDs to date, Steiger et al. (2009) found an increased prevalence of the high function allele and high-function homozygotes (L_A/L_A genotype) in a mixed sample of AN, BN and Eating Disorder Not Otherwise Specified (EDNOS) subjects. However, more fine-grained analyses suggested that the association may have been localized to those individuals displaying an inhibited/compulsive personality profile.

The preceding suggests another possible explanation for inconsistent findings on the association between 5-HTTLPR and BN, namely that, rather than being associated with risk of BN per se, 5-HTTLPR may be associated but with risk of comorbid psychopathological symptoms in BN. Consistent with this notion, in individuals with bulimia-spectrum disorders, 5-HTTLPR has been more consistently associated with severity of general psychopathological symptoms than with eating-specific ones. For example, 5-HTTLPR low function variants have been linked to increased affective instability, impulsivity, borderline personality disorder, and harm avoidance (Akkermann, Nordquist, Oreland, and Harro, 2010; Monteleone et al., 2006; Steiger et al., 2005) in women with BN-

spectrum disorders and (as mentioned above) 5-HTTLPR high-function variants have been associated with increased compulsivity and inhibition in women with EDs (Steiger et al., 2009). Such findings imply that 5-HTTLPR low function variants might be associated with traits or disorders of an affective-impulsive nature, whereas high function variants might be more closely associated with those of an anxious-compulsive type. Interestingly, similar findings have been found in non-ED populations, with studies generally showing low-function variants to be linked to disorders involving problems of affect or impulse-regulation—such as MDD (Joiner, Johnson, Soderstrom, & Brown, 2003; Lotrich & Pollock, 2004; Neumeister et al., 2002), alcohol use disorders (Lichtermann et al., 2000; Mannelli et al., 2005; Sander et al., 1997; 1998) and BPD (Lyons-Ruth et al., 2007; Ni et al., 2006)—and high-function variants associated with anxiety disorders, most prominently post-traumatic stress disorder (PTSD) (Grabe et al., 2009; Thakur, Joober, & Brunet, 2009) and obsessive-compulsive disorder (OCD) (Baca-Garcia et al., 2005; Bengel et al., 1999; Cavallini, Di Bella, Siliprandi, Malchiodi, & Bellodi, 2002; Hu et al., 2006). Taken together, such results suggest that, 5-HTTLPR allelic variations may be more closely associated with variations in comorbid-psychiatric symptoms in BN than with eating symptoms per se, with low function variants being associated with impulsive/affective traits or disorders and high function variants being associated with anxious/compulsive symptoms.

Polymorphisms of the Tryptophan Hydroxylase (TPH) Gene in BN. Another gene acting in the serotonin-system that is of potential interest in the study of BN is the tryptophan hydroxylase (TPH) gene. The TPH gene encodes the rate-limiting

biosynthetic enzyme in the serotonin pathway and regulates levels of 5-HT by converting tryptophan into 5-hydroxytryptophan, the direct precursor of 5-HT (Hennig, Reuter, Netter, Burk & Landt, 2005). Variations in the TPH gene could contribute to reduced 5-HT neurotransmission. Intron 7 (on the short arm of chromosome 11) is the site of the single nucleotide polymorphism (SNP) A779C, which has been shown to have functional relevance. Lower CSF 5-HIAA levels have been found in healthy men carrying the TPH A allele (Jonsson et al., 1997; Manuck et al., 1999). Since low 5-HT function has been implicated in BN, an association between the TPH A allele and BN has been posited. The only published study to examine the TPH A779C single nucleotide polymorphism (SNP) in BN reported that individuals carrying the AA genotype exhibited more disturbed binging behaviours and higher harm avoidance scores than did individuals with the CC genotype (Monteleone et al., 2007). The reported findings are consistent with data associating the A-allele with lower 5-HT function, which in turn has been associated with BN. Moreover, in non-ED samples the TPH A779C SNP has been associated with traits and behaviours often comorbid with BN and also associated with low 5-HT tone, such as impulsive aggression (Manuck et al., 1999), suicidal behaviour (Mann et al., 1997), and deliberate self-harm (Pooley, Houston, Hawton & Harrison, 2003). Taken together, research suggests that the TPH A779C SNP may play a role in predisposing individuals to a spectrum of impulsive-affective symptoms and disorders, including BN.

The interpretation of the preceding results is called into question by findings suggesting that TPH appears to be found exclusively in peripheral tissues and in

the pineal body (McKinney, Knappskog & Haavik, 2005). More recently, Walther and colleagues (2003) identified a second TPH isoform, designated as TPH-2, highly similar to the above-mentioned TPH gene (exhibiting 71% of amino acid identity), but expressed predominantly in the brain stem. Since this discovery, research in the field of mental illness has focused mainly on the TPH-2 gene. Investigations of the TPH-2 gene have yielded further support for the involvement of 5-HT genes in Axis-I disorders in which anomalies of 5-HT functioning have already been identified. For instance, researchers have found associations between certain polymorphisms or haplotypes of the TPH-2 gene and MDD (Zill, Baghai, et al., 2004; Zhou et al., 2005)), suicidality (Zill, Buttner, et al., 2004; Lopez de Lara et al., 2007; De Luca et al., 2005) and ADs (Zhou et al., 2005; Mössner et al., 2006). Possibly the most commonly studied SNP of the TPH-2 gene has been the TPH-2 G-703T (rs4570625) polymorphism. Associations of the TPH-2 G-703T polymorphism with psychopathological traits and syndromes that frequently co-occur with BN make it a good candidate SNP for study in BN. For example, studies have reported associations between the TPH-2 G-703T T-allele or T/T genotype and emotional instability (Brown et al., 2005; Canli, Congdon, Guktnecht, Constable & Lesch, 2005; Hermann et al., 2007), cluster B and C personality disorders (Gutknecht et al., 2006), harm avoidance (Reuter, Kuepper & Henning, 2007) and impulsivity (Reuter, Ott, Vaitl & Henning, 2007; Stoltenberg et al., 2006). In the only study to examine the role of genetic variation in TPH-2 SNPs in individuals with BN-spectrum disorders, Groleau and colleagues (unpublished findings) found lower perfectionism and compulsivity

scores in individuals carrying the T-allele of the TPH-2 G-703T polymorphism. Such findings, coupled with those in non-ED populations, suggest that the TPH-2 G-703T T allele may have implications for impulsive, affective and anxious traits in individuals with BN.

Gene-Environment Interactions.

It is widely accepted that nature and nurture interact to shape phenotype expression, with some disorders being more genetically based, and others more influenced by the environment (Plomin, Owen & McGuffin, 1994). Research exploring genetic and environmental factors in BN points to an important etiological role for both factors. However, few studies have examined gene-environment interactions in BN. The majority of research examining gene-environment interactions to date has been conducted in animals. Interesting findings associating childhood rearing experiences and genetic variations with trait and behaviour disturbances in rhesus monkeys has inspired investigation into possible interactions between genes and environment in human populations. Most of such research to date has been conducted in the field of depression. Again, promising findings showing that childhood trauma or stressful life events may interact with certain genetic variations to produce depressive symptoms and disorders encourages further investigation into gene-environment interactions. The subsequent paragraphs review the studies on gene-environment interactions in both the animal and human (depression) literature. Preliminary findings of gene-environment interactions in individuals with BN-spectrum disorders are also presented. Since most studies involving genes relevant to the 5-HT system have

studied 5-HTTLPR, the following review focuses exclusively on 5-HTTLPR-environment interactions.

5-HTTLPR-Environment Interactions in the Animal Literature. In one of the first studies to examine the interacting effects of 5-HTTLPR and environmental factors Bennett et al. (2002) found that peer reared, but not mother reared, rhesus macaques with a low function allele (s/l) had significantly lower concentrations of CSF 5HIAA than those with only high function alleles (l/l), suggesting that the low function allele of the 5-HTTLPR may predict decreased 5-HT activity in monkeys faced with developmental stressors. Similarly, Barr, Newman, Shannon et al. (2004) found that peer reared female macaques with a low function allele of 5-HTTLPR had higher adrenocorticotropic hormone ACTH levels during separation than both their mother reared low function (l/s) counterparts and high function (l/l) macaques, indicating that this group may be particularly vulnerable to the effects of stress. In addition, Champoux et al. (2002) found that both mother reared and nursery reared rhesus monkeys with a low function allele demonstrated increased affective responding (i.e., more distress) than monkeys with a high function allele, however, only nursery reared monkeys demonstrated lower orientation scores, thought to reflect increased distractibility or emotional arousal. Finally, Barr, Newman, Lindell, et al. (2004) found that peer reared female macaques carrying a low function allele showed higher levels of ethanol preference than other macaques and were most likely to progressively increase their levels of consumption across the course of the study, suggesting a possible interaction between 5-HTTLPR low function variants and childhood experience

in vulnerability to alcoholism. In sum, findings suggest that the low function allele of 5-HTTLPR may predict decreased 5-HT activity and increased stress reactivity, emotional instability and alcohol-seeking behaviour in rhesus monkeys, but that these effects are dependent upon the unique contributions of developmental stressors..

5-HTTLPR-Environment Interactions in MDD. In one of the first studies to examine the interacting effects of 5-HTTLPR and environmental factors in human populations, Caspi et al. (2003) found that the low function allele of 5-HTTLPR was associated with the development of depression, but only in adults with histories of child maltreatment or recent stressful life events. A second study in the field of depression, by Kaufman et al. (2004) found that carrying two low function alleles conferred vulnerability to depressive symptoms, but only in individuals with histories of childhood maltreatment. In a population-based sample of adult twins, Kendler, Kuhn, Vittum, Prescott and Riley (2005) found that individuals with two low function alleles were more likely to develop MDD in response to stressful life events than were those with a high function allele. Another important study by Zalsman et al. (2006) found that the low function allele of 5-HTTLPR was associated with more severe depression, both directly and via an interaction with stressful life events. A recent article by Caspi, Hariri, Holmes, Uher and Moffat (2010) reviews all human observational studies examining the interacting effects of 5-HTTLPR and stress on depression (up to summer 2009) and shows that positive findings have emerged from a variety of other studies using various research designs. In sum, findings suggest a diathesis

stress model in which 5-HTTLPR low function variants interact with stressful life events to confer vulnerability to depression.

5-HTTLPR-Environment Interactions in BN. Very few studies to date have examined 5-HTTLPR-environment interactions in BN. In the first study to do so Steiger et al. (2007) examined possible interaction effects implicating the 5-HTTLPR polymorphism and prior physical or sexual maltreatment in women with BN-spectrum disorders. Findings revealed that BN-spectrum women with a 5-HTTLPR low-function allele and a history of childhood physical or sexual abuse displayed the highest levels of associated psychopathology in the form of increased sensation seeking and insecure attachment, but no increases on eating symptoms. In a subsequent re-analysis of the data examining the bearing of 5-HTTLPR and prior maltreatment upon the validated, higher order personality-traits Emotional Dysregulation, Dissocial Behaviour, Compulsivity and Inhibition, Steiger et al. (2008) found that women with low function alleles and a history of abuse displayed the highest levels of Dissocial Behaviour. The present findings suggest that, in individuals with BN-spectrum disorders, 5-HTTLPR low function variants interact with childhood trauma to confer vulnerability to a range of "dramatic-erratic" psychopathological traits.

Taken together, findings from gene-environment studies in animals and humans suggest that 5-HTTLPR low function variants interact with stressful life events to confer vulnerability to an array of psychopathology, including mood instability, depression, sensation-seeking, and dissocial behaviour. Such findings

suggest that, in combination with stressful life events, 5-HTTLPR may be a non-specific vulnerability factor for psychopathology of an affective-impulsive nature.

Treatment Outcome.

Although BN responds to psychotherapy, research on treatment outcome shows unsatisfactory outcomes in many patients. Based on a recent review of outcome studies in bulimic individuals, Steinhausen and Weber (2009) concluded that close to 45% of patients show full recovery and that 27% improve considerably, but that nearly 23% show no improvements at all. Similarly, a meta-analysis of psychotherapy trials for BN led to the conclusion that approximately 40% of patients recover completely, but 60% maintain clinically significant BN symptoms post treatment (Thompson-Brenner, Glass & Westen, 2003). For trials involving Cognitive Behavioural Therapy (CBT)—regarded as the "treatment-of-choice" for BN—the recovery rate for treatment completers is on average 48% (Wilson, Grilo & Vitousek, 2007). Given a high percentage of patients who do not respond to treatment, researchers have tried to identify predictors of treatment outcome which, it is hoped, will point the way to more effective, individualized treatments.

Comorbidity and Treatment Outcome in BN. A common clinical perception is that individuals presenting with significant comorbidity respond less favourably to treatment for BN. However, research studies examining the effects of comorbidity on treatment outcome in individuals with BN have provided inconsistent support for this notion.

Axis-I Comorbidity and Outcome in BN. Studies examining the effect of Axis-I disorders on outcome in individuals with BN have yielded inconsistent findings. One study found that increased depressive symptoms at baseline significantly differentiated poor responders from good responders in a group treatment program for BN (Maddocks & Kaplan, 1991). Another study found that the presence of major depressive disorder increased the odds of poor outcome one year after completing a randomized clinical trial for BN (Bulik, Sullivan, Joyce, Carter, & McIntosh, 1998). However, other studies have found no effect of depression on bulimia-treatment outcome (Fairburn, Kirk, O'Connor, Anastasiades, & Cooper, P., 1987; Keel, Mitchell, Miller, Davis, & Crow, 1999). Similar inconsistencies exist among studies examining the effect of substance-use disorders on outcome in individuals with BN, with one study associating a history of substance abuse with poorer 10-year outcome (Keel et al., 1999), but other studies finding no association between substance abuse and treatment outcome in BN (Mitchell, Pyle, Eckert, & Hatsukami, 1990; Strasser, Pike, & Walsh, 1992. Of the few studies that have examined the effect of anxiety disorders on outcome in BN most studies showed no association of anxiety with treatment outcome (Bulik et al., 1998; Keel et al., 1999; Thiel, Züger, Jacoby, & Schübler, 1998).

Axis-II Comorbidity and Outcome in BN. Findings from studies examining the effect of Axis-II disorders on outcome in BN have produced similar inconsistencies to findings in Axis-I disorders. Most studies examining the effect of Axis-II comorbidity on outcome in BN have focused on Cluster B personality disorders, and most often borderline personality disorder (BPD) or borderline

phenomena. Studies using questionnaire measures to evaluate the effect of BPD symptoms on treatment outcome in individuals with BN have produced discrepant results, with findings from two studies showing no association of BPD symptoms with treatment outcome (Davis, Olmsted, & Rockert, 1992; Garner et al., 1990) and findings from two other studies finding that comorbid BPD symptoms predicted poorer treatment outcome after 1-year on both eating and general psychopathological symptoms (Johnson, Tobin, & Denis, 1990; Steiger, Thibaudeau, Leung, Houle, and Ghadirian, 1994)). Results from studies using structured interviews to assess BPD and Cluster B personality disorders have produced results suggesting a weak association or no association with outcome of eating symptoms. For example, Zeeck et al. (2007) found no differences between individuals with and without BPD in the reduction of eating or general psychopathology over the course of treatment for BN. Similarly, Norring (1993) found that, although patients with a borderline organization showed poorer outcome at 1-year follow-up than those without borderline symptoms, after 2 and 3 years there were no differences in outcome between the groups. Finally, Steiger and Stotland (1995) found that, when compared to individuals without BPD, patients with comorbid BPD showed significantly poorer outcome (at 3-month and 1-year follow-up) on general psychopathological symptoms, but only marginally poorer response on eating symptoms. Other researchers have examined the more general effect of personality disorders (PDs) on outcome in eating disorders. For example, Herzog, Keller, Lavori, Kenny, and Sacks (1992) reported that the presence of a personality disorder was associated with lower

rates of remission from BN symptoms after 9 months. In contrast, in a heterogeneous sample of individuals with EDs, Wonderlich, Fullerton, Swift, and Klein (1994) observed that subjects with comorbid PDs did not differ from those without PDs in outcome for eating symptoms after 4 or 5 years, however their psychopathological symptoms remained more severe. After a critical review of the literature examining the effect of personality disorders on treatment outcome for EDs, Grilo (2002) concluded that, in BN, personality disorders are more closely associated with the course of general-psychopathological symptoms than with the course of ED symptoms.

5-HTTLPR and Outcome in BN. More recently, researchers have turned their attention to genetic factors as possible modulators of outcome in individuals with BN. To our knowledge, the 5-HTTLPR polymorphism has been the only gene polymorphism examined in treatment outcome studies in BN to date. Although one study in an atypical "low comorbidity" inpatient sample of individuals with BN found no association between 5-HTTLPR and response of BN symptoms to pharmacologic treatment (Erzegovesi et al., 2004), other studies have linked 5-HTTLPR to treatment response in BN. For example, in a study of patients with BN undergoing a 12-week treatment with SSRIs plus nutritional counselling, Monteleone et al (2005) found a poorer response of bulimic symptoms to treatment in individuals carrying 5-HTTLPR low function variants. Similarly, in a sample of 98 individuals with BN-spectrum disorders undergoing psychotherapy at a specialized ED program Steiger et al. (2008) found an association between 5-HTTLPR low-function alleles and poorer treatment

responses on binge eating, anxiety and depression. Results appeared to indicate that serotonin-mediated genetic factors affect response to treatment in individuals with BN, regardless of type of therapy. Such findings are in line with research in non-ED samples demonstrating associations between 5-HTTLPR low function alleles and poor response to treatment in individuals undergoing treatment for MDD (Bocchio-Chiavetto et al., 2008; Lee, Lee, Lee, & Ryu, 2004; Serretti, Kato, De Ronchi, & Kinoshita, 2007; Smits et al., 2008; Yu, Tsai, Chen, Lin, & Hong, 2002), generalized social anxiety disorder (Stein, Seedat, & Gelernter, 2006) and PDs (Silva et al., 2010). Taken together, findings suggest a non-specific effect of 5-HTTLPR on treatment outcome in various psychiatric disorders.

Thesis Objectives

The reviewed literature suggests that although BN is defined by a specific set of eating symptoms, it is also characterized by a variety of more general psychiatric symptoms, which may be associated with different genetic and environmental etiologic factors. Some studies investigating the etiologic role of early childhood environment and genetic factors in BN have shown them to be more closely associated with risk of general psychopathology than with eating-specific pathology (e.g., Schmidt, Humfress, & Treasure, 1997; Steiger et al., 2005; 2007; 2009), suggesting that it may be important to take psychiatric comorbidity into account when examining potential etiologic factors for BN. The literature to date examining etiologic factors has largely neglected the role of associated comorbidity. Lumping heterogeneous subgroups of individuals (with

different comorbid symptom profiles) together in different proportions in different studies is likely to produce inconsistent findings across studies. A main aim of the current study is to examine genetic and environmental factors that may contribute to BN and tease apart to what extent such factors contribute to BN-specific symptoms versus more general associated comorbidity patterns.

Much as comorbidity patterns are variable in BN, so is treatment outcome—and we have yet to discover what factors contribute to different outcomes among individuals undergoing similar treatments for BN. The existing literature suggests that comorbidity may be a modulating factor in treatment outcome however methodological differences across studies and failure to examine the full gamut of comorbidity makes it difficult to make definite conclusions. A second factor recently hypothesized to effect treatment outcomes are genetic factors, in particular the 5-HTTLPR polymorphism. The current thesis explores the effects of a range of Axis-I and Axis-II comorbidity and genetic factors on treatment outcome for BN and aims to tease apart to what extent such factors contribute to outcome for BN-specific symptoms versus more general psychopathological symptoms.

All studies in the current thesis include individuals with BN-spectrum disorders—including both BN and EDNOS-BN—since findings from most studies have demonstrated no clinical differences between subthreshold and threshold BN variants (Fairburn and Cooper, 1984; Niego et al., 1997; Pratt et al., 1998).

Study 1. The first study used latent class analysis to explore the latent structure of Axis-I comorbidity in individuals with BN-spectrum disorders. LCA is a statistical clustering technique that offers several advantages over traditional cluster-analytic techniques, including probability-based classification estimated directly from the model. We sought to corroborate existing research demonstrating that individuals could be classified into "high" versus "low" psychiatric comorbidity subgroups (Duncan et al., 2005). Moreover, we extended previous findings by examining possible associations between latent-comorbidity patterns and putative vulnerability factors—including exposure to childhood sexual and physical abuse and genetic (i.e., 5-HTTLPR) variations.

Study 2. The second study follows from the first study in that it also examines possible associations between psychiatric comorbidity and putative vulnerability factors—including childhood sexual and physical abuse and genetic variations—in individuals with BN-spectrum disorders. However, it is different in several ways. First of all, we included a control group of women with no history of eating disorder in order to explore, not only differences within BN-spectrum individuals, but also potential differences on childhood abuse and genetic factors between individuals with BN-spectrum disorders and normal-eater control women. Secondly, as well as examining the 5-HTTLPR polymorphism we included a second gene polymorphism acting in the 5-HT system that has potential relevance for BN, the TPH-2 G-703T polymorphism. Finally, instead of examining associations between environmental and genetic factors and "high" versus "low" psychiatric comorbidity groups, the current study examined associations of

putative vulnerability factors with specific comorbid disorders (MDD, ADs, BPD, etc.). This allowed us to explore the extent to which environmental and genetic factors might be differentially associated, not only with severity of associated comorbidity, but also with the nature of psychiatric comorbidity (e.g., affective/impulsive disorders versus anxious/compulsive disorders).

Study 3. The third study explored how psychiatric comorbidity and genetic factors might influence treatment outcome for BN. The study utilized multilevel modeling analysis to examine treatment outcome in a sample of individuals undergoing a multimodal, 16-week treatment for BN at a specialized Eating Disorders Clinic. Multilevel modeling analysis is a generalization of the general linear model used in multiple regression, which allows for the specification of random and fixed effects and handles missing data without listwise deletion. The study had two major aims: The first was to evaluate the effect upon response to treatment of comorbid Axis-I and II disorders, identified at intake using structured clinical interviews, in individuals with BN-spectrum disorders. The second aim was to examine the effect of specific gene polymorphisms involved in the 5-HT system (i.e., 5-HTTLPR and TPH-2 G-703T) on response to treatment in the same group of individuals undergoing treatment.

Manuscript 1: Relevance of the 5-HTTLPR Polymorphism and Childhood Abuse to Increased Psychiatric Comorbidity in Women with Bulimia-Spectrum Disorders.

Richardson, J., Steiger, H., Schmitz, N., Joober, R., Bruce, K.R., Israel, M., Gauvin, L., Annestin, A., Dandurand, C., Howard, H., & de Guzman, R. (2008). Relevance of the 5-HTTLPR polymorphism and childhood abuse to increased psychiatric comorbidity in women with bulimia-spectrum disorders. *Journal of Clinical Psychiatry, 69,* 981-990.

Abstract

Objective: Individuals with bulimia nervosa have been shown to display heterogeneous profiles of comorbid psychiatric disturbance, possibly due to varying degrees of genetic and environmental vulnerability. Using information about comorbid psychiatric disturbances, we developed an empirically based classification of individuals with bulimia-spectrum disorders, and then explored whether or not the resulting phenotypes corresponded to variations in the serotonin transporter polymorphism (5HTTLPR) and exposure to childhood abuse. *Method*: Eighty-nine women with bulimia-spectrum disorders completed questionnaires assessing eating and general psychopathological symptoms, participated in interviews assessing Axis-I disorders and childhood abuse, and provided blood samples for genotyping. Data on lifetime Axis-I disorders were analyzed using latent class analysis and resulting classes were compared on eating and psychopathological symptoms, 5HTTLPR genotype, and childhood abuse. *Results*: The analysis yielded a model with two classes: a first class labelled "low comorbidity" (N= 59, 66%) characterized by a high likelihood of major depressive disorder and another class labelled "high comorbidity" (N= 30, 34%) characterized by a high likelihood of major depressive disorder, anxiety disorder, and substance-use disorders. The high-comorbidity class displayed significantly higher dieting preoccupations and conduct problems, and showed a greater likelihood of carrying the 5HTTLPR S allele and of childhood abuse than did the low-comorbidity class. *Conclusion*: The present results are consistent with previous findings identifying a subgroup of individuals with bulimia characterized

by high psychiatric comorbidity and suggest that the 5HTTLPR polymorphism and childhood trauma may both be pertinent to explaining the presence of greater psychiatric comorbidity in bulimia-spectrum disorders.

Introduction

Bulimia Nervosa (BN) has been linked to heterogeneous profiles of comorbid psychiatric disturbance. For instance, cluster-analytic studies of personality traits converge on the idea that the bulimic population includes at least two empirically distinguishable subgroups—one with relatively low comorbid personality pathology and another more "disturbed" group, displaying traits such as affective instability, sensation seeking, self-destructiveness and conduct problems (Goldner, Srikameswaran, Schroeder, Livesley, & Birmingham, 1999; Westen & Harnden-Fischer, 2001; Wonderlich et al., 2005). In a similar vein, using a latent class analysis based on comorbid Axis-I disorders in a sample of individuals with BN, Duncan and colleagues found a best-fitting 2-class solution implying a "low-comorbidity" class (characterized by major depressive disorder only), and a "high-comorbidity" class (characterized by a high likelihood of major depressive disorder, anxiety disorder, alcohol and drug dependence, antisocial personality disorder and concomitant impulsive behaviours). Such findings suggest that there exist at least two distinct bulimic phenotypes—one relatively intact, and another more disturbed subgroup. In the present study we sought to replicate such explorations in a sample of individuals with bulimia-spectrum disorders and to examine potential associations with environmental and constitutional factors that are thought to be linked to the etiology of BN—with the expectation that a more disturbed variant might implicate stronger doses of both environmental and constitutional vulnerabilities.

Childhood abuse in bulimia-spectrum disorders. One factor that has been thought to be causally linked to bulimic syndromes is childhood abuse. Data show that about 30% of adults with bulimia-spectrum disorders report a history of childhood sexual abuse, and 30% or more report a history of childhood physical abuse (Fullerton, Wonderlich, & Gosnell, 1995; Leonard, Steiger, & Kao, 2003; Wonderlich, Brewerton, Jocic, Dansky, & Abbott, 1997). It is noteworthy that although such rates are elevated compared to control individuals (without a psychiatric disorder), they are comparable to those found in other psychiatric patient groups (Steiger & Zanko, 1990; Welch & Fairburn, 1996)—suggesting that childhood abuse may not be a specific risk factor for BN, but rather, a factor that is generally linked to risk of psychopathology. Moreover, within various samples of individuals suffering psychiatric disorders, studies show that a history of childhood abuse coincides systematically with more complex comorbidity patterns (Langeland, Draijer, & van den Brink, 2004; Levitan, Rector, Sheldon, & Goering, 2003). Consistent with this notion, in individuals with bulimia-spectrum disorders, childhood abuse has been associated with increased self-destructiveness (Steiger, Gauvin, et al., 2001), submissiveness (Leonard, et al., 2003), and borderline personality disorder—a syndrome characterized by marked disturbances of self-, mood-, and impulse-regulation (Steiger, Jabalpurwala, & Champagne, 1996). Taken together, research suggests that childhood abuse is associated with vulnerability to psychiatric disorders in adulthood and that within any given disorder, including BN, abuse may contribute to a pattern of increased comorbid psychopathology.

Serotonin function in bulimia-spectrum disorders. Just as childhood abuse has been implicated as an environmental factor that may be etiologic for BN, the serotonin (5-hydroxytryptamine; 5-HT) system has been implicated as a neurobiological factor. Studies have consistently documented altered 5-HT system functioning in BN. For example, individuals with active BN have been shown to display reduced platelet binding of 5-HT uptake inhibitors (Marazziti, Macchi, Rotondo, Placidi, & Cassano, 1988; Steiger, Gauvin, et al., 2001; Steiger, Young, et al., 2001), reduced hypothalamic and thalamic 5-HT transporter availability (Tauscher et al., 2001) and diminished neuroendocrine responses to 5-HT precursors and agonists (Levitan, Kaplan, Joffe, Levitt, & Brown, 1997; Steiger, Gauvin, et al., 2001). Moreover, individuals recovered from BN and unaffected first-degree relatives of individuals with BN have been found to display similar serotonergic abnormalities (Kaye et al., 1998; Kaye et al., 2001; Steiger et al., 2006; Steiger, Richardson, et al., 2005). In line with the preceding, one study links the low function (S) allele of the serotonin transporter promoter polymorphism (5-HTTLPR)—thought to be associated with reduced transcription of 5-HT transporter protein (Lesch et al., 1996)—to BN (Di Bella, Catalano, Cavallini, Riboldi, & Bellodi, 2000). However, other studies have found incongruent findings, with one reporting an increased prevalence of the high function (L) allele (Monteleone et al., 2006) and two others reporting absence of association between 5-HTTLPR and BN (Lauzurica et al., 2003; Matsushita et al., 2004).

One possible explanation for such inconsistencies might be that 5-HTTLPR variations are associated, not with risk of BN per se, but with increased risk of comorbid psychiatric disturbance. In line with this hypothesis, within individuals with bulimia-spectrum disorders the 5-HTTLPR S allele has been observed to be a stronger predictor of severity of general psychopathological symptoms (e.g., affective instability, insecure attachment and borderline personality disorder) than it is of bulimia-specific symptoms (e.g., binge eating or purging) (Steiger, Joober, et al., 2005). Similar findings have shown associations of 5-HTTLPR S allele with impulsivity (Steiger et al., 2007) and dissocial behaviour (Steiger et al., 2008) in individuals suffering bulimic syndromes. Together, findings suggest that 5-HTTLPR may modulate risk, in BN, of more pronounced comorbid psychopathology.

The present study. The current study had 2 main aims: (1) to explore the extent to which we could corroborate the existence of distinct patterns of comorbid psychiatric disturbance in bulimia-spectrum disorders, and (2) to examine possible associations between psychiatric-comorbidity patterns and putative vulnerability factors—including exposure to childhood abuse and genetic (i.e., 5HTTLPR) variations. To do this, we applied latent class analysis (LCA) to lifetime DSM-IV Axis-I disorders. LCA is a statistical clustering technique that offers several advantages over traditional cluster-analytic techniques, including probability-based classification estimated directly from the model. Based on previous findings, we expected the LCA to produce at least 2 classes; one characterized by relatively low psychiatric comorbidity and another characterized

by a higher incidence of psychiatric comorbidity. Relevant research suggesting that comorbidity in BN may be more pertinent to explaining general psychopathological symptoms than eating specific ones (Steiger & Seguin, 1999) led us to hypothesize that a class with more Axis-I comorbidity would display increased general psychopathology, but no elevations on eating-specific symptoms. In addition, in light of previous literature showing that both a history of childhood abuse and 5-HTTLPR variations may be linked to more pronounced comorbidity in BN, we expected to find that a class with a higher likelihood of comorbid psychiatric disorders would also display a higher incidence of childhood abuse and greater genetic vulnerability.

Method

Participants

Written informed consent was obtained from all participants in this institutional ethics-board approved study. Participants included 89 women aged 17-49 years and with a body mass index (BMI) between 17.5 and 34.0 kg/m^2. These individuals were recruited from the active case register of a specialized Eating Disorders Program. Among the participants, 69 (77.5%) met DSM-IV criteria for BN-purging subtype, 4 (4.5%) for BN-nonpurging subtype, and 16 (18.0%) for a bulimia-spectrum eating disorder not otherwise specified (EDNOS) (binge eating or purging at less than the requisite twice weekly). Minimum binge frequency was 1 episode per month, over the past three months. We felt diagnostic variations to represent treatment-seeking women with BN, and note reports suggesting that threshold and sub-threshold variants of BN are equivalent on many clinical

dimensions (Fairburn & Harrison, 2003). Forty-one (46.1%) BN-spectrum women were on psychoactive medications at the time of testing. Limiting recruitment to unmedicated patients was impractical (and undesirable on grounds of representativeness). Statistical procedures were applied to examine whether LCA-based classes differed in frequency of psychiatric medication use.

Measures

Eating Pathology. The Eating Disorders Examination (EDE) (Fairburn & Cooper, 1993), a 62-item semi-structured clinical interview, was utilized to assess the presence/absence of a DSM-IV bulimia-spectrum eating disorder diagnosis and eating-disorder symptoms such as binge eating, vomiting, and purging frequencies. The EDE has good inter-reliability reliability (Fairburn & Cooper, 1993) and good discriminant validity in distinguishing between women with and without eating disorders (Cooper, Cooper, & Fairburn, 1989). To complement our assessment, we computed BMI (Kg/m^2) and added the Eating Attitudes Test-26 (EAT-26) (Garner, Olmstead, Bohr, & Garfinkel, 1982), a 26-item self-report questionnaire utilized to assess symptoms and concerns characteristic of eating disorders. The EAT-26 yields a global score and three subscales (Dieting, Bulimia and Food Preoccupation and Oral Control). The EAT-26 has been shown to have high internal consistency (.90) and a cut-off score of 20 reliably identifies clinical-range eating disturbances (Garner, et al., 1982).

General Psychopathology. Diagnosis of lifetime comorbid DSM-IV Axis-I disorders was accomplished using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I: First, Spitzer, Gibbon, & Williams, 1996) . The SCID-I

is an "industry standard" interview for assessing current and lifetime history of Axis–I disorders. Inter-rater reliability estimates calculated for a pseudo-randomly selected sample of SCID-I interviews (n=23), revealed the following inter-rater reliability estimates: major depressive disorder ($\kappa = .80$), anxiety disorder (excluding post-traumatic stress disorder) ($\kappa = 1.00$), alcohol use disorder (including abuse and dependence) ($\kappa = .83$), and drug use disorder (including abuse and dependence) ($\kappa = .86$). In the majority of cases in the current study (n=81), post-traumatic stress disorder (PTSD) was assessed using the Clinician-Administered Post-Traumatic Stress Disorder Scale (CAPS: Blake et al., 1995) (the other n=8 subjects were assesses using the SCID-I). The CAPS is a standard criterion measure of PTSD diagnostic status and symptom severity, exhibiting excellent convergent and discriminant validity, and reliability (Weathers, Keane, & Davidson, 2001). The Dimensional Assessment of Personality Pathology Disorder-Basic Questionnaire (DAPP-BQ: Livesley, Jackson, & Schroeder, 1991; Schroeder, Wormworth, & Livesley, 1992), a 290-item self-report measure consisting of 18 scales, was utilized to assess general psychopathological symptoms. We selected specific subscales that are frequently ascribed to eating disordered populations. The resulting battery measured Affective Instability, Stimulus Seeking, Conduct Problems, Compulsivity, Anxiousness, Social Avoidance, Insecure Attachment and Restricted Expression. Estimates of coefficient alpha for the DAPP-BQ range from .83 - .94, in both the general population and clinical samples (Livesley, Jang, & Vernon, 1998).

Childhood Abuse. Childhood abuse was assessed using the Childhood Trauma Interview (CTI: Fink, Bernstein, Handelsman, Foote, & Lovejoy, 1995), a roughly 30-minute structured interview on experiences of abuse prior to age 18. We used CTI severity indices (severity $\geq$ 3) to isolate experiences of unambiguous physical and sexual maltreatment occurring at or before age 18 (in conformity with the standard CTI protocol). Inter-rater reliability estimates were calculated for our index of abuse in a pseudo-randomly selected sample of CTI interviews (n=24), revealing $\kappa = .81$ for physical abuse and $\kappa = .91$ for sexual abuse. CTI indices have been shown to converge with other measures of abuse and construct validity is supported by logical associations with syndromes having theoretical links to trauma exposure (Fink, et al., 1995).

5-HTTLPR variations. The 5-HTTLPR polymorphism has traditionally been thought to be biallelic, with "long" (L) and "short" (S) variants respectively coding for high or low 5-HT transporter activity (Lesch, et al., 1996). However, recent findings suggest the existence of a low-frequency L-allele variant, L_G (an L allele with A→G SNP in its sequence), whose functioning may be comparable to that of the low-function, S allele (Hu et al., 2006; Zalsman et al., 2006). Such data imply that 5-HTTLPR may be triallelic, with S and L_G alleles regarded as "low-function" variants and L_A regarded as a "high-function" allele. In other words, a traditional biallelic classification may potentially underestimate the presence of low-function variants and overestimate the presence of high-function variants by classifying L_G as an L (or high-function) allele. Therefore, in the present study we

opted to examine 5-HTTLPR using both the traditional biallelic and novel triallelic models.

Genotyping. DNA samples, obtained from whole blood, were amplified by polymerase chain reaction (PCR) in a total volume of 20 µl, which contained 100ng 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 x PCR buffer, and 1 x Q solution (Qiagen). The forward primer (5'-ATG CCA GCA CCT AAC CCC TAA TGT-3') and reverse primer (5'-GG ACC GCA AGG TGG GCG GGA-3') were used to amplify a region encompassing 5-HTTLPR; long and short alleles were then resolved on a 2% agarose gel. The PCR protocol involved preheating the samples at 94^{o} C for 5 min, followed by 35 cycles of denaturation at 94^{o} C (30 sec), annealing at 64^{o} C (30 sec), and extension at 72^{o} C (45 sec), as well as a final hold of 5 min at 72^{o} C. The L_G and L_A alleles were subsequently studied by enzymatic digestion of 7 µl of the above mentioned PCR product using 5 U of MspI and incubating at 37^{o} C for a minimum of 3 hours. L_G and L_A alleles were then resolved on a 2% agarose gel.

Statistical Analyses

Latent Class Analysis (LCA).

The latent structure of psychiatric comorbidity in individuals with bulimia-spectrum disorders was examined by applying LCA to lifetime comorbid DSM-IV Axis-I diagnoses (dichotomized as present or absent), using Latent Gold software (Vermunt & Magidson, 2003). Disorders included in the LCA were major depressive disorder, anxiety disorder (including social phobia, agoraphobia,

panic disorder, generalized anxiety disorder, obsessive compulsive disorder and post-traumatic stress disorder), alcohol abuse/dependence, and substance abuse/dependence (including dependence or abuse of marijuana, stimulants, sedatives, cocaine, opiates and hallucinogens). LCA assumes that a set of latent classes exists that accounts for the pattern of observed covariation among a set of indicators measured at the categorical level. Information about the underlying class structure is conveyed through (a) latent class probabilities, which may be thought of as class prevalence estimates, and (b) conditional probabilities, which reflect the probability that an item is endorsed by an individual, given membership in a specific class. Initial estimation began with a one-class model. Latent classes were then added progressively, and resultant models compared using percentage classification error and information criteria—i.e., Bayesian information criterion (BIC); Akaike's information criterion (AIC)—that take into account both statistical goodness of fit and the number of parameters estimated to achieve a particular degree of fit.

Comparison of LCA-based classes.

Respondents were assigned to classes, using the modal probability, based on posterior probabilities derived from the latent class analysis. Resulting classes were compared on age, BMI, and prevalence of psychoactive medication use. Additionally, classes were compared on eating-disorder related variables, such as eating disorder diagnosis (threshold versus sub-threshold BN) and eating symptoms as measured by the EDE (binge eating days, binge eating episodes, vomiting episodes, and purging episodes per month in the past three months) and

the EAT-26 (Dieting, Bulimia and Food Preoccupation, Oral Control, and Total Score). Further comparisons were made to contrast classes on selected psychological variables from the DAPP (Affective Instability, Stimulus Seeking, Compulsivity, Anxiousness, Conduct Problems, Social Avoidance, Insecure Attachment, and Restricted Expression), presence or absence of childhood abuse (including sexual, physical and combined sexual or physical abuse before age 18), and 5-HTTLPR genotype and allele frequencies. In light of recent findings suggesting that 5-HTTLPR may be triallelic, 5-HTTLPR was examined using both the biallelic (S/S, S/L, L/L) and triallelic (S/S, S/L_G, L_G/L_G, L_G/L_A, S/L_A, L_A/L_A) models. Since the L_G allele has been shown to function in a way that is comparable to the low-function S allele, L_G and S alleles were grouped together under the label "Low" (to indicate that they are low-function variants) and L_A was labelled "High" (to indicate that it is a high-function variant), resulting in the triallelic classification: Low/Low (S/S, S/L_G and L_G/L_G), Low/High (L_G/L_A and S/L_A), and High/High (L_A/L_A).

Results

LCA

We examined 1- through 5-class LCA solutions reflecting loading of Axis-I disorders in potential sub-groups of BN. Based on information criteria (AIC = 446.64, BIC = 469.03 and classification error = 0.14) and previously published evidence (Duncan, et al., 2005) we selected a 2-class solution as having the best fit to the data.[1] Table 1 displays the conditional probabilities for each class as well as the overall latent class prevalence estimates. The majority of subjects fell into

class 1 (n=59, 66.3%), which featured a high conditional probability for major depressive disorder, a moderate conditional probability for anxiety disorder and low conditional probabilities for drug and alcohol abuse/dependence. We labelled class 1 the "low-comorbidity" class. A smaller proportion of subjects fell into class 2 (n=30, 33.7%), which displayed substantially more psychiatric comorbidity, with high conditional probabilities for all disorders. We labelled this class the "high-comorbidity" class.

Comparison of LCA-based classes.

T-tests revealed no significant differences between classes on age or BMI. Similarly, χ^2 analyses revealed no significant differences as to proportion of cases in the two classes using psychiatric medication or proportion of cases in the two classes with subthreshold (as opposed to threshold)-BN variants. Box and whisker plots revealed univariate outliers on EDE variables coding binge eating, vomiting and purging episodes. Outliers were transformed to the sample mean plus two standard deviations. The EAT-26 Oral Control subscale and binge eating, vomiting and purging episodes from the EDE were (as is common) found to be nonnormally distributed and were logarithmically transformed. T-tests revealed no significant differences between classes in binge eating days, or binge eating, vomiting or purging episodes, however a significant difference was observed on EAT-26 final score with the high-comorbidity class scoring higher than the low-comorbidity class (see table 2). When EAT-26 subscales were analyzed it was the Dieting subscale that significantly differentiated the two classes. No significant group differences were found on the Bulimia and Food Preoccupation and Oral

Control subscales (see table 2). On the DAPP-BQ, t-tests revealed a significant difference between classes on Conduct Problems with the high-comorbidity class, once again, scoring significantly higher than the low-comorbidity class. Significant differences were not obtained between classes on other selected personality trait subscales from the DAPP-BQ (see table 2).

Treating 5-HTTLPR in a biallelic fashion, frequencies of S/S, S/L, and L/L genotypes, respectively occurring in 22 (24.7%), 39 (43.8%) and 28 (31.5%) of our participants, were in conformity with Hardy-Weinberg equilibrium [$\chi^2_{(1)} = 1.27$, n.s.]. With a triallelic model, we observed S/S, S/L_G, S/L_A,, L_G/L_A, L_G/L_G and L_A/L_A genotypes, respectively to occur in 22 (24.7%), 5 (5.6%), 34 (38.2%), 6 (6.7%), 2 (2.2%), and 20 (22.5%) of our participants. Frequencies of Low/Low, Low/High, and High/High genotypes, respectively occurred in 29 (32.6%), 40 (44.9%) and 20 (22.5%) of our participants. Using the biallelic classification, a χ^2 test revealed a trend level difference in prevalence of genotypes between the high- and low-comorbidity classes, with the high-comorbidity class displaying a higher frequency of S/S and S/L genotypes and a lower frequency of L/L genotype (see Table 3). When S/S and S/L genotypes were grouped together to form a dichotomous S- versus no-S allele classification a significant difference between the two classes was revealed, with the high-comorbidity class displaying a significantly greater frequency of S allele than the low-comorbidity class (see Table 3). Although results pointed in the same direction, no significant genetic or allele effects were obtained in parallel analyses based on a triallelic model (see Table 3).

To ascertain whether or not the rate of S-allele carriers in our sample differed from that observed in the general population, we performed nonparametric chi-square tests comparing frequency of S allele in both high- and low-comorbidity classes to the frequency of S allele in primarily Caucasian, non-psychiatric samples from previous studies (66% S allele versus 34% no-S allele) (Gorwood, Batel, Ades, Hamon, & Boni, 2000; Greenberg et al., 2000; Lesch et al., 1996). We found that the low-comorbidity group was comparable to population norms ($\chi^2_{(1)} = 0.65$, $p = .419$), but that the high-comorbidity group was significantly different from the general population, displaying an increased frequency of S allele ($\chi^2_{(1)} = 4.02$, $p = .045$).

When examining history of childhood abuse in the two classes, χ^2 tests revealed no significant difference in the prevalence of sexual abuse, however a trend level difference was found in the prevalence of physical abuse—with the high-comorbidity class having experienced a higher prevalence of physical abuse in childhood than the low-comorbidity class (see Table 4). When childhood sexual and physical abuse were grouped together to form one variable (presence of physical or sexual abuse before age 18), a significant difference between the two classes was revealed, with the high-comorbidity class displaying a significantly greater incidence of childhood abuse than the low-comorbidity class (see table 4).

Since 5HTTLPR S allele and childhood abuse were both more prevalent in the high-comorbidity class we performed a hierarchical logistic regression analysis, aimed at isolating independent and interaction effects of these variables.

In this analysis, we entered genetic information at step 1 (G = presence or absence of biallelic 5HTTLPR S allele), childhood abuse information at step 2 (A = presence or absence of physical or sexual abuse before age 18), and the interaction between the two at step 3 (G x A). No significant G x A interaction effect was found, therefore a subsequent analysis tested for main effects alone. Results showed the S allele to be a significant predictor of class membership [OR= 3.19, 95% CI: 1.07-9.53, p = .037] at step 1. When abuse was added at step 2 the predictive power of the S allele was reduced [OR= 2.98, 95% CI: 0.97-9.16, p = .057] and abuse emerged as a significant predictor of class membership [OR= 3.42, 95% CI: 1.20-9.77, p = .022]. The risk of membership in the high-comorbidity class thus appeared to be associated with independent effects of genetic (S-allele) susceptibility and prior childhood abuse, with childhood abuse being a stronger predictor of class membership.

Discussion

To examine the latent structure of psychiatric comorbidity in a sample of individuals suffering bulimic syndromes, we applied latent class analysis to findings on comorbid DSM-IV Axis-I disorders. Using statistical information criteria and evidence from previous studies, a good-fitting solution revealed two classes; one larger class displayed a high probability of comorbid major depressive disorder only (and was hence labelled “low-comorbidity”), and a second smaller class had high probabilities for various disorders, including major depressive disorder, anxiety disorder, alcohol abuse/dependence and drug abuse/dependence (labelled “high-comorbidity”). The two-class structure of

psychiatric comorbidity found in the present study is strikingly similar to that found by Duncan and colleagues (2005) in a latent class analysis of comorbid psychiatric disorders in individuals with bulimia nervosa. Like ours, results revealed a two-class solution with one class characterized by major depressive disorder only and a second class characterized not only by a high prevalence of major depressive disorder, but also of comorbid anxiety disorder, alcohol and drug dependence and antisocial personality disorder. Taken together, such findings support the existence of at least two empirically distinguishable subgroups within the bulimic population—one relatively intact, and another, more psychiatrically disturbed.

Comparisons on eating symptoms revealed no differences between the high-and low-comorbidity groups with respect to binge eating and purging behaviours. However, the high-comorbidity class was found to have a significantly higher EAT-26 total score than the low-comorbidity class, with a specific elevation on the Dieting subscale. Although previous research suggests that comorbidity in bulimia nervosa may be more strongly associated with variations in psychopathological symptoms than eating-specific ones, some findings suggest that individuals with bulimia who display increased psychiatric comorbidity (e.g., personality disorders, major depression) also tend to display more maladaptive attitudes around dieting and drive for thinness, without displaying increased binge eating and purging behaviours (Steiger & Stotland, 1996; Sunday, Levey, & Halmi, 1993; Yates, Sieleni, & Bowers, 1989). A similar tendency appears to be indicated by our findings. A possible implication is that

drive to diet may be elevated in individuals with more severe associated psychopathology, whereas other eating symptoms (like binge and purge frequencies) may be more generally associated with having bulimia nervosa, and less associated with comorbid psychopathological symptoms.

The high-comorbidity class in the current study was also found to have significantly more conduct problems than the low-comorbidity class. Such findings are in line with those of Duncan and colleagues (2005) who found a high-comorbidity class to display higher likelihood of antisocial personality disorder—a disorder in which conduct problems are pathognomonic. In addition, in both our study and that of Duncan and colleagues (2005) the high-comorbidity class contained nearly every case of substance use disorder. Fittingly, previous research shows that individuals with bulimia and comorbid substance-use disorder display elevated rates of comorbid Axis I psychiatric disorders and conduct problems (Bulik, Sullivan, Carter, & Joyce, 1997; Duncan et al., 2006; Lilenfeld et al., 1997). Together, available findings support the existence of a relatively small (about 1/3rd of the population of bulimia sufferers) subgroup of individuals with bulimia, marked by a high likelihood of psychiatric comorbidity (especially substance use disorder) and increased conduct problems.

Aside from replicating previous observations, the present study introduces the novel element that individuals with bulimia-spectrum disorders and high comorbidity, when compared with individuals belonging to a more intact group, display more-marked susceptibilities, both environmental and genetic:

(1) With respect to environmental risks, the high-comorbidity class was found to display a greater prevalence of childhood abuse than the low-comorbidity class—a finding that is compatible with previous results showing formerly abused individuals with bulimia to display increased psychopathology in adulthood (Leonard, et al., 2003; Steiger, Gauvin, et al., 2001; Steiger, et al., 1996). Such findings also corroborate results obtained in nonbulimic psychiatric populations (e.g., individuals with depression, anxiety, alcohol abuse) linking a history of childhood abuse to more complex patterns of comorbid psychopathology (Langeland, et al., 2004; Levitan, et al., 2003). Such results converge upon the notion that within any given disorder—including bulimia nervosa—the presence of abuse in childhood contributes to a pattern of increased psychiatric comorbidity in adulthood.

(2) The high-comorbidity class was also found to display greater genetic vulnerability, in the form of greater likelihood of carrying the 5-HTTLPR S allele, relative to the low-comorbidity class. This finding is in line with previous research associating the 5-HTTLPR S allele with increased comorbid psychopathology and in particular, psychopathology of a dissocial nature (e.g., impulsivity, dissocial behaviour) in individuals with bulimia nervosa (Steiger, Joober, et al., 2005; Steiger et al., 2007; 2008). In addition, such findings are in line with literature in the field of substance abuse showing that type 2 or "dissocial" alcoholics (characterized by impulsivity, conduct problems, and deceitfulness) are more likely to have the S allele of 5-HTTLPR than type 1 alcoholics (a more intact subgroup) (Sander et al., 1998). Together, such findings

imply that the S allele of the 5-HTTLPR may be pertinent to explaining increased psychiatric comorbidity, in particular dissocial phenomena, in psychiatric disorders—like bulimia—in which such a component is present.

To the preceding, we add the following caveat: Since our sample is of modest size, any genotype-related effects we obtain must be regarded as preliminary and in need of replication. In addition, 5-HTTLPR S allele effects obtained are small, suggesting that if they are indeed repeatable, they must be understood to act within a much larger set of genetic and/or constitutional vulnerability factors that shape phenotypes. Finally, absence of a control group in the present study renders it impossible to ascertain whether or not our high-comorbidity group has a greater likelihood of carrying the S allele than would a group of subjects without an eating disorder from the same population. We do note, however, that our tests comparing S-allele rates in high- and low-comorbidity samples to those expected in a normal reference population suggest that the high-comorbidity group might be characterized by a higher-than-expected rate of S allele carriers. Although significant 5-HTTLPR genetic effects were not obtained in parallel analyses based on a triallelic model, we assume that the disparate results may be an artefact of limited statistical power, related to our sample size.

Why might women with bulimic symptoms and high psychiatric comorbidity show the combination of increased rates of childhood abuse and 5-HTTLPR S allele? We and other investigators have previously linked psychopathological manifestations in the bulimic population to underlying 5-HT

disturbances (for a review see Steiger, 2004). Furthermore, we have documented tendencies for previously abused bulimic women to display more pronounced serotonergic anomalies than do those without a history of abuse (Steiger, Gauvin, et al., 2001). Similarly, previous research has shown associations of 5HTTLPR S allele with altered 5-HT functioning (Lesch, et al., 1996). Based on the preceding, we specifically postulate that variants of BN characterized by marked psychiatric comorbidity may often implicate additive, 5-HT mediated, effects of developmental stressors and latent genetic propensities towards psychopathology. Alternatively, it remains possible that we observe a convergence among 5-HTTLPR S allele, childhood abuse and elevated psychopathology because the S allele actually increases risk of abuse—through such possible correlates as heightened psychopathology in potentially abusive, genetically disposed parents, or heightened conduct problems or risk-taking in genetically disposed children.

Conclusions. The results of the current study support the existence of heterogeneous comorbid-symptom profiles in BN, revealing one class of individuals with relatively low psychiatric comorbidity and a second class with greater psychiatric comorbidity and concomitant dissocial phenomena. Such findings corroborate those of previous studies suggesting that about one third of individuals with BN fall into a subgroup that is marked by increased lifetime psychiatric comorbidity (in particular comorbid substance use disorder) and heightened dissocial behaviour (Duncan, et al., 2005; Goldner, et al., 1999; Westen & Harnden-Fischer, 2001; Wonderlich, et al., 2005). In addition, the current study shows that within such a subgroup there is an increased prevalence

of 5-HTTLPR S allele and a higher incidence of childhood sexual or physical abuse, suggesting that genes and early environment may both be pertinent to explaining increased psychopathology in individuals with bulimia nervosa.

In identifying a more disturbed subgroup of individuals with bulimia, the present study may isolate factors of clinical importance. For this subgroup, interventions focused on eating symptoms may not be sufficient. For example, it has been shown that increased psychiatric comorbidity is associated with longer treatments and poorer outcomes in individuals with bulimia (Thompson-Brenner & Westen, 2005). In addition, a history of abuse—found to be more prevalent in the high-comorbidity subgroup in the present study—has been shown to be related to poorer treatment response and greater dropout rates in the treatment of eating disorders (Rodriguez, Perez, & Garcia, 2005). Furthermore, the 5-HTTLPR S allele—also found to be more prevalent in the high-comorbidity class—has been linked to poor response to pharmacological treatment in eating disorder patients (Monteleone et al., 2005). Such research findings beg the question: is the high-comorbidity subgroup identified in the present study the same group that does not get better with treatment? And, if so, are there ways of improving therapy (e.g., therapeutic adjuncts aimed at specific comorbid symptoms, posttraumatic therapy techniques, pharmacological support) so that individuals that fall into such a subgroup have more successful outcomes? Longitudinal outcome studies designed to test such questions could lead to more successful treatments for bulimia nervosa.

Footnotes

[1] 1- through 3-class solutions displayed similar AIC and BIC statistics (1 class model: AIC = 451.63, BIC = 461.59; 2 class model: AIC = 446.64, BIC = 469.03; 3 class model: AIC = 450.46, BIC = 485.30), and provided a relatively good fit to the data. Based on previously published results favouring a 2-class solution,(Duncan, et al., 2005) and the AIC obtained, we opted for the 2-class model as likely to be most informative.

Table 1. Latent Class Analysis: Conditional probabilities and class prevalence estimates for lifetime Axis-I comorbidity in women with bulimia-spectrum disorders.

	Class 1 Conditional Probability (SE)	**Class 2 Conditional Probability (SE)**
Major depressive disorder	0.65 (0.09)	0.67 (0.10)
Anxiety disorder	0.29 (0.10)	0.66 (0.11)
Alcohol abuse/dependence	0.09 (0.10)	0.52 (0.11)
Drug abuse/dependence	0.01 (0.03)	0.57 (0.18)
Class prevalence	0.56 (0.14)	0.44 (0.14)

Conditional probability = probability that an item is endorsed by an individual, given membership in a specific class.

Table 2. Eating and general psychopathological symptoms among women with bulimia-spectrum disorders (N=89) by LCA-based classes.

	Class 1 "Low-comorbidity" Mean (S.D.)	**Class 2 "High-comorbidity" Mean (S.D.)**	**t-test statistic (df), p value**
N = 89	N = 59	N = 30	
EDE: Binge days/month	16.43 (7.94)	14.86 (7.79)	t (87) = 0.89, p = .376
Binge episodes/ month	28.37 (23.93)	21.64 (18.68)	t (87) = 1.35, p = .182
Vomit episodes/ month	30.20 (37.19)	27.85 (33.40)	t (87) = 0.30, p = .764
Purge episodes/ month	34.56 (37.84)	32.56 (33.91)	t (87) = 0.25, p = .802
N = 88	N = 59	N = 29	
EAT-26 : Total Score	32.96 (12.87)	40.87 (11.41)	t (86) = -2.81, p = .006
Dieting	1.48 (0.63)	1.89 (0.54)	t (86) = -2.99, p = .004
Bulimia and Food Preoccupation	1.80 (0.72)	1.98 (0.61)	t (86) = -1.15, p = .254
Oral Control	0.42 (0.49)	0.63 (0.62)	t (86) = -1.74, p = .086
DAPP-BQ : Affective Instability	3.36 (0.80)	3.66 (0.85)	t (86) = -1.57, p = .121
Stimulus Seeking	2.79 (0.94)	2.98 (0.98)	t (86) = -0.87, p = .390
Conduct Problems	1.69 (0.59)	2.11 (0.66)	t (86) = -3.02, p = .003
Compulsivity	3.32 (0.67)	3.41 (0.73)	t (86) = -0.59, p = .557
Anxiousness	3.59 (0.88)	3.79 (0.98)	t (86) = -0.92, p = .364
Social Avoidance	3.25 (0.80)	3.13 (0.97)	t (86) = 0.58, p = .562
Insecure Attachment	2.72 (0.91)	2.91 (1.12)	t (86) = -0.87, p = .389
Restricted Expression	3.16 (0.80)	2.91 (0.76)	t (86) = 1.37, p = .173

Values reported for EDE binge, vomit, and purge episodes and the EAT-26 Oral Control subscale are actual values. Due to deviations from normality logarithmic transformations were performed and resulting analyses (not reported here) revealed similar results. Small differences in Ns reflect isolated missing values. S.D. = standard deviation; df = degrees of freedom; EDE = Eating Disorders Examination; EAT-26 = Eating Attitudes Test-26; DAPP-BQ = Dimensional Assessment of Personality Pathology Disorder-Basic Questionnaire

Table 3. 5HTTLPR (biallelic and triallelic) genotype and allele frequencies among women with bulimia-spectrum disorders (N=89) by LCA-based classes.

	Class 1 (N=59) "Low-comorbidity" N (%)	**Class 2 (N=30) "High-comorbidity" N (%)**	**χ^2 statistic $_{(df)}$, p value**
5HTTLPR Biallelic genotype			
L/L genotype	23 (39.0%)	5 (16.7%)	$\chi^2_{(2)} = 4.59$, p = .101
S/L genotype	23 (39.0%)	16 (53.3%)	
S/S genotype	13 (22.0%)	9 (30.0%)	
5HTTLPR Biallelic S allele			
No S allele (L/L)	23 (39.0%)	5 (16.7%)	$\chi^2_{(1)} = 4.59$, p = .032
S allele (S/S or S/L)	36 (61.0%)	25 (83.3%)	
5HTTLPR Triallelic genotype			
High/High genotype (L_A/L_A)	16 (27.1%)	4 (13.3%)	$\chi^2_{(2)} = 3.31$, p = .191
High/Low genotype (S/L_A or L_G/L_A)	27 (45.8%)	13 (43.3%)	
Low/Low genotype (S/S, S/L_G or L_G/L_G)	16 (27.1%)	13 (43.3%)	
5HTTLPR Triallelic Low-function allele			
No Low-function allele (L_A/L_A)	16 (27.1%)	4 (13.3%)	$\chi^2_{(1)} = 2.17$, p = .141
Low-function allele (S/L_A, L_G/L_A, S/S, S/L_G or L_G/L_G)	43 (72.9%)	26 (86.7%)	

Table 4. History of childhood physical or sexual abuse among women with bulimia-spectrum disorders (N=89) by LCA-based classes.

	Class 1 (N=59) "Low-comorbidity" N (%)	**Class 2 (N=30) "High-comorbidity" N (%)**	**χ^2 statistic $_{(df)}$, p value**
History of sexual abuse before age 18	16 (27.1%)	13 (43.3%)	$\chi^2_{(1)} = 2.38$, p = .123
History of physical abuse before age 18	25 (42.4%)	19 (63.3%)	$\chi^2_{(1)} = 3.50$, p = .062
History of sexual or physical abuse before age 18	31 (52.5%)	24 (80.0%)	$\chi^2_{(1)} = 6.35$, p = .012

Transition to Manuscript 2

Study 1 (Richardson et al., 2008) utilized latent class analysis to derive an empirically based classification of individuals with bulimia-spectrum disorders, based on information about comorbid psychiatric disturbances. Results revealed two classes: a "high comorbidity" class and a "low comorbidity" class. The two classes differed with respect to 5-HTTLPR variations and history of childhood abuse. Findings from Study 1 corroborate previous findings identifying a subgroup of individuals with bulimia characterized by high psychiatric comorbidity (Duncan et al., 2005). Aside from replicating previous observations, such findings introduce the novel element that individuals with bulimia-spectrum disorders and high comorbidity, when compared with individuals belonging to a more intact group, display more-marked susceptibilities, both environmental and genetic.

Study 2, which examined possible associations between Axis-I and Axis-II psychiatric comorbidity and putative environmental and genetic vulnerability factors in individuals with BN-spectrum disorders, extended findings from Study 1 in several ways. First of all, a control group of women with no history of eating disorder were recruited to the study in order to explore, not only differences within BN-spectrum individuals, but also potential differences on childhood abuse and genetic factors between individuals with BN-spectrum disorders and normal-eater control women. Secondly, instead of examining associations between environmental and genetic factors and "high" versus "low" psychiatric comorbidity groups, Study 2 examined associations of putative vulnerability

factors with specific comorbid disorders of interest (e.g., MDD, ADs, BPD). This allowed us to explore the extent to which environmental and genetic factors might be differentially associated, not only with severity of associated comorbidity, but also with the nature of psychiatric comorbidity (e.g., affective/impulsive disorders versus anxious/compulsive disorders). Finally, as well as examining the 5-HTTLPR polymorphism Study 2 included a second gene polymorphism acting in the 5-HT system that has potential relevance for BN, the TPH-2 G-703T polymorphism.

Manuscript 2: Childhood Abuse, Selected Serotonin Genes and Psychiatric Comorbidity in Women with Bulimia-Spectrum Disorders

Abstract

Objective: Although defined by eating disturbances, Bulimia Nervosa (BN) frequently co-occurs with other psychiatric disorders, including mood, anxiety, substance-use and personality disorders. Research suggests that variations in psychiatric comorbidity in individuals with BN may be partially explained by environmental and/or genetic factors. In the present study we examined Axis-I and Axis-II psychiatric comorbidity in individuals with bulimia-spectrum disorders (BSDs) and potential associations with childhood abuse and genetic factors—a polymorphism in the promoter region of the serotonin transporter gene, 5-HTTLPR, and a polymorphism in the promoter region of the tryptophan hydroxylase-2 gene, TPH-2 G(-703)T. *Method*: One hundred and two women with BSDs and 103 normal-eater control women participated in interviews assessing DSM-IV disorders and childhood sexual and physical abuse and provided blood samples for genotyping. Logistic regression analyses were used to examine whether or not genetic variations and childhood abuse predicted: 1) presence of a BSD, and 2) presence of comorbid Axis-I and Axis-II disorders, within individuals with BSDs. *Results*: Findings revealed that homozygosity for 5-HTTLPR high-function variants and history of childhood abuse were both associated with likelihood of having a BSD. However, more-fine-grained analyses, within women with BSDs, revealed that 5-HTTLPR high-function alleles were particularly relevant to the prediction of comorbid Anxiety Disorders and childhood abuse was particularly relevant to the prediction of comorbid Drug Abuse/Dependence. *Conclusion*: Our results are in line with previous findings in

eating- and non-eating-disordered populations that associate 5-HTTLPR high-function variants with anxiety-related disturbances and childhood adversity with substance-use disorders. Taken together, results suggest that 5-HTTLPR and childhood abuse, by influencing general psychiatric symptoms may, in turn, influence different clinical presentations in BN.

Introduction

Bulimia Nervosa (BN) is a severe eating disorder (ED), characterized by recurrent episodes of binge-eating, followed by compensation through vomiting, laxative/diuretic misuse or intensive exercise. Although defined by disturbances in eating behaviour, BN frequently co-occurs with other psychiatric disorders including mood, anxiety, substance-use and personality disorders. Mood Disorders (MDs) figure very prominently in BN, with studies reporting lifetime prevalence rates of 70-90% (Brewerton et al., 1995; Bulik, Sullivan, Carter, & Joyce, 1996; Hudson, Pope, & Yurgelon-Todd, 1988). Major Depressive Disorder (MDD) is the most common MD, with studies finding a history of MDD in 60-80% of individuals with BN (Brewerton et al., 1995; Godart et al., 2007; Herzog et al., 1999; Hudson et al., 1988). In addition, studies have found a history of Anxiety Disorder (AD) in 50-80% of bulimic subjects (Bulik et al., 1996; Garfinkel et al., 1995; Godart et al., 2003; Hudson et al., 2007) and of substance-use disorders (SUDs) in roughly a third (Garfinkel et al., 1995; Holderness, Brooks-Gunn, & Warren, 1994; Hudson et al., 2007; Lilenfeld et al., 1998). Possibly the strongest of the comorbid propensities in BN is that with personality disorders (PDs). A meta-analysis of studies examining DSM PDs in individuals with EDs found that 44% of individuals with BN met criteria for Cluster C (Avoidant, Dependent or Obsessive-Compulsive) PDs (characterized by anxious, fearful behaviour) and 44% met criteria for Cluster B (Borderline, Histrionic, Narcissistic or Antisocial) PDs (characterized by dramatic, erratic behaviours) (Rosenvinge, Martinussen & Ostensen, 2000). A recent review paper examining

10 studies, which included clinical BN samples and utilized clinical interview methods to diagnose PDs, found BPD and Avoidant Personality Disorder (AVPD) to be the two most common personality disorders in BN, with estimated prevalence rates of 21% and 19% respectively (Cassin & Von Ransen, 2005). Research suggests that genetic factors and developmental experiences both play a role in the etiology of BN. The literature to date examining etiologic factors in BN has largely neglected the role of associated psychiatric comorbidity. Lumping heterogeneous subgroups of individuals (with different comorbid symptom profiles) together in different proportions in different studies is likely to produce inconsistent findings across studies. The present study examines genetic factors and developmental experiences that may contribute to BN and aims to tease apart to what extent such factors contribute to BN versus more general associated psychiatric comorbidity.

Childhood abuse. One environmental factor that has been thought to be causally linked to BN is childhood abuse. Data show that about 30% of adults with bulimia-spectrum disorders (BSDs) report a history of childhood sexual abuse, and 50% or more report a history of childhood physical abuse (Leonard, Steiger, & Kao, 2003; Fullerton, Wonderlich, and Gosnell, 1995; Rorty, Yager, & Rossotto, 1994; Wonderlich, Brewerton, Jocic, Dansky, and Abbott, 1997). It is noteworthy that although such rates are elevated compared to individuals without a psychiatric disorder, they are comparable to those found in other psychiatric-patient groups (Steiger & Zanko, 1990; Welch & Fairburn, 1994)—suggesting that childhood abuse may not be a specific risk factor for BN, but rather, a factor

that is generally linked to risk of psychopathology. Nonetheless, childhood abuse might contribute indirectly to risk of BN by increasing generalized maladjustment, or activating generalized susceptibility factors. Consistent with this notion, in individuals with BSDs, childhood abuse has been associated with increased general psychopathology in the form of impulsivity (Myers et al., 2006), self-harming behaviours (Corstorphine, Waller, Lawson, & Ganis, 2007), dissocial behaviour (Steiger et al., 2009), BPD (Steiger, Jabalpurwala, & Champagne, 1996) and substance abuse (Corstorphine, Waller, Lawson, & Ganis, 2007)—without being associated with more severe eating symptoms. Taken together, research suggests that childhood abuse is likely a non-specific vulnerability factor for BN, possibly increasing risk for the disorder through activating general psychopathological susceptibilities, rather than eating-specific ones.

Serotonin function. Studies documenting altered serotonin (5-hydroxytryptamine; 5-HT) system functioning in BN have led to the hypothesis that 5-HT alterations may be causally linked to BN. For example, individuals with active BN have been shown to display reduced platelet binding of 5-HT uptake inhibitors (Marazziti, Macchi, Rotondo, Placidi, & Cassano, 1988; Steiger, Young, et al.2001), diminished neuroendocrine responses to 5-HT precursors and agonists (Levitan, Kaplan, Joffe, Levitt, & Brown, 1997; Steiger, Gauvin, et al., 2001), reduced hypothalamic and thalamic 5-HT transporter availability (Tauscher et al., 2001), and increased 5-HT_{1A} activity throughout the cortex and raphe regions—suggesting increased presynaptic autoreceptor activity and

decreased post-synaptic 5-HT availability (Tiihonen et al., 2004). The wish to clarify whether such alterations in 5-HT functioning are a consequence of nutritional influences (e.g., dietary restriction of the amino acid tryptophan used in 5-HT synthesis) or a pre-existing vulnerability factor for BN has led researchers to examine 5-HT in individuals who have fully recovered from BN, to remove the possible confound of nutritional factors. Studies in recovered individuals have revealed similar 5-HT abnormalities to those found in individuals with active EDs. For example, single photon emission computed tomography (SPECT) studies have shown reduced 5-HT_{2A} activity in the medial orbital frontal cortex of women who have recovered from BN (Kaye et al., 2001). Moreover, individuals recovered from BN and unaffected first-degree relatives of individuals with BN have been found to display similar reductions in platelet paroxetine binding (Steiger et al., 2005; Steiger et al., 2006).

The serotonin transporter promoter polymorphism (5-HTTLPR). In keeping with findings linking 5-HT dysfunction to BN, evidence has suggested relevance, in the etiology of BN, of gene polymorphisms that code for 5-HT system activity. The most extensively studied of such polymorphisms has been the serotonin transporter promoter polymorphism (5-HTTLPR). 5-HTTLPR is a 44-base pair insertion deletion polymorphism in the 5′ flanking regulatory region of the serotonin transporter gene, originally thought to have a "long" (L) and a "short" (S) variant, which differentially modulate transcriptional activity (Lesch et al., 1996). The S allele of 5-HTTLPR has been associated with reduced transcription of 5-HT transporter protein relative to the L allele (Heils et al., 1996;

Lesch et al., 1996). Recent findings, however, suggest the existence of a low-frequency L-allele variant, L_G (an L allele with A→G SNP in its sequence), whose functioning may be comparable to that of the low-function, S allele (Hu et al., 2006; Zalsman et al., 2006). Such data imply that 5-HTTLPR may be triallelic, with S and L_G alleles regarded as "low-function" variants (S') and L_A regarded as a "high-function" allele (L'). Studies examining the traditional biallelic model of 5-HTTLPR in BN have found incongruent findings, with one study reporting an increased prevalence of low function alleles in BN (DiBella, Catalano, Cavallini, Riboldi, & Bellodi, 2000), one reporting an increased prevalence of the high function allele (Monteleone et al., 2006) and two others reporting absence of association between 5-HTTLPR and BN (Lauzurica et al., 2003; Matsushita et al., 2004). One possible explanation for inconsistent findings is the fact that the biallelic model may be imprecise. A traditional biallelic classification may potentially underestimate the presence of low-function variants and overestimate the presence of high-function variants by classifying L_G as an L (or high-function) allele. In the only study to examine triallelic 5-HTTLPR in EDs to date, Steiger et al. (2009) found an increased prevalence of the high function allele and the high-function homozygotes (L_A/L_A genotype) in a mixed sample of AN, BN and Eating Disorder Not Otherwise Specified (EDNOS) subjects. However, more fine-grained analyses suggested that the association may have been localized to those individuals displaying an inhibited/compulsive profile (rather than a dissocial/impulsive or low psychopathology profile) as they were

significantly more likely than individuals in other groups to carry the high function allele and exhibit high-function homozygosity.

The preceding suggest that a possible explanation for inconsistent findings on the link between 5-HTTLPR and BN might be that 5-HTTLPR variations are associated, not with risk of BN per se, but with increased risk of comorbid psychiatric symptoms in BN. In line with this hypothesis, a previous study in our lab, examining the latent structure of psychiatric comorbidity in individuals with BSDs, found a higher frequency of 5-HTTLPR low-function alleles in a class characterized by high Axis-I comorbidity (about 1/3rd of the sample) as compared to a class with relatively low comorbid psychiatric disturbance (Richardson et al., 2008). In addition, in ED samples, 5-HTTLPR low function variants have been linked to increased psychopathology, in the form of affective instability, impulsivity, borderline personality disorder, dissocial behaviour and harm avoidance (Akkermann, Nordquist, Oreland, and Harro, 2010; Monteleone et al., 2006; Steiger et al., 2005). On the other hand, 5-HTTLPR high function variants have been associated with increased compulsivity and inhibition (Steiger et al., 2009). Similar findings have been found in non-ED populations, with studies generally showing low-function variants to be linked to disorders involving problems of affect or impulse-regulation—such as MDD (Joiner, Johnson, Soderstrom, & Brown, 2003; Lotrich & Pollock, 2004; Neumeister et al., 2002), substance use disorders (Lichtermann et al., 2000; Mannelli et al., 2005; Sander et al., 1997; 1998) and BPD (Lyons-Ruth et al., 2007; Ni et al., 2006)—and high-function variants associated with anxiety disorders (Grabe et al., 2009; Thakur,

Joober, & Brunet, 2009), most prominently obsessive-compulsive disorder (OCD) (Baca-Garcia et al., 2005; Bengel et al., 1999; Cavallini, Di Bella, Siliprandi, Malchiodi, & Bellodi, 2002; Hu et al., 2006). Taken together, such results suggest that, within BN, 5-HTTLPR allelic variations may differentially contribute to variations in comorbid symptom profiles, with low function variants predisposing individuals to impulsive/affective symptoms and disorders (e.g., MDD, substance use disorders, BPD) and high function variants predisposing individuals to anxious/compulsive symptoms and disorders (e.g., ADs).

The tryptophan hydroxylase-2 promoter polymorphism (TPH-2 G-703T). Another serotonin-system gene of potential interest in the study of BN is the tryptophan hydroxylase (TPH) gene. The TPH gene encodes the rate-limiting biosynthetic enzyme in the serotonin pathway and regulates levels of 5-HT by converting tryptophan into 5-hydroxytryptophan, the direct precursor of 5-HT (Hennig, Reuter, Netter, Burk & Landt, 2005). The only published study to examine the TPH gene in BN to date reported that individuals carrying the AA genotype of the TPH A779C single nucleotide polymorphism (SNP) exhibited more disturbed binging behaviours and higher harm avoidance scores than did individuals with the CC genotype (Monteleone et al., 2007). The reported findings are consistent with the fact that the A-allele should, in theory, be associated with lower 5-HT function (Manuck et al., 1999).

Implications of the preceding finding are called into question by findings suggesting that TPH appears to be found exclusively in peripheral tissues and in the pineal body (McKinney, Knappskog & Haavik, 2005). More recently, Walther

and colleagues (2003) identified a second TPH isoform, designated as TPH-2, highly similar to the above-mentioned TPH gene (exhibiting 71% of amino acid identity), but expressed predominantly in the brain stem. Investigations of the TPH-2 gene have yielded further support for the involvement of 5-HT genes in Axis-I disorders in which anomalies of 5-HT functioning have already been identified. For instance, researchers have found associations between certain polymorphisms or haplotypes of the TPH-2 gene and MDD (Zill, Baghai, et al., 2004; Zhou et al., 2005), suicidality (Zill, Buttner, et al., 2004; Lopez de Lara et al., 2007; De Luca et al., 2005) and ADs (Zhou et al., 2005; Mössner et al., 2006). Associations of the TPH-2 G-703T (rs4570625) polymorphism with psychopathological traits and syndromes that frequently co-occur with BN make it a good candidate SNP for study in BN. For example, studies have reported associations between the TPH-2 G-703T T-allele or T/T genotype and greater emotional instability (Brown et al., 2005; Canli, Congdon, Guktnecht, Constable & Lesch, 2005; Hermann et al., 2007), cluster B and C personality disorders (Gutknecht et al., 2006), harm avoidance (Reuter, Kuepper & Henning, 2007) and impulsivity (Reuter, Ott, Vaitl & Henning, 2007; Stoltenberg et al., 2006). In the only study to examine the role of genetic variation in TPH-2 SNPs in individuals with BSDs, Groleau and colleagues (unpublished findings) found lower perfectionism and compulsivity scores in individuals carrying the T-allele of the TPH-2 G-703T polymorphism. Such findings, coupled with those in non-ED populations, suggest that the TPH-2 G-703T T allele may have implications for impulsive, affective and anxious traits in individuals with BN.

The present study. The current study had 2 main aims: The first was to explore the extent to which childhood sexual or physical abuse and specific gene polymorphisms acting in the 5-HT system (i.e., 5-HTTLPR and TPH-2 G-703T) were associated with presence of a BSD. The second was to explore, within individuals with BSDs, possible associations between psychiatric (Axis-I and Axis-II) comorbidity and childhood abuse and genetic variations. Based on previous findings, we expected to find an association between childhood abuse and presence of a BSD. Furthermore, we expected to find increased experiences of childhood abuse in individuals with comorbid disorders, in particular those implicating impulsive or dissocial phenomena. Based on previous findings suggesting that genetic factors may be more pertinent to explaining general psychopathological symptoms than eating specific ones in individuals with BN (Akkermann et al., 2010; Steiger, 2005; 2009), we did not expect to find a direct association of either 5-HTTLPR or TPH-2 with the diagnosis of BSD per say. Instead, we expected to find an increased prevalence of 5-HTTLPR low-function variants in individuals with comorbid disorders characterized by affective or impulsive components and an increased prevalence of the 5-HTTLPR high-function allele in individuals displaying comorbid disorders characterized by anxious or compulsive traits. With respect to TPH-2, previous findings led us to expect possible associations with disorders that imply problems of affect, anxiety or impulse control.

Method

Participants

All participants in this institutional ethics-board approved study gave informed consent. Participants with EDs were recruited between May 2004 and January 2009 through a specialized ED program in Montreal, Quebec, Canada. Our ED sample consisted of 102 women, 67 (65.7%) meeting DSM-IV criteria for BN-Purging (BN-P) subtype, 6 (5.9%) for BN-Nonpurging (BN-NP) subtype, and 29 (28.4%) for a bulimia-spectrum EDNOS (EDNOS-BN). EDNOS-BN was defined as subjects who binged or purged at less than the requisite average of twice weekly over the past 3 months. EDNOS participants binged or purged at least 2 times per month, with average binge/purge frequencies in these cases being 2.26 (+/- 3.01) and 23.47 (+/- 28.91), respectively. One subject who engaged in subjective binge eating only, but at a high frequency (27 episodes/month), was also included in the EDNOS-BN group. Both BN and EDNOS-BN cases were included in the current study given previous results demonstrating no clinical differences between subthreshold and threshold BN variants (Fairburn and Cooper, 1984; Niego et al., 1997; Pratt et al., 1998).

We also recruited 103 normal-eater women through public media and university-based announcements, so as to produce a comparison group within a comparable age range to that found in our ED sample and including comparable proportions of student and non-student participants. Forty-two normal-eater subjects (40.8% of the sample) were recruited between May 2004 and January 2009. To increase our sample size, 61 control subjects (59.2% of the sample) were included from two previous studies conducted between February 1998 and April 2004. To confirm that there were no time effects that differentiated the older

and newer samples we performed analyses comparing the two samples on childhood abuse and 5-HTTLPR and TPH-2 G-703T genotypes. No significant differences were found between samples, confirming that the samples were similar with respect to experiences of abuse and frequency of genotypes. We thus felt the samples could be merged together for analytic purposes.

To be eligible for the normal-eater group, participants had to have a Body Mass Index (BMI) between 18 and 34, absence of clinical ED symptoms according to the ED Examination and no history of ED, psychotic disorder or bipolar disorder, according to an initial telephone screening. Control participants participated in face-to-face structured clinical interviews assessing current and past Axis-I (mood, anxiety and substance-use) and Axis-II disorders. Twenty-nine subjects were missing complete Axis-I and Axis-II data, mainly due to different methodologies in older studies. Of the remaining 74 participants with complete data, 13 control subjects showed some form of Axis-I or Axis-II psychiatric comorbidity within the past year. All analyses involving control subjects were first run in the complete (n=103) sample, then in a second set of analyses, we excluded the 13 individuals with some form of comorbidity in the past year and the 29 individuals missing complete Axis-I and Axis-II information in order to rule out any possible confounds of comorbidity in normal-eater women (see Results section).

All subjects were females between the ages of 18 and 50 (mean = 26.03 $\pm$ 6.51 for ED subjects and 24.60 $\pm$ 6.36 for control subjects) and had a Body Mass Index (BMI: Kg/m^2) between 17.5 and 39 (mean = 22.49 $\pm$ 4.76 for ED subjects

and 21.76 $\pm$ 2.47 for control subjects). No significant differences were found between control and ED women on either age or BMI.

The Quebec population (from which this sample was drawn) includes a large proportion of people from Western Europe. Consequently, our ED sample included mainly subjects of Caucasian descent (96 individuals, or 94.1% of the sample). Of the 96 Caucasian individuals 81 (84.4%) were of West-European descent, 5 (5.2%) of East-European descent, 2 (2.1%) of South-European descent, 6 (6.3%) of Middle-East descent, 1 (1.0%) of Latin-American descent, and 1 (1.0%) of South-Asian descent. The non-Caucasian participants (6 individuals, or 5.9% of the sample) included 1 individual of mixed West-European Caucasian/ Native American (aboriginal) descent, 1 of mixed West-European Caucasian/ Caribbean Black descent, 1 of mixed West-European Caucasian/ Asian descent, 1 of African Black descent, and 2 of Asian descent. To rule out possible confounding effects of ethnicity on results, all analyses were re-run excluding those 6 participants who were not of clear-cut, Caucasian descent (see Results section). We did not have full ethnicity data on all the normal-eater control participants, and therefore did no corresponding subject deletion in this group. However, as recruitment took place within the same Quebec population and the main analyses of the study concerned differences occurring within individuals with EDs alone, uncontrolled ethnic variations among normal-eater controls were unlikely to have represented a serious confound.

Measures

Eating Pathology. The Eating Disorder Examination (EDE: Fairburn & Cooper, 1993), a 62-item semi-structured clinical interview, was utilized to assess the presence/absence of a DSM-IV ED diagnosis. The EDE has good inter-rater reliability and good discriminant validity for differentiating women with and without an ED (Fairburn & Cooper, 1993). To complement our assessment, we computed BMI (Kg/m^2). For 3 participants missing the EDE interview, ED diagnosis was derived from their initial assessment with a psychiatrist at the Eating Disorders Program and was verified with questionnaire results from the Eating Disorder Examination Questionnaire (EDE-Q: Fairburn & Beglin, 1994). The EDE-Q uses 38 self-report questions (derived from the EDE) to assess presence and severity of criterion ED symptoms. The EDE-Q indices reportedly correspond well with those obtained using the EDE (Mond, Hay, Rodgers, & Owen, 2006).

General Psychopathology. Comorbid DSM-IV Axis-II disorders were assessed in individuals with or without EDs using the Structured Clinical Interview for DSM-IV Axis II disorders (SCID-II: First, Gibbon, Spitzer, Williams, & Benjamin, 2000). Within individuals with EDs, diagnosis of lifetime comorbid DSM-IV Axis-I disorders (mood, anxiety and substance use disorders) was accomplished using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I: First, Spitzer, Gibbon, & Williams, 1996). The SCID-I and SCID-II interviews are "industry standard" measures for assessing current and lifetime history of Axis-I and Axis-II disorders. Inter-rater reliability estimates calculated for a

pseudo-randomly selected sample of SCID-II interviews, revealed the following inter-rater reliability estimates for Axis-II disorders of interest (i.e., those disorders for which there were at least 20% of individuals with a positive lifetime diagnosis): AVPD (κ = .92, n=34), OCPD (κ = .85, n=34), and BPD (κ = .78, n=32). Inter-rater reliability estimates calculated for a sample of SCID-I interviews in a partially overlapping sample from a recently published study (Richardson et al., 2008) revealed the following inter-rater reliability estimates: MDD (κ = .80, n=23), AD (excluding post-traumatic stress disorder) (κ = 1.00, n=23), alcohol use disorder (including abuse and dependence) (κ = .83, n=23), and drug use disorder (including abuse and dependence) (κ = .86, n=23). For the majority of ED participants in the current study (92%) post-traumatic stress disorder (PTSD) was assessed using the Clinician-Administered Post-Traumatic Stress Disorder Scale (CAPS: Blake et al., 1995) (Due to a shift in research protocols that took place during the course of data collection for this study, the remaining 8% of participants were assessed using the SCID-I). The CAPS is a standard criterion measure of PTSD diagnostic status and symptom severity, exhibiting excellent convergent and discriminant validity, and reliability (Weathers, Keane, & Davidson, 2001). In normal-eater subjects, screening for DSM-IV Axis-I disorders was carried out, for the most part (n=56) using the SCID-I and CAPS. The remaining (n=49) control participants were screened for Axis-I disorders using a computerized version of the Diagnostic Interview Schedule, Version IV (DIS4).

Childhood Abuse. Childhood abuse was assessed using the Childhood Trauma Interview (CTI: Fink, Bernstein, Handelsman, Foote, & Lovejoy, 1995) a roughly 30-minute structured interview on experiences of abuse prior to age 18. We used CTI severity indices (severity $\geq$ 3) to isolate experiences of unambiguous physical and sexual maltreatment occurring at or before age 18 (in conformity with the standard CTI protocol). The variable utilized in the current study was an aggregate Abuse variable isolating experiences of physical or sexual abuse at or before age 18. Inter-rater reliability estimates calculated for a set of CTI interviews in a partially overlapping sample from a recently published study (Richardson et al., 2008), revealed the following inter-rater reliability estimates: Physical abuse (κ = .81, n=24), sexual abuse (κ = .91, n=24). CTI indices have been shown to converge with other measures of abuse and construct validity is supported by logical associations with syndromes having theoretical links to trauma exposure (Fink, et al., 1995).

Genotyping. DNA samples, obtained from whole blood, were amplified by polymerase chain reaction (PCR) in a total volume of 20 µl, which contained 100ng of genomic DNA, 200 µM of dNTPs, 10 pmol (or 5pmol for TPH-2) each of the forward and reverse primer, 1 U of Taq (or 0.5 U of Taq for TPH-2) DNA Polymerase (Qiagen, Alameda, CA), 1 x PCR buffer, and 1 x Q solution (Qiagen). Ten pmol each of the forward primer (5'-ATG CCA GCA CCT AAC CCC TAA TGT-3') and reverse primer (5'-GG ACC GCA AGG TGG GCG GGA-3') were used to amplify a region encompassing 5-HTTLPR. The L_G and L_A alleles were subsequently studied by enzymatic digestion of 7 µl of the above mentioned PCR

product using 5 U of MspI and incubating at 37 ^{o} C for a minimum of 3 hours. Five pmol each of the forward primer (5' TGC ATA GAG GCA TCA CAG GA 3') and reverse primer (5' TCT TAT CCC TCC CAT CAG CA 3') were used to amplify the DNA segment containing the single nucleotide polymorphism (SNP) TPH-2 G-703T (rs4570625). The PCR protocol involved preheating the samples at 94^{o} C for 5 min, followed by 35 cycles of denaturation at 94^{o} C (30 sec), annealing at 64^{o} C (30 sec), and extension at 72^{o} C (45 sec), as well as a final hold of 5 min at 72^{o} C. PCR product amplification was verified by running 5ul of the PCR product on a 2% agarose gel. The remaining product was then processed as per the ABI SNaPshot protocol, using primers designed for fluorescent dideoxy nucleotide termination. SNP analysis was carried out on the ABI 3100 genetic analyzer. Genotypes were determined automatically using the Genemapper software (Applied Biosystems) and consequently verified manually.

Statistical Analyses

Our first data-analytic step explored the effects Genotype (5-HTTLPR and TPH-2 G-703T) and childhood sexual or physical Abuse on presence/absence of BN-spectrum ED diagnosis. To do this we applied logistic regressions (testing the main effects of Genotype and Abuse) to the dichotomous variable "Presence of BSD" (defined as BSD versus Control). Our second data analytic step explored the effects of Genotype and Abuse on Axis-I and Axis-II psychiatric comorbidity within individuals with BSDs. Logistic regression analyses testing the main effects of 5-HTTLPR or TPH-2 G-703T Genotype and Abuse were applied to

Axis-I and Axis-II diagnoses (defined as Positive Lifetime Diagnosis versus No Positive Lifetime Diagnosis).

Results

Genotype frequencies. Frequencies (and percentages) of 5-HTTLPR low-function/low-function (S'/S'), low-function/high-function (S'/L'), and high-function/high-function (L'/L') genotypes, respectively occurring in 20 (19.6%), 56 (54.9%) and 26 (25.5%) of the bulimia-spectrum ED participants and 30 (29.4%), 58 (56.9%) and 14 (13.7%) of the normal-eater control participants, were in conformity with Hardy-Weinberg equilibrium [ED: $\chi^2_{(1)} = 1.26$, n.s.; Control: $\chi^2_{(1)} = 2.81$, n.s.]. Frequencies (and percentages) of TPH-2 G-703T G/G, G/T and T/T genotypes occurred respectively in 66 (64.7%), 34 (33.3%) and 2 (2.0%) of the ED participants, and 64 (62.7%), 31 (30.4%) and 7 (6.9%) of controls and were also in conformity with Hardy-Weinberg equilibrium [ED: $\chi^2_{(1)} = 1.01$, n.s.; Control: $\chi^2_{(1)} = 1.19$, n.s.]. One Control subject was missing 5-HTTLPR genotype information and one (different) control subject was missing TPH-2 G-703T genotype information.

Genotype and Abuse effects on "presence of BSD". In our first data-analytic step we applied logistic regressions to explore the effects of 5-HTTLPR or TPH-2 G-703T Genotype and Abuse on the dependent variable "Presence of BSD". Four separate logistic regression analyses were conducted. First, we examined the effect of 5-HTTLPR Genotype and Abuse on presence of BSD. 5-HTTLPR Genotype effects were assessed by creating two dummy variables (one coding S'/S' genotype and a second coding L'/L' genotype), with S'/L' genotype as the

reference category. Second, we examined the effect of 5-HTTLPR Alleles and Abuse on presence of BSD. Due to contradictory findings suggesting possible associations between 5-HTTLPR low-function S' variants and BN in one study, and 5-HTTLPR high-function L' variants and BN in two other studies, we examined the effects of both 5-HTTLPR S' and L' alleles on presence of BSD in two separate logistic-regression analyses (both also including the effect of Abuse), one in which we dichotomized the 5-HTTLPR genotype by presence (S'/S' and S'/ L') versus absence (L'/L') of S' variants and another in which we dichotomized 5-HTTLPR genotype by presence (L'/L' and S'/ L') versus absence (S'/S') of the L' variant. Finally, in a fourth analysis we examined the effect of the TPH-2 G-703T polymorphism and Abuse on presence of BSD. Based on previously reported functional evidence for the T allele (Brown et al., 2005; Canli et al., 2005; Hermann et al., 2007) and relatively low frequency of the T/T genotype (n=2) in the present sample we dichotomized the TPH-2 G-703T polymorphism by presence (T/T and G/T) versus absence (G/G) of the T Allele.

In the first logistic regression analysis a test of the full model against a constant only model was statistically significant ($\chi^2 = 24.879$, $p < .000$, $df = 3$), indicating that the predictors (5-HTTLPR Genotype and Abuse) as a set reliably distinguished between individuals with and without a BSD diagnosis. Prediction success overall was 66.7% (77.5% for BSD and 55.9% for Control). Table 1 displays coefficients (standard errors), Exp (B) (or odds ratios) and 95% confidence intervals, and significance levels for Wald statistics for each logistic regression analysis. In the first analysis the Wald criterion demonstrated that 5-

HTTLPR L'/L' Genotype contributed to prediction at a trend level (p= .071) and that Abuse made a significant contribution to prediction (p = .000). The odds ratio indicated that individuals with childhood abuse were 3.59 times more likely to have a BSD than those without a history of abuse. Figure 1 illustrates the proportion of BN-spectrum diagnosis in subjects with and without a history of childhood sexual or physical abuse. In the second logistic regression analysis a test of the full model against a constant only model was also statistically significant ($\chi^2 = 23.957$, $p < .000$, $df = 2$), indicating that the predictors (5-HTTLPR S' Allele and Abuse) as a set reliably distinguished between individuals with and without a BSD. Prediction success was 66.7% overall (77.5% for BSD and 55.9% for Control). As can be seen in Table 1, both 5-HTTLPR S' Allele (p=. 032) and Abuse (p= .000) made a significant contribution to prediction. In line with the trend in the first analysis suggesting that subjects with the 5-HTTLPR L'/L' genotype were more likely to have a BSD diagnosis, odds ratios indicated that individuals with S' alleles were 2.29 times less likely to have an ED than individuals with the L'/L' genotype. See Figure 2 for an illustration of the proportion of BN-spectrum diagnosis in individuals with and without the 5-HTTLPR L'/L' genotype. As in the first analysis, the odds ratio (3.60) also indicated that individuals with a history of childhood abuse were more likely to have a BSD than those without a history of childhood abuse. In the third and fourth logistic regression analyses tests of the full model against a constant only model were statistically significant ($\chi^2 = 21.512$, $p < .000$, $df = 2$; and $\chi^2 = 20.495$, $p < .000$, $df = 2$, respectively), indicating that the predictors reliably differentiated

eating and non-eating disordered individuals. Prediction success overall was, 65.2% (66.7% for BSD and 63.7.9% for Control) for the third analysis and 65.7% (66.7% for BSD and 64.7% for Control) for the fourth analysis. In both analyses only Abuse (p= .000 in each analysis) made a significant contribution to prediction. Neither 5-HTTLPR L' Allele or TPH-2 G-703T T Allele contributed significantly to prediction. Odds ratios for Abuse were similar to those obtained in previous analyses (3.51 and 3.69, respectively). All analyses were re-run replacing the preceding criteria for Abuse (physical or sexual abuse before age 18) with a measure of physical or sexual Abuse before age 14 to isolate abuse experiences that were likely before ED onset. Analyses including the revised abuse criteria yielded the same pattern of results. Furthermore, ancillary analyses excluding the 13 controls with some form of comorbidity in the past year and the 31 controls missing complete Axis-I and Axis-II information revealed the same pattern of results. Likewise, analyses excluding the 6 ED participants who were not of clear-cut, Caucasian descent rendered the same results. In sum, results suggest that both the experience of childhood physical or sexual abuse and being a 5-HTTLPR L' homozygote predict the presence of a BN-spectrum ED in adulthood.

Genotype and Abuse effects on psychiatric comorbidity in BSDs. Our second data-analytic step, including only BSD individuals, applied logistic regressions to explore the effects of 5-HTTLPR or TPH-2 G-703T Genotype and Abuse on lifetime Axis-I and Axis-II comorbidity. Four separate logistic regression

analyses were carried out for each disorder of interest, similar to those described in our first set of data analyses.

Table 2 shows frequencies of Axis-I and Axis-II disorders in BSD participants. To ensure an adequate sample size, we restricted our analyses to diagnoses for which there were at least 20% of individuals with a positive lifetime diagnosis. Axis-I disorders that had at least a 20% lifetime prevalence rate included: MDD, Panic Disorder, Drug Abuse/Dependence and Alcohol Abuse/Dependence. Since only one Anxiety Disorder (AD) was present in a sufficient number of individuals to support viable comparisons between individuals with and without a specific AD, we created a composite "Anxiety Disorder" variable (including Social Phobia, Agoraphobia, Panic Disorder, Generalized Anxiety Disorder, Obsessive Compulsive Disorder, Specific Phobia and Post-Traumatic Stress Disorder) to detect the presence of any AD versus no AD. Axis-II disorders with at least a 20% lifetime prevalence rate included: AVPD, OCPD and BPD.

Table 3 summarizes the models obtained with each logistic-regression analysis examining effects of Genotype and Abuse on lifetime history of AD. In the first logistic-regression analysis a test of the full model against a constant only model was statistically significant ($\chi^2 = 8.524$, $p = .036$, $df = 3$), indicating that the predictors (5-HTTLPR Genotype and Abuse) as a set reliably distinguished between individuals with and without a history of AD. Prediction success overall was 62.0% (91.5% for AD and 31.1% for No AD). As indicated in Table 3, only 5-HTTLPR S'/S' Genotype contributed significantly to prediction ($p= .020$). The

odds ratio shows that individuals with the S'/S' genotype were 4.41 times less likely to have a history of AD than individuals with the S'/L' genotype. In the second logistic regression analysis a test of the full model against a constant only model was not statistically significant ($\chi^2 = 2.275$, p = .321, df = 2), indicating that the predictors (5-HTTLPR S' Allele and Abuse) did not distinguish between individuals with and without a history of AD. In the third logistic regression analysis a test of the full model against a constant only model was statistically significant ($\chi^2 = 8.174$, p = .017, df = 2), indicating that the predictors (5-HTTLPR L' Allele and Abuse) reliably distinguished between individuals with and without a history of AD. Prediction success overall was 62.0% (91.5% for AD and 31.1% for No AD). Significance values indicated in Table 3 demonstrate that only 5-HTTLPR L' allele contributed significantly to prediction (p= .010). The odds ratio indicates that individuals with an L' allele were 4.87 times more likely to have a history of AD than individuals without an L' allele. See Figure 3 for an illustration of the proportion of a lifetime AD in subjects with and without the 5-HTTLPR L' Allele. In the fourth logistic regression analysis a test of the full model against a constant only model was not statistically significant ($\chi^2 = 1.016$, p = .602, df = 2), indicating that the predictors (TPH-2 G-703T T Allele and Abuse) did not distinguish between individuals with and without a history of AD. Taken together, findings demonstrated that the 5-HTTLPR high function L' allele was a significant predictor of lifetime AD in individuals with BSDs.

Table 4 summarizes the models obtained with each logistic regression analysis examining effects of Genotype and Abuse on lifetime history of Drug

Abuse/Dependence. In the first logistic regression analysis a test of the full model against a constant only model was significant at a trend level ($\chi^2 = 6.870$, p = .076, df = 3), indicating a tendency for the predictors (5-HTTLPR Genotype and Abuse) to distinguish between individuals with and without a history of drug dependence/abuse. Prediction success overall was 72.3% (0.0% for Drug abuse/dependence and 100.0% for No Drug abuse/dependence). Significance values in Table 4 demonstrate that only Abuse contributed significantly to prediction (p= .040). The odds ratio indicates that individuals with a history of childhood abuse were 3.45 times more likely to have a history of drug dependence/abuse than individuals without a history of childhood abuse. Figure 4 illustrates the proportion of drug dependence/abuse in individuals with and without a history of childhood sexual or physical abuse. In the second, third and fourth logistic regression analyses tests of the full model against a constant only model were statistically significant ($\chi^2 = 6.641$, p = .036, df = 2; $\chi^2 = 6.083$, p < .048, df = 2; and $\chi^2 = 8.500$, p < .014, respectively), indicating that the predictors reliably distinguished between individuals with and without a history of drug abuse/dependence. Prediction success overall was the same for all models, 72.3% (0.0% for Drug abuse/dependence and 100.0% for No drug abuse/dependence). In all analyses only childhood abuse (p= .040; p= .032; and p= .043, respectively) made a significant contribution to prediction. As can be observed in Table 4, odds ratios were similar to that observed in the first analysis. Neither 5-HTTLPR S' or L' Allele or TPH-2 G-703T T Allele contributed significantly to prediction. All analyses were re-run replacing the above criteria measure for Abuse (physical or

sexual abuse before age 18) with a measure of childhood physical or sexual abuse before age 14 in order to isolate abuse experiences that were likely before ED onset. Analyses including the revised abuse criteria revealed no significant effects of Abuse on the presence of Drug Dependence/Abuse. To summarize, results indicated that the experience of childhood physical or sexual abuse before age 18 predicted the presence of lifetime drug dependence or abuse in individuals with BN-spectrum EDs, however when abuse criterion were altered to isolate experiences of abuse before age 14 the predictive power of childhood abuse for drug use was no longer significant.

Finally, logistic regression analyses were carried out examining effects of Genotype and Abuse on lifetime history of MDD (see Table 5), Panic Disorder (see Table 6), Alcohol Abuse/Dependence (see Table 7), AVPD (see Table 8), OCPD (see Table 9) and BPD (see Table 10). Results revealed that neither Genes nor Abuse contributed significantly to predicting the lifetime presence any of the above-mentioned disorders in BN-spectrum subjects in the present sample. All analyses (in Tables 3-10) re-run to exclude the 6 ED participants who were not of clear-cut, Caucasian descent rendered the same pattern of results.

Discussion

The current study explored: 1) the extent to which the presence of childhood physical or sexual abuse and specific gene polymorphisms acting upon serotonin-system activity (5-HTTLPR and TPH-2 G-703T) were predictive of risk of BSDs, and 2) within individuals with BSDs, the extent to which childhood abuse and the above-mentioned gene polymorphisms were predictive of comorbid

Axis-I and Axis-II disorders. Findings showed the presence of physical or sexual abuse in childhood to be predictive of risk of BSDs. Within individuals with BSDs, more fine grained analyses revealed the presence of childhood abuse to be significantly associated with a history of Drug Abuse/Dependence, suggesting that childhood abuse is a particularly relevant predictor in individuals with SUDs and BSDs. With respect to genetic factors, results revealed that presence of the 5-HTTLPR S' allele predicted absence of BSDs. In line with this, presence of the 5-HTTLPR L'/L' genotype predicted (at a trend level) presence of BSDs, suggesting that 5-HTTLPR high-function alleles may be associated with risk of BSDs. More fine-grained analyses, within individuals with BSDs, showed that5-HTTLPR high-function alleles were associated with a history of ADs, suggesting that 5-HTTLPR high-function variants may be particularly relevant in individuals with ADs and BSDs. We found no effect of genes or childhood abuse on the presence of MDD, Panic Disorder, Alcohol Abuse/Dependence or PDs of interest (AVPD, OCPD and BPD). In sum, findings suggest that developmental (childhood abuse) and genetic (5-HTTLPR) factors are associated, to some extent, with risk of BN. However, such factors may be more relevant predictors of psychiatric comorbidity in individuals with BN, with childhood abuse predicting comorbid drug problems and 5-HTTLPR high-function variants predicting comorbid ADs.

Childhood abuse findings. Findings linking childhood physical or sexual abuse to risk of a BN-spectrum ED are consistent with previous results associating EDs with exposure to abuse (Dansky, Brewerton, Kilpatrick, & O'Neil, 1997; Leonard et al., 2003; Schmidt, Hunfress, & Treasure, 1997;

Wonderlich et al., 1997). In the current study, 66% percent of individuals with BN-spectrum EDs reported unwanted experiences of physical or sexual abuse before age 18, as compared to 36% percent of normal-eater subjects. It should be noted that although rates of abuse in individuals with BN were elevated compared to our control sample, rates we obtained are comparable to those found in other psychiatric patient groups (Steiger & Zanko, 1990; Welch & Fairburn, 1994)—suggesting that childhood abuse may not be a specific risk factor for BN, but rather, a non-specific risk factor for psychopathology in general. Bulik et al. (2001) hypothesized that childhood sexual abuse is a non-specific risk factor for various psychiatric disorders, possibly acting by lowering the threshold at which certain psychopathological traits—to which an individual is pre-disposed by other environmental or genetic factors risk factors—are expressed. Providing a more specific model, Steiger (2004) and Steiger & Bruce (2009) proposed that traumatic stressors during childhood may act to "amplify" latent serotonergic susceptibilities, that are then activated later in life by malnutrition-induced alterations in 5-HT functioning in individuals with BSDs. Within both perspectives, childhood abuse can be conceptualized as a non-specific risk factor that may confer risk for BN through amplifying general susceptibilities, rather than eating-specific ones, in individuals who are rendered susceptible by other risk agents, biological and environmental.

Findings from the present study associating experiences of childhood abuse with comorbid Drug Abuse/Dependence in individuals with BSDs provides further evidence for the hypothesis childhood abuse is not specific to risk of BN,

but more generally linked to psychopathology. Within individuals with BSDs, those with a history of childhood abuse were 3.45 times more likely to have a history of Drug Dependence/Abuse—findings that are in line with several previous studies associating childhood physical or sexual abuse with the presence of lifetime SUDs in subjects with EDs (Corstorphine et al., 2007; Deep, Lilenfeld, Plotnicov, Pollice, & Kaye, 1999; Dohm, Striegel-Moore, Wilfley, Pike, Hook, & Fairburn, 2002; Matsunaga et al., 1999). Findings associating childhood abuse with the presence of SUDs and BN have led some researchers to speculate that experiences of abuse in childhood may amplify characterological traits of poor impulse control or affective instability which may contribute to the development of both disorders (Deep et al., 1999). Other researchers have suggested that impulsive behaviours, like drug abuse or binge eating, may serve a dissociative function, allowing individuals who have experienced trauma to escape from the awareness of their experiences for a short time (Heatherton & Beaumeister, 1991). However, findings from the current study implying that substance use problems are connected to childhood abuse only when abuse consists of experiences occurring at a later age (before age 18 versus before age 14) open up the possibility that the direction of causality is opposite to the one that might be intuitive (i.e., abuse conferring vulnerability to SUDs). The present findings suggest that an association between drug abuse/dependence and childhood abuse could occur because drug abuse actually increases risk of abuse—possibly through such correlates as heightened conduct problems or impulsive behaviours—in people who are abusing drugs. However, lack of associations

between drug abuse/dependence and childhood abuse before age 14 may also be an artefact of less statistical power when isolating experiences of abuse under 14, rather than 18.

Genetic findings. Genetic findings from the current study suggest a positive relationship between the 5-HTTLPR L'/L' genotype and risk of BN. Such findings are in line with some previous studies examining the traditional biallelic model of 5-HTTLPR. In a study of non-clinical subjects, Matsushita et al. (2002) found a significantly higher frequency of the L/L genotype and the L allele in individuals who scored in the clinical range ($\geq$ 20) on the Eating Attitudes Test than in those in the non-clinical range. Similarly, in a sample of 125 women with BN and 94 control subjects, Monteleone et al. (2006) found a higher frequency of the L allele in individuals with BN. A more recent study, examining the triallelic model of 5-HTTLPR in a sample of 185 women with EDs (155 with a BN-spectrum ED and 30 with an AN spectrum ED) and 93 control women, found that the L'/L' genotype occurred significantly more frequently among ED subjects than among controls. However, further analyses within eating-disorder subjects revealed that the association may be attributable to the fact that individuals displaying an "inhibited/compulsive" profile (derived from latent class analysis) were more likely than other individuals to carry the L' allele and the L'/L' genotype (Steiger et al., 2009). Such findings, suggest that rather than predicting eating disorder symptoms per say, variations in 5-HTTLPR might be predictive of traits (like compulsivity) that may influence the manifestation of ED symptoms and expression.

Providing further evidence for the idea that 5-HTTLPR variants may predict comorbid psychopathology in individuals with BSDs, findings from the current study link the 5-HTTLPR L' allele to a history of Anxiety Disorder. Individuals with an L' allele were 4.87 times more likely to have a history of anxiety disorder than individuals without an L' allele. Such findings are in line with studies in non-ED populations linking high-function L' variants to generalized anxiety disorder (Grabe et al., 2009), PTSD (Thakur et al., 2009) and obsessive-compulsive disorder (Baca-Garcia et al., 2005; Bengel et al., 1999; Cavallini et al., 2002;Hu et al., 2006).

Taken together, findings associating 5-HTTLPR high function variants to risk of BN and ADs suggest that 5-HTTLPR may represent a genetic vulnerability factor for the development of both eating and anxiety disorders. Results from twin and family and twin studies suggesting that the disorders share a common underlying diathesis lend further evidence to such a hypothesis (Keel, Klump, Miller, McGue, & Iacono, 2005). How might 5-HTTLPR confer vulnerability to both EDs and ADs? Inconsistent evidence associating 5-HTTLPR with 5-HT transporter binding in the brain has led to the proposal that associations of the 5-HTTLPR polymorphism with clinical phenotypes may be due to developmental effects of 5-HTTLPR upon brain development, possibly in conjunction with early-life stress, rather than due to its direct effect on serotonin transporter binding (Parsey et al., 2006). Following from this, we speculate that presence of high- or low-function 5-HTTLPR alleles may have implications for the global propensity of an individual’s 5-HT system in childhood, leading to the

development of certain psychopathological traits that may make an individual more susceptible to develop psychiatric disorders in adulthood. More, specifically findings from the current study suggest that 5-HTTLPR high-function alleles may have implications for anxiety-related traits, which—likely in combination with environmental factors—may create vulnerability for both EDs and ADs in adulthood.

Limitations. Our sample is relatively small for the multivariate aspect of this exploration, and this may limit stability of findings and power to detect certain effects of potential interest. Since our sample is of modest size, any genotype-related effects we obtain must be interpreted cautiously, and regarded as preliminary and in need of replication. In addition, due to small sample size, in this study we have not addressed gene-environment interaction effects that may be relevant to the understanding of associations between genetic factors, childhood abuse and psychiatric disorders (Caspi et al., 2003; Kaufman et al., 2004; Steiger et al., 2007; 2008).

Conclusion. The present findings highlight the importance of considering comorbid psychopathology when examining developmental and genetic effects in individuals with EDs. Results show broad associations of childhood abuse and 5-HTTLPR with BSDs. However, more fine-grained analyses associating developmental and genetic factors with comorbid psychopathology leads us to hypothesize that such factors may indirectly influence eating-disorder risk and expression, by influencing personality-trait manifestations such as impulsivity or anxiety. This interpretation is consistent with general findings suggesting that 5-

HTTLPR variations and childhood abuse may be more powerful correlates of generalized psychopathological-trait variations seen in ED sufferers than they are of eating-symptom variations. Moreover, such interpretations suggest potentially important genetic and environmentally mediated pathways to ED expression through trait endophenotypes such as impulsivity and anxiety.

Table 1. Results of logistic regression analyses in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable Presence of bulimia-spectrum ED.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=204)			
Constant	-.713 (.254)**	.490	
S'/S'	-.346 (.361)	.708	.349-1.437
L'/L'	.723 (.400)†	2.060	.940-4.514
Abuse	1.277 (.299)***	3.586	1.994-6.450
5-HTTLPR S' Allele and Abuse (N=204)			
Constant	.007 (.370)	1.007	
S'	-.827 (.386)*	.437	.205-.931
Abuse	1.282 (.299)***	3.603	2.006-6.472
5-HTTLPR L' Allele and Abuse (N=204)			
Constant	-1.046 (.342)**	.351	
L'	.528 (.347)	1.695	.859-3.345
Abuse	1.254 (.296)***	3.505	1.962-6.261
TPH-2 G-703T T Allele and Abuse (N=204)			
Constant	-.695 (.246)**	.499	
T	.079 (.309)	1.082	.590-1.983
Abuse	1.306 (.297)***	3.692	2.065-6.602

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
$^{\dagger}p < .10$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

Table 2. Frequency of lifetime Axis-I and Axis-II diagnoses in individuals with bulimia-spectrum EDs. Differences in ns reflect isolated missing values.

Axis-I Diagnosis	**n total**	**n positive lifetime diagnosis**	**% positive lifetime diagnosis**
Major Depressive Disorder	96	72	75.0
Dysthimia	96	15	15.6
Manic Depressive Disorder	92	2	2.2
Panic Disorder	93	20	21.5
Agoraphobia	93	11	11.8
Obsessive Compulsive Disorder	92	13	14.1
Generalized Anxiety Disorder	92	17	18.5
Social Phobia	94	7	7.4
Specific Phobia	91	9	9.9
Post Traumatic Stress Disorder	87	5	5.7
Any Anxiety Disorder	92	47	51.1
Alcohol Abuse/Dependence	94	24	25.5
Drug Abuse/Dependence	94	26	27.7
Axis-II Diagnosis	**n total**	**n positive lifetime diagnosis**	**% positive lifetime diagnosis**
Dependent Personality Disorder	102	6	5.9
Avoidant Personality Disorder	102	22	21.6
Obsessive Compulsive Personality Disorder	102	29	28.4
Borderline Personality Disorder	102	28	27.5
Histrionic Personality Disorder	102	4	3.9
Narcissistic Personality Disorder	102	6	5.9
Antisocial Personality Disorder	102	3	2.9
Paranoid Personality Disorder	102	5	4.9
Schizotypal Personality Disorder	102	0	0.0
Schizoid Personality Disorder	102	2	2.0

Table 3. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of lifetime Anxiety Disorder.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=92)			
Constant	.016 (.441)	1.016	
S'/S'	-1.484 (.636)*	.227	.065-.788
L'/L'	.314 (.515)	1.370	.499-3.758
Abuse	.314 (.470)	1.368	.544-3.440
5-HTTLPR S' Allele and Abuse (N=92)			
Constant	.315 (.495)	1.370	
S'	-.675 (.493)	.509	.194-1.338
Abuse	.342 (.455)	1.407	.577-3.435
5-HTTLPR L' Allele and Abuse (N=92)			
Constant	-1.441 (.654)*	.237	
L'	1.583 (.615)*	4.872	1.459-16.263
Abuse	.276 (.465)	1.317	.530-3.274
TPH-2 G-703T T Allele and Abuse (N=92)			
Constant	-.282 (.410)	.755	
T	.368 (.452)	1.445	.596-3.506
Abuse	.305 (.451)	1.357	.561-3.284

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
†p < .10, *p < .05, **p < .01, ***p < .001

Table 4. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of lifetime Drug Abuse/Dependence.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=94)			
Constant	-1.819 (.586)**	.162	
S'/S'	.277 (.577)	1.320	.426-4.085
L'/L'	-.558 (.646)	.572	.161-2.029
Abuse	1.237 (.602)*	3.446	1.059-11.214
5-HTTLPR S' Allele and Abuse (N=94)			
Constant	-2.374 (.722)**	.093	
S'	.639 (.622)	1.894	.560-6.407
Abuse	1.233 (.601)*	3.432	1.056-11.150
5-HTTLPR L' Allele and Abuse (N=94)			
Constant	-1.577 (.681)*	.207	
L'	-.426 (.555)	.653	.220-1.940
Abuse	1.281 (.599)*	3.602	1.114-11.646
TPH-2 G-703T T Allele and Abuse (N=94)			
Constant	-1.593 (.561)**	.203	
T	-.937 (.567)†	.392	.129-1.191
Abuse	1.221 (.605)*	3.392	1.037-11.094

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
$^{\dagger}p < .10$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

Table 5. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of lifetime Major Depressive Disorder.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=96)			
Constant	.641 (.458)	1.898	
S'/S'	1.216 (.812)	3.373	.687-16.550
L'/L'	-.240 (.541)	.786	.272-2.272
Abuse	.520 (.501)	1.683	.630-4.493
5-HTTLPR S' Allele and Abuse (N=96)			
Constant	.411 (.512)	1.509	
S'	.501 (.524)	1.651	.592-4.607
Abuse	.500 (.494)	1.648	.626-4.339
5-HTTLPR L' Allele and Abuse (N=96)			
Constant	1.841 (.806)*	6.301	
L'	-1.296 (.790)	.274	.058-1.287
Abuse	.547 (.497)	1.727	.652-4.576
TPH-2 G-703T T Allele and Abuse (N=96)			
Constant	.711 (.438)	2.037	
T	.073 (.508)	1.076	.398-2.913
Abuse	.560 (.493)	1.751	.666-4.602

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
$^{\dagger}p < .10$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

Table 6. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of lifetime Panic Disorder.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=93)			
Constant	-1.178 (.513)*	.308	
S'/S'	-1.092 (.814)	.335	.068-1.655
L'/L'	-.268 (.601)	.765	.236-2.482
Abuse	.186 (.555)	1.204	.406-3.574
5-HTTLPR S' Allele and Abuse (N=93)			
Constant	-1.449 (.605)*	.235	
S'	.033 (.585)	1.033	.329-3.251
Abuse	.192 (.550)	1.211	.413-3.557
5-HTTLPR L' Allele and Abuse (N=93)			
Constant	-2.289 (.847)**	.101	
L'	1.011 (.796)	2.748	.578-13.073
Abuse	.212 (.552)	1.236	.419-3.644
TPH-2 G-703T T Allele and Abuse (N=93)			
Constant	-1.895 (.549)**	.150	
T	.953 (.524)†	2.593	.928-7.244
Abuse	.336 (.564)	1.399	.463-4.223

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
†$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$

Table 7. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of lifetime Alcohol Abuse/Dependence.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=96)			
Constant	-1.442 (.530)**	.236	
S'/S'	-.238 (.607)	.788	.240-2.592
L'/L'	-.640 (.637)	.527	.151-1.836
Abuse	.784 (.563)	2.190	.726-6.607
5-HTTLPR S' Allele and Abuse (N=96)			
Constant	-2.079 (.689)**	.125	
S'	.575 (.616)	1.778	.531-5.949
Abuse	.780 (.563)	2.182	.724-6.576
5-HTTLPR L' Allele and Abuse (N=96)			
Constant	-1.701 (.683)*	.182	
L'	.065 (.588)	1.067	.337-3.376
Abuse	.811 (.561)	2.250	.750-6.749
TPH-2 G-703T T Allele and Abuse (N=96)			
Constant	-1.668 (.535)**	.189	
T	.045 (.510)	1.046	.385-2.844
Abuse	.814 (.564)	2.258	.748-6.814

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
†p < .10, *p < .05, **p < .01, ***p < .001

Table 8. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of Avoidant Personality Disorder.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=102)			
Constant	-.815 (.452)†	.443	
S'/S'	-1.103 (.809)	.332	.068-1.619
L'/L'	-.158 (.565)	.854	.282-2.582
Abuse	-.420 (.505)	.657	.244-1.767
5-HTTLPR S' Allele and Abuse (N=102)			
Constant	-.976 (.535)†	.377	
S'	-.067 (.549)	.935	.319-2.745
Abuse	-.414 (.499)	.661	.248-1.760
5-HTTLPR L' Allele and Abuse (N=102)			
Constant	-1.929 (.811)*	.145	
L'	1.054 (.790)	2.869	.610-13.495
Abuse	-.404 (.501)	.668	.250-1.784
TPH-2 G-703T T Allele and Abuse (N=102)			
Constant	-1.138 (.447)*	.320	
T	.273 (.497)	1.314	.496-3.483
Abuse	-.397 (.499)	.672	.253-1.786

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
$^{\dagger}p < .10$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

Table 9. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of Obsessive Compulsive Personality Disorder.

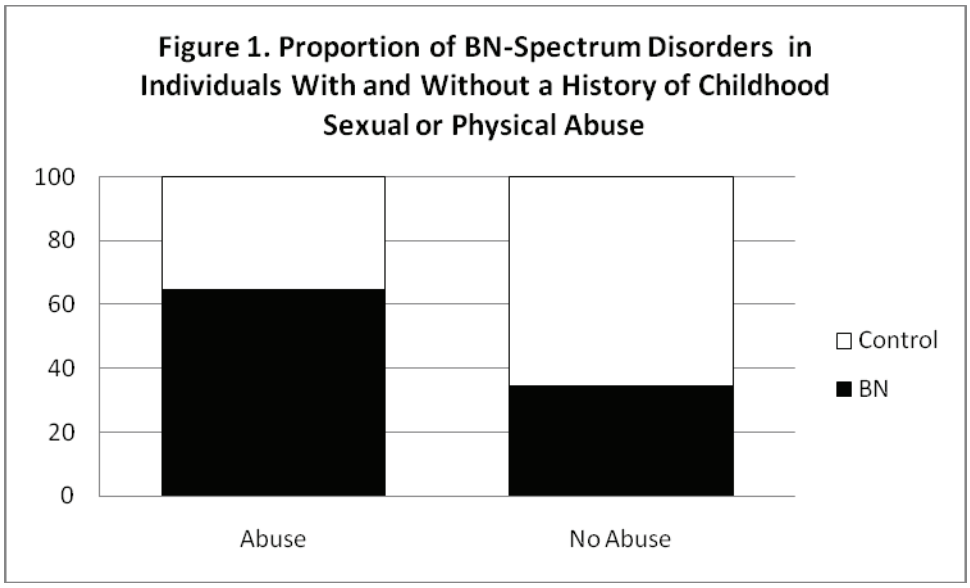
	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=102)			
Constant	-1.012 (.455)*	.364	
S'/S'	-.644 (.630)	.525	.153-1.804
L'/L'	-.209 (.531)	.811	.287-2.296
Abuse	.372 (.487)	1.451	.559-3.769
5-HTTLPR S' Allele and Abuse (N=102)			
Constant	-1.218 (.535)*	.296	
S'	.058 (.514)	1.060	.387-2.901
Abuse	.367 (.485)	1.444	.558-3.733
5-HTTLPR L' Allele and Abuse (N=102)			
Constant	-1.670 (.668)*	.188	
L'	.581 (.610)	1.788	.540-5.915
Abuse	.392 (.484)	1.480	.573-3.824
TPH-2 G-703T T Allele and Abuse (N=102)			
Constant	-1.171 (.447)**	.310	
T	-.019 (.464)	.981	.395-2.436
Abuse	.371 (.484)	1.450	.562-3.741

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
†p < .10, *p < .05, **p < .01, ***p < .001

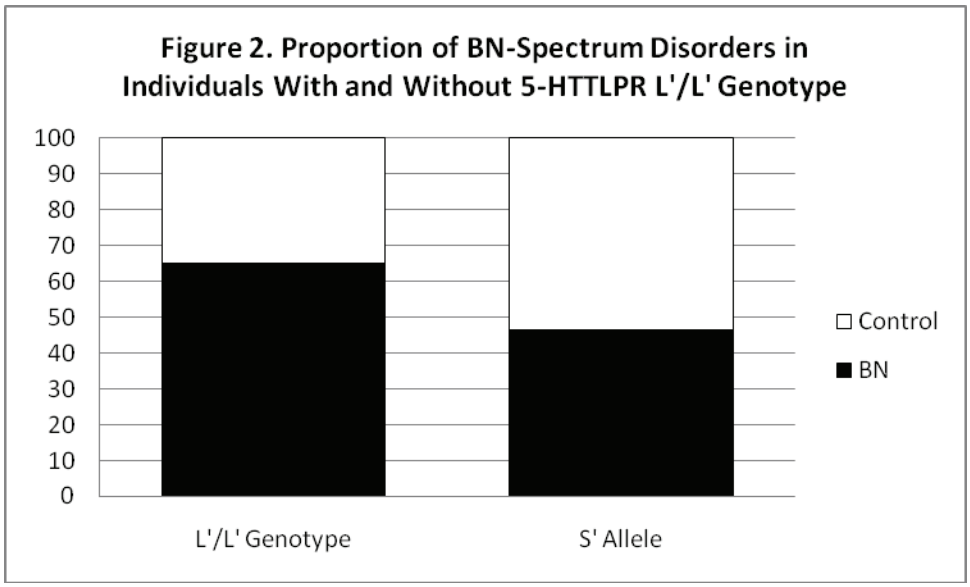
Table 10. Results of logistic regression analyses, in individuals with BSDs, in which main effects of Genotype (or Allele) and Abuse were regressed onto the dependent variable presence of Borderline Personality Disorder.

	Coefficient (SE)	OR	95% CI
5-HTTLPR Genotype and Abuse (N=102)			
Constant	-1.292 (.475)**	.275	
S'/S'	-.734 (.697)	.480	.123-1.880
L'/L'	.587 (.510)	1.798	.662-4.886
Abuse	.400 (.497)	1.491	.563-3.951
5-HTTLPR S' Allele and Abuse (N=102)			
Constant	-.702 (.502)	.495	
S'	-.752 (.492)	.472	.180-1.237
Abuse	.395 (.495)	1.484	.562-3.915
5-HTTLPR L' Allele and Abuse (N=102)			
Constant	-1.975 (.726)**	.139	
L'	.927 (.672)	2.527	.677-9.438
Abuse	.332 (.489)	1.394	.535-3.634
TPH-2 G-703T T Allele and Abuse (N=102)			
Constant	-1.200 (.449)**	.301	
T	.051 (.466)	1.053	.422-2.625
Abuse	.308 (.486)	1.360	.525-3.526

OR = Odds Ratio; 95% CI = 95% Confidence Interval for Odds Ratio
†p < .10, *p < .05, **p < .01, ***p < .001



Proportions are actual (rather than estimated) proportions in the sample.



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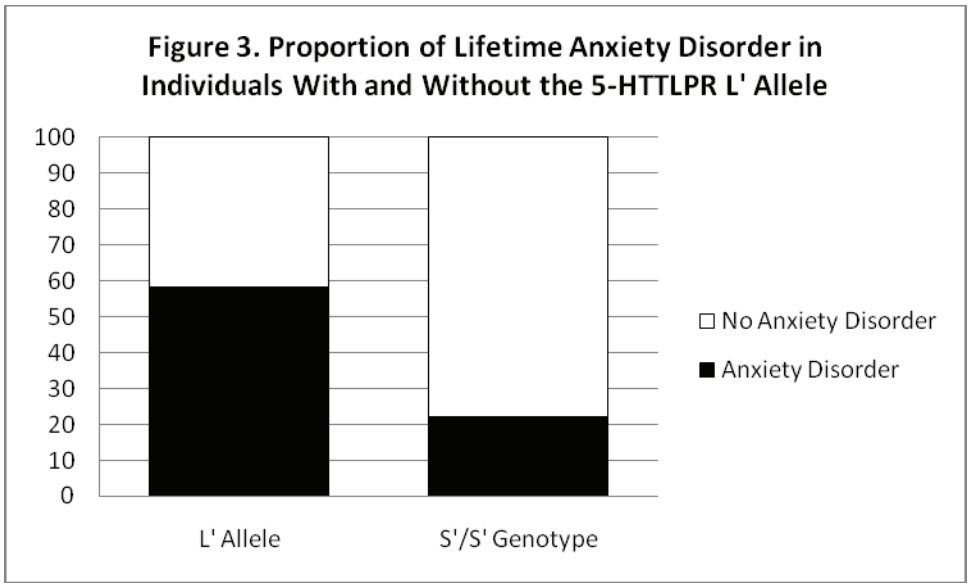

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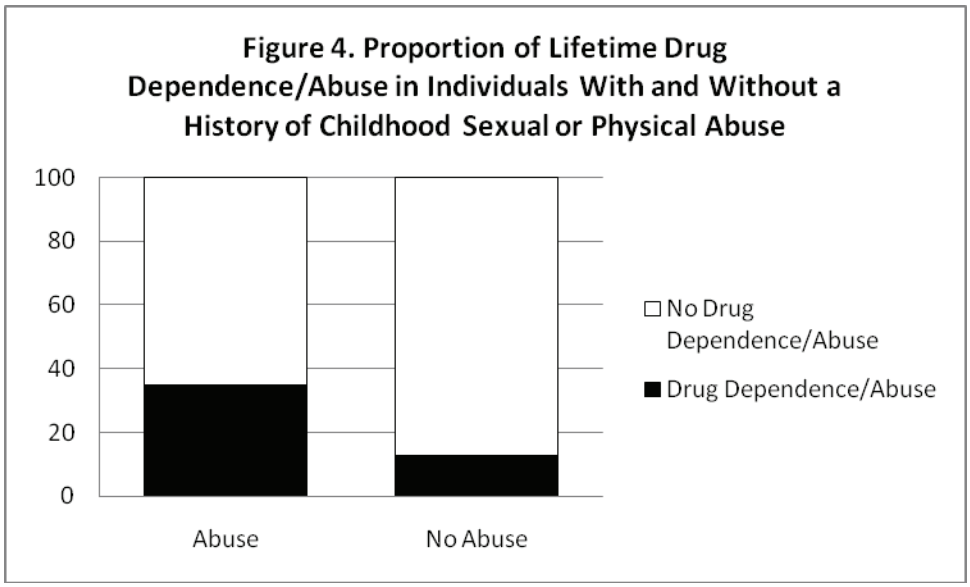


Proportions are actual (rather than estimated) proportions in the sample.

Transition to Manuscript 3

Study 1 and 2 in the current dissertation examined patterns of psychiatric comorbidity in individuals with bulimia-spectrum disorders and putative associations with environmental and genetic vulnerability factors. Findings showed that individuals with BN demonstrate significant comorbidity with other disorders and that heterogeneous subgroups exist based on severity of associated comorbidity. Moreover, different patterns of psychiatric comorbidity were shown to be associated with different genetic and environmental liabilities.

Much as comorbidity patterns are variable in BN, so is treatment outcome—and we have yet to discover what factors contribute to different outcomes among individuals undergoing similar treatments for BN. The existing literature suggests that psychiatric comorbidity may be a modulating factor in treatment outcome (e.g., Grilo, 2002; Steiger, Thibaudeau, Leung, Houle, and Ghadirian, 1994; Steiger & Stotland, 1995) however methodological differences across studies and failure to examine the full gamete of comorbidity makes it difficult to make definite conclusions. A second factor recently suggested to effect treatment outcome for BN are genetic factors, in particular the serotonin transporter promoter polymorphism (5-HTTLPR) (Monteleone et al., 2006; Steiger et al., 2008). Study 3 explores the effects of a range of Axis-I and Axis-II comorbidity and genetic factors on treatment outcome for BN and aims to tease apart to what extent such factors contribute to outcome for BN-specific symptoms versus more general psychopathological symptoms.

Manuscript 3: Influence of Psychiatric Comorbidity and Selected Serotonin-System Genes on Initial Treatment Response in Women with Bulimia-Spectrum Disorders

Abstract

Objective: Psychiatric comorbidity and genetic factors have both been shown to affect treatment response in individuals with Bulimia Nervosa (BN). We studied effects of Axis-I and Axis-II comorbidity and of selected genes affecting the serotonin (5-HT) system (a polymorphism in the promoter region of the 5-HT transporter gene, 5-HTTLPR, and a polymorphism in the promoter region of the tryptophan hydroxylase-2 TPH-2gene, G-703T) upon outcome in women undergoing specialized treatments for BN-spectrum disorders. *Method*: At the beginning of treatment, 103 women with BN-spectrum disorders participated in interviews assessing DSM-IV Axis-I and II disorders, provided blood samples for genotyping, and filled out questionnaires assessing eating and general psychopathological symptoms. The questionnaire evaluation was repeated after 4 months of treatment. Multilevel modeling was used to assess the effects on treatment outcome of genetic factors and of psychiatric comorbidity on Axes I and II. *Results*: Comorbid Major Depressive Disorder predicted higher depressive symptoms at baseline, but differences between depressed and non-depressed individuals tended to dissipate throughout treatment. Borderline Personality Disorder predicted higher impulsive symptoms, both at baseline and 4-months into treatment. In addition, Obsessive-Compulsive Personality Disorder (OCPD) predicted higher depressive symptoms at baseline and 4-months, as well as higher EAT-26 Bulimia symptoms at 4-months. Finally, 5-HTTLPR S'/ S' genotype predicted higher purge frequencies at baseline and 4-months. *Conclusion*: Results are consistent with previous findings suggesting that, in individuals with BN-

spectrum disorders, Axis-II disorders predict more severe general psychopathological symptoms that persist throughout treatment. Furthermore, our results suggest that the presence of comorbid OCPD may predict poorer response to treatment for bulimic symptoms. The present findings provide partial corroboration of previous results showing poorer treatment outcome for BN in individuals carrying 5-HTTLPR low-function (S') variants.

Introduction

Bulimia Nervosa (BN) is a severe eating disorder (ED), characterized by recurrent episodes of binge-eating and compensation (e.g., vomiting, laxative use, excessive exercise) and an intense preoccupation with body shape and weight. Although BN responds to psychotherapy, research on treatment outcome shows unsatisfactory outcomes in many patients. Based on a recent review of outcome studies in bulimic individuals, Steinhausen and Weber (2009) found that while close to 45% of patients show full recovery and 27% improve considerably, nearly 23% show no improvements at all. Similarly, a meta-analysis of psychotherapy trials for BN found that approximately 40% of patients recover completely and 60% maintain clinically significant BN symptoms post treatment (Thompson-Brenner, Glass, & Westen, 2003). For trials involving Cognitive Behavioural Therapy (CBT)—regarded as the "treatment-of-choice" for BN—the recovery rate for treatment completers is on average 48% (Wilson, Grilo, & Vitousek, 2007). Given the high percentage of patients who do not respond to treatment, researchers have tried to identify predictors of treatment outcome which, it is hoped, will point the way to more effective, individualized treatments.

Psychiatric comorbidity in BN. Due to high affinity with other psychiatric disorders, one factor that has been thought to predict poor treatment outcome for BN is psychiatric comorbidity. BN frequently co-occurs with comorbid mood, anxiety and substance-use disorders (Brewerton et al., 1995; Garfinkel et al., 1995; Godart et al., 2007; Hudson, Pope, & Yurgelon-Todd, 1988), as well as Cluster B (Borderline, Histrionic, Narcissistic or Antisocial) and Cluster C

(Avoidant, Dependent or Obsessive-Compulsive) personality disorders (Grilo, 2002; Rosenvinge, Martinussen, & Ostensen, 2000).

Axis-I comorbidity and outcome in BN. Studies examining the effect of mood disorders on outcome in individuals with BN have yielded inconsistent findings. In a study of 86 women with BN who completed a group treatment program for eating disorders, Maddocks and Kaplan (1991) found that increased depressive symptoms at baseline significantly differentiated poor responders from good responders. In addition, a study examining predictors of outcome one year after completing a randomized clinical trial for BN found that the presence of major depressive disorder increased the odds of poor outcome (Bulik, Sullivan, Joyce, Carter, & McIntosh, 1998). However, other studies have found no effect of depression on bulimia-treatment outcome (Fairburn, Kirk, O'Connor, Anastasiades, & Cooper, P., 1987; Keel, Mitchell, Miller, Davis, & Crow, 1999). Similar inconsistencies exist among findings on the effect of substance-use disorders for outcome in individuals with BN. One study examining 10-year outcome in BN associated a history of substance-use problems with poorer outcome (Keel et al., 1999). However, other studies found that a history of substance abuse had no implications for response to treatment (Mitchell, Pyle, Eckert, & Hatsukami, 1990; Strasser, Pike, & Walsh, 1992). Fewer studies have examined the effect of anxiety disorders on outcome in BN. Of the few outcome studies that have examined anxiety disorders one naturalistic study found that anxiety symptoms predicted bulimic symptoms 2.5 years later (Procopio, Holm-Denoma, Gordon, & Joiner, 2006), but most other studies showed no association

of anxiety with treatment outcome (Bulik et al., 1998; Keel et al., 1999; Thiel, Züger, Jacoby, & Schübler, 1998). Inconsistent associations between Axis-I comorbidity and outcome in BN suggest the need for further research in this area. Inconsistencies in previous research may be due to various factors, including differences in methods of assessment, criteria utilized for diagnosis, and recruitment methods across studies.

Axis-II comorbidity and outcome in BN. Most studies examining the effect of Axis-II comorbidity on outcome in BN have focused on Cluster B personality disorders and most often borderline personality disorder (BPD) or borderline phenomena. Two studies using the Borderline Syndrome Index (BSI) to evaluate symptoms in individuals with BN found that BSI scores were not predictive of treatment outcome (Davis, Olmsted, & Rockert, 1992; Garner et al., 1990). However, another study found that comorbid BPD symptoms (assessed using the BSI) predicted poorer treatment outcome at 1-year on both eating and general psychopathological symptoms (Johnson, Tobin, & Denis, 1990). Still another study, by Steiger, Thibaudeau, Leung, Houle, and Ghadirian (1994) found that bulimic patients with high BSI scores at baseline and 3-months responded less well to treatment (after 6 and 12 months) on both eating and psychopathological symptoms than patients who had low BSI scores or high scores at baseline only. Other studies, using structured interviews to assess BPD and Cluster B personality disorders have found results suggesting a weak association or no association with eating outcome. For example, Zeeck et al. (2007) found no differences between individuals with and without BPD in the

reduction of eating or general psychopathology over the course of treatment for BN. In a study examining 1-, 2- and 3-year outcome in a group of heterogeneous ED cases Norring (1993) found that, although patients showing a borderline organization showed poorer outcome on eating and psychopathological symptoms at 1-year follow-up, after 2 and 3 years they appeared to catch up to individuals without borderline features, and there were no differences in outcome between the groups. In another study, Steiger and Stotland (1995) found that, when compared to individuals without BPD, patients with comorbid BPD showed significantly poorer outcome (at 3-month and 1-year follow-up) on general psychiatric symptoms, but only marginally poorer response on eating symptoms. Other studies have examined the more general effect of personality disorders (PDs) on outcome in eating disorders. For example, Herzog, Keller, Lavori, Kenny, and Sacks (1992) reported that the presence of a personality disorder was associated with lower rates of remission from BN symptoms after 9 months. Similarly, a study examining Cluster B pathology in BN found that a high cluster B score (consisting of antisocial, borderline, histrionic and narcissistic features) predicted poor outcome (abstinence from purging) at 16 weeks and 1 year (Rossiter, Agras, Telch, & Schneider, 1992). In contrast, in a heterogeneous sample of individuals with EDs, Wonderlich, Fullerton, Swift, and Klein (1994) observed that subjects with comorbid PDs did not differ from those without PDs in outcome for eating symptoms after 4 or 5 years, however their psychopathological symptoms remained more severe. After a critical review of the literature examining the effect of personality disorders on treatment outcome for BN Grilo (2002)

concluded that personality disorders are more closely associated with the course of general psychopathological symptoms in EDs, than with the course of ED symptoms. In sum, studies examining Axis-II personality pathology as a predictor of outcome have produced inconsistent findings. Some research suggests that PDs show a stronger association with course of general psychiatric symptoms than with eating-specific ones. Few studies have examined personality pathology outside of cluster B disorders. With 44% of individuals meeting criteria for Cluster C pathology it is important that the effect of such disorders on treatment outcome be further studied.

Genes and comorbidity in BN. Due to associations with comorbidity and trait disturbances in BN, a second factor that has been thought to predict poor outcome for BN are genes. Relevant links with comorbidity have been found, in particular, with genes related to the serotonin (5-hydroxytryptamine; 5-HT) system. For example, the serotonin transporter promoter polymorphism (5-HTTLPR) has been linked to more severe disturbances in Axis-I comorbidity (Richardson et al., 2008) as well as personality and trait disturbances such as borderline personality disorder, affective instability, impulsivity, dissocial behaviour, harm avoidance and compulsivity in individuals with BN (Akkermann, Nordquist, Oreland, & Harro, 2010; Monteleone et al., 2006; Steiger et al., 2005; 2007; 2008; 2009). Although less well studied in BN, research in eating-disordered and non eating-disordered populations alike suggests that the G-703T (rs4570625) promoter polymorphism of the tryptophan hydroxylase-2 (TPH-2) gene may have implications for Axis-II comorbidity (Gutknecht et al., 2006) as

well as impulsive, affective and anxious traits (Brown et al., 2005; Canli, Congdon, Guktnecht, Constable, & Lesch, 2005; Groleau et al., unpublished findings; Hermann et al., 2007; Reuter, Kuepper, & Henning, 2007; Reuter, Ott, Vaitl, & Henning, 2007; Stoltenberg et al., 2006) in individuals with BN.

5-HTTLPR and outcome in BN. 5-HTTLPR is a 44-base pair insertion deletion polymorphism in the 5′ flanking regulatory region of the serotonin transporter gene, originally thought to have a "long" (L) and a "short" (S) variant, which differentially modulate transcriptional activity (Lesch et al., 1996). The S allele of 5-HTTLPR has been associated with reduced transcription of 5-HT transporter protein relative to the L allele (Heils et al., 1996; Lesch et al., 1996). Recent findings, however, suggest the existence of a low-frequency L-allele variant, L_G (an L allele with A→G SNP in its sequence), whose functioning may be comparable to that of the low-function, S allele (Hu et al., 2006; Zalsman et al., 2006). Such data imply that 5-HTTLPR may be triallelic, with S and L_G alleles regarded as "low-function" variants (S') and L_A regarded as a "high-function" allele (L'). Associations between 5-HTTLPR low function alleles and poor response to treatment have been demonstrated in individuals undergoing treatment for major depressive disorder (MDD) (Bocchio-Chiavetto et al., 2008; Lee, Lee, Lee, & Ryu, 2004; Smits et al., 2008; Yu, Tsai, Chen, Lin, & Hong, 2002) and confirmed by a meta-analysis of studies testing associations of 5-HTTLPR with treatment response for MDD (Serretti, Kato, De Ronchi, & Kinoshita, 2007). 5-HTTLPR low-function variants have also been associated with poor response to selective serotonin reuptake inhibitors (SSRIs) in patients

with generalized social anxiety disorder (Stein, Seedat, & Gelernter, 2006) and PDs (Silva et al., 2010).

Although one study in an atypical "low comorbidity" inpatient sample of individuals with BN found no association between 5-HTTLPR and response of BN symptoms to pharmacologic treatment (Erzegovesi et al., 2004), other studies have linked 5-HTTLPR to treatment response in BN. For example, in a study of patients with BN undergoing a 12-week treatment with SSRIs plus nutritional counselling, Monteleone et al. (2005) found a poorer response of bulimic symptoms to treatment in individuals carrying 5-HTTLPR low function variants. Similarly, in a sample of 98 individuals with BN-spectrum disorders undergoing psychotherapy at a specialized ED program Steiger et al. (2008) found an association between 5-HTTLPR low-function alleles and poorer treatment responses on binge eating, anxiety and depression. Results appear to indicate that serotonin-mediated genetic factors affect general response to psychotherapy in individuals with BN, regardless of type of therapy.

TPH-2 and outcome in BN. The TPH gene encodes the rate-limiting biosynthetic enzyme in the serotonin pathway and regulates levels of 5-HT by converting tryptophan into 5-hydroxytryptophan, the direct precursor of 5-HT (Hennig, Reuter, Netter, Burk & Landt, 2005). Variations in the TPH gene have been posited to contribute to altered 5-HT neurotransmission. Several studies have examined the association between TPH and response to treatment. These studies, focusing mainly on the effect of the TPH 218A/C polymorphism on SSRI response in individuals with MDD, have produced inconsistent results. Some

studies have found an association of the A allele with poorer response to SSRI antidepressants (Ham et al., 2007; Serretti, Zanardini, Cussin, et al., 2001; Serretti, Zanardini, Rossini, et al., 2001), and one study found an association of the C/C genotype with poorer response to SSRI and electro-convulsive therapy (ECT) treatment (Viikki et al., 2010). However, other studies have found no significant association between the TPH 218A/C polymorphism and antidepressant response (Ham et al., 2005; Peters, Slager, McGrath, Knowles, & Hamilton, 2004; Peters et al., 2009; Yoshida et al., 2002).

The relevance of studies examining TPH have recently been called into question by findings suggesting that TPH appears to be found exclusively in peripheral tissues and in the pineal body (McKinney, Knappskog, & Haavik, 2005). In 2003, Walther and colleagues identified a second TPH isoform, designated as TPH-2, highly similar to the above-mentioned TPH gene (exhibiting 71% of amino acid identity), but expressed predominantly in the brain stem. Few studies have examined the association of TPH-2 with response to treatment. One study, examining the TPH-2 rs1386494 polymorphism, found no association of TPH-2 with response to SSRI treatment in individuals with MDD (Illi et al., 2009). A second study, examining 5 TPH-2 polymorphisms, found TPH-2 rs2171363 homozygote (T/T and C/C) genotypes to be associated with poorer response to SSRIs in MDD patients (Tsai et al., 2009). To our knowledge no studies have examined the association of the TPH-2 G-703T polymorphism with treatment response and no studies have examined the association of TPH-2 with response to treatment in individuals with BN.

The present study. The present study had two major aims. The first was to evaluate the effect upon response to a multimodal, 16-week treatment of comorbid Axis-I and II disorders, identified at intake through structured clinical interviews, in individuals with BN-spectrum disorders. The second aim was to examine the effect of specific gene polymorphisms involved in the 5-HT system (i.e., 5-HTTLPR and TPH-2 G-703T) on response to treatment in the same group of individuals. Based on previous literature, we did not expect to find a strong effect of Axis-I comorbidity on treatment outcome. Research findings demonstrating a stronger association of PDs with outcome of general psychopathological symptoms than eating specific ones led us to expect an effect of Axis-II comorbidity on outcome for psychopathological, but not eating symptom measures. With respect to genes, we anticipated finding an effect of 5-HTTLPR on response to treatment, with low-function variants predicting poorer response on both eating and general psychopathological symptoms. Given its association with comorbid personality traits, we expected that we might find an effect of TPH-2 on response to treatment, in particular with respect to course of general psychopathological symptoms.

Method

Procedure

Women with BN-spectrum disorders (BSDs) were recruited to our study between May 2004 and January 2009 through consecutive consenting admissions to a specialized Eating Disorders program in Montreal, Quebec, Canada. All eligible participants who gave informed consent were contacted by a research

assistant at the beginning of therapy to inquire as to whether or not they would like to participate in structured clinical interviews, fill out a battery of questionnaires and partake in a blood draw. After 4-months of treatment participants were once again contacted and asked to complete questionnaires.

Participants

Participants included 103 women[1], 70 (68.0%) meeting DSM-IV criteria for BN-Purging (BN-P) subtype, 6 (5.8%) for BN-Nonpurging (BN-NP) subtype, and 27 (26.2%) for a bulimia-spectrum Eating Disorder Not Otherwise Specified (EDNOS)-BN. EDNOS-BN was defined as subjects who binged or purged at least once per month over the past 3 months, but at less than the requisite average of twice weekly. We felt our sample to be typical of treatment seeking women with BSDs and note previous findings demonstrating that BN and EDNOS-BN variants are equivalent on many clinical dimensions (Fairburn et al., 2007).

Subjects were between the ages of 18 and 49 (mean = 25.87 $\pm$ 6.56) and had a Body Mass Index (BMI: Kg/m^2) between 17.5 and 39 (mean = 22.82 $\pm$ 4.73). The Quebec population (from which this sample was drawn) includes a large proportion of people from Western Europe. Consequently, our sample included mainly subjects of Caucasian descent (98 individuals, or 95.1% of the sample). Of the 98 Caucasian individuals 82 (84.7%) were of West-European descent, 5 (5.1%) of East-European descent, 2 (2.0%) of South-European descent, 6 (6.1%) of Middle-East descent, 1 (1.0%) of Latin-American descent, and 1 (1.0%) of South-Asian descent. The non-Caucasian participants (5 individuals, or 4.9% of the sample) included 1 individual of mixed West-European Caucasian/

Native American (aboriginal) descent, 1 of mixed West-European Caucasian/ Asian descent, 1 of African Black descent, and 2 of Asian descent.

Measures

Structured Clinical Interviews (administered at Baseline only).

Eating Disorder Diagnosis. The Eating Disorders Examination (EDE: Fairburn & Cooper, 1993), a 62-item semi-structured clinical interview, was utilized to assess the presence/absence of a DSM-IV eating disorder diagnosis at baseline. The EDE has good inter-rater reliability, internal consistency (with alpha coefficients ranging from .68-.90) and good discriminant validity for distinguishing between women with and without eating disorders (Fairburn & Cooper, 1993). To complement our assessment, we computed BMI (Kg/m^2). For 5 participants missing the EDE interview at baseline, ED diagnosis was derived from their initial assessment with a psychiatrist at the Eating Disorders Program and was verified with questionnaire results from the Eating Disorders Examination Questionnaire (EDE-Q: Fairburn & Beglin, 1994). The EDE-Q indices reportedly correspond well with those obtained using the EDE (Mond, Hay, Rodgers, & Owen, 2006).

DSM-IV Axis-I Disorder Diagnosis. Current (past month) DSM-IV Axis-I disorders (MDD, Drug Abuse/Dependence, Alcohol Abuse/Dependence and Anxiety Disorders) were assessed at baseline using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I: First, Spitzer, Gibbon, & Williams, 1996). Since prevalence of any single anxiety disorder was too low to warrant viable statistical treatment, a composite Anxiety Disorder (AD) variable

(including social phobia, agoraphobia, panic disorder, generalized anxiety disorder, obsessive compulsive disorder, specific phobia and post-traumatic stress disorder) was created to detect the presence of any AD versus no AD. Inter-rater reliability estimates calculated for a sample of SCID-I interviews in a partially overlapping sample from a recently published study (Richardson et al., 2008) revealed the following inter-rater reliability estimates: MDD (κ = .80, n=23), AD (excluding post-traumatic stress disorder) (κ = 1.00, n=23), alcohol use disorder (including abuse and dependence) (κ = .83, n=23), and drug use disorder (including abuse and dependence) (κ = .86, n=23). For the majority of ED participants in the current study post-traumatic stress disorder (PTSD) was assessed using the Clinician-Administered Post-Traumatic Stress Disorder Scale (CAPS: Blake et al., 1995) (the remaining n=7 participants were assessed using the SCID-I). The CAPS is a standard criterion measure of PTSD diagnostic status and symptom severity, exhibiting excellent convergent and discriminant validity, and reliability (Weathers, Keane, & Davidson, 2001). The CAPS was utilized to assess PTSD (in most cases) instead of the SCID-I for the reason that it assesses symptoms in greater detail than the SCID-I—establishing frequency, intensity and duration information for each PTSD symptom.

DSM-IV Axis-II Disorder Diagnosis. DSM-IV Axis-II disorders were assessed at baseline using the Structured Clinical Interview for DSM-IV Axis II disorders (SCID-II: First, Gibbon, Spitzer, Williams, & Benjamin, 2000). Inter-rater reliability estimates calculated for a pseudo-randomly selected sample of SCID-II interviews drawn from the current sample revealed the following inter-rater

reliability estimates for Axis-II disorders of interest (i.e., those disorders for which there were at least 20% of individuals with a positive diagnosis): Avoidant PD (κ = .92, n=34), Obsessive-Compulsive PD (κ = .85, n=34), and BPD (κ = .78, n=32).

Questionnaire Measures (administered at Baseline and 4-Months).

Eating Symptoms. The Eating Disorders Examination Questionnaire (EDE-Q: Fairburn & Beglin, 1994), a 38-item self-report questionnaire (derived from the EDE), was used to assess presence and severity of the criterion eating disorder symptoms, binge eating, vomiting and purging. The EDE-Q has been demonstrated to have good internal consistency, test-retest reliability and stability over time (Luce & Crowther, 1999; Mond et al., 2004). The Eating Attitudes Test-26 (EAT-26: Garner, Olmstead, Bohr, & Garfinkel, 1982), a 26-item self-report questionnaire, was utilized to assess symptoms and concerns characteristic of eating disorders. The EAT-26 has been shown to have high internal consistency (.90) and a cut-off score of 20 reliably identifies clinical-range eating disturbances (Garner et al., 1982). The EAT-26 yields a Total score and three subscale scores (Dieting, Bulimia and Food Preoccupation and Oral Control). The Total Score and Dieting and Bulimia and Food Preoccupation subscales were utilized in the present study. The Oral Control subscale, a symptom measure more characteristic of Anorexia-spectrum eating disorders, showed little variance in this sample and thus was omitted from analyses. Internal consistency of scales ranges from .83-.90 (Garner et al., 1982).

General Psychopathological Symptoms. The Centre for Epidemiological studies for Depression (CES-D: Weissman, Sholomskas, Pottenger, Prusoff, & Locke, 1977) Scale was used to assess severity of Depressive symptoms. The CES-D Scale is a 20-item self-report scale designed to measure depressive symptomatology in the general population. It has high internal consistency and good construct validity, supported by correlations with other self-report measures of depression and by discrimination of clinical from non-clinical groups (Radloff, 1991). The Barrat Impulsivity Scale (BIS, version 11: Patton, Standford, & Barrat, 1995), a 30-item self-report questionnaire, was administered to assess Impulsivity. The BIS has been shown to have good internal consistency and to reliably discriminate between high and low impulsive groups (Patton et al., 1995).

Genotyping. DNA samples, obtained from whole blood, were amplified by polymerase chain reaction (PCR) in a total volume of 20 µl, which contained 100ng of genomic DNA, 200 µM of dNTPs, 10 pmol (or 5pmol for TPH-2) each of the forward and reverse primer, 1 U of Taq (or 0.5 U of Taq for TPH-2) DNA Polymerase (Qiagen, Alameda, CA), 1 x PCR buffer, and 1 x Q solution (Qiagen). Ten pmol each of the forward primer (5'-ATG CCA GCA CCT AAC CCC TAA TGT-3') and reverse primer (5'-GG ACC GCA AGG TGG GCG GGA-3') were used to amplify a region encompassing 5-HTTLPR. The L_G and L_A alleles were subsequently studied by enzymatic digestion of 7 µl of the above mentioned PCR product using 5 U of MspI and incubating at 37 ^{o} C for a minimum of 3 hours. Five pmol each of the forward primer (5' TGC ATA GAG GCA TCA CAG GA 3') and reverse primer (5' TCT TAT CCC TCC CAT CAG CA 3') were used to

amplify the DNA segment containing the single nucleotide polymorphism (SNP) TPH-2 G-703T (rs4570625). The PCR protocol involved preheating the samples at 94^{o} C for 5 min, followed by 35 cycles of denaturation at 94^{o} C (30 sec), annealing at 64^{o} C (30 sec), and extension at 72^{o} C (45 sec), as well as a final hold of 5 min at 72^{o} C. PCR product amplification was verified by running 5ul of the PCR product on a 2% agarose gel. The remaining product was then processed as per the ABI SNaPshot protocol, using primers designed for fluorescent dideoxy nucleotide termination. SNP analysis was carried out on the ABI 3100 genetic analyzer. Genotypes were determined automatically using the Genemapper software (Applied Biosystems) and consequently verified manually.

Treatment

Treatment was administered through a large-scale, specialized eating disorders program for adults. Psychotherapy was guided mainly by cognitive-behavioural principles with demonstrated efficacy in the treatment of BN and EDNOS-BN (Hay, Bacaltchuk, & Stefano, 2004). Treatments were offered in 4-month segments, with some patients invited (if indicated) to continue for a second 4-months. The current study assessed treatment outcome over the first 4-month segment of therapy. Of the 103 participants recruited from the clinic for the study (most, but not all, patients in the clinic agreed to participate in the study) 92 completed a 4-month span of treatment, 10 did not pursue the treatments offered and 1 was hospitalized. Of the 92 completers of a 4-month span of treatment, 81 provided assessment data both at the beginning of treatment and after 4 months.

All participants received individual therapy (mean ± SD = 12.46 ± 5.82, range = 2 to 29 sessions); 64 (79.0%) participated in weekly 1 ½ hour groups (mean ± SD = 10.16 ± 5.26, range = 1 to 19 sessions); 31 (38.3%) participated in 6- to 10-hour day treatments, 4 days per week (mean ± SD = 31.13 ± 20.52, range = 1 to 65 sessions); and 59 (72.8%) were on psychoactive medication either at onset or at some point throughout their treatment. Statistical procedures were used to control for effects of different types and amounts of therapy as well as medication use (see statistical analysis section).

Statistical Analyses

The principal analyses in the current study examined the influence of 1) psychiatric comorbidity (Axis-I and Axis-II disorders), and 2) genetic polymorphisms (5-HTTLPR and TPH-2 G-703T) upon response to treatment for BSDs. We used multilevel modeling analysis, a generalization of the general linear model used in multiple regression, which allows for the specification of random and fixed effects and handles missing data without listwise deletion (Raudenbusch & Bryk, 2002). Analyses were performed using HLM 6.04 software (Scientific Software International, Chicago, Ill., available at www.ssicentral.com). Repeated outcome measures assessing eating (EAT-26, binge days/month, vomit days/month, purge days/month), depressive (CES-D) and impulsive (BIS-11) symptoms (level-1 variables) were conceptualized as being nested within participants (level-2) and effects for each outcome measure were modeled across time. Time was modeled by creating a dummy variable

(level-1) with 0 indicating the beginning of treatment and 1 representing reports measured after the first 4 months of treatment.

Possible confounding effects of psychoactive medications were controlled using a level-1 variable that coded medication use as a dichotomous (present/absent) time-varying factor. Possible confounding effects of amount and type of psychotherapy were controlled in the following manner: Due to non-normal distributions of variables coding number of individual, group and day treatment sessions, variables were transformed into categorical indicators, creating 5 level-1, time-varying dummy variables: 1) a dummy variable contrasting people attending day treatments (6-hour day program or 10-hour day hospital) to those who did not, 2) 2 dummy variables contrasting people in the highest or middle tertile of "number of individual sessions" to those in the lowest tertile, and 3) 2 dummy variables contrasting people in the highest or middle tertile of "number of group sessions" to those in the lowest tertile. This approach has been used in a preceding paper examining treatment outcome in our lab (see Steiger et al., 2008).

Due to non-normal distributions outcomes for bingeing, vomiting and purging called for ordinal variables with 3 categories, created using tertiles coding "high", "medium" and "low" binge, vomit or purge days/month. Other outcomes called for continuous variables.

A first set of analyses, including no level-2 variables, examined the effect of treatment on all outcome measures. Analyses were performed using the model:

Model 1

Level-1 Model

$$Y_{ij} = \beta_{0j} + \beta_{1j}*(TIME2\_D) + e_{ij}$$

Level-2 Model

$$\beta_{0j} = \gamma_{00} + \upsilon_{0j}$$

$$\beta_{1j} = \gamma_{10}$$

A significant coefficient for the parameter γ_{10} reflected a significant change in symptoms at 4 months.

A second set of analyses included level-2 variables examining the effects of psychiatric comorbidity or genes on treatment outcome measures. Effects of psychiatric comorbidity were assessed by adding to both the intercept and the time dummy variable a level-2 variable that differentiated people with and without Axis-I or Axis-II psychiatric comorbidity within the past month. Similarly, effects of 5-HTTLPR and TPH-2 G-703T gene polymorphisms were assessed by adding to both the intercept and the time dummy variable a level-2 variable that differentiated people with and without a target genotype or allele. An example of the model used in the second set of analyses is as follows:

<u>Model 2</u>

Level-1 Model

$$Y_{ij} = \beta_{0j} + \beta_{1j}*(TIME2\_D) + e_{ij}$$

Level-2 Model

$$\beta_{0j} = \gamma_{00} + \gamma_{01}*(\text{Major Depressive Disorder } \textit{or} \text{ low function allele of 5-HTTLPR}) + \upsilon_{0j}$$

$\beta_{1j} = \gamma_{10} + \gamma_{11}$*(Major Depressive Disorder *or* low function allele of 5-HTTLPR)

Significant coefficients for the parameters γ_{01} and γ_{11} reflected effects of psychiatric comorbidity or genes at baseline or 4 months, respectively.

Axis-I and Axis-II disorders were dummy coded as "1" for presence of a disorder and "0" for no disorder. Comorbid disorders of interest included those disorders for which there were at least 20% of individuals with a positive current diagnosis. Disorders that had at least a 20% prevalence rate included MDD, AD, Avoidant PD (AVPD), Obsessive-Compulsive PD (OCPD), and BPD (see Table 1).

Effects of 5-HTTLPR genotype on treatment outcome were assessed by creating 2 dummy variables—one that represented the S'/S' genotype and another that represented the L'/ L' genotype, with the S'/ L' genotype serving as the reference category. Due to previous research suggesting that implications for treatment outcome might lie at the level of the allele (rather than the genotype) potential effects of 5-HTTLPR S' and L' alleles on treatment outcome were also examined. 5-HTTLPR alleles were modeled by creating dummy variables contrasting: 1) S' allele (S'/S' and S'/L') carriers to individuals with no S' allele (L'/ L'), and 2) L' allele (L'/L' and S'/L') carriers to individuals with no L' allele (S'/ S'). Effects of 5-HTTLPR S' and L' alleles were examined in separate multilevel modeling analyses. For TPH-2 G-703T, analyses examining the effect of genotype could not be carried out due to too few T/T carriers (n=1). Based on previously reported functional evidence for the T allele (Brown et al., 2005;

Canli et al., 2005; Hermann et al., 2007) and relatively low frequency of the T/T genotype (n=2) in the present sample we dichotomized the TPH-2 G-703T genotype by presence (T/T and G/T) versus absence (G/G) of the T variant.

In our sample of N=81 patients with baseline and 4-month assessment data, N=2 were missing genotype information. In the resulting N=79 patients, frequencies (and percentages) of 5-HTTLPR S'/L', S'/S', and L'/L' genotypes, respectively occurring in 40 (50.6%), 18 (22.8%) and 21 (26.6%) of participants were in conformity with Hardy-Weinberg equilibrium ($\chi^2_{(1)} = 0.16$, n.s.). Frequencies (and percentages) of TPH-2 G-703T G/G, G/T and T/T genotypes occurred respectively in 50 (63.3%), 28 (35.4%) and 1 (1.3%) of participants and were also in conformity with Hardy-Weinberg equilibrium ($\chi^2_{(1)} = 1.82$, n.s.). Conformity with Hardy-Weinberg equilibrium indicated that, in our sample, both genotype and allele frequencies—for 5-HTTLPR and TPH-2—have remained constant from generation to generation (i.e., no disturbing influences have been introduced).

A third set of analyses was performed, similar to the second set (including level-2 variables coding either psychiatric comorbidity or genes), but with the addition of level-1 covariates controlling for the effects of medication and amount of psychotherapy. An example of Model 3 is as follows:

Model 3

Level-1 Model

$Y_{ij} = \beta_{0j} + \beta_{1j}*(TIME2\_D) + \beta_{2j}*(PSYCH) + \beta_{3j}*(DAYTR\_D) +$

$\beta_{4j}*(INDTH\_D2) + \beta_{5j}*(INDTH\_D1) + \beta_{6j}*(OUTGR\_D2) +$

$\beta_{7j}*(OUTGR\_D1) + e_{ij}$

Level-2 Model

$\beta_{0j} = \gamma_{00} + \gamma_{01}*$(Major Depressive Disorder *or* low function allele of 5-HTTLPR) + υ_{0j}

$\beta_{1j} = \gamma_{10} + \gamma_{11}*$(Major Depressive Disorder *or* low function allele of 5-HTTLPR)

$\beta_{2j} = \gamma_{20}$

$\beta_{3j} = \gamma_{30}$

$\beta_{4j} = \gamma_{40}$

$\beta_{5j} = \gamma_{50}$

$\beta_{6j} = \gamma_{60}$

$\beta_{7j} = \gamma_{70}$

Significant coefficients for the parameters γ_{01} and γ_{11} reflected effects of psychiatric comorbidity or genes at baseline or 4 months, respectively, while taking into account variations in psychiatric medication use and amount of treatment.

A final set of analyses compared the group of treatment completers (n=92) to treatment dropouts (n=10) on age, BMI, binge, vomit and purge days/month, CES-D depressive symptoms, BIS-11 impulsivity, EAT-26 Total, Diet and Bulimia scales as well as rates of psychiatric comorbidity, 5-HTTLPR genotype

and allele frequencies and TPH-2 G-703T T allele frequency at baseline assessment.

Results

General Psychopathological Symptoms

Depressive Symptoms. Table 2 shows results of multilevel modeling analyses carried out for the outcome measure assessing depressive symptoms (i.e., CES-D). The table includes coefficients and standard errors (SEs) for analyses carried out using multilevel models 1, 2 and 3 (as described earlier). Results of model 1 analyses (shown in column 1 of Table 2) revealed that patients' depressive symptoms improved over the 4 months of treatment. Results of analyses examining effects of psychiatric comorbidity (Axis-I and Axis-II) on treatment response using model 2 (without covariates; shown in column 2 of Table 2) and model 3 (with covariates; shown in column 3 of Table 2) indicated effects of both Axis-I and Axis-II comorbidity. With respect to Axis-I comorbidity, individuals with comorbid MDD (not unexpectedly) had significantly higher baseline depressive symptoms than did individuals with no comorbid MDD. However, there was a trend ($p=.110$ Model 2; $p=.055$ Model 3) for depressive symptoms to decrease more in individuals with MDD from baseline to 4-month follow-up, thereby lessening symptom differences between the two groups at 4-months (see row 1 of Table 2). Figure 1 illustrates estimated means for individuals with and without MDD at baseline and 4-months. With respect to Axis-II comorbidity, individuals with comorbid OCPD had significantly higher baseline depressive symptoms than individuals with no comorbid OCPD. Moreover, the rate at which

depressive symptoms decreased in individuals with and without OCPD did not differ significantly, indicating that individuals with OCPD continued to have significantly higher depressive symptoms than did those without OCPD at 4 month follow-up (see row 4 of Table 2). Figure 2 illustrates estimated means for individuals with and without OCPD at baseline and 4-months. Similarly, individuals with comorbid BPD had significantly higher baseline depressive symptoms than individuals with no comorbid BPD, however this effect was no longer significant when covariates reflecting psychoactive medication use and amount of therapy were added to the model (see row 5 of Table 2). There were no effects of 5-HTTLPR or TPH-2 G-703T gene polymorphisms on depressive symptoms at baseline or 4-months (see rows 6-9 of Table 2).

Impulsivity. Table 3 shows results of multilevel modeling analyses (models 1, 2 and 3) carried out for the outcome measure assessing impulsivity (i.e., BIS). Results of model 1 analyses (shown in column 1 of Table 3) revealed that patients' impulsive symptoms improved over the 4 months of treatment. Results of analyses examining effects of psychiatric comorbidity (Axis-I and Axis-II) on treatment outcome (shown in columns 2 & 3; rows 1-5 of Table 3) indicated some effects of comorbidity. Although similar at baseline, impulsive symptoms in individuals with comorbid MDD decreased less throughout treatment, however such results were no longer significant when applying covariates controlling for medication use and amount of therapy (see row 1 of Table 3). With respect to Axis-II comorbidity, individuals with comorbid BPD had significantly higher baseline impulsive symptoms than did individuals without comorbid BPD.

Moreover, the rate at which impulsive symptoms decreased in individuals with and without BPD did not differ significantly, indicating that individuals with BPD continued to have significantly higher impulsive symptoms than those without BPD at 4 month follow-up (see row 5 of Table 3). Figure 3 illustrates estimated means for individuals with and without BPD at baseline and 4-months. There were no effects of 5-HTTLPR or TPH-2 G-703T gene polymorphisms on impulsive symptoms at baseline or 4-months (see rows 6-9 of Table 3).

Eating symptoms

EAT-26 Total Score. Table 4 shows results of multilevel modeling analyses carried out on the EAT-26 Total score. Results of model 1 analyses (shown in column 1 of Table 4) revealed that patients' EAT-26 scores improved over the 4 months of treatment. Results of analyses examining effects of psychiatric comorbidity (Axis-I and Axis-II) and gene polymorphisms (5-HTTLPR and TPH-2 G-703T) on treatment outcome (shown in columns 2 & 3 of Table 4) indicated no effects of psychiatric comorbidity or genetic variables on EAT-26 symptoms at baseline or 4-months.

EAT-26 Diet Score. Table 5 shows results of multilevel modeling analyses carried out on the EAT-26 Diet score. Results of model 1 analyses (shown in column 1 of Table 5) revealed that patients' EAT-26 Diet scores improved over the 4 months of treatment. However, similar to results obtained with the EAT-26 Total score, there were no effects of psychiatric comorbidity or genetic variables on EAT-26 Diet symptoms at baseline or 4-months (see columns 2 & 3 of Table 5).

EAT-26 Bulimia and Food Preoccupation. Table 6 shows results of multilevel modeling analyses carried out for the outcome measure EAT-26 Bulimia and Food Preoccupation. Results of model 1 analyses (shown in column 1 of Table 6) revealed that patients' EAT-26 Bulimia symptoms improved over the 4 months of treatment. Results of analyses examining effects of psychiatric comorbidity on treatment outcome (shown in columns 2 & 3; rows 1-5 of Table 6) indicated effects of comorbid OCPD. Individuals with OCPD had similar baseline EAT-26 Bulimia symptoms as individuals without OCPD, but symptoms decreased less in these individuals over time, suggesting that, with respect to bulimic symptoms, they responded less to treatment over the 4-month period (see row 4 of Table 6). Figure 4 illustrates estimated means for individuals with and without OCPD at baseline and 4-months. There were no effects of 5-HTTLPR or TPH-2 G-703T gene polymorphisms on EAT-26 Bulimia symptoms at baseline or 4-months (see rows 6-9 of Table 6).

EDE-Q Binge Days/Month. Table 7 shows results of multilevel modeling analyses carried out on the ordinal variable binge days/month. Results of model 1 analyses (shown in column 1 of Table 7) revealed that patients' binge eating symptoms improved over the 4 months of treatment. Results of analyses examining effects of psychiatric comorbidity and gene polymorphisms on treatment outcome (shown in columns 2 & 3 of Table 7) indicated no significant effects of psychiatric comorbidity or genetic variables on binge eating at baseline or 4-months.

EDE-Q Vomit Days/Month. Table 8 shows results of multilevel modeling analyses carried out on the ordinal variable vomit days/month. Results of model 1 analyses (shown in column 1 of Table 8) revealed that patients' vomiting symptoms improved over the 4 months of treatment. Similar to results obtained with binge eating, we found no effects of psychiatric comorbidity or genetic variables on vomiting symptoms at baseline or 4-months (see columns 2 & 3 of Table 8).

EDE-Q Purge Days/Month. Table 9 shows results of multilevel modeling analyses carried out for the ordinal variable purge days/month. Results of model 1 analyses (shown in column 1 of Table 9) revealed that patients' purging symptoms improved over the 4 months of treatment. Results of analyses examining effects of psychiatric comorbidity on treatment outcome indicated no effects of Axis-I or Axis-II psychiatric comorbidity on purging symptoms at baseline or 4-months (see columns 2 & 3; rows 1-5 of Table 9). Results of analyses examining effects of genetic variables on treatment outcome, however, revealed significant effects of 5-HTTLPR genotype. Individuals with the 5-HTTLPR S'/ S' genotype were more likely than individuals with the S'/ L' genotype to be in the high or moderate purge days/month category (as opposed to the low purge days/month category) at baseline. Moreover, there was no difference in the rate at which symptoms improved from baseline to 4 months in individuals with the S'/ S' and S'/ L' genotypes, indicating that S'/ S' individuals remained more likely than S'/ L' individuals to be in the high or moderate purge categories at 4 months (see row 6 of Table 9). Figure 5 illustrates estimated

percentages of individuals with S'/ L', L'/ L' and S'/ S' genotypes found in high, moderate and low purge groups at baseline and 4 months. Hypothesis tests were run in order to contrast individuals with the S'/ S' genotype to both individuals with the S'/ L' and the L'/ L' genotypes. Tests revealed significant differences between S'/ S' and S'/ L' carriers on purging frequency (as noted above). However, there were no significant differences between the S'/L' and L'/L' carriers with respect to purging frequency at baseline or 4 months. In sum, findings show that individuals with the S'/ S' genotype of 5-HTTLPR purge at a significantly higher frequency than individuals with the S'/ L', but not L'/L' genotype, at baseline and remain more symptomatic after 4 months of treatment.

T-tests comparing treatment completers (N=92) to treatment dropouts (N=10) on baseline measures of age, BMI and mean monthly binge, vomit and purge days revealed no significant differences between the two groups. At baseline assessment the 92 treatment completers had a mean (SD) age of 25.93 (6.77), a mean BMI of 22.71 (4.73) and mean monthly binge, vomit and purge days (averaged over the 3 months preceding baseline assessment) of 14.72 (9.33), 15.29 (11.21) and 20.44 (13.57), respectively. The 10 treatment drop-outs had a mean (SD) age of 25.40 (4.93), a mean BMI of 24.21 (4.87) and mean monthly binge, vomit and purge days of 10.67 (10.89), 19.20 (22.83) and 23.77 (22.52), respectively. T-tests revealed no significant differences between treatment completers and treatment drop-outs on continuous outcome variables at baseline, including depressive symptoms, impulsivity and EAT-26 total, diet and bulimia symptom measures. The treatment completers had mean (SD) depressive

symptoms of 31.02 (11.68), mean impulsive symptoms of 71.00 (11.04) and mean EAT-26 total, diet and bulimia symptoms of 37.62 (15.67), 1.71 (0.72) and 1.88 (0.73), respectively. The treatment drop-outs had mean (SD) depressive symptoms of 24.10 (14.45), mean impulsive symptoms of 67.56 (10.09) and mean EAT-26 total, diet and bulimia symptoms of 40.71 (12.58), 1.92 (0.53) and 1.91 (0.69), respectively. Chi squared analyses revealed no significant differences between treatment completers and dropouts on baseline Axis-I and Axis-II disorders. The respective numbers and percentages of individuals with psychiatric comorbidity in completer and dropout groups were as follows: Axis-I: N=42 (53%) versus N=4 (57%)[2], Axis-II: N=55 (60%) versus N=7 (70.0%). A final set of chi squared analyses, in 90 of the 92 treatment completers (2 participants were missing genetic information) and all of the treatment dropouts, revealed no significant differences between treatment completers and dropouts with respect to 5-HTTLPR genotype or allele frequencies or TPH-2 G-703T T allele frequency. Distribution of 5-HTTLPR genotypes and alleles and TPH-2 G-703T T allele in treatment completers were as follows: 5-HTTLPR genotype: S'/ L', N= 50 (55.6%), S'/ S', N=18 (20.0%), L'/ L', N=22 (24.4%); 5-HTTLPR S' allele: N=68 (75.6%); 5-HTTLPR L' allele: N=72 (80.0%); TPH-2 G-703T T allele: N=34 (37.8%). Distribution of 5-HTTLPR genotypes and alleles and TPH-2 G-703T T allele in treatment dropouts were as follows: 5-HTTLPR genotype: S'/ L', N= 6 (60.0%), S'/ S', N=2 (20.0%), L'/ L', N=2 (20.0%); 5-HTTLPR S' allele: N=8 (80.0%); 5-HTTLPR L' allele: N=8 (80.0%); TPH-2 G-703T T allele: N=2 (20.0%).

Discussion

In this study, we examined the effects on treatment response in individuals with BN-spectrum disorders of comorbidity on Axes I and II and of selected gene polymorphisms (5-HTTLPR and TPH-2 G-703T). In general, findings suggested that Axis-I disorders had little impact on treatment outcome. On the other hand, Axis-II comorbidity had significant effects on end of treatment status, both with respect to general-psychopathological symptoms (depression and impulsivity) and (in the case of OCPD) upon outcome of bulimic eating symptoms. Effects owing to genetic variables were more circumscribed; however, we found homozygosity for 5-HTTLPR low-function variants to predict more frequent purging behaviour that persisted throughout treatment. The ensuing discussion will describe results in more detail and highlight the ways in which current findings contribute to the empirical literature.

Effects of Axis-I comorbidity on treatment outcome. Our findings suggest that Axis-I disorders have a minimal impact on treatment outcome for BSDs. Although presence of comorbid MDD was observed to predict higher baseline depressive symptoms—a finding that is expected given that depressed mood is the hallmark symptom of MDD (American Psychiatric Association (APA), 2000)—such differences did not persist throughout treatment. We observed a trend for depressive symptoms to improve more in women with MDD over the 4 months of therapy. A possible implication of the preceding may be that depression is a "state-dependent" symptom that resolves along with improvements in eating symptoms in individuals with BSDs.

Effects of Axis-II comorbidity on treatment outcome. In contrast to Axis-I disorders, Axis-II disorders were observed to be a significant predictor of end of treatment status, for both general psychopathological and eating symptoms. The presence of comorbid BPD was found to predict more severe impulsivity both at baseline and end of treatment; however no effects on BPD were observed on eating symptoms. Such findings are in line with previous studies showing that BPD is more closely associated with the course of general psychopathological symptoms in BN, than with the course of ED symptoms (Steiger & Stotland, 1995). On the other hand, the presence of comorbid OCPD was found to predict more depressive symptoms at baseline and end of treatment as well as poorer response to treatment on bulimic eating symptoms. Such findings suggest that OCPD is associated with the course of both general psychopathological symptoms and eating-specific ones. Although few studies have examined the effect of OCPD on outcome in BN, our findings linking OCPD to poorer outcome are in line with those found in other eating disorder populations. For example, in individuals with binge eating disorder (BED) presence of Cluster C personality disorders has been found to predict both post-treatment negative affect and eating disorder psychopathology (Masheb & Grilo, 2008). In addition, in individuals with anorexia nervosa studies consistently find that obsessive-compulsive personality symptoms (Crane, Roberts, & Treasure, 2007; Lilenfeld, Wonderlich, Riso, Crosby, & Mitchell, 2006; Steinhausen, 2002) and perfectionism in particular (Bardone-Cone et al., 2007) predict poorer prognosis. Such findings perhaps suggest that symptoms of OCPD such as perfectionism, high personal standards

or compulsivity may make it particularly difficult to let go of food obsessions and preoccupations, or compulsive behaviours such as binge eating or purging during treatment.

In sum, findings examining effects of Axis-II disorders on response to treatment for BN suggest that they are significant predictors of end of treatment status. In contrast to Axis-I disorders, which appear to have a limited impact on post-treatment symptoms, Axis-II disorders show stable and enduring effects on symptoms. A possible implication here is that Axis-II disorders are "trait-like" syndromes that are likely to persist despite improvements in bulimic symptoms. Furthermore, findings suggest that, despite having effects on impulsive symptoms, BPD had no impact on outcome for eating symptoms. In contrast, the present findings add to a growing body of literature showing that OCPD may have important implications for outcome of both general psychopathological and eating symptoms in individuals with EDs.

Effects of genetic variables on treatment outcome. With respect to genetic effects, we observed only one effect of 5-HTTLPR on treatment outcome. Women who carried the S'/S' genotype had more purge days per month than did individuals with the S'/L' genotype, both at baseline and at 4 months, demonstrating an effect of 5-HTTLPR genotype on severity of purging symptoms that persisted throughout treatment. Such findings are in line with results from two previous studies showing poorer response to treatment in individuals with BN carrying 5-HTTLPR low-function variants (Monteleone et al., 2005; Steiger et al., 2008). Taken together, findings suggest that 5-HTTLPR low-function alleles may

have implications for response of bulimic symptoms to treatment. How might 5-HTTLPR impact outcome of bulimic symptoms in individuals with BN? We propose that low-function variants might confer vulnerability to certain personality traits (e.g., impulsivity) that, in turn, affect bulimic symptomatology. In ED and non-ED populations, 5-HTTLPR low-function variants have been repeatedly linked to impulsive traits and disorders (e.g., BPD, Substance Use Disorders) (Lichtermann et al., 2000; Mannelli et al., 2005; Sander et al., 1997; 1998; Steiger et al., 2005; 2007). Interestingly, studies have also shown that ED subjects who purge more display more associated psychopathology of an impulsive nature. For example, severity of purging has been related to impulse-control problems like substance abuse (Wiederman & Pryor, 1996), self-injury and suicide attempts (Favaro & Santonastaso, 1996), and borderline personality features (Tobin, Johnson, & Dennis, 1992) in individuals with eating disorders. A speculation from the above findings is that 5-HTTLPR may enhance purging by increasing the expression of psychopathological traits (like impulsivity) that may make individuals more susceptible to engage in purging behaviours.

Limitations. We would like to discuss some of the limitations of the current study. Most importantly, the naturalistic outcome design utilized in the current study creates the risk that effects owing to psychiatric comorbidity or genes may have been confounded by uncontrolled treatment variations. In order to control for such effects, statistical measures were applied to control for variations in amount of therapy and psychoactive medication use. Such statistics allow us to be more confident that comorbidity or genetic effects obtained were not

attributable to confounds owing to treatment factors. Similarly, there might be concern that attrition at 4-months may have affected outcome findings, in particular if attrition had been significantly associated with a specific form of psychiatric comorbidity or a specific genotype. Providing some reassurance that this was not the case, analyses comparing treatment completers to dropouts indicated no significant differences with respect to either psychiatric comorbidity or genetic variations. However, admittedly, power in these analyses was very limited.

Clinical Implications. In linking specific psychiatric comorbidity patterns and hereditary factors associated with the 5-HT system to treatment outcome for BN, our findings point to several potentially important clinical implications. Contrary to clinical speculations, results suggest that, for the most part, eating symptoms in individuals with psychiatric comorbidity (except OCPD) respond as well to treatment as those in individuals without comorbidity. Such findings suggest that, regardless of comorbidity, for many individuals traditional therapeutic techniques focused on eating symptoms may be sufficient for successful treatment of BN. However, a sizeable subgroup of individuals does not respond to traditional therapies. The present findings point to comorbid OCPD as one potential indicator of poor outcome for eating symptoms in individuals with BSDs. Clinicians should be sensitized to the fact OCPD patients may be in need of more enhanced treatments, possibly implicating strategies to address perfectionistic or obsessive/compulsive thoughts and behaviours that may be maintaining eating symptoms. In addition, results from the current study add to a

growing body of literature suggesting that 5-HTTLPR low-function variants may predict poorer outcome for BN. We propose that recovery from BSDs may be more challenging in individuals with 5-HTTLPR low-function alleles due to inherited problems of serotonin (5-HT) neurotransmission. Serotonin dysregulation may make it difficult to regulate problems of eating, mood and impulse control, all important components of successful treatment outcome. In such individuals, pharmacological support aimed at stabilizing 5-HT system functioning may be an important therapeutic adjunct.

Footnotes

[1] Fifty-four (52.4%) of the participants in the current study were part of the sample in Steiger et al.'s (2008) study. Differences in samples are due to increased sample size over time and differences in study protocols (e.g., structured clinical interviews were required at time 1 in the current study, but not in the previous study).

[2] Axis-I disorders included MDD, ADs, drug abuse/dependence and alcohol abuse/dependence. Axis-I comorbidity information was missing for: N=13 treatment completers and N=3 treatment dropouts.

Table 1. Frequency of comorbid Axis-I and Axis-II diagnoses at baseline in individuals with bulimia-spectrum disorders. Differences in ns reflect isolated missing values.

Axis-I Diagnosis	**n**	**n positive diagnosis**	**% positive diagnosis**
Major Depressive Disorder[a]	78	17	21.0
Anxiety Disorder[a]	72	23	28.4
Alcohol Abuse/Dependence	76	5	6.2
Drug Abuse/Dependence	79	1	1.2
Axis-II Diagnosis	**n**	**n positive diagnosis**	**% positive diagnosis**
Dependent Personality Disorder	81	5	6.2
Avoidant Personality Disorder[a]	81	19	23.5
Obsessive Compulsive Personality Disorder[a]	81	20	24.7
Borderline Personality Disorder[a]	81	24	29.6
Histrionic Personality Disorder	81	4	4.9
Narcissistic Personality Disorder	81	4	4.9
Antisocial Personality Disorder	81	2	2.5
Paranoid Personality Disorder	81	3	3.7
Schizotypal Personality Disorder	81	0	0.0
Schizoid Personality Disorder	81	2	2.5

[a] Only Axis-I and Axis-II diagnoses with at least a 20% prevalence rate were included in analyses.

Table 2. Results of multilevel modeling analyses examining: 1) Response to treatment for Depressive symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for Depressive symptoms, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

Depressive Symptoms	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=79	N=76	N=76
1) Intercept	30.72 (1.46)***	27.27 (1.56)***	24.46 (2.42)***
Major Depressive Disorder		14.23 (3.29)***	13.45 (3.22)***
Time	-6.35 (1.49)***	-4.94 (1.63)**	-5.50 (1.99)**
Major Depressive Disorder		-5.33 (3.32)	-6.15 (3.18)
		N=71	N=71
2) Intercept		30.71 (1.89)***	25.20 (2.76)***
Anxiety Disorder		1.31 (3.31)	-0.01 (3.17)
Time		-8.12 (1.74)***	-10.19 (2.23)***
Anxiety Disorder		3.32 (3.04)	4.16 (2.94)
Axis-II Comorbidity			
		N=79	N=79
3) Intercept		30.26 (1.68)***	26.76 (2.70)***
Avoidant PD		1.92 (3.41)	0.88 (3.36)
Time		-6.62 (1.73)***	-8.08 (2.19)**
Avoidant PD		1.07 (3.48)	2.05 (3.57)
4) Intercept		28.57 (1.60)***	25.91 (2.51)***
Obsessive-Compulsive PD		8.41 (3.16)*	7.73 (3.21)*
Time		-7.09 (1.75)***	-8.03 (2.18)**
Obsessive-Compulsive PD		2.79 (3.42)	2.34 (3.51)
5) Intercept		28.68 (1.67)***	25.57 (2.61)***
Borderline PD		6.76 (3.06)*	5.72 (3.01)
Time		-7.42 (1.76)***	-8.90 (2.27)***
Borderline PD		3.53 (3.25)	3.94 (3.35)
Genes			
		N=77	N=77
6) Intercept		31.13 (2.08)***	27.83 (3.02)***
5-HTTLPR S'/S' Genotype		-3.91 (3.68)	-2.69 (3.64)
5-HTTLPR L'/L' Genotype		2.73 (3.56)	2.13 (3.52)
Time		-7.80 (2.11)**	-8.94 (2.65)**
5-HTTLPR S'/S' Genotype		2.43 (3.75)	2.18 (3.81)
5-HTTLPR L'/L' Genotype		3.93 (3.72)	3.63 (3.79)
7) Intercept		33.85 (2.87)***	29.81 (3.74)***
5-HTTLPR S' Allele		-3.98 (3.35)	-2.94 (3.33)
Time		-3.87 (3.06)	-5.30 (3.45)
5-HTTLPR S' Allele		-3.15 (3.51)	-2.98 (3.61)

8) Intercept		27.22 (3.05)***	24.96 (3.52)***
5-HTTLPR L' Allele		4.83 (3.49)	3.40 (3.47)
Time		-5.37 (3.12)	-6.77 (3.43)
5-HTTLPR L' Allele		-1.25 (3.58)	-1.18 (3.65)
9) Intercept		31.34 (1.88)***	27.16 (2.90)***
TPH-2 T Allele		-1.16 (3.10)	0.13 (3.12)
Time		-6.70 (1.91)**	-7.85 (2.42)**
TPH-2 T Allele		1.01 (3.17)	0.06 (3.30)

*p < .05, **p < .01, ***p < .001

Inconsistent ns across models reflect isolated missing values.

Table 3. Results of multilevel modeling analyses examining: 1) Response to treatment for Impulsive symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for Impulsive symptoms, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

Impulsivity	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=78	N=76	N=76
1) Intercept	70.99 (1.28)***	71.09 (1.48)***	72.59 (2.11)***
Major Depressive Disorder		-0.72 (3.12)	-0.65 (3.09)
Time	-2.05 (0.96)*	-2.52 (0.98)*	-2.68 (1.21)*
Major Depressive Disorder		3.96 (1.95)*	2.73 (1.94)
		N=71	N=71
2) Intercept		71.04 (1.66)***	71.93 (2.32)***
Anxiety Disorder		-0.00 (2.92)	0.92 (2.90)
Time		-2.54 (1.05)**	-3.31 (1.34)*
Anxiety Disorder		2.63 (1.82)	1.75 (1.78)
Axis-II Comorbidity			
		N=78	N=78
3) Intercept		71.90 (1.46)***	74.42 (2.14)***
Avoidant PD		-3.77 (2.95)	-2.61 (2.92)
Time		-1.54 (1.10)	-2.14 (1.26)
Avoidant PD		-2.13 (2.22)	-2.46 (2.05)
4) Intercept		71.84 (1.48)***	74.09 (2.10)***
Obsessive-Compulsive PD		-3.39 (2.95)	-0.72 (2.96)
Time		-2.30 (1.10)*	-2.58 (1.28)*
Obsessive-Compulsive PD		1.03 (2.27)	-0.26 (2.10)
5) Intercept		68.94 (1.46)***	72.11 (2.09)***
Borderline PD		6.95 (2.68)*	7.18 (2.62)**
Time		-2.69 (1.13)*	-2.71 (1.32)*
Borderline PD		2.21 (2.09)	0.80 (1.98)
Genes			
		N=77	N=77
6) Intercept		70.13 (1.84)***	73.06 (2.46)***
5-HTTLPR S'/S' Genotype		3.13 (3.28)	1.93 (3.20)
5-HTTLPR L'/L' Genotype		-0.49 (3.14)	-0.33 (3.07)
Time		-1.97 (1.33)	-2.34 (1.53)
5-HTTLPR S'/S' Genotype		-2.55 (2.34)	-1.86 (2.17)
5-HTTLPR L'/L' Genotype		1.99 (2.38)	0.30 (2.23)
7) Intercept		69.64 (2.53)***	72.73 (3.07)***
5-HTTLPR S' Allele		1.47 (2.95)	0.91 (2.90)
Time		0.01 (1.97)	-2.12 (2.08)
5-HTTLPR S' Allele		-2.80 (2.25)	-0.84 (2.12)

8) Intercept		73.26 (2.69)***	74.99 (2.94)***
5-HTTLPR L' Allele		-3.30 (3.07)	-2.04 (3.02)
Time		-4.53 (1.92)*	-4.19 (1.90)*
5-HTTLPR L' Allele		3.18 (2.21)	1.95 (2.06)
9) Intercept		70.24 (1.61)***	72.45 (2.26)***
TPH-2 T Allele		1.35 (2.69)	2.34 (2.66)
Time		-3.15 (1.21)*	-3.61 (1.38)*
TPH-2 T Allele		2.72 (1.98)	2.17 (1.87)

*p < .05, **p < .01, ***p < .001

Inconsistent ns across models reflect isolated missing values.

Table 4. Results of multilevel modeling analyses examining: 1) Response to treatment for EAT-26 Total symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for EAT-26 Total, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

EAT-26 Total Score	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=79	N=76	N=76
1) Intercept	36.73 (1.87)***	35.70 (2.18)***	33.45 (3.37)***
Major Depressive Disorder		4.48 (4.59)	4.18 (4.50)
Time	-10.93 (1.69)***	-12.15 (2.03)***	-11.40 (2.58)***
Major Depressive Disorder		4.13 (4.11)	2.46 (4.13)
		N=71	N=71
2) Intercept		34.48 (2.44)***	31.50 (3.67)***
Anxiety Disorder		7.88 (4.26)	6.73 (4.17)
Time		-10.52 (2.27)***	-11.08 (2.94)***
Anxiety Disorder		-2.01 (3.95)	-1.73 (3.91)
Axis-II Comorbidity			
		N=79	N=79
3) Intercept		35.52 (2.14)***	33.26 (3.37)***
Avoidant PD		5.23 (4.42)	3.61 (4.33)
Time		-9.58 (1.93)***	-10.13 (2.45)***
Avoidant PD		-5.68 (3.94)	-3.55 (4.02)
4) Intercept		35.24 (2.12)***	33.75 (3.22)***
Obsessive-Compulsive PD		5.90 (4.20)	5.20 (4.19)
Time		-12.35 (1.97)***	-11.63 (2.43)***
Obsessive-Compulsive PD		5.18 (3.82)	5.29 (3.86)
5) Intercept		34.59 (2.22)***	32.62 (3.38)***
Borderline PD		7.01 (4.02)	5.65 (3.92)
Time		-10.67 (2.05)***	-10.06 (2.63)***
Borderline PD		-0.88 (3.69)	-1.99 (3.79)
Genes			
		N=77	N=77
6) Intercept		36.60 (2.67)***	34.76 (3.83)***
5-HTTLPR S'/S' Genotype		-4.76 (4.73)	-4.01 (4.65)
5-HTTLPR L'/L' Genotype		4.85 (4.56)	3.92 (4.49)
Time		-12.03 (2.40)***	-11.52 (2.95)***
5-HTTLPR S'/S' Genotype		3.21 (4.35)	2.30 (4.32)
5-HTTLPR L'/L' Genotype		1.90 (4.24)	1.18 (4.27)
7) Intercept		41.45 (3.69)***	38.42 (4.69)***
5-HTTLPR S' Allele		-6.38 (4.30)	-5.14 (4.25)
Time		-10.13 (3.49)**	-10.37 (3.91)**
5-HTTLPR S' Allele		-0.89 (4.02)	-0.51 (4.06)

8) Intercept		31.83 (3.91)***	30.62 (4.44)***
5-HTTLPR L' Allele		6.41 (4.47)	5.30 (4.41)
Time		-8.82 (3.61)**	-9.28 (3.90)*
5-HTTLPR L' Allele		-2.68 (4.11)	-2.01 (4.11)
9) Intercept		38.09 (2.39)***	34.76 (3.62)***
TPH-2 T Allele		-3.83 (3.99)	-1.45 (4.00)
Time		-12.22 (2.14)***	-11.40 (2.71)***
TPH-2 T Allele		3.87 (3.57)	1.39 (3.72)

*p < .05, **p < .01, ***p < .001

[a] EAT-26 = Eating Attitudes Test-26

Inconsistent ns across models reflect isolated missing values.

Table 5. Results of multilevel modeling analyses examining: 1) Response to treatment for EAT-26 Diet symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for EAT-26 Diet symptoms, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

EAT-26 Diet Score	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=79	N=76	N=76
1) Intercept	1.68 (0.09)	1.63 (0.10)***	1.48 (0.15)***
Major Depressive Disorder		0.24 (0.21)	0.22 (0.20)
Time	-0.48 (0.08)	-0.53 (0.09)***	-0.52 (0.12)***
Major Depressive Disorder		0.21 (0.19)	0.14 (0.19)
		N=71	N=71
2) Intercept		1.58 (0.11)***	1.41 (0.17)***
Anxiety Disorder		0.37 (0.19)	0.31 (0.19)
Time		-0.47 (0.10)***	-0.52 (0.14)***
Anxiety Disorder		-0.06 (0.18)	-0.04 (0.18)
Axis-II Comorbidity			
		N=79	N=79
3) Intercept		1.62 (0.10)***	1.48 (0.15)***
Avoidant PD		0.25 (0.20)	0.18 (0.20)
Time		-0.42 (0.09)***	-0.46 (0.11)***
Avoidant PD		-0.25 (0.18)	-0.17 (0.18)
4) Intercept		1.61 (0.10)***	1.51 (0.15)***
Obsessive-Compulsive PD		0.27 (0.19)	0.23 (0.19)
Time		-0.54 (0.09)***	-0.52 (0.11)***
Obsessive-Compulsive PD		0.211 (0.17)	0.21 (0.17)
5) Intercept		1.58 (0.10)***	1.46 (0.15)***
Borderline PD		0.31 (0.18)	0.24 (0.18)
Time		-0.47 (0.09)***	-0.46 (0.12)***
Borderline PD		-0.04 (0.17)	-0.08 (0.17)
Genes			
		N=77	N=77
6) Intercept		1.72 (0.12)***	1.60 (0.17)***
5-HTTLPR S'/S' Genotype		-0.35 (0.22)	-0.31 (0.21)
5-HTTLPR L'/L' Genotype		0.15 (0.21)	0.11 (0.20)
Time		-0.52 (0.11)***	-0.52 (0.13)***
5-HTTLPR S'/S' Genotype		0.21 (0.20)	0.17 (0.20)
5-HTTLPR L'/L' Genotype		0.01 (0.19)	-0.02 (0.19)
7) Intercept		1.87 (0.17)***	1.69 (0.21)***
5-HTTLPR S' Allele		-0.26 (0.20)	-0.20 (0.19)
Time		-0.51 (0.16)**	-0.54 (0.18)**
5-HTTLPR S' Allele		0.06 (0.18)	0.06 (0.18)

8) Intercept		1.37 (0.18)***	1.29 (0.20)***
5-HTTLPR L' Allele		0.40 (0.20)	0.34 (0.20)
Time		-0.32 (0.16)	-0.36 (0.18)*
5-HTTLPR L' Allele		-0.20 (0.19)	-0.17 (0.19)
9) Intercept		1.75 (0.11)***	1.57 (0.16)***
TPH-2 T Allele		-0.21 (0.18)	-0.11 (0.18)
Time		-0.55 (0.10)***	-0.53 (0.12)***
TPH-2 T Allele		0.21 (0.16)	0.11 (0.17)

*p < .05, **p < .01, ***p < .001

[a] EAT-26 = Eating Attitudes Test-26

Inconsistent ns across models reflect isolated missing values.

Table 6. Results of multilevel modeling analyses examining: 1) Response to treatment for EAT-26 Bulimia symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for EAT-26 Bulimia symptoms, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

EAT-26 Bulimia Score	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=79	N=76	N=76
1) Intercept	1.85 (0.09)***	1.81 (0.11)***	1.76 (0.17)***
Major Depressive Disorder		0.14 (0.22)	0.15 (0.22)
Time	-0.64 (0.10)***	-0.70 (0.12)***	-0.64 (0.15)***
Major Depressive Disorder		0.17 (0.25)	0.07 (0.25)
		N=71	N=71
2) Intercept		1.81 (0.12)***	1.69 (0.18)***
Anxiety Disorder		0.13 (0.21)	0.08 (0.21)
Time		-0.63 (0.14)***	-0.64 (0.17)***
Anxiety Disorder		-0.12 (0.24)	-0.09 (0.23)
Axis-II Comorbidity			
		N=79	N=79
3) Intercept		1.79 (0.10)***	1.75 (0.17)***
Avoidant PD		0.25 (0.22)	0.18 (0.22)
Time		-0.58 (0.12)***	-0.59 (0.15)***
Avoidant PD		-0.26 (0.24)	-0.14 (0.25)
4) Intercept		1.85 (0.10)***	1.83 (0.17)***
Obsessive-Compulsive PD		0.03 (0.21)	0.02 (0.21)
Time		-0.77 (0.12)***	-0.70 (0.14)***
Obsessive-Compulsive PD		0.50 (0.23)*	0.50 (0.23)*
5) Intercept		1.80 (0.11)***	1.76 (0.17)***
Borderline PD		0.18 (0.20)	0.13 (0.20)
Time		-0.64 (0.13)***	-0.58 (0.16)**
Borderline PD		.000 (0.23)	-0.08 (0.23)
Genes			
		N=77	N=77
6) Intercept		1.81 (0.13)***	1.78 (0.20)***
5-HTTLPR S'/S' Genotype		-0.01 (0.23)	0.00 (0.23)
5-HTTLPR L'/L' Genotype		0.15 (0.22)	0.11 (0.22)
Time		-0.72 (0.15)***	-0.67 (0.18)***
5-HTTLPR S'/S' Genotype		0.05 (0.26)	0.01 (0.26)
5-HTTLPR L'/L' Genotype		0.32 (0.26)	0.27 (0.26)
7) Intercept		1.96 (0.18)***	1.89 (0.24)***
5-HTTLPR S' Allele		-0.15 (0.21)	-0.11 (0.21)
Time		-0.40 (0.21)	-0.40 (0.23)
5-HTTLPR S' Allele		-0.30 (0.24)	-0.27 (0.25)

8) Intercept		1.80 (0.19)***	1.77 (0.22)***
5-HTTLPR L' Allele		0.06 (0.22)	0.04 (0.22)
Time		-0.66 (0.22)**	-0.66 (0.24)**
5-HTTLPR L' Allele		0.04 (0.25)	0.07 (0.25)
9) Intercept		1.94 (0.12)***	1.84 (0.19)***
TPH-2 T Allele		-0.26 (0.19)	-0.13 (0.20)
Time		-0.71 (0.13)***	-0.64 (0.16)***
TPH-2 T Allele		0.23 (0.22)	0.10 (0.23)

*p < .05, **p < .01, ***p < .001

[a] EAT-26 = Eating Attitudes Test-26

Inconsistent ns across models reflect isolated missing values.

Table 7. Results of multilevel modeling analyses examining: 1) Response to treatment for Binge Eating symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for Binge Eating symptoms, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

Binge Days/Month	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=81	N=78	N=78
1) Intercept	-0.33 (0.27)	-0.51 (0.30)	-0.36 (0.49)
Major Depressive Disorder		0.85 (0.65)	0.87 (0.67)
Time	-1.08 (0.31)**	-1.08 (0.36)**	-1.02 (0.44)*
Major Depressive Disorder		-0.19 (0.76)	-0.38 (0.79)
Threshold[a]	1.89 (0.21)***	1.86 (0.22)***	1.93 (0.23)***
		N=72	N=72
2) Intercept		-0.20 (0.34)	-0.08 (0.54)
Anxiety Disorder		-0.15 (0.59)	-0.08 (0.61)
Time		-0.86 (0.39)*	-0.90 (0.49)
Anxiety Disorder		-1.17 (0.72)	-1.33 (0.75)
Threshold		1.89 (0.23)***	1.97 (0.24)***
Axis-II Comorbidity			
		N=81	N=81
3) Intercept		-0.46 (0.31)	-0.48 (0.51)
Avoidant PD		0.56 (0.63)	0.63 (0.66)
Time		-0.90 (0.35)*	-0.97 (0.42)*
Avoidant PD		-0.95 (0.75)	-0.87 (0.78)
Threshold		1.93 (0.22)***	2.00 (0.23)***
4) Intercept		-0.51 (0.31)	-0.43 (0.49)
Obsessive-Compulsive PD		0.72 (0.63)	0.94 (0.66)
Time		-0.99 (0.35)**	-1.02 (0.43)*
Obsessive-Compulsive PD		-0.45 (0.74)	-0.48 (0.77)
Threshold		1.92 (0.22)***	2.00 (0.23)***
5) Intercept		-0.15 (0.32)	-0.16 (0.51)
Borderline PD		-0.61 (0.56)	-0.68 (0.57)
Time		-1.13 (0.37)**	-1.11 (0.45)*
Borderline PD		0.10 (0.67)	-0.14 (0.70)
Threshold		1.91 (0.22)***	1.99 (0.23)***
Genes			
		N=79	N=79
6) Intercept		-0.72 (0.39)	-0.75 (0.60)
5-HTTLPR S'/S' Genotype		1.14 (0.70)	1.05 (.072)
5-HTTLPR L'/L' Genotype		0.35 (0.64)	0.29 (0.66)
Time		-0.84 (0.44)	-0.80 (0.52)
5-HTTLPR S'/S' Genotype		-1.00 (0.80)	-0.99 (0.81)

5-HTTLPR L'/L' Genotype		0.07 (0.75)	-0.05 (0.77)
Threshold		1.99 (0.23)***	2.07 (0.24)***
7) Intercept		-0.36 (0.52)	-0.36 (0.70)
5-HTTLPR S' Allele		-0.01 (0.60)	0.01 (0.62)
Time		-0.75 (0.60)	-0.85 (0.68)
5-HTTLPR S' Allele		-0.38 (0.70)	-0.24 (0.73)
Threshold		1.94 (0.22)***	2.01 (0.23)***
8) Intercept		0.41 (0.59)	0.28 (0.69)
5-HTTLPR L' Allele		-1.01 (0.66)	-0.95 (0.68)
Time		-1.83 (0.68)**	-1.78 (0.73)*
5-HTTLPR L' Allele		1.02 (0.76)	0.97 (0.77)
Threshold		1.98 (0.23)***	2.05 (0.24)***
9) Intercept		-0.30 (0.34)	-0.35 (0.56)
TPH-2 T Allele		-0.16 (0.55)	-0.04 (0.58)
Time		-1.01 (0.39)*	-0.96 (0.47)*
TPH-2 T Allele		-0.05 (0.64)	-0.16 (0.67)
Threshold		1.94 (0.22)***	2.01 (0.23)***

*p < .05, **p < .01, ***p < .001

[a] Threshold = The threshold is defined as the difference between the unique intercepts for the probability functions of the first two categories of the ordinal outcome variable.

Inconsistent ns across models reflect isolated missing values.

Table 8. Results of multilevel modeling analyses examining: 1) Response to treatment for Vomiting symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for Vomiting symptoms, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

Vomit Days/Month	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=81	N=78	N=78
1) Intercept	-0.44 (0.31)	-0.35 (.034)	-0.61 (0.56)
Major Depressive Disorder		-0.40 (0.72)	-0.46 (0.76)
Time	-1.10 (0.32)**	-1.11 (0.37)**	-1.22 (0.46)**
Major Depressive Disorder		-0.06 (0.80)	-0.35 (0.84)
Threshold[a]	2.19 (0.25)***	2.25 (0.26)***	2.34 (0.27)***
		N=72	N=72
2) Intercept		-0.17 (0.41)	-0.30 (0.64)
Anxiety Disorder		-0.92 (0.69)	-1.01 (0.73)
Time		-1.03 (0.42)*	-1.06 (0.53)*
Anxiety Disorder		-0.58 (0.76)	-0.72 (0.79)
Threshold		2.35 (0.28)***	2.49 (0.30)***
Axis-II Comorbidity			
		N=81	N=81
3) Intercept		-0.35 (0.35)	-0.62 (0.57)
Avoidant PD		-0.42 (0.71)	-0.47 (0.75)
Time		-1.03 (0.36)**	-1.22 (0.44)**
Avoidant PD		-0.45 (0.80)	-0.38 (0.84)
Threshold		2.22 (0.25)***	2.29 (0.26)***
4) Intercept		-0.33 (0.35)	-0.63 (0.56)
Obsessive-Compulsive PD		-0.53 (0.70)	-0.53 (0.74)
Time		-1.25 (0.37)**	-1.41 (0.45)**
Obsessive-Compulsive PD		0.60 (0.76)	0.69 (0.80)
Threshold		2.23 (0.25)***	2.30 (0.26)***
5) Intercept		-0.32 (0.36)	-0.58 (0.57)
Borderline PD		-0.43 (0.64)	-0.54 (0.66)
Time		-1.09 (0.39)**	-1.24 (0.47)*
Borderline PD		-0.08 (0.70)	-0.25 (0.72)
Threshold		2.21 (0.25)***	2.29 (0.26)***
Genes			
		N=79	N=79
6) Intercept		-1.22 (0.45)**	-1.62 (0.69)*
5-HTTLPR S'/S' Genotype		1.48 (0.80)	1.57 (0.83)
5-HTTLPR L'/L' Genotype		1.17 (0.74)	1.07 (0.76)
Time		-1.06 (0.48)*	-1.17 (0.55)*
5-HTTLPR S'/S' Genotype		-0.21 (0.84)	-0.27 (0.86)

5-HTTLPR L'/L' Genotype		0.16 (0.78)	0.10 (0.81)
Threshold		2.41 (0.28)***	2.48 (0.29)***
7) Intercept		-0.04 (0.60)	-0.32 (0.81)
5-HTTLPR S' Allele		-0.70 (0.70)	-0.61 (0.72)
Time		-0.89 (0.62)	-1.01 (0.72)
5-HTTLPR S' Allele		-0.20 (0.73)	-0.16 (0.76)
Threshold		2.36 (0.27)***	2.43 (0.28)***
8) Intercept		0.27 (0.67)	-0.09 (0.78)
5-HTTLPR L' Allele		-1.06 (0.76)	-1.20 (0.79)
Time		-1.25 (0.70)	-1.44 (0.76)
5-HTTLPR L' Allele		0.28 (0.79)	0.30 (0.81)
Threshold		2.36 (0.27)***	2.43 (0.28)***
9) Intercept		-0.17 (0.40)	-0.48 (0.64)
TPH-2 T Allele		-1.03 (0.63)	-0.86 (0.67)
Time		-0.91 (0.41)*	-0.93 (0.49)
TPH-2 T Allele		-0.39 (0.69)	-0.56 (0.72)
Threshold		2.38 (0.27)***	2.46 (0.28)***

*p < .05, **p < .01, ***p < .001

[a] Threshold = The threshold is defined as the difference between the unique intercepts for the probability functions of the first two categories of the ordinal outcome variable.

Inconsistent ns across models reflect isolated missing values.

Table 9. Results of multilevel modeling analyses examining: 1) Response to treatment for Purging symptoms, 2) Effects of psychiatric comorbidity and genes on response to treatment for Purging symptoms, and 3) Effects of psychiatric comorbidity and genes on response to treatment while controlling for medication use and amount of therapy.

Purge Days/Month	**1)** Model 1 Coefficient (SE)	**2)** Model 2 Coefficient (SE)	**3)** Model 3 Coefficient (SE)
Axis-I Comorbidity			
	N=81	N=78	N=78
1) Intercept	-0.38 (0.29)	-0.54 (0.33)	-0.80 (0.55)
Major Depressive Disorder		0.80 (0.72)	0.84 (0.77)
Time	-1.11 (0.32)**	-0.94 (0.36)*	-0.97 (0.45)*
Major Depressive Disorder		-0.97 (0.81)	-1.32 (0.86)
Threshold[a]	2.09 (0.24)***	2.18 (0.25)***	2.29 (0.26)***
		N=72	N=72
2) Intercept		-0.52 (0.39)	-0.86 (0.62)
Anxiety Disorder		0.49 (0.67)	0.41 (0.71)
Time		-0.75 (0.40)	-0.78 (0.51)
Anxiety Disorder		-1.52 (0.75)*	-1.54 (0.78)
Threshold		2.24 (0.27)***	2.38 (0.29)***
Axis-II Comorbidity			
		N=81	N=81
3) Intercept		-0.28 (0.33)	-0.60 (0.55)
Avoidant PD		-0.48 (0.67)	-0.57 (0.71)
Time		-0.97 (0.36)**	-1.10 (0.43)*
Avoidant PD		-0.75 (0.79)	-0.62 (0.82)
Threshold		2.14 (0.24)***	2.22 (0.25)***
4) Intercept		-0.41 (0.33)	-0.69 (0.54)
Obsessive-Compulsive PD		0.11 (0.67)	0.12 (0.71)
Time		-1.12 (0.36)**	-1.21 (0.44)**
Obsessive-Compulsive PD		0.03 (0.75)	0.09 (0.79)
Threshold		2.11 (0.24)***	2.19 (0.25)***
5) Intercept		-0.22 (0.35)	-0.52 (0.55)
Borderline PD		-0.59 (0.61)	-0.74 (0.63)
Time		-1.11 (0.38)**	-1.17 (0.46)*
Borderline PD		-0.05 (0.69)	-0.23 (0.71)
Threshold		2.11 (0.24)***	2.20 (0.25)***
Genes			
		N=79	N=79
6) Intercept		-1.13 (0.43)*	-1.67 (0.66)*
5-HTTLPR S'/S' Genotype		1.65 (0.77)*	1.73 (0.80)*
5-HTTLPR L'/L' Genotype		0.99 (0.70)	0.91 (0.72)
Time		-1.01 (0.46)*	-1.07 (0.54)*
5-HTTLPR S'/S' Genotype		-0.35 (0.84)	-0.31 (0.85)

5-HTTLPR L'/L' Genotype		0.03 (0.77)	-0.10 (0.79)
Threshold		2.28 (0.26)***	2.36 (0.27)***
7) Intercept		-0.12 (0.58)	-0.49 (0.78)
5-HTTLPR S' Allele		-0.49 (0.67)	-0.42 (0.69)
Time		-0.97 (0.62)	-1.09 (0.71)
5-HTTLPR S' Allele		-0.10 (0.72)	0.04 (0.75)
Threshold		2.24 (0.25)***	2.31 (0.26)***
8) Intercept		0.53 (0.65)	0.03 (0.76)
5-HTTLPR L' Allele		-1.30 (0.73)	-1.41 (0.75)
Time		-1.34 (0.70)	-1.37 (0.75)
5-HTTLPR L' Allele		0.37 (0.79)	0.28 (0.81)
Threshold		2.25 (0.25)***	2.33 (0.27)***
9) Intercept		-0.38 (0.39)	-0.83 (0.63)
TPH-2 T Allele		-0.32 (0.61)	-0.05 (0.65)
Time		-0.71 (0.40)	-0.64 (0.48)
TPH-2 T Allele		-0.97 (0.68)	-1.28 (0.72)
Threshold		2.29 (0.26)***	2.40 (0.28)***

*p < .05, **p < .01, ***p < .001

[a] Threshold = The threshold is defined as the difference between the unique intercepts for the probability functions of the first two categories of the ordinal outcome variable.

Inconsistent ns across models reflect isolated missing values.

Figure 1. Estimated Means for Depressive Symptoms in Individuals with and Without Comorbid MDD at Baseline and 4-Month Follow-Up.

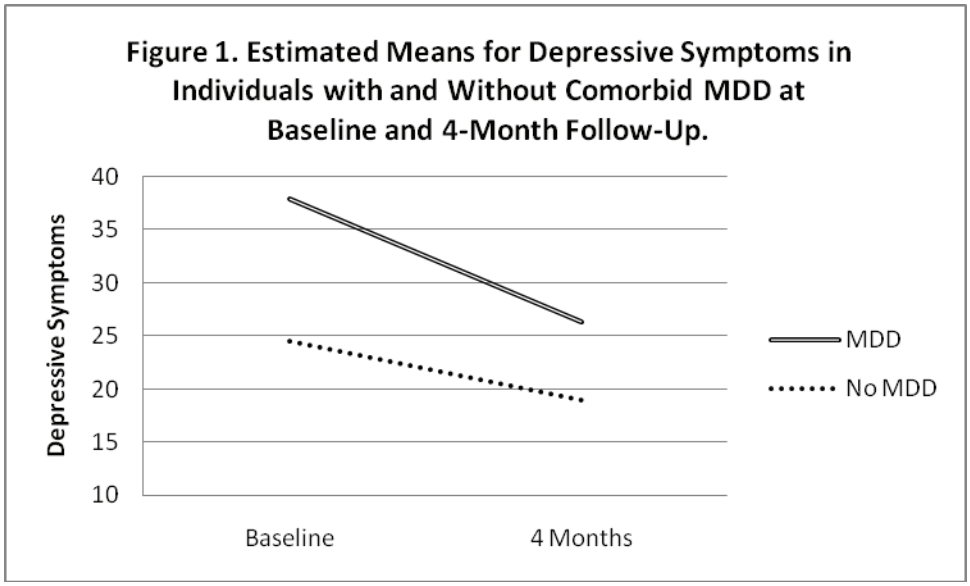
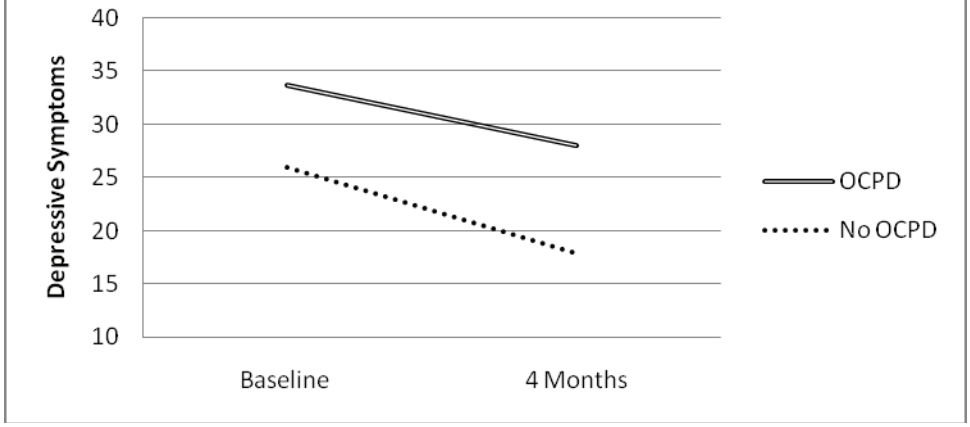


Figure 2. Estimated Means for Depressive Symptoms in Individuals with and Without Comorbid OCPD at Baseline and 4-Month Follow-UP.



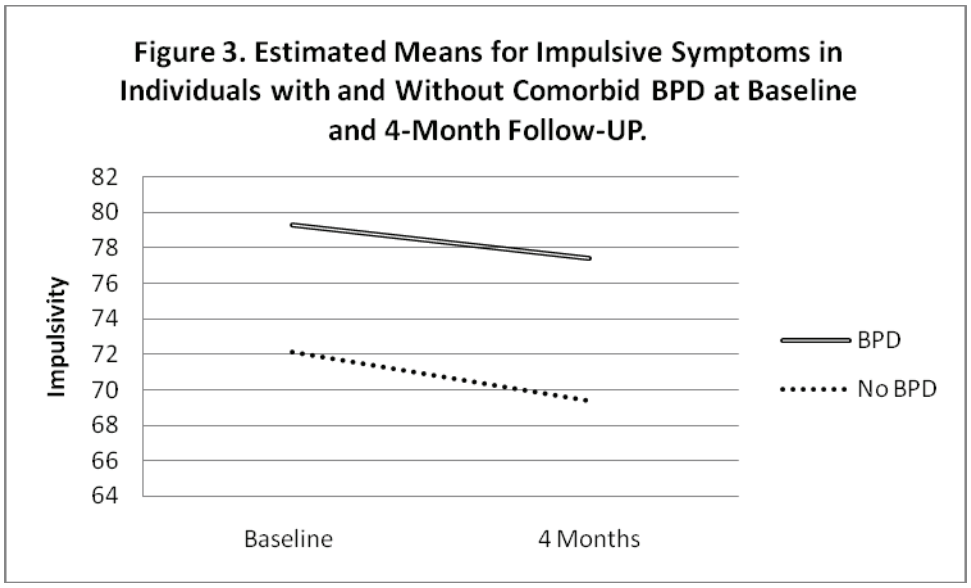


Figure 3. Estimated Means for Impulsive Symptoms in Individuals with and Without Comorbid BPD at Baseline and 4-Month Follow-UP.
Impulsivity
82
80
78
76
74
72
70
68
66
64
Baseline
4 Months
BPD
No BPD

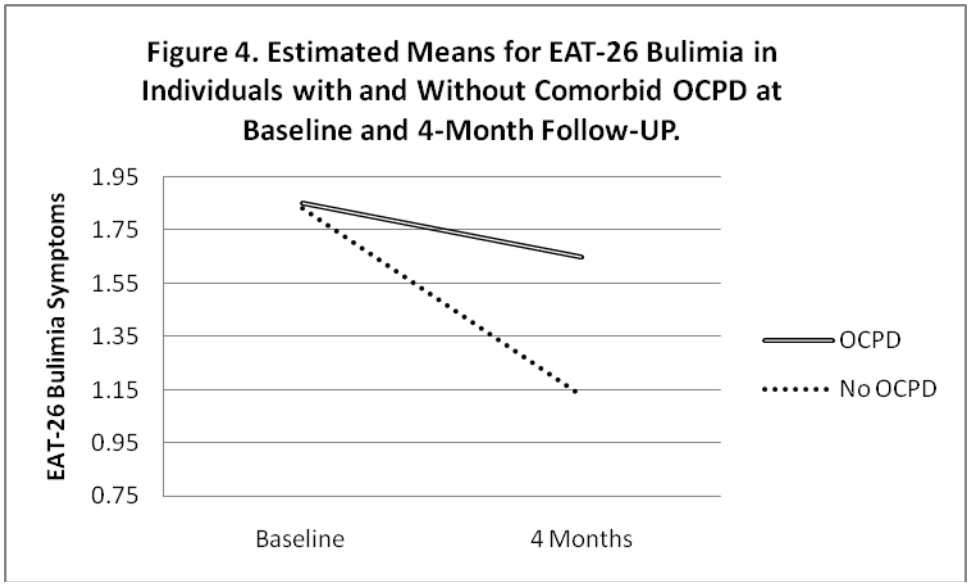


Figure 4. Estimated Means for EAT-26 Bulimia in Individuals with and Without Comorbid OCPD at Baseline and 4-Month Follow-UP.
EAT-26 Bulimia Symptoms
1.95
1.75
1.55
1.35
1.15
0.95
0.75
Baseline
4 Months
OCPD
No OCPD

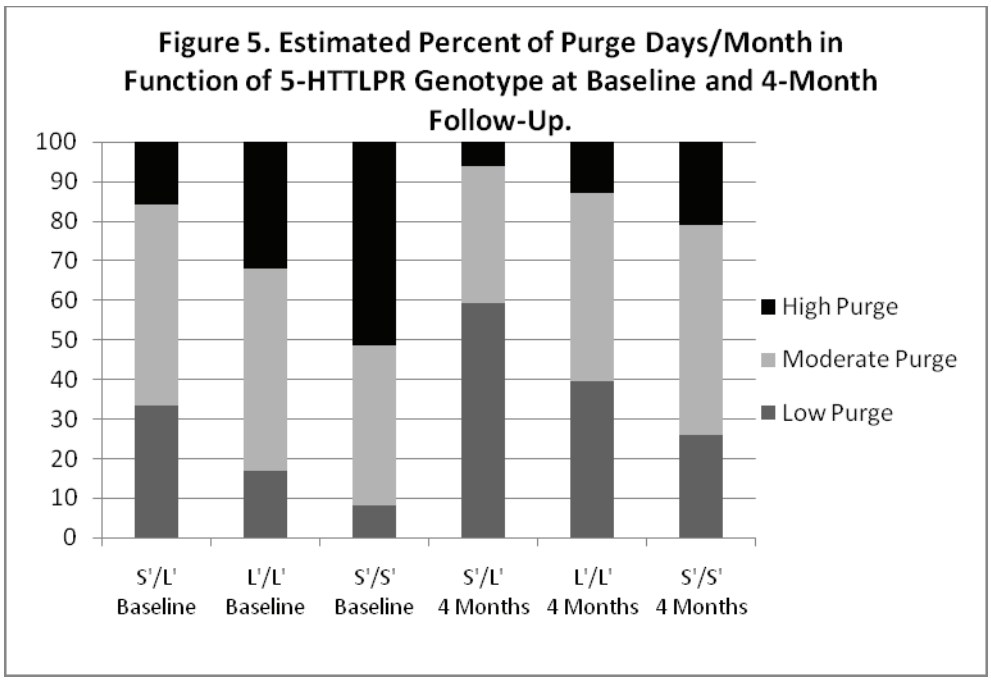


Figure 5. Estimated Percent of Purge Days/Month in Function of 5-HTTLPR Genotype at Baseline and 4-Month Follow-Up.
100
90
80
70
60
50
40
30
20
10
0
S'/L' Baseline
L'/L' Baseline
S'/S' Baseline
S'/L' 4 Months
L'/L' 4 Months
S'/S' 4 Months
High Purge
Moderate Purge
Low Purge

General Discussion

This dissertation had two major aims: The first was to explore how genetic factors and developmental experiences might be associated with patterns of psychiatric comorbidity in individuals with bulimia-spectrum disorders (BSDs); the second was to examine how psychiatric comorbidity and genetic factors might impact treatment outcome in bulimia-spectrum individuals. The dissertation consisted of three studies.

Study 1 derived an empirical classification of Axis-I psychiatric comorbidity in women with BSDs and investigated how distinct comorbidity classes might be associated with a) different genetic (i.e., 5-HTTLPR) susceptibilities, and b) different patterns of exposure to developmental risks (i.e., childhood abuse). Data on lifetime Axis-I disorders were analyzed using latent class analysis and resulting classes were compared on eating and psychopathological symptoms, 5-HTTLPR genotype, and childhood abuse. Results revealed two comorbidity-based classes: a smaller "high comorbidity" class characterized by a high likelihood of multiple comorbid Axis-I disorders and a larger "low comorbidity" class characterized by a high likelihood of comorbid major depressive disorder only. The high comorbidity class was characterized, in particular, by the presence of comorbid substance abuse/dependence and was found to display increased conduct problems. The two classes differed with respect to 5-HTTLPR variations and history of childhood abuse, with the high comorbidity class displaying a greater likelihood of carrying 5-HTTLPR low function alleles and more sexual or physical abuse in childhood. The results

described have been published in the Journal of Clinical Psychiatry (Richardson et al, 2008).

Study 2 consisted of two parts. First, we explored whether women with BSDs might display different genetic (i.e., 5-HTTLPR and TPH2 G-703T) susceptibilities and developmental risks (i.e., childhood abuse) than women with no past or present eating disorder (control women). Second, within bulimia-spectrum individuals, we examined possible differences in genetic and developmental history variables in individuals with different comorbid Axis-I and Axis-II disorders, including Major Depressive Disorder (MDD), Anxiety Disorders (ADs), Substance Use Disorders (SUDs), Borderline Personality Disorder (BPD), Obsessive-Compulsive Personality Disorder (OCPD) and Avoidant Personality Disorder (AVPD). Findings revealed that homozygosity for 5-HTTLPR high-function variants and a history of childhood abuse were both significantly associated with likelihood of having a BSD. Interestingly, when psychiatric comorbidity was examined within individuals with BSDs, 5-HTTLPR high-function alleles were found to predict the presence of comorbid ADs and childhood abuse was found to predict the presence of comorbid SUDs. The preceding results suggest that variations in genetic and developmental history variables are associated with different clinical presentations in individuals with BSDs.

Study 3 examined how psychiatric comorbidity (Axis-I and Axis-II) and genetic (i.e., 5-HTTLPR and TPH2 G-703T) variations might contribute to prediction of treatment response in individuals with BSDs. The findings

suggested that Axis-I comorbidity had little effect on response to treatment. On the other hand, Axis-II comorbidity (BPD and OCPD) had important implications for post-treatment symptoms, in particular with respect to general psychopathological symptoms (depression and impulsivity). BPD had no effect on response of eating symptoms to treatment; however OCPD had a significant effect on response of bulimic eating symptoms, suggesting that OCPD may have relevance for the outcome of both eating and general psychological symptoms in BSDs. With respect to genetic variables, homozygosity for 5-HTTLPR low-function variants predicted increased purging behaviour, which persisted throughout treatment.

The ensuing discussion will highlight the ways in which the present findings contribute to the empirical and theoretical literature. In addition, recommendations for future directions in research and clinical implications will be discussed.

Comorbidity

Findings from the current studies show a strong co-occurrence of bulimia-spectrum disorders with both Axis-I and Axis-II disorders.

Axis-I Disorders. Rates of Axis-I comorbidity in individuals with BSDs in the present studies are in line with those reported in previous research. For example, a history of MDD was found in 75% of BN-spectrum individuals (in Study 2), findings which are in line with reported rates of 60-80% for lifetime MDD in other clinical samples (Brewerton et al., 1995; Bulik, Sullivan, Carter, & Joyce, 1996; Godart et al., 2007; Herzog et al., 1999; Hudson, Pope, & Yurgelon-

Todd, 1988). Rates of lifetime ADs, occurring in about 50% of BN-spectrum women (in Study 2) are also in line with previous findings in clinical and community samples reporting prevalence rates of between 50-80% (Bulik et al., 1996; Garfinkel et al., 1995; Godart et al., 2003; Hudson, Hiripi, Pope, & Kessler, 2007). Similarly, rates of SUDs in 25-30% of individuals with BSDs (in Study 2) are similar to those obtained in previous studies showing that SUDs occur in roughly a third of individuals with Bulimia Nervosa (BN) (Garfinkel et al., 1995; Holderness, Brooks-Gunn, & Warren, 1994; Hudson et al., 2007; Lilenfeld et al., 1998). In sum, findings from the present studies are in line with previous reports showing an important co-occurrence of BSDs with mood, anxiety and substance-use disorders.

Axis-II Disorders. Findings from the current studies corroborate previous research showing a strong association of BN with both Cluster B (characterized by dramatic, erratic behaviours) and Cluster C (characterized by anxious, fearful behaviour) personality disorders. Although research on personality disorders in BN has tended to focus more on Cluster B disorders—likely due to comparisons with AN, which is more often associated with Cluster C disorders—individuals with BN show similar rates of Cluster C and Cluster B pathology (Rosenvinge et al., 2000). Previous studies have found BPD and AVPD to be the two most common personality disorders in BN; with estimated prevalence rates of 21% and 19% respectively (see Cassin & Von Ransen, 2005). Current findings are in line with previously reported prevalence rates, with BPD occurring in 27% and AVPD in 21% of BN-spectrum individuals (in Study 2). In the current studies we also

found a relatively high rate of OCPD, occurring in 28% of individuals (in Study 2), which is somewhat higher than estimated prevalence rates based on previous studies (9%), but in line with findings showing that OCPD is one of the most prominent PDs in BN (see Cassin & Von Ransen, 2005). Taken together, findings show an important co-occurrence of BSDs with both Cluster B and Cluster C personality pathology.

Cluster-Analytic Studies. The present findings add to a growing body of literature showing that there exists important heterogeneity, with respect to psychiatric comorbidity, in individuals with BSDs. The two-class structure of psychiatric comorbidity found in Study 1 of the current dissertation is strikingly similar to that found by Duncan et al (2005). As in their findings, we found a two-class solution with one class characterized by "low comorbidity" and a second class characterized a "high comorbidity". In both studies the low-comorbidity group showed a high likelihood of MDD only, whereas the high-comorbidity group displayed a high likelihood of MDD, ADs, SUDs, and antisocial personality disorder or traits. Such findings suggest that there are at least two empirically distinguishable subgroups of individuals with BSDs based on severity of associated psychiatric disorders. Cluster-analytic studies of personality traits in individuals with eating disorders (EDs) have also revealed empirically distinguishable subgroups. Most studies reveal three subgroups: an impulsive and emotionally dysregulated group; an anxious and compulsive group; and a relatively high-functioning group (Goldner, Srikameswaran, Schroeder, Livesly, and Birmingham, 1999; Steiger et al., 2009; Westen & Harnden-Fischer, 2001;

Goldner et al., 1999; Wonderlich et al., 2005). In contrast to individuals with Anorexia Nervosa (AN), who most often cluster into the anxious, compulsive subgroup, individuals with BN are found in all three subgroups (Goldner et al., 1999; Steiger et al., 2009; Westen & Harnden-Fischer, 2001; Goldner et al., 1999). Taken together, findings suggest that, whether at the level of personality traits or psychiatric disorders there exists important heterogeneity within BSDs that cannot be explained by ED diagnosis alone.

Etiologic Factors

Studies in the current dissertation show that different subgroups of individuals with BSDs appear to be associated with different genetic and developmental risk factors.

Childhood Abuse. Findings from Study 1 show that childhood sexual or physical abuse is significantly more prevalent in a subgroup of individuals with BSDs characterized by high psychiatric comorbidity, in particular drug abuse/dependence and conduct problems. Findings from Study 2 corroborate this result by demonstrating that childhood abuse predicts increased lifetime drug abuse/dependence (but not other disorders) in individuals with BSDs. Such findings are in line with previous results linking childhood trauma to SUDS (Corstorphine et al., 2007; Deep et al., 1999; Dohm et al., 2002; Matsunaga et al., 1999) and dissocial/impulsive behaviour (Corstorphine et al., 2007; Lacey, 1993; Steiger et al., 2009) in individuals with BN. Taken together findings from Studies 1 and 2 suggest that a history of childhood abuse may be etiologically relevant for

a subgroup of individuals with BSDs, characterized by SUDs and dissocial behaviour.

Genetic Factors. Findings from Study 1 suggest that, like childhood abuse, 5-HTTLPR low-function variants are significantly more likely to be found in a subgroup of individuals with BSDs characterized by high psychiatric comorbidity, in particular drug abuse/dependence and conduct problems. This observation corroborates previous reports linking 5-HTTLPR low-function alleles to disorders characterized by problems of impulse-control (e.g., SUDs and BPD) in non-eating disorder populations (Lichtermann et al., 2000; Lions-Ruth et al., 2007; Mannelli et al., 2005; Ni et al., 2006; Sander et al., 1997; 1998). Findings are also in line with studies associating 5-HTTLPR low-function variants with psychopathological symptoms of an impulsive/dissocial nature (e.g., affective instability, impulsivity, BPD and dissocial behaviour) in individuals with BSDs (Akkermann et al., 2009; Steiger et al., 2005; 2007; 2008). Whereas 5-HTTLPR low-function variants appear, in BN, to be associated with impulsive/dissocial characteristics, results from Study 2 suggest that high-function variants may be associated with anxious tendencies. 5-HTTLPR high function variants were associated with increased risk of comorbid ADs in individuals with BSDs, findings which are in line with one previous study linking 5-HTTLPR high-function alleles to increased compulsivity and inhibition in individuals with eating disorders (EDs) (Steiger et al., 2009). Furthermore, similar findings have been found in non-ED populations, with various studies showing high-function variants to be linked to anxiety disorders (Thakur et al., 2009; Grabe et al., 2009), most

prominently obsessive-compulsive disorder (OCD) (Bengel et al., 1999; Cavallini et al., 2002; Baca-Garcia et al., 2005; Hu et al., 2006; Steiger et al, 2009). In sum, findings suggest that variations in the 5-HTTLPR polymorphism may convey risk for general patterns of psychopathology in individuals with BSDs, with low-function variants possibly conveying risk to dissocial/impulsive symptoms and disorders and high-function variants conveying risk to anxious/compulsive phenomenon.

Findings linking variations in the 5-HTTLPR polymorphism to different psychological profiles in individuals with BSDs may have implications for why genetic findings often don't replicate. Most genetic association studies to date have compared frequencies of alleles or genotypes between those affected with BN and those unaffected. Comorbidity is either treated as an exclusion criteria (i.e., individuals with certain types of comorbid disorders are omitted) or neglected entirely. When individuals with comorbidity are omitted this creates an artificially homogeneous group of individuals with BN, which is not representative of the population as a whole. When comorbidity is not taken into account heterogeneous subtypes—such as anxious/inhibited bulimics versus impulsive/dissocial bulimics—may be grouped together in different proportions in different studies, thereby producing inconsistent findings across studies (Westen & Harnden-Fischer, 2001). The problem is particularly important when the same genetic risk factor—variations in 5-HTTLPR alleles—can manifest in opposite directions within the same diagnostic category—with high function alleles more prominent in anxious/inhibited individuals with BSDs and low-function alleles

more prominent in impulsive/dissocial individuals with BSDs. Accordingly, including psychiatric comorbidity as part of the behavioural phenotype in individuals with BSDs is important when examining genetic factors.

In conclusion, findings suggest that different subgroups of individuals with BSDs may have different etiologic risk factors. Childhood abuse appears to be a particularly relevant factor in a subgroup of individuals characterized by impulsive/dissocial traits and disorders. 5-HTTLPR allelic variations appear to have different implications for different subgroups, with low-function variants showing relevance in a subgroup characterized by impulsive/dissocial traits and disorders and high-function variants possibly having implications for anxiety traits and disorders.

Treatment Outcome

The present findings point to various important psychiatric and genetic predictors of treatment response in individuals with BSDs.

Effects of psychiatric comorbidity on treatment response. Results from Study 3 show significant affects of Axis-II comorbidity on response to treatment in individuals with BSDs, but limited effects of Axis-I comorbidity. Most previous studies examining effects of Axis-II pathology on response to treatment in BN have been limited by the fact that they have either focused solely on BPD or they have examined PDs as one general category, neglecting the heterogeneity that exists within the spectrum of PDs. The present study enhanced previous research by examining the effects, on treatment outcome, of a range of Cluster B and Cluster C disorders commonly found in BN. The present findings add to a

growing body of research showing that BPD predicts more severe post-treatment psychopathological symptoms (i.e., impulsivity) in individuals with BSDs, but has a limited effect on eating symptoms (Grilo, 2002; Steiger & Stotland, 1995; Wonderlich et al., 1994). OCPD, on the other hand, was found to predict more severe general psychopathology (i.e., depression) post-treatment and was also found to predict poorer response to treatment for bulimic eating symptoms. Such findings are particularly interesting as they are in line with results obtained in other ED populations (Anorexia Nervosa and Binge Eating Disorder) linking OCPD with poorer response to treatment (Crane et al., 2007; Lilenfeld et al., 2006; Masheb & Grilo, 2008; Steinhausen, 2002) and suggest that OCPD may be an important predictor of outcome for all eating-disorder subtypes. With respect to Axis-I disorders, findings add to an existing, albeit inconsistent, body of literature suggesting that Axis-I pathology has little or no impact on treatment response in individuals with BSDs. Although some studies have found effects of comorbid MDD or SUDs on treatment outcome for BN (Bulik et al., 1998; Keel et al., 1999; Maddocks & Kaplan, 1991) most studies have found no effects of Axis-I comorbidity (Fairburn et al., 1987; Keel et al., 1999; Mitchell et al., 1990; Strasser et al., 1992; Thiel et al., 1998). Together, findings show that Axis-I disorders have a limited impact on response to treatment for BSDs. An implication may be that Axis-I disorders are "state-dependent" symptoms that are likely to resolve along with improvements in BN symptoms. Axis-II disorders, on the other hand, appear to be a stronger predictor of treatment response in individuals with BSDs. This may reflect the fact that Axis-II disorders are more

"trait-like" in nature and thus likely to have a stable and enduring influence on symptoms.

Effects of genetic factors on treatment response. Findings from the present studies add to a growing body of literature associating 5-HTTLPR low function variants with poorer treatment response in individuals with BN. Findings from one previous study, for example, found that individuals with BN who were carriers of at least one 5-HTTLPR low function-allele showed less response (on binge-purging symptoms) to SSRI treatment than high-function homozygotes (Monteleone et al., 2006). In a subsequent study, Steiger et al. (2008) found that individuals carrying 5-HTTLPR low function variants responded less well to a multimodal treatment for BSDs than did high-function homozygotes, on binge eating symptoms. Findings from study 3 show that 5-HTTLPR low-function homozygotes displayed higher pre- and post-treatment purging symptoms than their heterozygote counterparts, adding further evidence to the hypothesis that 5-HTTLPR low-function variants predict poorer treatment outcome for BN. This is not to say that genes have a direct effect on treatment outcome, but more likely, they may impact outcome through activating more generalized susceptibilities in individuals with BN. For example, individuals with 5-HTTLPR low-function alleles may have inherited problems of serotonin (5-HT) neurotransmission, which make recovery from BN more challenging. In such individuals 5-HT dysregulation may make it more difficult to regulate problems of eating, mood and impulse control, all important components of successful treatment outcome.

In such a subgroup, pharmacological support aimed at stabilizing 5-HT system functioning may be an important therapeutic adjunct.

Future Directions

Findings from the current group of studies suggest that, in BSDs, individuals with different comorbid psychiatric profiles may differ with respect genetic liabilities, exposure to environmental risks, and treatment outcome. In showing this, we bring about several potentially important implications for future research.

When trying to understand etiology and treatment outcome in individuals with BN it is important to take into account two classes of agents: One factor relating to eating-specific pathology (i.e., eating habits, concerns about weight and shape) and another factor relating to more general aspects of psychopathology (i.e., mood, anxiety, impulsivity). Garner, Olmstead, Polivy and Garfinkel (1984) first proposed the two-component model of EDs, which suggests that EDs implicate both eating-specific and more general psychopathological components. As such, eating-specific disturbances alone are not enough to explain the etiology and course of an ED. In line with this, we propose that broadening the behavioural phenotype of BN to include psychopathological dimensions (such as concurrent problems in mood, anxiety and impulse control) when examining potential etiologic factors (e.g., genetic variations and developmental experiences) and factors that might contribute to treatment outcome will lead to a better understanding of the disorder and its treatment.

A broadening of the definition of BN to include associated psychopathological dimensions may be particularly important when examining candidate gene polymorphisms in the disorder. Researchers are moving more and more towards the idea that genes do not code for psychiatric disorders or specific symptoms per se, but instead may exert their effect at the level of more general psychopathological symptoms, which contribute to psychiatric disorders (Lindenberg, 2006). Findings from the present group of studies linking the 5-HTTLPR polymorphism to variations in psychiatric comorbidity in BSDs corroborates this idea and encourages the incorporation of psychopathological dimensions as part of the BN phenotype in future studies examining candidate gene polymorphisms in individuals with EDs.

For a more comprehensive understanding of how genetic factors may influence the etiology and course of BN an examination of interactions with environmental variables is warranted. The present group of studies identifies environmental factors (i.e., childhood trauma) that may have potential importance for the etiology of BN, however further studies are needed to examine how such variables might interact with genetic factors to contribute to the disorder. Very few studies to date have examined gene-environment interactions in individuals with BN. The few that have point to potentially interesting interactions between 5-HTTLPR and childhood abuse in the prediction of psychopathological symptoms, such as sensation seeking and dissocial behaviour in women with BSDs (Steiger et al., 2007; 2008). Future studies, with larger sample sizes, are

needed to corroborate such findings and further elucidate how genetic factors and environmental experiences might interact to predict risk of BN.

Clinical Implications

The present studies identify psychiatric comorbidity patterns and genetic factors that may help identify why some people with BSDs respond less well to traditional forms of psychotherapy. Study findings identifying a more psychiatrically disturbed subgroup of individuals with BN may isolate factors of clinical importance. Several authors have speculated that in such a subgroup interventions focused on eating symptoms may not be sufficient as they may be neglecting the fundamental maintaining mechanisms of the disorder, which may be linked to more general psychopathology (Steiger & Bruce, 2007; Westen & Harnden-Fischer, 2001). Adding empirical validation to the hypothesis that individuals with higher associated comorbidity do less favourably in treatment for BN, a study by Fairburn et al., (2009) examining response to Cognitive Behavioural Therapy (CBT), found that a subgroup with "complex" additional psychopathology did less well in therapy than a "less complex" subgroup.

In response to concerns that traditional CBT may not address the maintaining mechanisms underlying BN in some people, a more complex, enhanced form of CBT (CBT-E) was developed that addresses more general psychological problems that may maintain eating symptoms—mood intolerance, clinical perfectionism, low self-esteem and interpersonal difficulties (Fairburn, 2008). In the first study to examine the effectiveness of CBT-E Fairburn et al. (2009) found that within a subgroup of individuals with more complex

psychopathology those who received the enhanced CBT did better than those who received the traditional version of CBT. Interestingly though, the opposite pattern of findings emerged among less complex cases. Taken together, findings suggest that in a subgroup of individuals with BN with relatively circumscribed eating pathology a more traditional therapy focused on eating symptoms predicts the greatest response to treatment. On the other hand, adding adjunctive components to therapy aimed at specific comorbid psychopathological symptoms (e.g., mood intolerance, perfectionism) may enhance treatment response in a subgroup of individuals with BN characterized by greater psychopathology. Furthermore, evidence from our studies linking greater psychopathology to childhood abuse and hereditary factors associated with 5-HT function suggest that therapeutic adjuncts aimed at posttraumatic therapy techniques and pharmacological support may also be relevant in enhancing treatment response in a subgroup with more complex psychiatric comorbidity. Such findings highlight the importance of taking into account comorbid psychological profiles when devising treatment plans for individuals with BN.

Conclusions

Findings from the present group of studies emphasize the need to routinely test for subgroups within BN rather than assuming that BN is a homogeneous category. Study findings show that subgroups with different comorbid symptoms and disorders may display different environmental and genetic vulnerabilities and may respond differently to treatment for BN. Taken together, results highlight the

importance for both research and clinical practice to consider BN symptoms in the context of their comorbid symptoms and disorders.

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