Genetic Analysis of Bulimia Nervosa: Methods and Sample Description

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Accepted 3 September 2003

Abstract: Objective: Twin and family studies suggest that genetic variants contribute to the pathogenesis of bulimia nervosa (BN) and anorexia nervosa (AN). The Price Foundation has supported an international, multisite study of families with these disorders to identify these

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genetic variations. The current study presents the clinical characteristics of this sample as well as a description of the study methodology. **Method:** All probands met modified criteria for BN or bulimia nervosa with a history of AN (BAN) as defined in the 4th ed. of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 1994). All affected relatives met DSM-IV criteria for BN, AN, BAN, or eating disorders not otherwise specified (EDNOS). Probands and affected relatives were assessed diagnostically using both trained-rater and self-report assessments. DNA samples were collected from probands, affected relatives, and available biologic parents. **Results:** Assessments were obtained from 163 BN probands and 165 BAN probands. Overall, there were 365 relative pairs available for linkage analysis. Of the affected relatives of BN probands, 62 were diagnosed as BN (34.8%), 49 as BAN (27.5%), 35 as AN (19.7%), and 32 as EDNOS (18.0%). For the relatives of BAN probands, 42 were diagnosed as BN (22.5%), 67 as BAN (35.8%), 48 as AN (25.7%), and 30 as EDNOS (16.0%). **Discussion:** This study represents the largest genetic study of eating disorders to date. Clinical data indicate that although there are a large number of individuals with BN disorders, a range of eating pathology is represented in the sample, allowing for the examination of several different phenotypes in molecular genetic analyses. © 2004 by Wiley Periodicals, Inc. Int J Eat Disord 35: 556–570, 2004.

**Key words:** bulimia nervosa; anorexia nervosa; eating disorders; genetics; linkage analysis; affected relative pairs

**INTRODUCTION**

Bulimia nervosa (BN) is a disorder that most commonly occurs in women who are of normal body weight (American Psychiatric Association [APA], 1994). BN usually has its onset in adolescence or early adulthood and is characterized by repeated episodes of binge eating followed by inappropriate compensatory behaviors such as self-induced vomiting or misuse of laxatives. Individuals with BN place undue emphasis on weight and shape and often have comorbid disturbances of mood, anxiety, alcohol and other substance abuse, and extremes of impulse control (APA, 1994).

Developmental, social, and biologic processes influence the etiology of these disorders (Garner, 1993; Treasure & Campbell, 1994). However, the exact nature of these interactive processes is obscure. Cultural attitudes toward thinness are critical (Becker, Grinspoon, Klibanski, & Herzog, 1999), but they are not sufficient to account for pathogenesis. Dieting behavior is quite common in industrialized countries throughout the world, yet only an estimated 1.7%–2.5% of females in the general population have disordered eating behavior characterized as BN (APA, 1994). Instead, the introduction of Western ideals of thinness may serve to release a biologic propensity toward eating disorders, possibly by increasing behaviors such as dieting that may trigger the spiral of disordered eating.

Controlled family studies indicate that a vulnerability for BN is transmitted in families (Lilenfeld et al., 1998; Stein et al., 1999; Strober, Freeman, Lampert, Diamond, & Kaye, 2000). Because first-degree relatives share both genes and environments, family studies cannot differentiate genetic versus environmental causes for the observed familiality. However, with certain assumptions, studies of twins can disentangle these influences. Twin studies generally find that approximately 54%–83% of the variance in the liability to BN (Bulik, Sullivan, & Kendler, 1998; Kendler et al., 1991; Wade et al., 1999) and greater than 50% of the variability in anorexia nervosa (AN; Klump, Miller, Keel, McGue, & Iacono, 2001; Wade, Bulik, Neale, & Kendler, 2000) are attributable to the additive effects of genetic variants.
Although the confidence intervals on these estimates are generally wide, consistent findings across studies support moderate heritability of these disorders (Bulik et al., 1998). The eating disorder symptoms themselves also appear to be moderately heritable. Twin studies of binge eating, self-induced vomiting, and dietary restraint suggest that between 46% and 72% of the variance in liability of these behaviors is due to additive genetic effects (Klump, McGue, & Iacono, 2000; Sullivan, Bulik, & Kendler, 1998a). Likewise, pathologic attitudes, such as body dissatisfaction, eating and weight concerns, and weight preoccupation, show heritabilities of approximately 32%–72% (Klump et al., 2000; Rutherford, McGuffin, Katz, & Murray, 1993; Wade, Martin, & Tiggemann, 1998; Wade, Neale, et al., 1999), with some suggestion that weight concerns may be influenced by common environmental factors (Wade et al., 1998). Taken together, these findings suggest a significant genetic component to AN and BN, as well as the attitudes and behaviors that contribute to, and correlate with, clinical eating pathology.

It is important to emphasize that BN and AN are related disorders. Family studies (Lilenfeld et al., 1998; Strober et al., 2000) show that diagnoses of AN, BN, and eating disorders not otherwise specified (EDNOS) aggregate and are cross-transmitted in the first-degree relatives of subjects with AN or BN. Evidence from twin studies (Kendler et al., 1991; Weeks & Lathrop, 1995) shows that AN and BN may share genetic vulnerabilities. Moreover, approximately 50% of women with AN develop BN during the course of their illness and approximately 30% of women with BN report a history of AN (Bulik, Sullivan, Fear, & Pickering, 1997; Eckert, Halmi, Marchi, Grove, & Crosby, 1995; Garfinkel, Moldofsky, & Garner, 1980; Strober, Freeman, & Morrell, 1997). Finally, subthreshold forms of eating disorders lie on a continuum of liability with frank eating disorders (Kendler et al., 1991; Sullivan et al., 1998a). These findings suggest the existence of a broad eating disorder phenotype with possible shared genetic predispositions.

In the mid 1990s, the Price Foundation initiated the support of a multicenter, international collaborative group to study the genetics of eating disorders. From 1996 to 1998 the collaborative group collected 198 probands with AN and 237 affected relatives (Kaye et al., 2000) where the affected relative had AN or a broad-spectrum eating disorder. Our collaborative group then completed (1998–2000) the collection of BN affected relative pairs (ARP) reported in the current study. Our overarching aim has been to generate a combined AN and BN ARP dataset to understand factors that contribute to eating disorder risk as well as factors that distinguish subtypes.

Despite the evidence for heritability, psychiatric disorders are complex and the impact of any single gene is likely subtle. In our genetic studies of BN and AN, we employed several strategies to increase the likelihood of associating behavioral phenotypes with genetic variants. For example, clear entry and exclusion criteria were established for binging and purging and other eating disorder-related behaviors.

BN and AN could be a syndrome comprising multiple interrelated phenotypes with somewhat varying etiologies (Reichborn-Kjennerud et al., 2003). Therefore, a major focus of this investigation was to ensure that traits that might contribute to susceptibility to BN or AN were assessed, so that they could be utilized to enrich genetic analyses (Devlin, Bacanu, et al., 2002a). We targeted personality traits that are known to be prominent in individuals with BN and AN, but not restricted in their expression to the acute phase of the illness. Given that malnutrition can exacerbate certain traits (e.g. depression and obsessionality), we selected traits that have been identified in family members of BN (and AN) probands (Klump et al., 2000; Stein & Kaye, 2002), that persist after recovery (Casper, 1990; Collings & King, 1994; Fallon, Walsh, Sadik, Saoud, & Lukasik, 1991; Johnson-Sabine, Reiss, & Dayson, 1992; Kaye et al., 1998; Klump, Kaye, Plotnikov, Pollice, & Rao, 1999; Norring & Sohlberg, 1993; O’Dwyer, Lucey, & Russell, 1996;
Srinivasagam et al., 1995; Strober, 1980; Ward, Brown, Lightman, Campbell, & Treasure, 1998), and are moderately heritable (Tellegen et al., 1988). Several traits met these criteria, including harm avoidance, perfectionism, obsessionality, and negative emotionality (Bulik, Sullivan, Weltzin, & Kaye, 1995; Casper, 1990; Kleifield, Sunday, Hurt, & Halmi, 1994a, 1994b; Klump, Bulik, et al., 2000; O’Dwyer et al., 1996).

We provide an overview of the design and implementation of this multisite, international collaborative study designed to map genetic susceptibility loci involved in BN. Although similar in conceptualization to our previous study of AN (Kaye et al., 2000), the current study has several methodologic advancements that could enhance our ability to identify genes that contribute to the susceptibility to BN. We will focus on a description of the clinical characteristics of the BN ARP sample as well as on new or changed methods, but we will not repeat the description of methods included in our previous article on AN ARP (Kaye et al., 2000).

METHODS

Collaborative Arrangements

The current study was supported through funding provided by the Price Foundation under the principal direction of Walter H. Kaye (University of Pittsburgh). Wade Berrettini (University of Pennsylvania) provided direction for genetic methods. This initiative was developed through a cooperative arrangement among the Price Foundation, the University of Pittsburgh, and other academic sites in North America and Europe. The sites of collaborative arrangement, selected on the basis of experience in the assessment of eating disorders and geographic distribution, included the University of Pittsburgh, Cornell University, University of California at Los Angeles, University of Toronto, Rose-neck Hospital for Behavioural Medicine affiliated with the University of Munich, University of Pisa, University of North Dakota, University of Minnesota, Harvard University, and the University of Pennsylvania. Each site obtained institutional review board approval separately from its own institution’s human subjects committee.

Phenotypic Assessment

Inclusion criteria for probands and affected relatives were agreed upon through a series of consensus meetings involving all participating investigators. Acceptance into the study was not restricted by gender of the proband or affected relative and did not require active illness at the time of assessment. In addition, current use of medication did not affect the eligibility of probands or affected relatives.

For probands, acceptance into the study required that they had a lifetime diagnosis of BN, purging type, as defined in the 4th ed. of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 1994). Purging had to include regular vomiting, with other means of purging optional, and binging and vomiting must have occurred at least twice a week for a duration of at least 6 months. In addition, they were to be 13–65 years old and primarily of European descent. A current or lifetime history of AN was acceptable (i.e., BN with a history of AN [BAN]). Potential probands were excluded if they had a lifetime history of any of the following: mental retardation, dementia, organic brain syndromes, psychotic disorders, Turner’s syndrome, or any medical condition that could affect appetite, body weight, or eating (e.g., diabetes and thyroid conditions were excluded if the onset of the disease preceded the onset of the eating disorder). Bipolar I and Bipolar II were excluded only if symptoms of BN occurred exclusively during manic or hypomanic
episodes. Probands with neurologic disorders, such as seizure disorder, were excluded unless seizures developed as a result of trauma and clearly after the onset of the eating disorder. Individuals whose premorbid weight exceeded the body mass index (BMI) for the 95th percentile for their age and gender for a comparable epidemiologic sample (Hebebrand, Himmelmann, Heseker, Schafer, & Remschmidt, 1996) or whose high lifetime BMI > 35 were excluded from the study.

Affected relatives were biologically related to the proband (e.g., siblings, half siblings, cousins, aunts, uncles, grandparents, second cousins). Inclusion criteria for affected relatives were age between 13 and 65 years and at least one of the following lifetime eating disorder diagnoses: (1) DSM-IV BN, purging or nonpurging type; (2) DSM-IV AN, restricting type or binge eating/purging type (criteria were modified for this study to include individuals with and without amenorrhea); (3) EDNOS-1, defined as subclinical AN with the presence of at least two of the three criterion symptoms of low body weight, extreme fear of fatness, or body image disturbance (i.e., undue influence of body weight and shape on self-evaluation or denial of the seriousness of low body weight), no lifetime history of binge eating, and a lifetime ideal body weight (IBW) < 125% according to the Metropolitan Height and Weight Tables (1959); (4) EDNOS-2, defined as subthreshold BN in which the frequency or duration of eating binges and/or purging fell below the specified DSM-IV criteria (twice per week and 3 months, respectively); and (5) EDNOS-3, defined as a clinical mix, in which individuals of normal weight purged (e.g., vomited or abused laxatives, diuretics, enemas), fasted, or exercised excessively due to extreme fear of weight gain or undue influence of body weight on self-esteem, but did not binge eat. Exclusion criteria for affected relatives included all exclusion criteria listed for the probands with the following additional criteria: (1) monozygotic twin of the proband; (2) biologic parent with an eating disorder, unless there was another family member with whom the proband could be paired; and (3) a diagnosis of binge eating disorder as the only lifetime eating disorder diagnosis.

**Screening and Diagnostic Procedures**

An initial screening was performed to determine study suitability for each potential proband. A preliminary verification of the diagnosis of BN was undertaken and eating disorder histories on possible affected relatives were obtained. If probands satisfied all inclusion and no exclusion criteria and reported a possible history of an eating disorder in a nonparent, nonchild, or a nonmonozygotic twin blood relative, the proband was asked to discuss the study with the affected relative and obtain permission for study personnel to contact the relative to explain the study and screen for eligibility. If, upon initial screen, both proband and affected relative satisfied all inclusion and exclusion criteria, informed consent was obtained and both were scheduled to complete a series of clinical interviews. In addition, the proband and affected relative were told they would be mailed a packet of self-report assessments to be completed before the interviews. If participants were in close proximity to one of the multicenter sites, the interviews and blood draw were completed in person. Subjects who were unable to travel to a site were interviewed by phone and had their blood drawn at a local hospital or doctor’s office and sent to the site by overnight mail.

**Assessment Instruments**

Many of the same assessment instruments were used in both the AN and BN ARP studies (Table 1). However, some new instruments were added to the BN ARP study as described below.
Eating Disorder Phenotypes and General Clinical Information

For both studies, the lifetime diagnoses of eating disorders of probands and affected relatives were characterized by a modified version of the Structured Interview of Anorexia Nervosa and Bulimic Syndromes (SIAB; Fichter, Herpertz, Quadflieg, & Herpertz-Dahlmann, 1998). In addition, for the BN ARP study, eating disorder diagnoses were made by an expanded version of Module H of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), to obtain information such as age of onset, severity of illness, recovery status, and months since last eating disorder symptoms. The Yale-Brown-Cornell Eating Disorder Scale (YBC-EDS; Sunday, Halmi, & Einhorn, 1995) was used to assess the severity and types of core obsessions and compulsions specific to eating disorders. Additional information obtained from probands and affected relatives included gender, date of birth, height, and weight history. BMI (kg/m²) was based on self-reports of height and weight. Values were calculated for the current, minimum, and maximum BMI. Subjects responded to demographic questionnaires designed to elicit information about ethnicity, marital status, religious orientation, family structure, occupation, and education. The Eating Disorder Inventory-2 (EDI-2; Garner, 1990), which was included in the AN ARP study, was deleted from the BN ARP study because we determined that similar information was adequately characterized by other assessments and we wanted to make time for the addition of other new instruments.
**Personality and Temperament**

Several self-assessment instruments were used in both the AN and BN ARP studies. These included the Multidimensional Perfectionism Scale (MPS; Frost, Marten, Lahart, & Rosenblate, 1990) and the Temperament and Character Inventory (TCI; Cloninger, Svrakic, & Przybeck, 1993). The MPS assesses six specific dimensions of perfectionism, including concern over mistakes, high personal standards, high perceived parental expectations, high perceived parental criticism, doubts about quality of performance, and organization, order, and precision. The TCI measures seven dimensions of personality, including novelty seeking, harm avoidance, reward dependence, persistence, self-directedness, cooperativeness, and self-transcendence.

In addition, several new instruments were added to the BN ARP study. The Revised NEO Personality Inventory (NEO PI-R; Costa & McCrae, 1992), a 243-item questionnaire based on the Five-Factor model of personality, was added to obtain a measure of neuroticism. It measures the five major domains of personality (neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness), as well as the six facets or traits that define each domain and permit more detailed analysis. The NEO PI-R has shown excellent internal consistency and long-term stability. To characterize impulsivity, we added the Barrett Impulsivity Scale-11 (BIS-11; Barrett, 1983). The BIS is a 30-item self-report measure of impulsiveness, currently in its 11th revision. This scale provides three measures of impulsivity: motor, cognitive, and nonplanning. No measures, of which we are aware, specifically assess extremes of impulsivity in women. Bulik, Sullivan, Fear, and Joyce (1997) have shown that this measure successfully discriminates the degree of impulse control in subgroups of women with eating disorders.

Considerable data suggest that specific types of personality disorders may commonly cluster in subtypes of eating disorders, for example, obsessive-compulsive personality disorder (OCD) in restricting-type AN (Lilenfeld et al., 1998) and Cluster B disorders in multiimpulsive BN subjects (Rossiter, Agras, Telch, & Schneider, 1993). Therefore, the BN ARP subjects completed the Structured Clinical Interview for DSM-IV Personality Disorders (SCID-II; First et al., 1996), a semistructured clinical interview used to diagnose the DSM-IV personality disorders. We assessed only Cluster B and C personality disorders because Cluster A disorders occur much less commonly in women with eating disorders.

**Comorbid Psychiatric Disorders**

Both clinical and epidemiologic studies indicate that women with BN and AN, and their first-degree relatives, commonly suffer from comorbid anxiety disorders (particularly OCD) and major depression (Lilenfeld et al., 1998). These symptoms often persist after recovery from an eating disorder (Kaye et al., 1998; Stein & Kaye, 2002). In addition, women with BN and their family members have increased risk for substance use disorders (Bulik, 1987; Kaye et al., 1996). Twin studies suggest shared genetic liability between BN and major depression (Walters & Kendler, 1995) and between BN and panic disorder (Kendler et al., 1995). Therefore, we included a comprehensive assessment of mood disorders, anxiety disorders (including OCD), and substance use disorders in probands and relatives to further characterize potential phenotypic and genetic heterogeneity within the sample.

Similar to the AN ARP study, subjects in the BN ARP study completed the State-Trait Anxiety Inventory (STAI Form Y-1; Spielberger, Gorsuch, & Lushene, 1970) and the Yale-Brown Obsessive Compulsive Scale (Y-BOCS; Goodman et al., 1989). The STAI is a 40-item instrument that assesses states of anxiety both “at this moment” and how the individual “generally feels.” The Y-BOCS is a semistructured interview designed to
rate the presence and severity of obsessive thoughts and compulsive behaviors typically found among individuals with OCD.

New instruments added to the BN ARP study included the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), a 21-item questionnaire that has been widely used to assess the intensity and symptoms of depression in adolescents and adults. To characterize lifetime major Axis I disorders, such as depression, anxiety, and substance use disorders, subjects in the BN ARP study completed the SCID-I. The SCID-I is a semistructured clinician-administered interview designed to generate DSM-IV Axis I diagnoses. Interrater reliability for the SCID has been reported to be generally consistent with that of other diagnostic instruments for Axis I disorders (First et al., 1996). The Fagerstrom Test of Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) was used to obtain quantitative and diagnostic information about nicotine dependence. The FTND uses six of the original eight questions from the Fagerstrom Tolerance Questionnaire (FTQ; Fagerstrom & Schneider, 1989).

**Samples and Analysis**

Samples of blood were collected from each proband, affected relative, and participating biologic parent. DNA samples were extracted using Gentra Systems (Minneapolis, MN) reagents according to the manufacturer’s specifications. Kaye et al. (2000) provided a detailed discussion of the rationale for the current study design and planned genetic analysis. Our initial target of 400 affected sibling pairs/ARP was based on power analyses of Hauser, Boehnke, Guo, and Risch (1996). They showed that such a sample would yield good power to detect variants in genes having a modest impact on liability. Even though we did not meet this target, the power to detect genetic variants in genes having a modest impact on liability should be adequate.

**RESULTS**

Assessment data were collected from 360 probands and their families. All probands were female with a lifetime diagnosis of purging-type BN. By design, their compensatory behaviors must have included vomiting, but additional methods of purging were acceptable. In the final analysis, however, 8 probands did not meet modified DSM-IV criteria for purging bulimia. All had blood samples collected and 6 had at least one affected relative. These 8 subjects were excluded from the analyses. Of the 352 probands who met the study diagnostic criteria, 342 had blood samples collected and 331 had an affected relative, resulting in a total of 328 probands with blood samples collected who also had at least one affected relative in the study. These probands had 365 affected relatives, excluding parents, participating in the study. Of the affected relatives, 254 were full siblings to the proband and 111 had some other relationship. There were 318 nonindependent affected sibling pairs among the families, as well as numerous pairs of other, more complex relationships. Among the affected relatives, 15 were males with the following diagnoses: 3 AN, 3 BAN, 4 BN, 1 EDNOS-1, 1 EDNOS-2, and 3 EDNOS-3 subjects. DNA samples were collected from the biologic parents of the proband when available. Both parents participated in 22% of families and one parent participated in 19.5%. The majority of these families were included in the molecular analysis for the genome scan (97.6%). The DNA samples from the other families should prove useful for other linkage/association analyses. The remaining 2.4% were collected subsequent to the initiation of the scan.

Table 2 summarizes the eating disorder diagnoses of the proband ARP, after dividing probands into those with BAN or without such a history (BN). When only probands with
BN were considered, 34.8% of their relatives had a diagnosis of BN, whereas 27.5% had a diagnosis of BAN and 19.7% had a diagnosis of AN. When only BAN probands were considered, 22.5% of their relatives had a diagnosis of BN, whereas 35.8% had a diagnosis of BAN and 25.7% had a diagnosis of AN. It is apparent from these percentiles and the data in Table 2 that there is a tendency of concordance of diagnosis, with BN probands being somewhat more likely to identify other BN relatives and BAN probands being somewhat more likely to identify relatives with a history of AN. This trend is weakly significant (odds ratio \( OR = 2.0, df = 1, \chi^2 = 8.18, p = .029, \) number of pairs = 303).

There was no significant difference in age between BN and BAN probands (Table 3). As expected, BAN probands had significantly lower BMIs for measures of current, past minimum, and past maximum values.

When affected relatives were stratified by eating disorder subtype, measures of BMI differed significantly between strata (Table 4). BAN relatives were similar to AN relatives in terms of past minimum BMI. Given the diagnostic criteria for AN, it was expected that relatives with a history of AN would have a lower past minimum BMI, irrespective of additional eating disorder diagnoses. With regard to current and past maximum BMI, BAN relatives had a significantly lower BMI than BN relatives; they were significantly higher than AN relatives for past maximum BMI. BN relatives had significantly higher values than AN and BAN relatives on all measures of BMI.

When the AN and BN ARP datasets were combined (Table 5), there were a total of 594 relative pairs. With regard to pairs in which both had the same diagnosis, there were 86 AN-AN pairs, 67 BAN-BAN pairs, and 62 BN-BN pairs. In terms of mixed pairs, the most common pairs were AN-BAN \( (n = 107) \) followed by BAN-BN \( (n = 91) \) and then AN-BN \( (n = 82) \). A total of 1,188 individuals had fairly equally distributed numbers of AN, BAN, and BN diagnoses.

### DISCUSSION

The primary aim of the current study is to identify some of the genetic variants that influence susceptibility to BN. Our design has several strengths. First, by multisite,
multinational collaboration, we recruited a large sample of multiplex families despite the relatively low prevalence of BN in the general population. Second, by collecting DNA from available parents, we increased the power for linkage analysis, while also generating a substantial sample for family-based association analysis (Spielman, McGinnis, & Ewens, 1993). Third, by the use of structured diagnostic instruments and extensive training and reliability checks across sites, clear eating disorder diagnoses were obtained that will enable stratification on the basis of the presence or absence of certain core features. Fourth, by requiring that probands meet rigorous inclusion criteria, including vomiting as the primary compensatory behavior, and by using obesity as an exclusionary criterion, we attempted to ensure diagnostic homogeneity in the BN probands. Finally, by assessing a wide range of psychological traits and behaviors associated with BN, our objective was to enhance the search for genetic variants by using these traits as covariates (Devlin, Bacanu, et al., 2002; Devlin, Jones, Bacanu, & Roeder, 2002a) or quantitative endophenotypes in the linkage and association analyses.

Table 4. Characteristics of affected relatives (n = 365), stratified by eating disorder

<table>
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<tr>
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<th>AN ARP Study</th>
<th>BN ARP Study</th>
<th>Individual Subjects</th>
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<tr>
<td></td>
<td>AR N Pairs</td>
<td>AR N Pairs</td>
<td>Individual Subjects</td>
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<tr>
<td>AN</td>
<td>N = 83</td>
<td>N = 116</td>
<td>N AN N BAN N BN N EDNOS N Total</td>
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<tr>
<td>BAN</td>
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<td>86 172</td>
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<tr>
<td>BN</td>
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<td>EDNOS</td>
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<tr>
<td>Current BMI</td>
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<tr>
<td>Age (years)</td>
<td>26.6 ± 9.1</td>
<td>29.6 ± 9.7</td>
<td>29.0 ± 9.7 25.3 ± 10.1 27.0 ± 7.6 27.1 ± 8.5</td>
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<tr>
<td>Current BMI</td>
<td>19.1 ± 2.0</td>
<td>20.1 ± 2.5</td>
<td>23.0 ± 3.2 19.8 ± 1.5 22.6 ± 2.5 22.1 ± 2.8</td>
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<td>Past minimum BMI</td>
<td>15.2 ± 1.6</td>
<td>15.5 ± 1.8</td>
<td>19.6 ± 1.9 18.3 ± 0.9 19.9 ± 1.4 18.8 ± 1.8</td>
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<tr>
<td>Past maximum BMI</td>
<td>21.7 ± 2.5</td>
<td>22.8 ± 2.8</td>
<td>25.9 ± 3.4 21.0 ± 2.1 26.2 ± 2.9 23.9 ± 3.0</td>
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</tbody>
</table>

Note: BN = bulimia nervosa; BAN = bulimia nervosa with a history of anorexia nervosa; AN = anorexia nervosa; EDNOS = eating disorder not otherwise specified; BMI = body mass index. Group differences were calculated using generalized estimating equation analyses.

Table 5. Attributes of both the AN and BN ARP genetic studies

<table>
<thead>
<tr>
<th></th>
<th>AN ARP Study</th>
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<th>Individual Subjects</th>
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<td>AR N Pairs</td>
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<td>BAN</td>
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<td>BN</td>
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<tr>
<td>EDNOS</td>
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<td>91</td>
<td>91 91 91 91 91</td>
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<tr>
<td>Total</td>
<td>229</td>
<td>365</td>
<td>594 398 362 329 99 1188</td>
</tr>
</tbody>
</table>

Note: ARP = affected relative pair; AR = affected relatives; N = number of pairs or individuals; BN = bulimia nervosa; BAN = bulimia nervosa with a history of anorexia nervosa; AN = anorexia nervosa; EDNOS = eating disorder not otherwise specified.
There are also limitations to this study. Several of the questionnaires (e.g., BIS-11, FTND) were added to the assessment battery subsequent to the initial collection of data and, therefore, were not completed by all subjects. In addition, based on the low prevalence of BN, we had to expand inclusion criteria for affected relatives to include AN, BN, and EDNOS diagnoses. Inclusion of multiple diagnoses among relatives could reduce power to detect liability genes of small effect by introducing greater etiologic heterogeneity. We also recruited families from disparate locations, which could introduce additional genetic heterogeneity. However, results from our previous studies suggest that heterogeneity due to geographic separation will be small. Specifically, by evaluating the distributions of alleles at hundreds of genetic markers, Devlin et al. (2002a) found minimal differentiation among the seven populations sampled in our AN study (Kaye et al., 2000).

With regard to diagnostic heterogeneity of the BN ARP dataset, the majority of ARP have a lifetime diagnosis of BN (60.2%), with a much smaller percentage having a lifetime diagnosis of AN (22.7%) or EDNOS (17.0%). Aside from expected differences in BMI based on diagnostic categories, there are few significant differences among eating disorder subtypes in probands, relatives, and between probands and relatives.

Although we plan linkage and association analyses using the broad phenotype (i.e., the entire sample), we are considering other strategies to diminish etiologic heterogeneity. For example, when we used the broad diagnostic category for AN in a previous linkage study (Grice et al., 2002), we did not yield evidence for even suggestive linkage (Lander & Kruglyak, 1995) anywhere in the genome. However, by analyzing 37 families that had at least one pair of relatives with the narrow phenotype of restricting-type AN, we found evidence for linkage to chromosome 1p34 (Grice et al., 2002). Additional microsatellite genotyping increased evidence for linkage, with a nonparametric linkage score \( \frac{3.45}{(p/C24.00028)} \) near marker D1S255. Additional single nucleotide polymorphisms in candidate genes enhanced the linkage signal further (Bergen et al., in press).

Although we are considering linkage analysis using only narrow phenotypes, such as families with pairs diagnosed with BN (or BAN), we have some reservations. As is evident in Table 2, BN-BN and BAN-BAN pairs are not common, nor does proband status appear to be a potent discriminator of relative pair status. By contrast, when we recruited AN probands, 63.3% of the affected relatives had a diagnosis of AN (Kaye et al., 2000). Therefore, we continue to evaluate the data for critical subsets. For example, because clear behavioral indicators, such as self-induced vomiting, improve the reliability of the diagnosis of BN (Wade et al., 1999) and because vomiting behavior has been shown to be a strongly heritable component of the BN syndrome (Sullivan et al., 1998a, 1998b), an intriguing subset could be families in which multiple individuals exhibit severe and persistent self-induced vomiting.

Another avenue to diminish etiologic heterogeneity is through the use of covariates in the linkage analysis, an approach we used to good effect in our analysis of the AN linkage sample (Devlin et al., 2002a). In that study, we identified two covariates that appeared to create discernible subpopulations among the AN affected sibling pairs: drive for thinness and obsessionality. It seemed plausible to us that affected sibling pairs who are congruently high for these covariates could have a distinct etiologic basis compared with affected sibling pairs without such scores. When we incorporated these covariates into the affected sibling pair linkage analysis by using the methods of Devlin et al. (2002a,b), we found several regions of suggestive linkage: one close to genome-wide significance on Chromosome 1 (at 202 cM, D1S1660; logarithm of the odds [LOD] = 3.46, \( p = 0.00003 \)), another on Chromosome 2 (at 102 cM, D2S1779; LOD = 2.22; \( p = 0.00070 \)), and a third region on Chromosome 13 (at 102 cM, GATA121A08; LOD = 2.50; \( p = 0.00035 \)). We will explore the battery of
psychological traits and behaviors collected in the BN sample to determine if any show evidence for defining noteworthy subpopulations. If we find evidence for subpopulations defined by covariates, we will use these covariates in linkage analyses.

The AN ARP study previously reported (Kaye et al., 2000) and the current BN/BAN ARP study used similar diagnostic criteria for all of the eating disorders. In addition, many of the assessment instruments were used in both studies (Table 1). Finally, both samples were genotyped using the Weber Screening Set 9. These similarities should facilitate combining information over datasets. The total number of ARP and individual subjects for the combined datasets is shown in Table 5. Weighing against a simple combination of the data is the possibility of introducing even greater etiologic heterogeneity, which could overwhelm linkage signals. Therefore, although we plan to combine the AN and BN ARP datasets in some way, most likely through the use of common, informative covariates, the decision about exactly how they will be combined awaits further phenotypic analysis. We are currently investigating multivariate methods to explore the high dimensional phenotypic space to identify possible covariates.

Relatively little is known about risk factors in eating disorders and hypotheses for disentangling complex phenotypes remain speculative. Nonetheless, some intriguing hypotheses have been proposed, and they could be useful for frameworks for genetic analyses. For example, Westen and Harnden-Fischer (2001) proposed that three populations of eating disorder individuals exist, which cross subtype boundaries: a high functioning, perfectionistic group; a constricted, overcontrolled group; and an emotionally dysregulated, undercontrolled group. Studies from our laboratory are consistent with family (Lilenfeld et al., 1998; Strober et al., 2000) and twin data (Kendler et al., 1991; Walters & Kendler, 1995) suggesting that AN and BN share common susceptibility factors. For example, women who have recovered from AN and/or BN show a similar persistence and magnitude of body image distortion, perfectionism, anxiety, harm avoidance, obsessions with symmetry and exactness, as well as disturbances of serotonin activity (Kaye, 1997; Kaye et al., 1998).

Together, these data suggest that such clusters of traits create susceptibility to AN or BN, with additional factors determining the exact presentation (subgroup) of the eating disorder. For example, extreme self-control, asceticism, and inflexibility in restricting-type AN may be related to additional traits for OCD (Lilenfeld et al., 1998) and disturbances of dopamine systems that may modulate reward (Kaye, Frank, & McConaha, 1999).

In summary, the current project is the largest genetic study of eating disorders to date. Using the data collected in this study and our earlier study (Kaye et al., 2000), our goal is to refine the clinical picture of eating disorders and, in doing so, provide an additional theoretic framework for genetic analyses.

The authors thank the Price Foundation for the support of the clinical collection of subjects and genotyping. The authors are indebted to the participating families for their contribution of time and effort in support of this study and to Eva Gerardi for administrative support.

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