ORIGINAL ARTICLES

A Search for Susceptibility Loci for Anorexia Nervosa: Methods and Sample Description

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Background: *Eating disorders have not traditionally been viewed as heritable illnesses; however, recent family and twin studies lend credence to the potential role of genetic transmission. The Price Foundation funded an international, multisite study to identify genetic factors contributing to the pathogenesis of anorexia nervosa (AN) by recruiting affective relative pairs. This article is an overview of study methods and the clinical characteristics of the sample.*

Methods: *All probands met modified DSM-IV criteria for AN; all affected first, second, and third degree relatives met DSM-IV criteria for AN, bulimia nervosa (BN), or eating disorder not otherwise specified (NOS). Probands and affected relatives were assessed diagnostically with the Structured Interview for Anorexia and Bulimia. DNA was collected from probands, affected relatives and a subset of their biological parents.*

Results: *Assessments were obtained from 196 probands and 237 affected relatives, over 98% of whom are of Caucasian ancestry. Overall, there were 229 relative pairs who were informative for linkage analysis. Of the proband-relative pairs, 63% were AN-AN, 20% were AN-BN, and 16% were AN-NOS. For family-based association analyses, DNA has been collected from both biological parents of 159 eating-disordered subjects. Few significant differences in demographic characteristics were found between proband and relative groups.*

Conclusions: *The present study represents the first large-scale molecular genetic investigation of AN. Our successful recruitment of over 500 subjects, consisting of affected probands, affected relatives, and their biological parents, will provide the basis to investigate genetic transmission of eating disorders via a genome scan and assessment of candidate genes.* Biol Psychiatry 2000;47:794–803 © 2000 Society of Biological Psychiatry

Introduction

Anorexia nervosa (AN) is characterized by an obsessively morbid dread of body fat that drives, then sustains, an unrelenting avoidance of normal body weight (American Psychiatric Association 1994). It is widely assumed to have a complex, multifactorial etiology, yet psychological theories of pathogenesis have largely prevailed in explanatory paradigms put forward in recent decades. Likewise, social influences have been implicated in its causation in the light of mainstream cultural attitudes in industrialized countries favoring thinness as a beauty ideal.

Although a role for causal psychosocial factors is not in question, certain well-established observations regarding AN presentation argue persuasively for its qualitative distinction from mere extreme dieting, and against an etiological focus on nonbiological determinants alone in accounting for its development, progression, and morbidity. For example, weight-loss efforts in the general female population are frequent, yet AN is hardly a common illness—its lifetime prevalence among females is estimated to be 0.1%–0.7% (Hoek 1998). Second, in the majority of acutely ill patients, dietary restriction is highly entrenched, and resistance to initial therapeutic intervention is reflected in an unusually protracted time to achieve full remission of illness (Strober et al 1997). Third, AN appears to covary with other psychopathological risk

factors, as it occurs frequently in association with Axis I psychiatric disorders, mood and anxiety disorders in particular (Lilenfeld et al 1997), that have no evident connection to cultural attitudes relating to body weight. Fourth, neurobiological abnormalities have been documented that persist in a proportion of cases after normal body weight has been restored (Kaye and Strober 1999). These aspects of AN thus underscore convincingly the validity of comprehensive and integrative paradigms designed to elucidate the nature and identity of discrete risk and vulnerability factors in its etiology and pathogenesis.

Moreover, for eating disorders, several convergent lines of evidence suggest greater familial transmission of eating disorders than dieting, making a search for genes conferring susceptibility to AN warranted. First, psychometric studies (Brewerton et al 1993; Bulik et al 1995; Kaye and Strober 1999; O'Dwyer et al 1996; Sohlberg and Stober 1994; Srinivasagam et al 1995; Strober 1980; Vitousek and Manke 1994; Von Ranson et al in press) have consistently linked the illness to a cluster of moderately heritable (Heath et al 1994) personality and temperamental traits, specifically obsessionality, perfectionism, neophobia, and harm avoidance. In this regard, it has been speculated (Strober 1991, 1995; Vitousek and Manke 1994) that phenotypic similarities between these traits and the rigidly persevering, obsessional, and anxiety-reducing character of the anorexic's dietary restraint may be based on shared genetic and environmental factors. Second, recent controlled family studies (Gershon et al 1984; Lilenfeld et al 1998; Strober et al 1990, in press) have found a higher lifetime prevalence of AN or subthreshold cases of eating disorder in first-degree relatives of probands (3%–12%) than in the first-degree relatives of psychiatric and normal controls (0%–3.7%), and a higher prevalence of obsessional personality traits, suggesting that obsessional traits may manifest the effects of a genotype that increases susceptibility to AN. Consistent with family study findings are the results of twin studies (Holland et al 1984, 1988; Treasure et al 1989) which have obtained narrow sense heritability estimates ranging from 54% to 80%. Moreover, studies designed to model genetic and environmental effects on abnormal eating attitudes in large populations of clinically unaffected twins have shown that between 40% and 60% of the variance in liability to these behaviors is attributable to additive genetic influences (Klump et al, in press; Rutherford et al 1993; Wade et al 1998).

In this article, we present an overview of the design and implementation of a multisite, international collaborative study designed to map genetic susceptibility loci involved in AN. We also report on the clinical characteristics of our sample.

Methods and Materials

Collaborative Arrangements

Under the principal direction of Walter Kaye of the University of Pittsburgh, and supported through funding provided by The Price Foundation in Geneva, Switzerland, this initiative was developed through a cooperative arrangement between The Price Foundation, the University of Pittsburgh, and other academic sites in North America and Europe. The number and location of sites were determined by several factors. First, we needed academic collaborators who were experienced in assessing eating-disordered individuals in order to establish consistent behavioral assessment procedures across sites. Second, we had to find sites with records of 1000 to 1500 individuals who had previously been treated for AN over the past 10 or more years, because programs typically treat less than 100–200 AN patients per year. Thus, the only way to gather the necessary pool of subjects was to contact those people who had previously been in treatment at several well-established eating disorders treatment centers. Third, sites needed to be geographically distributed such that they could advertise and collect subjects from a region of several hundred miles or more. Six sites were identified that had well-established programs for the treatment of AN. The clinical sites included University of Pittsburgh (WK), Cornell University (KH), University of California at Los Angeles (MS), University of Toronto (AK and BW), University of London (JT), and University of Munich (MF). An additional site included for its expertise in molecular genetic research on psychiatric illness was at Thomas Jefferson University (WB). A small number of subjects were recruited at the Thomas Jefferson University site as well.

The core site at Pittsburgh, in collaboration with all of the investigators, developed a model protocol for ascertaining probands with AN who might have an affected blood relative (excluding parents), conducting psychological assessments, collecting peripheral blood samples for DNA, and obtaining informed consent. The model protocol was sent to each individual site to be modified as necessary by each site's principal investigators and Institutional Review Board (IRB). IRB approval was obtained separately by each site from its own institution's Human Subjects Committee. Additional mechanisms common to each site included identification of potential subjects through clinic databases, referral from clinicians with knowledge of the study, and advertisement in a variety of different media at local and national levels. Following informed consent and completion of all evaluative procedures, qualified subjects gave peripheral blood samples that were first maintained locally, then later shipped in bulk to Dr. Berrettini's laboratory for DNA extraction, genotyping, and quantitative genetic analyses. Under terms of this collaboration, all clinical and descriptive data collected at participating sites were transmitted to a Data Analytic Core at University of Pittsburgh for cleansing, storage, and statistical analysis.

Study Design

We initially entertained several different approaches to ascertaining a sample that was suitable for a genome-wide scan of susceptibility loci and for evaluating specific candidate genes. It was quickly decided that searching for single large affected kindreds or large extended multiplex families would be too labor

intensive and unjustifiably costly, because it was the shared experience of the investigators that such families were rare. Moreover, it seemed intuitively likely that reduced fertility in severely ill probands would diminish the likelihood of identifying a sufficiently large pool of multiplex families informative for linkage analysis (Risch 1983). We additionally assumed that AN, like other psychiatric disorders, was a complex phenotype with non-Mendelian inheritance. Based on these considerations, we decided that ascertainment of affected relative pairs would be an optimal genetic design in that the data can be analyzed with (genetic) model-free methods (Risch 1990a, 1990b, 1990c) and it is possible to accrue a sufficiently large sample for genetic analysis. To enhance the power of identity-by-descent (IBD)-based linkage analyses and to allow for family-based association tests of candidate gene polymorphisms (e.g., Spielman et al 1993), every effort was made to obtain blood from parents of each proband and affected relative.

Two controlled family studies of AN (Gershon et al 1984; Strober et al 1990) found an average 3% lifetime risk of AN in first-degree relatives of probands compared to 0% risk in roughly 1000 relatives of control subjects. Based on a sample of size 1000, with no observed cases, the true rate is estimated to be less than .003 with 95% confidence. This suggests an approximate relative risk of at least 10, perhaps much larger. Loci having large, individual effects on liability to complex traits have yet to be identified (see Corder et al 1993; Davies et al 1994; Pericak-Vance et al 1991; Tsai et al 1994 for possible exceptions). Based on these results and our own work on AN, we believe it is also highly unlikely that a single gene is responsible for mediating this relative risk in AN. Accordingly, we assumed that any single AN susceptibility locus increased relative risk by a small factor, probably between one- and two-fold.

Even if a liability locus were to confer a relative risk of 3, we would still need a substantial sample to achieve a significant finding of linkage between the gene and a proximate marker (or markers). For example, Hauser et al (1996) have shown that approximately 200 affected sibling pairs are needed to have >95% power to detect linkage (LOD score > 3, where the LOD is defined as the log base 10 of the likelihood ratio for linkage versus nonlinkage). On the other hand, if one is willing to take a lesser LOD score as defining regions of interest for further genetic study (*candidate regions*), then smaller samples are required. Based on considerations of cost of sample ascertainment and power, we decided a sample size of 200 affected relative pairs would be a reasonable initial goal. Such a sample gives us good-to-excellent power to define candidate regions. Our approach to finding candidate regions and their subsequent treatment will be described under "Genotyping and Genetic Analyses."

Diagnostic Categories

Agreement on inclusionary diagnostic criteria for probands and affected relatives was reached through a series of consensus meetings involving all participating Co-Principal Investigators. Acceptance into the study was not restricted by gender of proband or relative, and did not require active illness at the time of assessment. Only probands and affected relatives who were evaluated by personal interview were accepted into the study. For probands, our

ascertainment rule for acceptance into the study required they have the following: (1) an unequivocal lifetime "core" diagnosis of AN by DSM-IV criteria, waiving the single criterion of amenorrhea for 3 consecutive months, because some subjects were menstruating due to treatment with exogenous hormone replacement, and some were male; (2) age between 13 and 65 years; and (3) fulfillment of the criteria of AN for not less than 3 years prior to ascertainment. Potential probands were excluded if they had an onset of AN after age 25, or if they had a lifetime history of any of the following: organic brain syndrome; IQ less than 70; dementia, schizophrenia; bipolar illness; obesity; medical illness that could affect appetite, eating behavior, or body weight (e.g., diabetes); binge eating disorder (eating binges without inappropriate compensatory behaviors); and "regular" binge eating. The latter was defined as bingeing at least once weekly for 3 or more consecutive months. Binge eating is not uncommon in AN, developing in some 30%–50% of patients, most often within 3 years after onset of abnormal weight loss (Bulik et al 1997; Strober et al 1997). Nevertheless, our consensus opinion was that binge eating be excluded from the core phenotype so that selected probands comprised as diagnostically homogeneous a group as possible. The afore mentioned exclusion criteria were based on the expert clinical diagnosis of each sites' Co-Principal Investigator. No inclusion or exclusion criteria were applied to parents in the screening or selection of probands or affected relatives, so that affected relative pairs were ascertained without knowledge of parental history of eating disorder, or psychopathology in general.

Affection status of relatives ranged from narrow to broad. The narrow status entailed the same core phenotype of AN applied to proband selection, including the same exclusionary criteria. The broadened phenotype allowed for the presence of AN with binge eating, and also included bulimia nervosa (BN) defined by DSM-IV criteria, as well as one of three subthreshold eating disorder not otherwise specified (ED-NOS) diagnoses derived from an algorithm applied to our main assessment interview. These included subclinical AN, requiring at least two of the three criterion symptoms of low body weight, fear of fatness, or body image disturbance, undue influence of body weight and shape on self-evaluation, or denial of the seriousness of low body weight; subclinical bulimia nervosa, wherein the frequency or duration of eating binges and/or purging fell below the specified criteria (twice per week and 3 months, respectively); and subclinical mixed, including relatives who were normal weight but reported either purging behavior (self-induced vomiting or use of laxatives/diuretics) or excessive exercise or periods of fasting due to extreme fear of weight gain or undue influence of body weight on self-esteem. Exclusionary criteria applied to affected relatives included organic brain syndrome, IQ less than 70, dementia, schizophrenia, binge eating disorder, obesity, or any medical disorder that could affect appetite, body weight, or eating habits. The rationale for employing a broadened phenotype for selecting affected relatives was based on evidence from family studies (Lilenfeld et al 1998; Strober et al in press) that BN and ED-NOS diagnoses aggregated in first-degree relatives of AN probands, and evidence from twin studies (Kendler et al 1991; Walters and Kendler 1995) that AN and BN may share genetic vulnerabilities in common.

Evaluative Procedures

Potential subjects were first screened to determine study suitability. If a likely proband denied a family history of eating disorder, or refused permission to contact possibly ill relatives, the screening was terminated. Otherwise, a preliminary verification of the diagnosis of AN was undertaken and eating disorder histories on possibly affected relatives were obtained. If probands satisfied all inclusion and exclusion criteria and gave a history suggestive of eating disorder in a non-parent, non-child, and 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 for informed consent. Study personnel then contacted the relative who was screened for eligibility. If the relative fulfilled entrance criteria, both the proband and affected relative were scheduled for the complete battery of evaluative procedures. At the time that the interviews were scheduled, the proband and affected relative were told that they would be mailed a packet of self-rating assessments. They were asked to complete the assessments and bring the packet to the interview. For those probands and relatives who were not able to come to the satellite center for an in-person interview, the interview was conducted by telephone and the self-rating assessments were returned by mail. Blood samples were drawn on site for subjects whose interviews were done in person; subjects interviewed by phone had their blood drawn at a local hospital or doctor's office and sent to the site by overnight mail.

Assessment Instruments

The assessment battery was selected to facilitate diagnoses of eating disorders (AN, BN, ED-NOS) and to assess psychological and personality features that have been shown to be associated with, and may underlie vulnerability to, eating disorders. We were especially interested in capturing traits that tended to persist rather than being present only during the acute stage of the illness. A series of studies (Brewerton et al 1993; Bulik et al 1995; Kaye et al 1998; Kleifield et al 1993; O'Dwyer et al 1996; Srinivasagam et al 1995; Von Ranson et al in press) have used the Tridimensional Personality Questionnaire (or the revised version, the Temperament and Character Inventory), the Yale-Brown Obsessive Compulsive Scale (Y-BOCS), the Multidimensional Perfectionism Scale (MPS), and the State-Trait Anxiety Inventory (STAI) to assess personality and behavioral traits in subjects with eating disorders. These traits have included harm avoidance; obsessions with symmetry, order, and exactness; perfectionism; and anxiety. Compared to non–eating-disordered control subjects, these studies have found that women with AN and BN continued to display marked elevations on these traits after long-term recovery (e.g., more than 1 year without pathologic eating and with normal weight and normal menses). The persistence of these symptoms after recovery raises the possibility that these characteristics are premorbid traits that contribute to the pathogenesis of AN and BN. Additionally, heritability estimates (.26–.60) obtained from twin studies of these types of anxious, perfectionistic, and obsessive characteristics indicate moderate-to-substantial genetic influence (Carey and Gottesman

1981; Heath et al 1994; Rutherford et al 1993). For these reasons, we included measures of these characteristics in order to determine whether they are trait-disturbances that are both phenotypically and genotypically linked to eating pathology.

To follow is a general description of each of the personality and behavioral measures comprising our assessment battery. Psychometric findings from these measures will be the focus of future reports.

Trained Rater Assessments

STRUCTURED INTERVIEW OF ANOREXIA NERVOSA AND BULIMIC SYNDROMES (SIAB). This instrument was used for an assessment of a lifetime history of eating disorders among probands and affected relatives. The SIAB (Fichter et al 1998) is a detailed structured interview schedule that derives information relevant to lifetime severity of six psychopathologic factors, including body image and slimness ideal, social and sexual adjustment, mood disturbance, anxiety, bulimia, and laxative abuse. The internal consistency of the six SIAB subscales on the lifetime version of the instrument have been shown to be moderate to high, with Cronbach's α ranging from .64 to .89 (Fichter et al 1998). Likewise, the interrater reliability for these same subscales has been shown to be excellent, ranging between .80 and .90 (Fichter et al 1998). Subjects were asked to report "worst lifetime" symptoms.

A structured interview (Structured Interview of Anorexia Nervosa and Bulimic Syndromes; Fichter et al 1998; see below) was used to ascertain inclusion/exclusion criteria and the diagnosis of an eating disorder, but not for assessments of other axis I and II diagnoses. This compromise was necessary, owing to budget limitations. This omission may weaken the study in terms of the investigation of comorbid disorders; however, the omission of other axis I and II disorders should not compromise the diagnosis of AN, because AN is a relative homogenous disorder with little risk of false positive diagnoses.

YALE-BROWN OBSESSIVE COMPULSIVE SCALE (Y-BOCS). The Y-BOCS (Goodman et al 1989) is a semi-structured interview designed to rate the presence and severity of obsessive thoughts and compulsive behaviors typically found among individuals with obsessive-compulsive disorder (OCD). It has excellent interrater reliability (Goodman et al 1989) and is considered to be the "gold standard" for measuring obsessive-compulsive symptom severity (Pato et al 1994). Subjects were asked to report "worst lifetime" symptoms.

YALE-BROWN-CORNELL EATING DISORDER SCALE (YBC-EDS). The YBC-EDS (Sunday et al 1995) is similar to the Y-BOCS; however, it assesses core obsessions and compulsions specific to eating disorders (e.g., those related to food, eating, weight, and exercise). Excellent interrater reliability, internal consistency, and convergent validity have been demonstrated for the YBC-EDS (Mazure et al 1994). The YBC-EDS was modified with the authors of the instrument to assess "worst lifetime" symptoms, as well as current symptoms.

TRAINING FOR INTERVIEW ASSESSMENTS. The Data and Administrative Core at Pittsburgh developed a training

package consisting of readings and explanations of the rating instruments. Before interviewing subjects, each clinical interviewer completed a training program for the administration of the SIAB, the Y-BOCS, and the YBC-EDS. The training program included the following: 1) viewing videotapes of trained raters performing the assessments; 2) scoring another set of videotapes at an accepted standard of accuracy; 3) taping their own practice interviews, which were evaluated for accuracy. Subsequent to this training, every 10th interview was audiotaped for review by the project coordinator of the data core for drift prevention. Additionally, the interviewers at each site blindly rated tapes at 3-month intervals to ensure rating consistency across sites. These interviews were scored by the project coordinator of the data core.

To ensure accuracy of clinical identification of probands and relatives, there were several independent confirmations of diagnoses. First, all eating disorder diagnoses were confirmed by each Principal Investigator at each satellite after reviewing the SIAB. Second, the project coordinator of the data core independently reviewed every subject's SIAB interview to confirm diagnoses and ensure that all subjects met entrance criteria for the study.

Self-Report Assessments

EATING DISORDER INVENTORY-2 (EDI-2). The EDI-2 (Garner 1990) is a 91-item, standardized self-report measure consisting of 11 subscales that assess specific cognitive and behavioral dimensions of eating disorders: drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears, asceticism, impulse regulation, and social insecurity. The last three subscales are new to the revised edition of the EDI. The original EDI showed good internal consistency, as well as good convergent and discriminant validity (Garner et al 1983). Alpha coefficients for the eight original subscales range from .82 to .90. Internal consistency for the three new subscales is fair to good, with α coefficients between .70 and .80 (Garner 1990). The EDI has been used in numerous studies and has been found to successfully discriminate between subjects with and without eating disorders (Garner et al 1983). In order to assess for worst lifetime symptom expression, subjects were instructed to respond to the questions according to how they felt "at the time when your concerns about eating and weight were strongest."

STATE-TRAIT ANXIETY INVENTORY (STAI). The STAI (Spielberger et al 1970) is a widely used instrument for the assessment of anxiety. The state anxiety assessment asks subjects to report how they feel "at this moment," whereas the trait anxiety assessment asks subjects to report how they "generally feel." The internal consistency of both the state and trait assessments is high, ranging from .86 to .96.

MULTIDIMENSIONAL PERFECTIONISM SCALE (MPS). The MPS (Frost et al 1990) is a 35-item, factor-analytically developed self-rating instrument that consists of an overall assessment of perfectionism, as well as six specific dimensions of perfectionism. These dimensions are as follows: concern over

mistakes, high personal standards, high perceived parental expectations, high perceived parental criticism, doubt about quality of performance, and finally, organization, order and precision. The coefficients of internal consistency for the factor scales range from .77 to .93, and the reliability of the overall perfectionism scale is .90 (Frost et al 1990). The MPS has been found to successfully discriminate between subjects with and without eating disorders (Srinivasagan et al 1995). In order to assess for worst lifetime symptom expression, subjects were instructed to respond to the questions according to how they felt "at the time when your concerns about eating and weight were strongest."

TEMPERAMENT AND CHARACTER INVENTORY (TCI). The TCI (Cloninger et al 1993) is a 226-item factor-analytically developed self-rating instrument that measures seven dimensions of personality. The TCI is an extension of the Tridimensional Personality Questionnaire (TPQ; Cloninger et al 1991), which assesses the "temperament" dimensions of novelty seeking, harm avoidance, and reward dependence. These three personality dimensions were postulated to be genetically independent of one another (Cloninger et al 1991). The authors' original model has been extended to measure the additional "temperament" factor of persistence and the three "character" dimensions of self-directedness, cooperativeness, and self-transcendence. The internal consistency of all seven scales is high, ranging from .76 to .89 (Cloninger et al 1993).

BODY MASS INDEX (BMI). Current, past minimum, and past maximum BMI $(kg/(m)^2)$ were calculated from self-reports of height and weight.

Biological Assessments

Blood was collected from each proband, affected relative, and participating biological parent. Blood was drawn into ethylene-diaminetetra-acetic acid–treated tubes and frozen within 24 hours. DNA was extracted from whole frozen blood according to Lahiri and Nurnberger (1991).

Genotyping and Genetic Analyses

The genetic underpinnings of most common disorders are complex, with more than one gene affecting liability. The genetic underpinnings of eating disorders are unlikely to be an exception. With this observation in mind, we attempt to maximize our ability to detect liability genes by collecting a relatively large sample and by using a study design that features two complementary strategies, candidate gene analyses, and a comprehensive, nested genome scan.

GENOTYPING DATA. Our first pass screen of the genome uses a screening panel of 387 fluorescently tagged markers for use with the ABI sequencer, specifically the Weber Screening Set 9 (CMG Laboratories, Marshfield, WI). This is the same set used by the Center for Inherited Disease Research, a National Institutes of Health (NIH)-sponsored genotyping service (http://www.cidr.jhmi.edu/). We have chosen this set because of its ideal properties for a genome screen and because our results can

Table 1. Number of Probands and Affected Relatives from Each Site

| | Probands | Affected relatives | | |
		All relatives[a]	Full siblings	Other
Pittsburgh	38	47	35	12
New York	35	37	32	5
Los Angeles	30	33	22	11
Toronto	30	39	29	10
London	21	24	16	8
Munich	37	43	43	0
Philadelphia	5	6	6	0
Total	196	229	183	46

[a]Relatives who are unable to be used for linkage analyses on account of their relationship to the proband were excluded from the table ($n = 6$; 3 excluded affected mothers and 3 excluded affected daughters of probands).

be compared directly to the results from other genome screens using the same screening panel. This screening panel provides a slightly less than 10 cm grid of markers, with average marker heterozygosity of 0.77. The panel consists almost entirely of tri- and tetranucleotide repeats, which simplify scoring of alleles compared to dinucleotide repeats (i.e., less stutter artifact).

QUALITY CONTROL. All markers will be checked to determine if genotypes conform to Hardy-Weinberg (H-W) proportions. Violations of H-W can result from population genetic phenomena, but a frequent source of these violations is genotyping errors. Markers violating H-W will be scrutinized for genotyping errors. A serious concern for the genome scan is the true biological relationship of the reportedly full siblings. We will evaluate the biological relationships using the methods of Boehnke and Cox (1997), and extensions of that method.

GENOME SCAN. The nested genome analysis emphasizes a search strategy that we believe is highly efficient, as opposed to emphasizing statistical significance (see Elston et al 1996; Hauser et al 1996; Weeks and Lathrop 1995 for similar approaches). This emphasis is appropriate for complex disorders, because significant linkage findings are unlikely even with very large sample sizes. Our search strategy, in brief, is organized in three stages:

1. Analyze the genotypic data from the genome scan by multipoint methods. Use those analyses to rank regions of the genome for their potential for harboring a candidate gene.
2. Choose the most promising regions for further molecular and statistical analysis.
3. Finally, choose the most promising region(s) for intensive analysis of new markers, with preference given to Single Nucleotide Polymorphisms in "candidate genes."

Multipoint inheritance information will be extracted from our pedigrees using the methods developed by Kruglyak et al (1996), as implemented by GENEHUNTER. GENEHUNTER performs both parametric and nonparametric multipoint linkage analysis. Because little is known about the nature of the genetic model, we

Table 2. Eating Disorder Diagnoses of Proband-Affected Relative Pairs

Proband diagnosis	Affected relative diagnosis	Number of pairs	Percent of total pairs
AN	AN	145	63.3
AN	BN	47	20.4
AN	ED-NOS	37	16.1
Total		229	100.0

AN, anorexia nervosa; BN, bulimia nervosa; ED-NOS, eating disorder not otherwise specified.

are particularly interested in nonparametric measures of linkage. Under an assumed additive model for allelic effects on liability, we can improve the power of the nonparametric analysis and still use GENEHUNTER to perform the analysis (Kong and Cox 1997).

For any particular marker, the *identity-by-descent* or *ibd* relationship among sib-pairs usually can be inferred directly by genotyping the parents as well their children. For our relative pair analyses, we cannot always use this method, either because parents are not available or because we have more distant relatives. In these cases, *ibd* status must be inferred based on allele frequency distributions. Although too involved to be presented here, we will implement analyses that allow heterogeneity in marker allele frequency across sites.

Analyses searching for quantitative trait loci also are planned, with targeted traits being the heritable personality traits described earlier. Multivariate analyses will be performed to understand the underlying correlation structure of the measures of psychopathology and personality profiles, and determine whether a subset of these variables produce distinct clusters in our populations. In this case, the clustering may identify groups homogeneous for the traits *and* the genes underlying the trait dimensions. Guided by the covariance structure of the variables, linkage will be assessed for single and multiple quantitative traits (Amos et al 1997; Blangero and Almasy 1997).

ASSOCIATION ANALYSES. The association studies we plan use Affected Family-Based Controls (AFBAC; Thomson 1995) and the TDT test (e.g., Schaid 1996; Spielman and Ewens 1996; Spielman et al 1993). The advantages of the AFBAC sample and TDT analysis is that it takes into account population heterogeneity, in addition to being simple and powerful (Risch and Merikangas 1996). Martin et al (1997) point out that simple TDT tests in the situation of presumed linkage, which arise when candidate genes are tested after finding linkage, are anti-conservative. Consequently, we plan to apply the tests of Martin et al (1997) in this setting.

Several lines of evidence raise the possibility that disturbances of serotonergic, and perhaps dopaminergic, neuronal activity contribute to a vulnerability to develop an eating disorder. In brief, disturbances of these monoamine pathways persist after recovery (Kaye et al 1991; Kaye et al 1998 in press; Smith et al 1999). Any neurobiological abnormalities that persist after recovery may be trait-related and potentially contribute to the pathogenesis of the disorder. Four of six cohorts investigated

Table 3. Eating Disorder Diagnoses of Affected Relatives ($n = 229$) Stratified by Probands' Anorexia Nervosa Subtype

	Affected relatives				
	Anorexia nervosa (restricting) ($n = 55$)	Anorexia nervosa (purging) ($n = 31$)	Anorexia nervosa (bingeing)[a] ($n = 59$)	Bulimia nervosa ($n = 47$)	Eating disorder NOS ($n = 37$)
Probands					
Anorexia nervosa (restricting) $n = 107$	36 (16%)	14 (6%)	31 (14%)	30 (13%)	16 (7%)
Anorexia nervosa (purging) $n = 89$	19 (8%)	17 (8%)	28 (12%)	17 (7%)	21 (9%)

Percentages calculated using the total number of affected relatives ($n = 229$) in the denominator. NOS, not otherwise specified.
[a]Bingeing subtype of anorexia nervosa = bingeing only or bingeing and purging.

have found that a polymorphism ($-1438G/A$) in the promoter region of the gene for the 5-HT$_{2A}$ receptor is associated with AN (Campbell et al 1998; Collier et al 1997; Enoch et al 1998; Hinney et al 1998; Sorbi et al 1998). Thus, we will initially focus on determining whether variants of monoamine genes are associated with AN.

Results

We conducted psychological assessments and collected blood samples from 196 probands, 183 affected full siblings, and 46 other affected second- and third-degree relatives (see Table 1 for a breakdown by site). Because some probands had two or more affected relatives, there were a total of 229 relative pairs, *excluding* parent-child pairs. For the Pittsburgh, New York, Los Angeles, Toronto, London, Munich, and Philadelphia sites, we were able to collect DNA from both biological parents for 47, 21, 21, 28, 14, 17, and 3 of the probands and affected relatives, respectively, and from only one biological parent for 13, 17, 18, 10, 10, 19, and 2 of the probands and affected relatives, respectively. Information on ethnicity was obtained from the majority of families with the exception of subjects from the Munich and London sites; human subjects' concerns prohibited the distribution of the ethnicity questionnaire following collection of the genotypic and phenotypic data at these sites. Consequently, ethnicity was only obtained on 121 (62%) of the 196 families participating in the study. Of these families, 98.3% were of Caucasian ancestry; the remaining 1.7% were of mixed Caucasian and Asian, or mixed Caucasian and Native American ancestry. It is likely that the subjects recruited from the London and Munich sites were also predominantly of Caucasian ancestry.

Diagnostic and demographic characteristics of the sample are described in Tables 2–5. All probands had either restricting-type AN or purging-type AN. In approximately two thirds of the pairs, the affected relative also had a subtype of AN (Table 2). The diagnoses of specific proband-affected relative pairings are described in more detail in Table 3. There are no notable differences between

the probands diagnosed with AN-restricting and AN-purging in terms of the diagnosis of their affected relative. Likewise, there are no significant differences between the two proband groups in terms of age, gender, and measures of BMI (Table 4). When affected relatives are stratified by eating disorder subtype (Table 5), the groups of relatives did not differ significantly in age or gender; however, as expected, relatives with any type of AN diagnosis had significantly lower current, past minimum, and past maximum BMIs than those relatives with BN or ED-NOS.

Discussion

The primary aim of the present article was to provide the initial methodological overview of an affected relative pair investigation designed to identify genes that may influence susceptibility to anorexia nervosa. There are several strengths to this investigation. First, given the rarity of the condition, a coordinated multisite, multinational effort enabled collection of a sufficiently large sample for the task at hand. Second, our success at obtaining DNA from a large fraction of the biological parents increases the power of the genome scan and provides a substantial sample for family-based association analysis (Spielman et al 1993). Third, by using structured diagnostic methodology and providing close reliability checks across the sites, we have obtained clear eating disorder diagnoses that will

Table 4. Characteristics of Probands Stratified by Anorexia Nervosa Subtype

	Anorexia nervosa (restricting) ($n = 107$)	Anorexia nervosa (purging) ($n = 89$)	t value	p value
Age	27 ± 9	29 ± 10	-1.67	ns
Gender (% males)	9 (8%)	2 (2%)	FI = 3.28	.07
BMI current	18 ± 2	19 ± 3	-1.12	ns
BMI minimum (past)	14 ± 2	14 ± 3	-0.40	ns
BMI maximum (past)	21 ± 2	22 ± 3	-1.13	ns

BMI, Body Mass Index; FI, Fisher's statistic.

Table 5. Characteristics of Affected Relatives ($n = 229$) Stratified by Eating Disorder Subtype

	Anorexia nervosa (restricting) A ($n = 55$)	Anorexia nervosa (purging) B ($n = 31$)	Anorexia nervosa (bingeing)[a] C ($n = 59$)	Bulimia nervosa D ($n = 47$)	Eating disorder NOS E ($n = 37$)	F value	p value	Group differences[b]
Age (y)	30 ± 15	3 ± 12	26 ± 7	28 ± 9	27 ± 8	1.71	NS	
Gender (% males)	7 (13%)	1 (3%)	2 (3%)	3 (6%)	3 (8%)	FI = 4.44	NS	
BMI current	19 ± 3	19 ± 2	18 ± 3	23 ± 4	23 ± 4	24.55	.0001	D,E > A,B,C
BMI minimum (past)	15 ± 2	15 ± 1	15 ± 2	19 ± 1	19 ± 2	72.10	.0001	D,E > A,B,C
BMI maximum (past)	21 ± 3	23 ± 3	22 ± 3	25 ± 4	24 ± 3	16.56	.0001	D,E > A,C; D > B

FI, Fisher's statistic; NOS, not otherwise specified.
[a]Bingeing subtype = bingeing only or bingeing and purging.
[b]Group comparisons are significant at $p < .01$ using Scheffe's post-hoc tests.

enable stratification on the basis of the presence or absence of certain core features in the data analytic phase. Fourth, by requiring that probands suffered from AN for at least 3 years and using obesity as an exclusionary criterion, we have attempted to ensure diagnostic homogeneity in the proband group by minimizing the number of individuals who would be likely to cross over to a diagnosis of BN. Finally, by including a range of psychological assessments of behaviors and traits that have been documented to be associated with AN, we will be able to search for genes that influence susceptibility to these quantitative traits.

There are also limitations to the study that must be considered. Because of the rarity of the condition of AN, we were obliged to make certain concessions in designing the investigation. For example, it was essential to broaden the criteria for affected relatives to include AN, BN, and ED-NOS. Although this reduces the power to detect liability genes of small effect, it was an unavoidable decision based on the rarity of AN.

The second aim of this article was to describe the clinical characteristics of the sample. The majority (64%) of the 237 proband-affected relative pairs were both affected with AN, with a much smaller percentage of the pairs having an affected relative with BN (AN-BN; 20%) or ED-NOS (AN-EDNOS;16%). Comparisons among eating disorder subtypes in probands, relatives, and between probands and relatives, yielded few significant findings. Aside from expected differences in BMIs between AN and BN subjects, the group comparisons indicated that the probands and affected relatives are a relatively homogenous group. This homogeneity will enhance the chances of detecting liability genes.

To our knowledge, the current project is the first affected-relative pair study of eating disorders in the literature. It represents a large, international, collaborative effort across several sites known for their research and treatment of eating disorders. Molecular genetic analysis is now underway.

Several lines of evidence suggest that individuals with AN may have a trait-related disturbance of serotonin that could contribute to restricted eating, behavioral overcontrol, obsessive exactness, perfectionism, and negative affective states (Kaye et al 1991, 1993). This observation has led to study of specific candidate genes that may account for this suite of traits. Intriguingly, the first liability gene for eating disorders may have been discovered by this pursuit. Most, but not all studies (Campbell et al 1998; Collier et al 1997; Enoch et al 1998; Hinney et al 1997; Sorbi et al 1998) have found that a polymorphism ($-1438G/A$) in the promoter region of the gene for the 5-HT$_{2A}$ receptor is associated with AN. Continued assessment of this candidate is a subject of ongoing study.

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