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The Genetics of Anorexia Nervosa Collaborative Study: Methods and Sample Description

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Abstract

Objective—Supported by National Institute of Mental Health (NIMH), this 12-site international collaboration seeks to identify genetic variants that affect risk for anorexia nervosa (AN).

Method—Four hundred families will be ascertained with two or more individuals affected with AN. The assessment battery produces a rich set of phenotypes comprising eating disorder diagnoses and psychological and personality features known to be associated with vulnerability to eating disorders.

Results—We report attributes of the first 200 families, comprising 200 probands and 232 affected relatives.

Conclusion—These results provide context for the genotyping of the first 200 families by the Center for Inherited Disease Research. We will analyze our first 200 families for linkage, complete recruitment of roughly 400 families, and then perform final linkage analyses on the complete cohort. DNA, genotypes, and phenotypes will form a national eating disorder repository maintained by NIMH and available to qualified investigators.

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Keywords

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Introduction

Anorexia nervosa (AN) is characterized by the seemingly willful maintenance of low body weight, fear of weight gain, and indifference to the seriousness of the illness. It commonly arises during adolescence and occurs significantly more often in females than in males. Effective treatments for AN are few[1] and for many, the illness runs a chronic, relapsing course.[2–4] AN has a mortality rate of roughly 5% per decade[5] with a standardized mortality ratio of 10.5,[6] the highest of any psychiatric illness. Across psychiatric disorders, only schizophrenia accounts for more inpatient days than AN.[7] Improved understanding of the pathophysiology of AN will hopefully benefit attempts to develop effective treatment interventions and genetic studies comprise one critical step in achieving that goal.

The purpose of this article is to present the ascertainment methods and study design of the Genetics of Anorexia Nervosa Collaboration to provide detailed context for the recently completed genotyping of the first 200 families by Center for Inherited Disease Research (CIDR). To present this study, we provide background and rationale for our focus on the linkage analysis for AN including the following: (1) the nature of the AN phenotype; (2) associated features and personality characteristics; (3) family and twin studies; (4) previous linkage studies of eating disorders; and (5) details of the current investigation.

The Nature of the AN Phenotype

AN has an unusually stereotypic presentation with respect to sex, age of onset, premorbid and clinical characteristics, and disease course. Variations in consummatory patterns do exist, with some individuals maintaining an invariant profile of food restriction, whereas others exhibit binge eating and/or purging behavior. Few psychiatric disorders masquerade as AN, so diagnosis tends to be unambiguous. Nonetheless, variability exists within the diagnostic category on several dimensions, many of which are being explored as possible endo- or subphenotypes for the disorder.[8,9] For precisely this reason, as described later, in addition to diagnostic categories, we also incorporated rich phenotypic characterizations into our experimental methods.

Associated Characteristics and Personality in AN

Both individuals with AN and with bulimia nervosa (BN) display characteristic personality profiles of several traits each of which has been shown to be at least moderately heritable[10–15] Individuals with AN exhibit high levels of negative emotionality, obsessionality (OBS), perfectionism, inhibition, stress-reactivity, neuroticism, and harm avoidance.[16–24] Substantial evidence supports that many of these traits exist premorbidly, are heritable, are elevated in unaffected family members, persist after recovery from the disorder, and are independent of body weight.[9,17,19,20,24–28] Therefore, we postulate these traits confer liability to the development of AN. Furthermore, consistent with statistical and genetic theory, we postulate that genetic analyses targeting these quasi-continuous traits (possibly in conjunction with diagnostic categories) will have greater power, relative to diagnostic categories alone, for detecting genetic variation affecting risk for development of AN (e.g., Ref. 29–31). As described later, our extensive work on temperament in eating disorders[22,23,32–36] has informed our selection of our behavioral variables which we assume will be useful for linkage analyses.[37]

Family and Twin Studies

AN is highly familial.[38–40] The relative risk for AN in family members of probands with AN is 11.3.[39] This elevated risk places AN among the most familial of psychiatric disorders. Twin studies on European populations have yielded heritability estimates using various strategies. First, heritability of AN was estimated to be 58% (95% CI 0.33–0.84), in the context of a bivariate twin analysis with major depression.[41] Second, twin analyses were conducted for a single question of "have you ever had AN," yielding a heritability estimate of 48% (95% CI 0.27–0.65).[42] Third, broadening the definition of AN syndrome, Klump et al. reported the heritability to be 76% (95% CI 0.35–0.95).[43] A Swedish Twin Registry study of 31,406 twins born between 1935 and 1958 and diagnosed by clinical interview, hospital discharge diagnosis of AN, or cause of death certificate yielded a heritability estimate of 56% (95% CI 0.00–0.87) with the remaining variance attributable to shared environment (c^2 = 5% 95% CI 0.00–0.64) and unique environment (e^2 = 38% 95% CI 0.13–0.84).[27]

Linkage Studies of Eating Disorders

The purpose of a genomewide linkage study for a complex trait like AN is to identify the genomic regions that might harbor predisposing genes. Linkage does not require *a priori* assumptions about the nature and locations of genes involved in the etiology of AN.[44,45] Linkage analysis requires a large sample of pedigrees with multiply affected individuals.[46] Anonymous genetic markers across the genome are genotyped and, by virtue of how often marker alleles are shared by affected family members, are used to identify chromosomal regions that contain genetic variation affecting risk. Linkage approaches narrow the search space from the entire genome to one or several chromosomal regions (each perhaps 10–30 million base pairs). These regions can then be explored to identify genetic variation affecting risk.

One linkage sample for AN has been previously published by some members of this collaboration. The Price Foundation, a private, European-based foundation, supported a multicenter international collaboration to investigate the genetics of AN. One hundred and ninety two families were ascertained primarily from current and former patients of the participating treatment centers and from advertisements, using the following diagnostic criteria: all probands met modified DSM-IV criteria for AN; at least one additional affected first through fourth degree relative met DSM-IV criteria for AN, BN, or eating disorder not otherwise specified (EDNOS).[47] Blood for DNA was collected from all affected individuals and available biological parents. Factors potentially affecting susceptibility for AN were assessed with a battery of standardized and validated instruments. Using the Weber screening set, version 9 (Center for Medical Genetics, Marshfiled Medical Research Foundation) with markers dispersed across the genome at approximately 10 cM, and analyzing families in which at least two affected relative pairs had AN, restricting subtype (RAN) (n = 37 families, 32 sibling pairs of which 11 pairs had data for both parents) we found suggestive evidence for linkage (NPL score 5 3.03 at marker D1S3721 on chromosome 1p) according to Lander and Kruglyak criteria.[48] Genotyping additional regional variants in 1p for both linkage and association analyses amplified this signal beyond the Lander/Kruglyak threshold (p-value = .00002) for significant linkage.[49]

We also explored how behavioral covariates enhance the linkage signals. Devlin et al.[50] evaluated seven attributes thought to typify individuals with eating disorders in that they had to demonstrate the following: (1) be consistently related to eating pathology, (2) be heritable, and (3) indicate severity of some aspect of the disorder. Two variables, drive-for-thinness and OBS, each yielded a cluster of affected sibling pairs (total sibling pairs analyzed = 180) who had high and concordant values for these traits, whereas other sibling

pairs were notably discordant. Incorporation of these traits into covariate-based linkage analyses[51] yielded a significant additional linkage signal on 1q, with a LOD score of 3.46, marker D1S1660 (see Ref. 52) as well as two other suggestive linkage signals, one at 2p (LOD = 2.22), marker D2S1790 and another at 13q (LOD = 2.50), marker D13S894.

In further exploration of this linkage sample (154 affected sibling pairs) and an additional BN linkage sample (244 affected sibling pairs), Bulik et al.[37] thoroughly explored eating disorder-related traits. From more than 100 psychiatric, personality, and temperament phenotypes, they selected a parsimonious subset of attributes to incorporate into linkage analyses. Using a multilayer decision analysis, they chose variables relevant to eating disorder pathology with published evidence for heritability. OBS, age-at-menarche, and a composite anxiety measure (ANX) displayed features of heritable quantitative traits, such as normal distribution and familial correlation, and thus appeared ideal for quantitative trait locus linkage analysis. By contrast, some families showed highly concordant and extreme values for three variables-lifetime minimum Body Mass Index (lowest BMI attained during the course of illness), concern over mistakes (CM), and food-related obsessions (OBF). These distributions were consistent with a mixture of populations, and thus the variables were matched with covariate linkage analysis. The most compelling signals arose from the BN cohort. For the BN cohort, significant linkage signals arose on 4q21.1 (BMI), 14q21.1 (CM, OBF), 16p13.3 (CM). Suggestive linkages were detected at the following chromosomal locations: 1q31.1 (ANX), 3p23 (BMI), 4p15.33 (OBF), 4q35.2 (ANX), 5p15.3 (BMI), 8q11.23 (CM, OBF), 10p11.21 (CM), 10p13.1 (OBF), and 18p11.32 (OBF). For the AN cohort, the results for linkage were more modest. No result was genomewide significant, although there were some suggestive linkage findings: 4q13.1 (BMI), 6q21 (OBS), 9p21.3 (OBS), 11p11.2 (CM), 15q26.2 (OBF), and 17q25.1 (CM, OBF). While substantial linkage signals were not seen in both cohorts, more modest signals did coincide, defining other areas of suggestive linkage. These linkage findings are intriguing, but they require confirmation before substantial time and money are invested to identify critical genetic variation in the linkage regions. The approaches and methods that we developed for the PF studies have provided solid foundations from which to develop the analytic plans for the present investigation.

The Current Study: The Genetics of Anorexia Nervosa Collaborative Study

In 2001, the National Institute of Mental Health (NIMH) funded the Genetics of Anorexia Nervosa (GAN) collaborative study whose overarching goal was the detection and localization of genetic variation that increases susceptibility to AN and related phenotypes. The GAN collaboration incorporates a core site (University of Pittsburgh), 11 clinical sites (University of Pittsburgh; Weil Cornell Medical College; Roseneck Hospital for Behavioral Medicine Prien and Department of Psychiatry, University of Munich (LMU), Germany; University of California at Los Angeles; University of Toronto; Neuropsychiatric Research Institute, University of North Dakota; Laureate Psychiatric Hospital, Tulsa, OK; Sheppard Pratt, Towson, MD; University of Pennsylvania; Kings College London, Institute of Psychiatry, England; and University of Birmingham, England) and two data analytic sites (University of Pittsburgh and University of North Carolina at Chapel Hill). The primary aims of this study were: (1): to ascertain 400 families consisting of two or more affected individuals (i.e., multiplex families); (2); to perform a genome scan using up-to-date optimally informative markers with genotyping from the Center for Inherited Disease Research (CIDR); (3) to conduct linkage analyses on these data first focusing on the narrowly defined core phenotype (AN); (4) to analyze trait data to identify genetically meaningful phenotypes for linkage analyses; (5) to put all materials generated by this research, including DNA, genotypes, and phenotypes into a national eating disorder archival

database that will be made available to qualified investigators throughout the scientific community as stipulated by NIH.

Below, we describe the design and methods of the study and provide preliminary clinical descriptions of the GAN linkage sample.

Method

Screening and Diagnostic Procedures

Potential participants contacted the core or individual sites by phone or email in response to letters from treatment centers, advertisements, or word of mouth. A research associate at the site then performed an initial brief screen to determine a provisional diagnosis of anorexia nervosa (AN) and the presence of a suitable biological relative with possible AN. Probands were then asked to contact their relatives about the study to see if they were willing to be contacted by study staff. Probands provided informed consent to participate and permission for the contact of their willing affected relatives and parents in accordance with institutional review board requirements of each participating site. A similar brief screen was then conducted with an affected relative, after which the relative's informed consent was obtained. At that point, a site clinical interviewer assessed both members of the affected relative pair (ARP) with the Extended Screen, an elaboration of the eating disorders module of the Structured Clinical Interview for DSM-IV (SCID-I: Ref. 53), to confirm the DSM-IV diagnosis of AN and all other study inclusion and exclusion criteria. If the individuals met criteria for proband and affected paired relative, they were sent a packet of self-report assessments. An in-person interview was scheduled to complete the remaining diagnostic assessments for those who could easily travel to one of the sites, where the blood sample was also drawn. Those living further from the sites had their interviews conducted over the telephone (90%) and were asked to have their blood drawn at a local laboratory or physician's office using the kits provided for blood collection. The blood sample was then sent by overnight mail to the National Institute of Mental Health (NIMH)-sponsored repository for DNA and cell lines. We used our previous data[47,54] to determine whether significant differences existed between telephone and in-person interviews in the frequency with which various diagnoses are given. We compared the prevalence of all disorders assessed between telephone ($n = 932$) and in-person interviews ($n = 231$) and found excellent consistency: using tests, no significant differences emerged between telephone and in-person interviews on frequency of any diagnosis given (all p-values > .07).

After completion of the pair's interviews and collection of their blood samples, and with their permission, willing parents, affected or unaffected, as well as any additional affected relatives, were recruited. After providing informed consent, these affected relatives completed interviews, self-report assessments, and blood samples as had the pair. Unaffected parents, as determined by the screen, provided blood samples and completed self-report assessments but were not interviewed.

Inclusion and Exclusion Criteria

General Inclusion Criteria—Inclusion criteria for affected individuals and multiplex families were established by consensus of the study collaborators. Probands could be male or female, age 16 or older, ill or recovered. They must have met a lifetime diagnosis of DSM-IV AN, with or without amenorrhea, at least 3 years before study entry and by age 45. The amenorrhea criterion was waived because of its lack of applicability to males, the unreliability of its retrospective assessment in females, and replicated data indicating that individuals with and without amenorrhea do not differ meaningfully.[55,56] The threshold for low weight was defined as a BMI at or below 18 kg/m^2 for females and 19.6 kg/m^2 for

males, which corresponds to the 5th percentile BMI values of the National Health and Nutrition Examination Survey epidemiological sample of females and males, respectively, for the average age range (27–29 years) of the probands in our previous studies.[57] Probands were required to have at least one first, second, or third degree relative with AN, with the exception of parents and MZ twins who are noninformative for linkage, who was willing to participate in the study.

Specific Proband Inclusion Criteria—Probands were individuals with a lifetime diagnosis of AN, ill or recovered, predominantly of the restricting type because of our interest in replicating our previous linkage findings.[58] However, the clinical picture of AN is often protean and individuals who are primarily restrictors often experience some binge eating (either in the context of treatment or as a response to severe food restriction). No consensus definition exists on the optimal dividing line between those with restricting versus binge/purging AN. As we have previously noted,[47] because of the relative rarity of AN, we were obliged to make certain decisions in designing the investigation. Moreover, the boundaries between subtypes of eating disorders remain controversial. Thus, we included as probands individuals with AN who also purged, or who had occasional binge eating episodes, but not at the frequency or duration set forth by DSM-IV to indicate “regular” binge eating. In other words, probands were individuals who either reported no lifetime binge eating or purging (restricting anorexia nervosa; RAN); individuals who reported no “regular” binge-eating (defined according to the DSM-IV conceptualization of regular binge eating in BN, at least twice a week for a duration of at least 3 months) who may also have purged [AN(B)]; and individuals who reported having engaged in purging behaviors (vomiting, laxative or diuretic abuse) but no binge eating (purging anorexia nervosa; PAN). Substantial diagnostic crossover exists both across AN types as well as between the diagnoses of AN and BN.[59] Thus in the context of this study, we categorize probands with RAN, PAN, or AN(B); however, in reality, individuals who maintain a restricting profile are the exception rather than the rule. We did not include as probands those who at any time had met the diagnosis of BN or who reported regular binge eating when underweight. We required probands to have met the criteria for AN 3 years before study entry, ensuring that AN individuals who were unlikely to develop binge eating were appropriately classified, as research has shown that most binge eating develops within the first 3 years of illness in AN.[59–63] Table 1 presents the description and abbreviations of diagnostic inclusion categories for both probands and affected relatives.

Affected Relative Inclusion Criteria—Affected relatives must have met the same inclusion criteria as probands (i.e., met lifetime diagnostic criteria for some form of AN) except that regular binge-eating was permitted. Affected relatives were also required to have had a minimal duration of at least 3 months of AN before study entry. Additional affected relatives with the diagnosis of AN, BN, or Eating Disorder Not Otherwise Specified (EDNOS) were included as long as the family already had a fully ascertained proband and affected relative both having AN. EDNOS included three types: subthreshold AN (not quite meeting the low weight criterion for AN); subthreshold BN (binge eating and inappropriate compensatory behaviors at normal weight, but not meeting the frequency or duration criteria for BN); and inappropriate compensatory behaviors in the absence of either binge eating or low weight. All EDNOS groups also reported excessive concerns about weight and shape. There were no exclusion criteria for biological parents.

Exclusion Criteria—Potential probands were excluded from the study if they had a history of severe CNS trauma, psychotic disorder or developmental disability, or if they had a medical or neurological condition that could confound the diagnosis of AN. Subjects with a current substance use disorder were excluded only if, in the judgment of the interviewer,

they were unable to respond cogently to assessments. Those with a maximum lifetime BMI exceeding 30 kg/m^2 were also excluded to limit any potential obscuring genetic signals form obesity, as were those who did not speak either English or German. An Ascertainment Committee composed of four Principal Investigators and the Supervisor of Assessments reviewed all cases where any criteria were in question to assure the diagnosis of AN.

Clinical Assessment

The Genetics of Anorexia Nervosa (GAN) assessment battery evolved from our experience with the Price Foundation genetic studies,[47] with the major exception that the Diagnostic Interview for Genetic Studies (DIGS)[64] was used instead of the SCID to assess affective disorders, in accordance with other NIMH-sponsored genetic studies. Assessments were chosen by expert consensus to assess Axis I and II comorbidity and to measure the behavioral traits most important to the eating disorder phenotypes. (Table 2 presents the assessments used in the GAN study in comparison to those used in the Price Foundation investigations). The three previous Price Foundation investigations are detailed in the referenced publications and focused on: (1) anorexia nervosa affected relative pairs (AN ARP)[47]; (2) bulimia nervosa affected relative pairs (BN ARP)[65]; and (3) AN-trios which sampled probands with AN and their biological parents (726 AN probands as well as controls with no histories of eating disorders) (described in Ref. 54).

Eating Disorder Pathology—Three interviews were used to assess eating disorder pathology. The Extended Screening instrument, an expanded modified version of Module H of the SCID-I[53] was used to establish the diagnosis of AN, as well as assess for all other inclusion and exclusion criteria. In addition, The Structured Interview on Anorexia Nervosa and Bulimic Syndromes (SIAB)[66] was administered to confirm the eating disorder diagnosis and to obtain additional information on core eating disorder behaviors. Participants were asked to report worst lifetime symptoms.

The use of these instruments allowed us to classify individuals into eating disorder subtypes described earlier and in Table 1. The Yale-Brown-Cornell Eating Disorder Scale (YBC-EDS),[67] was used to assess core obsessions and compulsions specific to eating disorders (e.g., those related to food, eating, weight, and exercise) and to rate the current and lifetime severity of the eating disorder.

Comorbid Psychiatric Disorders—Other Axis I pathology was assessed by the Diagnostic Interview for Genetic Studies (DIGS 3.0/B) (DIGS[64]) (mood disorders and psychosis); the Structured Clinical Interview for Axis I Disorders (SCID-I) (Research Version)[53] (substance disorders, anxiety disorders, and, as mentioned earlier, eating disorders); the Yale-Brown Obsessive Compulsive Scale (Y-BOCS)[68] (presence and severity of obsessive thoughts and compulsive behaviors); and, sections on overanxious disorder and separation anxiety disorder (modified for DSM-IV criteria) from the Schedule for Affective Disorders and Schizophrenia-Lifetime Version, Childhood Anxiety (SADS-L).[69]

Personality traits were assessed with the Structured Clinical Interview for DSM-IV Axis II Disorders (SCID-II)[70] (clusters B and C) and a retrospective assessment of childhood perfectionism and rigidity, The Eatatelife Phenotype (EATATE), Version 2.1 January 19, 2001,[71] given the accumulating evidence that these traits often predate the onset of an eating disorder.[26,27]

Affected individuals and participating parents completed a self-report battery including: the Eating Disorders Inventory (EDI-2)[72] (drive for thinness, bulimia, and body dissatisfaction); the State-Trait Anxiety Inventory Form Y (STAI-Y)[73]; the Beck Depression Inventory First

Edition (BDI-I)[74]; and the Smoking and Quitting History Questionnaire, a revision of the Fagerstrom Test for Nicotine Dependence (FTND).[75]

Measures of personality and temperament included the following: The Multidimensional Perfectionism Scale (MPS)[76] assessing concern over mistakes (CM), personal standards, doubts about actions, perceived parental expectations, perceived parental criticism, and, organization; the Temperament and Character Inventory (TCI)[77] (novelty seeking, harm avoidance, reward dependence, persistence, self-directedness, cooperativeness, and selftranscendence); the Barratt Impulsivity Scale (BIS-11)[78] (three measures of impulsivity: motor, cognitive, and nonplanning).

In addition, mothers of affected individuals completed several questionnaires on prenatal events and childhood behaviors and temperament of their affected offspring. Although the data are retrospective, they tap aspects of personality that would have been present before the onset of the AN and may be another source of covariates for linkage analyses. Child Behavior Checklist for Ages 4–18 (CBCL/4-18)[79]: (internalizing and externalizing symptoms and behaviors); the Revised Dimensions of Temperament Survey (DOTS-R) [Windle M, Lerner R. Unpublished manuscript, 1985] (activity-general, activity-sleep, rhythmicity-sleep, rhythmicity-eating, and rhythmicity-habits); the Pregnancy Questionnaire (factors related to pregnancy and birth of the proband and affected relatives); the Infant Feeding Questionnaire (developed for the European Healthy Eating Project to assesses feeding patterns, aberrations, and digestive disturbances during infancy and early childhood).

Assessment Oversight

Clinical interviewers for the study were all masters or doctoral level psychologists or other mental health specialists, many of whom had participated in the preceding Price Foundation study. An initial 4-day central training session for all assessments was conducted in Pittsburgh. Psychologists from six of the sites provided training in the study instruments. Study manuals were sent to clinical interviewers before the training, then, each trainer provided a didactic session with a recorded sample interview, followed by discussion and role playing. After returning to their sites, clinical interviewers submitted recorded examples of their interviews to the respective trainers until an acceptable standard was achieved. The number of practice interviews submitted ranged from just two to as many as four, reflecting the difference in clinical interviewers' prior experience with these interviews. Upon certification with all interviews, clinical interviewers were permitted to begin interviewing study participants. Although the German clinical interviewers were fluent in English, a German psychiatrist previously trained in the interviews, who was working with the Pittsburgh Core, provided their certification training following the central training.

Best Estimate Diagnostic Procedures

Monthly teleconferences with the clinical interviewers were held to review any diagnostic issues or questions that arose at sites in order to promote diagnostic consensus across sites. Eating disorder diagnoses were reviewed by each site's investigator. After clinical interviewers scored and coded assessments, a final best estimate review of all Axis I and II diagnoses was conducted independently by one of two psychologists at the Pittsburgh Core. In cases where the reviewer's diagnosis differed from that of the interviewer, the reviewer and interviewer met by phone to discuss the case and arrive at a consensus diagnosis. When necessary the reviewer listened to the recording of the interview or requested the interviewer to call the subject to obtain additional information. The psychologist also checked for accuracy of coding. Finally, the Data Management Supervisor (DMS) compared diagnoses generated by computer algorithm with final best estimate diagnosis for a final diagnostic

check. All discrepancies were then resolved by consensus between the DMS and the reviewing psychologists. Three drift prevention exercises were conducted over the course of the study, in which a recorded assessment battery was sent to the clinical interviewers at all sites, who then submitted their own scoring and diagnoses. Based on these exercises, diagnostic consensus for mood disorders, anxiety disorders, and substance use disorders ranged from 0.80 to 1.00. Eating disorder diagnosis consensus was 1.00, while eating disorder subtype consensus was 0.93.

Blood Collection

Each participant provided a 30 cc sample of blood which was be placed in glass tubes (ACD additive, yellowtop) labeled only with their subject identification number, kept at room temperature, and sent within 24-48 h by Federal Express priority overnight mail to the NIMH-designated laboratory at Rutgers University for preparation and storage of DNA and lymphoblastoid cell lines. Cell lines and family pedigree information were sent to the Center for Inherited Diseases (CIDR) where genotyping was performed.

Data Analysis

All statistical analyses were conducted using SAS/STAT® 9.1 software.[80] tests were used to assess proband between-group differences for the prevalence of Axis I disorders. Analysis of variance was used to determine differences in mean values of age, BMI measures, EDI subscales and YBC-EDS subscales in the proband groups and in mean values of the age and BMI measures in affected relative groups with various AN subtypes. Because of the small number of male participants, Fisher's Exact tests were used to test gender differences between the various groups.

Results

Clinical Characteristics

The first 200 families include 432 affected individuals, 22 of whom were male. A total of 129 families have at least two individuals afflicted with RAN or PAN. Of these 200 families, 171 have two affected participating relatives; 27 have three participating affected relatives; 1 has four affected participating members; and 1 has five affected participating members. There are 158 families with at least two affected siblings, three families with affected halfsiblings, 19 families with affected cousins, and 20 families where the affected pair is aunt/niece. In addition to the ascertainment of affected relative pairs, we were successful in obtaining DNA from 94 (47%) of mothers and 64 (32%) of fathers of probands.

Table 3 presents the distribution of eating disorder subtypes across probands and affected relatives. All probands had a lifetime diagnosis of AN [RAN, PAN, or AN(B)], but none had a lifetime diagnosis of BN. Although all primary affected relatives had some subtype of AN, once the initial pair was complete, other affected relatives could be included. Across all affected relatives, more than 95% had a subtype of AN [RAN, PAN, AN(B), or ANBN].

Table 4 presents demographic characteristics and eating disorder characteristics of the three groups of probands. While participation was open to all who met criteria, 97.4% of the sample is of European ancestry. The age range of eating disordered participants is 16–76 years with a mean of 30.4 years (11.3). Age of onset of the eating disorder ranged from 10 to 42 years, with a mean of 17.3 years (4.4). The average lowest BMI for these individuals was 14.8 kg/m^2 (2.2) and the average highest BMI was 21.9 kg/m^2 (3.1). At the time of assessment, only 31.5% of the participants were considered fully remitted (defined as reporting the absence of any eating disorder symptoms in the 12 month period before

assessment). All others had experienced at least some eating disorder symptoms in the 12 months before assessment. In terms of core eating disorder symptoms and measures, as expected, probands with AN(B) reported higher minimum BMI values than either RAN or PAN probands. On the EDI bulimia subscale, AN(B) probands scored significantly higher than PAN probands who scored significantly higher than RAN probands. PAN probands scored significantly higher than RAN probands on EDI drive for thinness and body dissatisfaction subscales and the YBC-EDS worst rituals and worst preoccupations subscales.

Table 5 presents the comorbidity profiles of anxiety, affective, and substance use disorders in the probands of this sample. Overall, the proband sample was highly comorbid with 70% reporting having suffered from any childhood or adulthood anxiety disorder, 78% from any affective disorder, and 27% from alcohol or drug abuse or dependence.

Table 6 presents the affected relatives characterized by eating disorder subtypes. We excluded the three BN and six EDNOS relatives from analysis.

Conclusion

In this article, we provide an overview of the method and sample selection of an affected relative pair study designed to identify genes that may influence susceptibility to AN (Genetics of Anorexia Nervosa Collaborative Study or GAN). The assessment battery for GAN was selected to facilitate eating disorder diagnoses and to assess psychological and personality features that are associated with vulnerability to eating disorders. Previous reports from the Price Foundation Genetic Study of Anorexia Nervosa revealed several regions of suggestive and significant linkage in AN.[49,50,52,58] To replicate and extend these previous findings, we used similar ascertainment and assessment methods (Table 2).

All 200 families had at least two relatives affected by AN, comprising 200 probands and 232 affected relatives. The majority (95.0%) of the affected relatives had some form of AN, with a much smaller percentage having BN (1.3) or EDNOS (2.6%).

The three proband AN subtypes did not differ significantly on age, age of onset, sex (% female), current BMI, and maximum lifetime BMI. Individuals with AN(B) reported higher lifetime minimum BMIs than either RAN or PAN probands which is consistent with the presence of even limited binge eating.

In terms of self-report instruments, again as expected, individuals with AN(B) reported higher scores on the EDI bulimia scale than those with PAN or RAN reflecting the presence of limited binge eating. This also validates our groupings indicating that even limited binge eating can be captured by this scale. Other psychometric differences that emerged followed the pattern of probands with PAN scoring more pathologically than those with RAN on EDI drive for thinness and body dissatisfaction as well as YBC-EDS preoccupations and rituals. These results are consistent with previous observations of greater pathology in individuals with both low weight and purging behavior.[34] It is important to note that the scores of all three proband subgroups are higher than that expected in the healthy women.[25]

Comorbidity profiles also differed somewhat across the three proband subgroups. First, PAN and AN(B) probands reported greater major depressive disorder and drug abuse than those with RAN. Alcohol abuse was markedly greater in AN(B) than RAN. Finally, dysthymia and OCD were reported significantly more often in probands with PAN than in those with RAN. Together these findings are consistent with observations[34] that the presence of binge eating and purging is associated with greater comorbidity and underscore the importance of

developing analytic plans for linkage that carefully attend to the presence of binge eating in this sample.

Considerable strengths exist in the GAN sample. First, relatively rare conditions such as AN require coordinated multisite investigations. We have successfully established an efficient clinical network that has succeeded despite the substantial hurdles in collecting this initial sample of affected relative pairs. Second, DNA from the parents of the probands in the first 200 families will enhance power of the genome scan.[81] Third, our meticulous attention to diagnostic clarity and phenotyping and regular reliability checks across sites, allow for phenotypic clarity and complexity that will enable stratification on the basis of the presence or absence of certain core features in the data analytic phase. Fourth, within the bounds of practicality, we have attempted to create as diagnostically homogeneous a proband sample as possible. This included the requirement that probands suffered from AN at least 3 years before study entry (to minimize future crossover to BN) and using obesity as an exclusionary criterion. Finally, by including comprehensive phenotypic assessment, we will be able to include searches for genes that influence risk for the disorder by analysis of the diagnostic phenotype and by analysis of quantitative traits likely to map onto dimensional vulnerability to the disorder.

Limitations must also be considered. Because of the relative rarity of RAN and the frequency of diagnostic crossover,[60] we made certain concessions in designing the investigation. First, we allowed individuals with regular purging behavior (PAN) as well as individuals with limited binge eating [AN(B)] to enter as probands. Second, although all affected relative pairs had some form of AN, we broadened the inclusion criteria for additional affected relatives to include AN, BN, and EDNOS. Fortunately, given the thoroughness of our phenotyping, these individuals can be readily delineated. Given that the existing diagnostic criteria for eating disorders are infamous for their failure to “carve nature at its joints,” little guidance exists in the literature to project the magnitude of the potential impact of these broadened inclusion criteria.

The next steps for the GAN collaboration are to analyze the first 200 families for linkage, to complete the recruitment of roughly 400 families to the study, and then to perform final linkage analyses on the complete cohort. The Price Foundation linkage studies will be considered when interpreting the results from the GAN linkage studies. We plan to approach the linkage analyses similarly to that reported in Bulik et al.[37] and Bacanu et al.[52] Instead of genotyping Short Tandem Repeats as the linkage screening panel, a panel of roughly 6,000 single nucleotide polymorphisms (SNPs) will be genotyped. This new panel extracts more information about linkage, and thus should result in refined inference about linkage from our GAN families. By linkage analysis of GAN families, and in light of results from previous Price Foundation studies, we expect to define regions of the genome containing variation having a substantial impact on risk for AN.

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TABLE 1

Definitions of eating disorder subtypes used in the study

Abbreviation	Name	Description	Proband	Affected Relative
RAN	Anorexia nervosa restricting subtype	DSM-IV AN[a] with no lifetime history of binge eating or purging	Yes	Yes
PAN	Anorexia nervosa purging subtype	DSM-IV AN[a] with a lifetime history of: (1) purging behavior of any frequency, (2) no lifetime history of binge eating	Yes	Yes
AN(B)	Anorexia nervosa with limited binge eating	DSM-IV AN[a] with a lifetime history of: (1) limited binge eating defined as less that twice per week for three months (probands and affected relatives) *or regular binge eating* (*affected relatives only*), (2) with or without any purging behavior	Yes	Yes
ANBN	Lifetime anorexia nervosa and bulimia nervosa	Lifetime history of: (1) any DSM-IV AN[a] subtype, AND, at a different time, (2) DSM-IV BN	No	Yes
BN	Bulimia nervosa	Lifetime history of DSM-IV BN	No	Yes
EDNOS	Eating disorders not otherwise specified	• Subthreshold AN (the low weight criterion for AN not met); • Subthreshold BN (binge eating and inappropriate compensatory behaviors at normal weight, but not meeting the frequency or duration criterion for BN) • Inappropriate compensatory behaviors in the absence of either binge eating or low weight	No	Yes

[a] Amenorrhea not required for any anorexia nervosa diagnoses.

TABLE 2

Comparison of assessment instruments in the Price Foundation studies and GAN

Diagnosis/Trait	AN ARP	BN ARP	AN Trio	GAN
Eating disorder diagnosis	SIAB, 3rd revision	SIAB, 4th revision Module H (SCID)	SIAB 4th revision Module H (SCID)	SIAB 4th revision Module H (SCID)
Axis I	—	SCID-I	SCID-I	DIGS (Affective Disorders), SCID-I (Anxiety and Substance Disorders)
Axis II	—	SCID-II (clusters B and C)	SCID-II (clusters B and C)	SCID-II (clusters B and C)
Core eating disorder symptoms	YBC-EDS	YBC-EDS	YBC-EDS	YBC-EDS
Obsessions/compulsions	Y-BOCS	Y-BOCS	Y-BOCS	Y-BOCS
Childhood anxiety	—	—	SADS-L: Childhood anxiety	SADS-L: Childhood anxiety
Childhood perfectionism and rigidity and lifetime impulsivity	—	—	—	EATATELIFE phenotype
Self reports				
Core eating disorder symptoms	EDI-2	—	EDI-2, 1st 3 scales	EDI-2, 1st 3 scales
Depression	—	BDI-I	BDI-I	BDI-I
Anxiety	STAI-Y	STAI-Y	STAI-Y	STAI-Y
Perfectionism	MPS	MPS	MPS	MPS
Personality and temperament	TCI	TCI	TCI	TCI plus new items
	—	NEO-PI-R	NEO-PI-R	
Impulse control	—	BIS 11	BIS 11	BIS 11
Smoking history	—	FTND	FTND	FTND
Mother report on childhood behaviors	—		DOTS-R, CBCL, pregnancy questionnaire	DOTS-R, CBCL, pregnancy questionnaire, infant and childhood feeding questionnaire

Notes: AN ARP, Price Foundation Anorexia Nervosa Affected Relative Pairs Study; BN ARP, Price Foundation Bulimia Nervosa Affected Relative Pair Study; AN Trios, Price Foundation Anorexia Nervosa Trios Study; GAN, NIMH Genetics of Anorexia Nervosa Collaborative Study; SIAB, structured interview on anorexia nervosa and bulimic syndromes; SCID-I, structured clinical interview for DSM-IV Axis I Disorders; SCID-II, structured clinical interview for DSM-IV Axis II disorders; YBC-EDS, Yale-Brown-Cornell Eating Disorder Scale; Y-BOCS, Yale-Brown Obsessive Compulsive Scale; SADS-L, Schedule for Affective Disorders and Schizophrenia-Lifetime Version; EDI-2, Eating Disorders Inventory; BDI-I, The Beck Depression Inventory First Edition; STAI-Y, The State-Trait Anxiety Inventory Form Y; MPS, The Multidimensional Perfectionism Scale; TCI, The Temperament and Character Inventory; NEO-PI-R, The NEO-Personality Inventory-Revised; BIS 11, The Barratt Impulsivity Scale; FTND, Smoking and Quitting History Questionnaire with Fagerstrom Test of Nicotine Dependence; DOTS-R, Revised Dimensions of Temperament Survey; CBCL, Child Behavior Checklist for ages 4–18.

TABLE 3

Eating disorder diagnosis of affected relatives (n = 232) stratified by probands' anorexia nervosa subtype

Affected Relatives						
	RAN *n* = 102	**PAN *n* = 50**	**AN(B) *n* = 36**	**ANBN *n* = 35**	**BN *n* = 3**	**EDNOS *n* = 6**
Probands						
RAN *n* = 107	64 (27.6%)	29 (12.5%)	15 (6.5%)	14 (6.0%)	2 (0.9%)	4 (1.7%)
PAN *n* = 70	31 (13.4%)	17 (7.3%)	14 (6.0%)	15 (6.5%)	1 (0.4%)	1 (0.4%)
AN(B) *n* = 23	7 (3.0%)	4 (1.7%)	7 (3.0%)	6 (2.6%)	0 (0.0%)	1 (0.4%)

Notes: Percent represents percent of total pairs sample.

RAN, restricting anorexia nervosa; PAN, anorexia nervosa with purging; AN(B), anorexia nervosa with binge eating; ANBN, lifetime anorexia nervosa and bulimia nervosa; BN, bulimia nervosa; EDNOS, eating disorder not otherwise specified.

TABLE 4

Characteristics of probands stratified by anorexia nervosa subtype

	RAN *n* = 107	PAN *n* = 70	AN(B) *n* = 23	*F*-Value	*p*-Value	Group Differences
Age	28.7 (10.6)	29.4 (10.3)	27.9 (10.0)	0.20	ns	—
Eating disorder age of onset	17.1 (3.8)	16.4 (4.5)	17.5 (5.8)	0.71	ns	—
Eating disorder duration	8.5 (7.0)	10.2 (7.8)	8.7 (7.4)	1.16	ns	
Sex (%) males	6 (5.6%)	1 (1.4%)	1 (4.4%)	FI = 1.9	ns	—
BMI current	18.6 (2.6)	18.7 (2.5)	19.9 (2.4)	2.56	ns	—
BMI minimum (lifetime)	14.3 (1.9)	13.9 (1.9)	15.5 (1.9)	5.96	<.001	AN(B)>RAN, PAN
BMI maximum (lifetime)	21.3 (2.4)	21.6 (2.6)	22.0 (2.5)	0.74	ns	—
EDI Bulimia	0.81 (1.49)	1.75 (2.46)	4.05 (4.54)	17.56	<.001	AN(B)>PAN>RAN
EDI body dissatisfaction	14.0 (8.0)	18.1 (7.4)	18.0 (7.3)	6.73	.002	PAN>RAN
EDI drive for thinness	12.4 (7.2)	15.1 (5.9)	14.7 (6.1)	3.71	.026	PAN>RAN
YBC-EDS worst preoccupations	11.9 (2.8)	13.1 (2.4)	12.6 (2.1)	4.59	.011	PAN>RAN
YBC-EDS worst rituals	11.2 (3.2)	12.7 (2.4)	11.8 (2.9)	5.03	.007	PAN>RAN
YBC-EDS worst motivation to change	18.6 (5.4)	20.2 (4.3)	19.6 (3.4)	2.51	ns	—

Notes: BMI, body mass index; EDI, Eating Disorders Inventory II; YBC-EDS, Yale Brown Cornell Eating Disorder Scale; RAN, anorexia nervosa, restricting type; PAN, anorexia nervosa, purging type; AN(B), anorexia nervosa with binge eating.

TABLE 5

Comorbidity profiles across probands

Disorder	RAN % (*n*)	PAN % (*n*)	AN(B) % (*n*)	χ^2	*p*-Value	Group Differences
Childhood ANXIETY DISORDERS						
Overanxious	34.6 (36)	38.2 (26)	27.3 (6)	0.90	ns	—
Separation anxiety	5.8 (6)	10.3 (7)	0.0 (0)	—	—	—
Anxiety disorders						
Agoraphobia	2.9 (3)	1.5 (1)	4.4 (1)	—	—	—
GAD	11.5 (12)	20.6 (14)	4.4 (1)	4.79	ns	—
OCD	37.5 (39)	55.9 (38)	52.2 (12)	6.05	.049	RAN>PAN
Panic	9.6 (10)	13.4 (9)	17.4 (4)	1.33	ns	—
PTSD	8.6 (9)	20.9 (14)	9.1 (2)	5.74	ns	—
Social phobia	17.3 (18)	27.9 (19)	21.7 (5)	2.75	ns	—
Specific phobia	10.5 (11)	14.7 (10)	4.4 (1)	2.00	ns	—
Mood disorders						
MDD	64.4 (67)	83.6 (53)	87.0 (20)	10.1	.007	RAN>PAN, AN(B)
Depression NOS	1.0 (1)	0.0 (0)	0.0 (0)	—	—	—
Dysthymia	1.9 (2)	11.8 (8)	8.7 (2)	7.1	.029	RAN>PAN
Cyclothymia	0.0 (0)	0.0 (0)	0.0 (0)	—	—	—
Bipolar I	1.0 (1)	0 (0.0)	4.4 (1)	—	—	—
Bipolar II	1.0 (1)	1.5 (1)	0.0 (0)	—	—	—
Bipolar NOS	1.0 (1)	1.5 (1)	0.0 (0)	—	—	—
Substance use disorders						
Alcohol abuse/dependence	14.4 (15)	25.0 (17)	43.5 (10)	10.1	.006	RAN<AN(B)
Drug abuse/dependence	9.6 (10)	21.5 (14)	26.1 (6)	6.5	.039	RAN<PAN, AN(B)

Notes: GAD, generalized anxiety disorder; OCD, obsessive-compulsive disorder; PTSD, posttraumatic stress disorder; MDD, major depressive disorder; NOS, not otherwise specified; RAN, anorexia nervosa, restricting type; PAN, anorexia nervosa, purging type; AN(B), anorexia nervosa, with binge eating.

TABLE 6

Characteristics of affected relatives

	RAN *n* = 102	PAN *n* = 50	AN(B) *n* = 36	ANBN *n* = 35	*F*-Value	*p*-Value	Group Differences
Age	29.3 (10.7)	34.7 (13.6)	29.3 (10.5)	32.6 (9.6)	3.08	.029	PAN>RAN
Gender (%) males	11 (10.8%)	1 (2.0%)	1 (2.8%)	0 (0.0%)	0.0009[a]	.046	RAN>PAN, AN(B), ANBN
BMI current	19.7 (2.2)	19.8 (3.0)	19.3 (2.4)	19.9 (1.8)	0.38	ns	—
BMI minimum (lifetime)	15.2 (1.9)	14.8 (1.8)	14.9 (2.0)	15.5 (1.7)	1.06	ns	—
BMI maximum (lifetime)	21.7 (2.6)	22.1 (3.0)	22.0 (2.4)	23.3 (1.6)	3.14	.026	RAN<ANBN

[a]The table probability computed from the exact test.