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Association of Candidate Genes with Phenotypic Traits Relevant to Anorexia Nervosa

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Abstract

This analysis is a follow-up to an earlier investigation of 182 genes selected as likely candidate genetic variations conferring susceptibility to anorexia nervosa (AN). As those initial case-control results revealed no statistically significant differences in single nucleotide polymorphisms, herein we investigate alternative phenotypes associated with AN. In 1762 females using regression

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Conflict of interest.

The authors declare no conflict of interest.

analyses we examined: (1) lowest illness-related attained body mass index; (2) age at menarche; (3) drive for thinness; (4) body dissatisfaction; (5) trait anxiety; (6) concern over mistakes; and (7) the anticipatory worry and pessimism vs. uninhibited optimism subscale of the harm avoidance scale. After controlling for multiple comparisons, no statistically significant results emerged. Although results must be viewed in the context of limitations of statistical power, the approach illustrates a means of potentially identifying genetic variants conferring susceptibility to AN because less complex phenotypes associated with AN are more proximal to the genotype and may be influenced by fewer genes.

Keywords

covariates; eating disorders; association studies; personality; genetic

The challenge of identifying susceptibility loci associated with complex psychiatric traits is widely appreciated. Like other psychiatric disorders, anorexia nervosa (AN) is a complex phenotype whose etiology most likely encompasses multiple genes of small to moderate effect in combination with environmental factors. Although evidence supporting its familial recurrence risk and heritability is strong (Bulik, Slob-O'p't Landt, van Furth, & Sullivan, 2007; Bulik, et al., 2006; Bulik, et al., 2010; Strober, Freeman, Lampert, Diamond, & Kaye, 2000; Wade, Bulik, Neale, & Kendler, 2000), AN has no known pathophysiological markers and its diagnostic boundaries continue to be disputed (Hebebrand & Bulik, 2010). Additionally, certain behavioral correlates are associated with AN, including anxiety, perfectionism, and body dissatisfaction (Bulik, et al., 2005; Devlin, et al., 2002); however, variability within AN populations on these features also exists. Samples selected on the basis of the overarching AN diagnostic label remain heterogeneous. One approach to optimizing the search for loci involves focusing gene discovery studies on theory-driven intermediate behavioral phenotypes in order to rigorously delimit relevant sources of disease heterogeneity (Cardon & Palmer, 2003; Gershon & Goldin, 1986).

Carefully selected phenotypes can optimize genetic investigations by amplifying the genetic signal and relevance of association to the disease of interest. Past studies have applied quantitatively derived behavioral phenotypes associated with AN to refine linkage analyses (Bulik, et al., 2005; Devlin, et al., 2002). We now apply this approach to determine whether these same phenotypes would yield informative results in association analyses.

Previously, we reported the results of a case-control association study examining 5151 single nucleotide polymorphisms (SNPs) in 182 genes in which no statistically significant associations for any SNP were found across three increasingly stringent DSM-IV diagnostic groupings (Grice, et al., 2002; Pinheiro, et al., 2010). In the present secondary analyses, focusing on the same 182 genes, we conducted a case-control association analysis examining associations with the following quantitative phenotypes known to be associated with AN (Bulik, et al., 2005; Devlin, et al., 2002): (1) lowest illness-related attained body mass index (BMI); (2) age at menarche; (3) drive for thinness subscale of the Eating Disorders Inventory (EDI; Garner, Olmsted, & Polivy, 1983); (4) body dissatisfaction subscale of the EDI; (5) trait anxiety from the State Trait Anxiety Index (STAI; Spielberger, Gorsuch, & Luchene, 1970); (6) concern over mistakes from the Multidimensional Perfectionism Scale (MPS; Frost, Marten, Lahart, & Rosenblate, 1990); and (7) the anticipatory worry and pessimism vs. uninhibited optimism subscale of the harm avoidance scale from the Temperament and Character Inventory (TCI; Cloninger, Svrakic, & Przybeck, 1993). By including quantitative traits in our association study, rather than categorical diagnoses, we are capturing AN on a continuum of severity which may be more informative for genetic studies than discrete diagnostic categories.

MATERIALS and METHODS

Sample Selection for Association Studies

Participants—Female participants for this study came from three multisite international Price Foundation Genetic Studies of Eating Disorders: (1) AN Affected Relative Pair Study; (2) Bulimia Nervosa (BN) Affected Relative Pair Study; and (3) AN Trios (Kaye, et al., 2004; Kaye, et al., 2000). Informed consent was obtained from all study participants, and all sites received approval from their local Institutional Review Board. Sampling methods for the three original samples are briefly described below and are followed by the sampling method for the current study, which was derived from the three original studies.

For all studies, eating disorder history was assessed using the Structured Inventory for Anorexia Nervosa and Bulimic Syndromes (SIAB-EX), a semi-structured clinical interview designed to establish DSM-IV and ICD-10 eating disorder diagnoses (Fichter, Herpertz, Quadflieg, & Herpertz-Dahlmann, 1998). An expanded version of Module H of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID; First, Gibbon, Spitzer, Williams, & Benjamin, 1997) was used in the BN Affected Relative Pair Study and AN Trios to verify diagnoses.

AN Affected Relative Pair Study—The sample for this study included both probands and affected relatives. Probands met the following criteria: (1) between 13 and 65 years of age; (2) modified DSM-IV lifetime diagnosis of AN, waiving the criterion requiring amenorrhea for three consecutive months; (3) low weight that is/was less than the 5th percentile of BMI for age and gender according to the Hebebrand et al. (1996) chart of NHANES epidemiological sample; (4) eating disorder onset prior to age 25; and (5) fulfillment of the criteria of AN not less than three years prior to ascertainment. Affected relatives were defined as biological family members who were (1) between 13 and 65 years of age and (2) had lifetime diagnoses of modified DSM-IV AN (i.e., criterion D not required), lifetime diagnoses of DSM-IV BN, or eating disorders not otherwise specified (EDNOS). For a complete list of inclusion and exclusion criteria, see Kaye et al. (2000).

BN Affected Relative Pair Study—The sample for this study included probands and affected relatives. Probands met the following criteria: (1) between 13 and 65 years of age; and (2) DSM-IV diagnosis of BN - purging type, with the additional requirement of at least a six-month period of binge eating and vomiting at least twice a week. Affected relatives were defined as biological family members who were: (1) between the ages of 13 and 65 years; and (2) had a lifetime diagnoses of DSM-IV BN, modified DSM-IV AN (i.e., criterion D not required), or EDNOS. For the complete list of inclusion and exclusion criteria for probands and relatives, see Kaye et al. (2004).

AN Trios Study—The sample for this study included individuals with AN and their parents as well as a sample of control women. Probands met the following criteria: (1) between the ages of 13 and 65; (2) modified DSM-IV lifetime diagnosis of AN, with or without amenorrhea; (3) low weight that is/was less than 5th percentile of BMI for age and gender according to the Hebebrand and Himmellmann (1996) chart of NHANES epidemiological sample; (4) weight that is/was controlled through restricting and/or purging; (5) eating disorder onset prior to age 25; and (6) study diagnostic criteria were met at least three years prior to entry into the study. Potential participants were excluded if they reported a maximum BMI since puberty > 27 kg/m² for females and > 27.8 kg/m² for males.

Women in the control group were: (1) between the ages of 18-65 years; (2) at normal weight with lifetime adult BMI between 19 kg/m² and 27 kg/m² (BMI exclusions were designed to screen for eating disorders and obesity to be consistent with exclusion criteria in the eating

disorders groups); and (3) matched with eating disorder participants based on site, age range, ethnicity and highest educational level completed. Control participants were excluded if they: (1) reported history of an eating disorder or eating disordered behaviors, as defined by a score of 20 or higher on the Eating Attitudes Test (Garner, et al., 1983); (2) had a first degree relative with an eating disorder; or (3) had any psychiatric, alcohol or drug use disorder defined by the presence of an Axis I disorder on the SCID Screen Patient Questionnaire (First, et al., 1997). Participants in the control group completed the same battery of personality and symptom measures as probands.

Current Sample

For genotyping, participants were first chosen based on whether an adequate genomic DNA sample was available. Participants were then ordered into seven eating disorder categories using a diagnostic hierarchy (highest to lowest), regardless of whether they were probands or affected relatives. The female from each family with the diagnosis highest on the hierarchy was selected. From the original samples, 1085 participants with any of five AN subtypes based on lifetime history: (1) restricting AN; (2) AN with purging but no binge eating; (3) AN with binge eating with or without purging; (4) history of both AN and BN; and (5) subthreshold AN - no binge eating or purging, and 677 controls comprised the inclusion sample.

Single Nucleotide Polymorphisms (SNP) Quality Control Filters (QC)

A total of 6,568 SNPs was sent to Illumina for genotyping with their Custom Infinium Genotyping Beadchips platform. The design process failed for 480 SNPs and 237 SNPs failed genotyping resulting in 5,851 SNPs delivered. Quality control filters were then applied with a total of 700 SNPs failing for the following reasons (a SNP could fail for more than one reason): (1) minor allele frequency (MAF) < 0.01 (538 SNPs); (2) ≥ 2 Mendel errors in trios (1 SNP); (3) duplicates with ≥ 2 disagreements (111 SNPs); (4) missingness > 0.05 in cases or controls (24 SNPs); (5) missingness test in cases vs. controls $p < 0.01$ (4 SNPs); and (6) Hardy-Weinberg disequilibrium (HWD) exact $p < 0.01$ in controls (47 SNPs). A total of 5151 SNPs passed all QC steps and were included in the analyses (see supplemental material for a complete list of SNPs).

Measures

Based on criteria outlined by Bulik and colleagues (2005) seven quantitative phenotypes were selected: (1) lowest illness-related attained BMI; (2) age at menarche; (3) drive for thinness; (4) body dissatisfaction; (5) trait anxiety; (6) concern over mistakes; and (7) the anticipatory worry and pessimism vs. uninhibited optimism scale of the harm avoidance scale.

Clinical Phenotypes—Data for lifetime lowest attained BMI and age at menarche were obtained from SIAB-EX for DSM-IV and ICD-10 (Fichter, et al., 1998) for those with an eating disorder and from a demographic questionnaire for the control group.

Personality and Symptom Assessments—Drive for thinness and body dissatisfaction, two psychological features associated with AN, were from the EDI (Garner, et al., 1983), a 64-item self-report measure assessing behavioral dimensions of eating disorders. Trait anxiety was measured using the STAI-Y (Spielberger, et al., 1970), a widely used 40-item self-report measure assessing state and trait anxiety in adults. Concern over mistakes, which reflects the tendency to react negatively to mistakes, was assessed using the MPS (Frost, et al., 1990), a 35-item self-report scale comprised of six subscales. Harm avoidance was assessed using the anticipatory worry and pessimism vs. uninhibited

optimism subscale [Harm Avoidance 1(HA1)] of the TCI (Cloninger, et al., 1993), an assessment indexing multiple dimensions of personality.

Data Analyses

Statistical software R 2.9.1 (R Development Core Team, 2009), JMP 7.0 (SAS Institute Inc., 2004), and PLINK (Purcell, et al., 2007) were used for all analyses. Departures from normality were found, however the qualitative conclusions of the association test were unchanged when transformed data were used (data not shown). Prior to the association analyses we conducted a principal components analysis to test if the seven phenotypes could be reduced to one principal component, thereby decreasing the number of tests conducted. Each of the seven phenotypes contributed unique variability. Thus, each phenotype was analyzed separately.

For each phenotype, association was tested using the genotypes of individual SNPs under the additive model accounting for affection status and the interaction between the SNP and affection status. Logistic regression was used for binary variables and linear regression for continuous variables. The best model for each analysis was selected using a step-wise procedure based on AIC criterion. For each analysis, correction for multiple testing was accomplished using the local false discovery rate (FDR; Efron, Tibshirani, Storey, & Tusher, 2001) approach implemented in R (R/fdrtool; Strimmer, 2008).

Power Analysis—Power analysis was conducted using Genetic Analysis Package (Zhao, 2007) and Genetic Power Calculator (Purcell, et al., 2007). Under an additive genetic model assuming disease prevalence of 0.009, disease allele frequency of 0.4, and assuming type I error rate at 5×10^{-8} , our sample had 80% power to explain at least 2.2% of the variance for each quantitative trait (see Figure 1).

RESULTS

Demographics and Summary Statistics for Seven Quantitative Phenotypes

Across the total sample ($n=1762$), 24% ($n=415$) of participants were classified as restricting AN, 15% ($n=266$) as AN with purging but no binge eating, 7% ($n=132$) with AN with binge eating with or without purging, 15% ($n=266$) with AN and BN, and <1% ($n=6$) with subthreshold AN with no binge eating or purging. Thirty-eight percent ($n=677$) served as controls with no history of AN. Mean age at interview for the AN sample was 27 years ($SD=8.8$) and 26 years ($SD=8.3$) for the control sample ($p=.051$).

Table 1 presents means, standard deviations (SD), and tests of significance for the seven phenotypes in the AN and control samples. As expected, statistically significant differences emerged between the AN and control samples for all seven quantitative phenotypes. Compared with the control sample, the AN group had a lower mean value for lowest attained BMI and higher scores for age at menarche, drive for thinness, body dissatisfaction, trait anxiety, concern over mistakes, and the anticipatory worry and pessimism vs. uninhibited optimism subscale of the harm avoidance scale.

Association Analyses

Table 2 presents the SNPs with the lowest adjusted p-value and the associated gene for each of the seven quantitative phenotypes. After controlling for multiple comparisons, no statistically significant tests of association were found.

DISCUSSION

In this case-control association analysis using empirically selected quantitative phenotypes for AN, we sought to determine whether phenotypes used to refine linkage analyses (Bulik, et al., 2005; Devlin, et al., 2002) would yield informative results in association analyses. We failed to identify statistically significant associations after controlling for multiple comparisons.

Despite the rigor with which the intermediate phenotypes were selected (Bieling, Antony, & Swinson, 1998), it is possible that alternate phenotypes or endophenotypes would be more applicable for these analyses. Novel experimental approaches for defining endophenotypes for use in gene discovery studies of AN are in their infancy and the potential avenues for exploration are many. One approach applies neuroimaging genetics by measuring the effects of genetic variants on neural activation patterns in areas of the brain that modulate emotional and cognitive response to reward, threat, and habit patterns (Zhao, 2007), phenotypes which may be proximal to the extremes of motivational behavior and disordered habit patterns characteristic of AN. A second approach to defining endophenotypes for gene discovery is to apply neuropsychological deficits of possible relevance to AN, including set-shifting (Holliday, Tchanturia, Landau, Collier, & Treasure, 2005; Roberts, Tchanturia, Stahl, Southgate, & Treasure, 2007), impaired interoception and proprioception (Kaye, Fudge, & Paulus, 2009; Zucker, et al., 2007), or objective measures such as physical activity (Davis, Kennedy, Ravelski, & Dionne, 1994).

Lack of adequate power is a second possible explanation for our failure to find significant associations. Although our study had significant strengths, our analyses may have lacked sufficient power for identifying associations with small effects.

A third possible explanation is that the sample structure itself could obscure associations to a particular dependent variable. For example, associations might be modulated if the polymorphism had pleiotropic effects or if there were different relations between gene and dependent variable in different subgroups (i.e., there is a genetic association between restricting AN and drive for thinness but this association is not observed in those with AN with purging). Thus, the potential value of the approach taken here cannot be dismissed at this time.

Even though non-significant, the top SNP for drive for thinness (rs17719880) is worth mentioning given that it is located in the KCNN3 gene, which has previously been associated with AN and comorbid obsessive-compulsive disorder among a Jewish Israeli population (Koronyo-Hamaoui, et al., 2002; Koronyo-Hamaoui, et al., 2004). Further, the SNP with the lowest p-value for concern over mistakes (rs12744840) is located in the HCRTR1 gene—a gene involved in the regulation of feeding behavior (Sakurai, et al., 1998). Studies employing samples that are larger and potentially selected for extreme values of high and low eating disorder phenotypes will be needed to determine if any true association exists.

Despite the negative findings, we believe that the method using intermediate AN phenotypes deserves application in future genetic studies and that the rationale for a large scale unbiased search of risk variants is clear. As with other psychiatric disorders, AN is a complex disease thought to involve both genetic and environmental risk factors (Bulik, et al., 2006; Bulik, et al., 2010; Klump, Miller, Keel, McGue, & Iacono, 2001; Kortegaard, Hoerder, Joergensen, Gillberg, & Kyvik, 2001; Lilienfeld, et al., 1998; Striegel-Moore & Bulik, 2007; Strober, et al., 2000; Wade, et al., 2000). Previous genetic studies of AN based on candidate gene association and genome-wide linkage remain inconclusive (Bacanu, et al., 2005; Bulik, et al., 2007; Devlin, et al., 2002; Grice, et al., 2002; Slof-Op't Landt, et al., 2005). In the

advent of genomic technology advances, new experimental approaches have become available for the investigation of unbiased complex disorders regarding genomic location and type as well as allele frequency of genetic variants (Corvin, Craddock, & Sullivan, 2010; Gill, Donohoe, & Corvin, 2010). First, array-based genome-wide association studies (GWAS) have become a standard and have demonstrated the ability for identifying common risk variants of modest effects. Ample evidence demonstrates that AN is heritable and thus, there is a compelling rationale for applying GWAS methods to identify novel loci. Indeed, GWAS findings have identified associations of common SNPs and rare copy number variants (CNVs) with psychiatric disorders, including AN (Cichon, et al., 2009; Nakabayashi, et al., 2009; Stefansson, et al., 2008; Wang, et al., 2010). Twin studies report heritability estimates usually >50% (Bulik, et al., 2006; Klump, et al., 2001; Kortegeard, et al., 2001; Wade, et al., 2000). Further, recent findings suggest that both common SNPs and rare CNVs may confer genetic risk to AN (Wang, et al., 2010). Thus, there is plausible rationale for GWAS for AN. Second, high-throughput sequencing has become technically and financially practical and has the potential for identifying rare sequence variants (Lupski, et al., 2010; Roach, et al., 2010). Third, CNVs are detectable through either microarrays (Carter, 2007) or high-throughput sequencing (Medvedev, Stanciu, & Brudno, 2009) and could be included in the genetic study of AN. Last, to address sample size, a community effort is desired to conduct mega- and meta-analyses using as much data as can be obtained.

Some logical next steps include: (1) conducting research on AN phenotype refinement in order to optimize detection of genetic variants conferring risk to AN; (2) examining the alternate phenotypes presented in this paper, as well as using other refined phenotypes based on classification analyses and experimental study design, in larger samples; and lastly, (3) conducting unbiased genome-wide detection of genetic variations, such as GWAS, enabled by high-throughput genotyping and whole genome or Exome sequencing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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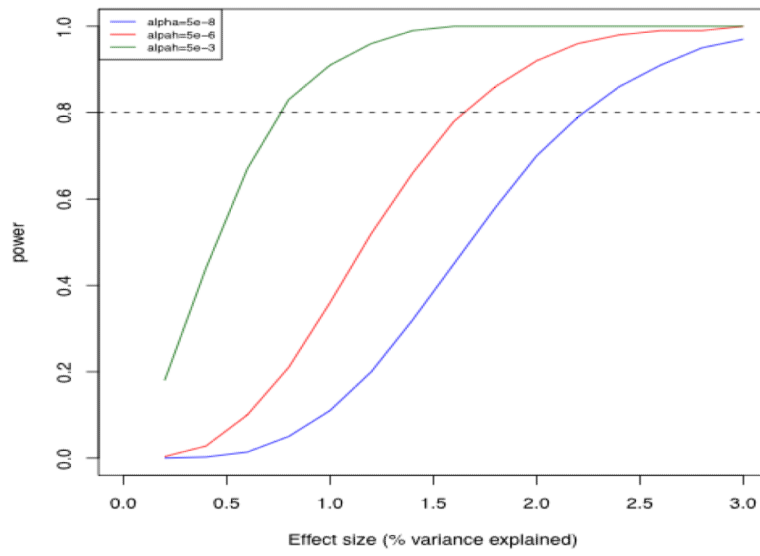


Figure 1. Power curves for quantitative phenotypes under an additive genetic model assuming disease prevalence of 0.009, disease allele frequency of 0.4, and type I error rate at 5×10^{-8} .

Table 1

Mean (SD) and tests of significance across the anorexia nervosa (AN) and control samples for the seven quantitative phenotypes used in the association analyses.

Variable	AN sample (n=1085)	Control sample (n=677)	t-test (p-value)
Lowest Attained BMI	14.2 (2.1)	20.4 (1.3)	78.30 (<.001)
Age at Menarche	13.2 (1.9)	12.7 (1.4)	-6.98 (<.001)
Drive For Thinness	15.3 (5.8)	0.5 (1.5)	-68.97 (<.001)
Body Dissatisfaction	18.1 (7.3)	3.4 (4.2)	-48.18 (<.001)
Trait Anxiety	52.7 (13.8)	29.5 (7.0)	-46.13 (<.001)
Concern Over Mistakes	33.0 (9.4)	15.9 (5.9)	-46.78 (<.001)
Anticipatory Worry and Pessimism vs. Uninhibited Optimism (Harm Avoidance)	6.8 (2.9)	2.8 (2.0)	-33.81 (<.001)

Note. SD=standard deviation. BMI=body mass index.

Table 2

Results of the single nucleotide polymorphism (SNP) with the lowest adjusted p-value from a total of 5151 SNPs from association analyses for each quantitative phenotype.

Variable	Lowest p-value (unadjusted)	Lowest p-value (FDR adjusted)	SNP	Gene	Chr	Base Pair
Lowest Attained BMI	0.000602	0.755471	rs11564710	INS	11	2156905
Concern over Mistakes	0.000165	0.755471	rs12744840	HCRTR1	1	31780194
Anticipatory Worry and Pessimism vs. Uninhibited Optimism (Harm Avoidance)	0.0000613	0.755471	rs7093673	GRK5	10	120972346
Trait Anxiety	0.000634	0.755471	rs16822416	DRD3	3	115373000
Trait Anxiety	0.000634	0.755471	rs3732783	DRD3	3	115373479
Drive for Thinness	0.000179	0.755471	rs17719880	KCNN3	1	151605004
Body Dissatisfaction	0.0000589	0.730628	rs7937452	GRM5	11	87866044
Age at Menarche	0.0000832	0.737012	rs11126453	TACR1	2	75280395

Note. SNP=single nucleotide polymorphism. FDR=false discovery rate. Chr=chromosome. BMI=body mass index.