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Association Study of 182 Candidate Genes in Anorexia Nervosa

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Abstract

We performed association studies with 5,151 SNPs that were judged as likely candidate genetic variations conferring susceptibility to anorexia nervosa (AN) based on location under reported linkage peaks, previous results in the literature (182 candidate genes), brain expression, biological plausibility, and estrogen responsivity. We employed a case–control design that tested each SNP individually as well as haplotypes derived from these SNPs in 1,085 case individuals with AN

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diagnoses and 677 control individuals. We also performed separate association analyses using three increasingly restrictive case definitions for AN: all individuals with any subtype of AN (All AN: n = 1,085); individuals with AN with no binge eating behavior (AN with No Binge Eating: n = 687); and individuals with the restricting subtype of AN (Restricting AN: n = 421). After accounting for multiple comparisons, there were no statistically significant associations for any individual SNP or haplotype block with any definition of illness. These results underscore the importance of large samples to yield appropriate power to detect genotypic differences in individuals with AN and also motivate complementary approaches involving Genome-Wide Association (GWA) studies, Copy Number Variation (CNV) analyses, sequencing-based rare variant discovery assays, and pathway-based analysis in order to make up for deficiencies in traditional candidate gene approaches to AN.

Keywords

INTRODUCTION

Anorexia nervosa (AN) is a potentially chronic illness with extremely high morbidity and mortality for which no generally agreed upon model of etiology presently exists [Sullivan, 1995; Birmingham et al., 2005]. In spite of the chronicity and high economic burden it confers, the effects of currently applied treatment approaches remain largely disappointing. Although psychosocial factors were long implicated in its etiology [Striegel-Moore and Bulik, 2007], a growing body of evidence now supports the role of genetic influences, although no specific variants have been definitively implicated as causal to date [Bulik et al., 2007]. In this light, however, the familiality of AN has now been well established, with first-degree female relatives of individuals with AN having an approximately 10-fold greater lifetime risk of having an eating disorder compared with relatives of unaffected individuals [Lilenfeld et al., 1998; Strober et al., 2000]. Heritability estimates for AN have ranged from 46% to 78% [Wade et al., 2000; Klump et al., 2001; Kortegaard et al., 2001; Bulik et al., 2006]. The convergent findings from family and twin studies have motivated efforts to identify loci that contribute to risk for AN through linkage and association studies.

A previous study from our group that applied linkage analysis to affected relatives with the restricting type of AN yielded significant evidence for a susceptibility locus in the 1p33–36 region [Grice et al., 2002]. In addition, enhancements to traditional linkage analyses that have incorporated key behavioral covariates such as drive for thinness and obsessionality have resulted in suggestive linkages on chromosomes 1, 2, and 13 [Devlin et al., 2002].

Specific candidate gene approaches have targeted neurotransmitter and neuropeptide systems, and have included a variety of serotonergic, epinephrine/norepinephrine and dopaminergic genes, genes coding for proteins associated with the melanocortin system, and genes for leptin, ghrelin, agouti-related protein, neuropeptide Y, opioids, cannabinoid receptors, potassium channels, brain derived neurotrophic factor, and reproductive hormones. Several reviews have been published detailing the results of these studies [Tozzi et al., 2002; Hinney et al., 2004; Klump and Gobrogge, 2005; Slof-Op 't Landt et al., 2005; Bulik et al., 2007]. Overall, association studies have yielded sporadically significant and typically non-replicated findings [Bulik et al., 2007] highlighting the methodological and statistical challenges associated with genetic studies of a relatively low prevalence disorder (e.g., small sample sizes, lack of power, multiple testing issues, and varying phenotypic definitions) [Balding, 2006]. As with other, and perhaps all, complex traits, it is likely that susceptibility to disease is comprised of multiple, relatively common genetic variants each of which confers only small to modest risk in conjunction with environmental factors, a model of disease that will require approaches that

increase the number of target genes and single nucleotide polymorphisms (SNPs) investigated. Nonetheless, in the literature on AN, some associations have been replicated, with the accumulated data suggesting the involvement of *HTR1D* and *OPRD1* [Bergen et al., 2003; Brown et al., 2006], SLC6A4 [Gorwood, 2004], and *BDNF* [Ribases et al., 2004; Gratacos et al., 2008].

Accordingly, we present herein the results of a case–control association study in a sample of 1,085 unrelated women with a lifetime history of AN and 677 unrelated controls ascertained in the multisite Price Foundation Genetic Studies of Eating Disorders initiative between 1996 and 2002 [Kaye et al., 2000, 2004; Reba et al., 2005]. We tested 5,151 SNPs as well as the derived haplotypes from these SNPs for association to three progressively more restrictive phenotypic definitions of anorexia nervosa. We selected a total of 182 candidate genes that had been previously identified in case–control candidate gene association analyses, genes under linkage peaks for anorexia nervosa and bulimia nervosa [Devlin et al., 2002; Grice et al., 2002; Bulik et al., 2003; Bacanu et al., 2005], genes with evidence of expression in the brain, biologically plausible genes, and genes that were known to be estrogen responsive from microarray studies.

MATERIALS AND METHODS

Participants

Participants of primarily European decent were from the three multisite international Price Foundation Genetic Studies of Eating Disorders: the Anorexia Nervosa Affected Relative Pair Study, the Bulimia Nervosa Affected Relative Pair Study, and the Anorexia Nervosa Trios Study [Kaye et al., 2000, 2004; Reba et al., 2005]. Each study was designed to identify susceptibility loci involved in risk for eating disorders. Informed consent was obtained from all study participants, and all sites received approval from their local Institutional Review Board. Brief descriptions of each study are provided below.

AN affected relative pair study—The sample for this study included both probands and affected relatives. Probands met the following criteria: (1) lifetime diagnosis of AN by DSM-IV criteria excluding the amenorrhea criterion because of the unreliability of its retrospective assessment in females, and replicated data indicating that individuals with and without amenorrhea do not differ meaningfully [Gendall et al., 2006; Poyastro Pinheiro et al., 2007]; (2) low weight that is/was less than the 5th percentile of body mass index (BMI) for age and gender according to the Hebebrand et al. [1996] NHANES chart; (3) ages 13 and 65 years; (4) eating disorder onset prior to age 25; and (5) having met criteria for AN not less than 3 years prior to ascertainment. Affected relatives were defined as biological family members who were (1) between the ages of 13 and 65 years, and (2) had lifetime eating disorder diagnoses of modified DSM-IV AN (again excluding amenorrhea), lifetime eating disorder diagnoses of DSM-IV bulimia nervosa (BN)-purging type or nonpurging type, or an eating disorder not otherwise specified (EDNOS)—sub-threshold AN, sub-threshold BN, or sub-threshold mixed (relatives who were normal weight but reported either purging behavior or excessive exercise or periods of fasting due to extreme fear of weight gain or undue influence of body weight on self-esteem). For the complete list of inclusion and exclusion criteria for probands and relatives, 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) DSM-IV diagnosis of BN—purging type, with the additional requirement of at least a 6-month period of binge eating and vomiting at least twice a week; and (2) ages 13 to 65. Affected relatives were defined as biological family members who were (1) between the ages of 13 and 65 years, and (2) had a lifetime eating

disorder diagnoses of modified DSM-IV AN (i.e., amenorrhea not required), DSM-IV BN—purging type or non-purging type, or eating disorder not otherwise specified EDNOS as defined above. For the complete list of inclusion and exclusion criteria for pro-bands 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 were required to meet the following criteria: (1) modified DSM-IV lifetime diagnosis of AN, with or without amenorrhea; (2) low weight that is/was less than 5th percentile of BMI for age and gender according to the Hebebrand et al. [1996] NHANES chart; (3) onset prior to age 25; (4) weight that is/was controlled through restricting and/or purging; (5) between the ages of 13 and 65; and (6) study diagnostic criteria were met at least 3 years prior to entry into the study. Potential affected participants were excluded if they reported maximum BMI since puberty >27 kg/m^2 for females and >27.8 kg/m^2 for males to limit any potential genetic signals from obesity. Parents were encouraged to participate regardless of age, weight, or psychiatric diagnosis.

In the AN Trios Study, healthy control women were recruited to serve as a comparison group for the affected individuals. These women were recruited by advertisements in the local communities of the participating sites. Participants in the control group were (1) between the ages of 18 and 65 years, (2) at normal weight with a lifetime adult BMI 19–27 kg/m^2, and (3) matched with the eating disorder participants based on site, age range, ancestry, and highest educational level completed. BMI exclusions were designed to screen for eating disorders (on the low end) and obesity on the upper end to be consistent with exclusion criteria in the eating disorders groups. Participants in the control group 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., 1982]; (2) had a first degree relative with an eating disorder; or (3) had any of the following as defined by the presence of an Axis I disorder on the Structured Clinical Interview for DSM-IV (SCID) Screen Patient Questionnaire [First et al., 1997]: mood disorders, anxiety disorders, alcohol dependence and abuse, other substance dependence and abuse, somatoform disorders, eating disorders, and schizophrenia and other psychotic disorders. Participants in the control group completed the same battery of self-report assessments as probands, which covered personality and symptom measures.

Participant Selection for Association Studies

From the three studies above, a total of 2,257 individuals were selected for these association studies. Participants were first chosen based on whether an adequate genomic DNA sample was available. Then, participants were ordered using a diagnostic hierarchy (highest to lowest), regardless of whether they were probands or affected relatives: restricting AN (RAN), AN with purging but no binging (PAN), AN with binge eating with or without purging (BAN), a lifetime history of both AN and BN (ANBN), subthreshold AN with no binging or purging, purging BN, non-purging BN, and subthreshold BN. The individual from each family with the diagnosis that was highest on the hierarchy was selected. These participants as well as 677 control women from the AN Trios Study were defined as the primary samples for inclusion. A secondary set of samples was selected based on the criteria above and each of these participants was related to one individual in the primary sample. Genomic DNA samples from these individuals were sent to Illumina for genotyping. Of these individuals, we removed 11 participants whose DNA samples failed genotyping; 220 participants who were included for quality control purposes; and 264 participants included to support pilot projects (27 males with AN, 121 first-degree relatives, and 116 individuals with eating disorder diagnoses other than AN). No participants were excluded due to excessive genotype missingness (all were ≤0.021). Thus the overall sample includes 1,762 female participants (1,085 cases and 677 controls). In this analysis

sample, there were 10 families with two second-degree relatives, 15 families with two third-degree relatives, and three families with two fourth-degree relatives.

Table I presents the abbreviations and definitions of illness on which we based our samples for analysis. We chose three increasingly restrictive definitions of AN based on the literature and previous linkage analysis results [Devlin et al., 2002;Grice et al., 2002]. For all definitions, participants were required to meet all DSM-IV criteria for AN except Criterion D, amenorrhea. The first analysis was conducted on the sample defined by the broadest definition of illness (All AN; N = 1,085). This sample included 415 individuals with RAN, 266 with PAN, 132 with BAN, 266 with ANBN, and 6 with subthreshold AN with no binging or purging. The second analysis was based on a sample defined as AN with No Binge Eating (N = 687) and included only individuals with AN subtypes that did not include regular binge eating. This definition of illness was chosen due to the fact that the presence of binge eating has been identified as a distinguishing feature within the AN diagnostic category [Herzog et al., 1996]. This sample comprised 415 individuals with RAN, 266 with PAN, and 6 with subthreshold AN with no binging or purging. The third analysis included the narrowest definition of illness, namely Restricting AN (N = 421), based on previous linkage analyses that identified significant and suggestive linkage signals based on a case definition of Restricting AN [Devlin et al., 2002;Grice et al., 2002]. This sample included 415 individuals with RAN and 6 individuals with subthreshold AN with no binging or purging. The AN with No Binge Eating sample was a subset of the All AN sample, and the Restricting AN sample was a subset of the AN with No Binge Eating sample.

Candidate Gene and SNP Selection

Candidate genes were selected by the investigators via a consensus process. First, genes residing under reported eating disorders linkage peaks were identified using SLEP [Konneker et al., 2008]. Second, an exhaustive search of the literature was conducted to identify plausible candidate genes based on previous findings reported in the eating disorders literature, published findings in other related disorders, and genes involved in pathways thought to be implicated in AN. This inclusive list was limited by selecting genes with evidence of expression in the brain and by genes that were shown to be estrogen responsive in microarray studies. Based on the RefSeq data base [Pruitt et al., 2007] accessed in 3/2007, these combined approaches yielded a list of 247 genes which were then necessarily prioritized by consensus to match available genotyping budget and winnowed to 190 plausible candidate genes. See supplemental material for the complete gene list.

SNP selection was conducted in two rounds. These 190 candidate genes were processed by TAMAL v2.0 [Hemminger et al., 2006], indicating that 186 could be uniquely mapped. SNPs were selected to tag common variation in Europeans (using TAGGER, aggressive tagging, r^2 threshold 0.8, and any coding SNPs forced in). Additional SNPs with MAF ≥0.05 in splice sites, exons (synonymous or non-synonymous), and highly conserved regions were selected along with SNPs in predicted promoter, regulatory, transcription factor binding, and micro RNA target sites. The final list comprised 186 genes and 6,568 SNPs and was sent to Illumina for genotyping with their Custom Infinium Genotyping Beadchips platform.

SNP Quality Control

Despite favorable design scores, the SNP assay design process failed at Illumina for 480 SNPs. An additional 237 SNPs failed genotyping and genotypes for 5,851 SNPs were delivered. Quality control (QC) filters were then applied and a total of 700 SNPs failed for the following reasons (note that a SNP could fail for more than one reason): minor allele frequency (MAF) <0.01 (538 SNPs); AN Trios (affected individual with two parents, parents only used for QC) with ≥2 Mendel errors (1 SNP); duplicate samples with ≥2 disagreements (111 SNPs); missing

genotypes in >0.05 of cases or controls (24 SNPs); differential genotype missingness in cases versus controls at P <0.01 (4 SNPs); and Hardy–Weinberg equilibrium (HWE) exact P <0.01 in controls (47 SNPs). The total number of SNPs passing all QC steps was 5,151. The total number of genes with SNPs passing all QC steps was 182. In addition, the plot of homozygosity over all SNPs showed no significant differences between cases and controls ($P = 0.35$) and no individual had marked elevated homozygosity. Finally, population stratification was assessed through genomic control [Devlin and Roeder, 1999]: lambda (λ) = 1.035 is consistent with an acceptably small amount of population stratification.

Statistical Analyses

PLINK [Purcell et al., 2007] was used to conduct all association analyses. The principal test for association was the 1 degree-of-freedom Cochran-Armitage trend test. For each analysis, multiple testing correction was accomplished using the false discovery rate approach [Benjamini and Hochberg, 1995; Benjamini et al., 2001]. Haplotype analyses using tagging SNPs were also performed. Haplotype blocks for these 5,151 SNPs were defined using the TAGGER method in Haploview 4.0 [Barrett et al., 2005]. The Haploview algorithm creates blocks if 95% of informative comparisons are in strong linkage disequilibrium. These blocks, ranging in size from 2 SNPs to 79 SNPs (chr 14), were then imported into PLINK for haplotype analysis. Because linkage disequilibrium was used to generate haplotype blocks, the sliding window method for haplotype analysis was not applied. Quanto [Gauderman, 2002] was used to approximate statistical power (0.80) given the following assumptions: two-tailed $P = 9.7 \times 10^{-6}$ (Bonferroni correction for 5,151 SNPs), lifetime morbid risk of AN of 0.009 [Hudson et al., 2007], and a log additive genetic model. For the broad phenotype, All AN, (1,085 AN cases, 677 controls), the minimum detectible genotypic relative risks were 1.8, 1.5, and 1.5 for minor allele frequencies of 0.10, 0.25, and 0.40. For the AN with No Binge Eating phenotype, (687 cases, 677 controls), the minimum detectible genotypic relative risks were 1.9, 1.6, and 1.5 for minor allele frequencies of 0.10, 0.25, and 0.40. For the narrow phenotype, Restricting AN, (421 cases, 677 controls), the minimum detectible genotypic relative risks are 2.0, 1.7, and 1.6 for minor allele frequencies of 0.10, 0.25, and 0.40.

RESULTS

Results for the All AN Phenotype

Table II presents the 25 most significant SNPs from the association analyses conducted on the broadest definition of illness, that is, the All AN phenotype. The λ for All AN was 1.01, close to unity, which is consistent with a lack of stratification artifacts. The minimum p value using the trend test was 4.7×10^{-5}, which corresponds to an FDR of 0.22, suggesting no significant differences between cases and controls. Table II also presents the p values and FDR for those top 25 SNPs from the analyses reported below for the AN with No Binge Eating and Restricting AN phenotypes for comparison. Figure 1a presents the QQ plot for this comparison. The QQ plot is based on P-values that correspond to the 1 degree-of-freedom Wald test on 5,151 autosomal SNPs by assuming an additive risk model. The observed values correspond closely to that expected by chance.

Results for the AN With No Binge Eating Phenotype

Table III presents the top 25 SNPs for analyses conducted on the AN with No Binge Eating sample. The λ for AN with No Binge Eating was 1.005, also close to unity, which is not consistent with the presence of stratification artifacts. Using the trend test, the minimum P-value was 1.9×10^{-4}, corresponding to an FDR of 0.62, suggesting no statistically significant differences between cases and controls. Figure 1b shows the QQ plot and again shows no deviation of observed from expected P-values.

Results for the Restricting AN Phenotype

Table IV presents the top 25 SNPs for analyses conducted on the Restricting AN sample. The λ for Restricting AN was 1.0. The minimum *P*-value using the trend test was 4.7×10^{-4}, which corresponds to an FDR of 0.91. Figure 1c depicts the QQ plot with no deviation of observed from expected *P*-values. Again, the observed *P*-value distribution corresponds closely to that expected by chance.

Haplotype Analyses

No haplotypes reached the statistical threshold of 5×10^{-7}. Results for the top 25 haplotypes for analyses conducted on each of the samples are available in the supplementary material.

DISCUSSION

Discovering genetic variants that confer risk to AN has the potential to illuminate pathways relevant to both etiopathogenesis and drug development efforts. However, to date replicable associations of candidate gene tagging and coding SNPs with diagnostic categories have mostly eluded the eating disorders field. The present study arguably represents the most comprehensive candidate-gene-based approach conducted in eating disorders. We performed association tests with SNPs and haplotypes to detect associations with a priori defined statistical thresholds to three eating disorder case–control datasets, where the cases were nested based on binging and purging. We studied a relatively large sample, selected high probability candidate genes, used tagging and coding SNPs, and applied conservative quality control and multiple test correction procedures. However, these association analyses yielded no statistically significant results.

The current study's failure to identify significant associations does not mean that associations do not exist. Although statistically nonsignificant, our top SNP is noteworthy in that the *GLP2R*, which is a member of the G protein-coupled receptor superfamily, is a receptor for a 33-amino acid proglucagon-derived peptide (GLP2), both expressed primarily in the gut. GLP2 has been shown to slow the ingestion and transit of food through the GI tract [Burrin et al., 2001]. While there is no evidence to suggest that systemic administration of GLP2 is associated with food intake, central administration of GLP2 in the lateral cerebral ventricle has been shown to inhibit food intake in rats [Tang-Christensen et al., 1996; Drucker, 1998]. GLP2 is also related to homeostatic control of human body weight in that GLP2 acts as a neurotransmitter linking the brainstem with the dorsal medial hypothalamic nucleus [Tang-Christensen et al., 2000]. Our second top SNP is associated with the *PAH* gene which codes for the enzyme phenylalanine hydroxylase [Scriver, 2007]. Phenylalanine hydroxylase is responsible for the conversion of phenylalanine to tyrosine. Phenylalanine is found in all proteins and some artificial sweeteners. Mutations in the *PAH* gene are most commonly associated with phenylketonuria (PKU), an inherited disorder which increases levels of phenylalanine in the blood [Erlandsen et al., 2003].

Finally, the top two SNPS located on chromosome 1 are also worth mentioning given that significant over-representation of *KCNN3* alleles with longer CAG repeats have been previously reported in AN patients [Koronyo-Hamaoui et al., 2002, 2004]. The small-conductance calcium-activated potassium channel gene *KCNN3* is expressed in brain; increased expression in the hippocampus has been associated with reduced long-term potentiation [Blank et al., 2003]. Although several studies have reported positive associations of increased CAG-repeat length of KCNN3 with schizophrenia and bipolar disorder, a recent meta-analysis casts doubt on this association, while leaving open the possibility that other aspects of this polymorphism may contribute to specific features of these disorders [Glatt et al., 2003].

This study had significant strengths including a five-nation cooperative and coordinated case and control ascertainment consortium, comprehensive phenotyping that was carefully designed and coordinated, and gene selection procedures designed to optimize findings. Nonetheless, the study was underpowered, limiting our ability to detect significant effects. After FDR corrections, the smallest *P*-value observed was 0.2125 for SNP rs4791362 on gene GLP2R for the All AN analyses. This corresponds to a relative risk of 1.3, 1.2, and 1.2 for minor allele frequencies of 0.10, 0.25, and 0.40. Using statistical power of 0.80, in a 1:1 unmatched case/ control study, the minimum sample sizes needed to obtain significance for these relative risks are approximately 4,050, 4,270, and 3,430, respectively, three to four times the number of the participants in this study. Lack of power is a common problem faced by consortia studying the genetics of relatively rare phenotypes such as AN. Statistical power was particularly weakened as case numbers dropped with the increasingly restrictive definitions of AN. Our approach to case definition was based on previous findings (i.e., stronger linkage signals with more restrictive case definitions). However, there are two concerns with narrowing the case definition: (1) statistical power is reduced, and (2) other case definition restrictions may lead to different results. In addition, although our strategy of selecting high-probability candidate genes was scientifically defensible, it is noteworthy that many genetic variants identified by genomewide association studies have not emerged from lists of "usual suspects" and have been genes previously not thought to be involved in the target disease etiology [Psychiatric GWAS Consortium (PGC), 2009].

Our results, in conjunction with the published inconsistent findings in single candidate-gene studies, underscore the critical need for larger samples but also additional analytic approaches to detect genotypic differences between individuals with and without AN. In this context, as we better appreciate the nature of diverse individual differences in trait characteristics, cognitions and affective processes, and symptomatic behaviors with broadly defined diagnostics classes, it will be essential for genetic analyses of AN to make better use of empirically derived phenotype selection criteria that integrate neural systems information with these behavioral data.

Following these points, several complementary approaches exist. One alternative is a genomewide association (GWA) scan on AN. GWA studies have been successful in identifying highly replicable associations for T1DM, T2DM, Crohn's disease, cardiovascular disease, prostate cancer, breast cancer, body mass index, nicotine addiction, and height [Bierut et al., 2007; Consortium, 2007; Frayling et al., 2007; Saxena et al., 2007; Scott et al., 2007; Berrettini et al., 2008; Thorgeirsson et al., 2008]. Whether similar successes will be realized in psychiatry remains unclear although many efforts are currently underway [Psychiatric GWAS Consortium (PGC), 2009]. Critical to note is that our approach focused on common variants (MAF >0.5%), and thus rare variation has been necessarily missed. Our results do not eliminate these genes as potential candidates for AN, but rather indicate that common variants located in them are unlikely to be the causal variants. Other approaches include searches for multiple rare variations via sequencing studies [Nejentsev et al., 2009], analysis of copy number variations [Walsh et al., 2008], accommodation and consideration of epigenomic factors [Peedicayil, 2004] and sophisticated multi-locus analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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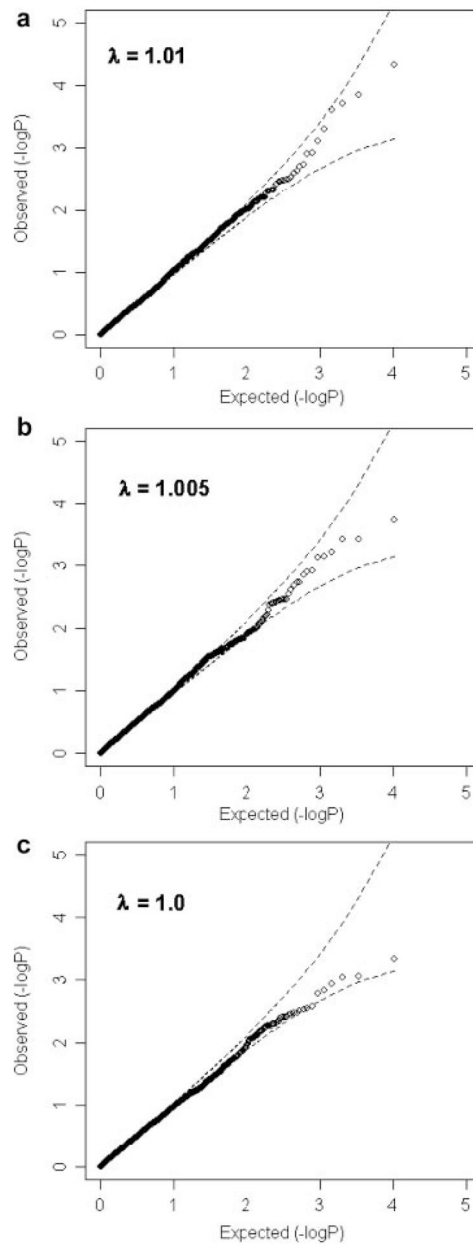
FIG. 1.
QQ plots for All AN (a), AN with No Binge Eating (b), and Restricting AN (c). The dashed lines show the expected 95% probability interval for ordered *P* values.

TABLE I

Abbreviations and Definitions of Illness Used in This Study

Abbreviations	Name	Included in which analyses?	Description
RAN	Anorexia nervosa restricting subtype	All AN AN with no binge eating Restricting AN.	DSM-IV AN[a] with no lifetime history of binge eating or purging
PAN	Anorexia nervosa purging subtype	All AN AN with no binge eating	DSM-IV AN[a] with a lifetime history of: (1) Purging behavior of any frequency. (2) No lifetime history of binge eating
BAN	Anorexia nervosa with binge eating	All AN	DSM-IV AN[a] with a lifetime history of: (1) Limited binge eating defined as less that twice per week for 3 months (probands and affected relatives) or regular binge eating (affected relatives only). (2) With or without any purging behavior
ANBN	Lifetime anorexia nervosa and bulimia nervosa	All AN	Lifetime history of: (1) Any DSM-IV AN[a] subtype AND, at a different time. (2) DSM-IV BN
Subthreshold AN no binge eating or purging	Eating disorders not otherwise specified	All AN AN with no binge eating Restricting AN	Subthreshold 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] No binge eating or purging

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

TABLE II

All Anorexia Nervosa (N = 1,085), Top 25 SNP Associations (Sorted by All AN Trend *P*) and Comparative Results for the Top 25 SNPs in the AN No Binge (n = 687) and Restricting AN (n = 421) Analyses

SNP	Gene	Chr.	Base pair	All AN trend *P*	FDR	AN no binge trend *P*	FDR	Restricting AN trend *P*	FDR
rs4791362	*GLP2R*	17	9654811	0.000047	0.2151	0.000187	0.6183	0.003652	1
rs1718312	*PAH*	12	101743655	0.000143	0.522	0.001392	0.9992	0.000894	0.99
rs2173114	*SLC18A1*	8	20087205	0.000192	0.6279	0.000377	0.8567	0.004892	1
rs10510057	*RGS10*	10	121301038	0.000242	0.7127	0.000727	0.9764	0.01541	1
rs2917928	*RGS10*	10	121305880	0.000506	0.9263	0.000618	0.9586	0.0133	1
rs10908435	*KCNN3*	1	151542467	0.000779	0.9819	0.000385	0.7979	0.000468	0.9101
rs3813720	*ADRB1*	10	115797006	0.001209	0.998	0.003955	1	0.00504	1
rs6876225	*SLC6A3*	5	1459036	0.001271	0.9986	0.004103	1	0.08563	1
rs2926832	*GRIA1*	5	153175865	0.001829	0.9999	0.002476	1	0.01683	1
rs219892	*GRIN2B*	12	13978866	0.001977	1	0.02561	1	0.1972	1
rs2247952	*QDPR*	4	17197045	0.002389	1	0.01455	1	0.05044	1
rs242924	*CRHR1*	17	41241147	0.002514	1	0.0021	1	0.00801	1
rs219873	*GRIN2B*	12	13967713	0.002926	1	0.02723	1	0.2048	1
rs11055693	*GRIN2B*	12	13986724	0.003169	1	0.01356	1	0.2835	1
rs2839671	*GAD2*	10	26545828	0.003233	1	0.01148	1	0.06423	1
rs11564771	*SLC6A3*	5	1451797	0.003404	1	0.01156	1	0.101	1
rs11577391	*KCNN3*	1	151530224	0.003405	1	0.004307	1	0.003372	1
rs2055940	*GABRA4*	4	46838841	0.003472	1	0.0515	1	0.05261	1
rs1390939	*SLC18A1*	8	20084992	0.003521	1	0.003975	1	0.03851	1
rs1055663	*FREQ*	9	130079012	0.003833	1	0.01167	1	0.02196	1
rs3733570	*QDPR*	4	17179702	0.003942	1	0.01843	1	0.01391	1
rs8190598	*GAD2*	10	26547601	0.004712	1	0.01556	1	0.07955	1
rs219931	*GRIN2B*	12	14000954	0.004803	1	0.04662	1	0.2621	1
rs2281845	*HTR5A*	7	154294432	0.004834	1	0.04802	1	0.07062	1
rs1718301	*PAH*	12	101773660	0.00497	1	0.02538	1	0.003914	1

AN, anorexia nervosa; SNP, single nucleotide polymorphism; Chr., chromosome; FDR, false discovery rate.

TABLE III

Anorexia Nervosa With No Binge Eating (N = 687), Top 25 SNP Associations (Sorted by Chromosome)

SNP	Gene	Chr.	Base pair	AN no binge trend P	FDR
rs11577391	*KCNN3*	1	151530224	0.004307	1
rs10908435	*KCNN3*	1	151542467	0.000385	0.8621
rs11589855	*KCNN3*	1	151568916	0.001268	0.9985
rs10920569	*ADORA1*	1	199830112	0.003464	1
rs11934028	*PPARGC1A*	4	23457198	0.003711	1
rs3104104	*UCP1*	4	141824083	0.00409	1
rs6876225	*SLC6A3*	5	1459036	0.004103	1
rs2926832	*GRIA1*	5	153175865	0.002476	1
rs1390939	*SLC18A1*	8	20084992	0.003975	1
rs2173114	*SLC18A1*	8	20087205	0.000377	0.8567
rs11783570	*CRH*	8	67266925	0.003481	1
rs13290443	*FREQ*	9	129994841	0.003615	1
rs4836698	*FREQ*	9	130061544	0.003458	1
rs3813720	*ADRB1*	10	115797006	0.003955	1
rs363294	*SLC18A2*	10	119033544	0.003588	1
rs10510057	*RGS10*	10	121301038	0.000727	0.9764
rs2917928	*RGS10*	10	121305880	0.000618	0.9586
rs1718312	*PAH*	12	1017436655	0.001392	0.9992
rs35917	*SLC6A2*	16	54234035	0.00286	1
rs4500724	*SLC6A2*	16	54240878	0.000744	0.9783
rs4791362	*GLP2R*	17	9654811	0.000187	0.6183
rs242924	*CRHR1*	17	41241147	0.0021	1
rs10491141	*NSF*	17	42147815	0.001844	0.9999
rs12947457	*NSF*	17	42153004	0.001844	0.9999
rs2427393	*NTSR1*	20	60796721	0.001199	0.9979

AN, anorexia nervosa; SNP, single nucleotide polymorphism, Chr., chromosome; FDR, false discovery rate.

TABLE IV

Restricting Anorexia Nervosa Only (N = 421), Top 25 SNP Associations (Sorted by Chromosome)

SNP	Gene	Chr.	hg17chromEnd	Restricting AN trend P	FDR
rs11577391	KCNN3	1	151530224	0.003372	1
rs10908435	KCNN3	1	151542467	0.000468	0.9101
rs11808053	KCNN3	1	151562120	0.003031	1
rs11589855	KCNN3	1	151568916	0.001464	0.9995
rs1866146	POMC	2	25292224	0.0046	1
rs9341189	IGFBP2	2	217346334	0.004159	1
rs4698599	QDPR	4	17154537	0.00088	0.9893
rs3104104	UCP1	4	141824083	0.002614	1
rs1363281	GABRP	5	170179876	0.003939	1
rs752596	GABRP	5	170187193	0.003078	1
rs2173114	SLC18A1	8	20087205	0.004892	1
rs11783570	CRH	8	67266925	0.003532	1
rs4836698	FREQ	9	130061544	0.002847	1
rs3813720	ADRB1	10	115797006	0.00504	1
rs363294	SLC18A2	10	119033544	0.005082	1
rs3009907	RGS10	10	121252444	0.005316	1
rs1718312	PAH	12	101743655	0.000894	0.99
rs1718301	PAH	12	101773660	0.003914	1
rs10778209	PAH	12	101786439	0.005419	1
rs3814843	CALM1	14	89942766	0.004848	1
rs6575134	CALM1	14	89946580	0.003907	1
rs4500724	SLC6A2	16	54240878	0.003396	1
rs4791362	GLP2R	17	9654811	0.003652	1
rs2020941	SLC6A4	17	25574084	0.001139	0.9972
rs4810145	GNAS	20	56829890	0.001656	0.9998

AN, anorexia nervosa; SNP, single nucleotide polymorphism, Chr., chromosome; FDR, false discovery rate.