Journal of Psychiatric Research 55 (2014) 77–86

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

The role of leptin, melanocortin, and neurotrophin system genes on body weight in anorexia nervosa and bulimia nervosa

Zeynep Yilmaz [a,b], Allan S. Kaplan [b,c,d], Arun K. Tiwari [e], Robert D. Levitan [c,d,f], Sara Piran [g], Andrew W. Bergen [h], Walter H. Kaye [i], Hakon Hakonarson [j], Kai Wang [k], Wade H. Berrettini [l], Harry A. Brandt [m], Cynthia M. Bulik [a,n], Steven Crawford [m], Scott Crow [o], Manfred M. Fichter [p,q], Katherine A. Halmi [r], Craig L. Johnson [s], Pamela K. Keel [t], Kelly L. Klump [u], Pierre Magistretti [v], James E. Mitchell [w,x], Michael Strober [y], Laura M. Thornton [a], Janet Treasure [z], D. Blake Woodside [d,aa], Joanne Knight [c,d,e], James L. Kennedy [c,d,e,*]

[a] Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[b] Clinical Research Department, Centre for Addiction and Mental Health, Toronto, Canada
[c] Institute of Medical Science, University of Toronto, Toronto, Canada
[d] Department of Psychiatry, University of Toronto, Toronto, Canada
[e] Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Canada
[f] Mood and Anxiety Program, Centre for Addiction and Mental Health, Toronto, Canada
[g] Faculty of Medicine, University of Ottawa, Ottawa, Canada
[h] Center for Health Sciences, SRI International, Menlo Park, CA, USA
[i] Department of Psychiatry, University of California, San Diego, CA, USA
[j] Joseph Stokes Jr. Research Institute, The Children's Hospital of Philadelphia, Philadelphia, PA, USA
[k] Department of Psychiatry, University of Southern California, Los Angeles, CA, USA
[l] Department of Psychiatry, Center of Neurobiology and Behavior, University of Pennsylvania, Philadelphia, PA, USA
[m] Department of Psychiatry, Sheppard Pratt Health System, Towson, MD, USA
[n] Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[o] Department of Psychiatry, University of Minnesota, Minneapolis, MN, USA
[p] Department of Psychiatry, University of Munich (LMU), Munich, Germany
[q] Roseneck Hospital for Behavioral Medicine, Prien, Germany
[r] Department of Psychiatry, Weill Cornell Medical College, New York, NY, USA
[s] Eating Recovery Center, Denver, CO, USA
[t] Department of Psychology, Florida State University, Tallahassee, FL, USA
[u] Department of Psychology, Michigan State University, East Lansing, MI, USA
[v] Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
[w] Department of Clinical Neuroscience, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND, USA
[x] Neuropsychiatric Research Institute, Fargo, ND, USA
[y] Department of Psychiatry, Semel Institute for Neuroscience and Human Behavior, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
[z] Department of Academic Psychiatry, King's College London, Institute of Psychiatry, London, United Kingdom
[aa] Eating Disorders Program, Toronto General Hospital, Toronto, Canada

ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form
5 March 2014
Accepted 4 April 2014

Keywords:
Anorexia nervosa
Bulimia nervosa

ABSTRACT

Objective: Although low weight is a key factor contributing to the high mortality in anorexia nervosa (AN), it is unclear how AN patients sustain low weight compared with bulimia nervosa (BN) patients with similar psychopathology. Studies of genes involved in appetite and weight regulation in eating disorders have yielded variable findings, in part due to small sample size and clinical heterogeneity. This study: (1) assessed the role of leptin, melanocortin, and neurotrophin genetic variants in conferring risk for AN and BN; and (2) explored the involvement of these genes in body mass index (BMI) variations within AN and BN.

* Corresponding author. Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario, M5T 1R8, Canada. Tel.: +1 416 535 8501x34987.
E-mail address: james_kennedy@camh.net.

http://dx.doi.org/10.1016/j.jpsychires.2014.04.005

Candidate gene association
Body weight
Melanocortins
Neurotrophins

Method: Our sample consisted of 745 individuals with AN without a history of BN, 245 individuals with BN without a history of AN, and 321 controls. We genotyped 20 markers with known or putative function among genes selected from leptin, melanocortin, and neurotrophin systems.
Results: There were no significant differences in allele frequencies among individuals with AN, BN, and controls. *AGRP* rs13338499 polymorphism was associated with lowest illness-related BMI in those with AN ($p = 0.0013$), and *NTRK2* rs1042571 was associated with highest BMI in those with BN ($p = 0.0018$).
Discussion: To our knowledge, this is the first study to address the issue of clinical heterogeneity in eating disorder genetic research and to explore the role of known or putatively functional markers in genes regulating appetite and weight in individuals with AN and BN. If replicated, our results may serve as an important first step toward gaining a better understanding of weight regulation in eating disorders.

1. Background

Indifference to extreme weight loss and low motivation to restore normal body mass are the *sine qua nons* of anorexia nervosa (AN) and the primary target of initial treatment (American Psychiatric Association, 2006). As the illness is often protracted, low BMI and the avoidance of eating to restore healthy weight are primary factors influencing high morbidity and mortality that distinguish this illness. Low weight (and the permissive factors involved) are of interest for additional reasons as these are key aspects of AN; moreover, low body weight is the primary distinguishing diagnostic feature separating AN from bulimia nervosa (BN; American Psychiatric Association, 2013) and is associated with other clinical phenotypes, anxiety in particular (Dellava et al., 2010; Thornton et al., 2011).

To date, the genetic risk architecture underlying eating disorders (EDs) remains largely unexplored; however, like most other psychiatric illnesses, the heritability of EDs appears to follow a non-Mendelian pattern, suggesting that large numbers of genes spanning multiple regions of the genome are involved in susceptibility. While a number of ED candidate gene studies have investigated neurotransmitter systems involved in motivated behaviors (Hinney et al., 1997; Gorwood et al., 2002; Hu et al., 2003; Ricca et al., 2004; Nisoli et al., 2007; Sorli et al., 2008; Frieling et al., 2010), the results have been unpersuasive. Other studies that focused on regulators of appetite and weight have not implicated specific and replicable polymorphisms or gene–phenotype associations (Hinney et al., 1998; Vink et al., 2001; Janeckova, 2001; Quinton et al., 2004; Cellini et al., 2006; Monteleone et al., 2006a; Dardennes et al., 2007), whereas a number of genes with effects on appetite and weight regulation have yet to be examined in EDs (Table 1). Similarly, although neurotrophin system genes have also been implicated in EDs in case–control studies (Ribases et al., 2003, 2004, 2005a, 2005b; Dmitrzak-Weglarz et al., 2007; Kaplan et al., 2008; Mercader et al., 2008), a recent meta-analysis has called into question the significance and the reliability of some of these findings (Brandys et al., 2013), while the other findings await replication. Furthermore, genome-wide association studies (GWAS) of obesity have identified new genetic variants with potential implication for ED phenotypes; for instance, common variants near the melanocortin 4 receptor (*MC4R*) gene have been repeatedly associated with BMI in obesity (e.g., Loos et al., 2008; Luan et al., 2009; Thorleifsson et al., 2009; Speliotes et al., 2010; Elks et al., 2010; Scherag et al., 2010; Beckers et al., 2011; Kvaloy et al., 2013). Although thus far this marker has not yielded positive findings in AN (Brandys et al., 2010), it requires further investigation. *MC4R* variants have also been associated with antipsychotic medication-induced weight gain (Malhotra et al., 2012; Chowdhury et al., 2013); however, the relevance of these variants with promising findings to ED phenotype variation currently remains unknown.

A complication in genetic studies of EDs is instability of the phenotype as the crossover between ED diagnoses, in particular from AN to BN, is upwards of 34–36% (Tozzi et al., 2005; Eddy et al., 2008), and most crossovers occur within five years from time of AN onset. By contrast, the BN-to-AN crossover is less common (Fichter and Quadflieg, 1997; Tozzi et al., 2005; Eddy et al., 2008). For this reason, clearly defining AN and BN phenotypes considering longitudinal course of illness is important to the design of genetic studies, as weight histories of AN and BN often diverge, and BN patients with prior AN histories usually report significantly lower current, maximum, and minimum BMIs than BN patients with without histories of AN (Kaye et al., 2004). Furthermore, premorbid obesity is more prevalent in those with BN compared with those with AN (33.2% vs. 4.6%, respectively; Villarejo et al., 2012), and a higher maximum lifetime BMI may be a predictor of AN to BN crossover (Monteleone et al., 2011).

The present study had two aims: first, to investigate single nucleotide polymorphisms (SNPs) with known or putative functions in the leptin, melanocortin, and neurotrophin system genes in individuals with AN, BN, and healthy controls; second, to explore the role of the selected candidate genes on illness-related minimum BMI, maximum lifetime BMI, and BMI at the time of ascertainment in each clinical group (AN and BN) separately.

2. Methods and materials

2.1. Sample selection

The main sample used for the selection of suitable cases was derived from the Price Foundation Consortium. All participants included in this collaborative initiative were carefully phenotyped, and these procedures and sample characteristics have been previously described in detail (Kaye et al., 2000, 2004; Jacobs et al., 2009). The present study consisted of a subgroup of female participants who either had AN with no history of BN (AN) or BN with no history of AN (BN; Supplementary Table 1). Minimum illness duration was three years for individuals in each diagnostic group to ensure stability of ED diagnosis. The AN group included individuals with the restricting (AN-R), binge/purge and purging (combined as AN-BP) subtypes. It was ensured that the individuals classified as AN-BP had no history of BN, i.e., regular binge eating and purging when not underweight.

Additional DNA samples from females with BN with no history of AN were selected from the Toronto Bulimia Nervosa Genetics Study (Supplementary Table 1), stored at the Centre for Addiction and Mental Health (CAMH) Neurogenetics Laboratory in Toronto, Canada. Recruitment criteria for this study closely followed those of the Price Foundation BN cases, and the details on recruitment have been published elsewhere (Yilmaz et al., 2011, 2012). Finally, DNA samples from female controls with no psychiatric history (as assessed by a self-report checklist) were obtained from the Toronto Centre for Applied Genomics. Since the controls were not screened for EDs, we only included individuals with a BMI between 19 kg/m^2

Table 1
Rationale for the inclusion of the candidate genes and SNPs in the study.

Gene name	Biological relevance	dbSNP#	SNP previously studied in EDs?	Rationale for SNP selection
Leptin receptor (*LEPR*)	Important anorexigenic hormone that regulates energy intake and expenditure, appetite, metabolism, and eating behavior; primarily expressed in adipose tissue	rs1137100	Quinton et al., 2004	Affects plasma soluble leptin receptor levels (Sun et al., 2010); preliminary ED findings that need replication (small sample size)
		rs1137101	Quinton et al., 2004	Affects plasma soluble leptin receptor levels (Sun et al., 2010); preliminary ED findings that need replication (small sample size)
Leptin (*LEP*)	Acts through the leptin receptor; a protein that controls fat-tissue mass via the hypothalamus effects on satiety and energy expenditure	rs7799039	Janas-Kozik et al., 2008	Affects mRNA expression and plasma leptin levels; preliminary ED findings that need replication (small sample size)
Ghrelin (*GHRL*)	Orexigenic peptide ligand of growth hormone secretagogue receptor; associated with the regulation of energy balance and food intake	rs696217	Ando et al., 2006; Dardennes et al., 2007; Cellini et al., 2006; Monteleone et al., 2006a; Monteleone et al., 2007; Kindler et al., 2011	Putative transcription factor-binding site; conflicting findings in EDs
		rs4684677	Cellini et al., 2006, Dardennes et al., 2007; Kindler et al., 2011	Putative splicing site; conflicting findings in EDs
Histamine receptor H1 (*HRH1*)	Leptin is known to partly exert its effects through HRH1 and facilitates the release of histamine via HRH1 in the hypothalamus; central histamine signaling is involved in the regulation of food intake and body weight	rs12490160	No	Putative transcription factor-binding site
		rs3732941	No	Putative miRNA binding site
Brain derived neurotrophic factor (*BDNF*)	Protein that supports the growth, survival, differentiation, and assigned function of neurons; involved in appetite suppression by downstream regulation of melanocortin signaling in the hypothalamus	rs6265	Ribases et al., 2003; Ribases et al., 2004; Ribases et al., 2005b; de Krom et al., 2005a; Monteleone et al., 2006b; Dmitrzak-Weglarz et al., 2007; Gratacos et al., 2007; Gelegen et al., 2008; Kaplan et al., 2008; Brandys et al., 2013	Affects the secretion and dendritic trafficking of BDNF protein (Chiaruttini et al., 2009); conflicting findings in EDs
		rs56164415	Ribases et al., 2003; Ribases et al., 2004; Ribases et al., 2005b; de Krom et al., 2005a; Dmitrzak-Weglarz et al., 2007; Dardennes et al., 2007; Mercader et al., 2008	Affects mRNA folding; possible splicing site; preliminary ED findings that need replication (small sample size); conflicting findings
Neurotrophic tyrosine kinase receptor type 2 (*NTRK2*)	Main receptor for brain derived neurotrophic factor; involved in appetite and weight regulation via its expression in the hypothalamus	rs1078947	Ribases et al., 2005a	Preliminary ED findings that need replication (small sample size)
		rs1187325	Ribases et al., 2005a	May affect the length and stability of the mRNA isoforms (Ribases et al., 2005a); preliminary ED findings that need replication (small sample size)
Neurotrophic tyrosine kinase receptor type 3 (*NTRK3*)	Major binding site and the physiologic receptor for neurotrophin 3, which affects the development of neurons expressing the *BDNF* gene	rs7180942	Mercader et al., 2008	Heterozygosity may reduce expression levels (Mercader et al., 2008); preliminary ED findings that need replication (small sample size)
Melanocortin 3 receptor (*MC3R*)	Similar to MC4R, heavily expressed in the hypothalamic regions of the brain; associated with increased fat mass despite decreased food intake when deficient in mice	rs6127698	No	Putative transcription factor-binding site
		rs3827103	de Krom et al., 2005b	Exonic; *in vitro* diminished functionality and expression of the receptor (Feng et al., 2005); preliminary ED findings that need replication (small sample size)
Melanocortin 4 receptor (*MC4R*)	Stimulation of brain melanocortin leads to a reduction in food intake and weight; leptin signals nutritional status to the hypothalamus by triggering melanocortin production through pro-opiomelanocortin neurons	rs17782313	Brandys et al., 2010	Preliminary ED findings that need replication; associated with obesity (e.g., Loos et al., 2008; Luan et al., 2009; Thorleifsson et al., 2009; Speliotes et al., 2010; Elks et al., 2010; Scherag et al., 2010; Beckers et al., 2011; Kvaloy et al., 2013)
		rs489693	No	Associated with antipsychotic medication-induced weight gain (Malhotra et al., 2012)
		rs8087522	No	Associated with antipsychotic medication-induced weight gain (Chowdhury et al., 2013)
Agouti related protein (*AGRP*)	A neuropeptide that suppresses melanocortin receptor activity, resulting in an increase in appetite and decrease in metabolic rate and energy expenditure	rs5030980	Vink et al., 2001; Dardennes et al., 2007	Preliminary ED findings that need replication (small sample size)
		rs13338499	No	Putative transcription factor-binding site; located upstream of *AGRP*; possible regulatory role

(*continued on next page*)

Table 1 (*continued*)

Gene name	Biological relevance	dbSNP#	SNP previously studied in EDs?	Rationale for SNP selection
Pro-opiomelanocortin (*POMC*)	Precursor anorexigenic polypeptide that is mainly expressed in the arcuate nucleus; associated with appetite regulation, as well as the secretion of glucocorticoids	rs1042571	No	Putative transcription factor-binding site; potential miRNA binding site; linked to obesity (Wang et al., 2012) and anthropometric measures such as waist-to-hip ratio, visceral and abdominal fat (Ternouth et al., 2011)

and 28 kg/m^2 to avoid extreme weight phenotypes (Supplementary Table 1).

All aspects of this research study were reviewed and approved by the CAMH Research Ethics Board and conducted in accordance with the Helsinki Declaration as revised in 1989. Informed consent for providing genetic materials and inclusion of these materials in future collaborative studies was obtained from all individuals whose DNA samples were included in our analysis.

2.2. Laboratory methods

Our genetic analysis focused on 11 candidate genes in the leptin (*LEPR, LEP, GHRL, HRH1*), melanocortin (*MC3R, MC4R, AGRP, POMC*), and neurotrophin (*BDNF, NTRK2, NTRK3*) systems. We pursued a targeted approach that focused on SNPs with known or putative function, as assessed by *in silico* analysis. This approach has a number of advantages over the tag SNP approach: first, the study of functional variants helps us make more biologically meaningful discoveries as to the effects of any genetic differences associated with the phenotype being studied; second, focusing on a small number of carefully selected loci reduces multiple testing and requires less stringent statistical correction. Two *in silico* tools were used: the National Institute of Environmental Health Sciences (http://snpinfo.niehs.nih.gov) and BrainArray (http://brainarray.mbni.med.umich.edu). On average, two markers per gene were selected. Priority was given to SNPs that have been studied in EDs, and a small number of SNPs without known function were also included based on the promising findings they have yielded in EDs despite small sample size (*NTRK2* rs1078947), obesity (*MC4R* rs17782313), antipsychotic medication-induced weight gain (*MC4R* rs489693 and rs8087522; Table 1).

Genomic DNA was extracted from whole blood for Price Foundation samples and from lymphocytes for Toronto BN cases and healthy controls using the high salt method (Lahiri and Nurnberger, 1991). All genotyping was performed using standard protocols for Applied Biosystems OpenArray® and ViiA™ 7 platforms at CAMH, blind to diagnosis.

2.3. Statistical analysis

Chi-square, *t*-test, and analysis of variance on anthropometric, demographic, and disease characteristics across AN, BN, and controls were performed using SPSS Statistics v17 (SPSS Inc., Chicago, USA, 2008). Quality control (QC) steps prior to data analysis consisted of checking for deviations from Hardy–Weinberg Equilibrium (HWE; cutoff $p < 0.01$), removal of SNPs with low minor allele frequency (MAF; <0.03) and low genotyping rate (<90%), and exclusion of individuals with low genotyping rate (<90%). For case–control analysis, genotype and ED diagnosis were treated as categorical variables. The chi-square test was performed for the case–control comparisons using PLINK (Purcell et al., 2007). Power calculations were carried out using Quanto v.1.2.4 (http://hydra.usc.edu/gxe), and we have over 90% power to detect an odds ratio as low as 1.5 (alpha = 0.05, two-tailed, MAF = 0.10, log additive model).

For the quantitative phenotypic analysis, we investigated the role of the genetic polymorphisms on three BMI measures: BMI at recruitment (curBMI), maximum lifetime BMI (maxBMI), and lowest illness-related BMI (minBMI). Quantitative data were analyzed separately in AN and BN using linear regression in PLINK. Age, age of onset, AN subtype (for AN only) and source (Price Foundation versus Toronto; for BN only) were entered as covariates, where appropriate, and we have over 80% power to detect a mean change of 0.6 kg/m^2 in BMI for the AN group (alpha = 0.05, two-tailed, MAF = 0.10, log additive model).

We corrected for multiple testing using Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD; Nyholt, 2004; Li and Ji, 2005). This method calculates the effective number of independent loci for the SNPs on the same gene using linkage disequilibrium (LD) information (Nyholt, 2004; Li and Ji, 2005). Once the effective number of independent loci was determined per gene, the adjusted alpha was calculated by dividing the uncorrected *p*-value of 0.05 by the effective number of independent SNPs. In our study, the effective number of independent SNPs was determined to be 18.75, setting the adjusted $p < 0.0027$. All statistical analyses were two-tailed.

3. Results

After applying the selection criteria, 787 AN cases, 267 BN cases, and 322 healthy controls were included. Following QC, 42 AN cases, 22 BN cases, and one control were removed due to low genotyping rate, bringing the final sample to 745 AN, 245 BN, and 321 controls. Of the AN cases, 369 had AN-R (49.5%), whereas 376 had AN-BP (50.5%). All AN and 128 BN cases (52.2%) came from the Price Foundation Consortium, and 117 BN cases (47.8%) came from the Toronto Bulimia Nervosa Genetics Study. Individuals removed due to low genotyping rate did not differ from those who passed QC in terms of demographic, anthropometric, and disease characteristics (results not shown).

In terms of sample characteristics (Table 2), controls were significantly older than AN and BN cases ($p < 0.0001$), whereas AN

Table 2
Characteristics of AN, BN, and control participants.

	AN ($n = 745$)	BN ($n = 267$)	Control ($n = 321$)	*F*	*p*
Age (years)[a,b]	26.1 ± 8.5	27.2 ± 8.3	49.4 ± 8.8	867.909	<0.0001
CurBMI (kg/m^2)[c]	18.05 ± 2.71	23.28 ± 3.01	23.60 ± 2.16	671.019	<0.0001
MinBMI (kg/m^2)[c]	13.82 ± 1.95	20.09 ± 1.54	–	−51.198[d]	<0.0001
MaxBMI (kg/m^2)[c]	21.07 ± 2.42	25.82 ± 3.06	–	−22.003[d]	<0.0001

[a] Age data missing for one AN and one BN case.
[b] Control > AN = BN.
[c] BMI data missing for four BN cases.
[d] Because minBMI and maxBMI information was not available for controls, independent *t*-test was run to compare AN and BN groups, and the statistic reported here is the *t*-value.

cases had a significantly lower mean curBMI compared with the other two groups ($p < 0.0001$). There were also significant differences between AN and BN groups in minBMI ($p < 0.0001$) and maxBMI ($p < 0.0001$).

We also observed a number of differences between AN-R and AN-BP subtypes. Cases in the AN-R group were younger ($M_{AN\text{-}R} = 25.0 \pm 8.5$, $M_{AN\text{-}BP} = 27.2 \pm 8.4$, $p < 0.0001$), weighed less at recruitment ($M_{AN\text{-}R} = 17.85 \pm 2.79$, $M_{AN\text{-}BP} = 18.25 \pm 2.62$, $p = 0.042$), and reported both lower minBMI and lower maxBMI ($M_{AN\text{-}R} = 13.66 \pm 1.89$, $M_{AN\text{-}BP} = 13.98 \pm 1.99$, $p = 0.025$; $M_{AN\text{-}R} = 20.86 \pm 2.42$, $M_{AN\text{-}BP} = 21.29 \pm 2.40$, $p = 0.013$; respectively). Age of onset for AN did not differ between subtypes ($M_{AN\text{-}R} = 16.2 \pm 3.2$, $M_{AN\text{-}BP} = 16.2 \pm 2.9$, $p = 0.758$). When BN cases were stratified by source, Price Foundation cases were older at recruitment ($M_{Price} = 29.0 \pm 9.6$, $M_{Toronto} = 25.2 \pm 6.7$, $p < 0.0001$) and had an earlier age of onset compared with Toronto cases ($M_{Price} = 17.0 \pm 3.7$, $M_{Toronto} = 18.0 \pm 4.2$, $p = 0.015$).

The minimum SNP genotype completion rate was 93%, with the majority of the SNPs reaching over 98%. None of the SNPs deviated from HWE in any of the three groups, and most of the SNPs were not correlated except for the SNPs in *LEPR* and *MC4R*, which were in moderate LD ($r^2 = 0.62$ and 0.77, respectively).

Results of the case–control comparisons are summarized in Supplementary Table 2. We did not find any evidence for differences in allele frequencies between AN and BN cases, AN and controls, or BN and controls. For the within-AN analysis of BMI, we entered age, age of onset of AN, and AN subtype as covariates. Although age of onset was comparable between subtypes, we chose to control for it since AN onset and weight suppression at an earlier age may act as a confounder in the analysis. Table 3 summarizes our findings involving curBMI, minBMI, and maxBMI in AN. None of the markers included in our analysis were linked to curBMI or maxBMI in AN. However, agouti related protein (*AGRP*) rs13338499 was significantly associated with minBMI ($p = 0.0013$; Table 3). Table 4 presents the results of our analysis in BN, where age, age of onset of BN, and source were entered as covariates. We did not find a significant association between the studied markers and curBMI or minBMI. However, we observed an association between neurotrophic tyrosine kinase receptor type 2 (*NTRK2*) rs1042571 and maxBMI in BN (Table 4), with each copy of the T allele being correlated with a mean maxBMI increase over 1 kg/m^2 ($p = 0.0018$).

4. Discussion

The case–control comparisons in the present study were designed to genotype a select number of SNPs with known or putative function in the leptin, melanocortin, and neurotrophin system genes in individuals with AN, BN, and healthy controls. Despite the methodological strengths of this study in maximizing phenotypic differences between AN and BN, we did not observe differences between the two ED groups in terms of frequencies of genetic variants included in the analysis. Furthermore, there were no differences in allele frequencies between AN, BN, and control groups. One possible reason for this lack of significant difference is that the control and BN sample sizes were too small and that these sample size limitations may have resulted in an underpowered analysis. In addition, it is also possible that the differences among AN, BN, and control groups may not be a function of vulnerability to sustained weight suppression, and future research should focus on different gene systems and ED-related phenotypes based on different *a priori* phenotypic hypotheses. Finally, it is possible that leptin-melanocortin-neurotrophin system genes may not confer risk for AN or BN.

In the AN group, *AGRP* rs13338499 was significantly associated with lowest illness-related BMI. To our knowledge, although the *AGRP* gene has been previously associated with body weight (Bonilla et al., 2006; Li et al., 2013) and AN (Vink et al., 2001; Dardennes et al., 2007), this is the first time this particular polymorphism has been studied in reference to BMI and EDs, and it is not in LD with any other *AGRP* locus previously investigated in EDs. This finding is intriguing on mechanistic and translational grounds, given that the *Agrp* knockout mouse is one of the earliest animal models of obesity, and AGRP administration ameliorates self-starvation and hyperactivity in rats (Kas et al., 2003; Hillebrand et al., 2006). In the case of acute AN, plasma AGRP levels are reported to be elevated (Moriya et al., 2006; Merle et al., 2011) and inversely correlated with BMI (Moriya et al., 2006). According to *in silico* analysis, rs13338499 is a putative transcription factor-binding site, and since it is located upstream of the *AGRP* gene, it may play a regulatory role. Considering the key orexigenic role AGRP plays through the hypothalamus, this finding further highlights the potential importance of the melanocortin system in weight regulation.

In the BN group, *NTRK2* rs1078947 T allele was associated with higher maximum lifetime BMI, a finding that is not in agreement with a previous report that found the C allele to be linked to a higher maximum BMI in AN (Ribases et al., 2005b). A few possible explanations exist for this discrepancy. First, since rs1078947 did not yield any significant associations with any of the three BMI measures in our 745 AN cases, the association reported in the first study might have been a false positive related to small sample size ($N = 83$). Second, it is possible that the AN group in the previous study may have included individuals with a history of BN, which may have led to the difference in the reported findings due to phenotypic heterogeneity. Furthermore, since the previous study was conducted using Spanish ancestry cases, the results could also be ancestry-specific. Replication studies are needed to understand the relationship between this particular marker and BMI in EDs. Via its expression in the hypothalamus, NTRK2 is involved in appetite and weight regulation; furthermore, peripheral and central administrations of NTRK2 agonists lead to appetite and weight suppression in animals and reduced obesity in *Bdnf* knockout mice (Xu et al., 2003). This marker is not predicted to have function *in silico* and was included in our study due to a previous preliminary association reported in AN (Ribases et al., 2005b), and our results combined with the previous findings further highlight the need for functional studies involving rs1078947.

Since a GWAS has been performed by the Price Foundation on the larger AN sample ($N = 1033$; Wang et al., 2011), we compared our results with the GWAS *p*-values for the SNPs included in our study. Out of the 20 SNPs analyzed in this study, only six overlapped with the GWAS, none of which was in the top 100 hits in the GWAS case–control analysis. It is also important to note that the GWAS included AN-R cases with and without BN history and did not look at quantitative traits such as BMI.

Despite the significant methodological strengths of this study, a number of limitations also merit consideration. For instance, controls were significantly older than AN and BN cases. However, it can be argued that the older age of the controls does not pose a risk to our findings since DNA sequence is independent of age. Another possible shortcoming involving controls is the lack of ED-specific screening; however, considering the low prevalence of EDs and that the AN and BN groups are enriched for any genetic risk factor for EDs, this is a conservative bias. We also did not have any lifetime BMI measures for controls, and although we only included controls within a certain BMI range, we cannot rule out history of obesity or underweight in this group. In the case of the ED groups, although AN sample size was one of the largest in ED candidate gene studies,

Table 3
Analysis of 20 leptin-melanocortin-neurotrophin system gene SNPs and BMI (in kg/m^2) in AN.

Gene	SNP	Genotype	$M \pm SD$			p		
			curBMI	minBMI	maxBMI	curBMI	minBMI	maxBMI
LEPR	rs1137100	G/G	17.96 ± 2.63	14.29 ± 2.02	20.96 ± 2.69	0.6923	0.9333	0.1834
		G/A	18.13 ± 2.62	13.72 ± 1.99	21.04 ± 2.41			
		A/A	18.00 ± 2.78	13.85 ± 1.91	21.10 ± 2.41			
LEPR	rs1137101	G/G	17.76 ± 2.40	14.05 ± 1.92	20.70 ± 2.40	0.2348	0.6239	0.0428
		G/A	18.15 ± 2.77	13.62 ± 1.96	21.17 ± 2.45			
		A/A	18.05 ± 2.80	13.97 ± 1.93	21.13 ± 2.39			
POMC	rs1042571	A/A	17.59 ± 2.84	13.89 ± 2.09	21.36 ± 2.30	0.8043	0.5856	0.8258
		A/G	18.19 ± 2.72	13.86 ± 1.84	21.07 ± 2.48			
		G/G	17.94 ± 2.74	13.75 ± 1.99	21.05 ± 2.45			
GHRL	rs4684677[a]	A/A	18.73 ± 2.12	14.62 ± 1.11	21.59 ± 1.74	0.9765	0.5865	0.8682
		A/T	17.99 ± 2.80	13.85 ± 1.84	20.95 ± 2.24			
		T/T	18.04 ± 2.72	13.79 ± 1.97	21.07 ± 2.44			
GHRL	rs696217	T/T	17.74 ± 1.88	13.87 ± 2.35	21.67 ± 2.43	0.08005	0.0786	0.0878
		T/G	18.48 ± 2.70	14.07 ± 2.07	21.46 ± 2.74			
		G/G	17.99 ± 2.72	13.78 ± 1.93	21.00 ± 2.36			
HRH1	rs12490160	G/G	18.42 ± 2.62	13.63 ± 1.61	20.75 ± 2.13	0.7484	0.7707	0.9613
		G/T	17.86 ± 2.82	13.84 ± 1.81	20.96 ± 2.48			
		T/T	18.09 ± 2.70	13.82 ± 1.99	21.11 ± 2.41			
HRH1	rs3732941	G/G	18.31 ± 1.62	14.12 ± 2.06	21.11 ± 1.75	0.04668	0.5845	0.4187
		G/A	17.71 ± 2.86	13.92 ± 1.87	20.94 ± 2.66			
		A/A	18.15 ± 2.67	13.79 ± 1.97	21.11 ± 2.36			
LEP	rs7799039	A/A	18.23 ± 3.02	13.59 ± 2.06	21.33 ± 2.58	0.4991	0.1125	0.3648
		A/G	18.01 ± 2.67	13.88 ± 1.84	20.96 ± 2.42			
		G/G	17.98 ± 2.54	13.88 ± 2.05	21.06 ± 2.29			
NTRK2	rs1187325	C/C	18.07 ± 2.81	13.71 ± 1.93	21.21 ± 2.66	0.7365	0.5963	0.1161
		C/G	18.01 ± 2.73	13.83 ± 1.97	21.11 ± 2.43			
		G/G	18.07 ± 2.67	13.81 ± 1.94	20.85 ± 2.23			
NTRK2	rs1078947	T/T	17.19 ± 2.65	13.92 ± 1.86	20.37 ± 2.73	0.1640	0.4541	0.1993
		T/C	18.02 ± 2.72	13.81 ± 2.01	21.14 ± 2.42			
		C/C	18.12 ± 2.72	13.84 ± 1.93	21.08 ± 2.41			
BDNF	rs6265	T/T	17.95 ± 2.01	14.49 ± 1.37	21.05 ± 1.95	0.8167	0.3437	0.5954
		T/C	18.07 ± 2.72	13.74 ± 2.02	20.96 ± 2.41			
		C/C	18.04 ± 2.75	13.82 ± 1.94	21.12 ± 2.45			
BDNF	rs56164415[a]	T/T	19.69 ± 1.78	14.29 ± 2.80	20.77 ± 1.47	0.1083	0.3602	0.8950
		T/C	17.42 ± 2.68	13.54 ± 2.05	21.06 ± 2.28			
		C/C	18.10 ± 2.70	13.85 ± 1.94	21.07 ± 2.44			
NTRK3	rs7180942	C/C	17.96 ± 2.63	14.29 ± 2.02	21.12 ± 2.43	0.6923	0.9333	0.3030
		C/T	18.13 ± 2.62	13.72 ± 1.99	21.16 ± 2.45			
		T/T	18.00 ± 2.78	13.85 ± 1.91	20.86 ± 2.38			
AGRP	rs5030980[a]	T/T	18.38 ± 2.70	10.76 ± 0.00	23.94 ± 0.00	0.0174	0.1066	0.6527
		T/G	18.05 ± 2.71	14.23 ± 1.86	21.28 ± 2.37			
		G/G	17.74 ± 2.72	13.79 ± 1.96	21.06 ± 2.43			
AGRP	**rs13338499**	G/G	14.79 ± 0.00	**14.89 ± 2.33**	21.66 ± 2.22	0.7789	**0.0013**	0.8917
		G/A	18.26 ± 2.76	**14.32 ± 1.86**	21.20 ± 2.21			
		A/A	18.05 ± 2.70	**13.70 ± 1.95**	21.03 ± 2.46			
MC4R	rs17782313	C/C	19.17 ± 2.51	13.88 ± 2.15	21.43 ± 2.69	0.6449	0.2203	0.0776
		C/T	18.12 ± 2.55	13.89 ± 1.79	21.15 ± 2.46			
		T/T	18.03 ± 2.73	13.77 ± 2.02	20.99 ± 2.36			
MC4R	rs489693	A/A	18.08 ± 2.79	13.87 ± 1.99	21.39 ± 2.82	0.1252	0.5680	0.0892
		A/C	18.27 ± 2.75	13.85 ± 1.82	21.12 ± 2.41			
		C/C	17.92 ± 2.68	13.79 ± 2.04	20.98 ± 2.36			
MC4R	rs8087522	A/A	18.19 ± 2.83	13.91 ± 1.97	21.04 ± 2.26	0.2651	0.0638	0.3360
		A/G	18.14 ± 2.73	13.88 ± 1.85	20.94 ± 2.46			
		G/G	17.96 ± 2.68	13.75 ± 2.04	21.18 ± 2.42			
MC3R	rs6127698	T/T	17.37 ± 2.86	13.76 ± 1.99	20.98 ± 2.46	0.2276	0.6160	0.3055
		T/G	18.11 ± 2.67	13.91 ± 1.89	20.96 ± 2.38			
		G/G	18.13 ± 2.71	13.70 ± 2.02	21.34 ± 2.46			
MC3R	rs3827103	G/G	17.80 ± 2.63	11.69 ± 1.27	20.58 ± 2.66	0.2974	0.1466	0.3058
		G/A	18.16 ± 2.69	13.87 ± 1.88	21.63 ± 2.59			
		A/A	18.04 ± 2.82	13.83 ± 1.96	20.99 ± 2.39			

Note 1: Age, age of onset, and AN subtype entered as covariates in log additive linear regression models.
Note 2: Corrected alpha set at 0.0027 for statistical significance. Results in bold represent statistical significance.
[a] MAF < 0.07.

BN and control groups were smaller, which may have reduced statistical power. Despite all individuals included in the study being of European ancestry, we did not genotype ancestry informative markers to control for population substructures. Finally, although we believe that one of the strengths of this study is the utilization of functional variants, genotyping a small number of SNPs and not using tag SNPs could be another criticism; it is possible that the risk loci for EDs located in these candidate genes are outside of the markers selected and that a tag SNP approach could provide better coverage of the genes.

If replicated, the present findings may have translational implications. For example, AGRP is the inverse agonist of melanocortins, and data suggest that melanocortin signaling may play a role in the regulation of circulating cholesterol: in rodents,

Table 4
Analysis of 20 leptin-melanocortin-neurotrophin system gene SNPs and BMI (in kg/m^2) in BN.

Gene	SNP	Genotype	*M* ± *SD*			*p*		
			curBMI	minBMI	maxBMI	curBMI	minBMI	maxBMI
LEPR	rs1137100	G/G	21.43 ± 8.23	20.39 ± 1.58	25.45 ± 2.29	0.5740	0.2701	0.6248
		G/A	22.82 ± 5.55	20.21 ± 1.48	26.18 ± 3.05			
		A/A	22.9 ± 4.15	19.97 ± 1.59	25.62 ± 3.14			
LEPR	rs1137101	G/G	22.73 ± 5.96	20.37 ± 1.45	25.58 ± 2.90	0.9011	0.2830	0.8491
		G/A	22.85 ± 4.91	20.03 ± 1.58	25.80 ± 3.05			
		A/A	22.43 ± 4.91	19.96 ± 1.42	25.85 ± 3.10			
POMC	rs1042571	A/A	24.49 ± 3.76	19.91 ± 1.16	26.92 ± 3.50	0.04465	0.6690	0.0159
		A/G	23.72 ± 3.28	20.18 ± 1.37	26.39 ± 3.26			
		G/G	22.36 ± 5.34	20.11 ± 1.64	25.51 ± 2.94			
GHRL	rs4684677[a]	A/A	23.30 ± 1.55	19.09 ± 0.38	24.62 ± 0.33	0.5120	0.9918	0.7216
		A/T	23.38 ± 2.41	20.20 ± 1.47	26.07 ± 2.54			
		T/T	22.67 ± 5.31	20.08 ± 1.56	25.78 ± 3.12			
GHRL	rs696217	T/T	23.04 ± 3.37	20.11 ± 1.59	28.66 ± 2.37	0.9337	0.7947	0.8488
		T/G	23.09 ± 3.17	20.02 ± 1.50	25.46 ± 3.18			
		G/G	22.69 ± 5.36	20.10 ± 1.56	25.83 ± 3.03			
HRH1	rs12490160	G/G	19.85 ± 2.36	17.99 ± 2.54	22.85 ± 1.77	0.3885	0.0647	0.2264
		G/T	22.61 ± 5.81	19.83 ± 1.35	25.56 ± 3.39			
		T/T	22.82 ± 4.91	20.17 ± 1.57	25.91 ± 2.98			
HRH1	rs3732941	G/G	25.21 ± 3.25	20.19 ± 1.54	27.29 ± 3.34	0.5918	0.6139	0.6820
		G/A	22.84 ± 2.70	20.19 ± 1.44	25.72 ± 2.89			
		A/A	22.64 ± 5.62	20.06 ± 1.59	25.80 ± 3.11			
LEP	rs7799039	A/A	23.4 ± 3.56	20.18 ± 1.69	25.73 ± 3.19	0.1018	0.0797	0.4461
		A/G	22.76 ± 6.03	20.26 ± 1.65	25.95 ± 3.10			
		G/G	22.34 ± 4.24	19.77 ± 1.20	25.69 ± 2.93			
NTRK2	rs1187325	C/C	22.74 ± 3.48	19.95 ± 1.87	25.29 ± 3.12	0.2841	0.4879	0.1866
		C/G	22.18 ± 6.68	20.17 ± 1.49	25.83 ± 3.03			
		G/G	23.40 ± 2.94	20.15 ± 1.48	26.06 ± 3.04			
NTRK2	**rs1078947**	T/T	25.70 ± 3.86	20.74 ± 2.16	**27.68 ± 2.88**	0.0773	0.0113	**0.0018**
		T/C	23.44 ± 5.22	20.47 ± 1.78	**26.63 ± 3.37**			
		C/C	22.36 ± 5.07	19.97 ± 1.44	**25.47 ± 2.90**			
BDNF	rs6265	T/T	22.09 ± 3.55	19.86 ± 1.04	23.99 ± 2.54	0.7843	0.1104	0.2622
		T/C	23.03 ± 2.71	19.85 ± 1.37	25.56 ± 2.97			
		C/C	22.64 ± 5.89	20.21 ± 1.62	25.98 ± 3.10			
BDNF	rs56164415[a]	T/T	20.20 ± 0.00	20.20 ± 0.00	24.27 ± 0.00	0.9172	0.8701	0.3244
		T/C	23.19 ± 2.60	20.19 ± 1.46	25.42 ± 2.89			
		C/C	22.72 ± 5.27	20.08 ± 1.56	25.87 ± 3.08			
NTRK3	rs7180942	C/C	23.04 ± 2.89	20.08 ± 1.41	25.51 ± 3.25	0.5053	0.2122	0.6312
		C/T	22.07 ± 6.48	19.87 ± 1.38	25.45 ± 2.29			
		T/T	23.62 ± 3.15	20.42 ± 1.78	26.18 ± 3.05			
AGRP	rs5030980[a]	T/T	–	–	–	0.8713	0.6000	0.7875
		T/G	22.76 ± 8.33	20.34 ± 1.40	26.36 ± 3.53			
		G/G	22.76 ± 4.70	20.07 ± 1.56	25.78 ± 3.02			
AGRP	rs13338499	G/G	22.11 ± 1.53	19.65 ± 0.66	25.18 ± 0.59	0.6047	0.6250	0.5617
		G/A	23.30 ± 6.53	20.32 ± 1.51	26.35 ± 3.17			
		A/A	22.64 ± 4.99	20.06 ± 1.57	25.76 ± 3.08			
MC4R	rs17782313	C/C	20.87 ± 8.52	20.10 ± 1.46	25.32 ± 2.73	0.5535	0.1953	0.2916
		C/T	22.65 ± 4.49	19.85 ± 1.44	25.78 ± 3.26			
		T/T	23.01 ± 4.95	20.24 ± 1.60	25.90 ± 2.99			
MC4R	rs489693	A/A	21.01 ± 6.55	19.59 ± 1.12	24.57 ± 2.42	0.2942	0.0318	0.0165
		A/C	22.81 ± 4.06	20.01 ± 1.49	25.85 ± 3.04			
		C/C	23.11 ± 5.57	20.29 ± 1.66	26.09 ± 3.16			
MC4R	rs8087522	A/A	23.64 ± 2.64	20.19 ± 1.44	26.14 ± 3.35	0.9886	0.8120	0.5316
		A/G	22.22 ± 6.10	20.01 ± 1.44	25.87 ± 3.03			
		G/G	23.05 ± 4.36	20.14 ± 1.65	25.74 ± 3.06			
MC3R	rs6127698	T/T	22.72 ± 5.60	20.12 ± 1.59	26.19 ± 3.52	0.2830	0.5759	0.2403
		T/G	23.11 ± 4.34	20.17 ± 1.63	25.78 ± 2.97			
		G/G	22.23 ± 5.77	19.95 ± 1.39	25.65 ± 2.91			
MC3R	rs3827103	G/G	23.91 ± 4.49	20.13 ± 1.70	27.93 ± 3.46	0.6214	0.8925	0.5723
		G/A	22.60 ± 5.97	20.02 ± 1.47	26.08 ± 3.48			
		A/A	22.78 ± 4.91	20.10 ± 1.56	25.75 ± 2.97			

Note 1: Age, age of onset, and source entered as covariates in log additive linear regression models.
Note 2: Corrected alpha set at 0.0027 for statistical significance. Results in bold represent statistical significance.
[a] MAF < 0.07.

inhibition of melanocortin system in the central nervous system leads to an increase in high-density lipoprotein cholesterol in a manner independent of food intake or body weight (Perez-Tilve et al., 2010). Interestingly, the top hit in the recent Price Foundation AN high-throughput sequencing study was in the *EPHX2* gene, known to influence cholesterol function (Scott-Van Zeeland et al., 2013). Considering that patients with AN often present with elevated cholesterol levels (Ohwada et al., 2006; Matzkin et al., 2006; Rigaud et al., 2009; Jauregui-Garrido et al., 2012), this clinical abnormality could be at least partially a sign of a disruption in the melanocortin system. Furthermore, future research on the possible use of AGRP and exogenous MC4R competitive antagonists

in the treatment of AN is needed. Our results also suggest a possible role for NTRK2 receptor agonists for individuals with BN who are overweight or obese. BDNF is the natural NTRK2 agonist, and we are not aware of any clinical studies investigating BDNF administration in BN or obesity. Interestingly, N-acetylserotonin, endogenous chemical intermediate of melatonin and serotonin, has been shown to mediate the antidepressive effects of selective serotonin reuptake inhibitors (SSRIs) through NTRK2 agonism (Jang et al., 2010). Considering that fluoxetine is approved for the treatment of BN and leads to a reduction in binge eating and purging (American Psychiatric Association, 2006), our findings are in line with the clinical evidence for SSRI use in the treatment of BN, and if replicated, these results may provide an alternate, non-serotonergic mechanism of action of SSRIs in BN through neurotrophin agonism.

Role of funding source

The present study was funded by research grants from the Ontario Mental Health Foundation (awarded to Drs. Kaplan and Levitan) and Province of Ontario Academic Health Science Centres Academic Funding Plan Innovation Fund (awarded to Drs. Kaplan and Kennedy). Dr. Yilmaz was funded by a Canadian Institutes of Health Research Doctoral Research Award (200910GSD-248658-174637) and is currently supported by the National Institutes of Health (NIH) Grant T32MH076694 (PI: Bulik). Dr. Tiwari is supported by the Brain & Behavior Research Foundation Young Investigator Award. None of these funding agencies had any influence on the design or results of the study.

Contributors

Drs. Kaplan, Levitan, Yilmaz, Kennedy, Bergen, Kaye, Berrettini, Brandt, Bulik, Crawford, Crow, Fichter, Halmi, Johnson, Keel, Klump, Magistretti, Mitchell, Strober, Thornton, Treasure and Woodside were involved in the recruitment of the eating disorder cases, biospecimen collection and DNA extraction. Drs. Yilmaz, Kaplan and Kennedy designed the present study and wrote the study protocol. Dr. Tiwari assisted with the marker selection and *in silico* analysis. With the help of Ms. Piran, Dr. Yilmaz prepared and genotyped the DNA samples. Drs. Hakonarson and Wang provided *p*-values from the previous Price Foundation-Children's Hospital of Pennsylvania GWAS for comparison of results. Drs. Yilmaz and Knight were involved in the statistical analysis, and Dr. Yilmaz prepared the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

Dr. Kennedy has received honoraria from Eli Lilly and Roche, whereas Dr. Levitan has received honorarium from Astra-Zeneca. Dr. Bergen is an employee of SRI International, and has received research, salary and travel support from the National Institutes of Health, from the Price Foundation, Ltd., and from the University of California, San Diego, through grants, study section service, and through professional service agreements. Dr. Bulik is a consultant for Shire Pharmaceuticals. Other authors have no financial interests to disclose.

Acknowledgments

The authors would like to thank the individuals who contributed DNA samples for genetic research, Ms. Sarah Gagliano for her assistance with statistical analysis, Ms. Natalie Freeman, Ms. Maria Tampakeras and Mr. Sajid Shaikh for their contributions to DNA preparation, and Ms. Misti Dowell for her help with the shipment of Price Foundation DNA samples.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2014.04.005.

References

American Psychiatric Association. Treatment of patients with eating disorders, third edition. Am J Psychiatry 2006;163:4–54.
American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Washington DC: American Psychiatric Association; 2013.
Ando T, Komaki G, Naruo T, Okabe K, Takii M, Kawai K, et al. Possible role of preproghrelin gene polymorphisms in susceptibility to bulimia nervosa. Am J Med Genet B Neuropsychiatr Genet 2006;141:929–34.
Beckers S, Zegers D, de Freitas F, Mertens IL, Van Gaal LF, Van Hul W. Association study of MC4R with complex obesity and replication of the rs17782313 association signal. Mol Genet Metab 2011;103:71–5.
Bonilla C, Panguluri RK, Taliaferro-Smith L, Argyropoulos G, Chen G, Adeyemo AA, et al. Agouti-related protein promoter variant associated with leanness and decreased risk for diabetes in West Africans. Int J Obes (Lond) 2006;30:715–21.
Brandys MK, van Elburg AA, Loos RJ, Bauer F, Hendriks J, van der Schouw YT, et al. Are recently identified genetic variants regulating BMI in the general population associated with anorexia nervosa? Am J Med Genet B Neuropsychiatr Genet 2010;153:695–9.
Brandys MK, Kas MJ, van Elburg AA, Ophoff R, Slof-Op't Landt MC, Middeldorp CM, et al. The Val66Met polymorphism of the BDNF gene in anorexia nervosa: new data and a meta-analysis. World J Biol Psychiatry 2013;14:441–51.
Cellini E, Nacmias B, Brecelj-Anderluh M, Badia-Casanovas A, Bellodi L, Boni C, et al. Case-control and combined family trios analysis of three polymorphisms in the ghrelin gene in European patients with anorexia and bulimia nervosa. Psychiatr Genet 2006;16:51–2.
Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P, Wu Y, et al. Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. Proc Natl Acad Sci 2009;106:16481–6.
Chowdhury NI, Tiwari AK, Souza RP, Zai CC, Shaikh SA, Chen S, et al. Genetic association study between antipsychotic-induced weight gain and the melanocortin-4 receptor gene. Pharmacogenomics J 2013;13:272–9.
Dardennes RM, Zizzari P, Tolle V, Foulon C, Kipman A, Romo L, et al. Family trios analysis of common polymorphisms in the obestatin/ghrelin, BDNF and AGRP genes in patients with anorexia nervosa: association with subtype, body-mass index, severity and age of onset. Psychoneuroendocrinology 2007;32:106–13.
de Krom M, Bakker SC, Hendriks J, van Elburg A, Hoogendoorn M, Verduijn W, et al. Polymorphisms in the brain-derived neurotrophic factor gene are not associated with either anorexia nervosa or schizophrenia in Dutch patients. Psychiatr Genet 2005a;15:81.
de Krom M, de Rijke CE, Hendriks J, van Engeland H, van Elburg AA, Adan RA. Mutation analysis of the agouti related protein promoter region and the melanocortin-3 receptor in anorexia nervosa patients. Psychiatr Genet 2005b;15:237.
Dellava JE, Thornton LM, Hamer RM, Strober M, Plotnicov K, Klump KL, et al. Childhood anxiety associated with low BMI in women with anorexia nervosa. Behav Res Ther 2010;48:60–7.
Dmitrzak-Weglarz M, Skibinska M, Slopien A, Szczepankiewicz A, Rybakowski F, Kramer L, et al. BDNF Met66 allele is associated with anorexia nervosa in the Polish population. Psychiatr Genet 2007;17:245–6.
Eddy KT, Dorer DJ, Franko DL, Tahilani K, Thompson-Brenner H, Herzog DB. Diagnostic crossover in anorexia nervosa and bulimia nervosa: implications for DSM-V. Am J Psychiatry 2008;165:245–50.
Elks CE, Loos RJ, Sharp SJ, Langenberg C, Ring SM, Timpson NJ, et al. Genetic markers of adult obesity risk are associated with greater early infancy weight gain and growth. PLoS Med 2010;7:e1000284.
Feng N, Young SF, Aguilera G, Puricelli E, Adler-Wailes DC, Sebring NG, et al. Co-occurrence of two partially inactivating polymorphisms of MC3R is associated with pediatric-onset obesity. Diabetes 2005;54:2663–7.
Fichter MM, Quadflieg N. Six-year course of bulimia nervosa. Int J Eat Disord 1997;22:361–84.
Frieling H, Romer KD, Scholz S, Mittelbach F, Wilhelm J, De Zwaan M, et al. Epigenetic dysregulation of dopaminergic genes in eating disorders. Int J Eat Disord 2010;43:577–83.
Gelegen C, van den Heuvel J, Collier DA, Campbell IC, Oppelaar H, Hessel E, et al. Dopaminergic and brain-derived neurotrophic factor signalling in inbred mice exposed to a restricted feeding schedule. Genes Brain Behav 2008;7:552–9.
Gorwood P, Ades J, Bellodi L, Cellini E, Collier DA, Di Bella D, et al. The 5-HT(2A) −1438G/A polymorphism in anorexia nervosa: a combined analysis of 316 trios from six European centres. Mol Psychiatry 2002;7:90–4.
Gratacos M, Gonzalez JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis

of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biol Psychiatry 2007;61:911–22.
Hillebrand JJ, Kas MJ, Scheurink AJ, van Dijk G, Adan RA. AgRP(83-132) and SHU9119 differently affect activity-based anorexia. Eur Neuropsychopharmacol 2006;16:403–12.
Hinney A, Ziegler A, Nothen MM, Remschmidt H, Hebebrand J. 5-HT2A receptor gene polymorphisms, anorexia nervosa, and obesity. Lancet 1997;350:1324–5.
Hinney A, Becker I, Heibult O, Nottebom K, Schmidt A, Ziegler A, et al. Systematic mutation screening of the pro-opiomelanocortin gene: identification of several genetic variants including three different insertions, one nonsense and two missense point mutations in probands of different weight extremes. J Clin Endocrinol Metab 1998;83:3737–41.
Hu X, Giotakis O, Li T, Karwautz A, Treasure J, Collier DA. Association of the 5-HT2C gene with susceptibility and minimum body mass index in anorexia nervosa. Neuroreport 2003;14:781–3.
Jacobs MJ, Roesch S, Wonderlich SA, Crosby R, Thornton L, Wilfley DE, et al. Anorexia nervosa trios: behavioral profiles of individuals with anorexia nervosa and their parents. Psychol Med 2009;39:451–61.
Janas-Kozik M, Stachowicz M, Mazurek U, Zajdel A, Wilczok A, Krupka-Matuszczyk I, et al. Preliminary study of the expression of genes connected with the orexigenic and anorexigenic system using microarray technique in anorexia nervosa. Neuropsychobiology 2008;57:116–20.
Janeckova R. The role of leptin in human physiology and pathophysiology. Physiol Res 2001;50:443–59.
Jang SW, Liu X, Pradoldej S, Tosini G, Chang Q, Iuvone PM, et al. N-acetylserotonin activates TrkB receptor in a circadian rhythm. Proc Natl Acad Sci 2010;107:3876–81.
Jauregui-Garrido B, Bolanos-Rios P, Santiago-Fernandez MJ, Jauregui-Lobera I. Lipid profile and cardiovascular risk in anorexia nervosa; the effect of nutritional treatment. Nutr Hosp 2012;27:908–13.
Kaplan AS, Levitan RD, Yilmaz Z, Davis C, Tharmalingam S, Kennedy JL. A DRD4/BDNF gene–gene interaction associated with maximum BMI in women with bulimia nervosa. Int J Eat Disord 2008;41:22–8.
Kas MJ, van Dijk G, Scheurink AJ, Adan RA. Agouti-related protein prevents self-starvation. Mol Psychiatr 2003;8:235–40.
Kaye WH, Lilenfeld LR, Berrettini WH, Strober M, Devlin B, Klump KL, et al. A search for susceptibility loci for anorexia nervosa: methods and sample description. Biol Psychiatry 2000;47:794–803.
Kaye WH, Devlin B, Barbarich N, Bulik CM, Thornton L, Bacanu SA, et al. Genetic analysis of bulimia nervosa: methods and sample description. Int J Eat Disord 2004;35:556–70.
Kindler J, Bailer U, de Zwaan M, Fuchs K, Leisch F, Grun B, et al. No association of the neuropeptide Y (Leu7Pro) and ghrelin gene (Arg51Gln, Leu72Met, Gln90Leu) single nucleotide polymorphisms with eating disorders. Nord J Psychiat 2011;65:203–7.
Kvaloy K, Kulle B, Romundstad P, Holmen TL. Sex-specific effects of weight-affecting gene variants in a life course perspective-the HUNT study, Norway. Int J Obes (Lond) 2013;37:1221–9.
Lahiri DK, Nurnberger Jr JI. A rapid non-enzymatic method for the preparation of HMWDNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444.
Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 2005;95:221–7.
Li P, Tiwari HK, Lin WY, Allison DB, Chung WK, Leibel RL, et al. Genetic association analysis of 30 genes related to obesity in a European American population. Int J Obes (Lond); 2013 Jul 31. http://dx.doi.org/10.1038/ijo.2013.140.
Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet 2008;40:768–75.
Luan J, Kerner B, Zhao JH, Loos RJ, Sharp SJ, Muthen BO, et al. A multilevel linear mixed model of the association between candidate genes and weight and body mass index using the Framingham longitudinal family data. BMC Proc 2009;3(Suppl. 7):S115.
Malhotra AK, Correll CU, Chowdhury NI, Muller DJ, Gregersen PK, Lee AT, et al. Association between common variants near the melanocortin 4 receptor gene and severe antipsychotic drug-induced weight gain. Arch Gen Psychiatry 2012;69:904–12.
Matzkin VB, Geissler C, Coniglio R, Selles J, Bello M. Cholesterol concentrations in patients with anorexia nervosa and in healthy controls. Int J Psychiatr Nurs Res 2006;11:1283–93.
Mercader JM, Saus E, Agüera Z, Bayés M, Boni C, Carreras A, et al. Association of NTRK3 and its interaction with NGF suggest an altered cross-regulation of the neurotrophin signaling pathway in eating disorders. Hum Mol Genet 2008;17:1234–44.
Merle JV, Haas V, Burghardt R, Döhler N, Schneider N, Lehmkuhl U, et al. Agouti-related protein in patients with acute and weight-restored anorexia nervosa. Psychol Med 2011;41:2183–92.
Monteleone P, Tortorella A, Castaldo E, Di Filippo C, Maj M. No association of the Arg51Gln and Leu72Met polymorphisms of the ghrelin gene with anorexia nervosa or bulimia nervosa. Neurosci Lett 2006a;398:325–7.
Monteleone P, Zanardini R, Tortorella A, Gennarelli M, Castaldo E, Canestrelli B, et al. The 196G/A (Val66Met) polymorphism of the BDNF gene is significantly associated with binge eating behavior in women with bulimia nervosa or binge eating disorder. Neurosci Lett 2006b;406:133–7.
Monteleone P, Tortorella A, Castaldo E, Di Filippo C, Maj M. The Leu72Met polymorphism of the ghrelin gene is significantly associated with binge eating disorder. Psychiatr Genet 2007;17:13–6.
Monteleone P, Di Genio M, Monteleone AM, Di Filippo C, Maj M. Investigation of factors associated to crossover from anorexia nervosa restricting type (ANR) and anorexia nervosa binge-purging type (ANBP) to bulimia nervosa and comparison of bulimia nervosa patients with or without previous ANR or ANBP. Compr Psychiatry 2011;52:56–62.
Moriya J, Takimoto Y, Yoshiuchi K, Shimosawa T, Akabayashi A. Plasma agouti-related protein levels in women with anorexia nervosa. Psychoneuroendocrinology 2006;31:1057–61.
Nisoli E, Brunani A, Borgomainerio E, Tonello C, Dioni L, Briscini L, et al. D2 dopamine receptor (DRD2) gene Taq1A polymorphism and the eating-related psychological traits in eating disorders (anorexia nervosa and bulimia) and obesity. Eat Weight Disord 2007;12:91–6.
Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004;74:765–9.
Ohwada R, Hotta M, Oikawa S, Takano K. Etiology of hypercholesterolemia in patients with anorexia nervosa. Int J Eat Disord 2006;39:598–601.
Perez-Tilve D, Hofmann SM, Basford J, Nogueiras R, Pfluger PT, Patterson JT, et al. Melanocortin signaling in the CNS directly regulates circulating cholesterol. Nat Neurosci 2010;13:877–82.
Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
Quinton ND, Meechan DW, Brown K, Eastwood H, Blakemore AI. Single nucleotide polymorphisms in the leptin receptor gene: studies in anorexia nervosa. Psychiatr Genet 2004;14:191–4.
Ribases M, Gratacos M, Armengol L, de Cid R, Badia A, Jimenez L, et al. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. Mol Psychiatry 2003;8:745–51.
Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, et al. Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations. Hum Mol Genet 2004;13:1205–12.
Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, et al. Association of BDNF with restricting anorexia nervosa and minimum body mass index: a family-based association study of eight European populations. Eur J Hum Genet 2005a;13:428–34.
Ribases M, Gratacos M, Badia A, Jimenez L, Solano R, Vallejo J, et al. Contribution of NTRK2 to the genetic susceptibility to anorexia nervosa, harm avoidance and minimum body mass index. Mol Psychiatry 2005b;10:851–60.
Ricca V, Nacmias B, Boldrini M, Cellini E, di Bernardo M, Ravaldi C, et al. Psychopathological traits and 5-HT2A receptor promoter polymorphism (−1438 G/A) in patients suffering from anorexia nervosa and bulimia nervosa. Neurosci Lett 2004;365:92–6.
Rigaud D, Tallonneau I, Verges B. Hypercholesterolaemia in anorexia nervosa: frequency and changes during refeeding. Diabetes Metab 2009;35:57–63.
Scherag A, Dina C, Hinney A, Vatin V, Scherag S, Vogel CI, et al. Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and German study groups. PLoS Genet 2010;6:e1000916.
Scott-Van Zeeland AA, Bloss CS, Tewhey R, Bansal V, Torkamani A, Libiger O, et al. Evidence for the role of EPHX2 gene variants in anorexia nervosa. Mol Psychiatry; 2013 Sep 3. http://dx.doi.org/10.1038/mp.2013.91.
Sorli JV, Frances F, Gonzalez JI, Guillen M, Portoles O, Sabater A, et al. Impact of the −1438G>a polymorphism in the serotonin 2A receptor gene on anthropometric profile and obesity risk: a case-control study in a Spanish Mediterranean population. Appetite 2008;50:260–5.
Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 2010;42:937–48.
Sun Q, Cornelis MC, Kraft P, Qi L, van Dam RM, Girman CJ, et al. Genome-wide association study identifies polymorphisms in LEPR as determinants of plasma soluble leptin receptor levels. Hum Mol Genet 2010;19:1846–55.
Ternouth A, Brandys MK, van der Schouw YT, Hendriks J, Jansson JO, Collier D, et al. Association study of POMC variants with body composition measures and nutrient choice. Eur J Pharmacol 2011;660:220–5.
Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 2009;41:18–24.
Thornton LM, Dellava JE, Root TL, Lichtenstein P, Bulik CM. Anorexia nervosa and generalized anxiety disorder: further explorations of the relation between anxiety and body mass index. J Anxiety Disord 2011;25:727–30.
Tozzi F, Thornton LM, Klump KL, Fichter MM, Halmi KA, Kaplan AS, et al. Symptom fluctuation in eating disorders: correlates of diagnostic crossover. Am J Psychiatry 2005;162:732–40.
Villarejo C, Fernandez-Aranda F, Jimenez-Murcia S, Penas-Lledo E, Granero R, Penelo E, et al. Lifetime obesity in patients with eating disorders: increasing prevalence, clinical and personality correlates. Eur Eat Disord Rev 2012;20:250–4.
Vink T, Hinney A, van Elburg AA, van Goozen SH, Sandkuijl LA, Sinke RJ, et al. Association between an agouti-related protein gene polymorphism and anorexia nervosa. Mol Psychiatry 2001;6:325–8.

Wang K, Zhang H, Bloss CS, Duvvuri V, Kaye W, Schork NJ, et al. A genome-wide association study on common SNPs and rare CNVs in anorexia nervosa. Mol Psychiatry 2011;16:949–59.
Wang F, Gelernter J, Kranzler HR, Zhang H. Identification of POMC exonic variants associated with substance dependence and body mass index. PLoS One 2012;7:e45300.
Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 2003;6:736–42.
Yilmaz Z, Kaplan AS, Zai CC, Levitan RD, Kennedy JL. COMT Val158Met variant and functional haplotypes associated with childhood ADHD history in women with bulimia nervosa. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:948–52.
Yilmaz Z, Kaplan AS, Levitan RD, Zai CC, Kennedy JL. Possible association of the DRD4 gene with a history of attention-deficit/hyperactivity disorder in women with bulimia nervosa. Int J Eat Disord 2012;45:622–5.