Eating disorders: the current status of molecular genetic research
Susann Scherag, Johannes Hebebrand, Anke Hinney

To cite this version:
Susann Scherag, Johannes Hebebrand, Anke Hinney. Eating disorders: the current status of molecular genetic research. European child adolescent psychiatry, 2009, 19 (3), pp.211-226. 10.1007/s00787-009-0085-9. hal-00550173

HAL Id: hal-00550173
https://hal.archives-ouvertes.fr/hal-00550173
Submitted on 24 Dec 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Eating disorders: the current status of molecular genetic research

Susann Scherag · Johannes Hebebrand · Anke Hinney

Abstract Anorexia nervosa (AN) and bulimia nervosa (BN) are complex disorders characterized by disordered eating behavior where the patient’s attitude towards weight and shape, as well as their perception of body shape, are disturbed. Formal genetic studies on twins and families suggested a substantial genetic influence for AN and BN. Candidate gene studies have initially focused on the serotonergic and other central neurotransmitter systems and on genes involved in body weight regulation. Hardly any of the positive findings achieved in these studies were unequivocally confirmed or substantiated in meta-analyses. This might be due to too small sample sizes and thus low power and/or the genes underlying eating disorders have not yet been analyzed. However, some studies that also used subphenotypes (e.g., restricting type of AN) led to more specific results; however, confirmation is as yet mostly lacking. Systematic genome-wide linkage scans based on families with at least two individuals with an eating disorder (AN or BN) revealed initial linkage regions on chromosomes 1, 3 and 4 (AN) and 10p (BN). Analyses on candidate genes in the chromosome 1 linkage region led to the (as yet unconfirmed) identification of certain variants associated with AN. Genome-wide association studies are under way and will presumably help to identify genes and pathways involved in these eating disorders. The elucidation of the molecular mechanisms underlying eating disorders might improve therapeutic approaches.

Keywords 5-HT2A receptor gene · Melanocortin 4 receptor gene · GWAS

Introduction

Anorexia nervosa (AN) and bulimia nervosa (BN) are complex eating disorders that are commonly defined by criteria either of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) [1] or the International Statistical Classification of Diseases and Related Health Problems (ICD-10) [2]. Formal genetic studies suggest a substantial genetic influence in these disorders [3–6]. Disturbances of neurotransmitter, neuropeptide, and neuroendocrine systems have been reported in acutely ill and patients who have been followed up for several years [7] so that an involvement of these systems in the etiology of eating disorders appears possible. As candidate gene approaches did not unequivocally identify susceptibility genes (alleles) for AN or BN, systematic model-free genome-wide linkage screens have been performed in order to identify unknown genes involved in the etiology of these eating disorders.

Twin and family studies

Twin studies can be powerful, but their methodology is complicated and their results are easily misjudged. Caveats regarding twin studies include ascertainment and power issues [8]. Use of a clinical sample for a twin study usually implies a greater severity of the illness; hence, the genetic loading might be different from that of patients from the general population. Additionally, factors like an equal environment assumption, as well as the generalizability of results need to be considered [9].
Evidence from family and twin studies suggests a substantial genetic contribution to the etiology of both AN and BN [4, 10–15]. Holland et al. [10] showed proband-wise concordance rates for AN of 0.71 for monozygotic twins and 0.1 for dizygotic twins. Heritability estimates based on these rates ranged from 0.86 to 0.98 for AN [11]. Higher concordance rates for monozygotic twins than for dizygotic twins have been shown in most twin studies. However, Walters and Kendler [16] could not detect a genetic component to AN. They analyzed an epidemiological female twin sample (n = 2,163); only a small number of twins were retrospectively diagnosed with AN. Surprisingly, concordance rates were higher for dizygotic than for monozygotic twins. Interestingly, co-twins of index cases with AN had significantly lower body mass index (BMI in kg/m²) and higher depression rates than co-twins of unaffected twins. Overlapping of the genetic predisposition to both BN and depression was found in the Virginia twin study [16]. Interestingly, Klump et al. [17] found that genetic effects were significantly more important for disordered eating during mid to late adolescence than during prepubertal adolescence. Puberty may therefore be a critical period to activate some of the genetic factors which make certain individuals more susceptible for the development of eating disorders. Klump et al. [15] identified 26 AN patients by interviewing 672 female 17-year-old twins. Biometrical analyses indicated that genetic and non-shared environmental factors accounted for 74 and 26% of the variance in AN, respectively. Based on data of the Swedish Twin Registry, Bulik et al. [18] estimate the heritability of AN at 56%, with the remaining variance attributable to shared environment (5%) and non-shared environment (39%).

Two controlled family studies have found an average 3% lifetime risk of AN in first-degree relatives of patients, being equivalent to an approximate relative risk of at least 10 [7, 19, 20]. AN was found to be infrequent in 1,831 relatives of 504 patients with eating disorders, whereas full and partial syndromes of these eating disorders aggregated in female relatives of both anorexic and bulimic probands (Strober et al. [21]). Relative risks were 11.3 and 12.3 for the full syndrome of AN in first-degree female relatives of patients with AN or BN, respectively [21], suggesting that specific genes can predispose to both eating disorders. The relative risks for BN were 4.2 and 4.4 for first-degree female relatives of patients with AN or BN, respectively. Overlapping of the genetic predisposition to both BN and depression was found in the Virginia twin study [22]. Interestingly, Klump et al. [17] found that genetic effects were significantly more important for disordered eating during mid to late adolescence than during prepubertal adolescence, accounting for 46% of variance from 14 to 18 years of age versus only 6% of variance at 11 years of age. Puberty may therefore be a critical period to activate some of the genetic factors which make certain individuals more susceptible to the development of eating disorders.

**Candidate gene studies**

Basically, two approaches are involved in the molecular genetic analysis of a complex phenotype: (1) association studies (case-control studies; Table 1), including genome-wide association studies (GWAS) and (2) family-based linkage studies. Both approaches have in general been used for molecular genetic studies of eating disorders, although GWAS are still lacking. The candidate gene approach relies on genetic, physiological, biochemical or pharmacological evidence to determine the involvement of a specific gene in the analyzed phenotype.

Candidate gene studies in eating disorders need to consider the following clinical observations: (a) the prevalence of AN and BN is considerably higher in females (ratio 9:1). (b) The manifestation periods for both AN and BN are predominantly in puberty and late adolescence, respectively. (c) Approximately 30% of patients with AN later on develop BN; the opposite sequence is less frequent. (d) There is a high rate of comorbidity with obsessive compulsive disorder, major depression and generalized anxiety disorder [7, 23, 24].

In general terms, the lower the numbers of regulatory pathways involved in a complex phenotype, the fewer genes are likely to be involved. An example of such a putative narrow pathway into AN is weight loss-induced dysregulation of the maintenance of a normal body weight, for whatever reason, during the critical age period. If, however, subtle perturbations in several different pathways lead to an eating disorder, greater the heterogeneity and smaller the effect of a predisposing allele is likely to be. Molecular genetic analyses of other complex disorders suggest that the genetic basis underlying eating disorders is polygenic; effect sizes of predisposing alleles are likely to be small [Hebebrand et al., this issue].

Neurobiological disturbances that persist after recovery might be trait-related and thus implicated in the etiology of the disorder. Studies pertaining to long-term follow-up of patients with AN suggest that disturbances of monoaminergic pathways and weight regulation continue after recovery [25–28]. These studies implicate that genes involved in the serotonergic and dopaminergic systems and in weight regulation can be perceived as candidates [29].
| Gene                                      | Variant                          | Test type | Evidence for association to AN | Evidence for association to BN | Study                          |
|-------------------------------------------|----------------------------------|-----------|--------------------------------|--------------------------------|--------------------------------|
| Catechol-O-methyltransferase (COMT)       | Val-158-Met                      | TDT       | Yes                            | ND                             | Frisch et al. [66]             |
|                                           | Val-158-Met                      | TDT       | Yes                            | ND                             | Michaelovsky et al. [68]       |
|                                           | Val-158-Met                      | CC        | Yes                            | Yes                            | Frieling et al. [150]          |
|                                           | Val-158-Met                      | CC        | Yes                            | No                             | Mikolajczyk et al. [69]        |
|                                           | Val-158-Met                      | TDT       | No                             | ND                             | Gabrovsek et al. [70]          |
| Dopamine D2 receptor (DRD2)               | TaqAI                            | CC        | Yes                            | Yes                            | Nisoli et al. [65]             |
|                                           | −141 Indel                       | TDT/CC    | Yes                            | ND                             | Bergen et al. [64]             |
| Dopamine D3 receptor (DRD3)               | Bal I polymorphism in exon 1     | CC        | No                             | ND                             | Bruins-Slot et al. [151]       |
| Dopamine D4 receptor (DRD4)               | 13 bp deletion                   | TDT       | No                             | ND                             | Hinney et al. [60]             |
|                                           | 48 bp deletion                   | Haplotype exon III VNTR, 120 bp repeat, 521C>T, 809A>G | TDT | Yes | ND | Bachner-Melman et al. [59] |
| Serotonin transporter (SERT, 5-HTT, SCL6A4) | 5-HTTLPR                         | CC        | Yes                            | ND                             | Kaplan et al. [63]             |
|                                           | 5-HTTLPR                         | CC        | No                             | ND                             | Karwautz et al. [61]           |
|                                           | 5-HTTLPR intron 2 VNTR           | CC        | Yes                            | ND                             | Matsushita et al. [49]         |
|                                           | 5-HTTLPR                         | CC        | ND                             | No                             | Lauzurica et al. [51]          |
|                                           | 5-HTTLPR                         | Meta-analysis | Yes                        | ND                             | Gorwood et al. [52]            |
|                                           | 5-HTTLPR                         | TDT       | No                             | ND                             | Urwin et al. [55]              |
|                                           | 5-HTTLPR                         | CC        | No                             | ND                             | Rybakowski et al. [40]         |
|                                           | 5-HTTLPR                         | CC        | No                             | Yes                            | Di Bella et al. [152]          |
|                                           | 5-HTTLPR                         | CC        | Yes                            | ND                             | Fumeron et al. [153]           |
|                                           | 5-HTTLPR                         | CC        | No                             | ND                             | Hinney et al. [48]             |
| 5-HT1B/1D receptor                       | 821G>C                           | CC        | Yes                            | ND                             | Levitan et al. [154]           |
|                                           | Phe-124-Cys                      | CC        | No                             | ND                             | Hinney et al. [155]            |
| 5-HT2A receptor                          | −1438 G/A                        | CC        | Yes                            | ND                             | Rybakowski et al. [40]         |
|                                           | −1438 G/A                        | CC        | No                             | ND                             | Ando et al. [42]               |
|                                           | −1438 G/A                        | CC        | No                             | Yes                            | Nishiguchi et al. [46]         |
|                                           | −1438 G/A                        | TDT       | No                             | ND                             | Gorwood et al. [45]            |
|                                           | −1438 G/A                        | Meta-analysis | Yes                        | ND                             | Gorwood et al. [52]            |
|                                           | −1438 G/A                        | CC        | Yes                            | No                             | Nacmias et al. [39]            |
|                                           | −1438 G/A                        | CC        | Yes                            | ND                             | Collier et al. [37]            |
|                                           | −1438 G/A                        | CC        | No                             | ND                             | Hinney et al. [156]            |
|                                           | −1438 G/A                        | CC        | No                             | ND                             | Campbell et al. [44]           |
|                                           | −1438 G/A                        | CC        | Yes                            | ND                             | Sorbi et al. [41]              |
| 5-HT2A receptor                          | −1438 G/A                        | CC        | Yes                            | No                             | Enoch et al. [38]              |
|                                           | −1438 G/A                        | CC        | No                             | No                             | Ziegler et al. [47]            |
|                                           | −1438 G/A                        | CC        | Yes                            | Yes                            | Ricca et al. [157]             |
|                                           | −1438 G/A                        | CC        | No                             | ND                             | Kipman et al. [96]             |
|                                           | Thr-25-Asn 102T>C, 516C>T, His-452-Tyr | CC     | No                             | ND                             | Hinney et al. [48]             |
| 5-HT3B receptor                          | Thr-25-Asn 102T>C, His-452-Tyr   | CC        | No                             | No                             | Nacmias et al. [39]            |
| Norepinephrine transporter gene (NET, SLC6A2) | Tyr129Ser                      | CC        | Yes                            | No                             | Hammer et al. [158]            |
|                                           | 4-bp ins/del in AAGG4 repeat island | TDT       | Yes                            | ND                             | Urwin et al. [55]              |
|                                           | Epistasis between 4-bp ins/del in AAGG4 repeat island and 5-HTTLPR | TDT | No | ND | Urwin et al. [55] |
Neurotransmitter systems implicated in eating disorders

Serotonergic system

Serotonin (5-hydroxytryptamine; 5-HT) is involved in a broad range of biological, physiological and behavioral functions [30, 31]. The neurotransmitter system includes tryptophan hydroxylase, the 5-HT transporter (SLC6A4 or 5-HTT) and 5-HT receptors. Several lines of evidence implicate the serotonergic system in body weight regulation and more specifically in eating behavior [30–32] and eating disorders [33, 34]. In cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA) levels were elevated in long-term weight-restored patients with AN or BN in comparison with controls, suggesting that hyperserotonergic function is a trait marker in eating disorders [35]. The increased serotonergic neurotransmission could also account for characteristic psychopathological features such as perfectionism, rigidity and obsessiveness frequently associated with AN [7, 23]. However, results of most of the molecular genetic studies pertaining to the serotonergic system were negative or equivocal see [36]. Two exceptions should be mentioned:

1. The A-allele of the promoter polymorphism −1438G>A of the 5-HT2A receptor gene was initially reported to be associated with AN [37]. Some of the subsequent studies confirmed this result [38–41], others did not [42–46]. Additionally, an early meta-

Table 1 continued

| Gene                                | Variant | Test type | Evidence for association to |
|-------------------------------------|---------|-----------|----------------------------|
| Neuropeptide FF (NFF)               |                                          |                          |
| Leptin                              | −1387G>A (promoter)                      | CC                      | No                        | No                        |
| Agouti related protein              | 526G>A (silent) in linkage disequilibrium with Ala-67-Thr | CC                      | Yes                       | ND                        |
| Pro-opiomelanocortin (POMC)         | Insertion of 9 bp between codon 73 and 74 | CC                      | No                        | ND                        |
| Brain-derived neurotrophic factor (BDNF) | −270C>T, Val-66-Met                        | CC                      | Yes                       | ND                        |
| Neuropeptide FF (NFF)               | −270C>T, Val-66-Met                        | FBAT                    | Yes                       | ND                        |
| Neurotrophic tyrosine kinase receptor type 3 (NTRK3) | rs7180942 | FBAT      | Yes                       | Yes                      |
| Cannabinoid receptor gene (CNR1)     | rs1049353 rs2180619 rs806379 rs1535255 rs2023239 | TDT/CC                  | No                        | ND                        |
| Fatty acid amide hydrolase (FAAH)    | rs3932816 rs324420 rs324419 rs873978 rs2295632 | TDT                     | No                        | ND                        |
| N-acylethanolamine-hydrolyzing acid amidase (NAAA) | rs2292534 rs4859567 rs10518142 rs6819442 | TDT                     | No                        | ND                        |
| Monoglyceride lipase (MGLL)          | rs8932942 | TDT       | No                        | ND                        |

TDT Transmission disequilibrium test, CC case–control study, ND no data
The promoter region of the serotonin transporter (5-HTTLPR) harbors a functional insertion/deletion polymorphism with two frequent alleles that were designated as short (*S) and long (*L) alleles. The frequency of the 5-HTTLPR *S allele has been assessed in AN [48–50] and BN [51], with conflicting results. A meta-analysis for AN indicates that the *S allele could represent a risk factor for AN [52].

Norepinephrine

In long-term weight-restored patients with AN, lower serum norepinephrine levels than in controls were measured [53]. A repeat polymorphism within the norepinephrine transporter (SLC6A2 or NET) promoter region alters a potential transcription factor binding site therefore presumably entailing altered norepinephrine reuptake. Preferential transmission of the L4 allele was detected in 87 Australian trios each comprising a patient with AN of the restricting type and both biological parents. These results suggested that L4 or a DNA variant in linkage disequilibrium with it doubles the risk to develop this type of AN [53]. Following up on previous studies [48, 53, 54] on single genes, Urwin et al. [55] investigated epistasis between the 5-HTT and NET in AN. However, epistasis between the 5-HTTLPR and the polymorphism within the NET promoter polymorphic region (NETpPR) was not observed.

Dopaminergic system

The dopaminergic system has been implicated in the pathophysiology of AN and BN [23, 56, 57]. For example, major symptoms related to AN like repulsion to food, weight loss, hyperactivity, distortion of body image, and obsessive–compulsive behavior have all been related to dopamine activity [58].

Association studies on the role of polymorphisms in the dopamine D4 receptor gene (DRD4), mainly the 7-repeat allele of the DRD4 exon 3 repeat, in AN have yielded positive [59] as well as negative results [60, 61]. The 7-repeat allele is seemingly relevant for binge eating disorder (BED) and BN: Levitan et al. [62] identified an elevated rate of binge eaters in a group of carriers of the 7-repeat allele. A similar finding was reported by Kaplan et al. [63] who showed that the 7-repeat allele of DRD4 contributes to weight gain in woman with BN. Bergen et al. [64] analyzed seven polymorphisms within the dopamine D2 receptor gene (DRD2) and reported nominal association for two of them and AN. However, Nisoli et al. [65] did confirm the association with AN or BN.

The catechol-O-methyltransferase gene (COMT) catalyzes brain catecholamine neurotransmitters such as dopamine and norepinephrine. An initial study suggested an influence of the Val158Met polymorphism on susceptibility to AN [66]. Data were confirmed in some [67–69] but not all studies [64, 70].

As most of the reported studies on the genetic influence of the dopaminergic system on the etiology of AN and BN did not have sufficient statistical power, these findings require replication in large independent samples or at least in a meta-analytical approach.

Body weight regulation: leptinergic–melanocortinergic system

There has been a tremendous increase in the number of molecular genetic studies pertaining to body weight regulation in the last 15 years see also [Hinney et al.]. Since its discovery in 1994, research has focused on leptin-mediated signaling pathways. Leptin is not only a key hormone implicated in the regulation of energy balance, but it is also a pleiotropic hormone involved in various neuroendocrine and behavioral alterations associated with profound changes in energy storage, including the adaptation of the organism to semi-starvation [71, 72].

Hypoleptinemia is a cardinal feature of acute AN, and in most studies the low leptin levels are typically below those of healthy gender- and age-matched controls and reflect the low fat mass, thus signaling energy depletion to the brain. In most studies, circulating levels of leptin were highly correlated with percent body fat [50, 73–78] and, to a lesser extent, with BMI on referral [26, 39, 50, 73, 76, 78]. In further studies patients who had recovered from eating disorders also had reduced serum leptin levels after adjustment for BMI and/or fat mass [79], suggesting that relative hypoleptinemia might be a trait marker in eating disorders. According to Frey et al. [25] females with a past history of AN (followed up 10-years after in-patient treatment) seemingly have a lower percent body fat and a trend to lower serum leptin levels than BMI-matched controls. However, other studies did not confirm such findings [80].

A mutation analysis of the coding region and part of the promoter region of the leptin gene in patients with AN, followed by case–control studies of the detected polymorphisms, yielded negative results [81]. Leptin receptor gene SNPs also do not appear to be associated with regulation of body weight or with AN [82].
Brain-derived neurotrophic factor (BDNF) mutations.

It is unlikely that BED is strongly associated with MC4R. Bdnf is expressed in the hypothalamic nuclei associated with weight regulation and feeding control. BDNF could result in an inadequate starvation signaling in the brain. In patients with AN three polymorphisms were detected in AGRP. The Ala67 allele was significantly enriched in patients with AN compared to controls [83]. In contrast, another study [84] observed over-transmission of the other allele (67Thr) to patients with AN (114 AN-trios). Hence, an influence of variations within AGRP on the development of AN is currently not shown unequivocally.

Melanocortin-4 receptor

A dominant form of obesity is conferred by mutations in MC4R that lead to a reduced receptor function e.g., [85–91]. Obesity has been identified as a risk factor for the development of BN [92]. Accordingly, genotypes predisposing to obesity might be detected more commonly in patients with BN than in controls not selected for body weight. A mutation screen in the MC4R of 81 BN patients revealed that a single extremely obese patient had a haploinsufficiency mutation in the MC4R. This was the first report on the detection of a validated genotype predisposing to obesity in a patient with BN [93]. Two further studies described that all identified obese carriers of MC4R variants were shown to have BED [94–96]. However, Branson et al. [94] included the Ile103 variant of the Val103Ile polymorphism as one of the risk alleles; this allele was however subsequently found to be negatively associated with obesity [95–97]. Furthermore, another study did not detect any MC4R mutation among extremely obese adults with BED [98]. Finally, in our own families with a total of 43 MC4R mutation carriers we found no evidence for elevated rates of binge eating behavior in carriers of MC4R mutations [99]. In conclusion, it is unlikely that BED is strongly associated with MC4R mutations.

Brain-derived neurotrophic factor (BDNF)

BDNF acts downstream of the MC4R [100]. It regulates synaptic efficiency through the modulation of key neurotransmitter systems previously shown to be involved in ED. BDNF is expressed in the hypothalamic nuclei associated with weight regulation and feeding control. Bdnf+/- mice show altered eating behavior, increased body weight and hypertrophic adipocytes. Humans with low serum BDNF levels display an aberrant eating behavior [101, 102]. BDNF levels might be involved in the severity of eating disorders (EDs) through the modulation of psychopathological traits that are associated with the ED phenotype [103]. Very recently, Ehrlich et al. [104] measured in acute patients with AN, in addition to hypoleptinemia, reduced peripheral BDNF concentrations. Short-term recovered patients had elevated BDNF levels compared with acutely underweight patients. This might be part of a regenerative process after biochemical and molecular neuronal injury and may also be related to the regulation of appetite and eating behavior [104]. Several studies investigated the role of the BDNF non-synonymous polymorphism Val66Met in the etiology of EDs. The 66Met variant was found to be associated with AN restricting type in a Spanish sample [105]. A confirmatory study revealed association with investigated eating disorder subtypes (restricting AN, binge eating/purging AN) in a case–control study of 1,142 Caucasian patients with EDs consistently recruited in six different centers from five European countries (France, Germany, Italy, Spain and UK) [106]. This finding was replicated by some [39, 107], but not all studies [84, 108, 109]. Beside BDNF, association studies of its receptors neurotrophic tyrosine kinase receptor types 2 and 3 (NTRK2 and NTRK3) revealed an association to eating disorders [110, 111]. These data point to the neurtrophin family as key regulators of eating behavior and as potential susceptibility factors for EDs.

Endocannabinoid system

One of the endogenous systems that, due to its therapeutic potential in the treatment of obesity [112], recently reached scientific interest for ED is the endocannabinoid system. Both exogenous and endogenous cannabinoids stimulate food intake through activation of the cannabinoid receptor 1 (CNR1). The central CNR1 as well as the major endocannabinoid degrading enzymes fatty acid amide hydrolase (FAAH), N-acylethanolamine-hydrolyzing acid amidase (NAAA) and monoglyceride lipase (MGLL) are implicated in mediating the orexigenic effects of cannabinoids. Initially, Siegfried et al. [43] investigated an (AAT)n repeat in the downstream flanking region of CNR1 and found that the 14-repeat allele was preferentially transmitted in the binge eating/purging AN group but not in the restricting AN group. The 13-repeat allele was slightly more often transmitted in the restricting AN group. As the sample comprised only 52 families, this finding should be viewed with caution in light of the small sample size. A second study on the endocannabinoid system in patients with AN pertained to the previously described (AAT)n repeat as well as a total of 15 SNPs in CNR1, FAAH, NAAA or MGLL in up to 91 German AN trios. Evidence for association (measured by transmission disequilibrium test) of any of the SNPs or the (AAT)n repeat in AN was not detected [113].
Genes with effect on BMI from genome-wide association studies

The neuroendocrine and molecular genetic pathways involved in body weight regulation might also be of major importance for eating disorders as suggested by identified common molecular mechanisms for, e.g., BN and obesity [29, 114, 115]. The first GWAS for BMI and obesity marked the beginning of a new hypothesis-free era to unravel the neurobiological mechanisms involved in body weight regulation e.g., [116–118]. Consequently, a recent report focussed on GWAS-based candidate gene variants [119] to test for association with AN and to investigate a combined effect of BMI-increasing alleles (as derived from the original GWA studies) on the risk of developing AN. However, association between individual SNPs and AN was not detected; combined effects of BMI-increasing alleles were also not found.

Genome-wide linkage studies

Despite considerable efforts hardly any of the candidate gene analyses (exceptions: 5HT2A receptor; BDNF; see Table 2) has yielded unequivocal and clearly confirmed evidence for the involvement of specific alleles in the etiology of eating disorders. The candidate gene approach in eating disorders is hampered by the fact that there is no clear-cut evidence implicating a specific regulatory system. In this situation a systematic genome-wide approach that does not rely on any a priori hypotheses as to the underlying genes offers a useful alternative to detect genes involved in the etiology of eating disorders.

Anorexia nervosa

For AN, an international, multi-site collaborative group (The Price Foundation Collaborative Group) [120] collected a large study group of 196 AN patients and 237 affected relatives [AN, BN or eating disorders not otherwise specified (EDNOS)]. The genome-wide linkage study [121] using 386 microsatellite markers, revealed a single non-significant multipoint nonparametric linkage score (NPL) above 1.5 on chromosome 4. NPL scores were also observed at additional markers on chromosome 4, 11, 13 and 15 [121]. Linkage analysis in a subset (n = 37) of families of individuals with restricting AN, the highest multipoint NPL score observed was 3.03, at marker D1S3721 on chromosome 1p. Using the same data set, an additional multipoint affected sibling pair (ASP) linkage analysis was performed specifically devised to incorporate covariates. By exploring seven attributes thought to typify individuals with eating disorders, two variables (drive-for-thinness and obsessionality) were identified, which delimit populations among the ASPs. For both of these traits, or covariates, there were clusters of ASPs who were highly concordant for these

Table 2  Linkage studies for eating disorders

| Chr | Position in cM | Multitpoint NPL (AN sample) | Multitpoint NPL (subsample with ANR) | Devlin et al. [122] AN sample | LOD score (covariate obsessionality in AN) | Bulik et al. [126] BN sample | MLS (BN sample) | Bacanu et al. [125] AN and BN samples | LOD (AN sample) | Position in cM | LOD (BN sample) |
|-----|---------------|-----------------------------|-------------------------------------|-------------------------------|------------------------------------------|-----------------------------|----------------|--------------------------------------|----------------|---------------|----------------|
| 1   | NR            | NR                          | NR                                 | NR                            | 1.06                                    | NR                          | NR             | 206 (1.549)                         | 208 (0.773)    |
| 2   | 186           | 0.12                        | 1.70                               | NR                            | 1.12                                    | NR                          | NR             | 134 (0.73)                          | NR             |
| 4   | 25            | 1.42                        | 1.82                               | NR                            | 1.10                                    | NR                          | NR             | 153 (1.052)                         | 179 (0.944)    |
| 6   | NR            | NR                          | NR                                 | NR                            | 24 (2.70)                               | NR                          | NR             | 206 (1.549)                         | 208 (0.773)    |
| 10  | NR            | NR                          | NR                                 | NR                            | 44 (2.92)                               | NR                          | NR             | 136 (0.406)                         | 100 (1.039)    |
| 11  | NR            | NR                          | NR                                 | NR                            | 29 (1.18)                               | NR                          | NR             | 15 (0.525)                          | NR             |
| 13  | 22            | 0.78                        | 1.70                               | NR                            | 22 (0.88)                               | NR                          | NR             | 15 (0.525)                          | NR             |

Results were included if at least one signal with a LOD >2 was detected or an initial signal was confirmed or narrowed down in the subanalyses for the same phenotype. Please note that the results of Grice et al. [121], Devlin et al. [122], and Bacanu et al. [125] are based on the same data set of families ascertained via a patient with AN or BN.

AN anorexia nervosa, ANR anorexia nervosa restricting type, BN bulimia nervosa, cM centi Morgan, NPL nonparametric linkage score, MLS multipoint maximum LOD scores, NR not reported.

Eur Child Adolesc Psychiatry (2010) 19:211–226 217
traits. When these covariates were incorporated into the ASP linkage analysis, both jointly and separately, several regions of suggestive linkage were found: one close to genome-wide significance on chromosome 1, chromosome 2 and on chromosome 13. By comparing these results with those implemented using more standard linkage methods, covariates that convey substantial information for the linkage analysis were found [122].

Initial candidate gene analyses pertaining to the linkage region on chromosome 1p36.3–34.3 described above have been published [123, 124]. Linkage analysis of polymorphisms in the serotonin 1D receptor gene (5-HT1DR) and the opioid delta receptor gene (OPRD1) together with 33 microsatellite markers in families including relative pairs concordantly affected with restricting AN (N = 37) substantially increased the evidence for linkage of this region to restricting AN. Statistically significant genotypic, allelic, and haplotypic association to AN was observed at 5-HT1DR and OPRD1 [123]. Brown et al. [124] conducted an independent association study to replicate this finding. One of 4 genotyped SNPs in 5-HT1D supported the hypothesis that the 5-HT1D gene is involved in susceptibility to AN, whereas in OPRD1 3 out of 6 investigated SNPs were found to be nominally associated to AN [124]. However, further analyses are required to validate these findings. Currently, there is no evidence to suggest that any of the polymorphisms examined has a functional consequence on the biological activity of 5-HT1DR and OPRD1 [124].

The same genome scan for AN was subsequently used to increase the likelihood of finding genetic variation conferring liability to eating disorders. Bacanu et al. [125] measured over 100 attributes thought to be related to liability to eating disorders in affected individuals from multiplex families of two cohorts: one recruited through a proband with AN (AN cohort); the other recruited through a proband with BN (BN cohort, see below). By a multilayer decision process based on expert evaluation and statistical analysis, six traits were selected for linkage analysis: obsessionality (OBS), age at menarche (MENAR) and anxiety (ANX) for quantitative trait locus (QTL) linkage analysis, and lifetime minimum BMI, concern over mistakes (CM) and food-related obsessions (OBF) for covariate-based linkage analysis. Results from the AN cohort were less compelling than those for BN (see below): The investigators detected two suggestive signals (OBS, ANX) for QTL linkage analysis and five suggestive signals (BMI, CM, OBF) for covariate-based linkage analysis. Despite multiple analyses, correction for multiple testing was not performed. Hence, it remains elusive if these peak regions indeed harbor gene variants underlying the genetic susceptibility to AN. Fine mapping or a confirmed positional candidate gene study have not been published yet.

Bulimia nervosa

A linkage analysis on 308 multiplex families with eating disorders that were identified through a proband with BN yielded a double peak, with the highest nonparametric multipoint maximum LOD score (MLS) on chromosome 10 [126]. Linkage analysis was performed in a subset of 133 families in which at least two affected relatives reported a symptom pattern that included self-induced vomiting. The highest linkage peak provided evidence for the presence of a susceptibility locus for BN on chromosome 10p [126]. Interestingly, this region already showed up in full and partial genome scans for obesity [54, 88, 127, 128]; attempts to identify the underlying gene variant(s) have however not led to clear-cut results [129, 130]. Nevertheless, a gene on chromosome 10p might well be involved in both obesity and BN. Another region on 14q met the criterion for genome-wide suggestive linkage for BN at 62 cM from p-ter [126]. The underlying genes for BN have not been described yet.

As described above, Bacanu et al. [125] determined over 100 attributes thought to be related to liability to eating disorders. The BN cohort produced the largest linkage signals, a QTL linkage analysis revealed four suggestive signals for MENAR and ANX; covariate-based linkage analyses revealed both significant and suggestive linkages for BMI, CM and OBF. Overlap between the two cohorts was minimal for substantial linkage signals. To our knowledge, candidate gene analyses for the chromosomal regions identified for BN have not been published yet.

Genome-wide association studies (GWAS)

Within the last 3 years the number of genetic association studies using large numbers of genetic markers (up to 1,000,000) to search for genetic variation underlying common diseases like obesity, diabetes, cardiovascular disease and cancer has increased dramatically. GWAS rely on the assumption that linkage disequilibrium (LD) enables one SNP to act as a surrogate marker for association to other sequence variants in the same region [131]. By genotyping a large number of SNPs, there is a good chance that at least one SNP will be in LD with common functional variant(s) relevant for the investigated phenotype. Genome-wide association studies represent a major step forward in the study of common genetic variation in complex diseases like eating disorders. Until today, several GWAS revealed previously unknown gene-disease associations, e.g., FTO and obesity [116, 117]. There are currently no published GWAS for eating disorders. To our knowledge, the first GWAS for AN will be conducted in the nearest future. One study is for instance funded by the

 Springer
Wellcome Trust and will soon be conducted by the International Wellcome Trust Case Control Consortium (WTCCC3) on 4,000 patients with AN and population-based controls.

Epigenetics

Epigenetic factors are thought to mediate, at least in part, the relationship between the genome and the environment. Research on epigenetic factors underlying eating disorders is at the very beginning. A first study revealed global DNA hypomethylation, but at the same time DNA hypermethylation of the alpha synuclein gene promoter in females with AN [132]. Two further studies investigated whether the mRNA expression of dopaminergic genes or of genes for the peptide hormones vasopressin and atrial natriuretic peptide (ANP) are altered in blood of patients suffering from eating disorders. It was also analyzed if these alterations can be explained by changes in the promoter specific DNA methylation of these genes [67, 133]. The primary results in a very small sample ($n = 46$) pointed to a disturbed expression of the dopaminergic genes and ANP. Further studies in independent samples are necessary to provide more insight into the epigenetic dysregulation of, e.g., dopaminergic neurotransmission in the pathophysiology of eating disorders.

Eating disorders sub(pheno)types and endophenotypes

The definition of ED sub(pheno)types and endophenotypes and their use for genetic association studies to reduce the heterogeneity in samples based on a clinical diagnostic category has been widely discussed, e.g., [9, 134]. Regarding sub(pheno)types we refer to the findings of Ribases et al. [104, 105, 114] already mentioned above. First studies showed that the 66Met variant of the BDNF Val66Met polymorphism was associated only with the restricting type of AN, whereas it was not associated with AN in general. Hence, a mere analysis of the gene in AN cases versus controls, at the given sample size, would have led to the wrong assumption that the gene is not involved in AN.

Bulik et al. [9] defined endophenotypes as measurable neurophysiological, biochemical, endocrinological, neuro-anatomical, cognitive, or neuropsychological components see also [135]. Additionally, they are heritable, cosegregate with a psychiatric clinical phenotype in the general population, are state independent, and are found in non-affected family members at a higher rate than in the general population [135–137]. Examples of candidate endophenotypes are impaired set-shifting [138], excessive exercise [139], weak coherence [140] or endophenotypes related to temperament like thin-ideal internalization, ineffectiveness, body dissatisfaction and sensitivity to punishment [141]. Specific susceptibility gene variants may underly endophenotypes, which in turn may predispose individuals to develop eating disorders and related conditions. To date standardized definitions for endophenotypes of eating disorders are still missing. As a consequence, results of studies investigating endophenotypes and genetic variants are hard to compare. Thus, their impact may become clearer once studies circumventing methodological flaws like small sample sizes, biases in sample ascertainment (e.g., population stratification) or the testing of multiple subsamples and subphenotypes without addressing the multiple testing issues are performed [142].

Gene × environment interactions

Both genetic and environmental factors contribute to the development of complex diseases like eating disorders. If a genetic factor requires the presence of an environmental factor (or vice versa) to result in increased risk, a situation of gene × environment ($G \times E$) interactions is given. Interacting genetic and environmental factors are distinguished by small to moderate effects. A number of genetic variants might affect several genes influencing vulnerability to eating disorders. As an example, an individual with genetic vulnerability to AN might become ill by exposure to high-risk environments like figure skating or fashion shows. While both the current knowledge on genetic risk factors and on environmental risk factors has been excellently reviewed previously [143] the knowledge on $G \times E$ is limited. To our knowledge, no studies have yet reported distinct findings on $G \times E$ interactions in eating disorders. In part this may be due to design challenges of $G \times E$ studies reviewed in [144, 145]. Following Dempfle et al. [144], the investigation of $G \times E$ interactions may be more rewarding for the detailed characterization of identified disease genes (i.e., at advanced stages of genetic research) as strong $G \times E$ interactions may have also contributed to the detection of the gene itself. To date, variations in the 5-HT2A receptor gene as well as in the $BDNF$ gene represent the most consistently supported genetic findings for eating disorders. As their genetic impact on the etiology of ED was solidly shown, they are presumably the best candidates to investigate interactions with environmental factors. In the future, this list will likely expand once the first large scale GWAS for eating disorders are conducted. Ideally, genetic research will help to improve the understanding of environmental risk factors for eating disorders [15, 143].
Conclusions and research directions

Similar to other complex disorders, genome-wide linkage studies for eating disorder have not led to unequivocally confirmed genes involved in these disorders. The same holds true for the candidate gene studies summarized above, which were often based on samples of small to moderate size. Variations in the 5-HT2A receptor gene as well as the Val66Met variant in BDNF might represent the first exceptions from this rule. Inadequate power to detect associations with small effect sizes and multiple post hoc comparisons are the most common and obvious reasons for contradictory findings. One has to bear in mind that eating disorders likely have a polygenic etiology, each gene having a relatively small effect. To address this issue, large-scaled meta-analyses or studies of sufficient sample size and more stringent significance levels [146] are needed. Recent success of genetic research into other psychiatric disorders [147] has demonstrated that large sample sizes encompassing several thousands of cases and controls are required to identify and validate molecular genetic findings.

Studies which focus on subphenotypes (e.g., restricting type of AN) may lead to more specific results see [104, 105, 114]. These findings will also require additional independent confirmations, but still they also show the value of carefully selected study groups and equally carefully derived candidate genes. The analyses of GWAS data will benefit from the candidate genes analyses performed so far. Although these analyses were mainly equivocal the list of analyzed genes for eating disorders can and will be used for the GWAS. These genes will be specifically and thoroughly analyzed in a complementary hypothesis-driven approach. Regarding body weight regulation, we might soon gain a deeper insight into the molecular mechanisms due to the success of large-scaled GWAS, e.g., [Hinney et al., this issue], 118, 148]. Furthermore, it can also be expected that some of the gene variants predisposing to obesity will play a role in the genetic susceptibility to eating disorders [29, 119].

In sum, GWAS have been a very successful tool for the identification of genes involved in various complex disorders and phenotypes, e.g., reviewed in [149]. The first GWAS for eating disorders are in progress and the results are eagerly awaited. Meanwhile, it is of crucial importance to recruit further patients with eating disorders in order to be in a solid position to confirm potential GWAS signals. In particular genes identified by epigenetic studies as well as studies focusing on well-defined endophenotypes will become more important to elucidate the etiology of eating disorders and to ultimately develop new treatment strategies.

Acknowledgments This work was supported by grants from the Federal Ministry of Education and Research (NGFNplus: 01GS0820 and EDNET: 01GV0905). We thank Dr. Carla I. G. Vogel for assistance with the tables and Dr. A. Scherag for helpful discussions and useful suggestions.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. American Psychiatric Association (APA) (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington (text revision)
2. World Health Organization (1992) ICD-10. International classification of mental and behavioural disorders. Clinical description and diagnostic guidelines, WHO, Geneva
3. Bulik CM, Slof-Op’t Landt MC, van Furth EF, Sullivan PF (2007) The genetics of anorexia nervosa. Annu Rev Nutr 27:263–275
4. Bulik CM, Sullivan PF, Wade TD, Kendler KS (2000) Twin studies of eating disorders: a review. Int J Eat Disord 27:1–20
5. Fairburn CG, Harrison PJ (2003) Eating disorders. Lancet 361:407–416
6. Gorwood P, Kipman A, Foulon C (2003) The human genetics of anorexia nervosa. Eur J Pharmacol 480:163–170
7. Kaye WH, Klump KL, Frank GK, Strober M (2000) Anorexia and bulimia nervosa. Annu Rev Med 51:299–313
8. Slof-Op’t Landt MC, van Furth EF, Meulenbelt I, Slagboom PE, Bartels M, Boomsma DI, Bulik CM (2005) Eating disorders: from twin studies to candidate genes and beyond. Twin Res Hum Genet 8:467–482
9. Bulik CM, Hebebrand J, Keski-Rahkonen A, Klump KL, Reichborn-Kjennerud T, Mazzeo SE, Wade TD (2007) Genetic epidemiology, endophenotypes, and eating disorder classification. Int J Eat Disord 40:S52–S60
10. Holland AJ, Hall A, Murray R, Russell GF, Crisp AH (1984) Anorexia nervosa: a study of 34 twin pairs and one set of triplets. Br J Psychiatry 145:414–419
11. Holland AJ, Sicotte N, Treasure J (1988) Anorexia nervosa: evidence for a genetic basis. J Psychosom Res 32:561–571
12. Kipman A, Gorwood P, Mouren-Simeoni MC, Adès J (1999) Genetic factors in anorexia nervosa. Eur Psychiatry 14:189–198
13. Klump KL, Kaye WH, Strober M (2001) The evolving genetic foundations of eating disorders. Psychiatr Clin North Am 24:215–225
14. Klump KL, Miller KB, Keel PK, McGue M, Iacono WG (2001) Genetic and environmental influences on anorexia nervosa syndromes in a population-based twin sample. Psychiatr Med 31:737–740
15. Klump KL, Wonderlich S, Lehoux P, Lilienfeld LR, Bulik CM (2002) Does environment matter? A review of nonshared environment and eating disorders. Int J Eat Disord 31:118–135
16. Walters EE, Kendler KS (1995) Anorexia nervosa and anorexia-like syndromes in a population-based female twin sample. Am J Psychiatry 152:64–71
17. Klump KL, Burt A, McGue M, Iacono WG (2007) Changes in genetic and environmental influences on disordered eating across adolescence. Arch Gen Psychiatry 64:1409–1415
18. Bulik CM, Sullivan PF, Tozzi F, Furberg H, Lichtenstein P, Pedersen NL (2006) Prevalence, heritability, and prospective risk factors for anorexia nervosa. Arch Gen Psychiatry 63:305–312 (erratum in Arch Gen Psychiatry 2008 65:1061)
21. Walters EE, Neale MC, Eaves LJ, Heath AC, Kessler RC
22. Walters EE, Neale MC, Eaves LJ, Heath AC, Kessler RC, Kendler KS (1992) Bulimia nervosa and major depression: a study of common genetic and environmental factors. Psychol Med 22:617–622
23. Kaye W, Strober M (1999) The neurobiology of eating disorders. In: Charney DS, Nestler EJ, Bunney BS (eds) Neurobiological foundations of mental illness. Oxford University Press, New York, pp 891–906
24. Koronyo-Hamaoui M, Danziger Y, Frisch A, Stein D, Leor S, Barkai G, Weizman A, Kak E (2002) Association between anorexia nervosa and the hSKCa3 gene: a family-based and case control study. Mol Psychiatry 7:82–85
25. Blundell JE, Lawton CL, Halford JC (1995) Serotonin, eating control study. Mol Psychiatry 7:82–85
26. Blundell JE, Lawton CL, Halford JC (1995) Serotonin, eating control study. Mol Psychiatry 7:82–85
27. Walters EE, Neale MC, Eaves LJ, Heath AC, Kessler RC, Kendler KS (1992) Bulimia nervosa and major depression: a study of common genetic and environmental factors. Psychol Med 22:617–622
28. Kaye WH, Blundell JE, Muller B, Ziegler A, Blum WF, Remschmidt H, Herpertz-Dahlmann BM (2000) Reduced body fat in long-term followed-up female patients with anorexia nervosa. J Psychiatr Res 34:83–88
29. Hebebrand J, Blum WF, Barth N, Coners H, Englaro P, Juul A, Ziegler A, Warnke A, Rascher W, Remschmidt H (1997) Leptin levels in patients with anorexia nervosa are reduced in the acute stage and elevated upon short-term weight restoration. Mol Psychiatry 2:330–334
30. Kaye WH, Berrettini W, Gwirtsman H, George DT (1990) Altered cerebrospinal fluid neuropeptide Y and peptide YY immunoreactivity in anorexia and bulimia nervosa. Arch Gen Psychiatry 47:548–556
31. Kaye WH, Greeno CG, Moss H, Fernstrom J, Fernstrom M, Lilienfeld LR, Weltzin TE, Mann JJ (1998) Alterations in serotonin activity and psychiatric symptoms after recovery from bulimia nervosa. Arch Gen Psychiatry 55:927–935
32. Hebebrand J, Remschmidt H (1995) Anorexia nervosa viewed as an extreme weight condition: genetic implications. Hum Genet 95:1–11
33. Blundell JE, Lawton CL, Halford JC (1995) Serotonin, eating behavior, and fat intake. Obes Res 3(Suppl 4):471S–476S
34. Halford JC, Blundell JE (2000) Separate systems for serotonin and leptin in appetite control. Ann Med 32:222–232
35. Wurtman RJ, Wurtman JJ (1996) Brain serotonin, carbohydrate-craving, obesity and depression. Adv Exp Med Biol 398:35–41
36. Brewerton TD, Jimerson DC (1996) Studies of serotonin function in anorexia nervosa. Psychiatry Res 62:31–42
37. Jimerson DC, Lesem MD, Kaye WH, Brewerton TD (1992) Low serotonin and dopamine metabolite concentrations in cerebrospinal fluid from bulimic patients with frequent binge episodes. Arch Gen Psychiatry 49:132–138
38. Kaye WH, Gwirtsman HE, George DT, Ebert MH (1991) Altered serotonin activity in anorexia nervosa after long-term weight restoration. Does elevated cerebrospinal fluid 5-hydroxyindoleacetic acid level correlate with rigid and obsessive behavior? Arch Gen Psychiatry 48:556–562
39. Hinney A, Friedel S, Remschmidt H, Hebebrand J (2004) Genetic risk factors in eating disorders. Am J Pharmacogenomics 4:209–223
40. Collier DA, Arranz MJ, Li T, Mupita D, Brown N, Treasure J (1997) Association between 5-HT2A gene promoter polymorphism and anorexia nervosa. Lancet 350:412
41. Sorbi S, Nacmias B, Tedde A, Ricca V, Mezzani B, Rotella CM (1998) 5-HT2A promoter polymorphism in anorexia nervosa. Lancet 351:1785
42. Ando T, Komaki G, Karibe M, Kawamura N, Hara S, Taki M, Naruo T, Kurokawa N, Takei M, Tatsuta N, Ohba M, Nazzo S, Kudo C, Ishikawa T (2001) 5-HT2A promoter polymorphism is not associated with anorexia nervosa in Japanese patients. Psychiatr Genet 11:157–160
43. Siegfried Z, Kanyas K, Latzer Y, Karni O, Bloch M, Lerer B, Berry EM (2004) Association study of cannabinoid receptor gene (CNR1) alleles and anorexia nervosa: differences between restricting and binging/purging subtypes. Am J Med Genet B Neuropsychiatr Genet 128B:126–130
44. Campbell DA, Sundaramurthy D, Markham AF, Pieri LF (1998) Lack of association between 5-HT2A gene promoter polymorphism and susceptibility to anorexia nervosa. Lancet 351:499
45. Loros J, Rosch T, Rosenkranz K, Fichter M, Herpertz-Dahlmann B, Remschmidt H, Hinney A (1999) Further lack of association between the 5-HT2A gene promoter polymorphism and susceptibility to eating disorders and a meta-analysis pertaining to anorexia nervosa. Mol Psychiatry 4:410–412
46. Hinney A, Barth N, Ziegler A, von Prittwitz S, Hamann A, Hennighausen K, Pirke KM, Heils A, Rosenkranz K, Roth H, Coners H, Mayer H, Herzog W, Siegfried A, Lehmkugl G, Pouskta F, Schmidt MH, Schäfer H, Grzeschik KH, Lesch KP, Lentes KU, Remschmidt H, Hebebrand J (1997) Serotonin transporter gene-linked polymorphic region: allele distributions in relationship to body weight and in anorexia nervosa. Life Sci 61:PL295–PL303
47. Matsushita S, Suzuki K, Murayama M, Shirakawa O, Higuchi S (2001) Association between 5HT2A receptor gene promoter region polymorphism and eating disorders in Japanese patients. Biol Psychiatry 50:123–128
48. Ziegler A, Hebebrand J, Görg T, Rosenkranz K, Fichter M, Herpertz-Dahlmann B, Remschmidt H, Hinney A (1999) Further lack of association between the 5-HT2A gene promoter polymorphism and susceptibility to eating disorders and a meta-analysis pertaining to anorexia nervosa. Mol Psychiatry 4:410–412
49. Hinney A, Barth N, Ziegler A, von Prittwitz S, Hamann A, Hennighausen K, Pirke KM, Heils A, Rosenkranz K, Roth H, Coners H, Mayer H, Herzog W, Siegfried A, Lehmkugl G, Pouskta F, Schmidt MH, Schäfer H, Grzeschik KH, Lesch KP, Lentes KU, Remschmidt H, Hebebrand J (1997) Serotonin transporter gene-linked polymorphic region: allele distributions in relationship to body weight and in anorexia nervosa. Life Sci 61:PL295–PL303
50. Matsushita S, Suzuki K, Murayama M, Nishiguchi N, Hishimoto K, Aoki T, Aida A, Shirakawa O, Higuchi S (2004) Serotonin transporter regulatory region polymorphism is associated with anorexia nervosa. Am J Med Genet B Neuropsychiatr Genet 128B:114–117
51. Sundaramurthy D, Pieri LF, Gape H, Markham AF, Campbell DA (2000) Analysis of the serotonin transporter gene linked polymorphism (5-HTTLPR) in anorexia nervosa. Am J Med Genet Genet 96:53–55
52. Lauzurica N, Hurtado A, Escartí A, Delgado M, Barrios V, Morandé G, Soriano J, Jáuregui I, González-Valdemoro MI, García-Camba E, Fuentes JA (2003) Polymorphisms within the...
promoter and the intron 2 of the serotonin transporter gene in a population of bulimic patients. Neurosci Lett 352:226–230.

52. Gorwood P (2004) Eating disorders, serotonin transporter polymorphisms and potential treatment response. Am J Pharmacogenomics 4:9–17.

53. Urwin RE, Bennett B, Wilcken B, Lampropoulos B, Beaumont P, Clarke S, Russell J, Tanner S, Nunn KP (2002) Anorexia nervosa (restrictive subtype) is associated with a polymorphism in the novel norepinephrine transporter gene promoter polymorphic region. Mol Psychiatry 7:652–657.

54. Price RA, Li WD, Bernstein A, Crystal A, Goldberg EM, Weisberg SJ, Zuckermann WA (2001) A locus affecting obesity in human chromosome region 10p12. Diabetologia 44:363–366.

55. Urwin RE, Bennett BH, Wilcken B, Beaumont PJ, Russell JD, Nunn KP (2003) Investigation of epistasis between the serotonin transporter and norepinephrine transporter genes in anorexia nervosa. Neuropsychopharmacology 28:1351–1355.

56. Barry VC, Klawans HL (1976) On the role of dopamine in the pathophysiology of anorexia nervosa. J Neural Transm 38:107–122.

57. Golden NH, Shenker IR (1994) Amenorrhea in anorexia nervosa. Neuroendocrine control of hypothalamic dysfunction. Int J Eat Disord 16:53–60.

58. Kaye WH, Bulik CM, Thornton L, Barbarich N, Masters K, Gratzacos M, Haigh R, Hebebrand J, Hinney A, Holliday I, Hu X, Karwautz A, Naemiais B, Ribases M, Reimschmidt H, Komel R, Sorbi S, Tomori M, Treasure J, Wagner G, Zhao J, Collier DA (2004) Combined family trio and case-control analysis of the COMT-ARVC gene region in Israeli anorexia nervosa family trios. Am J Med Genet B Neuropsychiatr Genet 139B:45–50.

59. Mikołajczyk E, Smiarowska M, Graywacz A, Samochowiec J (2006) Association of eating disorders with catechol-o-methyltransferase gene functional polymorphism. Neuropsychobiology 54:82–86.

60. Hinney A, Schneider J, Ziegler A, Lehmkohl G, Poustka A, Apter A, Weizman A (2005) Haplotype analysis of the COMT-ARVC gene region in Israeli anorexia nervosa family trios. Am J Med Genet B Neuropsychiatr Genet 139B:45–50.

61. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252.

62. Nemanov L, Golan M, Blank S, Gritsenko I, Ebstein RP (2007) The dopamine D4 receptor gene associated with binge eating and weight gain in women and body weight regulation in patients with anorexia nervosa before and during weight recovery. Am J Clin Nutr 81:889–896.

63. Davis C, Muglia P, Mackenzie B, Tharmalingam S, Kennedy DA, Treasure JL (2001) Individual-specific risk factors for anorexia nervosa: a pilot study using a discordant sister-pair design. Psychol Med 31:317–329.

64. Bergen AW, Yeager M, Welch RA, Haque K, Gajek JK, van den Bree MB, Mazzanti C, Nardi I, Fichter MM, Halmi KA, Davis C, Strober M, Treasure J, Woodside DB, Bulik CM, Bacanu SA, Devlin B, Berrettini WH, Goldman D, Kaye WH (2005) Association of multiple DRD2 polymorphisms with anorexia nervosa. Neuropsychopharmacology 30:1703–1710.

65. Nisoli E, Brunani A, Borgomainerio E, Tonello C, Dionis I, Briscini L, Redaelli G, Molinari E, Cavagnini F, Carruba MO (2007) 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 12:91–96.

66. Frisch A, Lauffer N, Danziger Y, Michaelovsky E, Leor S, Carel C, Stein D, Fennig S, Mimouni M, Apter A, Weizman A (2001) Association of anorexia nervosa with the high activity allele of the COMT gene: a family-based study in Israeli patients. Mol Psychiatry 6:243–245.

67. Frielings D, Bleich S, Otten J, Römer KD, Kornhuber J, de Zwaan M, Jacoby GE, Wilhelm J, Hillemecher T (2008) Epigenetic downregulation of atrial natriuretic peptide but not vasopressin mRNA expression in females with eating disorders is related to impulsivity. Neuropsychopharmacology 33:2605–2609.

68. Michaelovsky E, Frisch A, Leor S, Stein D, Danziger Y, Carel C, Fennig S, Mimouni M, Klaukk SM, Benner A, Poustka A, Apter A, Weizman A (2005) Haplotype analysis of the COMT-ARVC gene region in Israeli anorexia nervosa family trios. Am J Med Genet B Neuropsychiatr Genet 139B:45–50.

69. Mikołajczyk E, Smiarowska M, Graywacz A, Samochowiec J (2006) Association of eating disorders with catechol-o-methyltransferase gene functional polymorphism. Neuropsychobiology 54:82–86.
81. Hinney A, Bornscheuer A, Depenbusch M, Mierke B, Tölle A, Middeke K, Ziegler A, Roth H, Gerber G, Zamzow K, Ballauf A, Hamann A, Mayer H, Siegfried W, Lehmkuhl G, Pousta F, Schmidt MH, Hermann H, Herpertz-Dahlmann BM, Fichter M, Remschmidt H, Hebebrand J (1998) No evidence for involvement of the leptin gene in anorexia nervosa, bulimia nervosa, underweight or early onset extreme obesity: identification of two novel mutations in the coding sequence and a novel polymorphism in the leptin gene linked upstream region. Mol Psychiatry 3:539–543

82. Quinton ND, Meechan DW, Brown K, Eastwood H, Blakemore AE (2004) Single nucleotide polymorphisms in the leptin receptor gene: studies in anorexia nervosa. Psychiatr Genet 14:191–194

83. Vink T, Hinney A, van Elburg AA, van Goozen SH, Sandkuijl LA, Sinke RJ, Herpertz-Dahlmann BM, Hebebrand J, Remschmidt H, van Engeland H, Adan RA (2001) Association between an agouti-related protein gene polymorphism and anorexia nervosa. Mol Psychiatry 6:325–328

84. Dardennes RM, Zizzari P, Tolle V, Foulon C, Kipman A, Romo L, Iancu-Gontard D, Boni C, Sinet PM, Thérie Bluet M, Estour M, Mouren MC, Guelfi JD, Rouillon F, Gorwood P, Epelbaum J (2007) 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 32:106–113

85. Farooqi IS, Yeo GS, Keogh JM, Aminian S, Jebb SA, Butler G, Viskitkosky T, Hinney A, van Elburg AA, van Goozen SH, Sandkuijl LA, Sinke RJ, Herpertz-Dahlmann BM, Hebebrand J, Remschmidt H, van Engeland H, Adan RA (2001) Association between an agouti-related protein gene polymorphism and anorexia nervosa. Mol Psychiatry 6:325–328

86. Hinney A, Bornscheuer A, Depenbusch M, Mierke B, Tölle A, Middeke K, Ziegler A, Roth H, Gerber G, Zamzow K, Ballauf A, Hamann A, Mayer H, Siegfried W, Lehmkuhl G, Pousta F, Schmidt MH, Hermann H, Herpertz-Dahlmann BM, Fichter M, Remschmidt H, Hebebrand J (1998) No evidence for involvement of the leptin gene in anorexia nervosa, bulimia nervosa, underweight or early onset extreme obesity: identification of two novel mutations in the coding sequence and a novel polymorphism in the leptin gene linked upstream region. Mol Psychiatry 3:539–543

87. Hinney A, Hohmann S, Geller F, Reichwald K, Vogel C, Hess C, Gudermann T, Biber HM, Dierich P, Wichmann HE, Scherag A, Nguyen TT, Schlumberger P, Rief W, Herpertz S, Schäfer H (1999) Prevalence, spectrum, and functional characterization of melanocortin-4 receptor gene mutations in a representative population-based sample and obese adults from Germany. J Clin Endocrinol Metab 91:1761–1769

88. Hinney A, Hohmann S, Geller F, Vogel C, Hess C, Wermter AK, Brokamp B, Goldschmidt H, Siegfried W, Remschmidt H, Schäfer H, Fichter M, Remschmidt H, Hebebrand J (1999) Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. J Clin Endocrinol Metab 84:4258–4267

89. Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguet P (2000) Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. J Clin Invest 106:253–262

90. Vaisse C, Clement K, Guy-Grand B, Froguet P (1998) A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat Genet 20:113–114

91. Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O’Rahilly S (1998) A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat Genet 20:111–112

92. Fairburn CG, Welch SL, Doll HA, Davies BA, O’Connor ME (1997) Risk factors for bulimia nervosa. A community-based case-control study. Arch Gen Psychiatry 54:509–517

93. Hebebrand J, Fichter M, Gerber G, Görg T, Hermann H, Geller F, Schäfer H, Remschmidt H, Hinney A (2002) Genetic predisposition to obesity in bulimia nervosa: a mutation screen of the melanocortin-4 receptor gene. Mol Psychiatry 7:647–651

94. Branson R, Potoczna N, Král JG, Lentes KU, Hoehe MR, Horber FF (2003) Binge eating as a major phenotype of melanocortin 4 receptor gene mutations. N Engl J Med 348:1096–1103

95. Geller F, Reichwald K, Dempflle A, Illig T, Vollmert C, Herpertz S, Siffert W, Platzer M, Hess C, Gudermann T, Biber HM, Dierich P, Wichmann HE, Schäfer H, Hinney A, Hebebrand J (2004) Melanocortin-4 receptor gene variant I103 is negatively associated with obesity. Am J Hum Genet 74:572–581

96. Potoczna N, Branson R, Kral JG, Piec G, Steffen R, Ricklin T, Hoehe MR, Lentes KU, Horber FF (2004) Gene variants and binge eating as predictors of comorbidity and outcome in severe obesity. J Gastrointest Surg 8:971–981 (discussion 981–982)

97. Young EH, Wareham NJ, Farooqi S, Hinney A, Hebebrand J, Scherag A, O’Rahilly S, Barroso I, Sandhu MS (2007) The V103I polymorphism of the MC4R gene and obesity: population based studies and meta-analysis of 29 563 individuals. Int J Obes (Lond) 31:1437–1441

98. Herpertz S, Siffert W, Hebebrand J (2003) Binge eating as a phenotype of melanocortin 4 receptor gene mutations. N Engl J Med 349:606–609 (author reply 606–609)

99. Hebebrand J, Geller F, Dempflle A, Heinzl-Gutenbrunner M, Raab M, Gerber G, Wermter AK, Horro FR, Blundell J, Schäfer H, Remschmidt H, Herpertz S, Hinney A (2004) Binge-eating episodes are not characteristic of carriers of melanocortin-4 receptor gene mutations. Mol Psychiatry 9:796–800

100. Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, Tecott LH, Reichardt LF (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 6:736–742

101. Monteleone P, Fabrazzo M, Martiades V, Serritella C, Pannuto M, Maj M (2005) Circulating brain-derived neurotrophic factor is decreased in women with anorexia and bulimia nervosa but not in women with binge-eating disorder: relationships to comorbid depression, psychopathology and hormonal variables. Psychol Med 35:897–905

102. Saito S, Watanabe K, Hashimoto E, Saito T (2009) Low serum brain-derived neurotrophic factor (BDNF) and food intake regulation: a possible new explanation of the pathophysiology of eating disorders. Prog Neuropsychopharmacol Biol Psychiatry 33:312–316

103. Mercader JM, Fernández-Aranda F, Gratacos M, Ribasés M, Badia A, Villarejo C, Solano R, González JR, Vallejo J, Estivill X (2003) Binge eating as predictors of comorbidity and outcome of anorexia nervosa patients. Neuropsychobiology 48:312–316

104. Ehrlich S, Salbach-Andræ H, Eckart S, Merle JV, Burghardt R, Pfeiffer E, Franke L, Uebelhack R, Lehmkuhl U, Hellweg R (2009) Serum brain-derived neurotrophic factor and peripheral indicators of the serotonin system in underweight and weight-recovered adolescent girls and women with anorexia nervosa. J Psychiatry Neurosci 34:323–329

105. Ribasés M, Gratacos M, Fernández-Aranda F, Bellodi L, Boni C, Anderluh M, Cavallini MC, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Wagner G, Treasure J,
Collier DA, Estivill X (2004) Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations. Hum Mol Genet 13:1205–1212

107. Dmitrzak-Weglarz M, Skibinska M, Slopian A, Szczepankiewicz A, Rybakowski F, Kramer L, Hauser J, Rajewski A (2007) BDNF Met66 allele is associated with anorexia nervosa in the Polish population. Psychiatr Genet 17:245–246

108. Friedel S, Horro FF, Wermter AK, Geller F, Dempfle A, Reichwald K, Smidt J, Brönner G, Konrad K, Herpertz-Dahlmann B, Warnke A, Hemminger U, Linder M, Kiefl H, Goldschmidt HD, Siegfried W, Remschmidt H, Hinney A, Hebebrand J (2005) Mutation screen of the brain derived neurotrophic factor gene (BDNF): identification of several genetic variants and association studies in patients with obesity, eating disorders, and attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 132B:96–99

109. Rybakowski F, Dmitrzak-Weglarz M, Szczepankiewicz A, Skibinska M, Slopian A, Rajewski A, Hauser J (2007) Brain derived neurotrophic factor gene Val66Met and −270C/T polymorphisms and personality traits predisposing to anorexia nervosa. Neuro Endocrinol Lett 28:153–158

110. Mercader JM, Saus E, Aguera Z, Bayes M, Brönner G, Müller A, Bayés M, Boni C, Carreras A, Rybakowski F, Dmitrzak-Weglarz M, Szczepankiewicz A, Friedel S, Horro FF, Wermter AK, Geller F, Dempfle A, Dmitrzak-Weglarz M, Skibinska M, Slopien A, Szczepankiewicz A, Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RD, Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Nercessian A, Cellini E, de Cid R, Dierssen M, Escaramis G, Fernández-Arandea F, Forcano L, Gallego X, González JR, Gorwood P, Hebebrand J, Hinney A, Naemicias B, Puig A, Riabás M, Ricca V, Romeo L, Sorbi S, Versini A, Gratacos M, Estivill X (2008) Association of NTRK3 and its interaction with NGF suggest an altered cross-regulation of the neurotrophin signaling pathway in eating disorders. Hum Mol Genet 17:1234–1244

111. Riabás M, Gratacos M, Badia A, Jimenez L, Solano R, Vallejo J, Fernandez-Arandea F, Estivill X (2005) Contribution of NTRK2 to the genetic susceptibility to anorexia nervosa, harm avoidance and minimum body mass index. Mol Psychiatry 10:851–860

112. Di Marzo V, Bifulco M, De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 3(9):771–784

113. Müller TD, Reichwald K, Brönner G, Kirschner J, Nguyen TT, Scherag A, Herzog W, Herpertz-Dahlmann B, Lithner P, Meitinger T, Platzter M, Schäfer H, Hebebrand J, Hinney A (2008) Lack of association of genetic variants in genes of the endocannabinoid system with anorexia nervosa. Child Adolesc Psychiatry Ment Health 2:33

114. Riabas M, Gratacos M, Hernández-Español F, Bellodi L, Boni C, Andelruth M, Cristina Avalloni M, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel J, Naemicias B, Remschmidt H, Ricca V, Sorbi S, Tomori M, Wagner G, Treasure J, Collier DA, Estivill X (2005) 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 13:428–434

115. Thorleifsson G, Grice DE, Hálmsdóttir B, Hálmsdóttir L, Igelman M, Lindgren CM, Heid IM, Berndt S, Elliott A, Jackson AU, Laming C, Lettge G, Lim N, Lyon HN, McCarthy SA, Padakadis K, Qi L, Randall J, Roccasecca RM, Samra S, Scheet P, Weedon MN, Wheeler E, Zhao JH, Jacobs LC, Prokopenko I, Soranzo N, Tanaka K, Timpson NJ, Almgren P, Bennett A, Bergman RN, Bingham SA, Bonnycaust LL, Brown M, Burtt NP, Chines P, Collin S, Connell JM, Cooper C, Smith GD, Dennison EM, Deodhar P, Elliott P, Erdos MR, Estrada K, Evans DM, Gianniny L, Gieger C, Gillson CJ, Guiducci C, Hackett R, Hadley D, Hall AS, Havulinna AS, Hebebrand J, Hofman A, Isomaa B, Jacobs KB, Johnson T, Jousilahti P, Jovanovic Z, Khaw KT, Kraft P, Kuokkanen M, Kuusisto J, Laitinen J, Lakatta EG, Luan J, Luben RN, Mangino M, McArdle WL, Meitinger T, Mulas A, Munroe PB, Nairu S, Ness AR, Northstone K, O’Rahilly S, Purmann C, Rees MG, Riddler M, Ring SM, Rivadeneira F, Ruskonien A, Sandbu MS, Saramies J, Scott LJ, Scuteri A, Silander K, Sims MA, Song K, Stephens J, Stevens S, Stringham HM, Tung YC, Valle TT, Van Duijn CM, Viikmae J, Watanabe RM, Waterworth DM, Watkins N, Wellcome Trust Case Control Consortium, Witteman JC, Zeggini E, Zhai G, Zillikens MC, Altshuler D, Caulfield MJ, Chanock SJ, Farooqi IS, Ferrucci L, Guralnik JM, Hattersley AT, Hu FB, Jarvelin MR, Laakso M, Mooser V, Ong K, Ouwehand WH, Salomaa V, Samani NJ, Spector TD, Tuomi T, Tuomilehto J, Uda M, Uitterlinden AG, Wareham NJ, Deloukas P, Frayling TM, Group LC, Hayes RB, Hunter DJ, Mohlke KL, Peeton L, Schlessinger D, Strachan DP, Wichmann HE, McCarthy MI, Boehnke M, Barroso I, Abecasis GR, Hirschhorn JN, Genetic Investigation of ANthropometric Traits (GIANT) Consortium. Six novel loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41:25–34

116. Brandys MK, van Elburg AA, Loos RJ, Bauer F, Hendriks J, van der Schouw YT, Adan RA (2009) Are recently identified genetic variants regulating BMI in the general population associated with anorexia nervosa? Am J Med Genet B Neuropsychiatr Genet [Epub ahead of print]

117. Kaye WH, Lilenfeld LR, Berrettini WH, Strober M, Devlin B, Klump KL, Goldman D, Bulik CM, Halmi KA, Fichter MM, Kaplan A, Woodside DB, Treasure J, Plotnicov KH, Pollice C, Rao R, McConaha CW (2000) A search for susceptibility loci for anorexia nervosa incorporating behavioral covariates. Hum Mol Genet 9:2701–2709

118. Kaye WH, Berrettini WH (2002) Evidence for a susceptibility gene for anorexia nervosa on chromosome 1. Am J Hum Genet 70:787–792

119. Deavin B, Bacanu SA, Klump KL, Bulik CM, Fichter MM, Halmi KA, Kaplan A, Strober M, Treasure J, Woodside DB, Berrettini WH, Kaye WH (2002) Linkage analysis of anorexia nervosa incorporating behavioral covariates. Hum Mol Genet 11:689–696
153. Fumeron F, Betoulle D, Aubert R, Herbeth B, Siest G, Rigaud D (2001) Association of a functional 5-HT transporter gene polymorphism with anorexia nervosa and food intake. Mol Psychiatry 6:9–10

154. Levitan RD, Kaplan AS, Masellis M, Basile VS, Walker ML, Lipson N, Siegel GI, Woodside DB, Macciardi FM, Kennedy SH, Kennedy JL (2001) Polymorphism of the serotonin 5-HT1B receptor gene (HTR1B) associated with minimum lifetime body mass index in women with bulimia nervosa. Biol Psychiatry 50:640–643

155. Hinney A, Herrmann H, Löhr T, Rosenkranz K, Ziegler A, Lehmkühl G, Poustå F, Schmidt MH, Mayer H, Siegfried W, Remschmidt H, Hebebrand J (1999) No evidence for an involvement of alleles of polymorphisms in the serotonin1DBeta and 7 receptor genes in obesity, underweight or anorexia nervosa. Int J Obes Relat Metab Disord 23:760–763

156. Hinney A, Ziegler A, Nöthen MM, Remschmidt H, Hebebrand J (1997) 5-HT2A receptor gene polymorphisms, anorexia nervosa, and obesity. Lancet 350:1324–1325

157. Ricca V, Nacmias B, Cellini E, Di Bernardo M, Rotella CM, Sorbi S (2002) 5-HT2A receptor gene polymorphism and eating disorders. Neurosci Lett 323:105–108

158. Hammer C, Kapeller J, Endele M, Fischer C, Hebebrand J, Hinney A, Friedel S, Grataçós M, Estivill X, Fichter M, Fernández-Aranda F, Ehrlich S, Rappold G, Niesler B (2009) Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders. Pharmacogenet Genomics [Epub ahead of print]

159. Hinney A, Becker I, Heibüttl O, Nottebom K, Schmidt A, Ziegler A, Mayer H, Siegfried W, Remschmidt H, Hebebrand J (1998) 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 83:3737–3741