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Candidate gene polymorphisms in eating disorders

Anke Hinney *, Helmut Remschmidt, Johannes Hebebrand

Clinical Research Group, Department of Child and Adolescent Psychiatry, Philipps-University of Marburg, Hans-Sachs-Str. 6, D-35033 Marburg, Germany

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Abstract

Anorexia nervosa and bulimia nervosa are complex disorders characterized by disordered eating behaviour. Attitudes towards weight and shape as well as the perception of body shape are disturbed. A substantial genetic influence on these disorders has been suggested by formal genetic studies. Obsessive-compulsive behaviour, perfectionism and anxious personality traits seem to occur premorbidly in several patients. Disturbances of neurotransmitter, neuropeptide and neuroendocrine systems have been reported in acutely ill and followed-up patients. Hence, these systems might be involved in the etiology of these eating disorders.

Genetic studies on candidate genes have mainly focussed on the serotonergic system and on genes involved in body weight regulation. Up to now, polymorphisms and variations in various genes (e.g. genes for 5-HT receptors, leptin gene, melanocortin MC_4 receptor gene) have been assessed for association and transmission disequilibrium pertaining to anorexia nervosa and/or bulimia nervosa. Most of the studies yielded negative results. Four studies of a polymorphism (−1438 G/A) within the promoter of the 5-HT_{2A} gene (*5-HT$_{2A}$*) revealed an association of the A-allele to anorexia nervosa. However, three studies could not confirm this result. Furthermore, a meta-analysis did not support the positive association. Currently, combined efforts within the European Union will answer the question of whether or not the A-allele is involved in the predisposition to anorexia nervosa. A transmission disequilibrium test is being performed in about 300 trios consisting of a patient with anorexia nervosa and both parents. As candidate gene approaches did not unequivocally identify susceptibility genes (alleles) for anorexia nervosa or bulimia nervosa, systematic model-free genome-wide screenings should also be performed in order to identify currently unknown genes involved in eating disorders. This kind of approach has already been initiated for anorexia nervosa. Genetic research on eating disorders will hopefully lead to new pharmacological treatment strategies.

Keywords: 5-HT_{2A} receptor; Transmission disequilibrium; Association; Genome screening; Dopamine; Estrogen β receptor; Leptin

1. Formal genetic aspects

Recently, twin studies pertaining to eating disorders have been summarized and evaluated in order to delineate etiological factors in these disorders (Bulik et al., 2000). Twin studies can be powerful tools. However, their methodology can be complicated and their results can easily be misjudged. Caveats regarding twin studies primarily address the ascertainment and the statistical power. If a clinical sample is used for the twin study, one has to be aware of the greater severity of the illness in the clinical population; hence, the genetic loading might be different from that of the patients from the general population. Bulik et al. (2000) also pointed out that factors like zygosity, an equal environment assumption (similar exposure to environmental influences that are relevant to the analysed phenotype), as well as the generalizability of results (are twins comparable to singletons with respect to the phenotype studied?) need to be considered.

1.1. Anorexia nervosa

Evidence from family and twin studies suggests a genetic contribution to the etiology of anorexia nervosa (Hebebrand and Remschmidt, 1995; Kipman et al., 1999; Bulik et al., 2000). Holland et al. (1984) showed proband-wise concordance rates for anorexia nervosa 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 (Holland et al., 1988). Most twin studies have shown a higher concordance rate for monozygotic twins (approximately 0.44) than for dizygotic twins (approximately 0.13).

* Corresponding author. Tel.: +49-6421-2865361; fax: +49-6421-2863056.

E-mail address: hinneya@post.med.uni-marburg.de (A. Hinney).

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This difference implies that genetic factors, more so than shared familial environment, may explain why anorexia nervosa runs in families (Kipman et al., 1999). However, Walters and Kendler (1995) could not detect a genetic component to anorexia nervosa. They analysed a large epidemiological female twin sample ($n = 2163$), but with a rather small number of twins with retrospectively diagnosed anorexia nervosa. Interestingly, co-twins of twins with anorexia nervosa had significantly lower body mass index (BMI in kg/m^2) and higher depression rates than co-twins of unaffected twins. In their review of all studies, Bulik et al. (2000) stated that it was impossible to draw firm conclusions regarding the precise contribution of genetic and environmental factors to anorexia nervosa.

Controlled family studies found an average 3% lifetime risk of anorexia nervosa in first-degree relatives of patients compared to 0% in about 1000 relatives of control subjects (Gershon et al., 1984; Strober, 1991; Kaye et al., 2000b). This suggests an approximate relative risk of at least 10. It was assumed to be highly unlikely that a single gene is responsible for mediating this relative risk. Hence, it was expected that any single anorexia nervosa susceptibility locus increases the relative risk by a small factor, probably between one- and two-fold (Kaye et al., 2000b). Heritability estimates of 0.72 were calculated based on six controlled family studies, and estimates of 0.71 were given for 59 published twin studies (reviewed by Kipman et al., 1999).

Strober et al. (2000) recently published the results of a substantial family study (504 probands and 1831 of their relatives) pertaining to eating disorders. Anorexia nervosa was found to be rare in families, whereas full and partial syndromes aggregated in both anorexic and bulimic probands. Relative risks were 11.3 and 12.3 for the full syndrome of anorexia nervosa in first-degree female relatives of patients with anorexia nervosa and bulimia nervosa, respectively (Strober et al., 2000). In family studies, anorexia nervosa has been observed in both maternal and paternal relatives, thus seemingly indicating autosomal inheritance with a strongly reduced penetrance in males. Currently, there is no indication of a Carter effect, because female relatives of affected males have not been shown to have an increased risk compared to relatives of affected females. However, to our knowledge, this aspect has not been analysed systematically. Adoption studies for anorexia nervosa are lacking.

1.2. Bulimia nervosa

A genetic contribution to bulimia nervosa was revealed by twin studies. Significant contributions of both additive genetic effects (mean: 0.28–0.83) and of unique environmental factors (mean: 0.17–0.68) to liability to bulimia nervosa were calculated (reviewed in Bulik et al., 2000). The magnitude of the contribution of shared environment was less clear. However, in studies with the greatest statistical power, shared environment appeared to be less prominent than additive genetic factors (Bulik et al., 2000). Overlapping of the genetic predisposition to both bulimia nervosa and depression was found in the Virginia twin study (Walters et al., 1992). A recent family study (Strober et al., 2000) found that bulimia nervosa aggregated in families of diseased probands. The relative risks were 4.2 and 4.4 for first-degree female relatives of patients with anorexia nervosa and bulimia nervosa, respectively. These observations are consistent with findings from comparable previous studies (Strober et al., 2000). Adoption studies for bulimia nervosa are also lacking.

2. Statistical methods

Analysis of the genetic contribution to a complex phenotype generally involves three statistical procedures (i) association studies ('case-control-studies'), (ii) transmission disequilibrium tests and (iii) linkage studies.

Association studies measure the difference in allele frequency between patients (cases) and controls. Patients (cases) and controls have to be matched carefully for association studies, ethnicity being the most important. If both groups vary in more than the analysed parameter, a positive association could be due to the confounder. Anorexia nervosa and bulimia nervosa are complex diseases that are commonly defined by either the 'Diagnostic and Statistical Manual of Mental Disorders' criteria (DSM, American Psychiatric Association, 1994) or the 'International Classification of Mental and Behavioural Disorders' criteria (ICD, World Health Organization, 1992). Subtype, onset and severity of the disease can be used for association studies, to subcategorize the phenotype. However, if too many parameters are used to subdifferentiate eating disorders, the number of individuals in each group decreases substantially. Hence, power decreases as well. Furthermore, multiple comparisons will be required, so that appropriate statistical correction needs to be applied, again implying a reduction in power.

It is difficult to define an optimal control group for association studies in eating disorders. Exclusion of individuals who report a lifetime occurrence of an eating disorder appears to be a reasonable requirement. Ideally, preferentially female controls should have passed the critical manifestation age. A more accurate approach would entail exclusion of individuals with subclinical eating disorders, high anxiousness, obsessive-compulsive features and high depression scores. Large weight fluctuations, particularly during adolescence, could also be viewed as suboptimal aspects. Evidently, such an approach is cumbersome. Because of the low prevalence of eating disorders in the general population, some scientists propose the use of unscreened control populations.

It is unclear whether or not controls should be selected for body weight. Obese individuals differ greatly in weight

from patients with anorexia nervosa. Like patients with bulimia nervosa, obese controls might have binge eating episodes. Healthy underweight individuals might be considered as optimal controls for patients with anorexia nervosa, as they have only a slightly higher weight and thus differ mainly in psychopathology. Alternatively, in anorexia nervosa the controls' weights might not be important at all. Thus, premorbid body weights of patients have been shown to cover the entire weight range (Coners et al., 1999). In bulimia nervosa, premorbid obesity evidently occurs more frequently than can be expected by chance. Thus, in theory, in order to avoid the detection of obesity genes, an ideal control group should be matched for premorbid BMI. These brief considerations, pertaining to both psychopathology and weight, show the uncertainties inherent to the matching procedure.

Stratification problems inherent to association studies can be circumvented by use of the *transmission disequilibrium test* (TDT; Spielman et al., 1993). The transmission disequilibrium test uses family trios consisting of an index patient and both parents. The nontransmitted parental alleles serve as internal controls. The test requires heterozygous parents. The frequency of parental transmission of a given allele to the diseased child is compared to the frequency of its nontransmission. If the analysed allele is transmitted more often than expected by chance, this allele (or an allele in close proximity) is thought to predispose to the disease; if transmission is less frequent than expected, the allele seems to be protective (Spielman et al., 1993). The transmission disequilibrium test measures linkage in the presence of association. It is simple and powerful and takes genetic heterogeneity into account (Risch and Merikangas, 1996).

In *linkage analyses* specific markers are analysed within families affected by a particular disease. It is investigated whether a specific marker allele is shared (not shared) more often by the affected (unaffected) family members than expected by chance. Microsatellite markers that comprise simple repeat units (e.g. CA–CA–CA–CA–CA) are commonly used for these studies. It was recently suggested that whole-genome association studies using approximately 30,000 single nucleotide polymorphisms could be more efficient to capture disease genes (Abbott, 2000).

The standard scale for linkage studies is the LOD score, where LOD is defined as the logarithm ($\log_{10}$) of the likelihood ratio for linkage vs. nonlinkage. In complex disorders, a LOD score > 3.3 has been proposed as threshold for a positive linkage result (Lander and Kruglyak, 1995). If linkage between marker and disease is determined, the surrounding chromosomal region is thoroughly screened for candidate genes.

However, in the case of complex diseases, which involve many genes, association and linkage studies might have restricted power (Risch, 2000; Risch and Merikangas, 1996). Hence, if a mutation confers only a small effect to predisposition to the disease, individuals carrying this allele will have only a slightly elevated risk of developing the disorder.

It is generally agreed that sample sizes, both for association and linkage studies, need to be quite large to be allow detection of an effect of a specific allele on a complex phenotype. Currently, unknown genes can be identified by linkage studies, such as systematic model-free genome-wide screenings (Risch, 2000; Risch and Merikangas, 1996). Anorexia nervosa and bulimia nervosa are complex disorders with non-Mendelian inheritance. The use of an affected relative pair linkage approach has been assumed to be most efficient for anorexia nervosa. A large sample has already been ascertained (Kaye et al., 2000b).

3. Candidate gene approach

The candidate gene approach relies on genetic, physiological, biochemical or pharmacological evidence to show the involvement of a specific gene in the phenotype under consideration. Mutations, variations and polymorphisms affecting either the protein structure or expression appear of special interest.

Several lines of evidence raise the possibility that disturbances within the serotonergic (Kaye et al., 1990, 1998), dopaminergic (Kaye et al., 1999) or leptinergic system (Hebebrand et al., 1995, 1997; Frey et al., 2000) are implicated in the etiology of anorexia nervosa and bulimia nervosa, respectively. Any neurochemical or neurobiological disturbances that persist even after long-term recovery might be trait-related and implicated in the etiology of the disorder. Studies pertaining to long-term follow-up patients with anorexia nervosa showed that disturbances of monoaminergic pathways and weight regulation continue after recovery (Kaye et al., 1990; 1998; Hebebrand et al., 1997; Frey et al., 2000). Based on results of these studies, genes involved in the serotonergic and dopaminergic systems and in weight regulation can be perceived as candidate genes. Because psychopathological features and extremely low body weight are inseparable in anorexia nervosa, anorexia nervosa might be considered as an extreme weight condition (Hebebrand and Remschmidt, 1995). Weight regulation is also disturbed in several patients with bulimia nervosa.

Assessment of a candidate gene can additionally be based on the following clinical considerations: (i) The prevalence of anorexia nervosa and bulimia nervosa is considerably higher in females. (ii) The manifestation periods for both anorexia nervosa and bulimia nervosa are predominantly in puberty, late adolescence and early adulthood. (iii) Obsessive-compulsive behaviour is frequent in anorexia nervosa (Kaye and Strober, 1999; Kaye et al., 2000a). In general terms, the narrower the pathways into the respective eating disorder, the fewer genes are likely to be involved. An example of such a narrow pathway into anorexia nervosa is the dysregulation of maintenance of a

normal body weight upon weight loss, for whatever reason, during the critical age period. Thus, anorexia nervosa can set in not only after initiation of dieting, but also after a period of restricted energy intake, for example following an infection or an operation (Wakeling, 1985). If, however, several different pathways can lead to the eating disorder, the greater the heterogeneity and the smaller the effect of a predisposing allele is likely to be. If the genetic predisposition indeed encompasses several alleles each of which is associated with a relative risk < 2, it is doubtful that these findings will have an impact on clinical practice.

3.1. Neurotransmitters: serotonergic system

Serotonin (5-hydroxytryptamine; 5-HT) is involved in a broad range of biological, physiological and behavioural functions, such as motor activity, eating, mood, sleep, sex drive, thermoregulation, cardiovascular and respiratory activity (Blundell, 1992; Blundell et al., 1995; Simansky, 1996; Halford and Blundell, 2000).

Several lines of evidence implicate the serotonergic system in body weight regulation and more specifically in eating behaviour. Drugs that either directly or indirectly increase postsynaptic serotonergic stimulation routinely decrease hunger and the consumption of food in both rodents and humans (Blundell, 1992; Blundell et al., 1995; Simansky, 1996; Wurtman and Wurtman, 1996; Halford and Blundell, 2000). Vice versa, decreased serotonergic transmission appears to result in hyperphagia.

Knockout mice for the X-chromosomal 5-HT_{2C} receptor have a significantly elevated (13%) body weight compared with that of wild-type siblings (Tecott et al., 1995). Extensive serotonergic hyperinnervation has been detected in the central nervous system of the lethal neurodevelopmental mouse mutant *Anorexia* (*anx/anx*). The underly-

Table 1
Summary of association and transmission disequilibrium tests (TDT) pertaining to polymorphisms in different candidate genes in patients with anorexia nervosa (AN) or bulimia nervosa (BN)

Candidate gene	Analysed polymorphism or genetic marker	*p*-Values for association tests (or TDT) of the rare allele to AN	*p*-Values for association tests (or TDT) of the rare allele to BN	Reference(s)
β_3-adrenoceptor	Trp-64-Arg	NS (NS)	ND	Hinney et al. (1997b)
Dopamine D4 receptor	13 bp deletion	NS	ND	Hinney et al. (1999c)
	48 bp repeat	NS (NS)	ND	
Dopamine D3 receptor	*Bal*I polymorphism in exon 1	NS	ND	Bruins-Slot et al. (1998)
Estrogen β-receptor	1082G/A (silent)	0.04 nominal	NS	Rosenkranz et al. (1998a)
	1730A/G (silent)	NS	NS	
Leptin	−1387G/A (promoter)	NS (NS)[a]	NS[a]	Hinney et al. (1998b)[a]
Melanocortin MC_4 receptor	Val-103-Ile	NS	NS	Hinney et al. (1999b)
receptor	Ile-251-Thr	NS	NS	
Neuropeptid Y Y_1 receptor	*Pst*I-polymorphism within the first intron	NS	ND	Rosenkranz et al. (1998b)
Neuropeptid Y Y_5 receptor	1333G/A (silent)	NS	ND	Rosenkranz et al. (1998b)
Pro-opiomelanocortin	Insertion of 9 bp between codon 73 and 74	NS	ND	Hinney et al. (1998a)
5-$HT_{1D\beta}$ receptor	Phe-124-Cys	NS	ND	Hinney et al. (1999a)
5-HT_{2A} receptor	−1438G/A (promoter)	See Table 2	See Table 3	
	Thr-25-Asn	NS[a,b]	NS[b]	
	102T/C (silent)	NS[b]	NS[b]	
	516C/T (silent)	NS[b]	NS[b]	
	His-452-Tyr	NS[a,b]	NS[b]	
5-HT_{2C} receptor	Cys-23-Ser	NS[a,b,c]	NS[a,b]	Hinney et al. (1997c)[a], Nacmias et al. (1999)[b], Nacmias et al. (1999)[a] Burnet et al. (1999)[b], Hinney et al., unpublished data[c]
5-HT_7 receptor	Pro-279-Leu	NS	ND	Hinney et al. (1999a)
Serotonin transporter (SERT)	44 bp Del/Ins (promoter)	NS[a,b,c] (NS)[a]	< 0.000[b]	Hinney et al. (1997a),[a] Di Bella et al. (2000),[b] Sundaramurthy et al. (2000)[c]
Tryptophan hydroxylase	1095T/C (silent)	NS	ND	Han et al. (1999)
Uncoupling protein 2,3	Flanking microsatellite markers	S for certain alleles	ND	Campbell et al. (1999)

AN: Anorexia nervosa, BN: Bulimia nervosa; TDT: transmission disequilibrium test; S: significant; NS: non significant; ND: not determined.
[a] Refers to the references in the same row.
[b] Refers to the references in the same row.
[c] Refers to the references in the same row.

ing autosomal recessive mutation leads to starvation of preweanlings (Son et al., 1994).

The serotonergic system has been implicated in the development of eating disorders (Jimerson et al., 1992; Brewerton and Jimerson, 1996). In long-term weight-restored patients with anorexia nervosa or bulimia nervosa, 5-hydroxyindolacetic acid (5-HIAA) levels were elevated in cerebrospinal fluid in comparison with those of controls, suggesting that hyperserotonergic function is a trait marker in eating disorders (Kaye et al., 1990, 1998). The increased brain serotonin activity might pathophysiologically predispose to the development of eating disorders. In addition, increased serotonergic neurotransmission could account for characteristic psychopathological features, such as perfectionism, rigidity and obsessiveness frequently associated with anorexia nervosa (Kaye and Strober, 1999; Kaye et al., 2000a). The serotonergic system is regulated by tryptophan hydroxylase, the 5-HT transporter (SERT) and by several 5-HT receptors.

3.1.1. Serotonin transporter gene

The gene for the serotonin transport protein is expressed both peripherally and in the brain (Lesch et al., 1993; Ramamoorty et al., 1993). The key regulator of serotonergic transmission in the brain is the reuptake of extracellular serotonin by the SERT (Amara and Kuhar, 1993). Food restriction in rats is associated with a reduction in the density of the cortical serotonin transporters with unchanged transporter affinity (Zhou and Palmiter, 1995).

A common polymorphism upstream of the SERT coding region (long and short allele) was detected and was shown to confer different transcriptional efficiencies (Heils et al., 1996; Lesch et al., 1996). Cells homozygous for the long allele produced higher concentrations of SERT mRNA than did cells containing one or two copies of the short allele (Lesch et al., 1996), implying a dominant effect of the short allele. Although this polymorphism could have an effect on serotonin availability in vivo, association and transmission disequilibrium tests pertaining to this polymorphisms yielded negative results for anorexia nervosa (Table 1; Hinney et al., 1997a; Di Bella et al., 2000; Sundaramurthy et al., 2000). Di Bella et al. (2000) described significant differences in the distribution of both the alleles and the genotypes between patients with bulimia nervosa (N = 50) and controls (N = 120). Subjects bearing one or two copies of the short allele showed a seven-fold increased risk of bulimia (Di Bella et al., 2000). However, the authors also detected an unexplainable deviation from Hardy-Weinberg equilibrium in patients with bulimia nervosa. The positive association needs to be confirmed in an independent and larger sample. The use of a transmission disequilibrium test should be considered.

3.1.2. 5-HT receptors

Polymorphisms within different 5-HT receptor genes and the tryptophan hydroxylase gene have also been analysed. Results of studies that did not show an association to an eating disorder are summarized in Table 1.

Currently, four studio have described an association between the −1438 A-allele of the −1438 G/A polymorphism within the promoter region of the 5-HT_{2A} receptor gene and anorexia nervosa (Table 2; Collier et al., 1997; Enoch et al., 1998; Sorbi et al., 1998; Nacmias et al., 1999). However, three other studies could not confirm this association (Table 2; Hinney et al., 1997c; Campbell et al., 1998; Ziegler et al., 1999).

Two of the four studies with positive results (Sorbi et al., 1998; Nacmias et al., 1999) reported an association between the −1438 A allele and anorexia nervosa of the restricting subtype, whereas allele frequencies in patients

Table 2
Genotype-and allele frequencies of the −1438G/A polymorphism within the promoter region of the 5-HT_{2A} receptor gene in patients with anorexia nervosa and in controls

Study group	*N*	Origin	Genotype-wise			Allele-wise		*p*-Value	Reference
			−1438A/A	−1438G/A	−1438G/G	−1438A	−1438G		
Anorexia nervosa	81	British	25 (0.31)	33 (0.41)	23 (0.28)	83 (0.51)	79 (0.49)	0.02	Collier et al. (1997)
Control	226		34 (0.15)	117 (0.52)	75 (0.33)	185 (0.41)	267 (0.59)		
Anorexia nervosa	100	German	20 (0.20)	39 (0.39)	41 (0.41)	79 (0.40)	121 (0.61)	NS	Hinney et al. (1997c)
Control	355		62 (0.17)	177 (0.50)	116 (0.33)	301 (0.42)	409 (0.58)		
Anorexia nervosa	152	British	39 (0.25)	68 (0.45)	45 (0.30)	146 (0.48)	158 (0.52)	NS	Campbell et al. (1998)
Control	150		30 (0.20)	67 (0.45)	53 (0.35)	127 (0.42)	173 (0.58)		
Anorexia nervosa	77	Italian	23 (0.30)	41 (0.53)	13 (0.17)	87 (0.56)	67 (0.44)	0.005	Sorbi et al. (1998)
Control	107		10 (0.09)	56 (0.52)	41 (0.38)	76 (0.36)	138 (0.64)		
Anorexia nervosa	68	USA	17 (0.25)	35 (0.51)	16 (0.24)	69 (0.51)	67 (0.49)	0.0001	Enoch et al. (1998)
Control	69		6 (0.09)	38 (0.55)	25 (0.36)	50 (0.36)	88 (0.64)		
Anorexia nervosa	78	German	7 (0.09)	39 (0.50)[a]	32 (0.41)[a]	53 (0.34)[a]	103 (0.66)[a]	NS	Ziegler et al. (1999)
Control	170		21 (0.12)	89 (0.52)[a]	60 (0.35)[a]	131 (0.39)[a]	209 (0.61)[a]		
Anorexia nervosa	109	Italian	30 (0.28)	59 (0.54)	20 (0.18)	119 (0.55)	99 (0.45)	0.0001	Nacmias et al. (1999)
Control	107		10 (0.09)	56 (0.52)	41 (0.38)	76 (0.36)	138 (0.64)		

NS: nonsignificant.
[a] Ziegler et al., correction in press.

with the binge eating/purging subtype did not differ from those of the controls. Again, another study could not confirm this result (Ziegler et al., 1999).

Possible explanations for the inconsistent results include genetic heterogeneity and ethnic admixture (Hinney et al., 1997c). Enoch et al. (1998), for instance, analysed groups of patients from Italy and used US controls (not shown in Table 2). As the frequencies for the −1438 A/A-genotype vary from 8.7% in Italy (Enoch et al., 1998) to 20% in England (Campbell et al., 1998), it seems essential to analyse patients and controls of the same ethnic background. Aubert et al. (2000) found that the −1438 A allele was associated with lower energy intake and with a lower alcohol consumption in obese individuals, a finding which they assumed to be consistent with the higher frequency of the A allele in patients with anorexia nervosa.

Inconsistency of the findings made a meta-analysis necessary. No association was detected between the alleles of the −1438 A/G promoter polymorphism and anorexia nervosa (Ziegler et al., 1999; correction in press).

Hinney et al. (1997c) performed a transmission disequilibrium analysis, in addition to the 'case-control' study, of 57 trios consisting of a patient with anorexia nervosa and both parents. Among the limited number of heterozygous parents, no transmission disequilibrium of the alleles of the −1438 A/G promoter polymorphism could be detected. Currently, a combined group within the European Union (consortium sponsored by the EU-framework V 'Factors in healthy eating') is performing a transmission disequilibrium analysis of about 300 trios consisting of a patient with anorexia nervosa and both parents. Results will soon be available.

Association studies pertaining to the −1438 A/G promoter polymorphism and bulimia nervosa have consistently led to negative results (Table 3; Enoch et al., 1998; Nacmias et al., 1999; Ziegler et al., 1999).

Recently, in order to further investigate the 5-HT_{2A} receptor in patients with eating disorders, platelet [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) binding was studied in ten patients with anorexia nervosa, 23 patients with bulimia nervosa and 33 healthy controls. The results indicated that both patients with anorexia nervosa and patients with bulimia nervosa have enhanced 5-HT_{2A} receptor [^{3}H]LSD binding in the acute state (Spigset et al. 1999). However, in this study, the patients were not genotyped for the −1438 A/G promoter polymorphism. Hence, it is not clear how many of the patients with anorexia nervosa harboured the presumed 'anorexia nervosa-susceptibility −1438 A allele'. Additionally, there is no evidence of involvement of the −1438 A allele in bulimia nervosa. Thus, the results of Spigset et al. (1999) cannot readily be compared to those of association studies of the −1438 A/G promoter polymorphism of the 5-HT_{2A} receptor gene and eating disorders. Nevertheless, functional studies pertaining to the −1438 A/G promoter polymorphism appear warranted.

3.2. Neurotransmitters: dopaminergic system

The dopaminergic system has been implicated in the pathophysiology of anorexia nervosa (Barry and Klawans, 1976; Golden and Shenker, 1994; Kaye et al., 1999). Its role in feeding behaviour in general has been established (Terry et al., 1995; Yang et al., 1996). Dopamine-deficient (knock-out) mice become hypoactive and stop feeding soon after birth, indicating that dopamine is essential for movement and feeding (Zhou and Palmiter, 1995). Knock-out mice for the dopamine D4 receptor display locomotor supersensitivity to ethanol, cocaine and methamphetamine and are less active in the open field than normal littermates (Rubinstein et al., 1997). A human proband homozygous for a dopamine D4 receptor null mutation was obese (Nöthen et al., 1994). An additional finding implicating the dopamine D4 receptor in weight regulation is based on pharmacological evidence. Thus, clozapine which binds with a high affinity to the dopamine D4 receptor (Van Tol et al., 1991) can lead to increased food consumption followed by weight gain, both of which are associated with

Table 3
Genotype-and allele frequencies of the −1438G/A polymorphism within the promoter region of the 5-HT_{2A} receptor gene in patients with bulimia nervosa and in controls

Study group	*N*	Origin	Genotype-wise			Allele-wise		*p*-Value	Reference
			−1438A/A	−1438G/A	−1438G/G	−1438A	−1438G		
Bulimia nervosa	22	USA	1 (0.05)	13 (0.59)	8 (0.36)	15 (0.34)	29 (0.66)	NS	Enoch et al. (1998)
Control	69		6 (0.09)	38 (0.55)	25 (0.36)	50 (0.36)	88 (0.64)		
Bulimia nervosa	37	Italian	6 (0.16)	16 (0.43)	15 (0.41)	28 (0.38)	46 (0.62)		
Control	–		–	–	–	–	–		
Bulimia nervosa	99	German	18 (0.18)	55 (0.55)[a]	26 (0.26)[a]	91 (0.46)[a]	107 (0.54)[a]	NS	Ziegler et al. (1999)
Control	170		21 (0.12)	89 (0.52)[a]	60 (0.35)[a]	131 (0.39)[a]	209 (0.61)[a]		
Bulimia nervosa	59	Italian	10 (0.17)	35 (0.59)	14 (0.24)	55 (0.47)	63 (0.53)	NS	Nacmias et al. (1999)
Control	107		10 (0.09)	56 (0.52)	41 (0.38)	76 (0.36)	138 (0.64)		

NS: nonsignificant.
[a] Ziegler et al., correction in press.

an increased leptin secretion (Brömel et al., 1998). However, association studies on the role of polymorphisms in both the dopamine D3 and D4 receptor genes (*DRD3, DRD4*) in anorexia nervosa have yielded negative results (Table 1; Bruins-Slot et al., 1998; Hinney et al., 1999c).

3.3. Hormonal changes during female puberty

The female predominance of anorexia nervosa, bulimia nervosa and extreme obesity (Hebebrand and Remschmidt, 1995) has led to the hypothesis that sex hormones might be involved in the development of weight extremes and in the etiology of eating disorders. This is particularly so for anorexia nervosa, where both the female predominance and the frequent onset around puberty point to a possible involvement of female sex hormones in the development of this eating disorder (Young, 1990). Estrogen levels increase dramatically with the onset of puberty in females. An exaggerated sensitivity of brain structures to the rising estrogen levels possibly mediated by estrogen receptors might represent a predisposition to the development of anorexia nervosa. Similar mechanisms might play a role in the development of other eating disorders.

In rodents and primates, high estrogen levels have an anorectic effect. In ovarectomized rodents the increase in food intake and body weight gain can be reversed by estrogen treatment (Wade and Gray, 1979). These effects are thought to be mediated mainly via several possibly interacting hypothalamic mechanisms (Dagnault and Richard, 1997; Leibowitz et al., 1998). The estrogen β receptor gene (*ERβ*; Mosselman et al., 1996; Enmark et al., 1997) is, among other tissues, highly expressed in brain regions which are involved in weight regulation and energy expenditure (Shughrue et al., 1996; Osterlund et al., 1998).

A systematic mutation screening of the coding region of the *ERβ* revealed that the frequency of the 1082-G allele of a silent 1082 G/A polymorphism was increased in patients with anorexia nervosa compared to that of controls (obese and underweight) and of patients with bulimia nervosa. No evidence for an association between a second polymorphism (1730 A/G) in the *ERβ* and anorexia nervosa was found (Rosenkranz et al., 1998a). In the light of the observation of an association with only one of the two polymorphisms we assumed that the positive association is a chance finding, which does not imply a functional relationship. Nevertheless, an independent attempt to replicate the finding observed with the 1082-G allele should shed more light on the involvement of the *ERβ* in anorexia nervosa (Table 1).

3.4. Weight regulation

There has been a remarkable increase in the number of genetic studies pertaining to the regulation of body weight in the last few years. In principle, the relevant genes could be expressed centrally (e.g. hypothalamus) or peripherally (e.g. adipocytes).

3.4.1. Genes relevant for adipose tissue metabolism and other peripheral mechanisms

3.4.1.1. β3-adrenoceptor. Binding of catecholamines to β-adrenoceptors activates hormone-sensitive lipase, the key enzyme of lipolysis, through cAMP-dependent phosphorylation (Langin et al., 1991). Whereas in rodents expression is found in brown adipose tissue (Nahmias et al., 1991), in humans, the β_3-adrenoceptor is primarily expressed in visceral fat (Krief et al., 1993; Berkowitz et al., 1995).

Three initial studies described an association of the 64Arg allele of a Trp-64-Arg polymorphism within the β_3-adrenoceptor with (i) an increased capacity to gain weight in extremely obese individuals (Clément et al., 1995), (ii) insulin resistance (Widén et al., 1995) and (iii) time of onset of non-insulin dependent diabetes mellitus (Walston et al., 1995).

More than 70 association studies pertaining to this polymorphism and obesity, diabetes or related traits have been published so far. The results are rather conflicting; two meta-analyses even had opposite results (Fujisawa et al., 1998; Allison et al., 1998). Association and transmission disequilibrium tests pertaining to the Trp-64-Arg polymorphism and anorexia nervosa were negative (Table 1; Hinney et al., 1997b).

Uncoupling proteins are proton transport molecules, which can uncouple oxidative phosphorylation from ATP synthesis, resulting in dissipation of energy by production of heat. Uncoupling protein-1 is exclusively present in brown adipocytes (Klaus et al., 1991). The genes encoding for uncoupling protein-2 and uncoupling protein-3 are expressed in numerous tissues (Boss et al., 1997; Fleury et al., 1997; Gimeno et al., 1997; Vidal-Puig et al., 1997). Activation of β_3-adrenoceptor has been shown to increase the expression of uncoupling protein-2 and uncoupling protein-3 (Boss et al., 1999).

According to an as yet unreplicated study (Campbell et al., 1999), the chromosomal region containing the uncoupling protein-2/uncoupling protein-3 gene cluster is implicated in the predisposition to anorexia nervosa upon use of two microsatellite markers. Evidence of association was shown with an allele of one of the markers (D11S911), but not with any allele of the other marker (D11S916). Hence, it was speculated that a mutation within a candidate gene in close vicinity to this marker—possibly the uncoupling protein-2 or the uncoupling protein-3 gene-is in linkage disequilibrium with the respective allele of D11S911. Mice that overexpress the uncoupling protein-3 gene have been produced very recently. These mice are hyperphagic, but weigh less than their wild-type littermates (Clapham et al., 2000). Hence, uncoupling protein-3 might be involved in susceptibility to underweight in humans.

3.4.2. Leptinergic-melanocortinergic system

Cloning of the leptin gene in 1994 (Zhang et al., 1994) has lead to the unravelling of a major regulatory system involved in energy homeostasis. Recessive mutations leading to the absence of leptin or the hypothalamic leptin receptor result in extreme obesity in both humans and rodents (Zhang et al., 1994; Chen et al., 1996; Montague et al., 1997; Clément et al., 1998; Strobel et al., 1998).

Centrally, the leptin signal is transferred to various neurotransmitter systems. The melanocortinergic system is one of the main effectors (reviewed by Salton et al., 2000). At least two melanocortin receptors (melanocortin MC_3 receptor and melanocortin MC_4 receptor) and two classes of hormones are involved in the melanocortinergic system. Pro-opiomelanocortin (POMC) is a prohormone for melanocortins, such as α-melanocyte stimulating hormone (α-MSH) and adrenocorticotropin (ACTH). Melanocortins are melanocortin receptor agonists. In contrast, agouti-related peptide acts antagonistically at melanocortin receptors. Leptin increases the expression of *POMC* and decreases the expression of agouti-related peptide gene (*AGRP*) in the hypothalamic arcuate nucleus (Schwartz et al., 1997; Mizuno and Mobbs, 1999; Mizuno et al., 1998).

3.4.2.1. Leptin. Patients with anorexia nervosa have extremely low plasma levels of leptin (Hebebrand et al., 1995, 1997), thus signalling semi-starvation to the brain. Recently, it was shown that females with a past history of anorexia nervosa (followed up 10 years after in-patient treatment) have a lower percent body fat and a trend to lower serum leptin levels than the controls. It was concluded that body composition differs between long-term followed-up patients with anorexia nervosa and BMI- and gender-matched controls (Frey et al., 2000). A mutation analysis of the coding region and part of the promoter region of the leptin gene in patients with anorexia nervosa has yielded negative results (Hinney et al., 1998b). Hence, an involvement of the leptin gene in the etiology of anorexia nervosa seems unlikely.

3.4.2.2. Pro-opiomelanocortin (POMC). Melanocortins are derived from POMC-containing neurons of the hypothalamic arcuate nucleus (Schwartz et al., 1997; Thornton et al., 1997). Genetic defects within *POMC*, which prevent the production of α-MSH, can result in a monogenic endocrine disorder comprising obesity and adrenal insufficiency in addition to red hair pigmentation (Krude et al., 1998).

In addition to these relevant mutations, several lines of evidence suggest an influence of POMC-derived hormones in weight regulation (Cheung et al.,1997; Schwartz et al., 1997; Thornton et al., 1997). POMC-deficient mice are obese (Yaswen et al., 1999). Ectopic and constitutive expression of the endogenous antagonist at melanocortin receptors, the Agouti protein, is the basis of obesity in lethal-yellow mice (A^y/a, Fan et al., 1997). The respective mutation leads to a defect in POMC signalling in the brain, leading to resistance to the anorexigenic effects of leptin (Boston et al., 1997). Thus, enhancement in production, processing, or responsiveness to α-MSH may be a common feature in underweight. Hypothalamic POMC neurons, stimulated by leptin, may constitute a link between leptin and the melanocortin system (Mizuno et al., 1998). Variations and polymorphisms within *POMC* were not implicated in the etiology of anorexia nervosa or bulimia nervosa (Table 1; Hinney et al., 1998a).

3.4.2.3. Melanocortin MC_4 receptor. Dominant forms of obesity in humans and mice have been described to be conferred by loss of function mutations in *MC_4-R* (Huszar et al., 1997; Vaisse et al., 1998; Yeo et al., 1998; Hinney et al., 1999b; Sina et al., 1999; Farooqi et al., 2000; Vaisse et al., 2000). Inactivation of mouse *MC_3-R* results in increased fat mass, reduced lean body mass and higher feeding efficiency, despite hypophagia and maintenance of normal metabolic rates (Chen et al., 2000). Mice lacking both *MC_3-R* and *MC_4-R* become significantly heavier than *MC_4-R* knock-out mice. Nonredundant roles for these receptors in the regulation of energy homeostasis was the conclusion drawn (Chen et al., 2000). *MC_3R* is co-expressed with *POMC* in hypothalamic neurons of the arcuate nucleus, suggesting that melanocortin MC_3 receptor is an autoreceptor for α-MSH (Bagnol et al.,1999). Mutation screening of the coding region of *MC_4-R* in patients with anorexia nervosa and bulimia nervosa revealed two common polymorphisms in both study groups. Allele and genotype frequencies did not differ between these groups and probands of different weight extremes (Table 1; Hinney et al., 1999b).

3.4.2.4. Neuropeptide Y. Neuropeptide Y is one of the most abundant neuropetides in the brain and modulates numerous physiological processes including control of energy balance, blood pressure and circadian rhythm (Akabayashi et al., 1994; Munglani et al., 1996). These effects are mediated by several distinct neuropeptide Y receptors.

Neuropeptide Y is a potent stimulant of food intake after its administration into the paraventricular nucleus (Stanley and Leibowitz, 1984). It is predominantly expressed in the arcuate nucleus and lateral hypothalamus. Aberrations in the neuropeptide Y signalling pathway are predicted to result in hypertension, eating disorders and anxiety (Grundemar and Hakånson, 1994). Increased levels of neuropeptide Y are found in the cerebrospinal fluid of patients with anorexia nervosa or bulimia nervosa (Kaye et al., 1990) and in the hypothalamus of genetically obese (*ob/ob* mice, *db/db* mice) rodents (Chua et al., 1991; Wilding et al., 1993). Interestingly, knock-out mice for neuropeptide Y were shown to have both normal feeding behaviour and body weight (Erickson et al., 1996a). However, in *ob/ob* mice deficient in neuropeptide Y

($NPY^{-/-}ob/ob$ mice) the obesity syndrome is attenuated, thus implicating neuropeptide Y-containing neural pathways as mediators of hyperphagia, hypometabolism, and endocrine alterations resulting from chronic leptin deficiency (Erickson et al., 1996b).

The neuropeptide Y Y_5 receptor and neuropeptide Y Y_1 receptor in rats and humans are assumed to play a major role in neuropeptide Y-induced food intake (Gerald et al., 1996; Hu et al., 1996; Kushi et al., 1998; Pedrazzini et al., 1998). The neuropeptide Y Y_5 receptor receptor gene ($NPYY_5R$) is expressed in brain regions known to be involved in the central regulation of feeding behaviour, including the lateral hypothalamus, the paraventricular nucleus and the arcuate nucleus (Gerald et al., 1996). Knockout mice for both $NPYY_1R$ (Kushi et al., 1998; Pedrazzini et al., 1998) and $NPYY_5R$ (Marsh et al., 1998) are overweight.

A systematic mutation screen within the coding region of the $NPYY_5R$ revealed a rare (frequency < 1%) Glu-4-Ala variant in a single patient with anorexia nervosa. The allele was transmitted from the mother who had shown no history of an eating disorder as assessed in a psychiatric interview. This observation, in addition to the fact that in this family, a paternal relative also had anorexia nervosa, makes it appear unlikely that the described variant plays a significant role in the genetic predisposition to anorexia nervosa. Association and transmission disequilibrium studies pertaining to variations and polymorphisms within the $NPYY_1R$ and $NPYY_5R$ and anorexia nervosa were negative (Rosenkranz et al., 1998b).

4. Genome-wide screening

Despite major efforts, none of the candidate gene analyses has yielded unequivocal evidence for the involvement of specific alleles in the etiology of eating disorders. Most of the association and transmission disequilibrium tests were negative (Table 1). Single positive findings have not yet been confirmed. It has to kept in mind that molecular genetic studies pertaining to eating disorders have only recently been initiated on a larger scale. Furthermore, the candidate gene approach in anorexia nervosa is hampered by the fact that there is no clear cut evidence implicating a specific regulatory system. A systematic genome-wide approach needs to be applied in order to identify currently unknown susceptibility genes for anorexia nervosa or bulimia nervosa. Such a scan is usually performed by means of closely linked microsatellite markers. Relevant regions can be identified under consideration of both phenotypical information and marker data. For anorexia nervosa, an international, multisite collaborative group (The Price Foundation Collaborative Group; Kaye et al., 2000b) initiated the collection of a large study group of patients with anorexia nervosa and affected relatives. All of the 196 ascertained index patients, mostly of Caucasian origin met DSM-IV criteria for anorexia nervosa; all 237 affected relatives met DSM-IV criteria for anorexia nervosa, bulimia nervosa, or eating disorders not otherwise specified. There were 229 relative pairs informative for linkage analysis; 64% of the proband-relative pairs were anorexia nervosa–anorexia nervosa, 20% were anorexia nervosa–bulimia nervosa, and 16% were anorexia nervosa eating disorders not otherwise specified. A genome-wide screening of this study group, using at least 387 microsatellite markers, is in progress. An affected relative pair study will be used to identify linkage regions (Kaye et al., 2000b).

5. Conclusions and research directions

In forthcoming years, we will hopefully gain a deeper insight into the genetic basis of eating disorders. Analysis of the genetic mechanisms underlying weight regulation is now progressing very rapidly (e.g. Chagnon et al., 2000). Results of this field of genetic research might prove valuable for the analysis of eating disorders. Both sets of results in both fields will be relevant for pharmacological research. The genetic analysis of eating disorders will possibly help to define new drug targets and, therefore, lead to new treatment strategies.

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