Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 480 (2003) 13–20
www.elsevier.com/locate/ejphar

Refinement of behavioural traits in animals for the genetic dissection of eating disorders

Martien J.H. Kas[a,*], Annemarie A. Van Elburg[b], Herman Van Engeland[b], Roger A.H. Adan[a]

[a] *Department of Pharmacology and Anatomy, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands*
[b] *Department of Child and Adolescent Psychiatry, University Medical Centre Utrecht, The Netherlands*

Accepted 25 August 2003

Abstract

Both twin and family studies have revealed the involvement of genetic factors in disorders that affect the regulation of body weight, such as obesity and anorexia nervosa. However, pinpointing the genes that contribute to these human disorders has not yet been very successful. In contrast, genetic studies in animals have been basic for the identification of many genes involved in the regulation of various physiological processes of energy metabolism. We thus plan to review here ways in which findings from animal studies and what is known about behavioural diversity in the human population with eating disorders can be combined. This would probably optimise phenotype-based candidate gene analysis in humans.

Keywords: Anorexia nervosa; Phenotype; Obesity; Genotype; Trait; Hyperactivity; Anxiety

1. Gene hunting in a diverse human eating disorder population

The human population with eating disorders is highly diverse with many phenotypic differences both between and within eating disorders. Anorexia nervosa, for example, is a dramatic neuro-psychiatric disorder characterised by severe and selective restriction of food intake. This eating disorder, with a high prevalence among young adolescent females (15–19 years), results in extreme body weight loss and has a mortality rate of up to 15%. Anorexia nervosa is also characterised by other symptoms which, however, are not seen in the entire anorexia population. Behavioural hyperactivity, for instance, should be considered as an important characteristic of this disease (Bergh and Sodersten, 1996; Brewerton et al., 1995; Davis et al., 1997, 1999; Hebebrand et al., 2003), since it will accelerate body weight loss and has been observed in a large fraction of the anorexia nervosa patient population. Furthermore, most anorexia patients exhibit types of anxiety disorders that can vary greatly between patients (e.g., a general anxiety disorder, social phobia, or panic disorder (Brewerton et al., 1995; Bulik et al., 1997; Godart et al., 2002; Toner et al., 1988). In addition, lifetime compulsion and obsession phenotypes occur in a large sample, but not in all, of the anorexia nervosa population (Halmi et al., 2003). Thus, the expression of an eating disorder, such as anorexia nervosa, is not uniform and consists of many different phenotypes that are variable within the patient population.

In the last decade, rodent studies have helped with the identification of genes or genetic loci in the control of body weight (for review, see Brockmann and Bevova, 2002). The discovery of leptin (Zhang et al., 1994; Halaas et al., 1995) was a highly significant contribution in this respect and triggered the search for downstream leptin signalling components relevant to body weight control. Rodents that lack leptin production or that have no leptin receptors develop obesity with diabetes-like symptoms, such as hypersecretion of insulin. Leptin is a hormone released from adipose tissue and is a critical factor that signals starvation to the brain (Ahima et al., 1996). During starvation, reduced leptin receptor signalling in distinct brain regions will initiate appropriate physiological responses to fasting. These responses are carried out by activation of a highly integrated

* Corresponding author. Tel.: +31-30-253-8179; fax: +31-30-253-9032.
E-mail address: m.j.h.kas@med.uu.nl (M.J.H. Kas).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.08.088

Table 1
Association studies for anorexia nervosa

Gene	Allele	Reference
Agouti-related protein	Ala67 Thr	Vink et al., 2001
Catechol *O*-methyltransferase	the high activity allele of the Val158Met (Val high activity)	Frisch et al., 2001
δ-Opioid receptor	loci markers	Bergen et al., 2003
Estrogen ES_1 receptor	G1082A polymorphism	Eastwood et al., 2002
Estrogen ES_2 receptor	1421 T/C (silent)	Rosenkranz et al., 1998a
HsKCa3 channel	CAG repeat polymorphism	Koronyo-Hamaoui et al., 2002
Norepinephrine transporter gene	promotor region	Urwin et al., 2002
$5\text{-}HT_{1B}$ receptor	G861C polymorphism	Levitan et al., 2001
$5\text{-}HT_{1D}$ receptor	loci markers	Bergen et al., 2003
$5HT_{2A}$ receptor	1438G/A polymorphism	Collier et al., 1997
Serotonin transporter gene	44 bp Del/Ins (promoter)	Sundaramurthy et al., 2000
Ucoupling protein 2, 3	microsatellite markers	Campbell et al., 1999

Overview of genes (alleles) that have been associated with anorexia. Note that many associated genes (alleles) have not been confirmed yet or are also reported as not associated with the disorder (see Table 2).

network of signalling pathways, such as a hypothalamic circuitry of orexigenic and anorexigenic neuropeptide systems (reviewed by Hillebrand et al., 2002). As found in rodents, congenital leptin deficiency in humans is associated with an early onset of severe obesity (Montague et al., 1997).

Many of the systems identified to contribute to energy metabolism regulation have been tested individually for their involvement in human eating disorders by means of case control association studies. For example, candidate genes have been tested in anorexia nervosa patients versus a control population. Thus far, these approaches have not decisively identified susceptibility genes (alleles) for this disorder (Tables 1 and 2; Hinney et al., 2000). For example, in some studies, anorexia nervosa has been associated with gene polymorphisms in the estrogen receptor, $5\text{-}HT_{2A}$ receptor, or the serotonin transporter gene, but other association studies did not confirm these findings (see Tables 1 and 2). The questions thus remain as to whether the wrong genes were being tested, or whether human candidate gene analysis can be refined so as to yield useful results.

2. Refinement of phenotypes

The identification of gene function in complex behavioural disorders, such as eating disorders, requires refinement of behavioural phenotypes within the complex disorder. How far this refinement must go is still open, however, a recent study gives some clues (Branson et al., 2003). In this study, several behavioural phenotypes related to energy homeostasis, such as eating patterns, body fat, resting energy expenditure and serum leptin levels, were monitored in an obese population ($n = 469$). Binge eating was found to be a major phenotypic characteristic of subjects with a mutation in the melanocortin MC_4 receptor, a receptor considered important in food intake control (Fan et al., 1997; Huszar et al., 1997). In rodents, genetic deletion or pharmacological blockade of this melanocortin receptor results in hyperphagia and increased body weight. Thus, based on measurable phenotypic differences, subpopulations can be defined within the diverse disorder patient population. Applying a candidate gene approach to these classified phenotypic characteristics within a patient population may lead to optimisation of such a genetic approach. Animal studies may be a good starting point for the identification of gene function in refined behavioural phenotypes relevant to eating disorders.

3. An animal model for anorexia

Approaches other than studying food intake and body weight in genetically modified rodents such as leptin-deficient mice can help to identify genes. Induction of specific animal behaviour by environmental manipulation can contribute to the identification not only of genes, but of specific

Table 2
Association studies for anorexia nervosa

Gene	Allele	Reference
β_3-Adrenoceptor	Trp-64-Arg	Hinney et al., 1997b
Dopamine D_3 receptor	Bal I polymorphism in exon 1	Bruins-Slot et al., 1998
Dopamine D_4 receptor	13-bp deletion; 48-bp repeat	Hinney et al., 1999c
Estrogen ES_2 receptor	1730 A/G (silent)	Rosenkranz et al., 1998a
Estrogen ES_2 receptor	Several alleles	Eastwood et al., 2002
Leptin	− 1387 G/A (promoter)	Hinney et al., 1998b
Melanocortin MC_4 receptor	Val-103-Ile and Ile-251-Thr	Hinney et al., 1999b
Neuropeptide Y Y_1 receptor	Pst I-polymorphism within the first intron	Rosenkranz et al., 1998b
Neuropeptide Y Y_5 receptor	1333 G/A (silent)	Rosenkranz et al., 1998b
Pro-opiomelanocortin	Insertion of 9 bp between codon 73 and 74	Hinney et al., 1998a
$5\text{-}HT_{1D\beta}$ receptor	Phe-124-Cys	Hinney et al., 1999a
$5\text{-}HT_{2A}$ receptor	− 1438 G/A polymorphism	Campbell et al., 1998; Gorwood et al., 2002
$5\text{-}HT_{2C}$ receptor	Cys-23-Ser	Burnet et al., 1999
$5\text{-}HT_7$ receptor	Pro-279-Leu	Hinney et al., 1999a
Serotonin transporter gene	44 bp Del/Ins (promoter)	Hinney et al., 1997a; Urwin et al., 2003
Tryptophan hydroxylase	1095 T/C (silent)	Han et al., 1999
Tumor necrose factor-α	− 1031 T/C, − 863 C/A and − 857 C/T polymorphisms in the promoter	Ando et al., 2001

Overview of genes (alleles) that have not been associated with anorexia.

gene function in complex behaviours. For example, an inducible animal model for anorexia nervosa has been developed (Routtenberg and Kuznesof, 1967) and used for pharmacological intervention studies. This animal model, also referred to as activity-based anorexia or semi-starvation induced hyperactivity, is induced when food-restricted rodents are allowed access to running wheels. Rodents on a daily scheduled food restriction paradigm and without access to a running wheel will initially lose body weight, but can maintain their reduced body weight over an extended period. However, when these food-restricted animals also have voluntary access to running wheels, they will rapidly lose body weight, since they then will express behavioural hyperactivity on their running wheels. Although the function of this paradoxical behavioural hyperactivity is currently unknown, the animals develop symptoms reminiscent of those observed in anorexia nervosa patients. For instance, these animals will reduce their food intake over time, exhibit behavioural hyperactivity and extreme body weight loss, and will have much reduced body temperature and plasma leptin levels (Exner et al., 2000; Hebebrand et al., 1995, 2003; Kas et al., 2003; Routtenberg and Kuznesof, 1967).

A recent study with this animal model of anorexia showed that chronic infusion of leptin suppresses the development of behavioural hyperactivity that rodents display upon semi-starvation (Exner et al., 2000). This suggests that the induction of behavioural hyperactivity is partly driven by the reduction of plasma leptin levels during starvation. Since high levels of leptin normally will also further suppress food intake, a better understanding is needed of how increased leptin signalling may be beneficial during self-starvation.

In a comparable way, it was found that Agouti-related protein prevented self-starvation in these anorectic animals by maintaining body temperature (Kas et al., 2003). Agouti-related protein (AgRP) is a neuropeptide expressed in arcuate nucleus neurons of the hypothalamus, a major downstream target region in the brain for peripheral leptin (Elias et al., 1999; Arvaniti et al., 2001). Agouti-related protein is an inverse agonist of the melanocortin MC_4 receptor (Adan and Kas, 2003) and prevents self-starvation mainly by compensation of body temperature loss in these animals with scheduled food restriction. A polymorphism in the AgRP gene was shown to be associated with anorexia

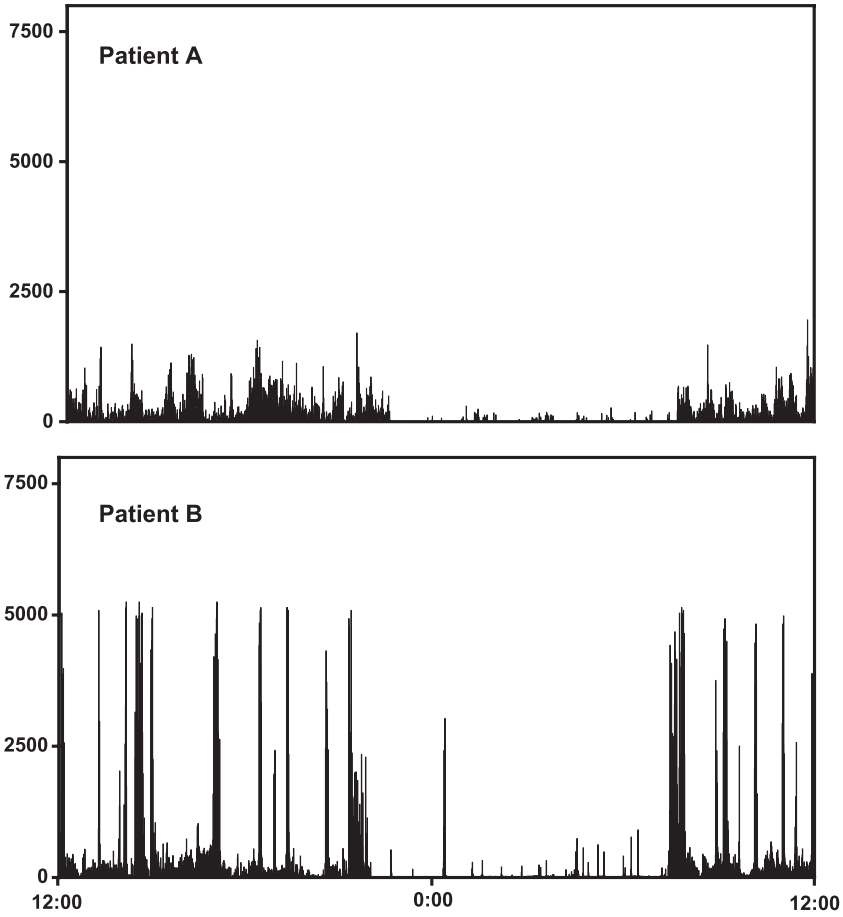

Fig. 1. Individual differences in the levels of locomotor activity expression in different anorexia nervosa patients with a comparable body mass index. The panels show the 24-h distributions of locomotor activity in a patient with low (Patient A) and in a patient with high (Patient B) levels of locomotor activity (*x*-axis: time of day; *y*-axis: activity level). Locomotor activity was monitored by means of an acti-watch (acti-watch AW4, Cambridge Neurotechnology, Cambridgeshire, United Kingdom).

nervosa (Vink et al., 2001). Although this association study in humans has not yet been confirmed for another population with anorexia, the findings in the animal model make it interesting to study body temperature changes in anorexia patients with the AgRP gene polymorphism. Alternatively, this polymorphism has been associated with a low body mass (Fan et al., 2002) that may relate to 'the drive for thinness', a potential risk factor for anorexia and bulimia nervosa (Fairburn and Harrison, 2003).

Extending the activity-based anorexia model from rats to mice will allow the genetic contribution to the development of activity-based anorexia to be studied. Although animal studies are promising for refinement of phenotypes in a standardised way, they will eventually help in the genetic dissection of eating disorders when phenotype refinement will be applied more extensively to the human population with eating disorders (Branson et al., 2003). In the case of anorexia nervosa, for example, hyperactive and non-hyperactive anorectics can be identified as subpopulations within the diverse anorexia patient population. Further measurement of behavioural characteristics should allow further refinement of the phenotypes. For instance, the use of continuous acti-watch recordings allows the distribution of behavioural hyperactivity throughout the 24-h day to be monitored and analysed. These continuous behavioural activity measurements make it easy to observe differences in behavioural activity levels and distribution (Fig. 1), which will lead to better classification of the patient population. Subdivision of the anorexia nervosa patient population based on relevant behavioural parameters will probably optimise candidate gene analysis.

4. State or trait phenomena

The aetiology of eating disorders is likely to be multifactorial with both environmental and genetic factors contributing to development of the disease. To determine the genetic contribution to certain phenotypes of the eating disorder, one should consider the difference between state and trait phenomena. State phenomena are a consequence of the disease. For example, severe food restriction in anorexia patients will lead to reduced thermogenesis (Bergh and Sodersten, 1996). However, certain characteristics of the anorexia patient are present before, during and after manifestation of the disorder. These latter characteristics are referred to as a trait and may reflect a heritable genetic variation leading to the phenotype and they may contribute to the development of the disease. Although information about the patient before or after onset of the disorder is often not available, it may be worthwhile obtaining this information. This will allow states and traits to be distinguished and facilitate identification of genes underlying these potential disease-specific traits (Fig. 2). Thus far, pre-morbid factors for anorexia nervosa, such as behavioural hyperactivity and anxiety (Brewerton et al., 1995; Bulik et al., 1997; Davis et al., 1997, 1999; Fairburn and Harrison, 2003; Godart et al., 2002; Toner et al., 1988), have been identified.

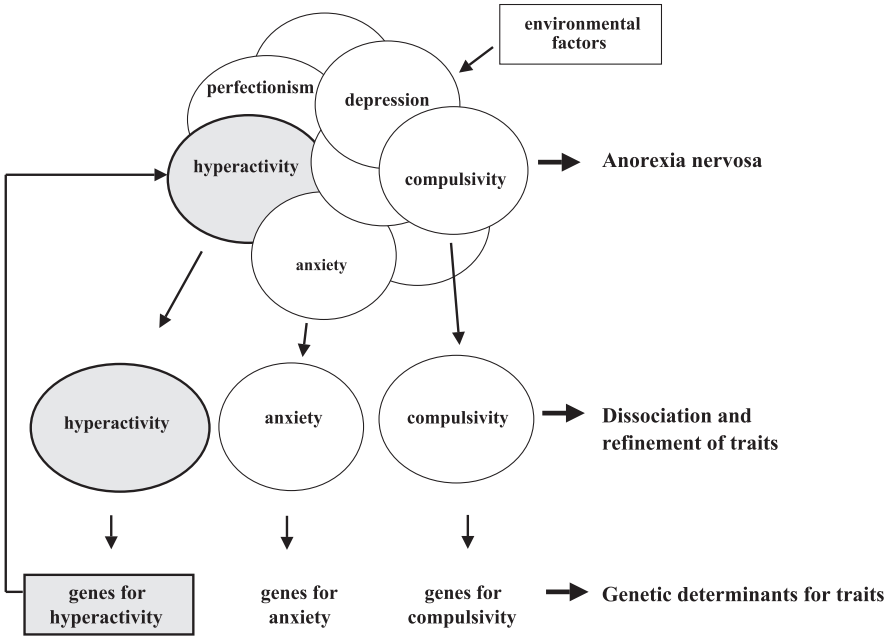

Fig. 2. Anorexia nervosa is a multi-factorial disease that likely results from complex gene–environment interactions. Identification of genes for this complex disorder requires dissociation of refined behavioural traits within this complex disorder (e.g., hyperactivity or traits/states that are yet unknown (illustrated by circles without text). Animal studies will highly facilitate the process of gene function determination as well as refinement of behavioural traits. Once genes and/or genetic pathways that determine the expression of a trait have been identified, association studies of candidate genes (e.g., genes for hyperactivity) in classified subpopulations with prominent expression of a trait (e.g., hyperactivity) can be performed.

5. Forward genetics

Besides applying reverse genetic strategies (going from genotype to phenotype), one could consider a forward genetic approach. This approach needs to be based on a stable heritable phenotype that can easily be assessed in several families with the disease. Combining the expression of this phenotype and the subsequent genetic mapping of segregating chromosomal regions will eventually lead to candidate genes for this particular phenotype. This forward genetic approach has been applied for various disorders, including anorexia nervosa (Bergen et al., 2003; Grice et al., 2002). Thus far, linkage studies of anorexia nervosa have revealed chromosomal regions that contain a wide variety of potential candidate genes (Table 3). Reduction of the amount of candidate genes in these relatively large chromosomal regions requires the testing of additional relevant families. Further, the biological function of candidate genes in these regions can be tested individually in animals with genetic defects in one of these genes.

6. Genes and genetic pathways

Identification of a gene for a refined behavioural phenotype will not always guarantee the identification of the candidate gene for a well-characterised subpopulation of patients. For example, genes can be expressed in defined cell groups of certain tissues and be part of an integrated network of regulatory mechanisms. Therefore, mutations in upstream and/or downstream signalling molecules of a particular candidate gene will likely cause phenotypes similar to those expected in individuals with mutations in the candidate gene. The orexin/hypocretin gene, for instance, is expressed in a very defined cell population of the hypothalamus and has been implicated in the regulation of feeding behaviour and arousal state (De Lecea et al., 1998; Sakurai et al., 1998). Terminals of these hypocretin/orexin producing neurons are spread throughout the brain (Peyron et al., 1998) and activate orexin/hypocretin receptors. A forward genetic study in dogs showed a hypocretin receptor and canine narcolepsy to be linked (Lin et al., 1999). A subsequent study in human narcoleptic patients, however, did not reveal an association between the hypocretin receptor gene and narcolepsy. Interestingly, post-mortem analysis revealed a lack of hypocretin/orexin production in hypothalamic neurons of narcoleptic patients when compared to those of controls (Peyron et al., 2000). This is a good example to show that searching for an association between a candidate gene (e.g., hypocretin receptor) and a certain phenotype (e.g., narcolepsy) may not be successful, since disruption of the expression of other genes (hypocretin/orexin) in the genetic pathway of the candidate gene may be responsible for the phenotype.

Table 3
Chromosomal regions and candidate genes in that region from a linkage study in restrictive anorexia nervosa patients by Grice et al. (2002) and Bergen et al. (2003)

Chromosome	Marker	Candidate genes in that region
1p34.2	D1S3721	Glutamate GLU_7 receptor Orexin/hypocretin receptor, Leptin receptor δ-Opioid receptor $5\text{-}HT_{1D}$ receptor
4q13.2	D4S2367	Oestrogen sulfotransferase
2q32.1	D2S1391	Inositol polyphosphate-1-phosphatase
4p15.3	D4S403	α_{2C} Adrenoceptor
8p23	D8S264	Lipoprotein lipase
16p11.2	D16S748	p8 Protein (regulation of glucagon transcription)

7. One gene with multiple functions

As indicated previously, the development of eating disorders is likely to be multi-factorial and results from complex gene–environment interactions. Regarding the genetic contribution to a complex disorder, multiple genetic defects may be needed to express multiple symptoms of a particular disorder. However, a single gene mutation may disrupt a wide variety of physiological processes that require appropriate regulation by a single protein encoded from this gene. For example, dopamine-deficient mice exhibit many problems that are mainly related to behavioural locomotion, including deficits in feeding and in exploration behaviour (Szczypka et al., 1999, 2001). A recent study showed that restoring local dopamine production in dopamine-deficient mice reinstated different behavioural components, depending on the brain region where dopamine was produced (Szczypka et al., 2001). Local dopamine production in dopamine-deficient mice was established by local injection of adeno-associated-viruses that achieved local expression of tyrosine hydroxylase, an essential enzyme for biosynthesis of dopamine, as well as guanosine-triphosphate (GTP) cyclohydrolase I, a co-factor required for tyrosine hydroxylase activity. Using this vector-directed gene expression technology, it was shown that restoration of dopamine production in the caudate putamen restored feeding and nest-building behaviour, whereas restoration of dopamine production in the nucleus accumbens re-established exploratory behaviour. Thus, disrupted signalling by a single transmitter (e.g., dopamine) leads to a combination of different defects in locomotor-related behaviours each of which depends on the brain region where the transmitter, dopamine, is produced. These findings indicate that multiple brain-region specific actions of a single neurotransmitter can be sufficient to explain multiple phenotypes within a complex disorder.

8. Animal studies in the genetics of eating disorders

We have now proposed several ways in which animal studies can contribute to the genetic dissection of complex behaviours in general and of eating disorders in particular. Refinement of behavioural phenotypes that mimic aspects of an eating disorder presents challenging opportunities for animal studies in this field. Once behavioural tests have been developed that allow dissociation of behavioural phenotypes within the complex behaviour, both forward and reverse genetic strategies can be applied to these phenotypes. One should keep in mind that behavioural phenotypes are regulated by integrated physiological processes and that the gene of interest interacts with down- and upstream signalling molecules that, upon genetic disruption, may induce similar phenotypes. Animal studies allow these genetic pathways to be identified following double mutant analysis. In addition, brain-region specificity of different gene functions can be addressed using transgenic approaches, such as vector-directed gene expression. Identification of genetic pathways that explain phenotypic differences within the diverse human eating disorder population will open opportunities for the understanding of the patho-physiology and for patient-specific treatment of these complex disorders. Solid interaction between clinical and basic neuroscientist will be needed, however, to address simultaneously the refinement of behavioural phenotypes in their patients and in animal populations.

References

Adan, R.A.H., Kas, M.J.H., 2003. Inverse agonism gains weight. Trends Pharmacol. Sci. 24, 315–321.

Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., Flier, J.S., 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382, 250–252.

Ando, T., Ishikawa, T., Kawamura, N., Karibe, M., Oba, M., Tatsuta, N., Hara, S., Takii, M., Naruo, T., Takei, M., Kurokawa, N., Nozoe, S., Kubo, C., Komaki, G., 2001. Analysis of tumor necrosis factor-alpha gene promoter polymorphisms in anorexia nervosa. Psychiatr. Genet. 11, 161–164.

Arvaniti, K., Huang, Q., Richard, D., 2001. Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. Neuroendocrinology 73, 227–236.

Bergen, A.W., Van den Bree, M.B., Yeager, M., Welch, R., Ganjei, J.K., Haque, K., Bacanu, S., Berrettini, W.H., Grice, D.E., Goldman, D., Bulik, C.M., Klump, K., Fichter, M., Halmi, K., Kaplan, A., Strober, M., Treasure, J., Woodside, B., Kaye, W.H., 2003. Candidate genes for anorexia nervosa in the 1p33–36 linkage region: serotonin 1D and delta opioid receptor loci exhibit significant association to anorexia nervosa. Mol. Psychiatry 8, 397–406.

Bergh, C., Sodersten, P., 1996. Anorexia nervosa, self-starvation and the reward of stress. Nat. Med. 2, 21–22.

Branson, R., Potoczna, N., Kral, J.G., Lentes, K.U., Hoehe, M.R., Horber, F.F., 2003. Binge eating as a major phenotype of melanocortin 4 receptor gene mutations. N. Engl. J. Med. 348, 1096–1103.

Brewerton, T.D., Stellefson, E.J., Hibbs, N., Hodges, E.L., Cochrane, C.E., 1995. Comparison of eating disorder patients with and without compulsive exercising. Int. J. Eat. Disord. 17, 413–416.

Brockmann, G.A., Bevova, M.R., 2002. Using mouse models to dissect the genetics of obesity. Trends Genet. 18, 367–376.

Bruins-Slot, L., Gorwood, P., Bouvard, M., Blot, P., Ades, J., Feingold, J., Schwartz, J.C., Mouren-Simeoni, M.C., 1998. Lack of association between anorexia nervosa and D3 dopamine receptor gene. Biol. Psychiatry 43, 76–78.

Bulik, C.M., Sullivan, P.F., Fear, J.L., Joyce, P.R., 1997. Eating disorders and antecedent anxiety disorders: a controlled study. Acta Psychiatr. Scand. 96, 101–107.

Burnet, P.W., Smith, K.A., Cowen, P.J., Fairburn, C.G., Harrison, P.J., 1999. Allelic variation of the 5-HT2C receptor (HTR2C) in bulimia nervosa and binge eating disorder. Psychiatr. Genet. 9, 101–104.

Campbell, D.A., Sundaramurthy, D., Markham, A.F., Pieri, L.F., 1998. Lack of association between 5-HT2A gene promoter polymorphism and susceptibility to anorexia nervosa. Lancet 351, 499.

Campbell, D.A., Sundaramurthy, D., Gordon, D., Markham, A.F., Pieri, L.F., 1999. Association between a marker in the UCP-2/UCP-3 gene cluster and genetic susceptibility to anorexia nervosa. Mol. Psychiatry 4, 68–70.

Collier, D.A., Arranz, M.J., Li, T., Mupita, D., Brown, N., Treasure, J., 1997. Association between 5-HT2A gene promoter polymorphism and anorexia nervosa. Lancet 350, 412.

Davis, C., Katzman, D.K., Kaptein, S., Kirsh, C., Brewer, H., Kalmbach, K., Olmsted, M.P., Woodside, D.B., Kaplan, A.S., 1997. The prevalence of high-level exercise in the eating disorders: etiological implications. Compr. Psychiatry 38, 321–326.

Davis, C., Katzman, D.K., Kirsh, C., 1999. Compulsive physical activity in adolescents with anorexia nervosa: a psychobehavioral spiral of pathology. J. of Nerv. Ment. Dis. 187, 336–342.

De Lecea, L., Kilduff, T.S., Peyron, C., Gao, X., Foye, P.E., Danielson, P.E., Fukuhara, C., Battenberg, E.L., Gautvik, V.T., Bartlett II, F.S., Frankel, W.N., Van den Pol, A.N., Bloom, F.E., Gautvik, K.M., Sutcliffe, J.G., 1998. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc. Natl. Acad. Sci. U. S. A. 95, 322–327.

Eastwood, H., Brown, K.M., Markovic, D., Pieri, L.F., 2002. Variation in the ESR1 and ESR2 genes and genetic susceptibility to anorexia nervosa. Mol. Psychiatry 7, 86–89.

Elias, C.F., Aschkenasi, C., Lee, C., Kelly, J., Ahima, R.S., Bjorbaek, C., Flier, J.S., Saper, C.B., Elmquist, J.K., 1999. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23, 775–786.

Exner, C., Hebebrand, J., Remschmidt, H., Wewetzer, C., Ziegler, A., Herpertz, S., Schweiger, U., Blum, W.F., Preibisch, G., Heldmaier, G., Klingenspor, M., 2000. Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. Mol. Psychiatry 5, 476–481.

Fairburn, C.G., Harrison, P.G., 2003. Eating disorders. Lancet 361, 407–416.

Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J., Cone, R.D., 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385, 165–168.

Fan, W., Marks, D.L., Haqq, A., Weissman, A., Rene, P., Cowley, M., Takahashi, K., Simerly, R., Low, M., Chagnon, Y., Cone, R.D., 2002. Mechanism of melanocortin action in the CNS. 5th Melanocortin Meeting Sunriver, OR. Abstract S011.

Frisch, A., Laufer, N., Danziger, Y., Michaelovsky, E., Leor, S., Carel, C., Stein, D., Fenig, 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.

Godart, N.T., Flament, M.F., Perdereau, F., Jeammet, P., 2002. Comorbidity between eating disorders and anxiety disorders: a review. Int. J. Eat. Disord. 32, 253–270.

Gorwood, P., Ades, J., Bellodi, L., Cellini, E., Collier, D.A., Di Bella, D., Di Bernardo, M., Estivill, X., Fernandez-Aranda, F., Gratacos, M., Hebebrand, J., Hinney, A., Hu, X., Karwautz, A., Kipman, A., Mouren-Simeoni, M.C., Nacmias, B., Ribases, M., Remschmidt, H., Ricca, V.,

Rotella, C.M., Sorbi, S., Treasure, J., 2002. EC Framework V 'Factors in Healthy Eating' consortium. The 5-HT(2A)-1438G/A polymorphism in anorexia nervosa: a combined analysis of 316 trios from six European centres. Mol. Psychiatry 7, 90–94.

Grice, D.E., Halmi, K.A., Fichter, M.M., Strober, M., Woodside, D.B., Treasure, J.T., Kaplan, A.S., Magistretti, P.J., Goldman, D., Bulik, C.M., Kaye, W.H., Berrettini, W.H., 2002. Evidence for a susceptibility gene for anorexia nervosa on chromosome 1. Am. J. Hum. Genet. 70, 787–792.

Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546.

Halmi, K.A., Sunday, S.R., Klump, K.L., Strober, M., Leckman, J.F., Fichter, M., Kaplan, A., Woodside, B., Treasure, J., Berrettini, W.H., Al Shabboat, M., Bulik, C.M., Kaye, W.H., 2003. Obsessions and compulsions in anorexia nervosa subtypes. Int. J. Eat. Disord. 33, 308–319.

Han, L., Nielsen, D.A., Rosenthal, N.E., Jefferson, K., Kaye, W., Murphy, D., Altemus, M., Humphries, J., Cassano, G., Rotondo, A., Virkkunen, M., Linnoila, M., Goldman, D., 1999. No coding variant of the tryptophan hydroxylase gene detected in seasonal affective disorder, obsessive-compulsive disorder, anorexia nervosa, and alcoholism. Biol. Psychiatry 45, 615–619.

Hebebrand, J., Van der Heyden, J., Devos, R., Kopp, W., Herpertz, S., Remschmidt, H., Herzog, W., 1995. Plasma concentrations of obese protein in anorexia nervosa. Lancet 346, 1624–1625.

Hebebrand, J., Exner, C., Hebebrand, K., Holtkamp, C., Casper, R.C., Remschmidt, H., Herpertz-Dahlmann, B., Klingenspor, M., 2003. Hyperactivity in patients with anorexia nervosa and in semistarved rats: evidence for a pivotal role of hypoleptinemia. Physiol. Behav. 79, 25–37.

Hillebrand, J.J., De Wied, D., Adan, R.A.H., 2002. Neuropeptides, food intake and body weight regulation: a hypothalamic focus. Peptides 23, 2283–2306.

Hinney, A., Barth, N., Ziegler, A., Von Prittwitz, S., Hamann, A., Hennighausen, K., Pirke, K.M., Heils, A., Rosenkranz, K., Roth, H., Coners, H., Mayer, H., Herzog, W., Siegfried, A., Lehmkuhl, G., Poustka, F., Schmidt, M.H., Schafer, H., Grzeschik, K.H., Lesch, K.P., Lentes, K.U., Remschmidt, H., Hebebrand, J., 1997a. Serotonin transporter gene-linked polymorphic region: allele distributions in relationship to body weight and in anorexia nervosa. Life Sci. 61, PL 295–303.

Hinney, A., Lentes, K.U., Rosenkranz, K., Barth, N., Roth, H., Ziegler, A., Hennighausen, K., Coners, H., Wurmser, H., Jacob, K., Romer, G., Winnikes, U., Mayer, H., Herzog, W., Lehmkuhl, G., Poustka, F., Schmidt, M.H., Blum, W.F., Pirke, K.M., Schafer, H., Grzeschik, K.H., Remschmidt, H., Hebebrand, J., 1997b. Beta 3-adrenergic-receptor allele distributions in children, adolescents and young adults with obesity, underweight or anorexia nervosa. Int. J. Obes. Relat. Metab. Disord. 21, 224–230.

Hinney, A., Becker, I., Heibult, O., Nottebom, K., Schmidt, A., Ziegler, A., Mayer, H., Siegfried, W., Blum, W.F., Remschmidt, H., Hebebrand, J., 1998a. 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.

Hinney, A., Bornscheuer, A., Depenbusch, M., Mierke, B., Tolle, A., Middeke, K., Ziegler, A., Roth, H., Gerber, G., Zamzow, K., Ballauff, A., Hamann, A., Mayer, H., Siegfried, W., Lehmkuhl, G., Poustka, F., Schmidt, M.H., Hermann, H., Herpertz-Dahlmann, B.M., Fichter, M., Remschmidt, H., Hebebrand, J., 1998b. 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.

Hinney, A., Herrmann, H., Lohr, T., Rosenkranz, K., Ziegler, A., Lehmkuhl, G., Poustka, F., Schmidt, M.H., Mayer, H., Siegfried, W., Remschmidt, H., Hebebrand, J., 1999a. 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.

Hinney, A., Schmidt, A., Nottebom, K., Heibult, O., Becker, I., Ziegler, A., Gerber, G., Sina, M., Gorg, T., Mayer, H., Siegfried, W., Fichter, M., Remschmidt, H., Hebebrand, J., 1999b. 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, 1483–1486.

Hinney, A., Schneider, J., Ziegler, A., Lehmkuhl, G., Poustka, F., Schmidt, M.H., Mayer, H., Siegfried, W., Remschmidt, H., Hebebrand, J., 1999c. No evidence for involvement of polymorphisms of the dopamine D4 receptor gene in anorexia nervosa, underweight, and obesity. Am. J. Med. Genet. 88, 594–597.

Hinney, A., Remschmidt, H., Hebebrand, J., 2000. Candidate gene polymorphisms in eating disorders. Eur. J. Pharmacol. 410, 147–159.

Huszar, D., Lynch, C.A., Fairchild-Huntres, sV., Dunmore, J.H., Fang, Q., Berkemeier, L.R., Gu, W., Kesterson, R.A., Boston, B.A., Cone, R.D., Smith, F.J., Campfield, L.A., Burn, P., Lee, F., 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88, 131–141.

Kas, M.J.H., Van Dijk, G., Scheurink, A.J., Adan, R.A.H., 2003. Agouti-related protein prevents self-starvation. Mol. Psychiatry 8, 235–240.

Koronyo-Hamaoui, M., Danziger, Y., Frisch, A., Stein, D., Leor, S., Laufer, N., Carel, C., Fennig, S., Minoumi, M., Apter, A., Goldman, B., Barkai, G., Weizman, A., Gak, E., 2002. Association between anorexia nervosa and the hsKCa3 gene: a family-based and case control study. Mol. Psychiatry 7, 82–85.

Levitan, R.D., Kaplan, A.S., Masellis, M., Basile, V.S., Walker, M.L, Lipson, N., Siegel, G.I., Woodside, D.B., Macciardi, F.M., Kennedy, S.H., Kennedy, J.L., 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.

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., Qiu, X., De Jong, P.J., Nishino, S., Mignot, E., 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 98, 365–376.

Montague, C.T., Farooqi, I.S., Whitehead, J.P., Soos, M.A., Rau, H., Wareham, N.J., Sewter, C.P., Digby, J.E., Mohammed, S.N., Hurst, J.A., Cheetham, C.H., Earley, A.R., Barnett, A.H., Prins, J.B., O'Rahilly, S., 1997. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387, 903–908.

Peyron, C., Tighe, D.K., Van den Pol, A.N., De Lecea, L., Heller, H.C., Sutcliffe, J.G., Kilduff, T.S., 1998. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J. Neurosci. 18, 9996–10015.

Peyron, C., Faraco, J., Rogers, W., Ripley, B., Overeem, S., Charnay, Y., Nevsimalova, S., Aldrich, M., Reynolds, D., Albin, R., Li, R., Hungs, M., Pedrazzoli, M., Padigaru, M., Kucherlapati, M., Fan, J., Maki, R., Lammers, G.J., Bouras, C., Kucherlapati, R., Nishino, S., Mignot, E., 2000. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat. Med. 6, 991–997.

Routtenberg, A., Kuznesof, A.W., 1967. Self-starvation of rats living in activity wheels on a restricted feeding schedule. J. Comp. Physiol. Psychol. 64, 414–421.

Rosenkranz, K., Hinney, A., Ziegler, A., Hermann, H., Fichter, M., Mayer, H., Siegfried, W., Young, J.K., Remschmidt, H., Hebebrand, J., 1998a. Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: identification of several genetic variants. J. Clin. Endocrinol. Metab. 83, 4524–4527.

Rosenkranz, K., Hinney, A., Ziegler, A., Von Prittwitz, S., Barth, N., Roth, H., Mayer, H., Siegfried, W., Lehmkuhl, G., Poustka, F., Schmidt, M., Schafer, H., Remschmidt, H., Hebebrand, J., 1998b. Screening for mutations in the neuropeptide Y Y5 receptor gene in

cohorts belonging to different weight extremes. Int. J. Obes. Relat. Metab. Disord. 22, 157–163.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozlowski, G.P., Wilson, S., Arch, J.R., Buckingham, R.E., Hayne, sA.C., Carr, S.A., Annan, R.S., McNulty, D.E., Liu, W.S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J., Yanagisawa, M., 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92, 573–585.

Sundaramurthy, D., Pieri, L.F., Gape, H., Markham, A.F., Campbell, D.A., 2000. Analysis of the serotonin transporter gene linked polymorphism (5-HTTLPR) in anorexia nervosa. Am. J. Med. Genet. 96, 53–55.

Szczypka, M.S., Rainey, M.A., Kim, D.S., Alaynick, W.A., Marck, B.T., Matsumoto, A.M., Palmiter, R.D., 1999. Feeding behavior in dopamine-deficient mice. Proc. Natl. Acad. Sci. U. S. A. 96, 12138–12143.

Szczypka, M.S., Kwok, K., Brot, M.D., Marck, B.T., Matsumoto, A.M., Donahue, B.A., Palmiter, R.D., 2001. Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. Neuron 30, 819–828.

Toner, B.B., Garfinkel, P.E., Garner, D.M., 1988. Affective and anxiety disorders in the long-term follow-up of anorexia nervosa. Int. J. Psychiatry Med. 18, 357–364.

Urwin, R.E., Bennetts, B., Wilcken, B., Lampropoulos, B., Beumont, P., Clarke, S., Russell, J., Tanner, S., Nunn, K.P., 2002. Anorexia nervosa (restrictive subtype) is associated with a polymorphism in the novel norepinephrine transporter gene promoter polymorphic region. Mol. Psychiatry 7, 652–657.

Urwin, R.E., Bennetts, B.H., Wilcken, B., Beumont, P.J., Russell, J.D., Nunn, K.P., 2003. Investigation of epistasis between the serotonin transporter and norepinephrine transporter genes in anorexia nervosa. Neuropsychopharmacology 28, 1351–1355.

Vink, T., Hinney, A., Van Elburg, A.A., Van Goozen, S.H., Sandkuijl, L.A., Sinke, R.J., Herpertz-Dahlmann, B.M., Hebebrand, J., Remschmidt, H., Van Engeland, H., Adan, R.A.H., 2001. Association between an agouti-related protein gene polymorphism and anorexia nervosa. Mol. Psychiatry 6, 325–328.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.