Genetic determinants of eating disorders

Slof-Op 't Landt, M.C.T.

Genetic Determinants of Eating Disorders

Rita Slof-Op ‘t Landt

Genetic determinants of Eating Disorders
Rita Slof-Op ‘t Landt
PhD thesis with summary in Dutch
ISBN 978-94-91211-49-2

Cover by Else Marie Bos Design (elsemariebos.nl)
Printed by Ipskamp drukkers

Genetic Determinants of Eating Disorders

Proefschrift

ter verkrijging van de graad Doctor aan de Universiteit van Leiden,
op gezag van Rector Magnificus prof. Mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 28 juni 2011
klokke 15.00 uur

door

Margarita Cornelia Theodora Slof-Op ’t Landt

geboren te Ter Aar in 1980

Promotiecommissie

Promotores	**Prof. Dr. P.E. Slagboom**
	Prof. Dr. D.I. Boomsma (Vrije Universiteit Amsterdam)
Co-promoter	**Dr. E.F. van Furth (Centrum Eetstoornissen Ursula)**
Overige leden	**Prof. Dr. R.A.H. Adan (Universiteit Utrecht)**
	Prof. Dr. C.M. Bulik (University of North Carolina, USA)
	Prof. Dr. F.G. Zitman

Contents

Chapter 1

Introduction

In this thesis, a series of studies are reported that investigated different aspects of the genetics of eating disorders. In general, three eating disorders are distinguished, anorexia nervosa (AN), bulimia nervosa (BN) and eating disorders not otherwise specified (EDNOS) (for DSM-IV (1994) criteria see Appendix A).

Anorexia nervosa

AN is characterized by a severely low body weight (85% or less than expected based on age and height) intense fear of weight gain, a distorted body image and amenorrhoea. Two subtypes of AN are distinguished: the restricting type, and the binge-eating/purging type. In contrast to the binge-eating/purging type, individuals with the restricting type do not regularly engage in eating binges and purging behaviors, like self-induced vomiting, or the misuse of laxatives or diuretics. Instead individuals with restricting type AN achieve and maintain low body weight through restriction of energy intake (fasting) and/or increased energy expenditure (excessive exercise).

Table 1.1 Prevalence and prognosis factors of eating disorders and other psychiatric disorders

	Prevalence[a]		Mortality		Course 4-10 years follow-up		
	Point (%)	Lifetime (%)	Crude mortality ratio (%)	Standardized mortality ratio	Recovery (%)	Improvement (%)	Chronicity (%)
AN	0.3	0.9-2.2	4.0-5.9	1.7-8.0	47	32	20
BN	1.0	1.5-1.7	0.32-3.9	1.7-9.3	66.5	21.6	11.9
EDNOS	-	-	5.2	1.8	-	-	-
BED	1.0	3.5	-	-	-	-	-

[a] Prevalences reported for women

AN mainly affects young women and generally starts during puberty or early adulthood. As can be seen in Table 1.1 prevalences for AN in women are low (Bulik et al., 2006; Hoek, 2006; Hoek & Van Hoeken, 2003; Hudson et al., 2007; Keski-Rahkonen et al., 2007). The mortality rate, on the other hand, is high in AN and the prognosis is poor, only half of the patients with AN fully recover (Crow et al., 2009; Harris & Barraclough, 1998; Nielsen et al., 1998; Steinhausen, 2002; Sullivan, 1995). Reported Standardized Mortality Ratios in AN are about twice as high in comparison to affective disorders (1.7), schizophrenia (1.6) and psychiatric illness in general (2.2) (Harris & Barraclough, 1998). So despite the rarity of AN, it is a severely debilitating disorder with a high impact.

Despite substantial efforts to identify causal pathways for AN, very little is known about the aetiology of this disorder. In longitudinal and cross-sectional studies, several risk factors have been identified for AN, including being female, dieting, negative self-evaluation and perfectionism (Bachner-Melman et al., 2007; Jacobi et al., 2004). In addition, results from both family and (population-based) twin studies suggest that genetic factors also play a substantial role in the development of AN (Bulik et al., 2006; Bulik et al., 2010; Slof-Op 't Landt et al., 2005), with heritability estimates ranging from 28 to 76%, as is shown in Table 1.2.

Table 1.2 Variance (%) accounted for by genetic, shared environmental and non-shared environmental factors of eating disorders and important related features in population-based twin studies.

	Genetic factors	Shared environmental factors	Non-shared environmental factors
AN	28-76	0-5	24-52
BN	30-83	0-41	17-50
Binge eating disorder	39-57	0-13	42-61
Self-induced vomiting	8-70	0	30-92
Perfectionism	29-42	0-19	58-68
Impulsivity	44-49	0	51-56

Bulimia nervosa

The main characteristics of BN are recurrent episodes of binge eating and inappropriate compensatory behaviors. In addition, an undue influence of body weight and/or shape on self-evaluation is present. In purging type BN self-induced vomiting, misuse of laxatives or misuse of diuretics are used in order to prevent weight gain. Excessive exercise and fasting are the main compensatory behaviors for nonpurging type BN.

Although slightly more common than AN, BN is also characterized by a low prevalence (Table 1.1; Fairburn & Beglin, 1990; Hoek & Van Hoeken, 2003; Hudson et al., 2007; Keski-Rahkonen et al., 2009). Almost 70% of patients with BN fully recover in a period of 4 to 10 years (Steinhausen & Weber, 2009). Despite the fact that the uncertainty of the estimates is larger, the mortality rates in BN appear to be two times lower than the rates reported in AN, but the reported Standardized Mortality Ratios again appears to be about 1.5 times higher than those reported for other mental illnesses (Crow et al., 2009; Harris & Barraclough, 1998; Papadopoulos et al., 2009; Steinhausen & Weber, 2009).

For BN, female sex, childhood obesity, negative self-evaluation, perfectionism and dieting are considered potent risk factors (Fairburn et al., 1997; Jacobi et al., 2004;

Lilenfeld et al., 2000). Furthermore, most twin studies have yielded substantial heritability estimates, ranging 30 to 83%, for BN in women (Slof-Op 't Landt et al., 2005). The remaining variance was primarily accounted for by non-shared environmental factors (Table 1.2). In sum, genetic factors also appear to contribute to the liability of BN.

Eating disorders not otherwise specified

The majority of eating disorder patients (about 60% of those representing for treatment) do not meet strict DSM-IV diagnostic criteria for either AN or BN, and therefore belong to the eating disorder not otherwise specified (EDNOS) category. The EDNOS category can be distinguished into three subcategories. The first category are cases that closely resemble AN or BN but just fail to meet their diagnostic threshold. The second category represents cases that show similar clinical features of AN and BN only in a different combination. Binge eating disorder (BED) is the third category of EDNOS, and is characterized by the occurrence of binge eating in the absence of regular compensatory behaviors (Fairburn & Bohn, 2005).

Only few epidemiological studies were performed for the total category of EDNOS. Therefore it is unknown how prevalent EDNOS is in the community (Table 1.1). Recently, a crude mortality rate of 5.2% was reported for EDNOS (Crow et al., 2009), indicating that the severity and clinical impact of this eating disorder is not less than that seen in AN or BN. BED is the most intensively studied category of EDNOS. As shown in Table 1.1, the prevalence reported for BED appears to be higher than the prevalences of both BN and AN (Hoek & Van Hoeken, 2003; Hudson et al., 2007).

Reported risk factors for BED are negative self-evaluation, stressful life events, adverse childhood experiences, childhood obesity and repeated exposure to negative comments from family members about shape, weight or eating (Fairburn et al., 1998; Pike et al., 2006; Striegel-Moore et al., 2005). As presented in Table 1.2, twin studies obtained moderate heritability estimates for BED (Javaras et al., 2008; Mitchell et al., 2010; Reichborn-Kjennerud et al., 2004b). No twin studies have been performed to evaluate the contribution of genetic factors to the liability of the total category of EDNOS.

Overlap among different types of eating disorders

It is clear that the different types of eating disorders share a number of attitudes and behaviors. It has even been hypothesized that eating disorders essentially share the same core psychopathology: over-evaluating eating, shape, weight and their control (Fairburn et

al., 2003). An important other symptom that is shared among the different types of eating disorders (AN purging type, BN, and EDNOS) is self-induced vomiting (SV). Individuals with an eating disorder engage in SV as a method to lose body weight or prevent weight gain, following regular meals or as a compensatory action following binge eating. Prevalence of vomiting within clinical samples of individuals with AN ranged between 31 and 39% (Ben-Tovim et al., 1989; Garner et al., 1993), whereas the rate in clinical samples of individuals with BN has been estimated to be over 90% (Ben-Tovim et al., 1989). Comparable to the eating disorders itself (Table 1.2), moderate to high heritability estimates have been shown for SV (Sullivan et al., 1998a; Wade et al., 2008b). It has been reported that the symptom of SV is associated with higher BMI, greater clinical severity and higher novelty seeking (Dalle Grave et al., 2009; Reba et al., 2005).

Table 1.3 Crossover rates (%) between the different types of eating disorders

	End diagnosis			
	AN	BN	EDNOS	BED
Start diagnosis				
AN	-	9-36	17	0
BN	4-27	-	22	1
EDNOS	10	16	-	-
BED	0	16.4	-	-

Eating disorders are not static diseases; rather there is a continuous process of diagnostic crossover between the different types. The crossover rate is the percentage of patients whose initial eating disorder diagnosis (AN,BN, EDNOS or BED) changes into a different eating disorder diagnosis. Crossover rates among the different types of eating disorders are presented in Table 1.3. In general, there is a considerable rate of cross-over between AN, BN and EDNOS, ranging between 4 and 36% (Eddy et al., 2008; Fichter & Quadflieg, 2007; Milos et al., 2005; Steinhausen & Weber, 2009; Tozzi et al., 2005). For BED the crossover to BN was around 16%, while the converse cross-over was close to zero. There was no crossover between BED and AN, and BED may thus be considered a different disease entity.

In addition to the cross-over rate between eating disorders, family studies have shown that AN and BN do not aggregate independently within families, the risk of developing both disorders is elevated in family members of individuals with an eating disorder (Lilenfeld et al., 1998; Strober et al., 2000). Therefore, it has been hypothesized that AN, BN, but also subthreshold forms of eating disorders (EDNOS) share risk and liability

factors. As shown in a Swedish twin study, approximately half of the genetic factors contributed to liability of both AN and BN (Bulik et al., 2010). To further explore the shared liability between eating disorders it would be interesting to investigate the genetic contribution of the variance in a combined phenotype of disordered eating behaviours and attitudes as well. The role of body weight should also be taken into account, since body weight is closely linked to eating behaviours and even appears to be a risk factor for the development of eating disorders (Jacobi et al., 2004). Unique genetic and environmental factors may determine the state of expression of the eating disorder (e.g. the restrictive fasting in AN or the self-induced vomiting seen in AN purging type, BN, and EDNOS). The shared genetic factors may influence predisposing behavioural features that are shared among the different types of eating disorders, like perfectionism and impulsivity.

Possible predisposing personality traits for eating disorders

A personality feature that consistently characterizes patients with AN and/or BN is perfectionism (Cassin & von Ranson, 2005). Perfectionism 'involves high standards of performance which are accompanied by tendencies for overly critical evaluations of one's own behavior' (Frost et al., 1990). Perfectionism is an important risk factor for both AN and BN, and although less pronounced it also appears to contribute to the vulnerability of BED (Fairburn et al., 1997; Jacobi et al., 2004; Lilenfeld et al., 2000; Striegel-Moore et al., 2005). As can be seen in Table 1.2, moderate to substantial heritability estimates have also been reported for perfectionism (Tozzi et al., 2004). In addition, elevated levels of perfectionism were observed in relatives of individuals with AN and BN (Lilenfeld et al., 2000; Woodside et al., 2002). These findings suggest that perfectionism may be of potential aetiological relevance for eating disorders.

Impulsivity has also regularly been associated with eating disorders. Impulsivity can be defined as the tendency to deliberate less than most people of equal ability before taking action (Dickman, 1990). High impulsivity appears to be a prospective risk factor to BN, whereas low impulsivity (or high control) seems to be associated with AN (Casper et al., 1992; Cassin & von Ranson, 2005; Claes et al., 2002; Steiger et al., 1991; Stice, 2002). Table 1.2 shows that almost half of the variance in impulsivity was accounted for by genetic factors (Hur & Bouchard, Jr., 1997; Pedersen et al., 1988; Seroczynski et al., 1999). In a large group of individuals with AN and BN Favaro et al. (Favaro et al., 2005) found that the presence of purging behaviour (self-induced vomiting and laxative use) was associated to impulsivity. Results from a large meta-analytic review support these findings (Fischer et al., 2008). In addition, Wade et al. (2008b) reported that novelty seeking, which

is closely related to impulsivity (Cloninger et al., 1993), predicted self-induced vomiting in a large twin study. In a meta-analysis all dimensions of impulsivity were significantly associated to bulimic symptoms (effect sizes r 0.08-0.38) (Fischer et al., 2008). A latent profile analysis in patients recovered from AN (both restricting as well as binge-purging type) and BN showed that a separate cluster could be identified based on impulsivity (Wagner et al., 2006). Thus, high impulsivity scores persist in some individuals after recovery from an eating disorder. It is possible that high impulsivity also precedes the onset of the eating disorder, indicating that this feature could also be of aetiological relevance.

Genetic study designs

Several approaches exist to identify genes involved in a trait with a heritable component (for an overview see Slagboom & Meulenbelt, 2002). Depending on the frequency and penetrance of the genetic variation underlying the disease a study design should be selected. Extended family designs are optimal to detect rare variants with high penetrance mutation by model based linkage. In linkage analysis, the segregation of alleles in families together with the affected phenotypes are investigated to localise genes that influence a quantitative trait. In linkage analyses LOD scores (logarithm of odds) are used to compare the likelihood of obtaining the test data if the two loci are indeed linked, to the likelihood of observing the same data purely by chance (Fulker & Cardon, 1994). Linkage studies can also be performed in affected sibling pairs, this design is optimal for detecting genetic variants with both a moderate effect and a moderate penetrance. Finally, common variants with possibly small effect sizes can be detected by performing association studies in a large group of unrelated cases and controls (Cardon & Bell, 2001). The aim of this approach is to find an ancestral risk allele that is shared among population cases with the disease, higher allele frequencies amongst cases versus controls. In the association analysis odds ratios or other measures of association are used to describe the strength of the association.

The genetic studies described above can further be distinguished into hypothesis based and hypothesis free approaches. In the hypothesis based approach the involvement of candidate genes in a certain trait or disorder are investigated. Candidate genes are selected because of their specific function or their involvement in a certain biological pathway. Depending on the number of candidate genes tested, moderate samples sizes are sufficient, and little genotyping resources have to be used. However, when the aetiology of a certain trait or disorder is largely unknown, applying a hypothesis free approach will enable the researchers to identify new genetic loci putatively involved in the trait. Due to technological advances the whole genome can now be scanned to investigate which area

may harbour susceptibility loci for the disorder of interest. Both genomewide linkage studies (in families or affected sibling pairs) and genomewide association studies belong to the hypothesis free approach. Genomewide linkage studies identify chromosomal regions that may harbour susceptibility loci for the trait under study. Genomewide association studies on the other hand identify actual genetic variants that may distinguish cases from controls.

Candidate pathways or genes

The serotonin pathway has mostly been indicated as relevant in the development of eating disorders, since it is involved in a broad range of relevant biological, physiological and behavioral functions, for example body weight regulation and eating behavior (Blundell, 1992; Blundell et al., 1995; Brewerton & Jimerson, 1996; Halford & Blundell, 2000; Kaye, 1997; Lucki, 1998; Monteleone et al., 2000; Simansky, 1996; Weltzin et al., 1994). In addition, serotonin might also contribute to the psychopathological features of eating disorders such as perfectionism, impulsivity and obsessionality (Carver & Miller, 2006; Hinney et al., 2000; Kaye, 1997; Kaye et al., 2000a). In both rodents and humans, drugs that either directly or indirectly increased postsynaptic serotonergic stimulation routinely decreased the consumption of food (Lucki, 1998; Simansky, 1996). In underweight and malnourished individuals with AN, levels of 5-hydroxyindolacetic acid (5-HIAA), a metabolite of serotonin, were reduced in cerebrospinal fluid compared to those of controls (Brewerton & Jimerson, 1996; Kaye et al., 2005b). Furthermore, the prolactin response to D-fen, which is an index of the functional activity of the central serotonin system, was reduced in underweight inidividuals with AN and in individuals with BN with high frequent binge episodes (two or more episodes a day) (Monteleone et al., 2000). In long-term weight-recovered patients with AN or BN, on the other hand levels of 5-HIAA in the cerebrospinal fluid were elevated (Kaye et al., 1991; Kaye et al., 1998). Whether these changes are a consequence or precede the disease onset has to be elucidated, for example by investigating the serotonin system in relatives of eating disorder patients. These results suggest that hyperserotonergic activity is a trait marker in eating disorders that could predispose to the development of the disorder.

In addition to genes belonging to the serotonin pathway, the involvement of many other candidate genes have been studied in eating disorders. Table 1.4 lists the candidate genes per pathway, the number of studies and the number of significant associations that have been evaluated. In addition, a large collaborative study investigated the association of 182 candidate genes in AN (Pinheiro et al., 2010). After accounting for multiple testing,

there were no statistically significant associations. Thirty six of these genes are also presented in Table 1.4. Many of the conducted studies had small sample sizes and inadequate statistical power to detect an effect. The studies in Table 1.4 were divided into large and small using a boundary of 80% statistical power. An elaborate review on the candidate gene studies conducted until 2005 can be found in chapter 2. In addition, the results from the large candidate gene studies performed during the last five years (2006-2010) are summarized in appendix B, references for the small studies from this same time-frame are given in appendix C.

Most association studies performed in the eating disorder field used a hypothesis based approach (listed in the top part of Table 1.4). So far the only association that has been observed in at least two large association studies was between brain-derived neurotrophic factor (*BDNF*) and AN. *BDNF* plays a key role in the survival, differentiation, and development of several central and peripheral neurons and is involved in synaptic plasticity (Huang & Reichardt, 2001; Lu, 2003). *BDNF* has been implicated in the pathophysiology of several psychiatric disorders, including mood disorders and schizophrenia (Angelucci et al., 2004; Duman, 2002; Nestler et al., 2002), and it is recognized as a regulator of satiety, appetite and weight regulation (Lebrun et al., 2006). Heterozygous *BDNF* knockout mice are obese and develop hyperphagia (Kernie et al., 2000). In addition, central administration of *BDNF* leads to severe, dose-dependent appetite suppression and weight loss (Pelleymounter et al., 1995). Thus increased function of *BDNF* may be a predisposing factor to develop AN.

In the bottom part of Table 1.4, the genes that were identified by a hypothesis free approach are listed. Genomewide linkage studies have demonstrated linkage peaks for AN on chromosomes 1p33-36 and 4q13 and for BN on chromosomes 10p13, and 14q22-23 (Bulik et al., 2003a; Devlin et al., 2002; Grice et al., 2002). Following up the genome-wide screen in AN (Grice et al., 2002), an association study was conducted focusing on candidate genes positioned under the linkage peak on chromosome 1p33-36 (Bergen et al., 2003). Three candidate genes were tested, namely the serotonin receptor 1D (*HTR1D*), opioid receptor delta 1 (*OPRD1*) and hypocretin receptor 1 (*HCRTR1*). The case-control study yielded a positive association between *HTR1D* and *OPRD1* polymorphisms in AN. These findings were confirmed in an independent association study (Brown et al., 2006).

Two genomewide association studies have been conducted in AN, results are listed in the bottom of Table 1.4. In a Japanese genomewide association study (Nakabayashi et al., 2009), two loci, namely 1q41 and 11q22 were significantly associated with AN. Neither of these loci showed a positive evidence of association with BN. The most significant

association was observed at a SNP located near the gene encoding spermatogenesis-associated protein 17.

Table 1.4 Candidate genes investigated in eating disorders, the number of studies and significant associations are divided into large (adequate statistical power) and small (statistically underpowered) studies.

Hypothesis based approach		Large studies		Small studies	
candidate pathways/genes	Position	N	Sign ass	N	Sign ass
Serotonin					
Serotonin receptor 1B (*HTR1B*)	6q13	1	0	1	0
Serotonin receptor 2A (*HTR2A*)	13q14-21	2	0	16	7
Serotonin receptor 2C (*HTR2C*)	Xq24	1	0	4	1
Serotonin receptor 3A (*HTR3A*)	11q23	2	1		
Serotonin receptor 3B (*HTR3B*)	11q23	2	1		
Serotonin receptor 7 (*HTR7*)	10q21-24	1	0	1	0
Serotonin transporter (*SLC6A4*)	17q11-12	1	0	10	6
Tryptophan hydroxylase (*TPH1*)	11p14-p15	1	0	2	0
Catecholamine					
Beta-3-adrenergic receptor (*ADRB3*)	8p11-12	2	0	1	0
Catechol-O-methyltransferase (*COMT*)	22q11	2	0	6	3
Dopamine receptor D2 (*DRD2*)	11q23	2	1		
Dopamine receptor D3 (*DRD3*)	3q13	1	0	1	0
Dopamine receptor D4 (*DRD4*)	11p15	2	1	2	0
Dopamine transporter (*SLC6A3*)	5p15	1	0	2	1
Mono amine oxidase A (*MAOA*)	Xp11	1	0	2	0
Norepineprhine transporter (*SLC6A2*)	16q12	2	1	1	1
Neuropeptide, feeding & energy regulation					
Agouti related protein (*AGRP*)	16q22	1	0	2	2
Cannabinoid receptor 1 (*CNR1*)	6q14-15	1	0	4	3
Cannobinoid receptor 2 (*CNR2*)	1p36	2	1		
Cholecystokinine (*CCK*)	3pter-p21	2	1		
Fatty acid amide hydrolase (*FAAH*)	1p34-35			2	1
Ghrelin (*GHRL*)	3p25-26	3	0	2	1
Leptin (*LEP*)	7q31	1	0	1	0
Leptin receptor (*LEPR*)	1q31	1	0	1	0
Melanocortin receptor 4 (*MCR4*)	18q22	2	0	1	0
Monogliceride lipase (*MGLL*)	3p21			1	0
N-acylethanolamine-hydrolyzing acid amidase (*NAAA*)	4q21			1	0
Neuropeptide Y receptor 1R (*NPY1R*)	4q31-32	1	0	1	0
Neuropeptide Y receptor 5R (*NPY5R*)	4q31-32	1	0	1	0
Proopiomelanocortine (*POMC*)	2q23	1	0	1	0

Hypothesis based approach		Large studies		Small studies	
candidate pathways/genes	Position	N	Sign ass	N	Sign ass
Uncoupling protein 2/3 (*UCP 2/3)*	11q13	1	0	3	1
Neurogenesis					
Brain-derived neuroptrophic factor (*BDNF*)	11p13	5	2	7	4
Neurotrophic tyrosine kinase receptor 2 (*NTRK2*)	9q22	1	0	1	1
Other candidate genes					
Armadillo repeat gene deleted in VCSF (*ARVCF*)	22q11	1	0		
Estrogen receptor 1 (*ESR1*)	6q25	2	1	1	0
Estrogen receptor 2 (*ESR2*)	14q	1	0	3	2
ETS variant gene 5 (*ETV5*)	3q28	1	0		
Fat mass- and obesity associated gene (*FTO*)	16q12	1	0		
Glucosamine-6-phosphate deaminase 2 (*GNPDA2*)	4p13	1	0		
Glutamate receptor (*GRIN2B*)	12p12	1	0	1	1
G-protein coupled receptor 55 (*GPR55*)	2q37	1	1		
Major histocompatibilty complex (*HLA*)	6q21			3	1
Mitochondrial carrier homolog 2(*MTCH2*)	11q12	1	0		
Neurol growth regulator 1 (*NEGR1*)	1p31	1	0		
Potassium channel (*KCNN3*)	1q21	1	0	3	3
Potassium channel tetramerisation domain (*KCTD15)*	19q13	1	0		
SH2B adaptor protein 1 (*SH2B1*)	16p11	1	0		
Transmembrane protein 18 (*TMEM18*)	2p25	1	0		
Tumor necrosis factor (*TNF*)	6q21			2	2
Hypothesis free approach		Large studies		Small studies	
genes	Position	N	Sign ass	N	Sign ass
Serotonin receptor 1D (*HTR1D*)	1p34-36	3	2		
Hypocretin receptor (*HCRTR1*)	1p34-36	2	0		
Opioid receptor delta-1 (*OPRD1*)	1p34-36	3	2		
A-Kinase anchor protein 6 (*AKAP6*)	14q12	1	1		
Cadherin 9 (*CDH9*)	5p14	1	1		
Cysteine- & glycine-rich protein 2 binding protein (*CSRP2BP*)	20p11	1	1		
Netrin G1 (*NTNG1*)	1p13	1	1		
Spermatogenesis-associated protein 17 (*SPATA17*)	1q41	2	1		
Zinc finger protein 804B (*ZNF804B*)	7q21	1	1		

This finding could not be confirmed in a second genomewide association study in individuals with AN and controls of European ancestry (Wang et al., 2010). In this study no SNP reached genome-wide significance, whereas top association signals were detected near genes encoding zinc protein 804B, cysteine- and glycine-rich protein 2 binding protein, netrin G1, a-kinase anchor protein and cadherin 9. In addition, the association between *OPRD1* and AN was confirmed, while suggestive evidence was obtained for involvement of *HTR1D* in AN.

Outline of this thesis

This thesis aims to answer the following questions:

1. Is disordered eating behaviour heritable and how much of this heritability is independent of BMI?
2. Are the genes encoding serotonin receptor 1D, stathmin, brain-derived neurotrophic factor and tryptophan hydroxylase 2 involved in anorexia nervosa and/or eating disorders characterized by self-induced vomiting?
3. Can genetic predisposition to high perfectionism and impulsivity explain an association between the tryptophan hydroxylase 2 gene and eating disorders?

Table 1.5 Study populations used in this thesis

Population	N	Description	Thesis question
GenED study	389	Female participants with an eating disorder (DSM-IV): 182 with AN, 149 with eating disorders characterized by self-induced vomiting	2 and 3
Netherlands Twin Registry (NTR):			
Young twins (YNTR)	2702	Adolescent twins and siblings (956 male twins, 1219 female twins, 239 brothers and 288 sisters)	1, for 2 and 3 (399 adolescent female twins)
Adult twins (ANTR)	250	Adult female twins and family members	2 and 3
Healthy controls:			
Questionnaire controls	240	Women without an eating disorder	3

To answer these questions data from three different populations were analyzed (see Table 1.5):

The Genetics of Eating Disorder (GenED) study was designed and initiated for this thesis to collect DNA, DSM-IV eating disorder diagnoses and extensive phenotype information from a large group of participants with an eating disorder. Participants were recruited through ten specialist eating disorder units throughout the Netherlands. Eating disorder diagnoses were made by experienced clinicians based on a semi-structured interview at intake. The phenotype information was comprised of questionnaire data on eating disorder symptoms and characteristics, impulsivity and perfectionism for all eating disorder cases. Furthermore, additional interview and questionnaire data were collected on eating disorder features, comorbid psychiatric disorders, personality and obsessionality, in a subgroup of participants who fulfilled DSM-IV criteria for anorexia (at least three years) or bulimia nervosa. First-degree family members from this group were also approached to participate in the GenED study.

In the late 1980s The Netherlands Twin Register (NTR) was established by recruiting young twins and multiples at birth and by approaching adolescent and young adult twins through city councils (Bartels et al., 2007; Boomsma et al., 2002; Boomsma et al., 2006). These twins, their parents and siblings participate in longitudinal survey studies concerning a wide variety of behavioral, psychological and lifestyle features, including disordered eating behavior (for items see Table 1.6), perfectionism and impulsivity. These phenotypic data were collected in a large sample of adolescent twins and siblings from the Young Netherlands Twin Registry (YNTR). In addition, DNA was collected in a subsample of adolescent female twins. Phenotypic data and DNA samples were also available for adult female twins and family members from the Adult Netherlands Twin Registry (ANTR).

Table 1.6 Disordered Eating Behavior (DEB) items

Items	Question
Dieting	Have you ever gone on a diet to lose weight or to stop gaining weight?
Fear of weight gain	How afraid are you to gain weight or become fat?
Importance of body weight or shape on self-evaluation	How important are body weight and/or shape in how you feel about yourself?
Binge eating	Have you ever had episodes of binge eating?

The third study population consists of a control group of adult women without eating disorders. This group was recruited through advertisements in magazines and via internet

websites. Questionnaire data on eating disorder symptoms and characteristics, impulsivity and perfectionism were collected, identical to the eating disorder participants and family members from the GenED study.

In chapter 2 an overview on the state of affairs of genetic research on family, twin and molecular genetic studies in the eating disorder field up till 2005 is given.

In chapter 3, we evaluated whether the Disordered Eating Behavior (DEB)-scale, used in the twin population of the NTR was comparable between adolescent men and women. This study provides a comprehensive overview of the different steps in multi-group discrete factor analyses accumulating into a model of complete measurement invariance with respect to sex, which were tested for the DEB-scale.

In chapter 4 it was investigated what proportion of heritability in DEB was attributable to genetic effects on body mass index (BMI), and what proportion was independent of these effects in a sample of twins and non-twin siblings from the YNTR. A bivariate genetic analysis of DEB and BMI was conducted to estimate the overlap between both traits and to disentangle the proportion of covariance due to shared and specific genetic and environmental factors.

In chapter 5, the association between four candidate genes and two types of eating disorders (AN and eating disorders characterized by SV) were evaluated in participants from the GenED study as cases and random twin-based controls from the NTR. Two candidate genes, namely brain-derived neurotrophic factor (*BDNF*) and serotonin receptor 1D (*HTR1D*) were selected because of previous promising associations in eating disorders. In addition, the genes encoding for tryptophan hydroxylase 2 (*TPH2*) and stathmin (*STMN1*) were selected. *TPH2* is the rate-determining enzyme in the synthesis of serotonin in the brain (Walther & Bader, 2003). *TPH2* was previously associated with depression and anxiety (Barnett & Smoller, 2009; Kim et al., 2009; Tsai et al., 2009; Zhang et al., 2006), which are both highly comorbid with eating disorders (Godart et al., 2000; Hudson et al., 2007; Kaye et al., 2004a; Raney et al., 2008). *STMN1* is located under the linkage peak for AN at 1p33-36. This gene is involved in the control of both learned and innate fear in mice (Shumyatsky et al., 2005), and fear and anxiety processing in humans (Brocke et al., 2010). Replication occurred in a meta-analysis with two additional independent eating disorder case-control samples from Germany and the Netherlands together providing 887 participants with AN, 306 participants with an eating disorder characterized by SV and 1914 controls.

In chapter 6 we explore the hypothesis that *TPH2* affects perfectionism or impulsivity which predisposes to the AN and or SV phenotype. First a phenotypic analysis was

performed to confirm the association between perfectionism, impulsivity and eating disorders, in participants from the GenED study and a control group of women without an eating disorder. Then genotypic analyses were conducted. First, four *TPH2* SNPs, that were previously associated to AN and/or SV, were tested for association with perfectionism and/or impulsivity in twin-based controls from the NTR. The SNPs that showed an association with perfectionism and/or impulsivity were subsequently tested for association with these features in the participants with an eating disorder.

In the final chapter of this thesis, a concise summary of the main findings is given followed by a discussion of the results in light of limitations, clinical implications and future research plans.

Chapter 2

Eating disorders: from twin studies to candidate genes and beyond

This chapter was previously published:
Slof-Op 't Landt, M.C.T., van Furth, E. F., Meulenbelt, I., Slagboom, P. E., Bartels, M., Boomsma, D. I., & Bulik, C. M. (2005). *Twin Research and Human Genetics, 8,* 467-482.

Abstract

Substantial effort has been put into the exploration of the biological background of eating disorders, through family, twin and molecular genetic studies. Family studies have shown that anorexia (AN) and bulimia nervosa (BN) are strongly familial, and that familial aetiologic factors appear to be shared by both disorders. Twin studies often focus on broader phenotypes or subthreshold eating disorders. These studies consistently yielded moderate to substantial heritabilities. In addition, there has been a proliferation of molecular genetic studies that focused on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 1994) AN and BN. Seven linkage regions have been identified in genome-wide screens. Many genetic association studies have been performed, but no consistent association between a candidate gene and AN or BN has been reported. Larger genetic association studies and collaborations are needed to examine the involvement of several candidate genes and biological pathways in eating disorders. In addition, twin studies should be designed to assist the molecular work by further exploring genetic determinants of endophenotypes, evaluating the magnitude of contribution to liability of measured genotypes as well as environmental risk factors related to eating disorders. In this manner twin and molecular studies can move the field forward in a mutually informative way.

Eating disorders are distinguished into anorexia nervosa (AN), bulimia nervosa (BN) and eating disorders not otherwise specified (EDNOS). The diagnostic features of AN include severe underweight and an intense fear of gaining weight or becoming fat. BN is characterized by recurrent episodes of binge eating and compensatory behaviors. Binge eating disorder, the most studied form of EDNOS, is characterized by the occurrence of binge eating in the absence of compensatory behaviors.

AN and BN mainly affect young women (90 to 95%) and generally start during puberty or early adulthood. The prevalence among women between 15 and 24 years of age is estimated to be 0.3% for AN and 1% for BN in industrialized countries (Hoek, 1993; Van Hoeken et al., 1998). Chronicity and mortality of patients with eating disorders are among the highest of all psychiatric illnesses (Harris & Barraclough, 1998; Nielsen et al., 1998; Sullivan, 1995). Long-term follow-up studies show a mortality rate of 5% per decade. Mortality from AN is five times higher than in a comparable age group (Harris & Barraclough, 1998). Recent studies show that even after 10 years, 20% of the individuals continue to fulfil diagnostic criteria for an eating disorder, while another 30% show partial symptoms (Steinhausen, 1999; Steinhausen, 2002).

Despite substantial efforts to identify causal pathways for AN and BN, very little is known about the etiology of eating disorders. In longitudinal and cross-sectional studies, several risk factors have been identified, including gender, elevated weight and shape concerns, negative body image, negative self-evaluation and dieting (Jacobi et al., 2004). For AN, high-level exercise and perfectionism have been identified as additional risk factors.

To explore the role of biology in the etiology of eating disorders, numerous studies have been performed (for a review see Bulik & Tozzi, 2004; Tozzi & Bulik, 2003). Family studies have been conducted to examine if relatives of patients with AN or BN have an increased risk for developing an eating disorder. Family studies cannot distinguish between the genetic and environmental contribution to familial aggregation. In contrast, in twin studies, the similarities and differences between monozygotic (MZ) and dizygotic (DZ) twins allow us to delineate the nature and magnitude of genetic and environmental influences for a particular trait. In the first section, we will review family and twin studies of AN and BN.

Evidence of heritability justifies efforts to identify genetic loci that influence the risk for a trait or disorder. The last two decades have witnessed a proliferation of linkage and association studies of eating disorders. In the majority of studies candidate genes were examined based on their hypothesized function in the etiology of eating disorders. The second part of this paper focuses on the genetic studies of eating disorders.

Family and Twin studies

All but one of the family studies of AN and BN (Logue et al., 1989) reported increased rates of eating disorders in relatives of patients (Biederman et al., 1985; Gershon et al., 1983; Grigoroiu-Serbanescu et al., 2003; Halmi et al., 1991; Herpertz-Dahlmann, 1988; Hudson et al., 1987; Kassett et al., 1989; Lilenfeld et al., 1998; Stern et al., 1992; Strober et al., 1985; Strober et al., 2000; Strober et al., 2001; Strober et al., 1990). Strober et al. (2000) performed the most extensive family study in eating disorders, using phenotypes based on Diagnostic and Statistical Manual of Mental Disorders (4th ed.; DSM-IV; American Psychiatric Association, 1994) criteria. They compared the rate of eating and other psychiatric disorders in the relatives of 152 restrictive AN patients, 171 BN patients and 181 healthy controls. The risk of developing AN was 11.4 times increased in relatives of AN patients compared to relatives of healthy controls, whereas the risk for BN in relatives of AN patients was 3.5 times increased. Relatives of BN patients had a 3.7 times increased risk for developing BN, and a 12.1 times increased risk for developing AN. Not only do these results suggest that AN and BN are familial, but also that familial etiological factors appear to be shared by AN and BN.

The first heritability studies in twins of AN and BN were clinical case reports and systematic studies of clinically ascertained twins (see the first section in Table 2.1; (Askevold & Heiberg, 1979; Fichter & Noegel, 1990; Holland et al., 1984; Holland et al., 1988; Hsu et al., 1990; Nowlin, 1983; Treasure & Holland, 1990). The criteria applied to define AN and BN varied considerably between these studies, and none were based on DSM-IV. Nevertheless, for AN and BN, the concordance of MZ twins was greater than for DZ twins, which was more pronounced in AN. Bulik et al. (2000), reanalyzed the clinical twin studies of BN and fitted a full ACE model (including genetic, common environmental and unique environmental factors) to the data from these reports. In this analysis, concordance rates of 46% in MZ and 26% in DZ twins resulted in a heritability estimate of 47% (95% confidence interval [CI] 0-66). It is important to note that clinical cases are likely to be more severely affected, and may differ from community cases in other ways that might bias the results of genetic investigations (Kendler, 1993). The results obtained in clinical studies may therefore not be extrapolated to the total population of affected individuals. But they may be suitable in detecting a pathway which leads to the onset of AN or BN.

Three different population-based twin registries assessed AN and/or BN, namely the Danish Twin registry (DTR; Kortegaard et al., 2001), the Minnesota Twin Family Study (MTFS; Klump et al., 2001) and the Virginia Twin Registry (VTR; Bulik et al., 1998;

Kendler et al., 1991; Kendler et al., 1995; Wade et al., 2000b; Walters et al., 1992; Walters & Kendler, 1995). Due to the low prevalence of both AN and BN, all studies used broader phenotypes to boost statistical power. The criteria used to define these broader phenotypes varied considerably between registries. Partial DSM-IV and Diagnostic and Statistical Manual of Mental Disorders (3rd ed., rev.; DSM-III-R; American Psychiatric Association, 1987) criteria were used in the MTFS and the VTR respectively, whereas the definitions used in the DTR were not based on DSM criteria at all. The reported prevalence for broad AN phenotypes ranged from 2.7% to 4.9% (Klump et al., 2001; Kortegaard et al., 2001; Wade et al., 2000b; Walters & Kendler, 1995), all exceeding the estimated population prevalence of 0.3% by far. For the broad BN phenotype the prevalences were around 4% (Bulik et al., 1998; Kendler et al., 1991; Kendler et al., 1995; Kortegaard et al., 2001), again considerably higher than the 1% population prevalence.

In the second section of Table 2.1, the best-fitting and full twin models of broad AN (if presented in the original study) are shown. In the best-fitting models, the heritability estimates in the DTR ranged from 48% to 52% (Kortegaard et al., 2001), and in the MTFS it was estimated at 76% (Klump et al., 2001). Moreover, Wade et al. (2000b) reported a heritability estimate of 58%, using bivariate analyses in the VTR. In all studies, the remaining variance was accounted for by unique environmental factors (Klump et al., 2001; Kortegaard et al., 2001; Wade et al., 2000b).

The best-fitting and full twin models of broad BN are listed in the final part of Table 2.1. Both univariate and bivariate twin analyses of broad BN (Bulik et al., 1998; Kendler et al., 1991; Kortegaard et al., 2001; Walters et al., 1992), consistently yielded high heritability estimates, ranging from 50% (Walters et al., 1992) to 70% (Bulik et al., 1998). In these studies, individual specific environmental components accounted for the remaining variance. In a multivariate analysis, examining BN in combination with five other psychiatric disorders (Kendler et al., 1995), a heritability estimate of 30% for broadly defined BN was obtained. Shared genetic factors were identified among BN, phobia and generalized anxiety disorder.

Statistical power can be enhanced by incorporating two occasions of measurement into a twin study. Bulik et al. (1998), applied this method using data from the first and third wave of data collection in the VTR to assess BN. The reliability between these two occasions of measurement was low (κ=.28), suggesting that single assessments of the lifetime history of broad BN are prone to error. When the error of measurement was incorporated into the structural equation twin model, a heritability of 83% was estimated for broad BN.

Table 2.1. Twin studies in eating disorders. Best-fitting and full twin models are shown including 95% confidence intervals, when reported in the original studies.

Subject	Sample size		Concordance		Model			Reference	Note
	MZ	DZ	MZ	DZ	a2	c2	e2		
Clinical studies									
AN	16	-	0.55	-				(Askevold et al., 1979)	
	24	2	0.59	0				(Nowlin, 1983)	Review, criteria unclear
	16	14	0.72	0.13				(Holland et al., 1984)	Female-female pairs, Crisp & Russel (1970)
	25	20	0.71	0.10	98 (se ±12)			(Holland et al., 1988)	Female-female pairs, ICD 9
	34	26	0.68	0.08	100	-	-	(Treasure et al, 1990)	Female-female pairs, EDE
BN	14	17	0.35	0.29	10	60	30	(Treasure et al., 1990)	Female-female pairs, EDE
	6	2	0.50	0				(Hsu et al., 1990)	Female-female pairs, DSM-III-R
	6	15	0.91	0.42				(Fichter et al., 1990)	Female-female pairs, DSM-III-R
Twin registries									
AN	590	440	0.10	0.22				(Walters et al., 1995)	VTR, wave 1, clinical broad AN
(broad)	196	105	0.40	0	74 (0-94)	0 (0-65)	27 (6-67)	(Klump et al., 2001)	MTFS, DSM-IV, full model
	196	105	0.40	0	76 (35-95)	-	24 (5-65)	(Klump et al., 2001)	MTFS, DSM-IV, best-fitting model
	190a	248a	0.18 (.10-.27)	0.07 (.02-.13)	48 (27-65)	-	52	(Kortegaard et al., 2001)	DTR, definition based on 1 item, best-fitting model
	190a	248a	0.25 (.18-.33)	0.13 (.08-.17)	52 (38-65)	-	36	(Kortegaard et al., 2001)	DTR, definition based on 2 items, best-fitting model
	597	433			28 (0-82)	27 (0-67)	45 (17-70)	(Wade et al., 2000)	VTR, DSM-III-R, bivariate analysis major depression, ra=0.81, full model
	597	433			58 (33-84)	-	42 (16-68)	(Wade et al., 2000)	VTR, DSM-III-R, bivariate analysis major depression, ra=0.58, best-fitting

BN	590	440	0.23	0.09	55	0	45	(Kendler et al., 1991)	VTR, wave 1, DSM-III-Rb, full model
(broad)	590	440	0.23	0.09	55	-	45	(Kendler et al., 1991)	VTR, wave 1, DSM-III-Rb, best-fitting model
	590	440	0.26	0.16	38	13	50	(Kendler et al., 1991)	VTR, wave 1, DSM-III-Rc, full model
	590	440	0.26	0.16	52	-	48	(Kendler et al., 1991)	VTR, wave 1, DSM-III-Rc, best-fitting model
	497	354			60	-	40	(Bulik et al., 1998)	VTR, wave 1, DSM-III-Rd, best-fitting model
	497	354			68	-	32	(Bulik et al., 1998)	VTR, wave 3, DSM-III-Rd, best-fitting model
	190a	248a	0.26 (.16-.35)	0.11 (.04-.17)	61 (44-75)	-	24	(Kortegaard et al., 2001)	DTR, best-fitting model
	590	440			35	22	43	(Walters et al., 1992)	VTR, wave 1, DSM-III-Rc, bivariate analysis major depression, ra=0.26, full model
	590	440			50	-	50	(Walters et al., 1992)	VTR, wave 1, DSM-III-Rc, bivariate analysis major depression, ra=0.46, best-fitting model
	590	440			30	41	29	(Kendler et al., 1995)	VTR, wave 1, DSM-III-Rc, multivariate analysis, best-fitting model
	497	354			83 (64-100)	-	17 (0-36)	(Bulik et al., 1998)	VTR, wave 1 & 3, DSM-III-Rd, incorporation of 2 occasions of measurement, best-fitting model

Note: Best-fitting and full twin models are shown including 95% confidence intervals when reported in the original studies.

Affected twin pairs: at least one twin endorsed one or more of the eating disorder questions.

Definition broad BN: definite and probable cases BN based on DSM-III-R.

Definition broad BN: definite, probable and possible cases BN based on DSM-III-R

Definition broad BN: all DSM-III-R criteria minus criterion D (minimum frequency of binge eating).

Due to the low prevalence of AN and BN, and subsequently the small numbers of affected twins in the VTR and MTFS samples, the statistical power, even when using broader phenotypes, was far from adequate in these studies as reflected in the broad confidence intervals around the heritability estimates (Neale et al., 1994). Although, the sample sizes in the DTR were much larger, the statistical power in this study was still insufficient and the diagnostic criteria suboptimal.

In summary, family studies have consistently shown that AN and BN are strongly familial. The twin studies in AN and BN are indicative of a modest to considerable role of genetic factors in the etiology of broadly defined eating disorders, component behaviours, and related traits. Due to the relatively low prevalence of AN and BN, whether a threshold DSM-IV diagnosis represents a more heritable phenotype than the broad definition phenotypes used remains unknown.

Molecular Genetic Studies

Linkage studies

In linkage analysis, the segregation of alleles in families together with the affected phenotypes is investigated to localize disease genes or, in general, genes that influence a quantitative trait (Slagboom & Meulenbelt, 2002). Relevant for the studies into AN and BN are the genome-wide marker data that are used to establish the extent of allele sharing in AN and BN affected sibling pairs by applying either a model-free or model-based linkage analysis method. To identify areas of the human genome that may harbour susceptibility loci for AN and BN, genome-wide screens have been conducted using polymorphic markers spanning all chromosomes, in a collaboration of the Price Foundation (Bulik et al., 2003a; Devlin et al., 2002; Grice et al., 2002; Kaye et al., 2000b; Kaye et al., 2004b).

For AN, probands were included in the study if they had a lifetime diagnosis of AN, based on DSM-IV criteria minus criterion D (amenorrhea) with a duration of at least 3 years, whereas relatives of AN probands needed to fulfill DSM-IV criteria for an eating disorder (either AN, BN or EDNOS). Using these criteria, a total of 229 affected relative pairs were recruited. To provide better identity by descent (IBD) estimation, DNA was collected from biological parents where possible (Kaye et al., 2000b).

The BN probands were required to have a lifetime diagnosis of BN, according to DSM-IV criteria, purging had to include regular vomiting, and bingeing and vomiting must have occurred at least twice a week for a duration of 6 months. To be included in the study, relatives were required to have a DSM-IV diagnosis of an eating disorder (either AN, BN

or EDNOS). A total of 365 affected relative pairs were recruited. Again DNA was collected from biological parents where possible (Kaye et al., 2004b).

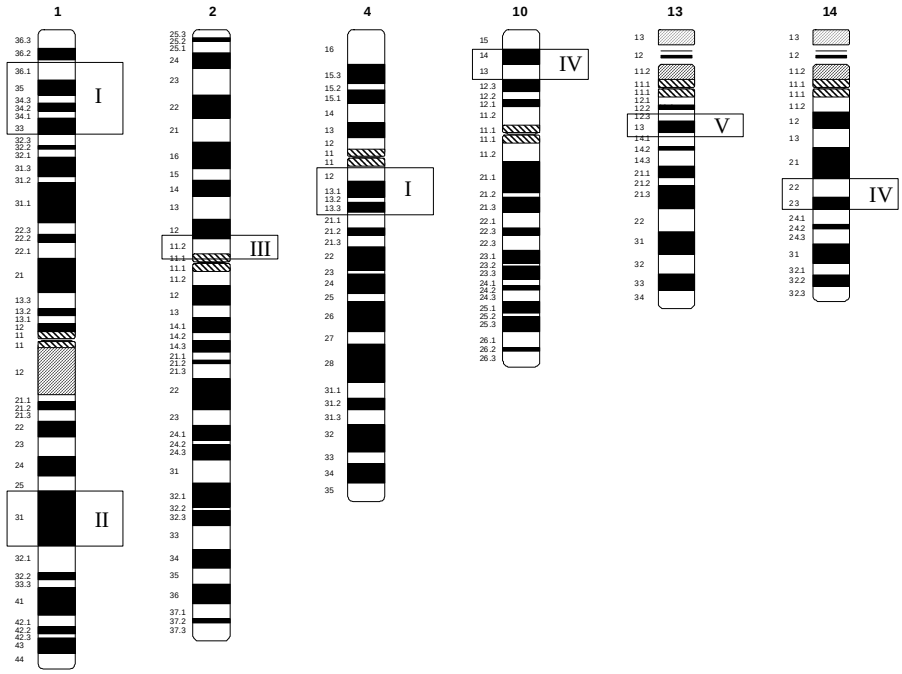


Figure 2.1. Linkage results of the genome-wide scans in eating disorders, with LOD-scores ≥ 1.97.

I Anorexia nervosa restrictive subtype (chromosome 1 NPL = 3.03, chromosome 4 NPL = 2.44)

II Anorexia nervosa, drive for thinness and obsessionality combined (LOD = 3.46)

III Anorexia nervosa and obsessionality (LOD = 2.22)

IV Bulimia nervosa (chromosome 10 MLS = 2.92, chromosome 14 MLS = 1.97)

V Anorexia nervosa and drive for thinness (LOD = 2.50)

Figure 2.1 shows the linkage regions that were obtained in genome-wide screens (linkage scores of 1.97 or greater) performed in these studies of AN and BN. In the total sample of AN affected relative pairs, none of the linkage peaks had a nonparametric linkage (NPL) score above 1.80. Linkage analysis performed in the restrictive subtype of AN (n = 37 families), however, yielded a linkage peak at 1p33-36 (NPL score = 3.03), providing suggestive evidence and a peak at 4q12-14 (NPL score = 2.44; Bergen et al., 2003; Grice et al., 2002). Within the AN sample, a quantative trait locus (QTL) analysis was subsequently performed with drive for thinness and obsessionality (Devlin et al., 2002) that showed three suggestive linkages on chromosome 1q31 (Logarithm of the Odds [LOD] score = 3.46 for

Drive for Thinness and Obsessionality combined), 2p11 (LOD score = 2.22 for Obsessionality), and 13q13 (LOD score = 2.50 for Drive for Thinness), respectively.

For the entire BN sample, three regions of linkage were reported, significant linkage was achieved on chromosome 10p13 (nonparametric multipoint maximum LOD score [MLS] = 2.92), and suggestive evidence for linkage was reported for 10p14 (MLS = 2.70) and 14q22-23 (MLS = 1.97). In addition, linkage analysis was performed in a subset of 133 families, in which at least two affected individuals reported regular vomiting behavior. This phenotype was chosen because vomiting is associated with a more reliable reporting of BN (Wade et al., 2000a), and because of its substantial heritability (Sullivan et al., 1998a). The analysis in this subset did not yield any different regions of linkage, but did increase the linkage peak on chromosome 10p13 (LOD score = 3.39).

Genetic Association Studies

In a genetic association study, candidate genes that are suggested to be involved in the pathophysiology of the disease can be investigated for their role in the onset of the trait. In such studies, the allele or genotype frequencies at markers, single nucleotide polymorphisms (SNP), are determined in affected individuals and compared to those of controls (either population- or family-based). Association studies are known as an effective approach to detect the effect of variants within candidate genes with relatively small effects (Slagboom & Meulenbelt, 2002).

A variety of genes have been tested in association studies in eating disorders. Most genes can be classified in three main biological pathways, namely the serotonin pathway, the catecholamine pathway, and the pathway involved in neuropeptide and feeding regulation. The serotonin pathway has been most intensively studied in eating disorders. It is involved in a broad range of biological, physiological and behavioral functions (Blundell, 1992; Blundell et al., 1995; Halford & Blundell, 2000; Lucki, 1998; Simansky, 1996). Serotonin is involved in body weight regulation and more specifically in eating behavior. In both rodents and humans, drugs that either directly or indirectly increased postsynaptic serotonergic stimulation routinely decreased the consumption of food (Lucki, 1998; Simansky, 1996). The serotonin pathway has also been implicated in the development of eating disorders (Brewerton & Jimerson, 1996; Kaye, 1997; Monteleone et al., 2000; Weltzin et al., 1994). In long-term weight-recovered patients with AN or BN, levels of 5-hydroxyindolacetic acid (5-HIAA), a metabolite of serotonin, were elevated in cerebrospinal fluid compared to those of controls (Kaye et al., 1991; Kaye et al., 1998). These results suggest that hyperserotonergic activity is a trait marker in eating disorders. It is hypothesized that increased brain serotonin activity could predispose to the development

of eating disorders. In addition, serotonin might also contribute to the psychopathological features of eating disorders such as perfectionism, rigidity and obsessionality (Hinney et al., 2000; Kaye, 1997; Kaye et al., 2000a).

Within the serotonin system, the serotonin 2A receptor gene (*HTR2A*) has received most attention in association studies. *HTR2A* is regulated by estrogen (Fink & Sumner, 1996), which could render a possible explanation why 90 to 95% of those affected by AN are female and why the onset of the disorder is peripubertal. In addition, it has been reported that recovered patients with AN and BN have a reduced *HTR2A* receptor binding compared to healthy controls (Frank et al., 2002; Kaye et al., 2001). These findings suggest that alterations in the *HTR2A* gene might be involved in the etiology of eating disorders.

The eating disorder field is characterized by the performance of many genetic association studies in small samples that often present contradicting results (for references see Appendix D). In the majority of the studies, AN and BN were diagnosed according to DSM-IV criteria. The general problem of these studies is their lack of statistical power. When recalculating the power of these studies, assuming a dominant model with an allele frequency of .10 and a relative risk of 2, 93% of the studies (53 out of the 57) do not have adequate statistical power to detect an effect. To obtain a power of 80% under these assumptions, at least 178 cases and 178 controls are required.

An example of the inconsistency in smaller studies is the G-1438A polymorphism in the promoter region of the *HTR2A* gene. In Caucasians, six case-control association studies reported a positive association of the G-1438A polymorphism and AN (Collier et al., 1997; Enoch et al., 1998; Nacmias et al., 1999; Ricca et al., 2002; Ricca et al., 2004; Sorbi et al., 1998). In these studies, a higher frequency of the –1438A allele and/or –1438 A/A genotype in anorectics compared to controls, was reported. However, six other studies, also performed in Caucasian populations, did not confirm this finding (Campbell et al., 1998; Hinney et al., 1997; Karwautz et al., 2001; Kipman et al., 2002; Rybakowski et al., 2004; Ziegler & Gorg, 1999). Finally, two Japanese studies also did not detect association between the G-1438A polymorphism and AN (Ando et al., 2001; Nishiguchi et al., 2001).

Meta analyses provide a quantitative approach for combining results from different studies on the same topic. Systematic meta-analyses can be a useful tool in estimating population-wide effects of genetic risk factors in human disease (Ioannidis et al., 2001). So in response to the apparent discrepancies mentioned above, three meta-analyses have been performed (Collier et al., 1999; Gorwood et al., 2003; Ziegler et al., 1999). Collier et al. (1999) conducted a meta-analysis on four association studies (Campbell et al., 1998; Collier et al., 1997; Enoch et al., 1998; Sorbi et al., 1998). In this analysis, a significant association between the -1438A/A genotype and AN, with an odds ratio of 2.29, was reported. Ziegler

et al. (1999) performed a meta-analysis based on six studies (Campbell et al., 1998; Collier et al., 1997; Enoch et al., 1998; Hinney et al., 1997; Sorbi et al., 1998; Ziegler & Gorg, 1999) including the four studies used in the first meta-analysis (Collier et al., 1999), and the study by Hinney et al. (1997) which did not have a normal weight control group. This meta-analysis did not reveal a significant association between the *HTR2A* promoter polymorphism and AN. The third meta-analysis (Gorwood et al., 2003) was based on nine studies (Campbell et al., 1998; Collier et al., 1997; Enoch et al., 1998; Hinney et al., 1997; Kipman et al., 2002; Nacmias et al., 1999; Nishiguchi et al., 2001; Sorbi et al., 1998; Ziegler & Gorg, 1999) including the studies used in the meta-analysis by Ziegler et al. (1999). One of the additional studies was a Japanese study (Nishiguchi et al., 2001). In this analysis, the frequency of the –1438A allele in patients with AN was significantly higher compared to controls.

An important issue concerning the meta-analyses is whether it is justified to combine the association studies. First, there is a difference in the type of control group used. Hinney et al. (1997) used a group of obese and a group of underweight participants as the control group, while all other studies used a normal weight control sample. Second, A-allele frequencies found in the Japanese controls (Nishiguchi et al., 2001), were higher (.54) than those reported in the Caucasian control samples (.30-.48). In addition, the frequencies of the -1438 A allele in the different Caucasian control samples shows substantial differences, ranging from .30 (Ziegler & Gorg, 1999) to .48 (Kipman et al., 2002). The difference of the -1438 A allele in AN patients is even greater, ranging from .29 (Ziegler & Gorg, 1999) to .57 (Sorbi et al., 1998). These fluctuating allele frequencies are almost inherent to small samples because none of the studies are able to give an accurate estimate of the allele frequencies in the population.

The performance of these meta-analyses does therefore not appear to solve the problem of interpreting the excess of these small inconsistent association studies in AN and BN. There is still a discrepancy between the results, and the choice of study-inclusion criteria remains arbitrary. A solution to this problem is the performance of larger genetic association studies with adequate statistical power to detect an effect. Two large collaborative studies have tested several candidate genes for association in both AN and BN (Table 2.2).

Following up their genome-wide screen in AN (Grice et al., 2002), the collaborative study mentioned previously performed an association study focusing on candidate genes positioned under the linkage peak on chromosome 1p33-36 (Bergen et al., 2003). For their association study, three candidate genes were tested namely the serotonin receptor 1D (*HTR1D*), opioid receptor delta 1 (*OPRD1*) and hypocretin receptor 1 (*HCRTR1*). The case-

control study (based on 196 cases, 98 controls and DSM-IV criteria) yielded a positive association between one *HTR1D* (C1080T) and three *OPRD1* (T8214C, G23340A, and A47821G) polymorphisms in AN. The subsequent Transmission Disequilibrium Test (TDT) analysis confirmed an association only for the *OPRD1* A47821G SNP. However, after correction for multiple testing only the association with the *HTR1D* C1080T polymorphism remained statistically significant.

Another large European collaboration tested the *HTR2A* (G-1438A SNP), the catechol-o-methyltransferase (*COMT*; Val-158-Met SNP), and the brain-derived neurotrophic factor (*BDNF*; C-270T and Val-66-Met SNPs) genes for association in eating disorders, defined according to DSM-IV criteria (Gabrovsek et al., 2004; Gorwood et al., 2002; Ribases et al., 2004; Ribases et al., 2005). A significantly higher frequency of the Met-66-Met genotype and the Met-66 allele was reported in both AN and BN cases compared to controls (Ribases et al., 2004; Ribases et al., 2005). None of the other polymorphisms were associated with eating disorders, although the TDT analysis did reveal an excess of transmission of the -270C/Met-66 haplotype in the *BDNF* gene in restrictive AN.

There is no overlap between the candidate genes studied in the two collaborations. Subsequently, the positive results reported for the candidate genes above are promising, but unless they are replicated in an independent sample, there is not enough evidence for a definite association. In addition, the only overlap between reported linkage regions and studied candidate genes is from the study by Bergen et al. (2003). Interestingly, the *HTR2A* gene is located near the linkage peak on chromosome 13q13.

In summary, the eating disorder field is characterized by an excess of small genetic association studies. In order to overcome the problems discussed above, association studies should be performed based on large sample sizes. Because of the low prevalence of eating disorders, it is not an easy task to collect large numbers of patients. The establishment of more collaborations between centers could help to solve this problem. Another important issue is the choice of appropriate candidate genes or biological pathways. Selection of candidate genes can be based on their biological function and/or their location within the genome. The serotonergic system remains one of the most intriguing biological pathways given the role that serotonin plays in appetite and mood regulation and given the recent findings from neuroimaging studies (Frank et al., 2002; Kaye et al., 1998; Kaye et al., 2001). However, little is known about the biological underpinnings of eating disorders, and many other biological pathways may be involved.

Table 2.2. Candidate gene studies performed by collaborations.

Gene	Polymorphism	Phenotype	N	P-value[a]	Reference	Note
Serotonin						
Serotonin Receptor 1D,	C1080T	AN	196	0.01	(Bergen et al.,	OR 2.63, TDT NS
HTR1D,		Controls	98	0.01 (geno)	2003)	USA, UK and Germany
1p36	A2190G	AN	196	NS	(Bergen et al.,	OR 1.37, TDT 0.04
		Controls	98		2003)	USA, UK and Germany
	T-628C	AN	196	NS	(Bergen et al.,	OR 0.72, TDT 0.01
		Controls	98		2003)	USA, UK and Germany
	T-1123C	AN	196	NS	(Bergen et al.,	OR 0.73, TDT 0.02
		Controls	98		2003)	USA, UK and Germany
Serotonin Receptor 2 A,	G-1438A	AN	316	NS	(Gorwood	TDT and HHRR, France,
HTR2A,13q14	(rs6311)		(trios)		et al., 2002)	Germany, UK, Italy and Spain
Catecholamine						
Catechol-o-methyltransferase,	Val-158-Met	AN	266	NS	(Gabrovsek	OR 0.98, TDT NS
COMT, 22q11	(rs4680)	Controls	418		et al., 2004)	Austria, Germany, Italy,
						Slovenia, Spain and UK
Neuropeptide & feeding regulation						
Hypocretin Receptor 1	C114T	AN	196	NS	(Bergen	Germany, UK and USA
Orexin 1 receptor,	(rs1056526)	Controls	98		et al., 2003)	
HCRTR1, 1p35	A846G	AN	196	NS	(Bergen	Germany, UK and USA
		Controls	98		et al., 2003)	
	A7757G	AN	196	NS	(Bergen	Germany, UK and USA
		Controls	98		et al., 2003)	
	C8793T	AN	196	NS	(Bergen	Germany, UK and USA
		Controls	98		et al., 2003)	
Opioid receptor delta-1	T80G	AN	196	NS	(Bergen	OR 0.98, TDT NS
OPRD1, 1p35	(rs1042114)	Controls	98		et al., 2003)	Germany, UK and USA

	T8214C	AN	196	0.045	(Bergen	OR 1.46, TDT NS
	(rs536706)	Controls	98		et al., 2003)	Germany, UK and USA
	G23340A	AN	196	0.046	(Bergen	OR 0.68, TDT NS
	(rs760589)	Controls	98		et al., 2003)	Germany, UK and USA
	A47821G	AN	196	0.01	(Bergen	OR 0.61, TDT 0.06
	(rs204081)	Controls	98	0.03 (geno)	et al., 2003)	Germany, UK and USA
	A51502T	AN	196	NS	(Bergen	OR 0.70, TDT 0.06
	(rs204076)	Controls	98		et al., 2003)	Germany, UK and USA
Other candidate genes						
Brain Derived	C-270T	AN unclassified	98	NS	(Ribases	France, Germany, Italy,
Neurotrophic Factor,		AN restrictive	347		et al., 2004)	Spain and UK
BDNF,		AN binge/purge	308			
11p13-14		BN	389			
		Controls	510			
		AN restrictive	219	NS	(Ribases	HRR / TDT
		AN binge/purge	140		et al., 2005)	Austria, France, Germany, Italy, Slovenia, Spain, UK
	Val-66-Met	AN unclassified	98	0.0008 (AN vs C; geno)	(Ribases	OR AN 1.37 (Met-allele)
	(rs6265)	AN restrictive	347	0.003 (ANr vs C; geno)	et al., 2004)	OR ANr 1.43 (Met-allele)
		AN binge/purge	308	0.012 (ANbp vs C;geno)		OR ANbp 1.29 (Met-allele)
		BN	389	<0.001 (BN vs C;geno)		OR BN 1.59 (Met-allele)
		Controls	510			France, Germany, Italy, Spain, UK
		AN restrictive	219	0.019	(Ribases	HRR and TDT
		AN binge/purge	140	(ANr vs C; HRR)	et al., 2005)	Austria, France, Germany , Italy, Slovenia, Spain, UK

[a.] P-values are reported for the allele-wise association of the polymorphism, unless stated otherwise.

Discussion

In the last decade, the number of studies focusing on the role of genetics in the etiology of eating disorders has increased enormously. This increase has been met by some successes in molecular genetic studies, although the majority of results remain inconclusive. Genome-wide screens have demonstrated linkage peaks for AN and BN on chromosomes 1p33-36, 4q13, 10p13, and 14q22-23. Furthermore, one collaborative study has incorporated behavioral covariates into their linkage analyses and identified three additional suggestive linkage peaks (chromosome 1q31, 2p11 and 13q13). To confirm the linkage findings, further replication studies are needed. Lander and Kruglyak (1995) propose that the significant linkage from one or a combination of initial studies should be confirmed in a further sample, preferably by an independent group of investigators. Since all of the linkage studies in eating disorders have been performed by the same international multicenter collaboration, replication in an independent sample is warranted.

Many genetic association studies have been conducted in eating disorders without any definite conclusion. Typical of the association studies in this field are the excess of small, discrepant studies. In the future, more SNPs should be measured per candidate gene to truly test a gene for association. For example in the *HTR2A* gene, the G-1438A polymorphism has been tested for association in 15 studies. Only two of these studies tested four other SNPs within this gene for association (Hinney et al., 1997; Nacmias et al., 1999), and none of the studies examined haplotypes. The only conclusion that can be drawn from these studies is that the G-1438A polymorphism is not associated with eating disorders however the *HTR2A* gene could still be involved. In addition to more SNPs per candidate gene, more candidate genes per pathway should also be examined. The linkage results in eating disorders should be used more extensively to identify new candidate genes and pathways. Because of the rarity of eating disorders, it is nearly impossible to identify biological pathways involved in the etiology by utilizing prospective study designs. The majority of the current candidate genes and biological pathways in eating disorders are adopted from research in other psychiatric disorders (e.g., the serotonin system in depression and anxiety disorders). The comorbidity between depression, anxiety disorders and eating disorders appears to have a substantial genetic component (Kendler et al., 1995; Rowe et al., 2002; Wade et al., 2000b; Walters et al., 1992), but this does not necessarily mean that the genes involved in these disorders will also play a role in AN and BN. Another method of identifying biological pathways possibly involved in the etiology of eating disorders is based on studies of individuals who have recovered from AN and BN.

One of the most striking features of the family, twin and molecular genetic research in eating disorders is the difference in phenotypes used in the various study designs. Practically all linkage and genetic association studies used DSM-IV criteria to define AN and BN. The strong familiality of the DSM-IV eating disorders has been demonstrated in family studies (Strober et al., 2000; Strober et al., 2001). However, twin studies, both clinical and population-based, have not been able to obtain an adequate number of affected twins to determine the heritability of eating disorders according to DSM-IV criteria. Twin studies did yield moderate to substantial heritability estimates for the broader eating disorder phenotypes. However, even when using broader, more prevalent phenotypes, the studies were still characterized by low statistical power.

One critical question is how best to integrate twin and molecular genetic studies when focusing on a relatively rare phenotype. On one hand there is interest in determining the contribution of genetic and environmental factors to liability to threshold DSM-IV diagnostic categories. This approach could tell us whether the threshold diagnoses in some way represent more genetically homogeneous entities than broader or subthreshold entities. Alternatively, greater genetic homogeneity may best be determined by focusing more on component behaviors rather than syndromes. There is no a priori reason to believe that the DSM diagnostic schema represent more 'genetic' syndromes than underlying core behaviors or traits. Indeed, Reichborn-Kjennerud et al (2004b; 2004a) have suggested that familial resemblance for different symptoms of bulimia nervosa, as codified by DSM-IV criteria, may have distinct sources. Binge-eating and vomiting represent more genetically mediated symptoms and undue importance of weight as an indicator of self-evaluation representing a more environmentally mediated symptom. Thus, our clinically determined diagnostic criteria may actually represent frequently co-occurring mixtures of genetically and environmentally influenced symptoms which could potentially obscure searches for susceptibility loci.

Moreover, several studies have demonstrated the presence of an underlying continuum of liability for eating disorders (Fairburn & Harrison, 2003; Hay & Fairburn, 1998; Kendler et al., 1991; Sullivan et al., 1998b; Walters & Kendler, 1995), and have indicated that the diagnostic entities and thresholds of the DSM-IV do not adequately capture the eating disordered behavior. Thus, both twin and molecular genetic studies should pay careful attention to the definition of phenotypes especially when dealing with a relatively rare set of disorders for which the underlying neurobiology is not yet fully understood.

Moving forward, what additional value might twin studies have in the field of eating disorders in the age of molecular genetics? Twin studies can be used to identify heritabilities and genetic determinants (loci) of eating disorder-related phenotypes

(endophenotypes) and risk factors or intermediate phenotypes for AN and BN. Loci for eating disorder-related traits can next provide new candidate gene loci to be tested for association in strict AN and BN phenotypes, applying a case-control design in collaborative studies.

The twin study design also enables exploration of gene-environment interactions. Most traditional genetic analyses assume that the effects of genes and environment are additive and that the impact of genetic factors is equal across diverse environments (Kendler, 2001). However two other forms of the joint effect of genes and environment in the etiology of psychiatric illness are also possible: genes may control sensitivity to the environment and genes may alter the probability of exposure to environments (Kendler & Eaves, 1986). Because of the complex nature of the etiology of eating disorders, gene-environmental interactions are expected. Due to the low prevalence of eating disorders, gene-environment interactions should be explored in endophenotypes and intermediate phenotypes of AN and BN.

In conclusion, how can we conduct future studies that maximize our chances of identifying genes and environmental factors that play a role in the development of AN and BN? First, sample size is of critical importance: large (collaborative) genetic studies should be conducted. Second, twin studies to identify heritabilities and genetic determinants of endophenotypes, risk factors and intermediate phenotypes associated with eating disorders should be applied. A combination of twin and genetic designs may lead to enhanced understanding of the complex manner in which genes and environment interact to increase risk for or confer protection against serious psychiatric conditions such as AN and BN.

Chapter 3

Sex differences in sum score may be hard to interpret: the importance of measurement invariance

This chapter was previously published:
Slof-Op 't Landt MC, Dolan, C. V., Rebollo-Mesa, I., Bartels, M., van Furth, E. F., Van Beijsterveldt, C. E., Meulenbelt, I., Slagboom, P. E., & Boomsma, D. I. (2009). *Assessment, 16,* 415-423.

Abstract

In most assessment instruments, distinct items are designed to measure a trait, and the sum score of these items serves as an approximation of an individual's trait score. In interpreting group differences with respect to sum scores, the instrument should measure the same underlying trait across groups (e.g., male/female, young/old). Differences with respect to the sum score should accurately reflect differences in the latent trait of interest. A necessary condition for this is that the instrument is *measurement invariant*. In the current study we illustrated a stepwise approach for testing measurement invariance with respect to sex in a 4-item instrument designed to assess disordered eating behavior (DEB-scale) in a large epidemiological sample (1195 men and 1507 women). Our approach can be applied to other phenotypes for which group differences are expected. Any analysis of such variables may be subject to measurement bias if a lack of measurement invariance between grouping variables goes undetected.

Questionnaires are often used to assess psychological and behavioral traits on a quantitative scale. Well-known examples are the Beck Depression Inventory (Beck et al., 1961), Eysenck EPQ scales (Eysenck & Eysenck, 1975) and, the Temperament and Character Inventory (Cloninger et al., 1993). In these assessment instruments, items are designed to measure an underlying trait or latent (i.e., unobserved) variable and scores on the items are summed to derive a total score on the trait of interest. The Diagnostic and Statistical Manual of Mental Disorders (4th ed., American Psychiatric Association, 1994) also employs a weighted sum score in diagnosing psychiatric disorders.

When comparing groups, it is vital that an instrument measures the same underlying trait across groups (e.g., male/female, young/old). Observed group differences in the sum scores should accurately reflect group differences with respect to the latent variable. A necessary condition for this is that the instrument displays measurement invariance with respect to the groups under consideration (Mellenbergh, 1989; Meredith, 1993). If there is a sex difference with respect to the latent trait, men should for example score lower on all the items of the instrument measuring this trait. If however, men score lower on all the items but one, this one item displays differential item functioning, and the scale is not measurement invariant with respect to sex (Dolan, 2000; Mellenbergh, 1989; Meredith, 1993; Millsap & Yun-Tein, 2004). In that case, group differences in sum scores reflect, at least in part, measurement bias. The interpretation of differences between groups with respect to the sum scores thus hinges on the establishment of measurement invariance, or at least on the understanding of the violations, if any, of measurement invariance. Ideally, differences in sum scores should reflect true differences in the latent variable that the psychometric instrument purports to measure.

Measurement invariance can be investigated by fitting a measurement model that relates item scores to the underlying trait(s) across groups. Several methods have been suggested for both continuous and categorical variables (Dolan, 2000; Mellenbergh, 1989; Meredith, 1993; Millsap & Yun-Tein, 2004; Muthen & Asparouhov, 2002; Muthén & Muthén, 2005). In the current study we described a stepwise approach that was derived from previous studies to investigate measurement invariance for ordered categorical items. Our goal was to provide a comprehensive overview of the different steps accumulating into a model of complete measurement invariance. To illustrate this approach, we investigated whether a four item instrument, designed to measure disordered eating behavior is measurement invariant with respect to sex. As eating disorders mainly affect young women (90 – 95% of cases) (Fairburn & Harrison, 2003; Hoek, 1993; Van Hoeken et al., 1998), one might expect sex differences in the endorsement of the four eating disorder items.

Multi-group discrete factor analyses were applied to test whether the disordered eating behavior instrument is measurement invariant with respect to sex.

Method

Participants

All participants were registered with the Netherlands Twin Registry, which is maintained at the Department of Biological Psychology at the VU University in Amsterdam (Bartels et al., 2007; Boomsma et al., 2006). In this study, we used data from the 1986-1992 birth cohorts. In January 2005, questionnaires were sent to adolescent twins (mean age 15.2, *SD*=1.3) and their non-twin siblings (mean age 16.7, *SD*=2.8). The twins and siblings were asked to complete a survey containing items relevant for eating disorders. Questionnaires were sent to 2000 families. A total of 2175 twins (twin response rate 54.4%) and 527 siblings from 1144 families returned the questionnaire (family response rate 57.2 %). The total sample consisted of 1195 men and 1507 women (956 male twins, 1219 female twins, 239 brothers and 288 sisters, respectively), mean age was 15.5 (*SD*=1.8).

Measures

Participants filled out a self-report questionnaire containing measures of health and behavior (Bartels et al., 2007; Boomsma et al., 2006). The eating disorder section included four items: 1) dieting (Q: Have you ever gone on a diet to lose weight or to stop gaining weight?); 2) fear of weight gain (Q: How afraid are you to gain weight or become fat?); 3) importance of body weight or shape on self-evaluation (Q: How important are body weight and/or shape in how you feel about yourself?); 4) binge eating (Q: Have you ever had episodes of binge eating?). Responses were given on five point Likert-scales, ranging from ‘never’ to ‘always’ for dieting (DIET), from ‘not afraid’ to ‘extremely afraid’ for fear of weight gain (FEAR), from ‘not important’ to ‘most important’ for importance of body weight and shape on self-evaluation (ISE), and from ‘never’ to ‘more than once a week’ for binge eating (BE). For the multi-group confirmatory factor analyses it was essential that, for every item, each category was endorsed by both groups. Because none of the men reported that they were always on a diet, the fourth and fifth categories of the dieting item were merged. As a consequence, three items with five categories and one item with four categories were used in the analyses.

Data Analysis

We performed multi-group confirmatory factor analyses to establish whether the four eating disorder items formed a uni-dimensional scale, and whether the scale was measurement invariant with respect to sex. To conduct a confirmatory factor analysis, a minimum of three items is required. Measurement invariance with respect to sex held if the probability of a certain response on a given item was the same for all participants with the same value on the underlying trait (disordered eating behavior [DEB]) regardless of the sex of the participant. This definition gave rise to a highly constrained multi-group factor model (Chen et al., 2005; Meredith, 1993; Millsap & Yun-Tein, 2004). To establish measurement invariance, we fitted several increasingly restrictive models derived from approaches described in previous studies (Dolan, 2000; Mellenbergh, 1989; Meredith, 1993; Millsap & Yun-Tein, 2004; Muthen & Asparouhov, 2002; Muthén & Muthén, 2005), cumulating in this highly constrained model.

In the first step, a saturated model was fitted to the data simply to obtain estimates of the item thresholds and the polychoric correlation among items. To this end, we assumed that a latent continuous variable, called the liability, was underlying the responses to each discrete item. Assuming the liability underlying each item was standard normally distributed, the discrete responses were modeled to items by estimating thresholds on the standard normal distributions of the liability (3 thresholds for the DIET item, and 4 thresholds for the other three items). The positions of these thresholds determined the marginal response probabilities of each item. In addition, the (polychoric) correlations among the liability underlying the four items were estimated. Thresholds and correlations were estimated separately in men and women.

In the second model it was tested whether the four items were uni-dimensional in men and women. The four continuous latent liabilities were regressed on a single common factor, without imposing any equality constraints over sex. Thresholds in men were constrained to equal those in the women. By imposing this constraint, the thresholds were estimated on a common metric. The distribution of the liability for each item was standard normal in the women as in model 1. In the men the means and variances of the liability underlying the four items were estimated freely. Thus, in this step we fitted a single factor model to the correlation matrix of the liabilities in the women, and a single factor model to the covariance matrix of the liabilities in the men. In both sexes, the common factor was scaled to have a mean of zero and a variance of one (i.e., standard scaling constraints in the common factor model). By estimating all the factor loadings freely, the item reliability in the women and the men were obtained separately. Note that these reliability estimates need not be equal over sex.

In model 3, the factor loadings were constrained to be equal over sex. This constraint allowed estimation of the variance of the common factor in one group (men), while retaining the scaling constraint (variance of factor equal to one) in the other group (women). We thus allowed for a difference in common factor variance between men and women. This model included sex differences in the residual variances of the items, in the liability means and in the common factor variance.

In model 4 mean liabilities (intercepts) in the male sample were constrained at zero, and the common factor mean was estimated. As before, the mean liabilities and common factor mean were fixed to zero in women. In the preceding model the estimated mean in liabilities in men gave an indication of the sex differences per item. By fixing these intercepts at zero in men, while freely estimating the mean of the common factor, any sex difference in means of the liabilities was explained by a difference in the mean of the common factor, i.e. a difference with respect to the latent variable of interest.

In model 5, we added the final constraint of 'invariance of residual variances over sex'. As a consequence, the amount of the variance in the separate items that was not explained by the common factor was constrained to be equal in the women and men. This model represented full measurement invariance. Note that in this model any observed sex difference in the observed test scores was attributable to a difference with respect to the latent variable that we purported to measure. With respect to the interpretation of sex differences in test scores, model 5 represented the ideal. Model 4 represented a weaker form of invariance in which sex differences in the residuals were permitted. Model 4 was still useful as it allowed us to interpret sex differences in the mean scale score as a manifestation of a mean difference with respect to the latent variable. Weaker forms of measurement invariance are entertained in the literature (e.g., model 3: equality of factor loadings), but we did not consider these to be sufficient for the interpretation of sex differences with respect to the test scores (Meredith, 1993).

All analyses were performed in Mplus 4.0 (Muthen & Asparouhov, 2002; Muthén & Muthén, 2005). Because our sample consisted of families, the individual cases were not independent. To correct for the effect of this dependence on the standard errors and overall goodness of fit indices, we used the Weighted Least Square with mean adjusted Chi-square test statistics (WLSM) in combination with the 'Complex' option in Mplus. The latter corrects the statistical effect of clustering on the results. Rebollo et al. (2006) found this method to be satisfactory to correct for dependency due to family grouping.

As suggested by Schermelleh-Engel, Moosbrugger and Müller (2003), several fit statistics were used to evaluate the fit of the models; hierarchical Chi-square tests, the comparative fit index (CFI), and the root mean square error of approximation (RMSEA).

For the hierarchical Chi-square test, the difference between the Chi-square test statistics obtained for each model yielded a new Chi-square value with degrees of freedom equal to the difference in the number of parameters in the two models. In the WLSM approach in Mplus, the reported Chi-squares were mean adjusted and a scaling correction factor was applied for each model. As a consequence, in calculating the Chi-square difference test, scaling correction factors had to be entered into the equation (Asparouhov & Muthen, 2006). According to the principle of parsimony, models with fewer parameters are preferred, if they do not give a significant deterioration of the fit. Significance can be determined on statistical grounds, but in structural equation modeling, rules of thumb are usually used (Schermelleh-Engel et al., 2003). The CFI ranges from zero to one with higher values indicating better fit; for a good model fit the CFI should be above 0.97, and values greater than 0.95 indicate an acceptable fit (Schermelleh-Engel et al., 2003). The RMSEA is a measure of closeness of fit, and provides a measure of discrepancy per degree of freedom. A value of 0.05 or smaller indicates a close fit, and values between 0.05 and 0.08 indicate an acceptable fit (Jöreskog, 1993; Schermelleh-Engel et al., 2003).

There were 257 persons (n=127 men and n=130 women) who completed the survey twice with an interval of six months. Retest data obtained in this group will serve to estimate stability of the test scores. The reliability of the eating disorder items was estimated separately in men and women. Polychoric correlations between the two occasions of measurement were calculated for each item using Mplus.

Results

To evaluate how often the different eating disorder attitudes and behaviors were endorsed, we calculated the frequencies of the item scores greater than three in the adolescent twins and their non-twin siblings for the four items. These frequencies showed significant sex differences for three features (p<0.001). For the DIET item, 0.4% of the men compared to 3.4% of the women had been on a diet often or always. Few men (1.3%) reported being very or extremely afraid to gain weight or become fat (FEAR). In women this item was endorsed more often with 8.7%. A large proportion of both men and women reported that “their body weight and or shape played an important role in how they felt about themselves” (ISE). The frequency of this feature was 40.9% in the women compared to 26.8% in the men. No sex differences were found for the BE item, 5.1% of the women and 5.5% of the men reported having binge eating episodes at least once a week.

In model 1 polychoric correlations among items, and the thresholds for each item were estimated per sex. These are reported in Table 3.1. Small to moderate correlations between

the items were found in both sexes. Although the magnitude of the correlations differed between groups, similar patterns were observed with the highest correlation between DIET and FEAR and the lowest between ISE and BE. The thresholds of the liabilities represent the cut-points of the response categories in the corresponding ordinal items on a sex-specific z-scale. The mainly positive thresholds indicate that the majority of women and men did not engage in eating disordered behaviors and/or attitudes.

Table 3.1 Correlations and thresholds for women and men (saturated model).

	DIET[b]	FEAR[c]	ISE[d]	BE[e]
Correlations[a]				
DIET[b]	1.00	0.59 (0.52,0.66)	0.39 (0.30,0.48)	0.41 (0.31,0.51)
FEAR[c]	0.53 (0.38,0.67)	1.00	0.59 (0.54,0.64)	0.33 (0.24,0.41)
ISE[d]	0.27 (0.13,0.40)	0.39 (0.29,0.48)	1.00	0.27 (0.19,0.36)
BE[e]	0.22 (0.03,0.41)	0.20 (0.06,0.34)	0.16 (0.06,0.27)	1.00
Women				
Threshold 1	0.68 (0.57,0.78)	-0.43 (-0.52,-0.33)	-1.54 (-1.68,-1.40)	0.64 (0.54,0.74)
Threshold 2	1.36 (1.24,1.49)	0.67 (0.57,0.77)	-0.56 (-0.66,-0.46)	1.12 (1.00,1.23)
Threshold 3	1.83 (1.66,1.99)	1.36 (1.24,1.49)	0.23 (0.14,0.32)	1.63 (1.48,1.78)
Threshold 4	-	2.13 (1.93,2.33)	1.88 (1.70,2.06)	2.08 (1.87,2.29)
Men				
Threshold 1	1.62 (1.44,1.80)	0.72 (0.60,0.83)	-0.94 (-1.06,-0.83)	0.95 (0.83,1.07)
Threshold 2	2.29 (2.02,2.56)	1.71 (1.52,1.89)	-0.10 (-0.20,0.001)	1.27 (1.13,1.40)
Threshold 3	2.64 (2.25,3.02)	2.24 (1.97,2.51)	0.58 (0.48,0.69)	1.60 (1.44,1.76)
Threshold 4	-	2.71 (2.28,3.14)	1.94 (1.73,2.15)	1.87 (1.68,2.06)

[a] The correlations in the women are listed above the diagonal, the correlations in the men are listed below the diagonal. Numbers in parentheses represent 95% confidence intervals.
The thresholds are estimated on a sex-specific z-scale.
[b] DIET: Dieting
[c] FEAR: Fear of weight gain
[d] ISE: Importance of body weight or shape in self-evaluation
[e] BE: Binge eating

In Table 3.2, fit statistics of the nested models are given. Model 2, which tested whether one factor could account for the correlations among the four eating disorder variables, fitted significantly worse compared to model 1 according to the chi-square. However, both the RMSEA and the CFI indicated a good fit of this model. The parameter estimates of model 2 are presented in Table 3.3. The factor loadings of DIET and BE were comparable between men and women. On the other hand, the factor loading in the men for FEAR was higher and for ISE was lower compared to the women. The least reliable item was BE, while the FEAR item had the highest reliability.

Table 3.2 Model fit statistics

Model	χ2	df	CFI [a]	RMSEA [b]	CM [c]	Δχ2 [d]	Δdf [e]	*p*
Model 1 (saturated)	0.00	0	1.00	0.00	-	-	-	-
Model 2 (one factor model)	37.98	11	0.99	0.04	1	37.98	11	0.0001
Model 3	35.62	14	0.99	0.03	2	2.32	3	0.51
Model 4	101.07	17	0.96	0.06	3	50.99	3	0.0001
Model 5 (full measurement invariance)	246.53	21	0.90	0.09	4	99.57	4	0.0001

[a] CFI: Comparative Fit Index

[b] RMSEA: Root Mean Square of Error of Approximation

[c] CM: Compared to model

[d] Δχ2: Chi-square test statistic between two models adjusted for scaling correction factor

[e] Δdf: degrees of freedom for the Chi-square difference test

The estimates of the mean liability in men were all significantly lower than zero. As these means were fixed to zero in the women, we established, as expected, that the men scored lower than the women on all eating disorder items. The estimated variances of the liability of FEAR, ISE, and BE were significantly smaller than one in the men. The variances were fixed at one in the women.

Table 3.3 Parameter estimates for model 2 in the female reference group and the male group.

	DIET[a]	FEAR[b]	ISE[c]	BE[d]
Women				
Factor loading	0.68 (0.60, 0.75)	0.88 (0.81, 0.94)	0.66 (0.59, 0.72)	0.44 (0.35, 0.52)
Mean	0	0	0	0
Variance	1	1	1	1
Reliability	0.46	0.77	0.43	0.19
Men				
Factor loading	0.69 (0.35, 1.03)	0.97 (0.71, 1.24)	0.55 (0.41, 0.70)	0.45 (0.19, 0.71)
Mean	-1.11 (-1.83, -0.39)	-1.30 (-1.56, -1.05)	-0.44 (-0.57, -0.31)	-0.84 (-1.28, -0.40)
Variance	0.91 (0.59, 1.24)	0.84 (0.70, 0.98)	0.85 (0.77, 0.93)	0.65 (0.50, 0.79)
Reliability	0.48	0.94	0.30	0.20

Numbers in parentheses represent 95% confidence intervals for the factor loadings and residual variances

[a] DIET: Dieting

[b] FEAR: Fear of weight gain

[c] ISE: Importance of body weight or shape in self-evaluation

[d] BE: Binge eating

The Chi-square test statistic suggested some violation of uni-dimensionality (model 2). But because both the RMSEA and the CFI indicated a good fit, the invariance of factor

loadings across sexes was tested next. For this model, all three fit statistics indicated a good fit. The estimate of variance of the common factor (disordered eating behavior (DEB)) in the male group was 0.96. Given the 95% confidence interval (CI) of 0.62 and 1.30, we concluded that the variance was not significantly different between the men and women in model 3.

In model 4, the mean of the liabilities were constrained to be zero in men (as they were in women). The mean of the common factor was fixed to zero in the women, as before, and estimated freely in the men. This model did not fit very well in comparison to model 3. The Chi-square test statistic indicated a significantly worse fit for this model. However, the fit was acceptable according to the RMSEA and the CFI. The estimated common factor mean in the men was -0.99, which differed significantly from zero (95% CI -1.18 - -0.80). In other words, the mean of DEB was lower in men than in women (factor mean fixed at zero).

Because the fit of model 4 was acceptable based on the RMSEA and the CFI, the final model of complete measurement invariance was tested. In this fifth model, the residual variances were also constrained to be equal across the groups. The Chi-square statistic indicated deterioration in fit compared to model 4. In addition, the CFI and the RMSEA indicated a bad fit. This implied that the eating disorder items were not fully measurement invariant with respect to sex. The variances presented in Table 3.3, give an indication of which item might be underlying this bad fit. The variance of BE showed the largest deviation from 1, suggesting that the greatest difference between both groups in residual variance was observed for this item.

Finally the stability of the item responses and the DEB total score were considered. The four eating disorder items were moderately to highly correlated over a period of six months. The polychoric correlation was 0.59 (95% CI 0.28-0.89) for DIET, 0.75 (95% CI 0.59-0.90) for FEAR, 0.56 (95% CI 0.41-0.71) for ISE, and 0.74 (95% CI 0.55-0.93) for BE in men. In women, the polychoric correlation was 0.75 (95% CI 0.60-0.89) for DIET, 0.67 (95% CI 0.55-0.79) for FEAR, 0.43 (95% CI 0.27-0.59) for ISE, and 0.58 (95% CI 0.42-0.74) for BE.

Discussion

In most assessment instruments, distinct items are designed to measure a trait, and the sum score of these items serves as an approximation of an individual’s trait score. The interpretation of differences between groups with respect to these sum scores hinges on the establishment of measurement invariance. Ideally, differences in sum scores should reflect

true differences in the latent variable that the psychometric instrument purports to measure. If there is a lack of measurement invariance, group differences in sum scores reflect, at least in part, measurement bias.

We described a stepwise multi-group confirmatory factor analysis to investigate measurement invariance for categorical items with respect to a grouping variable. Previously, several methods have been reported to test for measurement invariance both for continuous and categorical items (Dolan, 2000; Mellenbergh, 1989; Meredith, 1993; Millsap & Yun-Tein, 2004; Muthen & Asparouhov, 2002; Muthén & Muthén, 2005). All these methods cumulated in an identical highly constrained model in which strict factorial invariance, or complete measurement invariance, was tested. However, the number and order of the constraints in the intermediate models differed between the reported methods. In contrast to previous studies, our analysis began by fitting a saturated model to the data, to obtain estimates of the polychoric correlation among items and the thresholds for each item. The second model, which tested for uni-dimensionality of the items, was more comparable to the baseline models described by other groups (Millsap & Yun-Tein, 2004; Muthen & Asparouhov, 2002; Muthén & Muthén, 2005), although there was a difference in the constraints. In our model, thresholds were constrained across groups, while factor loadings were estimated freely. This enabled us to calculate the reliability of the separate item scores. Means and variances of the liabilities provided insight in the between-group differences. In the third model, both item thresholds and factor loadings were constrained to be equal across groups. The between-group differences in this model were represented by the residual variances of the items, the liability means and by the common factor variance. In addition to the previous constraints, the liability means were constrained at zero in all groups in model 4. Within this model, any group difference in the means of the latent indicators would be explained by a difference in the mean of the common factor. This model represented a weaker form of invariance in which group differences were permitted in the residuals, and was similar to the third model described by Millsap et al. (2004). The final model of strict factorial invariance, added the constraint of invariance of residual variances over groups; i.e. the amount of the variance in each item that was not explained by the common factor was constrained to be equal in the groups.

The method was illustrated by investigating whether a scale comprised of four eating disorder items was measurement invariant with respect to sex. The model of full measurement invariance with respect to sex (model 5), did not fit the data well. If this model had fitted, the probability of a certain response on a given item would have been the same for all participants with the same value on the underlying trait (DEB) regardless of the sex of the participant. However, this was not the case. The underlying common factor might

not be the only source of difference between the sexes with respect to the four items. The sum score based on the four eating disorder items therefore cannot be taken to represent exactly the same underlying trait in men and women. This means that sex differences in this sum score might be due to measurement bias instead of a true difference in the underlying trait.

What implication does this finding have for existing eating disorder measurement instruments? We acknowledge that a scale consisting of four items might not be ideal to measure the underlying latent trait in eating disorders. However, in large epidemiological studies such as becoming common for gene finding, short scales might be a requirement to obtain phenotyping in sufficiently large samples. With the selection of the items we have tried to capture a variety of eating disorder symptoms. Three of the items (FEAR, ISE and BE) used in this study are based on eating disorder criteria from the Diagnostic and Statistical Manual of Mental Disorders (4^{th} ed., American Psychiatric Association, 1994). The fourth item (DIET) has been identified as a potent risk factor (Jacobi et al., 2004). However, one eating disorder symptom, compensatory behavior, is missing in our assessment instrument.

There has been a lot of debate about whether eating disorders are dimensional like proposed in the "continuum of eating disorders" (Fairburn & Harrison, 2003; Hay & Fairburn, 1998), or whether they are discrete syndromes (Williamson et al., 2005). Some studies suggest that eating disorders can be conceptualized as having at least two latent features (Williamson et al., 2002; Williamson et al., 2005); binge eating, and general psychopathology. Accordingly, the FEAR, DIET, and ISE items would load on one factor, and the BE item would load on a second factor. The correlations presented in Table 3.1, however, show substantial correlations between DIET and BE, especially in women (0.41). Bulimic behavior has been correlated with dieting and body concerns in several other studies (Williamson et al., 2005), although this correlation appears to exist exclusively in nonclinical samples. Since our sample is also nonclinical, this may be the cause of the high correlation between DIET and BE. Hence, the factor structure discussed above might not be suitable in nonclinical groups. On the other hand, the low reliability of the BE item and the fact that the variance of this item showed the largest deviation from one in model 2, might be supportive of the two factor structure underlying eating disorders. However, investigating partial measurement invariance by omitting the final constraints on the BE item did not lead to a model of strict factorial invariance for the remaining three items.

The finding of a lack of strict factorial invariance in the 4-item DEB scale might not generalize to existing eating disorder scales. However, this form of measurement invariance has never been tested in the eating disorder field. Many studies have used both exploratory

and confirmatory factor analysis to test whether existing measurement instruments have the same factor structure across, for example, different types of patients, and different ethnic groups, and to establish different factors within eating disorders (Calugi et al., 2006; Fernandez et al., 2006; Hrabosky et al., 2008; Lee et al., 2007; Peterson et al., 2007; Varnado et al., 1995; Wade et al., 2008a; Williamson et al., 2002; Williamson et al., 2005). Until now, only one study has investigated measurement equivalence (Warren et al., 2008). Warren et al. tested for the equivalence of factor loadings for the Body Shape Questionnaire in American and Spanish women with and without an eating disorder diagnosis. For a subscale of 10 items, the constraint of invariant factor loadings fitted the data well. However, because the intercepts were not constrained to equivalence in this study, the scores in the different groups may not have the same origin (Chen et al., 2005). Thus, differences on factor means between groups could still be caused by measurement bias.

The responses to the four eating disorder items were fairly stable over a six month period, with correlations ranging from 0.43 for ISE in the women to 0.75 for FEAR in the men and DIET in the women. The prevalence for the DIET, FEAR, and BE item were low to moderate. The prevalence of ISE was substantially higher. Comparable rates were found in other population-based studies in adolescents with the exception of the DIET item, which had a lower prevalence (Kjelsas et al., 2004; Neumark-Sztainer et al., 2007; Rowe et al., 2002; Silberg & Bulik, 2005). Because of the low endorsement rates of dieting in the men, we had to merge the fourth and fifth category for the DIET item. As a consequence, the number of response frequencies differed between the four items. This difference in response categories does not appear to impact the results. When all items are merged into four or even three categories, the same results were found throughout the different steps of the confirmatory factor analyses. Comparable correlations, thresholds and factor loadings for the four items were found. In addition, the model of weak measurement invariance (model 4) remained the best-fitting model.

The framework we presented in this paper can serve as a valuable tool for examining the psychometric qualities of other interviews and questionnaires with respect to sex. In addition, other kinds of grouping variables (e.g. age, level of education) can also be studied using this method. An advantage of our approach is that it provides a better understanding of the consequences of the different constraints per model. As a consequence, it gives a better insight into the violations of measurement invariance, and the underlying causes of this measurement bias. It is essential to test for measurement invariance before sum scores or scale scores are used to compare groups. This is not only the case in the eating disorder field, but applies to other fields of research as well.

Chapter 4

Genetic influences on disordered eating behavior are largely independent of BMI

This chapter was previously published:
Slof-Op 't Landt M.C.T., Bartels, M., van Furth, E. F., Van Beijsterveldt, C. E., Meulenbelt, I., Slagboom, P. E., & Boomsma, D. I. (2008).
Acta Psychiatrica Scandinavica, 117, 348-356.

Abstract

Introduction: Prior studies suggest eating disorders and related characteristics are moderately to substantially heritable. We are interested in identifying genes underlying disordered eating behavior (DEB), and want to know how much of the genetic influence underlying DEB is attributable to genetic influences on BMI.
Method: Bivariate analyses were performed, in adolescent twins and siblings from the Netherlands Twin Registry, to estimate the genetic and environmental contributions for DEB, BMI and their overlap.
Results: Shared genetic risk factors explained the overlap between BMI and DEB (genetic correlation was 0.43 in women, 0.51 in men). DEB was highly heritable in women (a^2=0.65; a^2 independent of BMI=0.53) and moderately heritable in men (a^2=0.39; a^2 independent of BMI=0.29). BMI was highly heritable in both men (a^2=0.76) and women (a^2=0.80).
Conclusion: The entire correlation between DEB and BMI was explained by shared genetic risk, but the majority of genetic influences on DEB were due to genetic effects independent of BMI.

Despite substantial efforts to identify causal pathways for anorexia and bulimia nervosa, very little is known about the aetiology of eating disorders. In longitudinal and cross-sectional studies, several risk factors have been identified, including gender, elevated weight and shape concerns, negative body image, negative self-evaluation, dieting and childhood obesity (Jacobi et al., 2004).

Various family and twin studies have been performed to explore causes of individual differences in the development and stability of eating disorders, a variety of eating disorder symptoms, and related characteristics. In population-based twin studies, the heritability estimates for these different phenotypes in women ranged from zero to 0.82, but on average a moderate heritability of around 0.40 was estimated (Bulik et al., 1998; Bulik et al., 2003b; Bulik et al., 2006; Holland et al., 1988; Keski-Rahkonen et al., 2005; Klump et al., 2000; Klump et al., 2003; Neale et al., 2003a; Reichborn-Kjennerud et al., 2003; Reichborn-Kjennerud et al., 2004b; Reichborn-Kjennerud et al., 2004a; Rowe et al., 2002; Rutherford et al., 1993; Slof-Op 't Landt et al., 2005; Sullivan et al., 1998a; Wade et al., 1998). In men, heritability estimates ranged from 0 to 0.51, with an average heritability estimate of 0.20 (Keski-Rahkonen et al., 2005; Reichborn-Kjennerud et al., 2003; Reichborn-Kjennerud et al., 2004b; Reichborn-Kjennerud et al., 2004a; Rowe et al., 2002). Only one study focussed on the overlap between eating attitudes, behavior and body weight in adolescent female twins (Klump et al., 2000). This is an interesting overlap to investigate, since body weight might be a risk factor for the development of eating disorders (Jacobi et al., 2004).

We herein report the results of a bivariate twin study on disordered eating behavior (DEB) and body mass index (BMI) in a Dutch population sample of adolescent male and female twins. To overcome the drawbacks and limitations of the previous studies (such as small sample sizes, inadequate power, and the use of categorical data; e.g. see (Slof-Op 't Landt et al., 2005, review)), we used a large sample of twins and siblings aged 11-18 years. DEB was measured in a more continuous fashion. Four items on different eating disorder features were used to calculate a sum score. Three items used in this study are based on eating disorder criteria from the DSM-IV (1994). The fourth item, dieting, was added to assess an important risk factor for the development of eating disorders (Jacobi et al., 2004). Prior work has shown that these four items could be accounted for by one underlying latent factor in a confirmatory factor analysis (Slof-Op 't Landt et al., 2009). However, the DEB items were not measurement invariant with respect to sex, indicating that this scale might not measure the same trait in men and women (Slof-Op 't Landt et al., 2009). Therefore the genetic analyses were performed separately in men and women.

The aim of the current study is to investigate how much of the heritability in DEB is attributable to genetic effects on body mass index (BMI), and how much of it is

independent of these effects. Because we would like to identify genes that influence DEB in the future, investigating the overlap between DEB and BMI may shed some light on possible biological pathways involved in DEB. We performed a bivariate analysis using both traits, to estimate the overlap between DEB and BMI and to disentangle the proportion of variance due to shared and specific genetic and environmental factors.

Materials and methods

Sample

All participants were registered with the Netherlands Twin Registry (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam. Young twins (YNTR) are registered at birth by their parents, who are approached through 'birth felicitation' services. During the first years of their lives the parents are the primary sources of information on their development. Twins are categorized by birth cohort and data collection is cohort driven. Nationwide data collection of all families is by mailed surveys. Parents of twins receive questionnaires when their twins are aged 1, 2, 3, 5, 7, 10, and 12 years of age. At ages 7, 10, and 12, teacher data are also collected, after written permission is given by the parents. When the twins are 14, 16, and 18 they receive a self report questionnaire, used in the current study (Bartels et al., 2007; Boomsma et al., 2006). For this study data from the 1986-1992 birth cohorts were used. In January 2005, questionnaires were sent to 14-, 16- and 18-year old twins and their non-twin siblings. The twins and siblings were asked to complete a survey containing items relevant for eating disorders. Questionnaires were sent to 2000 families. A total of 2131 twins and 517 siblings from 1121 families returned the questionnaire (family response rate 56.1 %).

Zygosity was determined for 461 same-sex twin pairs by DNA analysis or blood group polymorphisms. For all other same-sex twin pairs, zygosity was determined by discriminant analysis, using longitudinal questionnaire items. Agreement between zygosity assignment by the replies to the longitudinal questionnaire and zygosity determined by DNA markers/blood typing was around 93% (Rietveld et al., 2000).

The final sample consisted of 474 monozygotic twin pairs (194 male (MZM) and 280 female (MZF) pairs), 310 dizygotic twin pairs (140 male (DZM) and 170 female (DZF) pairs), and 45 incomplete twin pairs (22 men and 23 women). The sibling group was comprised of 69 brothers and 115 sisters.

Measures

The Dutch Health Behaviour Questionnaire is a self-report instrument containing direct measures of several health and behavior features, including a number of eating disorder characteristics and self report of height and weight. Based on the self-reported height and weight, the body mass index (BMI = weight [kg] / height^2 [m]) was used as a measure of relative body weight in this study.

The eating disorder section included four items: 1) dieting (have you ever gone on a diet to loose weight or to stop gaining weight?); 2) fear of weight gain (how afraid are you to gain weight or become fat?); 3) importance of body weight or shape on self-evaluation (how important are body weight and/or shape in how you feel about yourself?); 4) eating binges (have you ever had eating binges?). Responses were given on a five point scale. The scores on the four items were summed to calculate disordered eating behavior (DEB). If one of the four eating disorder items was missing, the sum score was also missing.

Prior work has shown that these four items could be accounted for by one underlying latent factor in a confirmatory factor analysis (Slof-Op 't Landt et al., 2009). In comparing groups or parallel use of data from different groups, such as men and women, it is important that an instrument measures the same underlying latent (unobserved) trait in these groups. Observed group differences in the sum scores should accurately reflect group differences with respect to the latent variable. A necessary condition for this is that the instrument displays measurement invariance with respect to the groups under consideration (Mellenbergh, 1989; Meredith, 1993). Formally, measurement invariance requires that the distribution of the item scores, conditional on only the trait score equals the distribution of the item scores, conditional on both the trait score and group membership. If for example men score lower on average on one item than women without actually scoring lower on the total scale (underlying trait), this item is said to lack measurement invariance. In that case, observed group differences in sum scores might not be caused by true differences in the underlying trait, but by measurement bias. Prior analyses have shown that the four eating disorder items were not measurement invariant with respect to sex. This implies that the sum score based on these items cannot be taken to present exactly the same trait in men and women. Therefore all analyses were performed separately in men and women.

Statistical Analyses

Age-effects for both DEB and BMI were expected (Klump et al., 2000; Schousboe et al., 2003), therefore we first calculated the correlations between both traits and age in the two sex groups. For the descriptive statistics, we tested whether the means and variances for DEB and BMI were equal between the twins and siblings in men and women.All analyses

were performed using the software package Mx (Neale et al., 2003b). The means were corrected for age in all genetic analyses.

In the next step, the phenotypic correlation between DEB and BMI was calculated. Subsequently we calculated twin correlations, twin-sibling, and cross-twin/sib cross- trait correlations. The correlations provide an initial indication of genetic and environmental effects on DEB, BMI, and their overlap. By constraining the DZ twin correlations and the twin-sib correlation to be equal the presence of a specific twin environment is tested. Monozygotic (MZ) twin pairs are genetically (nearly) identical, whereas dizygotic twin and sibling pairs share on average 50% of their segregating genes. Therefore, if the MZ twin correlation is substantially larger than the DZ twin and twin-sib correlations genetic influence is implied. Shared family environmental factors (for example religion, socioeconomic level and parenting style) will make family members relatively more similar and will create differences between families. If the MZ and DZ twin correlation are similar and both statistically significant, shared environmental influence is suggested. Finally, the importance of non-shared environmental influences can be seen from the extent to which the MZ twin correlations differ from one. This influence stands for the impact of all environmental factors influencing only one of the twin pair (for example illness, trauma or relationships with peers). In addition, the pattern of cross-twin cross-trait correlations for MZ twins and DZ twins and siblings indicates to what extent the covariance between the traits is influenced by genetic or environmental components. Finally, if a twin specific environment is implied if DZ twin correlations are significantly higher than twin-sib correlations.

The Cholesky Decomposition or triangular decomposition, is used for the bivariate genetic model fitting. The Cholesky decomposition decomposes the phenotypic statistics into genetic, shared environmental, and nonshared environmental contributions. In other words the pattern of the factor loadings on the latent genetic and environmental factors reveals a first insight into the etiology of covariances between DEB and BMI. Since the saturated model is fully parameterized, it yields the best possible fit to the input matrices.

The bivariate Cholesky decomposition model contained two latent factors for A, C and E respectively (per individual), of which the variances were constrained to be one. In Figure 4.1 the path diagram of this model is shown. Correlation coefficients are represented by curved lines with an arrow at each end. Within a twin- or sibling pair the C component for a trait is identical for each member (correlation coefficient of one), the E component is uncorrelated. A on the other hand, is identical for MZ twins but the correlation is 0.5 for DZ twins or sibling pairs. BMI loaded on the first latent factors A, C and E.

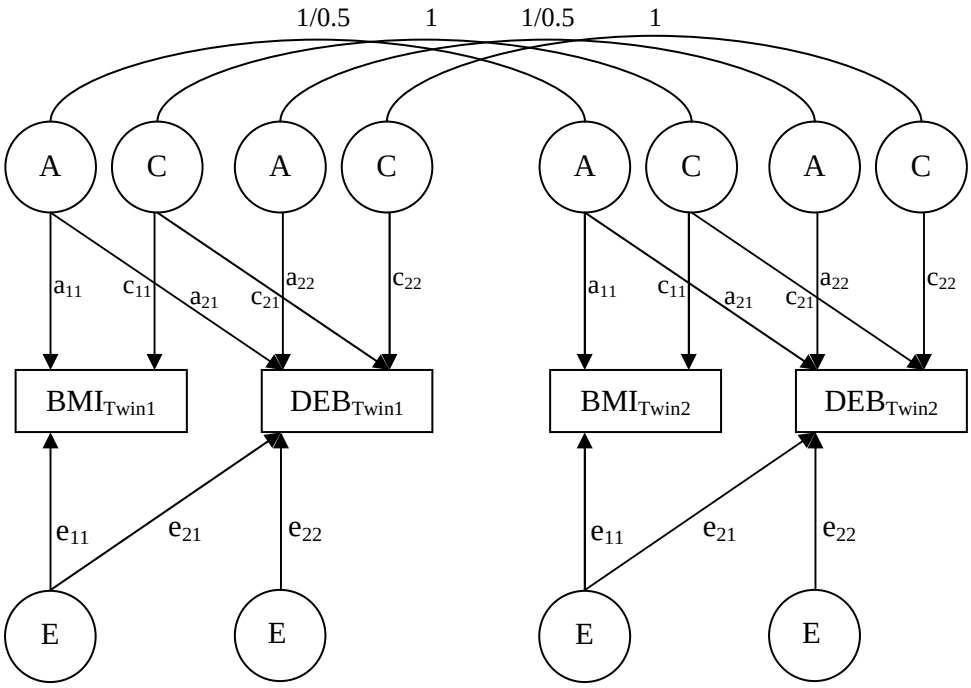


Figure 4.1. The bivariate Cholesky model for BMI and DEB, represented for a twin or sibling pair. Correlation coefficients are represented by curved lines with an arrow at each end. Variance in each phenotype is assumed to be determined by the additive combination of three latent factors: additive genetic effects (A), shared environmental effects (C) and nonshared environmental effects (E). BMI loaded on the first latent factors A, C and E. The additive genetic, shared environmental and nonshared environmental variance in DEB scores are partitioned into those components attributable to the genetic and environmental effects on BMI (a_{21}, c_{21}, e_{21}) and residual components that are independent of the genetic and environmental effects of BMI (a_{22}, c_{22}, e_{22}).

The phenotypic variance for BMI is represented by the sum of squared estimates of factor loadings (i.e., $(a_{11}^2) + (c_{11}^2) + (e_{11}^2)$). DEB loaded on both factors, and the sum of the squared factor loadings (i.e., $(a_{21}^2 + a_{22}^2) + (c_{21}^2 + c_{22}^2) + (e_{21}^2 + e_{22}^2)$) represented the phenotypic variance for this trait. The heritability of BMI and DEB will be estimated by:

$$a^2_{BMI} = a_{11}^2 / (a_{11}^2 + c_{11}^2 + e_{11}^2)$$
$$a^2_{DEB} = (a_{21}^2 + a_{22}^2) / (a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$$

When multiplying the factor loadings on the first latent factors (i.e., $(a_{11} \times a_{21}) + (c_{11} \times c_{21}) + (e_{11} \times e_{21})$), the covariance between BMI and DEB is derived. Based on the covariance, genetic and environmental correlations between the two traits can be calculated (see below).

Genetic correlation: $r_g = (a_{11} \times a_{21}) / (\sqrt{a_{11}^2} \times \sqrt{(a_{21}^2 + a_{22}^2)}$

Common environmental correlation: $r_c = (c_{11} \times c_{21}) / (\sqrt{c_{11}^2} \times \sqrt{(c_{21}^2 + c_{22}^2)}$

Unique environmental correlation: $r_e = (e_{11} \times e_{21}) / (\sqrt{e_{11}^2} \times \sqrt{(e_{21}^2 + e_{22}^2)}$

Based on the estimated heritability for DEB and the genetic correlation, the heritability estimates for DEB dependent on BMI (a_{21}^2) and independent on BMI (a_{22}^2) can be determined by:

$$a_{21} = r_g \times (a_{21} + a_{22}) = rg \times a_{DEB}$$

$$a_{22} = a_{DEB} - (rg \times a_{DEB})$$

We fitted models by the method of maximum likelihood to data from all twins and siblings, separately in women and men, beginning with a full bivariate ACE model. Subsequently, parameters (a_{21}, c_{21}, e_{21},) were dropped from the model to test if the covariance between traits can be attributed to shared genes (a_{21},) or overlapping C or E influences. Twice the difference in log-likelihood between two models yields a statistic that is asymptotically distributed as a Chi-square statistic with degrees of freedom equal to the difference in the number of estimated parameters in the two models. This statistic can be used to test the tenability of the constraints associated with the more constrained model. According to the principle of parsimony, models with fewer estimated parameters are preferred if they do not give a significant deterioration of the fit (p>0.05)..

Based on the twin and twin-sibling correlations estimated in this study, we performed power analyses in Mx. We calculated the power to test for the significance of the different paths of A (a_{11},a_{21},a_{22}) and/or C (c_{11},c_{21},c_{22}) in a bivariate model with a significance level α of .05 for the phenotypes (DEB and BMI). In addition, we calculated the statistical power to test whether the genetic correlation between the two phenotypes was statistically different from one or zero in the bivariate model. A genetic correlation of one indicates that identical genes are underlying the genetic influence on the traits. A genetic correlation of zero, means that genetic influences on the traits are totally independent from each other. This analysis was based on the results of the full bivariate model.

Results

Based on the independent analyses in women and men, results for women and men are presented separately.

Table 4.1 Descriptive statistics for DEB and BMI in women (upper part) and men (lower part) per zygosity

	DEB					BMI				
	N	Mean[a]	Var	Min	Max	N	Mean[a]	Var	Min	Max
Women										
MZF firstborn	280	8.0	6.1	4.0	18.0	266	20.1	8.4	14.2	34.6
MZF second born	278	7.7	6.2	4.0	17.0	268	19.7	7.7	14.7	36.4
MZF sister	64	8.2	5.5	4.0	16.0	61	20.9	8.8	14.0	30.8
DZF firstborn	172	7.9	5.7	4.0	16.0	169	20.3	8.3	14.5	32.7
DZF second born	173	7.9	6.1	4.0	19.0	169	20.2	7.9	15.5	33.2
DZF sister	49	8.1	7.8	4.0	15.0	47	20.5	8.3	13.7	29.9
Men										
MZM firstborn	194	6.3	3.4	4.0	13.0	183	20.0	8.4	13.0	34.0
MZM second born	190	6.3	3.4	4.0	12.0	190	19.7	6.5	14.1	34.0
MZM brother	41	6.6	4.6	4.0	14.0	40	20.9	8.2	16.2	34.6
DZM firstborn	145	6.5	3.0	4.0	13.0	140	19.7	5.6	15.1	30.3
DZM second born	141	6.3	2.5	4.0	12.0	139	19.3	3.9	13.8	26.2
DZM brother	26	**5.7**[b]	2.0	4.0	10.0	26	**21.2**[b]	5.9	15.7	28.4

MZF = monozygotic females, DZF = dizygotic females

MZM= monozygotic males, DZM= dizygotic males

[a] Unadjusted means, in the analyses means were adjusted for age

[b] The mean for both DEB and BMI of the DZM brothers was not equal to the means in the remaining males.

Women

In the women both BMI and DEB showed a significant correlation with age, r=0.27 (95% CI 0.19-0.34) and r=0.14 (95% CI 0.06-0.21) respectively. BMI and DEB scores increased with increasing age. The descriptive statistics for the female sample are presented in the upper part of Table 4.1. Means (adjusted for age), and variances of DEB and BMI were equal in the female twins and siblings ($\chi^2_8 = 5.54$; $p = 0.70$). The phenotypic correlation between BMI and DEB was 0.32 (95% CI 0.25-0.38) in women. Table 4.2 displays the correlations and cross-correlations for BMI and DEB in MZ twins, and same-sex DZ twins/twin-sibling pairs in the women. All the MZ correlations, both cross-twin and cross-twin cross-trait, were substantially higher than the DZ/twin-sibling correlations. In other words, genetic influence is implied in DEB, BMI, and the overlap between these traits. DZ twin correlations and twin-sibling correlations could be constrained to be equal ($\chi^2_{18} = 26.72$; $p = 0.08$).

Table 4.2 Correlations and cross-correlations for DEB and BMI in monozygotic twins, and in same-sex dizygotic twins or twin-sibling pairs. Women are presented in the upper part of the table, men in the lower part.

		MZ		DZ / same-sex siblings	
		DEB	BMI	DEB	BMI
Women	DEB	**0.67** (0.60, 0.72)		**0.21** (0.10, 0.32)	
	BMI	*0.29* (0.20, 0.37)	**0.80** (0.76, 0.84)	*0.15* (0.07, 0.24)	**0.30** (0.19, 0.40)
Men	DEB	**0.38** (0.26, 0.49)		**0.25** (0.12, 0.37)	
	BMI	*0.24* (0.15, 0.33)	**0.76** (0.70, 0.81)	*0.23* (0.13, 0.32)	**0.34** (0.21, 0.45)

95% confidence intervals are shown in parentheses

In Table 4.3, the parameter estimates and fit statistics for the full model and the best-fitting model, from the bivariate twin analyses, are presented. The AE model, with genetic influences explaining the overlap between BMI and DEB (a_{21}) gave the best fit to the data. Both BMI and DEB were highly heritable in women. The total phenotypic correlation between BMI and DEB was due to shared genetic influences with an r_g of 0.43 (95% CI 0.34- 0.52) in women.

Table 4.3 Fit statistics and parameter estimates of the full and best-fitting model of bivariate Cholesky analysis of BMI and DEB in female same-sex twins and siblings.

	Fit statistics					
	-2ll[a]	df	$\Delta\chi^2$ [b]	Δdf [c]		
ACE;	9034.13	1983	-	-		
a_{12}, c_{12}, e_{12}						
AE; a_{12}	**9035.54**	**1987**	**1.42**	**4**		
	a^2		c^2		e^2	
	BMI	DEB	BMI	DEB	BMI	DEB
ACE;	0.80	0.65	0.00	0.00	0.20	0.35
a_{12}, c_{12}, e_{12}	(0.71, 0.84)	(0.55, 0.71)	(0, 0.08)	(0, 0.08)	(0.16, 0.25)	(0.29, 0.42)
AE; a_{12}	**0.80**	**0.65**	**-**	**-**	**0.20**	**0.35**
	(0.75, 0.84)	**(0.58, 0.71)**			**(0.16, 0.25)**	**(0.29, 0.42)**

95% confidence intervals shown in parentheses

[a] -2ll: -2 log likelihood

[b] $\Delta\chi^2$: Chi-square test statistic between two models

[c] Δdf: degrees of freedom for the Chi-square difference test

For the women, the statistical power to test for the significance of the different paths of A (a_{11},a_{21},a_{22}) was 1.00 in the bivariate analyses. In addition, the power to test whether r_g

was significantly different from zero or one, was also 1.00. This means that we had sufficient power to decompose the variance and covariance in BMI and DEB.

Men

The correlation between age and BMI was 0.35 (95% CI 0.26-0.42) in men, between age and DEB a non significant correlation of 0.08 (95% CI -0.01-0.16) was obtained. In the lower part of Table 4.1, the descriptive statistics for the male sample are listed. Not all means of DEB and BMI were equal between twins and siblings. The mean of DEB was lower, while the mean of BMI was higher in the DZM brothers compared to the other male twins and siblings ($\chi^2_6 = 8.33$; $p = 0.22$). In the subsequent analyses, we therefore used different means for the DZM brothers.

The phenotypic correlation between BMI and DEB was 0.28 (95% CI 0.21-0.36). The lower part of Table 4.2 displays the correlations and cross-correlations for BMI and DEB in MZ twins, and same-sex DZ twins or twin-sibling pairs estimated in the male sample. The correlations for BMI and DEB were substantially higher in the MZ than in the DZ/twin-sibling pairs in men. The cross-twin cross-trait correlation, however, was quite similar in the MZ and DZ/twin-sibling pairs. DZ twin correlations and twin-sibling correlations could be constrained to be equal ($\chi^2_{18} = 26.52$; $p = 0.09$).

Table 4.4. Parameter estimates and fit statistics of the full and best-fitting model of bivariate Cholesky analysis of BMI and DEB in male same-sex twins and siblings.

	Fit statistics					
	-2ll[a]	df	$\Delta\chi^2$ [b]	Δdf [c]		
ACE;	6023.62	1440	-	-		
a_{12}, c_{12}, e_{12}						
AE; a_{12}	**6026.35**	**1444**	**2.72**	**4**		
	a^2		c^2		e^2	
	BMI	DEB	BMI	DEB	BMI	DEB
ACE;	0.69	0.21	0.07	0.16	0.24	0.35
a_{12}, c_{12}, e_{12}	(0.52 0.79)	(0.00, 0.45)	(0, 0.23)	(0, 0.37)	(0.19, 0.30)	(0.29, 0.42)
AE; a_{12}	**0.76**	**0.39**	**-**	**-**	**0.24**	**0.35**
	(0.70, 0.81)	**(0.28, 0.49)**			**(0.19, 0.30)**	**(0.29, 0.42)**

95% confidence intervals shown in parentheses

[a] -2ll: -2 log likelihood

[b] $\Delta\chi^2$: Chi-square test statistic between two models

[c] Δdf: degrees of freedom for the Chi-square difference test

For the bivariate Cholesky decomposition analyses, the AE model with genetic components explaining the overlap (a_{21}), gave the best fit to the data. In Table 4.4, the parameter estimates as well as the fit statistics are mentioned for the full and best-fitting models in the male sample. DEB was moderately heritable in men, whereas BMI was a highly heritable trait. The total phenotypic correlation between BMI and DEB was due to shared genetic influences with an r_g of 0.51 (95% CI 0.37-0.64) in men.

The statistical power to test for the significance of the different paths of A (a_{11},a_{21},a_{22}) was 1.00 in the male sample. However, the power to test whether r_g between BMI and DEB was statistically different from one was only 0.58, while the power to test if r_g was significantly different from zero was 0.99 in the AE model. This means, that we had limited power to estimate the size of r_g accurately.

How much of the genetic influence on DEB is independent of BMI?
In both women and men the estimated genetic correlations indicated that about half of the genetic factors that influence BMI also influence DEB. But what does this mean for the heritability? How much of the heritability estimate in DEB is attributable to genetic influences on BMI, and how much is independent of it? Based on the genetic correlation we can calculate the heritability of DEB independent of genetic influences on BMI. For women this leads to a heritability estimate of 0.53, in the men an independent heritability of 0.29 was obtained. These results show that the majority of genetic influence on DEB is independent of genetic influences on BMI.

Discussion

Twin-, cross-twin, and twin-sibling correlations indicated that a large part of the variance in both DEB and BMI was explained by genetic factors, and that genetic components were underlying the overlap between DEB and BMI in women. The bivariate analysis showed that DEB is a highly heritable trait in women (a^2=0.65) and moderately heritable in men (a^2=0.39), whereas BMI is highly heritable in both women (a^2=0.80) and men (a^2=0.76). In addition, additive genetic factors were responsible for the total overlap between the two characteristics, yielding a genetic correlation of 0.43 in women and 0.51 in men. Despite the overlap between BMI and DEB, the majority of the genetic influences on DEB were due to genetic effects that are independent of BMI in women as well as men.

Klump et al. (2000) used a bivariate Cholesky decomposition analysis to examine the genetic and environmental contributions to BMI and several scales from the Eating Disorder Inventory (EDI) in adolescent female twins. In this study heritability estimates

ranged from 0.02 to 0.45 in 11-year old twins, and 0.52 to 0.63 in 17-year old twins for the EDI scales, and from 0.78 to 0.84 for BMI in both 11-year and 17-year old twins. Genetic correlations between 0.38 and 0.97 in 11-year old twins and between 0.33 and 0.60 in 17-year old twins were estimated for BMI and the different scales of the EDI. Despite the difference in age and the use of different assessment instruments, our results in the women were comparable to the estimates in the 17-year old twins from this study. In addition, results from the current study are comparable to adult population-based univariate twin studies that have investigated genetic and environmental contributions to BMI (Schousboe et al., 2003), and eating disorder related characteristics (Bulik et al., 1998; Bulik et al., 2003b; Bulik et al., 2006; Holland et al., 1988; Keski-Rahkonen et al., 2005; Klump et al., 2000; Klump et al., 2003; Neale et al., 2003a; Reichborn-Kjennerud et al., 2003; Reichborn-Kjennerud et al., 2004b; Reichborn-Kjennerud et al., 2004a; Rowe et al., 2002; Rutherford et al., 1993; Sullivan et al., 1998a; Wade et al., 1998).

The majority of the variance in DEB was explained by genetic factors in women, while unique environmental factors had the largest influence in men. Since eating disorders are more common in women, items used to asses symptoms and features related to these disorders are also mainly developed for women. The scale we used might not be measuring the same underlying trait in men and women (Slof-Op 't Landt et al., 2009), the differences in heritability estimates between the sexes in the current study can therefore be indicative of a true difference in disordered eating behavior, but might also be due to measurement bias. None of the previously performed twin studies examining eating disorder related characteristics (Keski-Rahkonen et al., 2005; Reichborn-Kjennerud et al., 2003; Reichborn-Kjennerud et al., 2004b; Reichborn-Kjennerud et al., 2004a; Rowe et al., 2002) in both men and women, have tested whether the items used to asses the phenotype measured the same trait in both sexes. As a consequence it is not clear if the reported differences and similarities between male and female heritability estimates are due to measurement bias or true sex differences in disordered eating behavior.

The genetic correlation of 0.43 in women and 0.51 in men obtained in this study, indicates that approximately 50% of the genetic factors that influence BMI also influence DEB. Because DEB and BMI are related with each other, it would be interesting to disentangle the direction of causation of the overlap between these characteristics. Genetic influences on for example metabolism may be causal to weight gain that eventually leads to disturbed eating behavior. Genetic influences on DEB may alternatively be causal to a disturbed eating profile, leading to fluctuations in weight. Eventually we would like to identify genes that are underlying DEB. Therefore, we are planning to test the causal hypothesis in future studies, to further clarify the underlying aetiology of the overlap

between BMI and DEB. Several approaches can be taken to disentangle the direction of causation, for example phenotypic causation models (Duffy & Martin, 1994; Heath et al., 1993) and the co-twin control design (Cederlof et al., 1977; Kendler et al., 1993). The first method is a nested model of the bivariate Cholesky decomposition, in this approach the correlated traits need to have different modes of inheritance. In the co-twin control design relative risks for DEB would be compared between unrelated individuals discordant for BMI, DZ twins discordant for BMI, and MZ twins discordant for BMI.

The power analysis revealed that our sample size was sufficient to detect genetic and shared environmental effects on BMI and DEB (both dependent and independent from BMI) in men and women. Our female sample size also was sufficient to estimate the genetic correlation between BMI and DEB correctly. In the men, we had limited statistical power to estimate this correlation. The small difference between the cross-twin cross-trait correlations in the male MZ and DZ/twin-sib pairs gave a first indication for this lack of power. As a consequence, there is a possibility that the overlap between BMI and DEB is not solely due to genetic factors in men, but that common environmental factors also play a role.

A concern with regard to our study is the selection of the eating disorder features, and the comparability of this phenotype with other studies. Three items used in this study are based on DSM-IV (1994) criteria for eating disorders. The fourth item, dieting, was added to assess an important risk factor for the development of eating disorders (Jacobi et al., 2004). Within the eating disorder field a broad variety of assessment instruments is used to assess eating disorders and eating disorder-related phenotypes. A majority of these assessment instruments is based on DSM-IV criteria, indicating that our broad phenotype is probably fairly comparable to these phenotypes. However, one eating disorder symptom is missing in our phenotype, namely compensatory behavior. Heritabilities of 0.50 for compensatory behavior in 17-year old female twins (Klump et al., 2000) and 0.70 for self-induced vomiting in adult female twins (Sullivan et al., 1998a) have been found. Based on these findings, the inclusion of compensatory behaviors in our phenotype might not influence the results found for the women in the current study. However, we do not know what the consequences for the heritability estimates in the men would be, especially since significant gender differences have been reported for a variety of compensatory behaviors like self-induced vomiting, laxative use and fasting (Anderson & Bulik, 2004).

The current study provides further evidence that genetic components are underlying disordered eating behavior in both men and women. Part of these genetic components are influencing both BMI and disordered eating behavior, while the majority of genetic effects influencing disordered eating behavior is independent of the genetic effects that influence

BMI. In future studies, we hope to identify genes that are involved in this eating disorder phenotype by performing genetic association studies.

Chapter 5

Association study in eating disorders: TPH2 associates with anorexia nervosa and self-induced vomiting

This chapter was previously published:

Slof-Op 't Landt, M. C. T., Meulenbelt, I., Bartels, M., Suchiman, H. E. D., Middeldorp, C. M., Houwing-Duistermaat, J. J., van Trier, J., Onkenhout, E. J., Vink, J. M., Van Beijsterveldt, C. E. M., Brandys, M. K., Sanders, N., Zipfel, S., Herzog, W., Herpertz-Dahlmann, B., Warnke, A., Fleischhaker, C., Zeeck, A., de Zwaan, M., Herpertz, S., Ehrlich, S., van Elburg, A. A., Adan, R. A. H., Scherag, S., Hinney, A., Hebebrand, J., Boomsma, D. I., van Furth, E. F., & Slagboom, P. E. (2011).

Genes, Brian and Behavior, 10, 236-243.

Abstract

Twin studies suggest that genetic factors play a substantial role in anorexia nervosa (AN) and self-induced vomiting (SV), a key symptom that is shared among different types of eating disorders. We investigated the association of 25 single nucleotide polymorphisms (SNPs), capturing 71 to 91% of the common variance in candidate genes stathmin (*STMN1*) , serotonin receptor 1D (*HTR1D*), tryptophan hydroxylase 2 (*TPH2*) and brain-derived neurotrophic factor (*BDNF*), with AN and eating disorders characterized by SV.
First, allele frequencies of all SNPs were compared between a Dutch case group (182 AN, 149 eating disorders characterized by SV) and 607 controls. Associations rendering *p*-values <0.05 from this initial study were then tested for replication in a meta-analysis with two additional independent eating disorder case-control samples, together providing 887 AN participants, 306 participants with an eating disorder characterized SV, and 1914 controls. A significant effect for the minor C-allele of *TPH2* rs1473473 was observed for both AN (OR=1.30, 95% CI 1.08-1.57, $p<0.003$) and eating disorders characterized by SV (OR=1.52, 95% CI 1.28-2.04, $p<0.006$). In the combined case group a dominant effect was observed for rs1473473 (OR=1.38, 95% CI 1.16-1.64, $p<0.0003$). The meta-analysis revealed that the *TPH2* polymorphism rs1473473 was associated with a higher risk for AN, eating disorders characterized by SV and for the combined group.

Eating disorders (ED) are debilitating diseases with high chronicity and mortality rates (Crow et al., 2009; Steinhausen, 2002; Steinhausen & Weber, 2009). Genetic influences appear to be considerable for ED, with heritability estimates ranging from 28 to 83% in women (Bulik et al., 2006; Slof-Op 't Landt et al., 2005). A common and frequently occurring symptom in individuals with ED is self-induced vomiting (SV). This symptom was associated with greater clinical severity (Dalle Grave et al., 2009; Reba et al., 2005), and also appears to be heritable (8-72%) (Sullivan et al., 1998a; Wade et al., 2008b).

Despite the multitude of performed molecular genetic studies in ED, no specific genes have been definitively implicated as causal, although several promising candidate genes exist (Scherag et al., 2010; Slof-Op 't Landt et al., 2005). To retain adequate statistical power we selected four of these candidate genes to test for association in a case-control design. The selected genes were serotonin receptor 1D (*HTR1D*), tryptophan hydroxylase 2 (*TPH2*), stathmin (*STMN1*) and brain-derived neurotrophic factor (*BDNF*).

HTR1D and *TPH2* belong to the serotonin pathway. Serotonin is involved in a broad range of functions, including body weight regulation, eating behavior and mood (Lucki, 1998). Furthermore, the functional activity of the serotonin system appears to be altered in both current as well as recovered ED patients (Ehrlich et al., 2010; Kaye, 2008; Kaye et al., 2005a). *HTR1D* is located under the linkage peak for anorexia nervosa (AN) at 1p33-36 (Grice et al., 2002), and was significantly associated to AN in two independent studies (Bergen et al., 2003; Brown et al., 2006). *TPH2* encodes the rate-determining enzyme in the synthesis of serotonin tryptophan hydroxylase in the brain (Walther & Bader, 2003), and was previously associated with depression and anxiety (Barnett & Smoller, 2009; Kim et al., 2009; Tsai et al., 2009; Zhang et al., 2006).

STMN1 is also located under the linkage peak for restrictive AN (Grice et al., 2002), and was associated to fear processing and anxiety in both mice and humans (Brocke et al., 2010; Shumyatsky et al., 2005).

Finally, the involvement of *BDNF* in ED was reported by two large collaborative studies, that showed an association between AN and the functional Val-66-Met polymorphism (Ribases et al., 2004; Ribases et al., 2005). This finding was replicated by some but not all subsequent studies (Scherag et al., 2010).

In general, consistent associations in the ED field are lacking, possibly due to small sample sizes and the limited number of polymorphisms assessed (Scherag et al., 2010; Slof-Op 't Landt et al., 2005). In the current study we selected 25 tagging SNPs across the four genes and tested them for association with AN (N=182) and participants (N=149) with an ED characterized by self-induced vomiting (SV). Replication occurred in a meta-analysis

with two additional independent ED case-control samples from Germany and the Netherlands together providing 887 AN cases, 306 SV cases and 1914 controls.

Methods and Materials

Participants

This study was approved by each national ethics committee. All participants (and if underage, their parents) gave written informed consent.

Three hundred and eighty nine female ED patients were recruited through ten specialist ED units throughout the Netherlands (the GenED study). All participants fulfilled DSM-IV (Diagnostic and Statistical Manual of Mental Disorders 4th edition) criteria for an ED, made by experienced clinicians based on a semi-structured interview at intake, and via the self-report eating disorder examination questionnaire (EDEQ; Fairburn & Beglin, 1994). For AN, criterion D - amenorrhea for three consecutive months- was discarded because some of the participants despite having AN continue to menstruate (for example, due to treatment with oral contraceptives). Of the 389 cases, 182 fulfilled the DSM-IV criteria (excluding criterion D) for AN. Based on the EDEQ (q14: Over the past 28 days, how many times have you made yourself sick (vomit) as a means of controlling your shape and weight?) and assessment interviews (current and past slef-induced vomiting), we defined a subgroup of ED cases (N=149) who reported regular self-induced vomiting (SV). Frequencies of mean rates of self-induced vomiting were 30% 2 to 8 times per month, 40% 8 to 20 times per month, and 30% more than 20 times per month. Participants with SV fulfilled the following DSM-IV diagnoses AN (N=64), boulimia nervosa (N=74) and ED not otherwise specified (N=11) (see Table 5.1). Thus the two groups were partly overlapping, with 64 participants belonging to both groups.

Random controls come from the population-based Netherlands Twin Registry (NTR), which was established in the late 1980s at the VU University in Amsterdam, the Netherlands. Data on the multiples (twins or triplets) and their families have been collected every two to three years in longitudinal survey studies (Boomsma et al., 2002). Subsamples of the multiples were invited to participate in experimental and laboratory studies and donate their DNA (Boomsma et al., 2006). For the current study, one woman per family served as control, yielding a control group of 607 unrelated women (Middeldorp et al., 2010).

For the meta-analysis additional sample collections were used from Essen (The EDNET and Essen study, Germany) and Utrecht (The Netherlands) (see Table 5.1). The EDNET and Essen sample consisted of 420 female participants with AN according to

DSM-IV criteria and 189 normal weight controls (75 men and 114 women; females with ED were excluded) (Muller et al., 2008). The Utrecht sample consisted of 481 female participants diagnosed with an eating disorder, 285 participants fulfilled DSM-IV criteria for AN and 157 participants reported regular self-induced vomiting. These two groups were partly overlapping, with 56 participants belonging to both groups. As a control population measured and imputed genotype data from the female control group of the GAIN GWA study were used (Boomsma et al., 2008). This group comprised 1118 unrelated female participants from the NTR who were at low liability for major depressive disorder. The GAIN control group was independent of the initial NTR control group.

Table 5.1 Cases and controls

				DSM-IV Eating disorder diagnosis				
Cases and controls	Total N	Age (SD)	Overlap AN & SV	AN-R	AN-BP	AN-P	BN	EDNOS
GenED								
NTR Controls	607	25.4 (13.6)						
GenED AN	182	28.7 (9.9)	64	108	35	39	-	-
GenED SV	149	28.9 (9.9)	64	-	29	35	74	11
EDNET-Essen								
EDNET-Essen Controls	189	24.6 (2.5)						
EDNET-Essen AN	420	21.4 (9.1)	-	152	N.A.	N.A.	-	-
Utrecht								
Gain GWA NTR Controls	1118	44.0 (13.7)						
Utrecht AN	285	22.9 (4.8)	56	213	N.A.	N.A.	-	-
Utrecht SV	157	23.8 (5.7)	56	-	N.A.	N.A.	37	63

AN= anorexia nervosa, AN-R= anorexia nervosa restriciting type, AN-BP= anorexia nervosa binge purging type, AN-P= anorexia nervosa purging type (without binge eating), SV= eating disorders characterized by self-induced vomiting, BN=bulimia nervosa, EDNOS=eating disorders not otherwise specified, N.A.=data not available

SNP selection and genotype measurements

Genomic DNA was isolated from buccal swabs for the case group from the GenED study and for part of the NTR control group (39%). For the EDNET-Essen, and the Utrecht samples, genomic DNA was isolated from blood samples.

Table 5.2 Selected SNPs per candidate gene

Gene	SNP	Remarks
STMN1	rs12037513	The 3 SNPs genotyped capture
	rs807055	11 out of 12 (91%) alleles of
	rs807062	STMN1 at r^2 >=0.8
HTR1D	rs605367	The 2 tagging SNPs (rs676643
	rs6300	and rs674386) genotyped
	rs676643	capture 9 out of 10 (90%)
	rs674386	alleles of HTR1D at r^2 >=0.8
TPH2	rs10748185	The 10 SNPs genotyped
	rs2129575	capture 108 out of 148 (72%)
	rs17110489	alleles of TPH2 at r^2 >=0.8
	rs7305115	
	rs1007023	
	rs4760820	
	rs1473473	
	rs3903502	
	rs12231356	
	rs4474484	
BDNF	rs7124442	The 8 SNPs genotyped capture
	rs6265	38 out of 53 (71%) alleles of
	rs11030107	BDNF at r^2 >=0.8
	rs7103873	
	rs11030123	
	rs17309930	
	rs2049048	
	rs1491851	

HTR1D SNPs were selected based on previous association studies in AN (Bergen et al., 2003; Brown et al., 2006). For *BDNF*, *STMN1*, and *TPH2* tagging SNPs were selected from HapMap Public Release #19 applying the efficient multimarker method with r^2>0.8 and minor allele frequency (MAF)>0.05 as implemented in the HapMap web browsers (http://www.hapmap.org; de Bakker et al., 2005). Two of the selected *HTR1D* SNPs

(rs676643 and rs674386) were also present as tagging SNPs in the HapMap database. In Table 5.2 the selected SNPs and coverage rate per candidate gene are listed.

Multiplex genotyping assays were designed using Assay Designer software (Sequenom, San Diego, CA). SNPs were genotyped by mass spectrometry (the homogeneous MassARRAY system; Sequenom, San Diego, CA) using standard conditions. PCR reactions were carried out in a final volume of 5 µl and contained standard reagents and 2.5 ng of genomic DNA. Genotypes were assigned by using Genotyper version 3 software (Sequenom, San Diego, CA).

Genotype call rates for each multiplex were checked within the cohorts. Samples with call rate <75% were excluded from further analyses in the datasets. Success rates of the SNPs ranged from 97.9 to 100% for the GenED case group, and from 87.3 to 100% for the NTR control group. Between 6 and 10% of the samples were genotyped in duplicate and checked for concordance. Duplicate genotyping error rates were 0.07% in the case group, 0.2% for the control sample.

For the GAIN GWA controls, genomic DNA was isolated from blood samples. Individual genotyping was conducted by Perlegen Sciences (Mountain View, CA, USA) using a set of four proprietary, high-density oligonucleotide arrays (Sullivan et al., 2009). SNPs were imputed by Abecasis' MACH (v1). For the imputed SNPs the average maximum posterior probability was calculated. This measure represents how much uncertainty there is for the imputation of each SNP, ranging from 0 (high uncertainty) to 1 (low uncertainty).

Statistical analyses

The χ^2 test for Hardy-Weinberg equilibrium (HWE) was calculated in the NTR controls using the HWE program of LINKUTIL (http://linkage.rockefeller.edu/ott/linkutil.htm). To investigate the association of the 25 SNPs from four candidate genes we applied a two-stepped approach. First, allele frequencies for all SNPs were compared between cases from the GenED study and controls from the NTR. SNPs that showed nominal significant association (p<0.05) with either AN or SV in the first step, were tested for replication in a meta-analysis with the two additional independent case-control samples (EDNET and Essen, and Utrecht).

Differences in allele frequencies were compared and tested for significance by Pearson's chi-square test with SPSS version 15 software (SPSS, Chicago, IL). For the meta-analysis, the fixed- and random-effects model of DerSimonian and Laird (DerSimonian & Laird, 1986) was used to estimate summary odds ratio's (ORs), as implemented in R (http://www.r-project.org/, package meta). The heterogeneity was

quantified using the I2 statistic for inconsistency (Higgins & Thompson, 2002) and its statistical significance was tested with the $\chi 2$ distributed Cochran Q statistic (Lau et al., 1997). I^2 describes the proportion of variation that is unlikely to be due to chance and is considered large for values over 50% (Higgins & Thompson, 2002). Two tailed *p*-values are reported for all analyses.

Power calculations were performed in Quanto version 1.2.4 (2009). Instead of adjusting *p*-values a priori for multiple testing, nominal *p*-values are provided in order to allow the reader to interpret the level of significance. The results from the final analyses were corrected for multiple testing by using an interface developed by Nyholt (2004), available at http://genepi.qimr.edu.au/general/daleN/SNPSpD/. Given the fact that the linkage disequilibrium (LD) structure among the SNPs was not independent, adjusting the *p*-value for the actual number of tests would be overly stringent and result in a loss of power. With this method the *p*-values were therefore adjusted for the estimated number of *independent* SNPs tested. Calculation of the number of independent SNPs (also called the effective number of SNPs; M_{eff}) was based on the number of eigenvalues of the *n* x *n* correlation matrix of allele frequencies of SNPs using equation 5 by Li and Ji (2005).

Results

SNP association analysis

In the NTR control group none of the SNPs revealed a departure from HWE ($p>0.01$). Depending on the MAF of the SNP, this initial study had adequate power (85% power at an alpha level of 0.05, log-additive or allelic model) to detect effects sizes ranging between 1.45 and 1.8 for AN and ranging between 1.48 and 1.85 for SV.

The results of the association analysis in the initial study (GenED cases and NTR controls) are presented in Table 5.3. A nominal significant association ($p<0.05$) was observed for *TPH2* rs1473473 in AN as well as SV. This SNP was followed-up in the meta-analysis.

Exploratory association analyses were performed in the restricting type AN subgroup (N=108) of the GenED study and the NTR controls. The results of these analyses are presented in supplementary Table S5.1. No significant association was observed for any of the 25 SNPs

Table 5.3 Minor allele frequencies (MAF) for each SNP in cases of the GenED study and NTR controls

				Control	AN			SV		
			DNA	(n=607)	(n=182)			(n=149)		
Gene	Position	SNP	change	MAF	MAF	X^2	*p*	MAF	X^2	*p*
STMN1	1.p36.11	rs12037513	A>G	0.35	0.32			0.33		
		rs807055	C>T	0.43	0.39			0.37	3.14	0.08
		rs807062	G>C	0.25	0.26			0.24		
HTR1D	1.p36.12	rs605367	T>C	0.31	0.33			0.33		
		rs6300	A>G	0.10	0.10			0.07		
		rs676643	G>A	0.16	0.15			0.16		
		rs674386	G>A	0.29	0.30			0.30		
TPH2	12.q21.1	rs10748185	G>A	0.49	0.45			0.46		
		rs2129575	G>T	0.26	0.25			0.24		
		rs17110489	T>C	0.26	0.27			0.24		
		rs7305115	G>A	0.41	0.41			0.41		
		rs1007023	T>G	0.12	0.15			0.16	3.38	0.07
		rs4760820	C>G	0.43	0.40			0.38	2.84	0.09
		rs1473473	T>C	0.14	0.18	4.26	0.04	0.19	4.82	0.03
		rs3903502	C>T	0.39	0.42			0.41		
		rs12231356	C>T	0.08	0.05	3.41	0.07	0.07		
		rs4474484	G>A	0.35	0.36			0.37		
BDNF	11p14.1	rs7124442	T>C	0.33	0.29			0.28		
		rs6265	C>T	0.19	0.19			0.20		
		rs11030107	A>G	0.27	0.23			0.24		
		rs7103873	G>C	0.46	0.49			0.48		
		rs11030123	G>A	0.11	0.10			0.10		
		rs17309930	C>A	0.20	0.20			0.18		
		rs2049048	G>A	0.16	0.13			0.17		
		rs1491851	C>T	0.46	0.45			0.46		

AN=anorexia nervosa, SV=eating disorders characterized by self-induced vomiting

Reported results are comparisons between allele frequencies (1df), *p*-values <0.1 are shown only.

Meta-analysis

The *TPH2* SNP rs1473473 was genotyped in the EDNET and Essen and the Utrecht case-control samples. In the GAIN GWA control group this SNP was imputed. The average maximum posterior probability, which represents how much uncertainty there is for the imputation of a SNP, was 0.99 for *TPH2* rs1437473. For the meta-analysis, genotype data was available for a total of 2,987 individuals (887 AN cases, 306 SV cases, 1914 controls) which provides adequate power (85% power at an alpha level of 0.05, log-additive or allelic

model, MAF of 0.16) to detect effect sizes higher than 1.25 for AN and higher than 1.4 for SV.

Table 5.4 Meta-analysis of *TPH2* SNP rs1473473 in anorexia nervosa and eating disorders characterized by self-induced vomiting

	SNP rs1473473				SNP rs1473473			
	AN				SV			
Study	OR	CIL	CIR	*p*	OR	CIL	CIR	*p*
GenED	1.39	1.02	1.92	0.040	1.46	1.04	2.04	0.029
Utrecht	1.25	0.98	1.60	0.067	1.24	0.91	1.70	0.176
Ednet-Essen	1.11	0.81	1.51	0.53	-	-	-	-
Meta-analysis	**1.25**	**1.06**	**1.47**	**0.009**	**1.34**	**1.06**	**1.69**	**0.013**

AN=anorexia nervosa, SV=eating disorders characterized by self-induced vomiting , CIL=lower 95% confidence interval, CIR=upper 95% confidence interval. Number of AN and SV cases per study: GenED AN N=182, SV N=149; Utrecht AN N=285, SV N=157; EDNET-Essen AN N=420, SV N=0.

Table 5.4 shows ORs, their 95% CI and *p*-values within the individual case-control samples and the subsequent meta-analyses. For the minor C-allele (frequency 0.16) of *TPH2* SNP rs1473473 a significant association was observed in the meta-analyses with both AN and SV. We observed an OR of 1.25 (95% CI 1.06-1.47, $p<0.009$) for AN, and an OR of 1.34 (95% CI 1.06-1.69, $p<0.013$) for SV. There was no significant evidence for heterogeneity of the effect in the AN or SV analyses ($p=0.58$, $I^2= 0\%$ and $p=0.50$, $I^2=0\%$).

Table 5.5 Genotype counts *TPH2* rs1473473 for the three case-control samples

	AN			SV			Control		
	Genotype (n)			Genotype (n)			Genotype (n)		
Case-control sample	11	12	22	11	12	22	11	12	22
GenED	123	52	7	95	52	2	447	125	18
Utrecht	187	90	8	95	49	3	789	300	29
EDNET-Essen	266	128	16	-	-	-	130	50	9

The OR for the combined group of AN and/or SV cases (n=1073) was 1.24 (95% CI 1.06-1.44, $p<0.006$). We could not observe significant evidence for heterogeneity of the effect ($p=0.38$, $I^2= 0\%$) between the different case-control samples. Based on the genotype frequencies of the *TPH2* SNP rs1473473 (presented in Table 5.5) we expected a dominant effect to be underlying the association. Therefore, we evaluated the association with this SNP in the combined case-group under a dominant genotypic model.

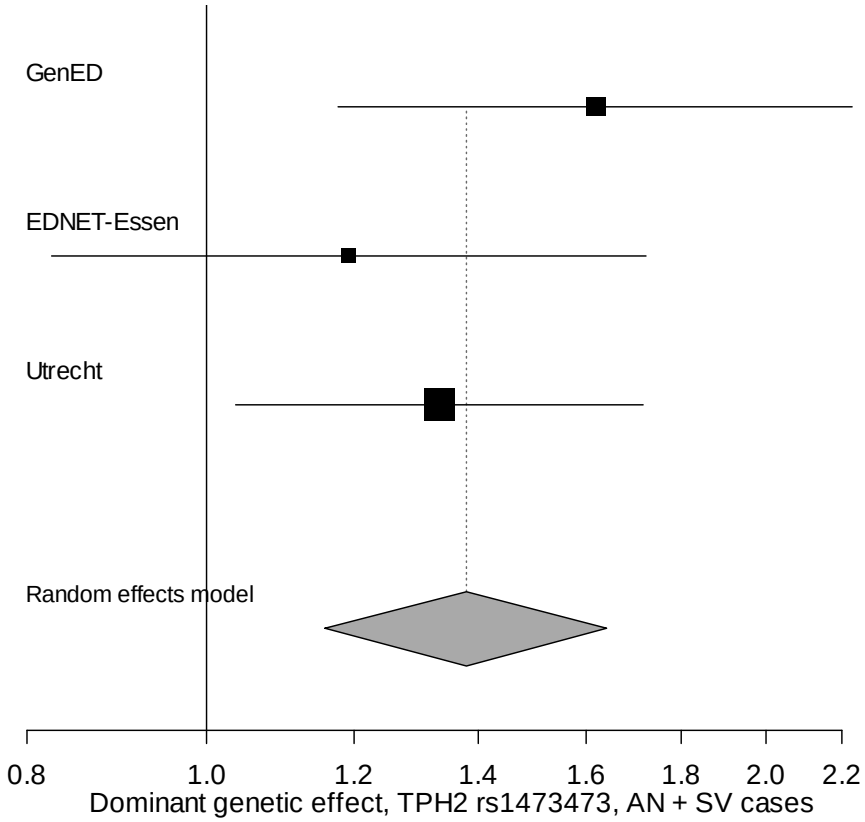


Figure 5.1 Random effect plot of the association between *TPH2* rs1473473 and the combined AN/SV cases under a dominant genotypic model.
Results: GenED: OR=1.62 (95% CI 1.18-2.23); EDNET-Essen: OR=1.19 (95% CI 0.83-1.72); Utrecht OR=1.33 (95% CI 1.04-1.72); Random effect model total: OR=1.38 (95% CI 1.16-1.64, p<0.0003)

Figure 5.1 represents the results of this association. Homo- and/or heterozygous carriers of the minor allele of rs1473473 had an increased probability of either AN or SV (OR=1.38, 95% CI 1.16-1.64, p<0.0003). Again, no evidence for heterogeneity was observed (p=0.44, I^2= 0%). As there is a general tendency for initial studies to overestimate effect sizes, we tested sensitivity of the association by excluding the discovery sample (GenED cases and NTR controls). Under the dominant genotypic model, carriers of the minor allele of rs1473473 had an OR of 1.29 (95%CI 1.05-1.59, p<0.018) among the two replication case-control samples. In supplementary Figure S5.1, the LD-plot between *TPH2* rs1473473 and the nine other selected *TPH2* tagging SNPs is depicted.

Because the LD structure among the SNPs was not completely independent, adjusting the *p*-value for the actual number of tests would be overly stringent and result in a loss of power. By using the interface developed by Nyholt (2004), the number of independent SNPs in our study was estimated to be 23.5. This led to an experiment-wide significance threshold of $p<0.002$. Thus the observed dominant effect of rs1473473 in the final analysis in the combined AN-SV group remained significant after adjustment for multiple testing. However, the observed effects in the separate AN and SV analyses did not remain significant. In this case, the method by Nyholt (2004) was still conservative since not all 25 SNPs were measured in the additional EDNET and Essen, and Utrecht case-control samples.

Discussion

This is the first study to report that *TPH2* SNP rs1473473 is significantly associated to AN and ED characterized by SV. When the two ED case groups are combined, a dominant genotypic model for rs1473473 shows that carriers of the minor allele of rs1473473 had a higher risk of AN or SV (OR=1.38, 95% CI 1.16-1.64, $p<0.0003$). This SNP tags an LD block that spans across part of the *TPH2* gene, and is ended by a recombination hotspot on one side. Therefore it is highly likely that this SNP is in LD with a functionally relevant variant(s) in the *TPH2* gene. The *TPH2* gene encodes the main rate-limiting enzyme in the synthesis of serotonin in the brain (Zill et al., 2007). Serotonin is involved in satiety, anxious and obsessional behavior, mood, and impulse control, features all linked to ED (Kaye, 2008; Lucki, 1998). In long-term recovered ED participants elevated 5-hydroxyindoleacetic acid levels in cerebrospinal fluid were detected (Kaye, 2008; Kaye et al., 2005c). This is the major metabolite of serotonin in the brain and body and is thought to reflect extracellular serotonin concentrations. This finding thus could be indicative of an 'overactive' serotonin system in ED, which in turn could be caused by an increased function of the *TPH2* gene. *TPH2* was also one of 182 candidate genes that were tested for association by comparing in total 5151 SNPs between 1085 AN cases and 677 controls (Pinheiro et al., 2010). After accounting for multiple testing, there were no statistically significant associations for any individual SNP (including *TPH2*). Rs1473473 is not in LD with known *TPH2* mutations (Haavik et al., 2008). *TPH2* SNPs in LD with rs1473473 however, have been associated with a suicidal mental condition in Finnish men (Zhou et al., 2005), with antidepressant response in depressive patients (Peters et al., 2004), and with allelic mRNA expression imbalance in sections of the human pons (Lim et al., 2007),

indicating that genetic variation at this locus may contribute to mental conditions and could influence gene function.

To retain adequate statistical power, the current study only covered a selection of candidate genes for ED. To replicate previous results in ED, we selected genes for which association was observed and confirmed in studies with an adequate sample size. Both *HTR1D* and *BDNF* fulfilled these criteria, although we acknowledge that inclusion of the gene encoding the opioid delta receptor (*OPRD1*) would also have been appropriate (Bergen et al., 2003; Brown et al., 2006; Ribases et al., 2004; Ribases et al., 2005). Because of previous inconsistent results the Serotonin Receptor 2A and the Serotonin Transporter genes were not included in our selection (for a review see Bulik et al., 2007b; Slof-Op 't Landt et al., 2005). Besides replication of previous results, the current study also aimed to evaluate the involvement of two unexplored candidate genes for ED. Like *HTR1D* and *OPRD1*, *STMN1* was located under the linkage peak of restrictive AN (1p33-36) (Grice et al., 2002). Because the associations with *HTR1D* and *OPRD1* only explained part of the linkage, it was expected that additional candidate genes could underlie the linkage peak (Bergen et al., 2003). *TPH2* was selected because of the link between serotonin and ED. The role of *TPH2* in the synthesis of serotonin (Zill et al., 2007), makes it a plausible candidate gene for ED. Thus far no other genes have been analysed in the GenED study.

A note concerning our study populations is the fact that the EDNET and Essen control population was limited in size and consisted of both men and women. However, no difference in the allele frequency of rs1473473 between sexes was observed, in either the German controls or the GAIN GWA control group (Boomsma et al., 2008). So it is unlikely that this has interfered with our results. Another remark with regard to the German sample is the lack of information regarding self-induced vomiting. Finally, the NTR control group were random controls, not selected based on for example liability to psychiatric disorders or social economic status. Due to the low prevalence of ED in the general population we do not think that this has affected our results.

Another concern is the issue of multiple testing. We acknowledge that if we correct for multiple testing in the GenED study, the association with rs1473473 does not remain significant. However, if we perform permutation analysis in this study the global *p*-value for the association between the *TPH2* gene and SV and AN is still trend significant ($p<0.10$). Therefore we do think that the decision to follow-up the association of *TPH2* SNP rs1473473 in the additional cohorts was justified.

The reported association between the functional *BDNF* Val-66-Met polymorphism (rs6265) and AN was not replicated in this study (Ribases et al., 2004; Ribases et al., 2005). However, this result is in line with several other studies which also could not confirm this

association (Dardennes et al., 2006; de Krom et al., 2006; Dmitrzak-Weglarz et al., 2007; Friedel et al., 2005; Koizumi et al., 2004; Mercader et al., 2007).

Previously two studies have reported significant association between *HTR1D* SNPs (including rs6300 and rs674386) and AN (Bergen et al., 2003; Brown et al., 2006). We did not detect any allele frequency differences between controls and AN cases in the four SNPs that were examined. Considering the strength of the previous association and the allele frequency, we should have had sufficient power to detect an effect of rs6300. For rs674386 on the other hand, statistical power was lower (60%), and the association may have been missed due to this reason.

No consistent associations were observed for the other positional candidate gene, *STMN1*. Despite its position under the linkage peak for AN, it might not be involved in ED. However, since linkage was observed in the restrictive subtype of AN, it is also possible that an effect of this gene is only apparent in this specific eating disorder subgroup. The exploratory analyses in restrictive AN (N=108) of the GenED study and the NTR controls (supplementary Table S5.1), also did not reveal an association with *STMN1*. This exploratory study had adequate power (85% power at an alpha level of 0.05, log-additive or allelic model) to detect effects sizes around 1.6 for restricting AN. Thus, the association may have been missed due to limited statistical power.

For the first time candidate genes in ED characterized by self-induced vomiting were evaluated. We selected this phenotype because there is no *a priori* reason to believe that the DSM diagnostic schema represent more 'genetic' syndromes than underlying core behaviors or traits. A distinctive eating disorder symptom that is shared among different types of ED is self-induced vomiting. Prevalences of vomiting within clinical samples ranged between 31 and 39% for AN (Ben-Tovim et al., 1989; Garner et al., 1993), and even over 90% in BN (Ben-Tovim et al., 1989). The reliability of the measurement of this behavior and the heritability of self-induced vomiting has also been demonstrated (Sullivan et al., 1998a; Wade et al., 2008b). Other symptoms that are shared among ED are binge eating and the undue influence of weight and shape on self-evaluation. Binge eating has a substantial heritability but is less reliably measured (Bulik et al., 1998; Reichborn-Kjennerud et al., 2003; Sullivan et al., 1998a; Wade et al., 2000a; Wade et al., 2008b). The undue influence of body weight appears to be more environmentally mediated (Reichborn-Kjennerud et al., 2004a; Wade & Bulik, 2007).

Many genetic studies in AN have been performed, mainly in small populations measuring only one or a few SNPs (Bulik et al., 2007b). In the current study we used a large population of AN cases. We selected 25 SNPs to capture the majority of the common variation within four candidate genes (*STMN1*, *HTR1D*, *TPH2* and *BDNF*). Our two-step

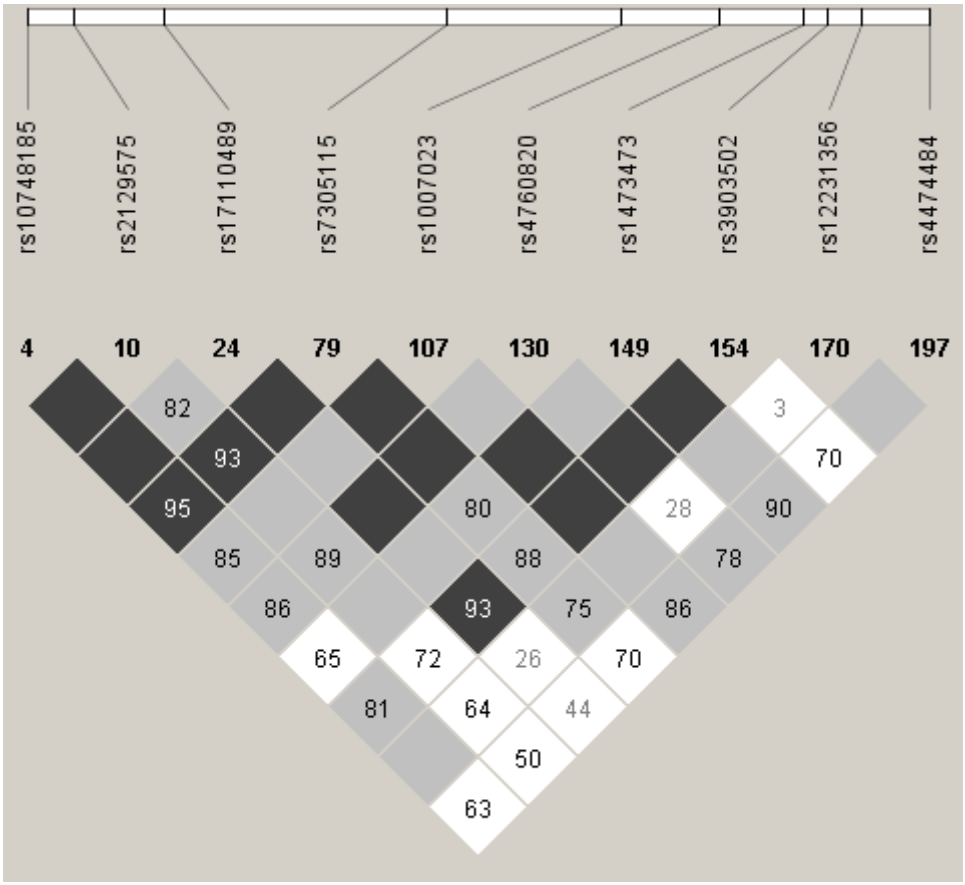
approach gave us the opportunity to explore association with all 25 SNPs in the first step, and to evaluate the initial findings in two additional independent case-control samples. This approach has led to a robust association of the *TPH2* SNP rs1473473. The minor allele of this SNP was associated with a higher risk for AN, SV and for the combined group. It is interesting that the same SNP was associated with both types of ED. Although there was overlap between the two types of ED, 13% of the 887 AN cases also belonged to the SV group, the effect of rs1473473 is also present in the independent AN and SV groups. It has been hypothesized that AN, BN, and also subthreshold forms of ED share at least some risk and liability factors (Kaye, 2008; Strober et al., 2000). In a Swedish twin study, approximately half of the genetic factors contributed to liability to both AN and BN (Bulik et al., 2010). Our current finding is consistent with this hypothesis. For future studies we aim to establish the effect of genetic variation at the *TPH2* gene on behaviors underlying different types of ED, like perfectionism, impulsivity or obsessive-compulsiveness (Kaye, 2008).

Supplementary table S5.1 Minor allele frequencies (MAF) for each SNP in restricting AN cases of the GenED study and NTR controls

				Control (n=607)	Restricting AN (n=108)		
Gene	Position	SNP	DNA change	MAF	MAF	X^2	*p*
STMN1	1.p36.11	rs12037513	A>G	0.35	0.31		
		rs807055	C>T	0.43	0.42		
		rs807062	G>C	0.25	0.29		
HTR1D	1.p36.12	rs605367	T>C	0.31	0.33		
		rs6300	A>G	0.10	0.11		
		rs676643	G>A	0.16	0.17		
		rs674386	G>A	0.29	0.29		
TPH2	12.q21.1	rs10748185	G>A	0.49	0.44		
		rs2129575	G>T	0.26	0.27		
		rs17110489	T>C	0.26	0.27		
		rs7305115	G>A	0.41	0.41		
		rs1007023	T>G	0.12	0.14		
		rs4760820	C>G	0.43	0.42		
		rs1473473	T>C	0.14	0.18	2.77	0.09
		rs3903502	C>T	0.39	0.42		
		rs12231356	C>T	0.08	0.06		
		rs4474484	G>A	0.35	0.37		
BDNF	11p14.1	rs7124442	T>C	0.33	0.29		
		rs6265	C>T	0.19	0.22		
		rs11030107	A>G	0.27	0.24		
		rs7103873	G>C	0.46	0.45		
		rs11030123	G>A	0.11	0.11		
		rs17309930	C>A	0.20	0.19		
		rs2049048	G>A	0.16	0.12		
		rs1491851	C>T	0.46	0.44		

AN=anorexia nervosa.

Reported results are comparisons between allele frequencies (1df), *p*-values <0.1 are shown only.



Supplementary figure S5.1 Linkage disequilibrium (LD) plot for the ten *TPH2* tagging SNPs, based on HapMap. D' values are presented (if absent D'=1), color scheme dark grey: strong evidence of LD ; light gray: uninformative; white: strong evidence of recombination.

Chapter 6

Genetic variation at the TPH2 gene influences impulsivity in addition to eating disorders

This chapter was previously submitted:
Slof-Op 't Landt, M. C. T., Bartels, M., Slagboom, P.E.,Boomsma, D. I., van Furth, E. F., & Meulenbelt I.(2011). *Psychiatric Genetics*
.

Abstract

Objective: Genes are involved in anorexia nervosa (AN) and a key symptom of different types of eating disorders (ED), self-induced vomiting (SV). Perfectionism and impulsivity are possible predisposing personality features for ED. Could genetic variation in the Tryptophan Hydroxylase 2 (*TPH2*) gene, previously associated with AN and SV (Slof-Op 't Landt et al., 2011), explain the overlap between ED, perfectionism and impulsivity?
Method: Perfectionism and impulsivity scores were compared between 324 participants with ED and 240 controls. Subsequently, the genetic association between four *TPH2* SNPs and perfectionism/impulsivity was evaluated. First in a random twin-based control group (N=512), and secondly within ED participants.
Results: ED participants report higher levels of perfectionism and impulsivity than controls. The minor alleles of *TPH2* rs1473473 (OR=1.49, p=0.04) and rs1007023 (OR=1.60, p=0.02) were more frequent in impulsive controls, but also in impulsive participants with ED (OR=1.79, p=0.05 and OR=1.83, p=0.02, respectively).
Discussion: Genetic variation at the *TPH2* gene appeared to affect impulsivity which in turn might predispose to the ED phenotype.

Eating disorders have chronicity and mortality rates which are among the highest in the field of psychiatry (Nielsen et al., 1998). The most familiar types of eating disorders are anorexia nervosa (AN) and bulimia nervosa (BN). However, the majority of eating disorder patients (about 60%) do not meet strict DSM-IV diagnostic criteria for either of these types, and therefore belong to the eating disorder not otherwise specified (EDNOS) category (Fairburn & Bohn, 2005). An important symptom that is shared among the different types of eating disorders (AN purging type, BN, and EDNOS) is self-induced vomiting (SV), which has been associated with greater clinical severity and higher novelty seeking (Dalle Grave et al., 2009; Reba et al., 2005).

Despite substantial efforts to identify causal pathways for AN and BN, very little is known about the aetiology of eating disorders. In longitudinal and cross-sectional studies, several risk factors have been proposed, including being a woman, elevated weight and shape concerns, negative self-evaluation, dieting, perfectionism and possibly impulsivity (Fairburn et al., 1997; Fairburn et al., 1998; Jacobi et al., 2004; Stice, 2002). Population-based twin studies have yielded moderate to substantial heritability estimates (8%-83%) for eating disorders and self-induced vomiting in women (Bulik et al., 2006; Slof-Op 't Landt et al., 2005; Sullivan et al., 1998a; Wade et al., 2008b).

In this paper we explore the hypothesis that part of the heritability of eating disorders may be explained by heritable aspects of perfectionism and impulsivity, which are consistently associated to AN and /or BN (Cassin & von Ranson, 2005). Both perfectionism and impulsivity remained present after recovering from an eating disorder (Bastiani et al., 1995; Kaye et al., 1998; Lilenfeld et al., 2000; Srinivasagam et al., 1995; Wagner et al., 2006). In addition, elevated levels of perfectionism were observed in relatives of individuals with AN and BN (Lilenfeld et al., 2000; Woodside et al., 2002). These findings suggest that perfectionism and impulsivity may be of potential aetiological relevance for eating disorders.

The Multidimensional Perfectionism Scale (MPS) developed by Frost et al. (Frost et al., 1990) is one of the most widely used instruments to measure perfectionism. This scale distinguishes six dimensions of perfectionism, the core three dimensions were moderate to substantially heritable (0.29 to 0.42) (Tozzi et al., 2004). The Dickman Impulsivity Inventory (DII; (Dickman, 1990) distinguishes two forms of impulsivity (dysfunctional and functional). This same distinction was observed in a factor analysis based on the DII, the Eysenck Impulsiveness Questionnaire, and the Barrat Impulsiveness Scale in a Flemish population (Bastiaens et al., 2003). For the DII scale the highest factor loadings and highest internal consistency was observed. A substantial heritability of approximately 45% has been estimated for different impulsivity measures (Hur & Bouchard, Jr., 1997; Pedersen et

al., 1988; Seroczynski et al., 1999). To obtain perfectionism and impulsivity scores in a large random control group, we have selected two items from the Youth Self Report (YSR) (Levinson, 2005; Verhulst et al., 1997) questionnaire, to represent these behaviours in the current study.

Recently we observed an association between tryptophan hydroxylase 2 (*TPH2*) SNP rs1473473 and AN (p=0.04) as well as ED characterized by SV (p=0.03) (Slof-Op 't Landt et al., 2011). Trend significant associations were observed for TPH2 rs12231356, rs1007023 and rs4760820 (p<0.09). In a subsequent meta-analysis with two additional independent case-control populations the association between rs1473473 and both disorders (AN p=0.009, SV p=0.01) was replicated. The *TPH2* gene encodes tryptophan hydroxylase in the brain (Zill et al., 2007). This enzyme catalyses the formation of 5-hydroxytrypthophan, which is the first and rate-determining step in the biosynthesis of the neurotransmitter serotonin. In individuals with long-term recovered AN and BN elevated 5-hydroxyindoleacetic acid levels in cerebrospinal fluid were detected (Kaye et al., 1991; Kaye et al., 1998). This is the major metabolite of serotonin in the brain and body and is thought to reflect extracellular serotonin concentrations. This finding thus could be indicative of an ‘overactive’ serotonin system in eating disorders, which in turn could be caused by an increased function of the *TPH2* gene. It has been hypothesized that a disturbance of serotonin activity may create a vulnerability for a cluster of symptoms that are common to both AN and BN, for example perfectionism and impulsivity/control (Bruce et al., 2005; Kaye et al., 1998; Racine et al., 2009; Steiger et al., 2001; Steiger et al., 2004; Steiger et al., 2005).

In the current study, we investigated whether genetic variation at the *TPH2* gene explains the overlap between eating disorders, perfectionism and impulsivity, by performing three analyses. Firstly, extensive phenotypic analyses were conducted to evaluate the association between the perfectionism and impulsivity phenotypes, as measured by the MPS and DII, and AN and SV in 324 participants with eating disorders from the GenED study and 240 controls without an eating disorder. DNA was not collected for this control group. Secondly, to study the involvement of the *TPH2* gene in perfectionism and impulsivity items (YSR) in the absence of disease, four *TPH2* SNPs were evaluated in a second control group consisting of random twin-based controls (N=512) from the Netherlands Twin Registry (NTR). Thirdly, for the SNPs that showed an association with impulsivity or perfectionism in the NTR controls, we tested if this association was also present within the participants with eating disorders from the GenED study for which the *TPH2* genotypes were available (N=267).

Methods

Participants

This study was approved by the ethics committee of the VU University and by the ethics committee for mental health instutions in the Netherlands (METiGG). All participants (and if underage, their parents) gave written informed consent. In this study we distinguish three groups (Table 6.1): 1. participants with eating disorders from the GenED study, for whom extensive perfectionism and impulsivity phenotypes (MPS, DII, and YSR) and DNA was available; 2. controls without an eating disorder, for whom extensive perfectionism and impulsivity phenotypes (MPS and DII) but no DNA was available; 3. random twin-based controls from the NTR, for whom less extensive perfectionism and impulsivity phenotypes (YSR) and DNA was available.

The GenED study.

Female eating disorder patients (N=322) were recruited through 10 specialist eating disorder units throughout the Netherlands (the GenED study). DSM-IV eating disorder diagnoses were made by experienced clinicians based on a semi-structured interview at intake, and by the self-report eating disorder examination questionnaire (Fairburn & Beglin, 1994). Of the 322 participants, 218 fulfilled modified DSM-IV criteria for AN (excluding criterion D, amenorrhea). Based on the eating disorder examination questionnaire and assessment interviews, we defined a subgroup of participants (n=189) who reported regular self-induced vomiting (SV). These two groups were partly overlapping, 85 participants belonged to both groups, thus the eating disorder group was comprised of 133 independent participants with AN, and 104 independent participants with SV.

DNA was collected for all the participants of the GenED study. However, *TPH2* SNPs were only genotyped in part of the participants with eating disorders (as can be seen in Table 6.1). For the genetic analyses in the eating disorder group, *TPH2* genotype data was available for 267 participants with either AN or SV.

Controls

A healthy control group was recruited through advertisements in magazines and via internet websites. The questionnaires collected in the GenED study were send to 276 women, 252 women returned the questionnaire (response rate 91.3%). Twelve women were excluded because they reported that they had a current or past eating disorder. A total of 240 women without an eating disorder were used in the current study for phenotypic comparison to participants with AN and SV. No DNA was collected from these participants.

Netherlands Twin Registry (NTR) controls

The NTR was established in the late 1980s at the VU University in Amsterdam, the Netherlands. Data on the multiples (twins or triplets) and their family members have been collected every two to three years in longitudinal survey studies (Bartels et al., 2007; Boomsma et al., 2002). Subsamples of the multiples were invited to participate in experimental and laboratory studies and provide their DNA (Boomsma et al., 2006). Genotype and phenotype data for a total of 512 random unrelated women from the NTR were analyzed.

Table 6.1 Sample sizes and available phenotype and genotype data in the different participating groups

Participating Groups	MPS Perfectionism	DII Impulsivity	YSR Perfectionism	YSR Impulsivity	*TPH2* genotypes
GenED study					
Participants with eating disorders (AN and/or SV)	322	315	89	89	267
Controls					
Controls without an eating disorder	240	233	-	-	-
Netherlands Twin Registry (NTR)					
Twin-based controls	-	-	512	484	512

MPS=Multidimensional Perfectionism Scale, DII= Dickman Impulsivity Inventory, YSR=Youth Self Report

Measures

Phenotypes

An overview of measurement instruments available in the participating groups in this study is presented in Table 6.1. The Multidimensional Perfectionism Scale (MPS) by Frost (1990) is a 36-item questionnaire which distinguishes six dimensions of perfectionism: Concern over Mistakes (the negative reaction to mistakes and the tendency to interpret mistakes as equivalent to failure), Personal Standards (the settings of high standards and the importance placed on these standards for self-evaluation), Parental Expectations (the believe that one's parents set very high goals), Parental Criticism (the believe that one's parents are overly critical), Doubt about Actions (the tendency to doubt about the ability to accomplish tasks), and Organization (the importance and preference for order and organization). Responses were given on five point Likert-scales, ranging from 'strongly disagree' to 'strongly agree'. The coefficients of internal consistency for the factor scales ranged from 0.77 to 0.93 (Frost

et al., 1990). In our control group without eating disorders the internal consistency coefficients were comparable and ranged from 0.79 to 0.91.

The Dickman Impulsivity Inventory (DII, Dickman, 1990) is a 23-item questionnaire with responses in a true/false answer format. This instrument distinguishes two forms of impulsivity: Dysfunctional Impulsivity (the tendency to engage in rapid, error-prone information processing in situations where this is nonoptimal) and Functional Impulsivity (the tendency to engage in rapid, error-prone information processing when such a strategy is rendered optimal). The coefficient of internal consistency was 0.85 for the DI subscale and 0.74 for the FI subscale (Dickman, 1990). In our controls without eating disorders the internal consistency coefficients were 0.75 for the DI subscale and 0.77 for the FI subscale.

The Young Adult Self Report (YASR; Achenbach, 1990) and the Youth Self Report (YSR; Levinson, 2005; Verhulst et al., 1997) questionnaires belong to the Achenbach System of Empirically Based Assessment (ASEBA, www.aseba.org), which provides age adjusted instruments to assess similar facets of maladaptive functioning from 1.5 to 90 years. Responses were given on three point scale, with the code 0 if the item was not true, 1 for sometimes true, and 2 for often true. For the current study two items (item 32: I feel that I have to be perfect, and item 41: I act without stopping to think) from the YSR/YASR questionnaire were used to measure perfectionism and impulsivity.

For the genetic analyses in the eating disorder group, data from the YSR perfectionism, YSR impulsivity, MPS perfectionism and DII impulsivity measures were available to test for association with the *TPH2* SNPs.

Genotype measurements

Genomic DNA was isolated from buccal swabs for the participants with eating disorders from the GenED study and for part of the controls from the Netherlands Twin Registry (39%), for the remaining 61% of the genotype controls genomic DNA was isolated from blood samples. Multiplex genotyping assays were designed using Assay Designer software (Sequenom, San Diego, CA). SNPs were genotyped by mass spectrometry (the homogeneous MassARRAY system; Sequenom, San Diego, CA) using standard conditions. PCR reactions were carried out in a final volume of 5 µl and contained standard reagents and 2.5 ng of genomic DNA. Genotypes were assigned by using Genotyper version 3 software (Sequenom, San Diego, CA).

In a previous study (Slof-Op 't Landt et al., 2011), ten *TPH2* tagging SNPs were selected from HapMap Public Release #19. We observed and replicated an association between the minor allele of *TPH2* SNP rs1473473 and a higher risk for both AN (OR=1.30, 95% CI 1.08-1.57, $p<0.003$) and SV (OR=1.52, 95% CI 1.28-2.04, $p<0.006$). Furthermore,

trend significant associations were observed for three additional *TPH2* SNPs in participants from the GenED study, rs12231356 was associated with AN (OR=0.59, 95% CI 0.35-1.01, $p<0.05$), rs1007023 (OR=1.40, 95% CI 0.98-2.01, $p<0.06$) and rs4760820 (OR=0.80, 95% CI 0.62-1.04, $p<0.09$) with SV. In the current study, genotypes for these four SNPs were available for 267 participants with eating disorders and 512 controls from the NTR.

Study design

In the current study we investigated whether genetic variation at the *TPH2* gene explains the overlap between eating disorders, perfectionism and impulsivity, by performing three analyses. In the phenotypic analyses, we tested whether perfectionism and impulsivity, as measured by the MPS and DII scales, are different in participants with an eating disorder (AN or SV) from the GenED study compared to controls without an eating disorder (see Table 6.1). In addition to the MPS and DII scales, the YSR perfectionism and impulsivity items were obtained in a subset of the participants with eating disorders (N=89). In these participants, we tested the overlap between the different MPS and DII scales and the YSR items.

In the genetic analyses, we tested whether the four *TPH2* SNPs that we found previously to be associated to eating disorders (rs1007023, rs4760820, rs1473473, and rs12231356; Slof-Op 't Landt et al., 2011) were associated to perfectionism or impulsivity, as measured by the YSR items, in the random twin-based control group from the NTR (N=512).

In the final analyses, we tested whether the *TPH2* SNPs, that were associated with perfectionism or impulsivity in the NTR controls, also showed such associations in the participants with eating disorders from the GenED study. These analyses were performed for the YSR items, and the MPS and DII measures that were substantially correlated to these items.

Statistical Analyses

Data from the MPS and DII scales were tested for normality. Natural logarithm transformations were calculated for nonnormally distributed data. ANOVA's were performed to calculate the differences between the participants without eating disorders and the participants with eating disorders (AN, SV, and the combined group), and to compare the two independent eating disorder groups (AN and SV) with each other.

The overlap between the YSR perfectionism and impulsivity items and the subscales of the MPS and DII in a subset of the participants with eating disorders (N=89) was evaluated

by calculating Pearson correlations. The MPS and/or DII scales that were correlated (above 0.5) to the YSR perfectionism or impulsivity item were used in the final genetic analyses.

In the two genetic analyses, Pearson's Chi-Square statistics were calculated to compare response frequencies of perfectionism and impulsivity between carriers and non-carriers of the minor allele of the *TPH2* SNPs, and between the different *TPH2* genotypes. In the NTR controls these analyses were performed for the YSR items, whereas the YSR items, the MPS and the DII subscales were tested in the participants from the GenED study. All statistical analyses were performed in SPSS version 16 (SPSS, Chicago, IL).

Results

Phenotypic analyses

The controls without eating disorders (N=240) were on average four years older (mean age=31.7, SD=11.3) than the participants with AN (mean age=27.1, SD=9.4), and with SV (mean age=27.8, SD=9.8). As expected, the BMI was significantly different between the controls (mean BMI=22.4, SD=3.4) and the participants with AN (mean BMI=16.6, SD=2.9). Within the control group a significant effect for age on the perfectionism scales Personal Standards and Parental Criticism was observed, therefore the subsequent case-control analyses were adjusted for age.

As can be seen in Table 6.2, the participants with eating disorders scored significantly higher on all perfectionism scales and the Dysfunctional Impulsivity scale (except for participants with AN), whereas the score on Functional Impulsivity was significantly lower in participants with eating disorders (p-values ranging from 0.003 to 5.8×10^{-50}). Within the eating disorder group, the participants with AN scored significantly higher than the participants with SV on the perfectionism scales Personal Standards ($t(232)=3.063$, $p<0.01$) and Organization ($t(234)=3.882$, $p<0.01$). Dysfunctional Impulsivity scores, on the other hand, were significantly higher in the participants with SV ($t(232)=-3.923$, $p<0.01$). When performing nonparametric tests instead of applying natural log-transformations identical results were observed (data not shown).

As expected the participants with eating disorders scored extremely different on the perfectionism and impulsivity scales compared to the controls without an eating disorder. Because the differences between participants with AN and with SV were minimal, and the scores in comparison to the controls went in the same direction, the combined AN/SV group was used in the subsequent genetic analyses.

Table 6.2. Comparison of MPS and DII scales between Controls and independent AN, independent SV, AN and/or SV combined

	Controls (N=240) Mean (SD)	AN (N=133) Mean (SD)	*p*	SV (N=104) Mean (SD)	*p*	AN / SV (N=322) Mean (SD)	*p*
MPS:							
CM	15.7 (6.2)	30.9 (9.0)	**	28.6 (9.5)	**	30.4 (9.1)	**
PS[1]	17.2 (6.0)	26.8 (5.7)	**	24.6 (7.2)	**	26.0 (6.5)	**
PE	7.3 (3.4)	9.5 (5.1)	*	9.3 (4.7)	*	9.5 (4.9)	**
PC[1]	5.4 (2.7)	8.1 (4.2)	**	8.4 (4.4)	**	8.4 (4.4)	**
DA	7.2 (3.1)	13.0 (4.0)	**	12.5 (4.6)	**	13.1 (4.1)	**
O	19.5 (5.2)	24.4 (5.3)	**	21.6 (5.6)	*	23.4 (5.6)	**
DII:							
FI	6.6 (2.7)	3.7 (2.8)	**	4.2 (2.8)	**	3.8 (2.8)	**
DI	1.8 (2.2)	2.3 (2.8)	N.S.	3.7 (3.5)	*	3.0 (3.2)	*

MPS=Multidimensional Perfectionism Scale: CM=Concern over Mistakes; PS=Personal Standards; PE=Parental Expectations; PC=Parental Criticism; DA=Doubt about Actions; O=Organization. DII=Dickman Impulsivity Inventory: FI=Functional Impulsivity; DI=Dysfunctional Impulsivity.

[1] Analyses in PC and PS corrected for age

* $p < 5 \times 10^{-4}$

** $p < 5 \times 10^{-10}$

The two YSR items measuring perfectionism and impulsivity were also present in a subset of the eating disorder group (N=89). Table 6.3 presents the Pearson's correlations between the different MPS and DII scales and the YSR items. For the YSR perfectionism item the highest correlation was observed with the MPS scales Concern over Mistakes (r=0.53) and Personal Standards (r=0.51). The correlation between the YSR impulsivity item and the Dysfunctional Impulsivity scale of the DII was 0.61.

Table 6.3 Pearson correlations between YSR items and MPS and DII scales in participants with eating disorders (N=89)

	MPS						DII	
YSR-item	CM	PS	PE	PC	DA	O	FI	DI
Perfectionism	0.53**	0.51**	0.01	0.05	0.38**	0.21*		
Impulsivity							0.01	0.61**

MPS=Multidimensional Perfectionism Scale: CM=Concern over Mistakes; PS=Personal Standards; PE=Parental Expectations; PC=Parental Criticism; DA=Doubt about Actions; O=Organization. DII=Dickman Impulsivity Inventory: FI=Functional Impulsivity; DI=Dysfunctional Impulsivity.

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Genetic analyses in twin-based controls

The NTR control group had a mean age of 29.3 (SD=14.3) and a mean BMI of 22.9 (SD=4.1). For the YSR perfectionism response frequencies were 47.7% score 0, 41.6% score 1, and 9.3% score 2. The response frequencies for the YSR impulsivity item were 45.9% score 0, 49.6% score 1, and 4.5% score 2. Because less than five percent of the controls reported an impulsivity-score of 2, the responses were merged into two answer categories (absent/present, Impulsivity 1 / 2).

In Table 6.4 the genotype counts and minor allele frequencies of the four *TPH2* SNPs are shown per perfectionism category. As a comparison the MAF in participants with AN and/or SV are also listed in this table.

Table 6.4 Genotype counts and minor allele frequencies (MAF) for the four *TPH2* SNPs per perfectionism category within a control sample (as reference MAF in combined AN/SV group are included)

		Rs1007023	Rs476820	Rs1473473	Rs12213156
Perfectionism 0	Genotype 11 (n)	200	77	187	203
(N=244)	Genotype 12 (n)	34	126	43	39
	Genotype 22 (n)	10	37	10	1
	MAF	0.11	0.42	0.13	0.08
Perfectionism 1	Genotype 11 (n)	154	72	150	178
(N=213)	Genotype 12 (n)	54	101	55	33
	Genotype 22 (n)	4	38	5	2
	MAF	0.15	0.42	0.16	0.09
Perfectionism 2	Genotype 11 (n)	10	14	43	50
(N=55)	Genotype 12 (n)	4	28	11	5
	Genotype 22 (n)	1	13	1	0
	MAF	0.12	0.49	0.12	0.05
Association	Geno *p*	**0.02**	0.51	0.21	0.61
	Allele *p*	0.26	0.34	0.47	0.35
AN/SV (N=267)	MAF	0.15	0.39	0.19	0.06

For rs1007023 a significant difference in genotype frequency was observed between the three perfectionism categories. However, no allelic association was observed and the genotypic effect appeared to be due to a higher frequency of heterozygotes in the 'middle perfectionism' category compared to lower frequencies of heterozygotes in both the 'no perfectionism' and 'high perfectionism' group, and is not considered robust.

For impulsivity (Table 6.5) two SNPs showed a significant association. Both genotypic and allelic associations were observed for rs1007023. The minor allele of this SNP was more frequent in impulsive controls compared to non impulsive controls (OR=1.60, 95% CI 1.08-2.36, p=0.02). Furthermore, the minor allele of rs1473473 was also more frequent in the impulsive controls (OR=1.49, 95% CI 1.02-2.17, p=0.04) compared to the non-impulsive controls. The linkage disequilibrium (LD) between the two *TPH2* SNPs was high

(D'= 0.95, r^2=0.74), therefore haplotype analyses with these SNPs were performed in New Thesias (http://ecgene.net/genecanvas/uploads/THESIAS3.1). The combined haplotype of the minor alleles was significantly more frequent in the impulsive controls compared to the non-impulsive controls (OR=1.58, 95% CI 1.05-2.37, *p*=0.03).

Table 6.5 Genotype counts and minor allele frequencies (MAF) for the four TPH2 SNPs per impulsivity category within a control sample (as reference MAF in combined AN/SV group are included)

		Rs1007023	Rs476820	Rs1473473	Rs12213156
Impulsivity 0	Genotype 11 (n)	179	66	172	188
(N=222)	Genotype 12 (n)	41	117	42	33
	Genotype 22 (n)	2	37	4	1
	MAF	0.10	0.43	0.12	0.08
Impulsivity 1 / 2	Genotype 11 (n)	195	90	187	221
(N=262)	Genotype 12 (n)	54	125	62	39
	Genotype 22 (n)	13	43	11	2
	MAF	0.15	0.41	0.16	0.08
Association	Geno *p*	**0.03**	0.50	0.13	0.91
	Allele *p*	**0.02**	0.43	**0.04**	0.85
AN/SV (N=267)	MAF	0.15	0.39	0.19	0.06

Genetic analysis in participants with an eating disorder

The YSR impulsivity item and *TPH2* genotypes were available for 79 participants with AN and/or SV. The reported frequencies for YSR impulsivity were 34.2% score 0, 50.6% score 1, and 15.2% score 2. In accordance with the NTR controls, the responses on the YSR impulsivity item were merged into two answer categories (absent/present, Impulsivity 1 / 2). In the eating disorder group the minor alleles of both rs1007023 and rs1473473 appeared to be more frequent in the impulsive participants compared to the non-impulsive participants (14.8% vs 25.0%), although this effect did not reach significance (OR=1.97, 95% CI 0.80-4.59, *p*=0.14).

In the final analyses, it was evaluated whether the *TPH2* SNPs rs1007023 and rs1473473 were also associated to the Dysfunctional Impulsivity scale in the eating disorder group. The responses on this impulsivity scale were also merged into two answer categories, 31% of the participants reported that impulsivity was absent whereas 69% reported that impulsivity was present. The minor alleles of both rs1007023 and rs1473473 were more frequent in the impulsive participants compared to the non-impulsive participants (rs1007023: 10.6% vs 17.5%, OR=1.79 95% CI 1.01-3.17, *p*=0.05; rs1473473: 13.1% vs 21.6%, OR=1.83, 95% CI 1.08-3.08, *p*=0.02). The combined haplotype of the minor alleles was significantly more frequent in the impulsive participants with eating disorders compared to the non-impulsive participants (OR=2.12, 95% CI 1.11-4.04, *p*=0.02).

Because the LD structure among the *TPH2* SNPs was not independent, adjusting the *p*-value for the actual number of tests would be overly stringent. An interface developed by Nyholt (2004; http://genepi.qimr.edu.au/general/daleN/SNPSpD/); was used to determine experiment-wide significant *p*-values for the analyses in the control women ($p<0.017$), and the ED patients ($p<0.041$). Thus, the association between impulsivity and rs1007023 in the control women ($p=0.018$) came close to significance, while the association between rs1473473 and impulsivity in the patients ($p=0.02$) remained significant after correction for multiple testing.

Discussion

In the current study it was shown that perfectionism and impulsivity are related to AN and SV. Because the hypothesis of the study was based upon the assumed relation between these features and eating disorders (Cassin & von Ranson, 2005), we first confirmed this association in a sample consisting of 324 participants with eating disorders and 240 controls without an eating disorder. To study the involvement of *TPH2* in perfectionism or impulsivity in the absence of disease, the relation between the *TPH2* SNPs and a perfectionism and impulsivity item was subsequently evaluated in a random twin-based control group. It was shown that the *TPH2* SNP rs1473473 that was associated with a higher risk of both AN as well as SV previously (Slof-Op 't Landt et al., 2011), was associated with higher impulsivity in the controls (OR =1.49, 95% CI 1.02-2.17, $p=0.04$). In addition, the nearby located rs1007023 showed an even stronger association with higher impulsivity (OR=1.60, 95% CI 1.08-2.36, $p=0.02$). In our previous study, a trend association was observed between this SNP and a higher risk of SV, although this association could not be replicated in two additional case-control samples. Finally, in the combined AN/SV group an association between Dysfunctional Impulsivity (DII) and *TPH2* rs1007023 (OR=1.79, 95% CI 1.01-3.17, $p=0.05$) and rs1473473 (OR=1.83, 95% CI 1.08-3.08, $p=0.02$) was observed.

The observed significant difference between participants with AN and participants with SV in the perfectionism scales Personal Standards and Organization, and in the Dysfunctional Impulsivity scale was in accordance with the study by Reba et al. (2005). They also reported that individuals with eating disorders who endorse vomiting scored lower on the Personal Standards and Organization scales of the MPS compared to participants who do not vomit. Furthermore, both Reba et al. (2005) and Dalle Grave et al. (2009) have shown significantly higher scores on the Temperament and Character Inventory scale Novelty Seeking in individuals who endorse vomiting compared to those

without vomiting or purging. Impulsivity is also measured as a subscale of the Novelty Seeking scale (Cloninger et al., 1993). Whiteside et al. (2001) showed that this Impulsivity subscale of the Temperament and Character Inventory loads on the same underlying factor as the Dysfunctional Impulsivity scale, suggesting that these scales measure the same underlying construct.

The haplotype analyses revealed that the association between impulsivity and the combined haplotype of rs1007023 and rs1437473 was not stronger than the effect observed for rs1007023 alone in the controls. In the participants with eating disorders however, the association between the combined haplotype was stronger than the effect observed for both SNPs separately. It appears that the presence of an eating disorder influences the association between *TPH2* and impulsivity. However, the difference could also be caused by the difference in measurement instrument used. Despite the considerable correlation between the YSR impulsivity item and the Dysfunctional Impulsivity scale, these scales might not measure the same underlying construct. Another possibility is that both rs1007023 and rs1437473 are in LD with a third genetic variant that underlies the associations with eating disorders and impulsivity.

It is interesting that rs1473473, a *TPH2* SNP associated to both AN and SV (Slof-Op 't Landt et al., 2011), was linked to impulsivity in healthy controls and individuals with eating disorders, while the results in Table 6.2 indicate that Dysfunctional Impulsivity scores in AN did not differ from healthy controls. This might be explained by the relatively small sample sizes. The association between AN and Dysfunctional Impulsivity may have been small, hence there was insufficient power to detect this effect.

Impulsivity has been linked to alterations in serotonin activity in both non-clinical populations and in eating disordered groups (Bruce et al., 2005; Racine et al., 2009; Steiger et al., 2001; Steiger et al., 2004; Steiger et al., 2005). In addition, trend significant (p<0.07) associations were observed between three other *TPH2* SNPs (rs1352250, rs10879352, and rs1487275) and cognitive impulsivity in children with ADHD (Oades et al., 2008). There is substantial LD between these SNPs and rs1007023 and rs1473473, D' ranges from 0.9 to 1.0 and r^2 is between 0.21 and 0.46. Furthermore, genetic variation at the *TPH2* gene have been associated with suicide attempts (Yoon & Kim, 2009; Zhou et al., 2005; Zill et al., 2004), response inhibition (Stoltenberg et al., 2006), behaviors that are linked to impulsivity (Congdon & Canli, 2008). Indicating that genetic variation at this locus may contribute to mental conditions characterized by impulsivity like SV.

The current study has several limitations. First, with the present study design we could not test whether the association between impulsivity and *TPH2* was underlying the previously reported association between eating disorders and *TPH2*. Because YSR

impulsivity was only measured in a small subset of the participants with eating disorders, we did not have sufficient power to adjust the previous case-control association analyses with impulsivity scores. Another approach to determine a causal relation is to evaluate, in a longitudinal prospective design, whether carriers of the minor alleles of rs1007023 and rs1473473 who are impulsive will also develop an eating disorder more frequently than non-carriers or non-impulsive carriers. As mentioned above, there was a difference in measurement instruments used in the twin-based controls from the NTR and the participants with eating disorders from the GenED study. Because we were not able to collect DNA from the healthy control group used in the phenotypic analyses, we could not evaluate whether an association with Dysfunctional Impulsivity was also present in controls without an eating disorder, which is subject for further research. Finally, sample sizes of the eating disorder groups were modest. Hence, we might have missed associations due to suboptimal statistical power.

In the present study we have reported that two of the previously identified susceptibility SNPs for eating disorders in *TPH2* showed an association with impulsivity in a twin-based control group. Carriers of the minor alleles of rs1473473 and rs1007023 did not only have a higher risk for SV and/or AN, but were also more prone to higher impulsivity. Interestingly, these same two SNPs were also associated with high impulsivity in individuals with eating disorders. Genetic variation at the *TPH2* gene thus appears to affect impulsivity which in turn might predispose to the AN and or SV phenotype. In future studies we hope to explore this link further.

Chapter 7

General discussion

Eating disorders have a debilitating effect on the lives of people who suffer from these disorders, and the people surrounding them. The mortality rate is considerable and the prognosis is poor. Relapse rates are high, and after four to ten years of follow-up a substantial part of the patients has not recovered (Crow et al., 2009; Harris & Barraclough, 1998; Nielsen et al., 1998; Papadopoulos et al., 2009; Steinhausen, 2002; Steinhausen & Weber, 2009; Sullivan, 1995). Eating disorders have a large heritable component, and identification of susceptibility genes underlying these disorders will clarify which pathophysiologic mechanisms and pathways contribute to their aetiology. In the future, knowledge regarding the genetic aetiologies may open pathways for novel drug targets, enhance treatment and improve prevention. Also, an increase in knowledge regarding the extent to which disorders are influenced by genetic vulnerability may help to decrease the stigma associated with such disorders (Klump et al., 2009).

However, it is clear that the genetics of eating disorders is still in an early phase. Despite the fact that since the publication of our review (chapter 2) several twin (Bulik et al., 2006; Bulik et al., 2010) and a multitude of molecular genetic studies have been performed (see Appendix B and C), the conclusions regarding those studies remain the same. Studies have mainly been characterized by small sample sizes, inadequate statistical power and the use of diagnostic categories for phenotype assessment.

In the series of genetic studies described in this thesis, I tried to improve issues such as study design, use of phenotypes, phenotype measurements, choice of candidate genes, and genotype measurements. The main findings from our studies will be discussed in this chapter.

Study design

Figure 7.1 summarizes the design of the novel study described in this thesis. There were three groups of participants who took part in different phases of the study:

1. participants from the Genetics of Eating Disorder (GenED) study, this study was initiated as part of this thesis to collect DSM-IV eating disorder diagnoses, DNA and extensive phenotype information from a large group of participants with an eating disorder throughout the Netherlands;
2. population-based sample of twins and family members from the Netherlands Twin Registry (NTR); and
3. control participants without an eating disorder.

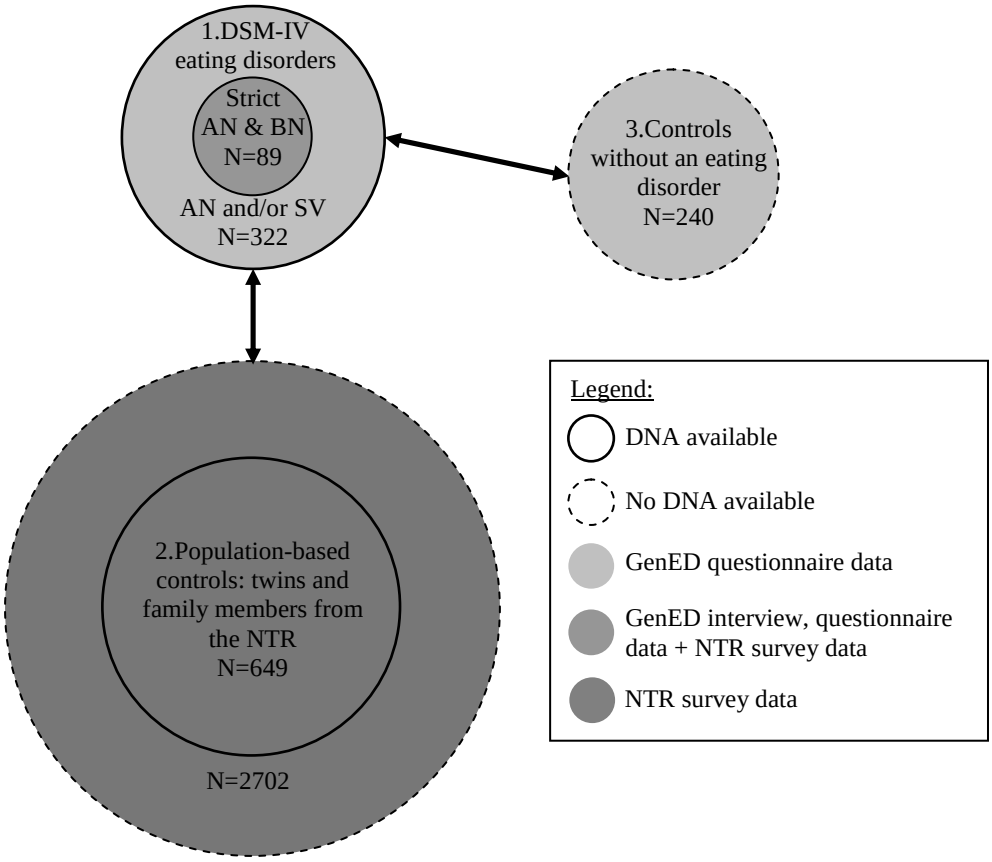


Figure 7.1 Study design used in this thesis

This design can be used for heritability studies (in group 2) and genetic association studies (in groups 1 and 2). Elaborate phenotype data were collected for the different groups by administering questionnaires and interviews. Therefore, it was not only possible to examine the genetics of DSM-IV eating disorder diagnoses, but also of related traits and possible endophenotypes like perfectionism and impulsivity (Bulik et al., 2007a).

Heritability of disordered eating behaviour

In the adolescent twins from the NTR, the Disordered Eating Behavior (DEB) scale was devised and used instead of categorical DSM-IV (1994) eating disorder diagnoses. To enhance the statistical power, an extended twin design including an additional sibling per family was applied. In addition, the psychometric quality of the DEB-scale was evaluated

by testing for measurement invariance with respect to sex (chapter 3), before performing the bivariate genetic analysis. In both adolescent men and women the different disordered eating behaviors and attitudes measured by the DEB-scale were explained by one underlying factor. This is in line with the overlap and the hypothesized shared liability between the different eating disorders. The requirements of complete measurement invariance with respect to sex could not be met for the DEB-scale. Thus scores on the DEB-scale cannot be taken to represent exactly the same underlying trait in men and women. As a consequence the genetic analysis described in chapter 4 were performed separately in men and women. We were the first to evaluate complete measurement invariance in the eating disorder field. Thus, despite the fact that sum scores on eating disorder scales are used widely to compare for example men and women, eating disorder cases and controls, or different age and ethnic groups, measurement invariance was never established for any of these grouping variables. In other words, reported similarities and differences between these groups could be a consequence of measurement bias instead of a genuine resemblance or difference in the underlying trait.

Genetic bivariate analyses as presented in chapter 4 showed that genetic factors explained a considerable part of the variance in disordered eating behavior (DEB). Heritability estimates for DEB were 0.65 in women and 0.39 in men. BMI was highly heritable in women (heritability =0.80) and men (heritability =0.76). In addition, additive genetic factors were responsible for the total overlap between the two characteristics, yielding a genetic correlation of 0.43 in women and 0.51 in men. Despite the overlap between BMI and DEB, a large part of the genetic influences on DEB was due to genetic effects that are independent of BMI in women as well as men. The heritability estimates for DEB are in line with the heritability estimates previously reported for eating disorders and eating disorder symptoms (ranging from 7 to 83%, see chapter 1).

The four DEB items are related to body weight, and in general body weight appears to be a risk factor for the development of eating disorders, especially bulimia nervosa (Jacobi et al., 2004). Therefore it is noteworthy that the genetic influence on DEB was largely independent of BMI, indicating that there are genes involved in DEB that do not seem to have an effect on body weight. On the other hand, part of the genetic factors that influence BMI also influence DEB. It would be interesting to disentangle the direction of causation of the overlap between these characteristics. Genetic influences on for example metabolism may be causal to weight gain that eventually leads to disturbed eating behavior. Genetic influences on DEB may alternatively be causal to a disturbed eating profile, leading to fluctuations in weight. Several approaches can be taken to disentangle the direction of

causation, for example phenotypic causation models (Duffy & Martin, 1994; Heath et al., 1993) and the co-twin control design (Cederlof et al., 1977; Kendler et al., 1993).

A concern with regard to the four item DEB-scale is that it might not be ideal to measure the underlying latent trait in eating disorders. However, in large epidemiological studies such as becoming common for gene finding, short scales might be a requirement to obtain phenotyping in sufficiently large samples. With the selection of the items we have tried to capture a variety of eating disorder symptoms. Three of the items (fear of weight gain, importance of body weight and shape for self-evaluation and binge eating) used in this study are based on eating disorder criteria from the DSM-IV. The fourth item (dieting) has been identified as a potent risk factor (Jacobi et al., 2004). One eating disorder symptom, compensatory behavior, is missing in our assessment instrument. Since heritability estimates for compensatory behavior and self-induced vomiting in female twins (0.50-0.70) were comparable to the estimate for DEB (Klump et al., 2000; Sullivan et al., 1998a), the inclusion of compensatory behaviors in our phenotype might not influence the results found for the women in the current study. However, we do not know what the consequences for the heritability estimates in the men would be, especially since significant gender differences have been reported for a variety of compensatory behaviors like self-induced vomiting, laxative use and fasting (Anderson & Bulik, 2004).

Genetic association study

In a two step design, the association between four candidate genes and eating disorders was investigated in chapter 5. Eating disorders included anorexia nervosa (AN) as well as eating disorders characterized by self-induced vomiting (SV). The four candidate genes were serotonin receptor 1D (*HTR1D*), stathmin (*STMN1*), brain-derived neurotrophic factor (*BDNF*) and tryptophan hydroxylase 2 (*TPH2*). To replicate previous results in ED, genes were selected for which association was observed and confirmed in studies with an adequate sample size (as discussed in Chapter 2). Both *HTR1D* and *BDNF* fulfilled these criteria (Bergen et al., 2003; Brown et al., 2006; Ribases et al., 2004; Ribases et al., 2005). Besides replication of previous results, we also aimed to evaluate the involvement of two unexplored candidate genes for ED. *STMN1* was located under the linkage peak of restrictive AN (1p33-36) (Grice et al., 2002). Because the associations with *HTR1D* and Opioid Receptor Delta-1 (genes located under the same peak) only explained part of the linkage, it was expected that additional candidate genes could underlie the linkage peak (Bergen et al., 2003). *TPH2* was selected because of the link between serotonin and eating disorders (Kaye, 2008; Lucki, 1998). To overcome the drawbacks of previous association

studies in which candidate genes were tested by single SNPs , 25 tagging SNPs were selected based on HapMap to capture the majority of the common variation within the four candidate genes. Between 71 and 91% of common variation within the four genes was captured by the tagging SNPs. In addition, replication of association was performed in two additional independent case-control samples from the Netherlands and Germany, making this one of the largest association studies performed thus far with 887 AN cases, 306 SV cases and 1914 controls.

In the first step allele frequencies of the 25 SNPs were compared between the GenED case groups (AN and SV cases) and random controls from the NTR. No association was observed for any of the *BDNF*, *HTR1D* and *STMN1* SNPs. A nominal significant association ($p<0.05$) was observed for *TPH2* rs1473473 in AN as well as SV. Subsequently this association was replicated in a meta-analysis with two additional independent eating disorder case-control samples. In the meta-analysis, the minor allele of *TPH2* SNP rs1473473 was significantly more frequent in AN cases (OR=1.25, 95% CI 1.06-1.47, $p<0.009$) and SV cases (OR=1.34, 95% CI 1.06-1.69, $p=0.013$) compared to controls. We are the first to observe an association with a genetic variant in the *TPH2* gene and eating disorders.

The *TPH2* gene encodes the main rate-determining enzyme in the synthesis of serotonin in the brain (Zill et al., 2007). Serotonin is involved in satiety, anxious and obsessional behavior, mood, and impulse control, features all linked to eating disorders (Kaye, 2008; Lucki, 1998). In long-term recovered AN and bulimia nervosa (BN) patients elevated 5-hydroxyindoleacetic acid levels in cerebrospinal fluid were detected (Kaye et al., 1991; Kaye et al., 1998). This is the major metabolite of serotonin in the brain and body and is thought to reflect extracellular serotonin concentrations. This finding thus could be indicative of an ‘overactive’ serotonin system in eating disorders, which in turn could be caused by an increased function of the *TPH2* gene.

The *TPH2* gene was also one of 182 candidate genes that were investigated for association with AN in another large collaborative study (Pinheiro et al., 2010). In this study 5151 SNPs (43 SNPs within the *TPH2* gene) were evaluated in 1085 participants with AN and 677 controls. After accounting for multiple testing, there were no statistically significant associations for any individual SNP. No association was found between the measured *TPH2* SNPs and AN, but rs1473473 was not genotyped in this study. The extent of the linkage disequilibrium between rs1473473 and the genotyped SNPs is not known, a small linkage disequilibrium could explain why the association with *TPH2* was not found in this study.

The LD block that *TPH2* rs1473473 tags spans across part of the *TPH2* gene, and is ended by a recombination hotspot on one side. Therefore it is highly likely that this SNP is in LD with a functionally relevant variant(s) in the *TPH2* gene. Rs1473473 is not in LD with known *TPH2* mutations (Haavik et al., 2008). *TPH2* SNPs in LD with rs1473473 however, have been associated with a suicidal mental condition in Finnish men (Zhou et al., 2005), with antidepressant response in depressive patients (Peters et al., 2004), and with allelic mRNA expression imbalance in sections of the human pons (Lim et al., 2007), indicating that genetic variation at this locus may contribute to mental conditions and could influence gene function.

To find yet unidentified functionally relevant variants with an effect in eating disorders, the *TPH2* gene could be sequenced in eating disorder cases and family members, for example by exon resequencing to detect rare or low frequency variants of medium effect (Gloyn & McCarthy, 2010; Johansen et al., 2010). Since a multitude of genes belong to the serotonin pathway, measuring the presence and activity of serotonin either in blood or in cerebrospinal fluid will probably not be a good representation of the *TPH2* gene per se (Kaye et al., 1998; Savelieva et al., 2008). A reliable way to evaluate the activity of the *TPH2* gene is by measuring mRNA expression levels in the brain (Lim et al., 2007). A difficulty of this approach is that brain tissue is required. When investigating gene expression in eating disorders it is important to differentiate between the secondary effects of malnutrition (for example due to fasting in AN) from effects of the disorder itself. The inclusion of a recovered eating disorder group in these studies therefore seems to be a prerequisite, which makes this approach even more difficult. Translational models might offer a solution for this problem.

Previously, a full phenotypic evaluation of the *TPH2* knock-out mouse was performed (Savelieva et al., 2008). The mice appeared largely normal both in appearance as well as behavior. In general the results indicated that the role of serotonin appears to be modulatory instead of essential in behavior. However, the results of one behavioral test suggested increased obsessive/compulsive behavior in the *TPH2* knock-out mice. This is an interesting finding, since a substantial comorbidity between eating disorders and obsessive-compulsive disorder is present (Bulik et al., 1997; Lilenfeld et al., 1998).

This is the first genetic study that used SV as a phenotype. This phenotype was chosen because there is no *a priori* reason to believe that a DSM eating disorder diagnosis represents a more ‘genetic’ syndrome than underlying core behaviors or traits. Twin studies have shown that binge-eating and vomiting represent more genetically mediated symptoms (heritability estimates ranging between 8 and 80), while genetic factors did not appear to have any effect (heritability estimate of 0) on the undue influence of weight and shape on

self-evaluation represents (Bulik et al., 1998; Reichborn-Kjennerud et al., 2003; Reichborn-Kjennerud et al., 2004b; Reichborn-Kjennerud et al., 2004a; Sullivan et al., 1998a; Wade et al., 2008b; Wade & Bulik, 2007). Because the reliability of the measurement of SV is high and the heritability of this behaviour is higher, as opposed to binge eating, it was decided to select cases based on this core behaviour in the study described in chapter 5 (Sullivan et al., 1998a; Wade et al., 2000a).

The selection of candidate genes in chapter 5 represents a choice from a larger set of possibilities. Another plausible candidate gene would have been Opioid Receptor Delta-1, since association with this gene was also observed and confirmed in two large studies (Bergen et al., 2003; Brown et al., 2006). This was not the case for the genes encoding Serotonin Receptor 2A and the Serotonin Transporter, which have been studied most intensively in eating disorders (see Table 1.4). Of course many alternatives exist for the novel candidate genes *STMN1* and *TPH2* that were currently selected. However, with the sample sizes of the current study design (Figure 7.1) only a few candidate genes could be evaluated with a good coverage rate while retaining adequate statistical power.

Despite this selection, the statistical power in the GenED study was limited. Depending on the minor allele frequency the study had adequate power (85% power at an alpha level of 0.05, log-additive or allelic model) to detect effects sizes ranging between 1.45 and 1.8 for AN and ranging between 1.48 and 1.85 for SV. This could explain why previously reported associations between *BDNF*, *HTR1D* and AN were not observed in this study (Bergen et al., 2003; Brown et al., 2006; Ribases et al., 2004; Ribases et al., 2005). The meta-analysis of *TPH2* rs1473473 had adequate power (85% power at an alpha level of 0.05, log-additive or allelic model) to detect effect sizes of at least 1.25 for AN and 1.4 for SV. This approach has led to a robust association of the *TPH2* SNP rs1473473.

Performing candidate gene studies is an outdated approach to identify susceptibility genes for eating disorders. However, at the time that this study was designed it was the available method for performing this kind of research. Since the aetiology of eating disorders is largely unknown, genomewide association studies are more appropriate for finding candidate genes in these disorders. These studies rely on the assumption that linkage disequilibrium enables one SNP to act as a surrogate marker for association to other sequence variants in the same region (Freimer & Sabatti, 2007). Currently the Genetic Consortium of Anorexia Nervosa is conducting a genomewide association study in a large group of female participants who meet DSM-IV criteria for AN and a group of female controls, all of European ancestry. The GenED study also takes part in this consortium. Although this is a huge step forward in identifying plausible candidate genes, the heterogeneity of eating disorders, including AN, should be kept in mind. Substantial

differences in genetic and environmental contributions to component *symptoms* of AN suggests that we may be obscuring our ability to detect loci that contribute to risk by focusing on a contrived and heterogeneous condition. Incorporating additional phenotypes within this study might lead to interesting findings, although the multiple testing issue should be taken into account. Additional phenotypes can be core symptoms of eating disorders, like binge eating and SV, but also personality features associated to eating disorders, like perfectionism and impulsivity.

The link between TPH2 and personality features

The substantial heritability of AN and SV may in part be explained by heritable aspects of perfectionism and impulsivity, which are consistently associated to eating disorders (Cassin & von Ranson, 2005). Both perfectionism and impulsivity remained present after recovering from an eating disorder (Bastiani et al., 1995; Kaye et al., 1998; Lilenfeld et al., 2000; Srinivasagam et al., 1995; Wagner et al., 2006). In addition, elevated levels of perfectionism were observed in relatives of individuals with AN and BN (Lilenfeld et al., 2000; Woodside et al., 2002). These findings suggest that perfectionism and impulsivity may be of potential aetiological relevance for eating disorders.

Chapter 6 explored the hypothesis that genetic variation in the TPH2 gene explains part of the overlap between eating disorders, perfectionism and impulsivity. In the phenotypic analyses, earlier observations that patients with AN and/or SV score different from healthy controls on perfectionism and impulsivity, as measured by the Multidimensional Perfectionism Scale (Frost et al., 1990) and the Dickman Impulsivity Inventory (Dickman, 1990) were confirmed. To study the involvement of four TPH2 SNPs, that were associated to AN or SV previously (chapter 5), in perfectionism and/or impulsivity in the general population, genetic association analyses were performed in a random twin-based control group (N=512) with perfectionism and impulsivity items from the Youth Self-Report (Levinson, 2005; Verhulst et al., 1997). The minor allele of rs1473473 (OR =1.49, 95% CI 1.02-2.17, p=0.04) and rs1007023 (OR=1.60, 95% CI 1.08-2.36, p=0.02) were more frequent in impulsive controls. In the eating disorder case group (N=267), an association to Dysfunctional Impulsivity, a subscale from the Dickman Impulsivity Inventory, was observed for both rs1007023 (OR=1.79, 95% CI 1.01-3.17, p=0.05) and rs1473473 (OR=1.83, 95% CI 1.08-3.08, p=0.02). The degree of perfectionism was not associated to genetic variation at the TPH2 gene.

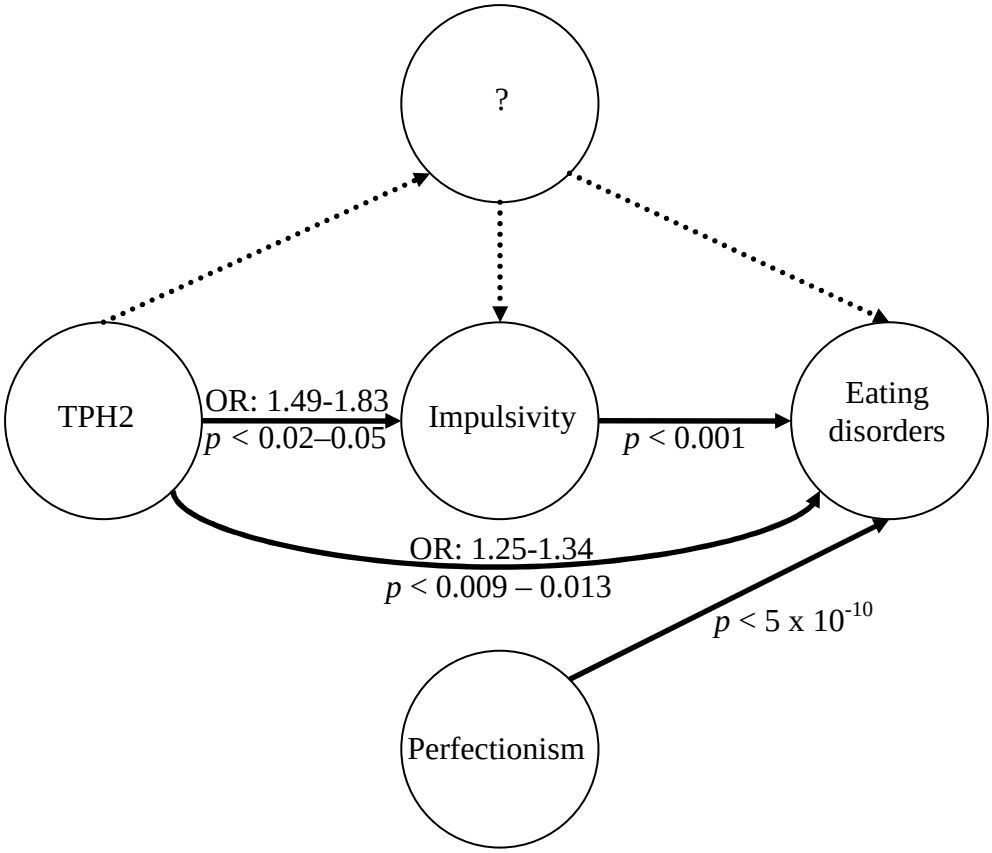


Figure 7.2 The link between TPH2, impulsivity and eating disorders. The observed associations between TPH2 and impulsivity (chapter 6), TPH2 and eating disorders (chapter 5), impulsivity and eating disorders (chapter 6), and perfectionism and eating disorders (chapter 6) are depicted with the arrows. The TPH2 gene leads to higher impulsivity which in turn might make people more susceptible for an eating disorder. An alternative explanation, a third factor that is affected by TPH2, and which in turn influences both impulsivity and eating disorders is presented by the dashed arrows.

In Figure 7.2 the results from the analyses are summarized. Both impulsivity and perfectionism were associated to eating disorders. TPH2 showed an association with eating disorders (AN and SV) and with impulsivity. Possibly there is a causal relation, thus that genetic variation at the TPH2 gene leads to higher impulsivity which in turn will make people more susceptible for developing an eating disorder. Another explanation is that TPH2 affects another unknown factor, which influences both impulsivity and eating disorders. To evaluate whether the association between TPH2 and impulsivity is underlying the association with eating disorders a longitudinal prospective study design (following up carriers of the minor alleles of rs1007023 and rs1473473) is preferred. However, because of

the low prevalence of eating disorders and the complex nature, it will be difficult to conduct this study. To overcome this, a more prevalent intermediate phenotype, for example DEB discussed in chapter 3 and 4, could be used. Another approach to test for causality is by adjusting the case-control association analysis for the impulsivity phenotype. Because the Youth Self Report impulsivity item was only measured in a small subset of the eating disorders cases, we did not have sufficient power to adjust the previous case-control association analyses with impulsivity scores.

In addition to perfectionism and impulsivity, there are other established risk factors for eating disorders that might predispose to their development, like negative self-evaluation/low self-esteem, negative body image and weight concerns (Jacobi et al., 2004). It would be interesting to find out whether one or several shared factors are underlying this overlap between personality features and eating disorders, and whether these factors have a genetic or an environmental background. Large population-based twin studies can be used to investigate these questions.

A limitation of the study design used in chapter 6 is that although there is overlap in the features that were phenotyped in the cases from the GenED study and the NTR controls, the questionnaires used to measure these features are not identical (Figure 7.1). Extensive perfectionism and impulsivity phenotypes were available for the participants with an eating disorder, whereas the available phenotypes in the NTR controls were based on single items. It is possible that despite the considerable correlation between these instruments they do not measure the same underlying construct. By recruiting the control women without eating disorders we tried to overcome this limitation. The questionnaires administered to this group were identical to the questionnaires used in the GenED study. However, because no DNA was collected from these women the utility of this group was limited (Figure 7.1). Therefore the association with Dysfunctional Impulsivity scale could not be evaluated in controls without an eating disorder.

The observed differences in perfectionism and impulsivity scores between participants with an eating disorder and controls without an eating disorder indicate that currently ill individuals are more impulsive and highly perfectionistic. Although measurement invariance with respect to disease state was not tested (the framework described in chapter 3 can be used to do this), it is likely that the scales do not measure exactly the same underlying trait in both groups (both within patient subgroups and between patients and controls). For example, someone who regularly engages in self-induced vomiting (a quite dysfunctional impulsive behavior), may have another opinion or standard on dysfunctional impulsivity compared to somebody who does not engage in self-induced vomiting. These persons will probably respond in a different matter preceding their illness or after recovery.

The previously reported elevated levels of perfectionism and impulsivity in recovered patients however, point out that the differences in perfectionism and impulsivity are not entirely disease state-dependent and may be an underlying trait preceding eating disorder onset (Bastiani et al., 1995; Kaye et al., 1998; Srinivasagam et al., 1995; Wagner et al., 2006).

Future Perspectives

In future research identical phenotypes will be available for a substantial group of cases from the GenED study and for random participants from the NTR. This will increase the possibilities for conducting studies into genetic associations, phenotype correlations and the way these influence the development of eating disorders. A major limitation of the study design thus far used is that questionnaires used in GenED and NTR controls were not identical (Figure 7.1). Both studies are however still ongoing which enable us to complete the data.

It will be interesting to evaluate the influence of disease-state on the performance of the used measurement instruments, by investigating measurement invariance (as discussed in chapter 3). For the Multidimensional Perfectionism Scale (Frost et al., 1990) and the Dickman Impulsivity Inventory (Dickman, 1990) these analyses can be performed in the cases from the GenED study and the controls without an eating disorder (group 1 and 3 depicted in Figure 7.1). In the future, it will also be possible to test for measurement invariance in the combined sample of GenED cases and NTR controls, not only for perfectionism and impulsivity but also for other personality features which are measured within the NTR survey.

Besides perfectionism and impulsivity there are other personality features that possibly predispose to the development of eating disorders, like negative self-evaluation/low self-esteem, negative body image and weight concerns (Jacobi et al., 2004). Because different types of eating disorders are characterized by a different combination of these features (for example high perfectionism, low self-esteem and negative body image in AN), it will be interesting to examine the overlap of these features, including the overlap with different types of eating disorders or different eating disorder symptoms (e.g. binge eating, SV). Within the twin sample of the NTR it will be possible to examine whether these features have a common background, and whether genetic or environmental influences are underlying this overlap. Furthermore the twin design can be used to disentangle the direction of causation, for example phenotypic causation models (Duffy & Martin, 1994; Heath et al., 1993) and the co-twin control design (Cederlof et al., 1977; Kendler et al.,

1993). The first method is a nested model of the bivariate Cholesky decomposition, in this approach the correlated traits need to have different modes of inheritance. In the co-twin control design relative risks for one feature (e.g. perfectionism) would be compared between unrelated individuals discordant for another feature (e.g. impulsivity), DZ twins discordant for impulsivity, and MZ twins discordant for impulsivity.

The next step is conducting genomewide association studies to identify genetic loci that influence the susceptibility for eating disorders, but also the susceptibility for the combined phenotype of predisposing personality features. As was shown in chapter 6, perfectionism and impulsivity scores are different in eating disorder cases compared to controls, and the same is expected for the personality features mentioned above. In order to detect genes that are involved in these personality features in the absence of disease, genomewide association studies should be performed in the twins from the NTR or in family members of eating disorder cases. As part of the GenED study first degree family members of participating individuals with an eating disorder are also approached to participate in the study. Currently, phenotype and genotype data of 161 relatives of 64 eating disorder cases have been collected. Besides for genetic association studies, this family sample can also be used to perform phenotypic studies. It can be questioned whether family members of eating disorder cases show elevated levels of for example perfectionism and impulsivity. Furthermore, in the combined sample of cases and relatives it can be examined whether these features are familial.

When genetic loci are identified by the genomewide association studies, these genes will have to be followed-up. In the first step more (preferably) functionally relevant variants within that gene will be measured. Subsequently, the gene will be sequenced to find yet unidentified variants that can explain the observed association. A recommended approach for genes associated to eating disorders is exon resequencing in severe cases and matched healthy controls, to detect rare or low frequency variants of medium effect (Gloyn & McCarthy, 2010; Johansen et al., 2010). In addition to newly identified genes based on genomewide association studies, the same approach will be used to follow up the association with the TPH2 gene in the future.

Once it is established that a gene is involved in either eating disorders or a predisposing personality feature, the activity and function of this gene can be tested by using translational models, like knock-out mice. Possibly these mice show a difference in behavior compared to wild-type mice, which can be relevant for the studied phenotype in humans. In addition to knock-out mice, the activity-based anorexia (ABA) or semi-starvation induced hyperactivity model (Kas et al., 2009), a translational model for AN, can be used to test the activity of the identified gene.

Finally, in the future more studies regarding gene-environment interaction (G x E), whereby a person's genes may influence how sensitive he or she is to the effects of the environment, should be performed. This interaction could be a potential explanatory model in eating disorders (Bulik, 2005). Despite the fact that practically every young girl in Western society is exposed to cultural standards of slenderness and attractiveness and the majority of them is or has been on a diet, only a small number ever develops an eating disorder. Possibly dieting will only trigger the development of eating disorders in girls with a large genetic vulnerability for these disorders. The underlying molecular processes of G x E, with genes mediating an individual's risk to for example stressful life events, may be elucidated by studying epigenetics (Feinberg, 2008; Schroeder et al., 2010). Epigenetics is the study of inherited changes in phenotype or gene expression caused by mechanisms other than changes in the primary underlying DNA sequence. These epigenetic alterations are potentially reversible and accessible for drug treatment. Recent findings suggest that the transmission of vulnerability for depression from parent to offspring could occur, in part, through the epigenetic modification of genomic regions that are implicated in the regulation of stress response and related processes such as neurogenesis, like the glucocorticoid receptor and brain-derived neurotrophic factor (McGowan et al., 2009; Schroeder et al., 2010). Expectancies with relation to benefits for future personalized diagnostics and therapies for psychiatric disorders from this relatively new form of genetic research are high. Epigenetic changes might also be involved in eating disorders. To examine these changes epigenetic studies can be performed in currently ill or recovered patients with eating disorders.

Clinical implications

Genetic research as discussed in this thesis will improve the knowledge regarding the genetic background of eating disorders. Once the pathways involved in these disorders and in the associated personality features are elucidated, prevention and treatment can be improved. Phenotypic studies into the shared background of eating disorders and personality features will enable us to make a risk profile for eating disorders. This will increase the chance of early recognition, and improve prevention strategies.

The knowledge that certain genes are involved in eating disorders could be used to predict the outcome of the disease if genetic variants will be found with sufficient predictive effect sizes. Carrying a number of risk alleles might worsen the prognosis of the eating disorder, because specific behaviors or eating disorder features are influenced by these genes (Hetrick et al., 2008; Maguire, 2008). Treatment programs could be adjusted

and improved based on this knowledge. Furthermore, knowledge regarding the genetic aetiologies may open pathways for novel drug targets. Since the prevalence of eating disorders is relatively low, pharmaceutical companies may not invest in the development of medications to cure or mask genetic effects specific for these disorders. However, since there is evidence for shared genetic vulnerability between eating disorders, depression and anxiety (Keel et al., 2005; Kendler et al., 1995; Wade et al., 2000b; Wade et al., 2004), genes that contribute to this spectrum of disorders might become targets for medical interventions.

Also, an increase in knowledge regarding the extent to which disorders are influenced by genetic vulnerability may help to decrease the stigma associated with such disorders. It can relief the burden of guilt with which many parents of eating disorder patients struggle (Enten & Golan, 2009; Jacobi et al., 2004; Le Grange et al., 2010). In addition, this knowledge could also change and ameliorate the attitudes towards individuals suffering from them (Crisafulli et al., 2008; Crisp, 2005; Stewart et al., 2006).

Chapter 8

Summary

In this thesis, a series of studies were conducted to investigate different aspects of the genetics of eating disorders. Eating disorders are distinguished into anorexia nervosa (AN), bulimia nervosa (BN) and eating disorders not otherwise specified (EDNOS). Chronicity and mortality of patients with eating disorders are among the highest of all psychiatric illnesses. Despite substantial efforts to identify causal pathways for AN and BN, very little is known about the aetiology of eating disorders

Chapter 2 reviews the different studies that have been performed to explore the biological background of eating disorders. Family studies have shown that AN and BN are strongly familial, and that familial aetiologic factors appear to be shared by both disorders. Twin studies mainly focussed on broader phenotypes or subthreshold eating disorders. These studies consistently yielded moderate to substantial heritability estimates for both AN (ranging between 48 and 76%) and BN (ranging between 30 and 83%). However, due to the low prevalence of both disorders, and subsequently the small numbers of affected twins, the statistical power, even when using broader phenotypes, was far from adequate. Genomewide screens have demonstrated linkage peaks for AN on chromosomes 1p33-36 and 4q13, for AN including behavioral covariates on chromosomes 1q31, 2p11 and 13q13, and for BN on chromosomes 10p13, and 14q22-23. Many genetic association studies have been conducted in eating disorders, in which genetic variation within a candidate gene is compared between cases and controls. Nearly all of these studies did not lead to any definite conclusion. Typical of the association studies in this field is the excess of small, discrepant studies.

In chapter 3 we evaluated whether the disordered eating behavior (DEB)-scale was comparable between men and women. We described five different steps of a multi-group discrete factor analysis accumulating into a model of complete measurement invariance, which was applied in a sample of 1195 adolescent men and 1507 adolescent women from the Netherlands Twin Registry (NTR). DEB was the sum score of four items on clinical features from different eating disorders: dieting, fear of weight gain, importance of body weight or shape on self-evaluation and binge eating. For DEB, the model of full measurement invariance with respect to sex (model 5), did not fit the data well. If this model had fitted, the probability of a certain response on a given item would have been the same for all participants with the same value on the underlying trait (DEB) regardless of the sex of the participant. However, this was not the case. The underlying common factor might not be the only source of difference between the sexes with respect to the four items. The sum score based on the four eating disorder items therefore cannot be taken to represent exactly the same underlying trait in men and women. This means that sex differences in this

sum score might be due to measurement bias instead of a true difference in the underlying trait.

In chapter 4 we reported the results of a bivariate twin study on DEB and body mass index (BMI) in 474 monozygotic twin pairs (194 male and 280 female pairs), 310 dizygotic twin pairs (140 male and 170 female pairs), and 45 incomplete twin pairs (22 men and 23 women) from the NTR. The sibling group was comprised of 69 brothers and 115 sisters. Because the DEB items were not measurement invariant with respect to sex (chapter 3), the genetic analyses were performed separately in men and women. Twin-, cross-twin, and twin-sibling correlations indicated that a large part of the variance in both DEB and BMI was explained by genetic factors, and that genetic components were underlying the overlap between DEB and BMI in women. The bivariate analysis showed that DEB is a highly heritable trait in women (a^2=0.65) and moderately heritable in men (a^2=0.39), whereas BMI is highly heritable in both women (a^2=0.80) and men (a^2=0.76). The remaining variance in both traits was explained by unique environmental factors. In addition, additive genetic factors were responsible for the total overlap between the two characteristics, yielding a genetic correlation of 0.43 in women and 0.51 in men. Despite the overlap between BMI and DEB, the majority of the genetic influences on DEB were due to genetic effects that are independent of BMI in women as well as men.

Based on the overview of genetic studies presented in chapter 2 and the update given in chapter 1, it is clear that the serotonin pathway has mostly been indicated as relevant in the development of eating disorders, because of its involvement in a broad range of biological, physiological and behavioral functions, for example body weight regulation, eating behavior, perfectionism, impulsivity and obsessionality. But the involvement of many other candidate genes has also been studied in eating disorders. So far the only association with a 'hypothesis based' candidate gene that has been observed in at least two large association studies was between brain-derived neurotrophic factor and AN. From a 'hypothesis free' approach the associations with two candidate genes have been observed in at least two large studies, namely serotonin receptor 1D and opioid receptor delta 1.

In chapter 5, we evaluated the association of 25 SNPs from four candidate genes serotonin receptor 1D (HTR1D), stathmin (STMN1), brain-derived neurotrophic factor (BDNF) and tryptophan hydroxylase 2 (TPH2), with both AN and eating disorders characterized by self-induced vomiting (SV). First, we performed genetic association analyses in cases from the GenED study (182 AN cases and 149 SV cases) and random controls from the NTR (N=607). A nominal significant association (p<0.05) was observed for TPH2 rs1473473 in AN as well as SV. This SNP was subsequently tested for replication in a meta-analysis with two additional independent eating disorder case-control samples

from Germany and the Netherlands together providing 887 AN cases, 306 SV cases and 1914 controls. For the minor C-allele (frequency 0.16) of TPH2 SNP rs1473473 a significant association was observed in the meta-analyses with both AN and SV. We observed an OR of 1.25 (95% CI 1.06-1.47, $p<0.009$) for AN, and an OR of 1.34 (95% CI 1.06-1.69, $p=0.013$) for SV. The OR for the combined group of AN and/or SV cases (n=1073) was 1.24 (95% CI 1.06-1.44, $p<0.006$). Based on the genotype frequencies of the TPH2 SNP rs1473473 we expected a dominant effect to be underlying the association. Therefore, we evaluated the association with this SNP in the combined case-group under a dominant genotypic model. Homo- and/or heterozygous carriers of the minor allele of rs1473473 had a higher risk of either AN or SV (OR=1.38, 95% CI 1.16-1.64, $p<0.0003$). The TPH2 gene encodes the main rate-determining enzyme in the synthesis of serotonin in the brain (Zill et al., 2007). Serotonin is involved in satiety, anxious and obsessional behavior, mood, and impulse control, features all linked to eating disorders (Kaye, 2008; Lucki, 1998).

Chapter 6 explored the hypothesis that genetic variation in the TPH2 gene (associated to a higher risk for AN and/or SV in chapter 5), explains the overlap between eating disorders, perfectionism and impulsivity by performing three analyses. In the extensive phenotypic analyses, we confirmed earlier observations that participants with AN and/or SV score different from healthy controls on perfectionism and impulsivity as measured by the Multidimensional Perfectionism Scale and Dickman Impulsivity Inventory. To study the involvement of four TPH2 SNPs in perfectionism and impulsivity in the absence of disease, genetic analyses were performed in a random twin-based control group (N=512). We observed an association with the Youth Self Report item on impulsivity for two SNPs. The minor allele of rs1473473 (OR =1.49, 95% CI 1.02-2.17, $p=0.04$) and rs1007023 (OR=1.60, 95% CI 1.08-2.36, $p=0.02$) were more frequent in impulsive controls. Next, we tested whether these two SNPs were also associated to impulsivity (as measured by the Dickman Impulsivity Inventory) in an eating disorder case group (N=267). An association was observed for both rs1007023 (OR=1.79, 95% CI 1.01-3.17, $p=0.05$) and rs1473473 (OR=1.83, 95% CI 1.08-3.08, $p=0.02$). Genetic variation at the TPH2 gene thus appeared to affect impulsivity which in turn might predispose to the AN and/or SV phenotype.

Chapter 9

Nederlandse samenvatting

References

Appendices

Publications

Curriculum vitae

Genetische factoren bij eetstoornissen

Het is nog onvoldoende bekend waarom mensen eetstoornissen ontwikkelen. Wel is gebleken dat er niet één oorzaak is, maar dat verschillende factoren een rol spelen bij het ontstaan van deze ziekten. Eén van deze factoren is erfelijkheid. In dit proefschrift hebben we meerdere studies uitgevoerd om meer te leren over de rol van erfelijkheid en genen in relatie tot eetstoornissen.

Eetstoornissen hebben grote negatieve gevolgen op het leven van mensen die eraan lijden en van mensen in hun omgeving. De mortaliteit van deze stoornissen is hoog en de prognose is slecht. Deze ziekten worden gekenmerkt door een grote kans op terugval na behandeling en een groot deel van de patiënten met een eetstoornis is vier tot tien jaar later nog niet hersteld. In het handboek voor psychiatrische stoornissen (DSM-IV) worden drie eetstoornisdiagnoses onderscheiden, namelijk anorexia nervosa, boulimia nervosa en eetstoornis niet anderszins omschreven (zie kader 9.1). Het grootste deel van de eetstoornispatiënten (ongeveer 60%) behoort tot deze laatste groep.

Er is veel overlap in de symptomen van de verschillende eetstoornissen. Zo worden verschillende vormen van eetgestoord gedrag en attitudes gedeeld tussen eetstoornissen, zoals bijvoorbeeld de rol die lichaamsvorm en gewicht spelen in de manier waarop men zichzelf beoordeelt en zelfopgewekt braken. Daarnaast is het niet ongebruikelijk dat mensen tijdens hun leven voldoen aan verschillende eetstoornis-diagnoses. Over risicofactoren is bekend dat het vrouwelijk geslacht, zwaarlijvigheid in de kindertijd, ernstige bezorgdheid over gewicht en lichaamsvorm, negatieve zelf-evaluatie (laag zelfvertrouwen), lijnen, perfectionisme en mogelijk impulsiviteit betrokken zijn bij het ontstaan van eetstoornissen.

Het menselijk lichaam bestaat uit verschillende weefsels (huid, spier, bloed, vet etc.), die opgebouwd zijn uit cellen. In iedere celkern ligt het erfelijk materiaal (DNA) verdeeld over 23 chromosoomparen opgeslagen. Een gen bestaat uit een stukje DNA binnen een chromosoom en draagt de informatie voor een specifieke erfelijke eigenschap in een cel. Alle genen samen bepalen het functioneren van de cellen waaruit de mens is opgebouwd. Genen zijn grotendeels volledig identiek tussen mensen, maar er is ook variatie. Deze variatie kan de functie van het gen beïnvloeden, maar dat hoeft niet.

Studie opzet

In dit proefschrift wordt een serie studies beschreven waarin de verschillende aspecten van genetica van eetstoornissen onderzocht worden. In totaal zijn drie verschillende studie

populaties gebruikt om de onderzoeken in uit te voeren. Allereerst de 'Genetics of Eating Disorders' (GenED) populatie, bestaande uit een grote groep Nederlandse eetstoornispatiënten. De tweede studiepopulatie bestaat uit tweelingen en hun familieleden van het Nederlands Tweelingen Register (NTR). Als derde is een populatie van volwassen Nederlandse vrouwen zonder een eetstoornis gebruikt.

Kader 9.1 Kenmerken verschillende eetstoornissen

Anorexia nervosa wordt gekenmerkt door ernstig ondergewicht, een intense angst voor gewichtstoename en een gestoorde beleving van gewicht of lichaamsvorm. Anorexia nervosa wordt onderverdeeld in twee subtypes: het beperkende type en het eetbuien/purgerende type. In tegenstelling tot het eetbuien/purgerende type, zijn personen met het beperkende type niet geregeld bezig met eetbuien of purgerende maatregelen, zoals zelf-opgewekt braken of het misbruik van laxeermiddelen.

Belangrijke symptomen van *boulimia nervosa* zijn het herhaaldelijk optreden van eetbuien (gemiddeld 2x per week in de afgelopen 3 maanden) gevolgd door inadequaat compensatoir gedrag (zoals braken, misbruiken van laxeermiddelen en overmatig bewegen) om de toename in gewicht te voorkomen. Bovendien wordt het oordeel over zichzelf in onevenredige mate bepaald door lichaamsvormen en/of gewicht. Bij boulimia nervosa wordt een onderscheid gemaakt tussen het purgerende type (regelmatig bezig met o.a. zelfopgewekt braken of misbruik van laxeermiddelen) en het niet-purgerende type (andere inadequate compensatoire gedragingen zoals vasten of overmatige lichaamsbeweging worden toegepast).

De groep patiënten met een *eetstoornis niet anderszins omschreven* bestaat uit: 1. personen die niet voldoen aan één van de criteria voor anorexia of boulimia nervosa; 2. personen die een combinatie hebben van criteria van zowel anorexia als boulimia nervosa; 3. personen met een eetbuistoornis, gekenmerkt door herhaaldelijke episodes van eetbuien (gemiddeld twee keer per week in de afgelopen 6 maanden) in afwezigheid van inadequaat compensatoir gedrag.

Overzicht ander onderzoek naar genetische achtergrond eetstoornissen

Hoofdstuk 2 is een overzichtsartikel van studies naar de genetische achtergrond van eetstoornissen die zijn uitgevoerd tot en met 2005. Familie studies laten zien dat anorexia en boulimia nervosa vaak binnen bepaalde families voorkomen. Daarnaast is het risico op

het krijgen van zowel anorexia als boulimia nervosa verhoogd in familieleden van personen die één van beide stoornissen hebben. In tweelingstudies worden de overeenkomsten en verschillen tussen ééneiïge (genetisch identiek) en tweeeiïge (genetisch gezien 50% gelijk) tweelingparen gebruikt om te berekenen hoe groot de erfelijke en omgevingsinvloeden zijn voor een bepaalde aandoening. Uit deze studies blijkt dat 48 tot 76% van de individuele variatie in anorexia nervosa verklaard wordt door genetische factoren, voor boulimia nervosa ligt dit percentage tussen de 30 en 83%.

Om chromosomale locaties op te sporen waarin zich nog onbekende belangrijke genen bevinden die het ontstaan van anorexia en boulimia nervosa kunnen verklaren, zijn zogenoemde koppelingsstudies uitgevoerd. Dergelijke koppelingsstudies worden gedaan binnen families waarin de ziekte vaker voorkomt, bijvoorbeeld in aangedane broer en/of zusparen. Omdat 50% van het DNA tussen broers en zussen gelijk is, is de verwachting dat zodra beide zussen anorexia nervosa hebben ze ook genvarianten zullen delen die anorexia nervosa veroorzaken. Door heel veel van dit soort paren uit verschillende families te onderzoeken kan er worden bekeken welk deel van het DNA door al deze paren vaker gedeeld wordt dan wat op basis van toeval wordt verwacht. Zo kunnen er bepaalde chromosomale gebieden worden gevonden die ‘gekoppeld’ zijn aan de ziekte. In eerder uitgevoerde koppelingsstudies is voor anorexia nervosa bewijs voor koppeling gevonden in gebieden op chromosoom 1, 2, 4, en 13, en voor boulimia nervosa op chromosoom 10 en 14.

Associatie studies worden uitgevoerd om te onderzoeken of een specifiek gen, waarvan wordt gedacht dat het een rol speelt bij een bepaalde ziekte, ook werkelijk betrokken is bij deze ziekte. In deze studies wordt onderzocht of varianten van dit gen vaker voorkomen bij een groep aangedane personen (patiëntengroep) in vergelijking tot een groep niet-aangedane personen (controlegroep). In dat geval is er sprake van associatie. Er zijn veel associatiestudies uitgevoerd in de verschillende eetstoornissen. Doordat in de meeste uitgevoerde associatie studies in eetstoornissen kleine patiënten- en controlegroepen zijn onderzocht, zijn er veel tegenstrijdige resultaten gevonden. Op basis van grotere associatiestudies lijken serotonine receptor 1D, opioid receptor D1 en brain-derived neurotrophic factor geassocieerd te zijn met eetstoornissen.

Vergelijkbaarheid vragenlijst eetgestoord gedrag

In *hoofdstuk 3* wordt een methode beschreven om de vergelijkbaarheid van een meetinstrument (vragenlijst/interview) tussen groepen (op basis van geslacht, leeftijd etc.) te onderzoeken. Als toepassing van deze methode werd onderzocht of mannen en vrouwen

de ‘disordered eating behavior’ (DEB)-schaal op dezelfde wijze invullen of interpreteren. De DEB-schaal is opgebouwd uit vier losse items die ieder een aspect van eetgestoord gedrag meten, namelijk: lijnen, angst voor gewichtstoename, belang van lichaamsgewicht en –vorm bij zelf-evaluatie en eetbuien. Door de score op deze vier items op te tellen, krijg je een totaal score die iets zegt over de mate van ‘verstoring van eetgedrag’ van die persoon. Het idee is dat mensen die geen of weinig ‘eetgestoord’ gedrag vertonen een lagere score op alle vier de items hebben dan mensen die meer of veel ‘eetgestoord’ gedrag vertonen, ongeacht hun geslacht. Als nu bijvoorbeeld blijkt dat de ‘niet-eetgestoorde’ mannen op slechts drie van de vier items laag scoren, terwijl de ‘niet-eetgestoorde’ vrouwen op alle vier de items laag scoren, dan is er dus een sekse-verschil in hoe de DEB-schaal de mate van ‘eetgestoordheid’ meet. Met andere woorden de DEB-schaal is dan niet meetinvariant met betrekking tot geslacht. Om deze meetinvariantie van de DEB-schaal te testen, werd een discrete factor analyse bestaande uit vijf opeenvolgende stappen uitgevoerd in een steekproef van 1195 adolescente mannen en 1507 adolescente vrouwen van het NTR. De DEB-schaal bleek niet volledig meetinvariant te zijn, het is onduidelijk of gevonden sekse-verschillen in de DEB score werkelijke verschillen tussen mannen en vrouwen weerspiegelen in ‘eetgestoordheid’ of een gevolg zijn van de meetafwijking in de schaal. Op basis van de DEB-schaal zijn mannen en vrouwen dus niet vergelijkbaar, en in toekomstige analyses moeten deze groepen apart worden meegenomen.

Erfelijkheid eetgestoord gedrag en BMI

Is de mate van eetgestoord gedrag erfelijk, en in welke mate zijn genetische factoren die van invloed zijn op deze mate van eetgestoord gedrag ook van invloed op BMI? De tweelingstudie die uitgevoerd is om deze vragen te beantwoorden wordt beschreven in *hoofdstuk 4*. Hiervoor werden gegevens van een grote groep tweelingen (474 ééneiïge, 310 tweeeiïge tweelingparen en 45 losse tweelingen) en hun broers en zussen (69 broers, 115 zussen) van het NTR gebruikt. Omdat de DEB-schaal niet meetinvariant was met betrekking tot geslacht (hoofdstuk 3) werden de analyses apart uitgevoerd voor mannen en vrouwen. Uit de bivariate tweelinganalyses bleek dat 65% van de individuele variatie in eetgestoord gedrag verklaard werd door genetische factoren in vrouwen, in mannen was dit percentage 38%. De erfelijkheid van BMI was hoog in zowel vrouwen (80%) als mannen (76%). De overlap tussen de mate van eetgestoord gedrag en BMI werd verklaard door genetische factoren, er werd een genetische correlatie van 0.43 in vrouwen en 0.51 in mannen gevonden. Ondanks deze overlap, werd het merendeel van de genetische invloed

op de mate van eetgestoord gedrag verklaard door genetische factoren die geen rol speelde bij BMI in mannen en vrouwen.

Associatie studie van kandidaatgenen in anorexia nervosa en zelfopgewekt braken

In *hoofdstuk 5* wordt een associatie studie beschreven waarin de rol van verschillende kandidaatgenen in anorexia nervosa en in eetstoornissen gekenmerkt door zelfopgewekt braken worden onderzocht. Zelfopgewekt braken is een belangrijk klinisch symptoom bij verschillende eetstoornissen, dat in hoge mate erfelijk bepaald blijkt te zijn. In deze studie werden vier kandidaatgenen onderzocht, serotonine receptor 1D (*HTR1D*), brain-derived neurotrophic factor (*BDNF*), tryptophaan hydroxylase 2 (*TPH2*) en stathmine (*STMN1*). *HTR1D* en *BDNF* werden gekozen op basis van resultaten uit eerdere studies. *TPH2* speelt een belangrijke rol binnen het serotonine systeem. Dit systeem is betrokken bij verschillende biologische, fysiologische en gedragsfuncties die een rol kunnen spelen bij de ontwikkeling van een eetstoornis, zo is serotonine van invloed op de regulatie van lichaamsgewicht, eetgedrag, maar ook op psychische kenmerken zoals perfectionisme, impulsiviteit en obsessief gedrag. *STMN1* ligt in een gebied op chromosoom 1 waarmee eerder koppeling was gevonden met anorexia nervosa. Verder speelt dit gen een rol in angst en angstgevoelens bij zowel muizen als mensen.

Voor de associatie studie werden 25 varianten binnen de vier kandidaatgenen (*HTR1D*, *BDNF*, *TPH2* en *STMN1*) in twee patiëntgroepen van de GenED populatie (182 patiënten met anorexia nervosa en 149 patiënten met een eetstoornis gekenmerkt door zelfopgewekt braken) vergeleken met 607 vrouwen van het NTR. Voor één variant (rs1473473) binnen het *TPH2* gen werd een associatie gevonden met beide patiëntgroepen. Voor replicatie, werd vervolgens onderzocht of deze variant ook een associatie liet zien in een meta-analyse met twee andere onafhankelijke eetstoornis populaties uit Duitsland en Nederland. De studiepopulatie voor deze analyse bestond inclusief de GenED populatie uit totaal 887 anorexia nervosa patiënten, 306 patiënten met een eetstoornis gekenmerkt door zelfopgewekt braken en 1914 vrouwen uit de algemene bevolking. In deze analyse bleek rs1473473 ook geassocieerd met anorexia nervosa en eetstoornissen gekenmerkt door zelfopgewekt braken. Voor de zeldzame variant van rs1473473 werd een odds ratio van 1.25 voor anorexia nervosa en een odds ratio van 1.34 voor zelfopgewekt braken gevonden. Dit betekent dat dragers van de zeldzame variant een verhoogd risico hebben op het ontwikkelen van anorexia nervosa en eetstoornissen gekenmerkt door zelfopgewekt braken. *TPH2* encodeert het enzym dat de mate bepaald waarin serotonine wordt gevormd in de hersenen.

Perfectionisme en impulsiviteit bij de associatie tussen eetstoornissen en TPH2

Hoofdstuk 6 onderzoekt de hypothese dat genetische variatie in het *TPH2* gen (geassocieerd met eetstoornissen, hoofdstuk 5) de overlap tussen perfectionisme, impulsiviteit en het voorkomen van eetstoornissen kan verklaren. Allereerst werd getoetst of patiënten met een eetstoornis (GenED populatie) anders scoren op perfectionisme- en impulsiviteitsvragenlijsten in vergelijking met volwassen vrouwen zonder een eetstoornis. Patiënten met een eetstoornis bleken zeer perfectionistisch en impulsief te zijn in vergelijking met de groep zonder eetstoornis. Vervolgens werd binnen een groep van 512 vrouwen van het NTR de associatie tussen vier *TPH2*-varianten en een enkel perfectionisme- en impulsiviteitsitem getoetst. Twee varianten (rs1473473 en rs1007023) waren geassocieerd met een hogere impulsiviteitscore (odds ratio's 1.49 en 1.60). Ten slotte werd onderzocht of deze twee varianten ook een associatie met impulsiviteit lieten zien in een groep van 267 eetstoornispatiënten.Voor beide genvarianten werd een associatie gevonden met hogere impulsiviteitsscores (rs1007023, odds ratio 1.79; rs1473473, odds ratio 1.83). Genetische variatie in het *TPH2* gen lijkt dus invloed te hebben op impulsiviteit, wat de kwetsbaarheid op het ontstaan van anorexia nervosa of eetstoornissen gekenmerkt door zelfopgewekt braken kan beïnvloeden.

Conclusie en toekomst

In *hoofdstuk 7* worden de belangrijkste bevindingen uit de voorgaande hoofdstukken besproken, verder worden mogelijkheden voor toekomstig onderzoek en klinische implicaties toegelicht. Het is duidelijk dat het genetisch onderzoek bij eetstoornissen, vergeleken met andere psychische en somatische aandoeningen, nog in de kinderschoenen staat. Het veld wordt nog steeds gekenmerkt door vele kleine associatie studies naar bepaalde kandidaatgenen, met tegenstrijdige bevindingen als resultaat. Doordat eetstoornissen weinig voorkomen onder de bevolking, is het noodzakelijk dat onderzoeksgroepen wereldwijd meer samenwerken om onderzoek te kunnen doen in grotere groepen. Daarnaast maakt de opkomst van genoomwijde associatie studies het uitvoeren van kandidaatgen studies achterhaald. Met name wanneer er nog weinig over de etiologie van een ziekte bekend is, zoals bij eetstoornissen, zijn genoomwijde associatie studies een goede methode omdat genetische varianten verspreid over het hele genoom (alle chromosomen) in één keer worden getoetst op associatie. Binnenkort worden de resultaten

van een grote genoomwijde associatie studie in anorexia nervosa verwacht, uitgevoerd door het Genetic Consortium of Anorexia Nervosa waarvan de GenED studie ook deel uitmaakt.

De bevinding dat een variant binnen het *TPH2* gen geassocieerd is met anorexia nervosa en met eetstoornissen gekenmerkt door zelfopgewekt braken lijkt robuust. Het is niet duidelijk wat de functie van variant rs1473473 is. Heeft deze variant zelf invloed op de activiteit van het gen, of weerspiegelt het de werking van een functionele variant in de buurt? Om deze vraag te beantwoorden zal het *TPH2* gen beter onderzocht moeten worden, bijvoorbeeld door meer varianten binnen dit gen te meten of door het gen te sequencen (het uitlezen van het hele stuk DNA waaruit het gen is opgebouwd). Verder is het ook interessant om de activiteit van dit gen te onderzoeken door de zogenaamde expressie in de hersenen te meten.

Naast het genotype is het belangrijk om naar de fenotypes gerelateerd aan eetstoornissen kijken. De verwachting is dat genen niet van invloed zullen zijn op een volledige eetstoornis diagnose, maar eerder een effect zullen hebben op onderliggende gedragskenmerken, zoals perfectionisme, zelfopgewekt braken en impulsiviteit (zie hoofdstuk 5 en 6). Naast perfectionisme en impulsiviteit zijn er ook andere gedragskenmerken gerelateerd aan eetstoornissen, zoals negatieve zelf-evaluatie/laag zelfvertrouwen, negatief lichaamsbeeld en zorgen over het gewicht. In de toekomst is het belangrijk om te onderzoeken of de overlap tussen deze gedragskenmerken en eetstoornissen wordt verklaard door één of meerdere onderliggende factoren, en in hoeverre deze factoren door genetische of omgevingsinvloeden worden verklaard. Tweelingstudies kunnen hierin een belangrijke bijdrage leveren.

Een ander belangrijk aspect is het meetinstrument van het betreffende (gedrags)kenmerk. Als in grote groepen mensen vragenlijsten of interviews worden afgenomen om de mate van aanwezigheid van een bepaald kenmerk vast te stellen, is het wel essentieel dat het meetinstrument vergelijkbaar is tussen bijvoorbeeld man en vrouw, oud en jong, maar ook tussen ziek en gezond. Met onze studie (beschreven in hoofdstuk 3) waren we de eerste die binnen het eetstoornisveld hebben gekeken naar de meetinvariantie. Dus ondanks het feit dat somscores van eetstoornisschalen vaak worden gebruikt om groepen te vergelijken, is nooit eerder vastgesteld of deze schalen wel werkelijke verschillen laten zien of dat het om een meetafwijking gaat.

Op korte termijn zijn de klinische implicaties van het genetisch onderzoek in eetstoornissen niet zo groot. Het kan helpen om het stigma omtrent eetstoornissen te verminderen en om het schuldgevoel van ouders te verlichten. Op de lange termijn, als meer duidelijk is over de etiologie en betrokkenheid van specifieke genen bij eetstoornissen, kunnen preventie-activiteiten en behandeling worden verbeterd (zgn.

‘matched care’). Betere risicoprofielen op basis van genen en persoonlijkheidskenmerken zouden kunnen worden opgesteld voor eetstoornissen, zodat de kans op een vroege ontdekking van de stoornis groter wordt. Daarnaast zouden genen kunnen worden gebruikt als voorspellers van het verloop en de uitkomst van de ziekte. De behandeling zou hier vervolgens op aangepast kunnen worden.

References

Diagnostic and statistical manual of mental disorders, 4th edition (1994). (4 ed.) Washington: American Psychiatric Press.

Achenbach, T. M. (1990). *The Young Adult Self Report*. Burlington, VT: University of Vermont, Dept of Psychiatry.

Anderson, C. B. & Bulik, C. M. (2004). Gender differences in compensatory behaviors, weight and shape salience, and drive for thinness. *Eating Behaviors, 5*, 1-11.

Ando, T., Komaki, G., Karibe, M., Kawamura, N., Hara, S., Takii, M., Naruo, T., Kurokawa, N., Takei, M., Tatsuta, N., Ohba, M., Nozoe, S., Kubo, C., & Ishikawa, T. (2001). 5-HT2A promoter polymorphism is not associated with anorexia nervosa in Japanese patients. *Psychiatric Genetics, 11*, 157-160.

Angelucci, F., Mathe, A. A., & Aloe, L. (2004). Neurotrophic factors and CNS disorders: findings in rodent models of depression and schizophrenia. *Progress in Brain Research, 146*, 151-165.

Askevold, F. & Heiberg, A. (1979). Anorexia nervosa--two cases in discordant MZ twins. *Psychotherapy & Psychosomatics, 32*, 223-228.

Asparouhov, T. & Muthen, B. (2006). Robust chi square difference testing with mean and variance adjusted test statistics. *Mplus Web Notes, No.10*.

Bachner-Melman, R., Lerer, E., Zohar, A. H., Kremer, I., Elizur, Y., Nemanov, L., Golan, M., Blank, S., Gritsenko, I., & Ebstein, R. P. (2007). Anorexia nervosa, perfectionism, and dopamine D4 receptor (DRD4). *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 144B*, 748-756.

Barnett, J. H. & Smoller, J. W. (2009). The genetics of bipolar disorder. *Neuroscience, 164*, 331-343.

Bartels, M., Van Beijsterveldt, C. E. M., Stroet, T. M., Hudziak, J. J., & Boomsma, D. I. (2007). Young- Netherlands Twin Register (Y-NTR); A longitudinal multiple informant study of problem behavior. *Twin Research and Human Genetics, 10*, 3-12.

Bastiaens, T., Vertommen, H., & Braspenning, N. (2003). Impulsiviteit bij een niet-klinische proefgroep. *Diagnostiek-wijzer, 6*, 2-17.

Bastiani, A. M., Rao, R., Weltzin, T., & Kaye, W. H. (1995). Perfectionism in anorexia nervosa. *International Journal of Eating Disorders, 17*, 147-152.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Archives of General Psychiatry, 4*, 561-571.

Ben-Tovim, D. I., Subbiah, N., Scheutz, B., & Morton, J. (1989). Bulimia: symptoms and syndromes in an urban population. *Australian and New Zealand Journal of Psychiatry, 23*, 73-80.

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. *Molecular Psychiatry, 8*, 397-406.

Biederman, J., Rivinus, T., Kemper, K., Hamilton, D., MacFadyen, J., & Harmatz, J. (1985). Depressive disorders in relatives of anorexia nervosa patients with and without a current episode of nonbipolar major depression. *American Journal of Psychiatry, 142,* 1495-1497.

Blundell, J. E. (1992). Serotonin and the biology of feeding. *American Journal of Clinical Nutrition, 55,* 155S-159S.

Blundell, J. E., Lawton, C. L., & Halford, J. C. (1995). Serotonin, eating behavior, and fat intake. *Obesity Research, 3 Suppl 4,* 471S-476S.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., Posthuma, D., van Beijsterveldt, T. C., Hudziak, J. J., Bartels, M., & Willemsen, G. (2006). Netherlands Twin Register: from twins to twin families. *Twin Research and Human Genetics, 9,* 849-857.

Boomsma, D. I., Vink, J. M., van Beijsterveldt, T. C., de Geus, E. J., Beem, A. L., Mulder, E. J., Derks, E. M., Riese, H., Willemsen, G. A., Bartels, M., van den, B. M., Kupper, N. H., Polderman, T. J., Posthuma, D., Rietveld, M. J., Stubbe, J. H., Knol, L. I., Stroet, T., & van Baal, G. C. (2002). Netherlands Twin Register: a focus on longitudinal research. *Twin Research, 5,* 401-406.

Boomsma, D. I., Willemsen, G., Sullivan, P. F., Heutink, P., Meijer, P., Sondervan, D., Kluft, C., Smit, G., Nolen, W. A., Zitman, F. G., Smit, J. H., Hoogendijk, W. J., van, D. R., de Geus, E. J., & Penninx, B. W. (2008). Genome-wide association of major depression: description of samples for the GAIN Major Depressive Disorder Study: NTR and NESDA biobank projects. *European Journal of Human Genetics, 16,* 335-342.

Brewerton, T. D. & Jimerson, D. C. (1996). Studies of serotonin function in anorexia nervosa. *Psychiatry Research, 62,* 31-42.

Brocke, B., Lesch, K. P., Armbruster, D., Moser, D. A., Muller, A., Strobel, A., & Kirschbaum, C. (2010). Stathmin, a gene regulating neural plasticity, affects fear and anxiety processing in humans. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 153B,* 243-251.

Brown, K. M., Bujac, S. R., Mann, E. T., Campbell, D. A., Stubbins, M. J., & Blundell, J. E. (2006). Further Evidence of Association of OPRD1 & HTR1D Polymorphisms with Susceptibility to Anorexia Nervosa. *Biologic Psychiatry, 61,* 367-373.

Bruce, K. R., Steiger, H., Joober, R., Kin, N. M., Israel, M., & Young, S. N. (2005). Association of the promoter polymorphism -1438G/A of the 5-HT2A receptor gene with behavioral impulsiveness and serotonin function in women with bulimia nervosa. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 137,* 40-44.

Bulik, C. M. (2005). Exploring the gene-environment nexus in eating disorders. *Journal of Psychiatry & Neuroscience, 30,* 335-339.

Bulik, C. M., Devlin, B., Bacanu, S. A., Thornton, L., Klump, K. L., Fichter, M. M., Halmi, K. A., Kaplan, A. S., Strober, M., Woodside, D. B., Bergen, A. W., Ganjei, J. K., Crow, S., Mitchell, J., Rotondo, A., Mauri, M., Cassano, G., Keel, P., Berrettini, W. H., & Kaye, W. H. (2003a). Significant linkage on chromosome 10p in families with bulimia nervosa. *American Journal of Human Genetics, 72,* 200-207.

Bulik, C. M., Hebebrand, J., Keski-Rahkonen, A., Klump, K. L., Reichborn-Kjennerud, T., Mazzeo, S. E., & Wade, T. D. (2007a). Genetic epidemiology, endophenotypes, and

eating disorder classification. *International Journal of Eating Disorders, 40 Suppl,* S52-S60.
Bulik, C. M., Slof-Op 't Landt, M. C. T., van Furth, E. F., & Sullivan, P. F. (2007b). The genetics of anorexia nervosa. *Annual Review of Nutrition, 27,* 263-275.
Bulik, C. M., Sullivan, P. F., Fear, J. L., & Joyce, P. R. (1997). Eating disorders and antecedent anxiety disorders: a controlled study. *Acta Psychiatrica Scandinavica, 96,* 101-107.
Bulik, C. M., Sullivan, P. F., & Kendler, K. S. (1998). Heritability of binge-eating and broadly defined bulimia nervosa. *Biologic Psychiatry, 44,* 1210-1218.
Bulik, C. M., Sullivan, P. F., & Kendler, K. S. (2003b). Genetic and environmental contributions to obesity and binge eating. *International Journal of Eating Disorders, 33,* 293-298.
Bulik, C. M., Sullivan, P. F., Tozzi, F., Furberg, H., Lichtenstein, P., & Pedersen, N. L. (2006). Prevalence, heritability, and prospective risk factors for anorexia nervosa. *Archives of General Psychiatry, 63,* 305-312.
Bulik, C. M., Sullivan, P. F., Wade, T. D., & Kendler, K. S. (2000). Twin studies of eating disorders: a review. *International Journal of Eating Disorders, 27,* 1-20.
Bulik, C. M., Thornton, L. M., Root, T. L., Pisetsky, E. M., Lichtenstein, P., & Pedersen, N. L. (2010). Understanding the relation between anorexia nervosa and bulimia nervosa in a Swedish national twin sample. *Biologic Psychiatry, 67,* 71-77.
Bulik, C. M. & Tozzi, F. (2004). Genetics in eating disorders: state of the science. *CNS Spectrums, 9,* 511-515.
Calugi, S., Grave, R. D., Ghisi, M., & Sanavio, E. (2006). Validation of the body checking questionnaire (BCQ) in an eating disorders population. *Behavioural and Cognitive Psychotherapy, 34,* 233-242.
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.
Cardon, L. R. & Bell, J. I. (2001). Association study designs for complex diseases. *Nature Review Genetics, 2,* 91-99.
Carver, C. S. & Miller, C. J. (2006). Relations of serotonin function to personality: current views and a key methodological issue. *Psychiatry Research, 144,* 1-15.
Casper, R. C., Hedeker, D., & McClough, J. F. (1992). Personality dimensions in eating disorders and their relevance for subtyping. *Journal of the American Academy of Child and Adolescent Psychiatry, 31,* 830-840.
Cassin, S. E. & von Ranson, K. M. (2005). Personality and eating disorders: a decade in review. *Clinical Psychology Review, 25,* 895-916.
Cederlof, R., Friberg, L., & Lundman, T. (1977). The interactions of smoking, environment and heredity and their implications for disease etiology. A report of epidemiological studies on the Swedish twin registries. *Acta Medica Scandinavica Supplement, 612,* 1-128.
Chen, F. F., Sousa, K. H., & West, S. G. (2005). Testing measurement invariance of second-order factor models. *Structural Equation Modeling, 12,* 471-492.
Claes, L., Vandereycken, W., & Vertommen, H. (2002). Impulsive and compulsive traits in eating disordered patients compared with controls. *Personality and Individual Differences, 32,* 707-714.

Cloninger, C. R., Svrakic, D. M., & Przybeck, T. R. (1993). A psychobiological model of temperament and character. *Archives of General Psychiatry, 50,* 975-990.

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.

Collier, D. A., Sham, P. C., Arranz, M. J., Hu, X., & Treasure, J. (1999). Understanding the genetic predisposition to anorexia nervosa. *European Eating Disorders Review, 7,* 96-102.

Congdon, E. & Canli, T. (2008). A neurogenetic approach to impulsivity. *Journal of Personality, 76,* 1447-1483.

Crisafulli, M. A., Von, H. A., & Bulik, C. M. (2008). Attitudes towards anorexia nervosa: the impact of framing on blame and stigma. *International Journal of Eating Disorders, 41,* 333-339.

Crisp, A. (2005). Stigmatization of and discrimination against people with eating disorders including a report of two nationwide surveys. *European Eating Disorders Review, 13,* 147-152.

Crow, S. J., Peterson, C. B., Swanson, S. A., Raymond, N. C., Specker, S., Eckert, E. D., & Mitchell, J. E. (2009). Increased mortality in bulimia nervosa and other eating disorders. *American Journal of Psychiatry, 166,* 1342-1346.

Dalle Grave, R., Calugi, S., & Marchesini, G. (2009). Self-induced vomiting in eating disorders: Associated features and treatment outcome. *Behaviour Research and Therapy, 47,* 680-684.

Dardennes, R. M., Zizzari, P., Tolle, V., Foulon, C., Kipman, A., Romo, L., Iancu-Gontard, D., Boni, C., Sinet, P. M., Therese, B. M., Estour, B., Mouren, M. C., Guelfi, J. D., Rouillon, F., Gorwood, P., & Epelbaum, J. (2006). 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.

de Bakker, P. I., Yelensky, R., Pe'er, I., Gabriel, S. B., Daly, M. J., & Altshuler, D. (2005). Efficiency and power in genetic association studies. *Nature Genetics, 37,* 1217-1223.

de Krom, M., Hendriks, J., Hillebrand, J., van Elburg, A., & Adan, R. (2006). A polymorphism in the 3' untranslated region of the CCK gene is associated with anorexia nervosa in Dutch patients. *Psychiatric Genetics, 16,* 239.

DerSimonian, R. & Laird, N. (1986). Meta-analysis in clinical trials. *Controlled Clinical Trials, 7,* 177-188.

Devlin, B., Bacanu, S. A., Klump, K. L., Bulik, C. M., Fichter, M. M., Halmi, K. A., Kaplan, A. S., Strober, M., Treasure, J., Woodside, D. B., Berrettini, W. H., & Kaye, W. H. (2002). Linkage analysis of anorexia nervosa incorporating behavioral covariates. *Human Molecular Genetics, 11,* 689-696.

Dickman, S. J. (1990). Functional and dysfunctional impulsivity: personality and cognitive correlates. *Journal of Personality and Social Psychology, 58,* 95-102.

Dmitrzak-Weglarz, M., Skibinska, M., Slopien, A., Szczepankiewicz, A., Rybakowski, F., Kramer, L., Hauser, J., & Rajewski, A. (2007). BDNF Met66 allele is associated with anorexia nervosa in the Polish population. *Psychiatric Genetics, 17,* 245-246.

Dolan, C. V. (2000). Investigating Spearman's hypothesis by means of multi-group confirmatory factor analysis. *Multivariate Behavioral Research, 35,* 21-50.

Duffy, D. L. & Martin, N. G. (1994). Inferring the direction of causation in cross-sectional twin data: theoretical and empirical considerations. *Genetic Epidemiology, 11*, 483-502.

Duman, R. S. (2002). Synaptic plasticity and mood disorders. *Molecular Psychiatry, 7 Suppl 1*, S29-S34.

Eddy, K. T., Dorer, D. L., Franko, D. L., Tahilani, K., Thompson-Brenner, H., & Herzog, D. B. (2008). Diagnostic crossover in anorexia nervosa and bulimia nervosa: Implications for DSM-V. *American Journal of Psychiatry, 165*, 245-250.

Ehrlich, S., Franke, L., Scherag, S., Burghardt, R., Schott, R., Schneider, N., Brockhaus, S., Hein, J., Uebelhack, R., & Lehmkuhl, U. (2010). The 5-HTTLPR polymorphism, platelet serotonin transporter activity and platelet serotonin content in underweight and weight-recovered females with anorexia nervosa. *European Archives of Psychiatry and Clinical Neuroscience, 260*, 483-490.

Enoch, M. A., Kaye, W. H., Rotondo, A., Greenberg, B. D., Murphy, D. L., & Goldman, D. (1998). 5-HT2A promoter polymorphism -1438G/A, anorexia nervosa, and obsessive-compulsive disorder. *Lancet, 351*, 1785-1786.

Enten, R. S. & Golan, M. (2009). Parenting styles and eating disorder pathology. *Appetite, 52*, 784-787.

Eysenck, H. J. & Eysenck, S. B. G. (1975). *Manual of the eysenck personality questionnaire*. San Diego: Digits.

Fairburn, C. G. & Beglin, S. J. (1990). Studies of the epidemiology of bulimia nervosa. *American Journal of Psychiatry, 147*, 401-408.

Fairburn, C. G. & Beglin, S. J. (1994). Assessment of eating disorders: interview or self-report questionnaire? *International Journal of Eating Disorders, 16*, 363-370.

Fairburn, C. G. & Bohn, K. (2005). Eating disorder NOS (EDNOS): an example of the troublesome "not otherwise specified" (NOS) category in DSM-IV. *Behaviour Research and Therapy, 43*, 691-701.

Fairburn, C. G., Doll, H. A., Welch, S. L., Hay, P. J., Davies, B. A., & O'Connor, M. E. (1998). Risk factors for binge eating disorder: a community-based, case-control study. *Archives of General Psychiatry, 55*, 425-432.

Fairburn, C. G. & Harrison, P. J. (2003). Eating disorders. *Lancet, 361*, 407-416.

Fairburn, C. G., Welch, S. L., Doll, H. A., Davies, B. A., & O'Connor, M. E. (1997). Risk factors for bulimia nervosa. A community-based case-control study. *Archives of General Psychiatry, 54*, 509-517.

Fairburn, C. G., Cooper, Z., & Shafran, R. (2003). Cognitive behaviour therapy for eating disorders: a "transdiagnostic" theory and treatment. *Behaviour Research and Therapy, 41*, 509-528.

Favaro, A., Zanetti, T., Tenconi, E., Degortes, D., Ronzan, A., Veronese, A., & Santonastaso, P. (2005). The relationship between temperament and impulsive behaviors in eating disordered subjects. *Eating Disorfders The Journal of Treatment & Prevention, 13*, 61-70.

Feinberg, A. P. (2008). Epigenetics at the epicenter of modern medicine. *Jama-Journal of the American Medical Association, 299*, 1345-1350.

Fernandez, S., Malacrne, V. L., Wifley, D. E., & McQuaid, J. (2006). Factor structure of the Bulimia Test-Revised in college women from four ethnic groups. *Cultural Diversity and Ethnic Minority Psychology, 12*, 403-419.

Fichter, M. M. & Noegel, R. (1990). Concordance for bulimia nervosa in twins. *International Journal of Eating Disorders, 9,* 255-263.

Fichter, M. M. & Quadflieg, N. (2007). Long-term stability of eating disorder diagnoses. *International Journal of Eating Disorders, 40 Suppl,* S61-S66.

Fink, G. & Sumner, B. E. (1996). Oestrogen and mental state. *Nature, 383,* 306.

Fischer, S., Smith, G. T., & Cyders, M. A. (2008). Another look at impulsivity: a meta-analytic review comparing specific dispositions to rash action in their relationship to bulimic symptoms. *Clinical Psychology Review, 28,* 1413-1425.

Frank, G. K., Kaye, W. H., Meltzer, C. C., Price, J. C., Greer, P., McConaha, C., & Skovira, K. (2002). Reduced 5-HT2A receptor binding after recovery from anorexia nervosa. *Biologic Psychiatry, 52,* 896-906.

Freimer, N. B. & Sabatti, C. (2007). Human genetics: variants in common diseases. *Nature, 445,* 828-830.

Friedel, S., Fontenla, H. F., Wermter, A. K., Geller, F., Dempfle, A., Reichwald, K., Smidt, J., Bronner, G., Konrad, K., Herpertz-Dahlmann, B., Warnke, A., Hemminger, U., Linder, M., Kiefl, H., Goldschmidt, H. P., 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. *American Journal of Medical Genetics, 132,* 96-99.

Frost, R. O., Marten, P., Lahart, C., & Rosenblate, R. (1990). The dimensions of perfectionism. *Cognitive Therapy & Research, 14,* 449-468.

Fulker, D. W. & Cardon, L. R. (1994). A sib-pair approach to interval mapping of quantitative trait loci. *American Journal of Human Genetics, 54,* 1092-1103.

Gabrovsek, M., Brecelj-Anderluh, M., Bellodi, L., Cellini, E., Di Bella, D., Estivill, X., Fernandez-Aranda, F., Freeman, B., Geller, F., Gratacos, M., Haigh, R., Hebebrand, J., Hinney, A., Holliday, J., Hu, X., Karwautz, A., Nacmias, B., Ribases, M., Remschmidt, H., Komel, R., Sorbi, S., Tomori, M., Treasure, J., Wagner, G., Zhao, J., & Collier, D. A. (2004). Combined family trio and case-control analysis of the COMT Val158Met polymorphism in European patients with anorexia nervosa. *American Journal of Medical Genetics, 124B,* 68-72.

Garner, D. M., Garner, M. V., & Rosen, L. W. (1993). Anorexia nervosa "restricters" who purge: implications for subtyping anorexia nervosa. *International Journal of Eating Disorders, 13,* 171-185.

Gershon, E. S., Schreiber, J. L., Hamovit, J. R., Dibble, E. D., Kaye, W. H., Nurnberger, J. I. et al. (1983). Anorexia nervosa and major affective disorders associated in families: a preliminary report. In S.B.Guze, F. J. Earls, & J. E. Barret (Eds.), *Childhood psychopathology and development* (pp. 279-284). New York: Raven Press.

Gloyn, A. L. & McCarthy, M. I. (2010). Variation across the allele frequency spectrum. *Nature Genetics, 42,* 648-650.

Godart, N. T., Flament, M. F., Lecrubier, Y., & Jeammet, P. (2000). Anxiety disorders in anorexia nervosa and bulimia nervosa: co-morbidity and chronology of appearance. *European Psychiatry, 15,* 38-45.

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). The 5-HT(2A) -1438G/A polymorphism in anorexia nervosa: a combined analysis of 316 trios from six European centres. *Molecular Psychiatry, 7*, 90-94.

Gorwood, P., Kipman, A., & Foulon, C. (2003). The human genetics of anorexia nervosa. *European Journal of Pharmacology, 480*, 163-170.

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. *American Journal of Human Genetics, 70*, 787-792.

Grigoroiu-Serbanescu, M., Magureanu, S., Milea, S., Dobrescu, I., & Marinescu, E. (2003). Modest familial aggregation of eating disorders in restrictive anorexia nervosa with adolescent onset in a Romanian sample. *European Child & Adolescent Psychiatry, 12 Suppl 1*, I47-I53.

Haavik, J., Blau, N., & Thony, B. (2008). Mutations in human monoamine-related neurotransmitter pathway genes. *Human Mutation, 29*, 891-902.

Halford, J. C. & Blundell, J. E. (2000). Separate systems for serotonin and leptin in appetite control. *Annals of Medicine, 32*, 222-232.

Halmi, K. A., Eckert, E., Marchi, P., Sampugnaro, V., Apple, R., & Cohen, J. (1991). Comorbidity of psychiatric diagnoses in anorexia nervosa. *Archives of General Psychiatry, 48*, 712-718.

Harris, E. C. & Barraclough, B. (1998). Excess mortality of mental disorder. *British Journal of Psychiatry, 173*, 11-53.

Hay, P. & Fairburn, C. (1998). The validity of the DSM-IV scheme for classifying bulimic eating disorders. *International Journal of Eating Disorders, 23*, 7-15.

Heath, A. C., Kessler, R. C., Neale, M. C., Hewitt, J. K., Eaves, L. J., & Kendler, K. S. (1993). Testing hypotheses about direction of causation using cross-sectional family data. *Behavior Genetics, 23*, 29-50.

Herpertz-Dahlmann, B. (1988). [Familial incidence of affective diseases in patients with anorexia nervosa]. *Zeitschrift für Kinder und Jugend Psychiatrie, 16*, 14-19.

Hetrick, S. E., Parker, A. G., Hickie, I. B., Purcell, R., Yung, A. R., & McGorry, P. D. (2008). Early identification and intervention in depressive disorders: towards a clinical staging model. *Psychotherapy & Psychosomatics, 77*, 263-270.

Higgins, J. P. & Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine, 21*, 1539-1558.

Hinney, A., Remschmidt, H., & Hebebrand, J. (2000). Candidate gene polymorphisms in eating disorders. *European Journal of Pharmacology, 410*, 147-159.

Hinney, A., Ziegler, A., Nothen, M. M., Remschmidt, H., & Hebebrand, J. (1997). 5-HT2A receptor gene polymorphisms, anorexia nervosa, and obesity. *Lancet, 350*, 1324-1325.

Hoek, H. W. (1993). Review of the epidemiological studies of eating disorders. *International Review of Psychiatry, 5*, 61-74.

Hoek, H. W. (2006). Incidence, prevalence and mortality of anorexia nervosa and other eating disorders. *Current Opinion in Psychiatry, 19*, 389-394.

Hoek, H. W. & Van Hoeken, D. (2003). Review of the prevalence and incidence of eating disorders. *International Journal of Eating Disorders, 34*, 383-396.

Holland, A. J., Hall, A., Murray, R., Russell, G. F., & Crisp, A. H. (1984). Anorexia nervosa: a study of 34 twin pairs and one set of triplets. *British Journal of Psychiatry, 145,* 414-419.

Holland, A. J., Sicotte, N., & Treasure, J. (1988). Anorexia nervosa: evidence for a genetic basis. *Journal of Psychosomatic Research, 32,* 561-571.

Hrabosky, J. I., White, M. A., Masheb, R. M., Rothschild, B. S., Burke-Martindale, C. H., & Grilo, C. M. (2008). Psychometric evaluation of the eating disorder examination-questionnaire for bariatric surgery candidates. *Obesity, 16,* 763-769.

Hsu, L. G., Chesler, B. E., & Santhouse, R. (1990). Bulimia nervosa in eleven sets of twins: A clinical report. *International Journal of Eating Disorders, 9,* 275-282.

Huang, E. J. & Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annual Review of Neuroscience, 24,* 677-736.

Hudson, J. I., Hiripi, E., Pope, H. G., Jr., & Kessler, R. C. (2007). The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biologic Psychiatry, 61,* 348-358.

Hudson, J. I., Pope, H. G., Jr., Jonas, J. M., Yurgelun-Todd, D., & Frankenburg, F. R. (1987). A controlled family history study of bulimia. *Psychological Medicine, 17,* 883-890.

Hur, Y. M. & Bouchard, T. J., Jr. (1997). The genetic correlation between impulsivity and sensation seeking traits. *Behavior Genetics, 27,* 455-463.

Ioannidis, J. P., Ntzani, E. E., Trikalinos, T. A., & Contopoulos-Ioannidis, D. G. (2001). Replication validity of genetic association studies. *Nature Genetics, 29,* 306-309.

Jacobi, C., Hayward, C., de Zwaan, M., Kraemer, H. C., & Agras, W. S. (2004). Coming to terms with risk factors for eating disorders: application of risk terminology and suggestions for a general taxonomy. *Psychological Bulletin, 130,* 19-65.

Javaras, K. N., Laird, N. M., Reichborn-Kjennerud, T., Bulik, C. M., Pope, H. G., Jr., & Hudson, J. I. (2008). Familiality and heritability of binge eating disorder: results of a case-control family study and a twin study. *International Journal of Eating Disorders, 41,* 174-179.

Johansen, C. T., Wang, J., Lanktree, M. B., Cao, H., McIntyre, A. D., Ban, M. R., Martins, R. A., Kennedy, B. A., Hassell, R. G., Visser, M. E., Schwartz, S. M., Voight, B. F., Elosua, R., Salomaa, V., O'Donnell, C. J., linga-Thie, G. M., Anand, S. S., Yusuf, S., Huff, M. W., Kathiresan, S., & Hegele, R. A. (2010). Excess of rare variants in genes identified by genome-wide association study of hypertriglyceridemia. *Nature Genetics, 42,* 684-687.

Jöreskog, K. G. (1993). Testing structural equation models. In K.A.Bollen & J. Scott Long (Eds.), *Testing structural equation models* (pp. 294-317). Newbury Park: Sage Publications.

Karwautz, A., Rabe-Hesketh, S., Hu, X., Zhao, J., Sham, P., Collier, D. A., & Treasure, J. L. (2001). Individual-specific risk factors for anorexia nervosa: a pilot study using a discordant sister-pair design. *Psychological Medicine, 31,* 317-329.

Kas, M. J., Kaye, W. H., Foulds, M. W., & Bulik, C. M. (2009). Interspecies genetics of eating disorder traits. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 150B,* 318-327.

Kassett, J. A., Gershon, E. S., Maxwell, M. E., Guroff, J. J., Kazuba, D. M., Smith, A. L., Brandt, H. A., & Jimerson, D. C. (1989). Psychiatric disorders in the first-degree

relatives of probands with bulimia nervosa. *American Journal of Psychiatry, 146*, 1468-1471.

Kaye, W. (2008). Neurobiology of anorexia and bulimia nervosa. *Physiology & Behavior, 94*, 121-135.

Kaye, W. H. (1997). Anorexia nervosa, obsessional behavior, and serotonin. *Psychopharmacology Bulletin, 33*, 335-344.

Kaye, W. H., Bailer, U. F., Frank, G. K., Wagner, A., & Henry, S. E. (2005a). Brain imaging of serotonin after recovery from anorexia and bulimia nervosa. *Physiology & Behavior, 86*, 15-17.

Kaye, W. H., Bulik, C. M., Thornton, L., Barbarich, N., & Masters, K. (2004a). Comorbidity of anxiety disorders with anorexia and bulimia nervosa. *American Journal of Psychiatry, 161*, 2215-2221.

Kaye, W. H., Devlin, B., Barbarich, N., Bulik, C. M., Thornton, L., Bacanu, S. A., Fichter, M. M., Halmi, K. A., Kaplan, A. S., Strober, M., Woodside, D. B., Bergen, A. W., Crow, S., Mitchell, J., Rotondo, A., Mauri, M., Cassano, G., Keel, P., Plotnicov, K., Pollice, C., Klump, K. L., Lilenfeld, L. R., Ganjei, J. K., Quadflieg, N., & Berrettini, W. H. (2004b). Genetic analysis of bulimia nervosa: Methods and sample description. *International Journal of Eating Disorders, 35*, 556-570.

Kaye, W. H., Frank, G. K., Bailer, U. F., & Henry, S. E. (2005b). Neurobiology of anorexia nervosa: clinical implications of alterations of the function of serotonin and other neuronal systems. *International Journal of Eating Disorders, 37 Suppl*, S15-S19.

Kaye, W. H., Frank, G. K., Bailer, U. F., Henry, S. E., Meltzer, C. C., Price, J. C., Mathis, C. A., & Wagner, A. (2005c). Serotonin alterations in anorexia and bulimia nervosa: New insights from imaging studies. *Physiology & Behavior, 85*, 73-81.

Kaye, W. H., Frank, G. K., Meltzer, C. C., Price, J. C., McConaha, C. W., Crossan, P. J., Klump, K. L., & Rhodes, L. (2001). Altered serotonin 2A receptor activity in women who have recovered from bulimia nervosa. *American Journal of Psychiatry, 158*, 1152-1155.

Kaye, W. H., Greeno, C. G., Moss, H., Fernstrom, J., Fernstrom, M., Lilenfeld, L. R., Weltzin, T. E., & Mann, J. J. (1998). Alterations in serotonin activity and psychiatric symptoms after recovery from bulimia nervosa. *Archives of General Psychiatry, 55*, 927-935.

Kaye, W. H., Gwirtsman, H. E., George, D. T., & Ebert, M. H. (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? *Archives of General Psychiatry, 48*, 556-562.

Kaye, W. H., Klump, K. L., Frank, G. K., & Strober, M. (2000a). Anorexia and bulimia nervosa. *Annual Review of Medicine, 51*, 299-313.

Kaye, W. H., Lilenfeld, L. R., Berrettini, W. H., Strober, M., Devlin, B., Klump, K. L., Goldman, D., Bulik, C. M., Halmi, K. A., Fichter, M. M., Kaplan, A., Woodside, D. B., Treasure, J., Plotnicov, K. H., Pollice, C., Rao, R., & McConaha, C. W. (2000b). A search for susceptibility loci for anorexia nervosa: methods and sample description. *Biologic Psychiatry, 47*, 794-803.

Keel, P. K., Klump, K. L., Miller, K. B., McGue, M., & Iacono, W. G. (2005). Shared transmission of eating disorders and anxiety disorders. *International Journal of Eating Disorders, 38*, 99-105.

Kendler, K. S. (1993). Twin studies of psychiatric illness. Current status and future directions. *Archives of General Psychiatry, 50*, 905-915.

Kendler, K. S. (2001). Twin studies of psychiatric illness: an update. *Archives of General Psychiatry, 58*, 1005-1014.

Kendler, K. S. & Eaves, L. J. (1986). Models for the joint effect of genotype and environment on liability to psychiatric illness. *American Journal of Psychiatry, 143*, 279-289.

Kendler, K. S., MacLean, C., Neale, M., Kessler, R., Heath, A., & Eaves, L. (1991). The genetic epidemiology of bulimia nervosa. *American Journal of Psychiatry, 148*, 1627-1637.

Kendler, K. S., Neale, M. C., MacLean, C. J., Heath, A. C., Eaves, L. J., & Kessler, R. C. (1993). Smoking and major depression. A causal analysis. *Archives of General Psychiatry, 50*, 36-43.

Kendler, K. S., Walters, E. E., Neale, M. C., Kessler, R. C., Heath, A. C., & Eaves, L. J. (1995). The structure of the genetic and environmental risk factors for six major psychiatric disorders in women. Phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism. *Archives of General Psychiatry, 52*, 374-383.

Kernie, S. G., Liebl, D. J., & Parada, L. F. (2000). BDNF regulates eating behavior and locomotor activity in mice. *The EMBO Journal, 19*, 1290-1300.

Keski-Rahkonen, A., Bulik, C. M., Neale, B. M., Rose, R. J., Rissanen, A., & Kaprio, J. (2005). Body dissatisfaction and drive for thinness in young adult twins. *International Journal of Eating Disorders, 37*, 188-199.

Keski-Rahkonen, A., Hoek, H. W., Linna, M. S., Raevuori, A., Sihvola, E., Bulik, C. M., Rissanen, A., & Kaprio, J. (2009). Incidence and outcomes of bulimia nervosa: a nationwide population-based study. *Psychological Medicine, 39*, 823-831.

Keski-Rahkonen, A., Hoek, H. W., Susser, E. S., Linna, M. S., Sihvola, E., Raevuori, A., Bulik, C. M., Kaprio, J., & Rissanen, A. (2007). Epidemiology and course of anorexia nervosa in the community. *American Journal of Psychiatry, 164*, 1259-1265.

Kim, Y. K., Lee, H. J., Yang, J. C., Hwang, J. A., & Yoon, H. K. (2009). A tryptophan hydroxylase 2 gene polymorphism is associated with panic disorder. *Behavior Genetics, 39*, 170-175.

Kipman, A., Bruins-Slot, L., Boni, C., Hanoun, N., Ades, J., Blot, P., Hamon, M., Mouren-Simeoni, M., & Gorwood, P. (2002). 5-HT(2A) gene promoter polymorphism as a modifying rather than a vulnerability factor in anorexia nervosa. *European Psychiatry, 17*, 227-229.

Kjelsas, E., Bjornstrom, C., & Gotestam, K. G. (2004). Prevalence of eating disorders in female and male adolescents (14-15 years). *Eating Behaviors, 5*, 13-25.

Klump, K. L., Bulik, C. M., Kaye, W. H., Treasure, J., & Tyson, E. (2009). Academy for eating disorders position paper: eating disorders are serious mental illnesses. *International Journal of Eating Disorders, 42*, 97-103.

Klump, K. L., McGue, M., & Iacono, W. G. (2000). Age differences in genetic and environmental influences on eating attitudes and behaviors in preadolescent and adolescent female twins. *Journal of Abnormal Psychology, 109*, 239-251.

Klump, K. L., McGue, M., & Iacono, W. G. (2003). Differential heritability of eating attitudes and behaviors in prepubertal versus pubertal twins. *International Journal of Eating Disorders, 33,* 287-292.

Klump, K. L., Miller, K. B., Keel, P. K., McGue, M., & Iacono, W. G. (2001). Genetic and environmental influences on anorexia nervosa syndromes in a population-based twin sample. *Psychological Medicine, 31,* 737-740.

Koizumi, H., Hashimoto, K., Itoh, K., Nakazato, M., Shimizu, E., Ohgake, S., Koike, K., Okamura, N., Matsushita, S., Suzuki, K., Murayama, M., Higuchi, S., & Iyo, M. (2004). Association between the brain-derived neurotrophic factor 196G/A polymorphism and eating disorders. *American Journal of Medical Genetics, 127B,* 125-127.

Kortegaard, L. S., Hoerder, K., Joergensen, J., Gillberg, C., & Kyvik, K. O. (2001). A preliminary population-based twin study of self-reported eating disorder. *Psychological Medicine, 31,* 361-365.

Lander, E. & Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics, 11,* 241-247.

Lau, J., Ioannidis, J. P., & Schmid, C. H. (1997). Quantitative synthesis in systematic reviews. *Annals of Internal Medicine, 127,* 820-826.

Le Grange, D., Lock, J., Loeb, K., & Nicholls, D. (2010). Academy for Eating Disorders position paper: the role of the family in eating disorders. *International Journal of Eating Disorders, 43,* 1-5.

Lebrun, B., Jean, A., Bariohay, B., & Moyse, E. (2006). Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. *Autonomic Neuroscience, 126-127,* 30-38.

Lee, S. W., Stewart, S. M., Striegel-Moore, R. H., Lee, S., Ho, S. Y., Lee, P. W. H., Katzman, M. A., & Lam, T. H. (2007). Validation of the eating disorder diagnostic scale for use with Hong kong adolescents. *International Journal of Eating Disorders, 40,* 569-574.

Levinson, D. F. (2005). Meta-analysis in psychiatric genetics. *Current Psychiatry Reports, 7,* 143-151.

Li, J. & Ji, L. (2005). Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity, 95,* 221-227.

Lilenfeld, L. R., Kaye, W. H., Greeno, C. G., Merikangas, K. R., Plotnicov, K., Pollice, C., Rao, R., Strober, M., Bulik, C. M., & Nagy, L. (1998). A controlled family study of anorexia nervosa and bulimia nervosa: psychiatric disorders in first-degree relatives and effects of proband comorbidity. *Archives of General Psychiatry, 55,* 603-610.

Lilenfeld, L. R., Stein, D., Bulik, C. M., Strober, M., Plotnicov, K., Pollice, C., Rao, R., Merikangas, K. R., Nagy, L., & Kaye, W. H. (2000). Personality traits among currently eating disordered, recovered and never ill first-degree female relatives of bulimic and control women. *Psychological Medicine, 30,* 1399-1410.

Lim, J. E., Pinsonneault, J., Sadee, W., & Saffen, D. (2007). Tryptophan hydroxylase 2 (TPH2) haplotypes predict levels of TPH2 mRNA expression in human pons. *Molecular Psychiatry, 12,* 491-501.

Logue, C. M., Crowe, R. R., & Bean, J. A. (1989). A family study of anorexia nervosa and bulimia. *Comprehensive Psychiatry, 30,* 179-188.

Lu, B. (2003). BDNF and activity-dependent synaptic modulation. *Learning & Memory, 10,* 86-98.

Lucki, I. (1998). The spectrum of behaviors influenced by serotonin. *Biologic Psychiatry, 44,* 151-162.

Maguire, S. (2008). Staging anorexia nervosa: conceptualizing illness severity. *Early Intervention in Psychiatry, 2,* 3-10.

McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonte, B., Szyf, M., Turecki, G., & Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience, 12,* 342-348.

Mellenbergh, G. J. (1989). Item bias and item response theory. *International Journal of Educational Research, 13,* 127-143.

Mercader, J. M., Ribases, M., Gratacos, M., Gonzalez, J. R., Bayes, M., de Cid, R., Badia, A., Fernandez-Aranda, F., & Estivill, X. (2007). Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. *Genes, Brain and Behavior, 6,* 706-716.

Meredith, W. (1993). Measurement invariance, factor analysis and factorial invariance. *Psychometrika, 58,* 525-543.

Middeldorp, C. M., Slof-Op 't Landt, M. C. T., Medland, S. E., Van Beijsterveldt, C. E. M., Bartels, M., Willemsen, G., Hottenga, J. J., de Geus, E. J. C., Suchiman, H. E. D., Neale, M. C., Slagboom, P. E., & Boomsma, D. I. (2010). Anxiety and depression in children and adults:influence of serotonergic and neurotrophic genes? Genes, Brain and Behavior 9, 808-816.
Ref Type: Journal (Full)

Millsap, R. E. & Yun-Tein, J. (2004). Assessing Factorial Invariance in Ordered-Categorical Measures. *Multivariate Behavioral Research, 39,* 479-515.

Milos, G., Spindler, A., Schnyder, U., & Fairburn, C. G. (2005). Instability of eating disorder diagnoses: prospective study. *British Journal of Psychiatry, 187,* 573-578.

Mitchell, K. S., Neale, M. C., Bulik, C. M., Aggen, S. H., Kendler, K. S., & Mazzeo, S. E. (2010). Binge eating disorder: a symptom-level investigation of genetic and environmental influences on liability. *Psychological Medicine,* 1-8.

Monteleone, P., Brambilla, F., Bortolotti, F., & Maj, M. (2000). Serotonergic dysfunction across the eating disorders: relationship to eating behaviour, purging behaviour, nutritional status and general psychopathology. *Psychological Medicine, 30,* 1099-1110.

Muller, T. D., Hinney, A., Scherag, A., Nguyen, T. T., Schreiner, F., Schafer, H., Hebebrand, J., Roth, C. L., & Reinehr, T. (2008). 'Fat mass and obesity associated' gene (FTO): no significant association of variant rs9939609 with weight loss in a lifestyle intervention and lipid metabolism markers in German obese children and adolescents. *BMC Medical Genetics, 9,* 85.

Muthen, B. & Asparouhov, T. (2002). Latent variable analysis with categorical outcomes: multiple-group and growth modeling in Mplus. *Mplus Web Notes, No. 4*.

Muthén, L. K. & Muthén, B. O. (2005). *Mplus User's guide*. (Third edition ed.) Los Angeles, CA: Muthén & Muthén.

Nacmias, B., Ricca, V., Tedde, A., Mezzani, B., Rotella, C. M., & Sorbi, S. (1999). 5-HT2A receptor gene polymorphisms in anorexia nervosa and bulimia nervosa. *Neuroscience Letters, 277,* 134-136.

Nakabayashi, K., Komaki, G., Tajima, A., Ando, T., Ishikawa, M., Nomoto, J., Hata, K., Oka, A., Inoko, H., Sasazuki, T., & Shirasawa, S. (2009). Identification of novel candidate loci for anorexia nervosa at 1q41 and 11q22 in Japanese by a genome-wide association analysis with microsatellite markers. *J.Hum.Genet., 54,* 531-537.

Neale, B. M., Mazzeo, S. E., & Bulik, C. M. (2003a). A twin study of dietary restraint, disinhibition and hunger: an examination of the eating inventory (three factor eating questionnaire). *Twin Research, 6,* 471-478.

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. (2003b). *Mx: Statistical modeling*. Richmond: Department of psychiatry.

Neale, M. C., Eaves, L. J., & Kendler, K. S. (1994). The power of the classical twin study to resolve variation in threshold traits. *Behavior Genetics, 24,* 239-258.

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron, 34,* 13-25.

Neumark-Sztainer, D., Wall, M., Haines, J., Story, M., & Eisenberg, M. E. (2007). Why does dieting predict weight gain in adolescents? Findings from project EAT-II: a 5-year longitudinal study. *Journal of the American Dietetic Association, 107,* 448-455.

Nielsen, S., Moller-Madsen, S., Isager, T., Jorgensen, J., Pagsberg, K., & Theander, S. (1998). Standardized mortality in eating disorders--a quantitative summary of previously published and new evidence. *Journal of Psychosomatic Research, 44,* 413-434.

Nishiguchi, N., 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. *Biologic Psychiatry, 50,* 123-128.

Nowlin, N. S. (1983). Anorexia Nervosa in twins: case report and review. *Journal of Clinical Psychiatry, 44,* 101-105.

Nyholt, D. R. (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *American Journal of Human Genetics, 74,* 765-769.

Oades, R. D., Lasky-Su, J., Christiansen, H., Faraone, S. V., Sonuga-Barke, E. J., Banaschewski, T., Chen, W., Anney, R. J., Buitelaar, J. K., Ebstein, R. P., Franke, B., Gill, M., Miranda, A., Roeyers, H., Rothenberger, A., Sergeant, J. A., Steinhausen, H. C., Taylor, E. A., Thompson, M., & Asherson, P. (2008). The influence of serotonin- and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. *Behavioral and Brain Functions, 4,* 48.

Papadopoulos, F. C., Ekbom, A., Brandt, L., & Ekselius, L. (2009). Excess mortality, causes of death and prognostic factors in anorexia nervosa. *British Journal of Psychiatry, 194,* 10-17.

Pedersen, N. L., Plomin, R., McClearn, G. E., & Friberg, L. (1988). Neuroticism, extraversion, and related traits in adult twins reared apart and reared together. *Journal of Personality and Social Psychology, 55,* 950-957.

Pelleymounter, M. A., Cullen, M. J., & Wellman, C. L. (1995). Characteristics of BDNF-induced weight loss. *Experimental Neurology, 131,* 229-238.

Peters, E. J., Slager, S. L., McGrath, P. J., Knowles, J. A., & Hamilton, S. P. (2004). Investigation of serotonin-related genes in antidepressant response. *Molecular Psychiatry, 9,* 879-889.

Peterson, C. B., Crosby, R. D., Wonderlich, S. A., Joiner, T., Crow, S. J., Mitchell, J. E., Bardone-Cone, A. M., Klein, M., & lw Grange, D. (2007). Psychometric properties of the Eating Disorder Examination-Questionnaire: Factor structure and internal consistency. *International Journal of Eating Disorders, 40,* 386-389.

Pike, K. M., Wilfley, D., Hilbert, A., Fairburn, C. G., Dohm, F. A., & Striegel-Moore, R. H. (2006). Antecedent life events of binge-eating disorder. *Psychiatry Research, 142,* 19-29.

Pinheiro, A. P., Bulik, C. M., Thornton, L. M., Sullivan, P. F., Root, T. L., Bloss, C. S., Berrettini, W. H., Schork, N. J., Kaye, W. H., Bergen, A. W., Magistretti, P., Brandt, H., Crawford, S., Crow, S., Fichter, M. M., Goldman, D., Halmi, K. A., Johnson, C., Kaplan, A. S., Keel, P. K., Klump, K. L., La, V. M., Mitchell, J. E., Strober, M., Rotondo, A., Treasure, J., & Woodside, D. B. (2010). Association study of 182 candidate genes in anorexia nervosa. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 135B,* 1070-1080.

Racine, S. E., Culbert, K. M., Larson, C. L., & Klump, K. L. (2009). The possible influence of impulsivity and dietary restraint on associations between serotonin genes and binge eating. *Journal of Psychiatric Research, 43,* 1278-1286.

Raney, T. J., Thornton, L. M., Berrettini, W., Brandt, H., Crawford, S., Fichter, M. M., Halmi, K. A., Johnson, C., Kaplan, A. S., LaVia, M., Mitchell, J., Rotondo, A., Strober, M., Woodside, D. B., Kaye, W. H., & Bulik, C. M. (2008). Influence of overanxious disorder of childhood on the expression of anorexia nervosa. *International Journal of Eating Disorders, 41,* 326-332.

Reba, L., Thornton, L., Tozzi, F., Klump, K. L., Brandt, H., Crawford, S., Crow, S., Fichter, M. M., Halmi, K. A., Johnson, C., Kaplan, A. S., Keel, P., LaVia, M., Mitchell, J., Strober, M., Woodside, D. B., Rotondo, A., Berrettini, W. H., Kaye, W. H., & Bulik, C. M. (2005). Relationships between features associated with vomiting in purging-type eating disorders. *International Journal of Eating Disorders, 38,* 287-294.

Rebollo, I., de Moor, M. H., Dolan, C. V., & Boomsma, D. I. (2006). Phenotypic factor analysis of family data: correction of the bias due to dependency. *Twin Research and Human Genetics, 9,* 367-376.

Reichborn-Kjennerud, T., Bulik, C. M., Kendler, K. S., Roysamb, E., Maes, H., Tambs, K., & Harris, J. R. (2003). Gender differences in binge-eating: a population-based twin study. *Acta Psychiatrica Scandinavica, 108,* 196-202.

Reichborn-Kjennerud, T., Bulik, C. M., Kendler, K. S., Roysamb, E., Tambs, K., Torgersen, S., & Harris, J. R. (2004a). Undue influence of weight on self-evaluation: a population-based twin study of gender differences. *International Journal of Eating Disorders, 35,* 123-132.

Reichborn-Kjennerud, T., Bulik, C. M., Tambs, K., & Harris, J. R. (2004b). Genetic and environmental influences on binge eating in the absence of compensatory behaviors: a population-based twin study. *International Journal of Eating Disorders, 36,* 307-314.

Ribases, M., Gratacos, M., Fernandez-Aranda, F., Bellodi, L., Boni, C., Anderluh, M., Cavallini, M. C., 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, D. A., & Estivill, X. (2004). Association of BDNF with anorexia,

bulimia and age of onset of weight loss in six European populations. *Human Molecular Genetics, 13,* 1205-1212.

Ribases, M., Gratacos, M., Fernandez-Aranda, F., Bellodi, L., Boni, C., Anderluh, M., Cristina, C. 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, R., Nacmias, B., Remschmidt, H., Ricca, V., Sorbi, S., Tomori, M., Wagner, G., Treasure, J., Collier, D. A., & Estivill, X. (2005). Association of BDNF with restricting anorexia nervosa and minimum body mass index: a family-based association study of eight European populations. *European Journal of Human Genetics, 13,* 428-434.

Ricca, V., Nacmias, B., Boldrini, M., Cellini, E., Di Bernardo, M., Ravaldi, C., Tedde, A., Bagnoli, S., Placidi, G. F., Rotella, C. M., & Sorbi, S. (2004). Psychopathological traits and 5-HT(2A) receptor promoter polymorphism (-1438 G/A) in patients suffering from Anorexia Nervosa and Bulimia Nervosa. *Neuroscience Letters, 365,* 92-96.

Ricca, V., Nacmias, B., Cellini, E., Di Bernardo, M., Rotella, C. M., & Sorbi, S. (2002). 5-HT2A receptor gene polymorphism and eating disorders. *Neuroscience Letters, 323,* 105-108.

Rietveld, M. J., Der Valk, J. C., Bongers, I. L., Stroet, T. M., Slagboom, P. E., & Boomsma, D. I. (2000). Zygosity diagnosis in young twins by parental report. *Twin Research, 3,* 134-141.

Rowe, R., Pickles, A., Simonoff, E., Bulik, C. M., & Silberg, J. L. (2002). Bulimic symptoms in the Virginia Twin Study of Adolescent Behavioral Development: correlates, comorbidity, and genetics. *Biologic Psychiatry, 51,* 172-182.

Rutherford, J., McGuffin, P., Katz, R. J., & Murray, R. M. (1993). Genetic influences on eating attitudes in a normal female twin population. *Psychological Medicine, 23,* 425-436.

Rybakowski, F., Slopien, A., Dmitrzak-Weglarz, M., Czerski, P., Hauser, J., & Rajewski, A. (2004). Association study of 5-HT2A receptor gene polymorphism in anorexia nervosa in Polish population. *Archives of Psychiatry & Psychotherapy, 6,* 53-59.

Savelieva, K. V., Zhao, S., Pogorelov, V. M., Rajan, I., Yang, Q., Cullinan, E., & Lanthorn, T. H. (2008). Genetic disruption of both tryptophan hydroxylase genes dramatically reduces serotonin and affects behavior in models sensitive to antidepressants. *PLoS.One., 3,* e3301.

Scherag, S., Hebebrand, J., & Hinney, A. (2010). Eating disorders: the current status of molecular genetic research. *European Child & Adolescent Psychiatry, 19,* 211-226.

Schermelleh-Engel, K., Moosbrugger, H., & Müller, H. (2003). Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures. *Methods of Psychological Research Online, 8,* 23-74.

Schousboe, K., Willemsen, G., Kyvik, K. O., Mortensen, J., Boomsma, D. I., Cornes, B. K., Davis, C. J., Fagnani, C., Hjelmborg, J., Kaprio, J., De Lange, M., Luciano, M., Martin, N. G., Pedersen, N., Pietilainen, K. H., Rissanen, A., Saarni, S., Sorensen, T. I., van Baal, G. C., & Harris, J. R. (2003). Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. *Twin Research, 6,* 409-421.

Schroeder, M., Krebs, M. O., Bleich, S., & Frieling, H. (2010). Epigenetics and depression: current challenges and new therapeutic options. *Current Opinion in Psychiatry*.

Seroczynski, A. D., Bergeman, C. S., & Coccaro, E. F. (1999). Etiology of the impulsivity/aggression relationship: genes or environment? *Psychiatry Research, 86,* 41-57.

Shumyatsky, G. P., Malleret, G., Shin, R. M., Takizawa, S., Tully, K., Tsvetkov, E., Zakharenko, S. S., Joseph, J., Vronskaya, S., Yin, D., Schubart, U. K., Kandel, E. R., & Bolshakov, V. Y. (2005). stathmin, a gene enriched in the amygdala, controls both learned and innate fear. *Cell, 123,* 697-709.

Silberg, J. L. & Bulik, C. M. (2005). The developmental association between eating disorders symptoms and symptoms of depression and anxiety in juvenile twin girls. *Journal of Child Psychology and Psychiatry*.

Simansky, K. J. (1996). Serotonergic control of the organization of feeding and satiety. *Behavioural Brain Research, 73,* 37-42.

Slagboom, P. E. & Meulenbelt, I. (2002). Organisation of the human genome and our tools for identifying disease genes. *Biologic Psychology, 61,* 11-31.

Slof-Op 't Landt, M. C., Meulenbelt, I., Bartels, M., Suchiman, E., Middeldorp, C. M., Houwing-Duistermaat, J. J., van Trier J., Onkenhout, E. J., Vink, J. M., Van Beijsterveldt, C. E., Brandys, M. K., Sanders, N., Zipfel, S., Herzog, W., Herpertz-Dahlmann, B., Klampfl, K., Fleischhaker, C., Zeeck, A., de, Z. M., Herpertz, S., Ehrlich, S., van Elburg, A. A., Adan, R. A., Scherag, S., Hinney, A., Hebebrand, J., Boomsma, D. I., van Furth, E. F., & Slagboom, P. E. (2011). Association study in eating disorders: TPH2 associates with anorexia nervosa and self-induced vomiting. *Genes, Brain and Behavior, 10,* 236-243.

Slof-Op 't Landt, M. C., van Furth, E. F., Meulenbelt, I., Slagboom, P. E., Bartels, M., Boomsma, D. I., & Bulik, C. M. (2005). Eating disorders: from twin studies to candidate genes and beyond. *Twin Research and Human Genetics, 8,* 467-482.

Slof-Op 't Landt, M. C. T., Dolan, C. V., Rebollo-Mesa, I., Bartels, M., van Furth, E. F., Van Beijsterveldt, C. E., Meulenbelt, I., Slagboom, P. E., & Boomsma, D. I. (2009). Sex differences in sum scores may be hard to interpret: the importance of measurement invariance. *Assessment, 16,* 415-423.

Sorbi, S., Nacmias, B., Tedde, A., Ricca, V., Mezzani, B., & Rotella, C. M. (1998). 5-HT2A promoter polymorphism in anorexia nervosa. *Lancet, 351,* 1785.

Srinivasagam, N. M., Kaye, W. H., Plotnicov, K. H., Greeno, C., Weltzin, T. E., & Rao, R. (1995). Persistent perfectionism, symmetry, and exactness after long-term recovery from anorexia nervosa. *American Journal of Psychiatry, 152,* 1630-1634.

Steiger, H., Gauvin, L., Israel, M., Kin, N. M., Young, S. N., & Roussin, J. (2004). Serotonin function, personality-trait variations, and childhood abuse in women with bulimia-spectrum eating disorders. *Journal of Clinical Psychiatry, 65,* 830-837.

Steiger, H., Joober, R., Israel, M., Young, S. N., Ng Ying Kin, N. M., Gauvin, L., Bruce, K. R., Joncas, J., & Torkaman-Zehi, A. (2005). The 5HTTLPR polymorphism, psychopathologic symptoms, and platelet [(3)H-] paroxetine binding in bulimic syndromes. *International Journal of Eating Disorders, 37,* 57-60.

Steiger, H., Puentes-Neuman, G., & Leung, F. Y. (1991). Personality and family features of adolescent girls with eating symptoms: evidence for restricter/binger differences in a nonclinical population. *Addictive Behaviors, 16,* 303-314.

Steiger, H., Young, S. N., Kin, N. M., Koerner, N., Israel, M., Lageix, P., & Paris, J. (2001). Implications of impulsive and affective symptoms for serotonin function in bulimia nervosa. *Psychological Medicine, 31,* 85-95.

Steinhausen, H. C. (1999). Eating disorders. In H.C.Steinhausen & F. C. Verhulst (Eds.), *Risk and outcomes in developmental psychopathology* (pp. 210-230). Oxford: Oxford University Press.

Steinhausen, H. C. (2002). The outcome of anorexia nervosa in the 20th century. *American Journal of Psychiatry, 159,* 1284-1293.

Steinhausen, H. C. & Weber, S. (2009). The outcome of bulimia nervosa: findings from one-quarter century of research. *American Journal of Psychiatry, 166,* 1331-1341.

Stern, S. L., Dixon, K. N., Sansone, R. A., Lake, M. D., Nemzer, E., & Jones, D. (1992). Psychoactive substance use disorder in relatives of patients with anorexia nervosa. *Comprehensive Psychiatry, 33,* 207-212.

Stewart, M. C., Keel, P. K., & Schiavo, R. S. (2006). Stigmatization of anorexia nervosa. *International Journal of Eating Disorders, 39,* 320-325.

Stice, E. (2002). Risk and maintenance factors for eating pathology: a meta-analytic review. *Psychological Bulletin, 128,* 825-848.

Stoltenberg, S. F., Glass, J. M., Chermack, S. T., Flynn, H. A., Li, S., Weston, M. E., & Burmeister, M. (2006). Possible association between response inhibition and a variant in the brain-expressed tryptophan hydroxylase-2 gene. *Psychiatric Genetics, 16,* 35-38.

Striegel-Moore, R. H., Fairburn, C. G., Wilfley, D. E., Pike, K. M., Dohm, F. A., & Kraemer, H. C. (2005). Toward an understanding of risk factors for binge-eating disorder in black and white women: a community-based case-control study. *Psychological Medicine, 35,* 907-917.

Strober, M., Freeman, R., Lampert, C., Diamond, J., & Kaye, W. (2000). Controlled family study of anorexia nervosa and bulimia nervosa: evidence of shared liability and transmission of partial syndromes. *American Journal of Psychiatry, 157,* 393-401.

Strober, M., Freeman, R., Lampert, C., Diamond, J., & Kaye, W. (2001). Males with anorexia nervosa: a controlled study of eating disorders in first-degree relatives. *International Journal of Eating Disorders, 29,* 263-269.

Strober, M., Morrell, W., Burroughs, J., Salkin, B., & Jacobs, C. (1985). A controlled family study of anorexia nervosa. *Journal of Psychiatric Research, 19,* 239-246.

Strober, M., Lampert, C., Morrell, W., & Burroughs, J. (1990). A controlled family study of anorexia nervosa: Evidence of familial aggregation and lack of shared transmission with affective disorders. *International Journal of Eating Disorders, 9,* 239-253.

Sullivan, P. F. (1995). Mortality in anorexia nervosa. *American Journal of Psychiatry, 152,* 1073-1074.

Sullivan, P. F., Bulik, C. M., & Kendler, K. S. (1998a). Genetic epidemiology of binging and vomiting. *British Journal of Psychiatry, 173,* 75-79.

Sullivan, P. F., Bulik, C. M., & Kendler, K. S. (1998b). The epidemiology and classification of bulimia nervosa. *Psychological Medicine, 28,* 599-610.

Sullivan, P. F., de Geus, E. J., Willemsen, G., James, M. R., Smit, J. H., Zandbelt, T., Arolt, V., Baune, B. T., Blackwood, D., Cichon, S., Coventry, W. L., Domschke, K., Farmer, A., Fava, M., Gordon, S. D., He, Q., Heath, A. C., Heutink, P., Holsboer, F., Hoogendijk, W. J., Hottenga, J. J., Hu, Y., Kohli, M., Lin, D., Lucae, S., Macintyre, D. J., Maier, W., McGhee, K. A., McGuffin, P., Montgomery, G. W., Muir, W. J., Nolen,

W. A., Nothen, M. M., Perlis, R. H., Pirlo, K., Posthuma, D., Rietschel, M., Rizzu, P., Schosser, A., Smit, A. B., Smoller, J. W., Tzeng, J. Y., van, D. R., Verhage, M., Zitman, F. G., Martin, N. G., Wray, N. R., Boomsma, D. I., & Penninx, B. W. (2009). Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. *Molecular Psychiatry, 14,* 359-375.

Tozzi, F., Aggen, S. H., Neale, B. M., Anderson, C. B., Mazzeo, S. E., Neale, M. C., & Bulik, C. M. (2004). The structure of perfectionism: a twin study. *Behavior Genetics, 34,* 483-494.

Tozzi, F. & Bulik, C. M. (2003). Candidate genes in eating disorders. *Current Drug Targets.CNS and Neurological Disorders, 2,* 31-39.

Tozzi, F., Thornton, L. M., Klump, K. L., Fichter, M. M., Halmi, K. A., Kaplan, A. S., Strober, M., Woodside, D. B., Crow, S., Mitchell, J., Rotondo, A., Mauri, M., Cassano, G., Keel, P., Plotnicov, K. H., Pollice, C., Lilenfeld, L. R., Berrettini, W. H., Bulik, C. M., & Kaye, W. H. (2005). Symptom fluctuation in eating disorders: correlates of diagnostic crossover. *American Journal of Psychiatry, 162,* 732-740.

Treasure, J. & Holland, A. J. (1990). Genetic vulnerability to eating disorders: evidence from twin and family studies. In H.Remschmidt & M. H. Schmidt (Eds.), *Anorexia nervosa* (pp. 59-68). Toronto: Hogrefe & Huber.

Tsai, S. J., Hong, C. J., Liou, Y. J., Yu, Y. W., Chen, T. J., Hou, S. J., & Yen, F. C. (2009). Tryptophan hydroxylase 2 gene is associated with major depression and antidepressant treatment response. *Progress in Neuropsychopharmacology & Biological Psychiatry, 33,* 637-641.

Van Hoeken, D., Lucas, A. R., & Hoek, H. W. (1998). Epidemiology. In H.W.Hoek, J. Treasure, & M. Katzman (Eds.), *The integration of neurobiology in the treatment of eating disorders* (pp. 97-126). London: Wiley.

Varnado, P. J., Williamson, D. A., & Netemeyer, R. (1995). Confirmatory factor analysis of eating disorder symptoms in college women. *Journal of Psychopathology and Behavioral Assessment., 17,* 69-79.

Verhulst, F. C., van Ende, J., & Koot, H. M. (1997). *Handleiding voor de Youth Self Report*. Rotterdam: Afdeling Kinder- en Jeugdpsychiatrie, Sophia Kinderziekenhuis / Academisch Ziekenhuis Rotterdam / Erasmus Universiteit Rotterdam.

Wade, T., Martin, N. G., & Tiggemann, M. (1998). Genetic and environmental risk factors for the weight and shape concerns characteristic of bulimia nervosa. *Psychological Medicine, 28,* 761-771.

Wade, T. D. & Bulik, C. M. (2007). Shared genetic and environmental risk factors between undue influence of body shape and weight on self-evaluation and dimensions of perfectionism. *Psychological Medicine, 37,* 635-644.

Wade, T. D., Bulik, C. M., & Kendler, K. S. (2000a). Reliability of lifetime history of bulimia nervosa. Comparison with major depression. *British Journal of Psychiatry, 177,* 72-76.

Wade, T. D., Bulik, C. M., Neale, M., & Kendler, K. S. (2000b). Anorexia nervosa and major depression: shared genetic and environmental risk factors. *American Journal of Psychiatry, 157,* 469-471.

Wade, T. D., Bulik, C. M., Prescott, C. A., & Kendler, K. S. (2004). Sex influences on shared risk factors for bulimia nervosa and other psychiatric disorders. *Archives of General Psychiatry, 61,* 251-256.

Wade, T. D., Byrne, S., & Bryant-Waugh, R. (2008a). The eating disorder examination: Norms and construct validity with young and middle adolescent girls. *International Journal of Eating Disorders, 41,* 551-558.

Wade, T. D., Treloar, S., & Martin, N. G. (2008b). Shared and unique risk factors between lifetime purging and objective binge eating: a twin study. *Psychological Medicine, 38,* 1455-1464.

Wagner, A., Barbarich-Marsteller, N. C., Frank, G. K., Bailer, U. F., Wonderlich, S. A., Crosby, R. D., Henry, S. E., Vogel, V., Plotnicov, K., McConaha, C., & Kaye, W. H. (2006). Personality traits after recovery from eating disorders: do subtypes differ? *International Journal of Eating Disorders, 39,* 276-284.

Walters, E. E. & Kendler, K. S. (1995). Anorexia nervosa and anorexic-like syndromes in a population-based female twin sample. *American Journal of Psychiatry, 152,* 64-71.

Walters, E. E., Neale, M. C., Eaves, L. J., Heath, A. C., Kessler, R. C., & Kendler, K. S. (1992). Bulimia nervosa and major depression: a study of common genetic and environmental factors. *Psychological Medicine, 22,* 617-622.

Walther, D. J. & Bader, M. (2003). A unique central tryptophan hydroxylase isoform. *Biochemical Pharmacology, 66,* 1673-1680.

Wang, K., Zhang, H., Bloss, C. S., Duvvuri, V., Kaye, W., Schork, N. J., Berrettini, W., & Hakonarson, H. (2010). A genome-wide association study on common SNPs and rare CNVs in anorexia nervosa. *Molecular Psychiatry*.

Warren, C. S., Cepeda-Benito, A., Gleaves, D. H., Moreno, S., Rodriquez, S., Fernandez, M. C., Fingeret, M. C., & Pearson, C. A. (2008). English and Spanish versions of the Body Shape Questionnaire: Measurement equivalence across ethnicity and clinical status. *International Journal of Eating Disorders, 41,* 265-272.

Weltzin, T. E., Fernstrom, M. H., & Kaye, W. H. (1994). Serotonin and bulimia nervosa. *Nutrition Reviews, 52,* 399-408.

Whiteside, S. P. & Lynam, D. R. (2001). The Five Factor Model and impulsivity:using a structural model of personality to understand impulsivity. *Personality and Individual Differences, 30,* 669-689.

Williamson, D. A., Gleaves, D. H., & Stewart, T. M. (2005). Categorical versus dimensional models of eating disorders: An examination of the evidence. *International Journal of Eating Disorders, 37,* 1-10.

Williamson, D. A., Womble, L. G., Smeets, M. A. M., Netemeyer, R. G., Thaw, J. M., Kutlesic, V., & Gleaves, D. H. (2002). Latent structure of eating disorder symptoms: A factor analytic and taxometric investigation. *American Journal of Psychiatry, 159,* 412-418.

Woodside, D. B., Bulik, C. M., Halmi, K. A., Fichter, M. M., Kaplan, A., Berrettini, W. H., Strober, M., Treasure, J., Lilenfeld, L., Klump, K., & Kaye, W. H. (2002). Personality, perfectionism, and attitudes toward eating in parents of individuals with eating disorders. *International Journal of Eating Disorders, 31,* 290-299.

Yoon, H. K. & Kim, Y. K. (2009). TPH2 -703G/T SNP may have important effect on susceptibility to suicidal behavior in major depression. *Progress in Neuropsychopharmacology & Biological Psychiatry, 33,* 403-409.

Zhang, X., Beaulieu, J. M., Gainetdinov, R. R., & Caron, M. G. (2006). Functional polymorphisms of the brain serotonin synthesizing enzyme tryptophan hydroxylase-2. *Cellular and Molecular Life Sciences, 63,* 6-11.

Zhou, Z., Roy, A., Lipsky, R., Kuchipudi, K., Zhu, G., Taubman, J., Enoch, M. A., Virkkunen, M., & Goldman, D. (2005). Haplotype-based linkage of tryptophan hydroxylase 2 to suicide attempt, major depression, and cerebrospinal fluid 5-hydroxyindoleacetic acid in 4 populations. *Archives of General Psychiatry, 62,* 1109-1118.

Ziegler, A. & Gorg, T. (1999). 5-HT2A gene promoter polymorphism and anorexia nervosa. *Lancet, 353,* 929.

Ziegler, A., Hebebrand, J., Gorg, 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. *Molecular Psychiatry, 4,* 410-412.

Zill, P., Buttner, A., Eisenmenger, W., Moller, H. J., Ackenheil, M., & Bondy, B. (2007). Analysis of tryptophan hydroxylase I and II mRNA expression in the human brain: a post-mortem study. *Journal of Psychiatric Research, 41,* 168-173.

Zill, P., Buttner, A., Eisenmenger, W., Moller, H. J., Bondy, B., & Ackenheil, M. (2004). Single nucleotide polymorphism and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene in suicide victims. *Biologic Psychiatry, 56,* 581-586.

Appendix A: DSM-IV criteria for eating disorders

Anorexia nervosa

A. Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g., weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
B. Intense fear of gaining weight or becoming fat, even though underweight.
C. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight.
D. In postmenarcheal females, amenorrhea, i.e., the absence of at least three consecutive menstrual cycles. (A woman is considered to have amenorrhea if her periods occur only following hormone, e.g., estrogen, administration.)

Specify type:

Restricting Type: during the current episode of Anorexia Nervosa, the person has not regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)
Binge-Eating/Purging Type: during the current episode of Anorexia Nervosa, the person has regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Bulimia nervosa

A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:
   (1) eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances
   (2) a sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating)

B. Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
C. The binge eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for 3 months.
D. Self-evaluation is unduly influenced by body shape and weight.
E. The disturbance does not occur exclusively during episodes of Anorexia Nervosa.

Specify type:
Purging Type: during the current episode of Bulimia Nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas
Nonpurging Type: during the current episode of Bulimia Nervosa, the person has used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas

Eating disorder not otherwise specified
Eating disorder not otherwise specified includes disorders of eating that do not meet the criteria for any specific eating disorder.

1. For female patients, all of the criteria for anorexia nervosa are met except that the patient has regular menses.
2. All of the criteria for anorexia nervosa are met except that, despite significant weight loss, the patient's current weight is in the normal range.
3. All of the criteria for bulimia nervosa are met except that the binge eating and inappropriate compensatory mechanisms occur less than twice a week or for less than 3 months.
4. The patient has normal body weight and regularly uses inappropriate compensatory behavior after eating small amounts of food (e.g., self-induced vomiting after consuming two cookies).
5. Repeatedly chewing and spitting out, but not swallowing, large amounts of food.
6. Binge-eating disorder is recurrent episodes of binge eating in the absence if regular inappropriate compensatory behavior characteristic of bulimia nervosa.

Appendix B: Overview of genetic association studies performed since 2005 with adequate statistical power.

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
Serotonin						
Serotonin	Rs652783	RAN	122	NS	(1)	UK
Receptor 1D,		BPAN	104			
HTR1D, 1p36		Controls	678			
	Rs604030	RAN	122	NS	(1)	UK
		BPAN	104			
		Controls	678			
	T-1123C	RAN	122	0.03	(1)	ANr vs ctrl OR 1.44
	(rs674386)	BPAN	104			UK
		Controls	678			
	Rs856510	RAN	122	0.02, 0.04 (geno)	(1)	ANr vs ctrl OR 1.51
		BPAN	104			UK
		Controls	678			
Serotonin	p.L459L	German AN	265	NS	(2)	Germany, Spain
Receptor 3A,	(exon 9)	German BN	91			
HTR3A, 11q23		German Controls	191			
		Spanish AN	78			
		Spanish BN	119			
		Spanish Controls	331			

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	c.-42C>T (rs1062613)	German AN German BN German Controls Spanish AN Spanish BN Spanish Controls	265 91 191 78 119 331	German sample: ANr vs ctrl 0.04 (geno, N=138)	(2)	Pooled data NS Germany, Spain
	IVS1-19G>A (rs1176722)	German AN German BN German Controls Spanish AN Spanish BN Spanish Controls	265 91 191 78 119 331	German: 0.03 (geno) ANr vs ctrl 0.006 (geno, N=138) BN vs ctrl 0.01 BNP vs ctrl 0.008 (geno, N=80)	(2)	Pooled data NS Germany, Spain
	IVS3+7A>C	German AN German BN German Controls Spanish AN Spanish BN Spanish Controls	265 91 191 78 119 331	NS	(2)	Germany, Spain
Serotonin Receptor 3B, HTR3B, 11q23	p.Y192S (rs1176744)	German AN German BN German Controls Spanish AN Spanish BN Spanish Controls	265 91 191 78 119 331	German: 0.004 (geno) ANr vs ctrl 0.007 (genotypic, N=138)	(2)	Pooled ANr vs ctrl p<0.002 Germany, Spain

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	c.-104_-102 delAGA (‘5 untransl region)	German AN	265	NS	(2)	Germany, Spain
		German BN	91			
		German Controls	191			
		Spanish AN	78			
		Spanish BN	119			
		Spanish Controls	331			
	IV6+72A>G	German AN	265	NS	(2)	Germany, Spain
		German BN	91			
		German Controls	191			
		Spanish AN	78			
		Spanish BN	119			
		Spanish Controls	331			
Catecholamine						
Beta 3 adrenergic receptor, ADRB3, 8p11-12	Trp64Arg	AN	96	NS	(3)	Japan
		BN	116			
		Controls	284			
Dopamine D2 Receptor, DRD2, 11q23	-141C/Indel (rs1799732)	AN	191	NS	(4)	TDT 0.01 USA, UK and Germany
		Parents & aff rel	457			
		Controls	98			
	T2730C (rs1800498)	AN	191	NS	(4)	USA, UK and Germany
		Parents & aff rel	457			
		Controls	98			

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	C932G (rs1801028)	AN Parents & aff rel Controls	191 457 98	NS	(4)	USA, UK and Germany
	C939T (rs6275)	AN Parents & aff rel Controls	191 457 98	NS	(4)	USA, UK and Germany
	C957T (rs6277)	AN Parents & aff rel Controls	191 457 98	NS	(4)	TDT 0.006 USA, UK and Germany
	Rs6278	AN Parents & aff rel Controls	191 457 98	ANp vs ctrl 0.04 (geno, N=88)	(4)	USA, UK and Germany
	C10620T (rs1800497)	AN Parents & aff rel Controls	191 457 98	ANp vs ctrl 0.05 (geno, N=88)	(4)	USA, UK and Germany
Dopamine D4 Receptor, DRD4, 11p15	C-521T	AN (trios) Controls (fam)	202 418	0.009	(5)	TDT Israel
	C-616G	AN (trios) Controls (fam)	202 418	NS	(5)	TDT Israel
	A-809G	AN (trios) Controls (fam)	202 418	NS	(5)	TDT Israel
	120 bp tandem repeat dupl	AN (trios) Controls (fam)	202 418	NS	(5)	TDT Israel

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	Exon III repeat	AN (trios)	202	NS	(5)	TDT
		Controls (fam)	418			Israel
Noradrenaline transporter, SLC6A2, 16q12	S4/L4 (promoter)	RAN	67	NS	(6)	Austria, UK
		BPAN	48			
		AN subtype n.a.	27 (trios)			
Neuropeptide & feeding regulation						
Cholecystokinin, CCK, 3p21	Rs6791019	AN	165	NS	(7)	Netherlands
		Controls	283			
	Rs7611677	AN	165	NS	(7)	Netherlands
		Controls	283			
	Rs6809785	AN	165	NS	(7)	Netherlands
		Controls	283			
	Rs6801844	AN	165	NS	(7)	Netherlands
		Controls	283			
	Rs11129946	AN	165	0.0001 (geno)	(7)	AC genotype OR 2.64
		Controls	283			Netherlands
Ghrelin, GHRL, 3p25-26	Gln90Leu	AN	366	NS	(8)	Austria, France,
		BN	326			Germany, Italy,
		AN and BN (trios)	529			Slovenia, Spain,
		Controls	342			and UK

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	Leu72Met	AN Controls	196 98	NS	(8)	Austria, France, Germany, Italy, Slovenia, Spain, and UK
	Arg51Gln	AN Controls	196 98	NS	(8)	Austria, France, Germany, Italy, Slovenia, Spain, and UK
	T171C (rs495225)	AN BN Controls	96 116 284	BN vs ctrl 0.04	(3)	Japan
	Rs17700633	AN Controls	267 1636	NS	(9)	Netherlands
	Rs17782313	AN Controls	267 1636	NS	(9)	Netherlands
Opioid receptor delta-1 OPRD1, 1p35	Rs569356	RAN BPAN Controls	122 104 678	0.007, 0.0003 (geno) 0.05, 0.04 (geno) AN vs ctrl 0.0008, 0.001 (geno)	(1)	AN vs ctrl OR 1.67, ANr vs ctrl OR 1.77 ANbp vs ctrl OR 1.57 UK
	Rs204047	RAN BPAN Controls	122 104 678	NS	(1)	UK

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	Rs204055	RAN	122	NS	(1)	UK
		BPAN	104			
		Controls	678			
	Rs2298896	RAN	122	NS	(1)	UK
		BPAN	104			
		Controls	678			
	Rs521809	RAN	122	0.02 (geno)	(1)	UK
		BPAN	104			
		Controls	678			
	Rs4654327	RAN	122	0.03, 0.03 (geno)	(1)	ANr vs ctrl OR 1.42
		BPAN	104			UK
		Controls	678			
Other candidate genes						
Brain Derived Neurotrophic Factor, BDNF, 11p13-14	C-270T	AN	195	NS	(10)	Netherlands
		Schizofrenia	273			
		Controls	580			
	Val-66-Met (rs6265)	AN	195	NS	(10)	Netherlands
		Schizofrenia	273			
		Controls	580			
	20 kb upstr ATG	AN	195	NS	(10)	Netherlands
		Schizofrenia	273			
		Controls	580			

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	33 kb downstr exon 2	AN Schizofrenia Controls	195 273 580	NS	(10)	Netherlands
	Rs1488830	AN Controls	267 1636	NS	(9)	Netherlands
	Rs925946	AN Controls	267 1636	NS	(9)	Netherlands
Cannabinoid receptor 2, CNR2, 1p36	R63Q	AN BN Controls	94 111 1867	ED vs ctrl 0.04	(11)	ED vs ctrl OR 1.24 Japan
Estrogen receptor 1, ESR1, 6q25	Rs488133	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs11155819	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs12199722	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs188405	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs726281	French AN (fam) German RAN (fam)	321 41	French AN 0.02 French RAN 0.005 German RAN 0.03	(12)	France and Germany
	Rs3020407	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
	Rs17081994	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs2981712	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs3020371	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs2228480	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
	Rs3798577	French AN (fam) German RAN (fam) French women (population-based)	321 41 693	French RAN 0.02 ED in population-based women 0.008	(12)	France and Germany
	Rs2295193	French AN (fam) German RAN (fam)	321 41	French AN 0.02 French RAN 0.007	(12)	France and Germany
	Rs2252837	French AN (fam) German RAN (fam)	321 41	NS	(12)	France and Germany
ETS variant gene 5, ETV5, 3q28	Rs7647305	AN Controls	267 1636	NS	(9)	Netherlands
Fat mass- and obesity associated gene, FTO, 16q12	Rs1121980	AN Controls	267 1636	NS	(9)	Netherlands

Gene	Polymorphism	Phenotype	N	*p*-value[a]	Ref	Note
Glucosamine-6-phosphate deaminase 2, GNPDA2, 4p13	Rs10938397	AN Controls	267 1636	NS	(9)	Netherlands
G-Protein coupled receptor 55, GPR55, 2q37	Gly195Val (rs3749073)	AN Controls	235 1244	0.02	(13)	OR 1.31 OR 2.41 (p<0.005) Val195 homozygotes Japan
Potassium channel tetramerisation domain, KCTD15, 19q13	Rs368794	AN Controls	267 1636	NS	(9)	Netherlands
Mitochondrial carrier homolog 2, MTCH2, 11q12	Rs10838738	AN Controls	267 1636	NS	(9)	Netherlands
Neurol growth regulator 1, NEGR1, 1p31	Rs2568958	AN Controls	267 1636	NS	(9)	Netherlands
SH2B adaptor protein 1, SH2B1, 16p11	Rs7498665	AN Controls	267 1636	NS	(9)	Netherlands
Transmembrane protein 18, TMEM18, 2p25	Rs6548238	AN Controls	267 1636	NS	(9)	Netherlands

AN=Anorexia Nervosa, BN=Bulimia Nervosa, RAN= Restrictive Anorexia Nervos, BPAN= Binge-purge Anorexia Nervosa

Appendix B: Reference list large genetic association studies since 2005

1. Brown, K. M., Bujac, S. R. et al. (2006). Further Evidence of Association of OPRD1 & HTR1D Polymorphisms with Susceptibility to Anorexia Nervosa. *Biologic Psychiatry, 61,* 367-373.
2. Hammer, C., Kapeller, J. et al. (2009). Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders. *Pharmacogenet Genomics, 19,* 790-799.
3. Miyasaka, K., Hosoya, H. et al. (2005). Association of ghrelin receptor gene polymorphism with bulimia nervosa in a Japanese population. *Journal of Neural Transmission, 113,* 1279-1285.
4. Bergen, A. W., Yeager, M. et al. (2005). Association of Multiple DRD2 Polymorphisms with Anorexia Nervosa. *Neuropsychopharmacology, 30,* 1703-1710.
5. Bachner-Melman, R., Lerer, E. et al. (2007). Anorexia nervosa, perfectionism, and dopamine D4 receptor (DRD4). *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 144B,* 748-756.
6. Hu, X., Karwautz, A. et al. (2007). No association between a promoter polymorphism in the noradrenaline transporter gene and anorexia nervosa. *Psychiatric Genetics, 17,* 247-248.
7. de Krom, M., Hendriks, J. et al. (2006). A polymorphism in the 3' untranslated region of the CCK gene is associated with anorexia nervosa in Dutch patients. *Psychiatric Genetics, 16,* 239.
8. Cellini, E., Nacmias, B. et al. (2006). Case-control and combined family trios analysis of three polymorphisms in the ghrelin gene in European patients with anorexia and bulimia nervosa. *Psychiatric Genetics, 16,* 51-52.
9. Brandys, M. K., van Elburg, A. A. et al. (2010). Are recently identified genetic variants regulating BMI in the general population associated with anorexia nervosa? *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 153B,* 695-699.
10. de Krom M., Bakker, S. C. et al. (2005). Polymorphisms in the brain-derived neurotrophic factor gene are not associated with either anorexia nervosa or schizophrenia in Dutch patients. *Psychiatric Genetics, 15,* 81.
11. Ishiguro, H., Carpio, O. et al. (2010). A nonsynonymous polymorphism in cannabinoid CB2 receptor gene is associated with eating disorders in humans and food intake is modified in mice by its ligands. *Synapse, 64,* 92-96.

12. Versini, A., Ramoz, N. et al. (2010). Estrogen receptor 1 gene (ESR1) is associated with restrictive anorexia nervosa. *Neuropsychopharmacology, 35,* 1818-1825.
13. Ishiguro, H., Onaivi, E. S. et al. (2011). Functional polymorphism in the GPR55 gene is associated with Anorexia Nervosa. *Synapse, 65*, 103-108.

Appendix C: References of small genetic association studies in eating disorders from 2005 to present

Arias, F., Ampuero, I., Sagredo, O., Almodovar, F., Sanchez, S., Gorgojo, J. J. et al. (2009). Lack of association between polymorphisms in cannabinoid receptor gene (CNR1) and fatty acid amide hydroxylase gene (FAAH) and eating disorders in a preliminary study. *Psychiatric Genetics, 19,* 336.

Dardennes, R. M., Zizzari, P., Tolle, V., Foulon, C., Kipman, A., Romo, L. et al. (2006). 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.

Dmitrzak-Weglarz, M., Skibinska, M., Slopien, A., Szczepankiewicz, A., Rybakowski, F., Kramer, L. et al. (2007). BDNF Met66 allele is associated with anorexia nervosa in the Polish population. *Psychiatric Genetics, 17,* 245-246.

Dmitrzak-Weglarz, M., Slopien, A., Rybakowski, F., Czerski, P. M., Hauser, J., & Rajewski, A. (2005). An association study of the dopamine transporter (DAT) gene and catechol-O-methyltransferase (COMT) gene in anorexia nervosa in the Polish population. *Archives of Psychiatry and Psychotherapy, 7,* 5-12.

Kanbur, N., Mesci, L., Derman, O., Turul, T., Cuhadaroglu, F., Kutluk, T. et al. (2008). Tumor necrosis factor alpha-308 gene polymorphism in patients with anorexia nervosa. *Turkish Journal of Pediatrics., 50,* 219-222.

Kim, Y. R., Woo, J. M., Heo, S. Y., Kim, J. H., Lim, S. J., & Yu, B. H. (2009). An Association Study of the A218C Polymorphism of the Tryptophan Hydroxylase 1 Gene with Eating Disorders in a Korean Population: A Pilot Study. *Psychiatry Investigation, 6,* 44-49.

Koronyo-Hamaoui, M., Frisch, A., Stein, D., Denziger, Y., Leor, S., Michaelovsky, E. et al. (2007). Dual contribution of NR2B subunit of NMDA receptor and SK3 Ca(2+)-activated K(+) channel to genetic predisposition to anorexia nervosa. *Journal of Psychiatric Research, 41,* 160-167.

Martaskova, D., Slachtova, L., Kemlink, D., Zahorakova, D., & Papezova, H. (2009). Polymorphisms in serotonin-related genes in anorexia nervosa. The first study in Czech population and metaanalyses with previously performed studies. *Folia Biologica (Praha), 55,* 192-197.

Mercader, J. M., Ribases, M., Gratacos, M., Gonzalez, J. R., Bayes, M., de Cid, R.

et al. (2007). Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. *Genes, Brain and Behavior, 6,* 706-716.

Michaelovsky, E., Frisch, A., Leor, S., Stein, D., Danziger, Y., Carel, C. et al. (2005). Haplotype analysis of the COMT-ARVCF gene region in Israeli anorexia nervosa family trios. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 139,* 45-50.

Mikolajczyk, E., Smiarowska, M., Grzywacz, A., & Samochowiec, J. (2006). Association of eating disorders with catechol-o-methyltransferase gene functional polymorphism. *Neuropsychobiology., 54,* 82-86.

Monteleone, P., Bifulco, M., Di, F. C., Gazzerro, P., Canestrelli, B., Monteleone, F. et al. (2009). Association of CNR1 and FAAH endocannabinoid gene polymorphisms with anorexia nervosa and bulimia nervosa: evidence for synergistic effects. *Genes, Brain and Behavior, 8,* 728-732.

Monteleone, P., Tortorella, A., Castaldo, E., Di Filippo, C., & Maj, M. (2006). No association of the Arg51Gln and Leu72Met polymorphisms of the ghrelin gene with anorexia nervosa or bulimia nervosa. *Neuroscience Letters, 398,* 325-327.

Monteleone, P., Tortorella, A., Castaldo, E., & Maj, M. (2006). Association of a functional serotonin transporter gene polymorphism with binge eating disorder. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics, 141B,* 7-9.

Muller, T. D., Reichwald, K., Bronner, G., Kirschner, J., Nguyen, T. T., Scherag, A. et al. (2008). Lack of association of genetic variants in genes of the endocannabinoid system with anorexia nervosa. *Child and Adolescent Psychiatry and Mental Health, 2,* 33.

Rybakowski, F., Dmitrzak-Weglarz, M., Szczepankiewicz, A., Skibinska, M., Slopien, A., Rajewski, A. et al. (2007). Brain derived neurotrophic factor gene Val66Met and -270C/T polymorphisms and personality traits predisposing to anorexia nervosa. *Neuro Endocrinology Letters, 28,* 153-158.

Urwin, R. E. & Nunn, K. P. (2005). Epistatic interaction between the monoamine oxidase A and serotonin transporter genes in anorexia nervosa. *European Journal of Human Genetics, 13,* 370-375.

Appendix D: References of small genetic association studies in eating disorders until 2005

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. *Psychiatric Genetics, 11,* 161-164.

Ando, T., Kodama, N., Ishikawa, T., Naruo, T., Tachikawa, N., Nozaki, T., Okabe, K., Takeuchi, K., Masuda, A., Kawamura, N., & Komaki, G. (2004). Uncoupling protein-2/uncoupling protein-3 gene polymorphism is not associated with anorexia nervosa. *Psychiatric Genetics, 14,* 215-218.

Ando, T., Komaki, G., Karibe, M., Kawamura, N., Hara, S., Takii, M., Naruo, T., Kurokawa, N., Takei, M., Tatsuta, N., Ohba, M., Nozoe, S., Kubo, C., & Ishikawa, T. (2001). 5-HT2A promoter polymorphism is not associated with anorexia nervosa in Japanese patients. *Psychiatric Genetics, 11,* 157-160.

Biederman, J., Rivinus, T. M., Herzog, D. B., Harmatz, J. S., Shanley, K., & Yunis, E. J. (1984). High frequency of HLA-Bw16 in patients with anorexia nervosa. *American Journal of Psychiatry, 141,* 1109-1110.

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. *Biologic Psychiatry, 43,* 76-78.

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. *Psychiatric Genetics, 9,* 101-104.

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. *Molecular Psychiatry, 4,* 68-70.

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.

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.

Di Bella, D., Cavallini, M. C., Riboldi, C., Riboldi, S., & Bellodi, L. (1998). Genetic studies in eating disorders. *American Journal of Medical Genetics, 81,* 523-523.

Di Bella, D. D., Catalano, M., Cavallini, M. C., Riboldi, C., & Bellodi, L. (2000). Serotonin transporter linked polymorphic region in anorexia nervosa and bulimia nervosa. *Molecular Psychiatry, 5,* 233-234.

Eastwood, H., Brown, K. M., Markovic, D., & Pieri, L. F. (2002). Variation in the ESR1 and ESR2 genes and genetic susceptibility to anorexia nervosa. *Molecular Psychiatry, 7,* 86-89.

Enoch, M. A., Kaye, W. H., Rotondo, A., Greenberg, B. D., Murphy, D. L., & Goldman, D. (1998). 5-HT2A promoter polymorphism -1438G/A, anorexia nervosa, and obsessive-compulsive disorder. *Lancet, 351,* 1785-1786.

Friedel, S., Fontenla, H. F., Wermter, A. K., Geller, F., Dempfle, A., Reichwald, K., Smidt, J., Bronner, G., Konrad, K., Herpertz-Dahlmann, B., Warnke, A., Hemminger, U., Linder, M., Kiefl, H., Goldschmidt, H. P., 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. *American Journal of Medical Genetics, 132,* 96-99.

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. *Molecular Psychiatry, 6,* 243-245.

Fuentes, J. A., Lauzurica, N., Hurtado, A., Escarti, A., Barrios, V., Morande, G., Soriano, J., Jauregui, I., Gonzalez-Valdemoro, M. I., & Garcia-Camba, E. (2004). Analysis of the -1438 G/A polymorphism of the 5-HT2A serotonin receptor gene in bulimia nervosa patients with or without a history of anorexia nervosa. *Psychiatric Genetics, 14,* 107-109.

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. *Molecular Psychiatry, 6,* 9-10.

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. *Biologic Psychiatry, 45,* 615-619.

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. (1997). Serotonin transporter gene-linked polymorphic region: allele distributions in relationship to body weight and in anorexia nervosa. *Life Sciences, 61*, L-303.

Hinney, A., Becker, I., Heibult, O., Nottebom, K., Schmidt, A., Ziegler, A., Mayer, H., Siegfried, W., Blum, W. F., 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. *The Journal of Clinical Endocrinology and Metabolism, 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. (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. *Molecular 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. (1999). No evidence for an involvement of alleles of polymorphisms in the serotonin1Dbeta and 7 receptor genes in obesity, underweight or anorexia nervosa. *International Journal of Obesity Related Metabolic Disorders, 23*, 760-763.

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. (1997). Beta 3-adrenergic-receptor allele distributions in children, adolescents and young adults with obesity, underweight or anorexia nervosa. *International Journal of Obesity Related Metabolic Disorders, 21*, 224-230.

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. (1999). Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. *The Journal of Clinical Endocrinology and Metabolism, 84*, 1483-1486.

Hinney, A., Schneider, J., Ziegler, A., Lehmkuhl, G., Poustka, F., Schmidt, M. H., Mayer, H., Siegfried, W., Remschmidt, H., & Hebebrand, J. (1999). No evidence for involvement of polymorphisms of the dopamine D4 receptor gene in anorexia nervosa, underweight, and obesity. *American Journal of Medical Genetics, 88,* 594-597.

Hinney, A., Ziegler, A., Nothen, M. M., Remschmidt, H., & Hebebrand, J. (1997). 5-HT2A receptor gene polymorphisms, anorexia nervosa, and obesity. *Lancet, 350,* 1324-1325.

Hu, X., Giotakis, O., Li, T., Karwautz, A., Treasure, J., & Collier, D. A. (2003). Association of the 5-HT2c gene with susceptibility and minimum body mass index in anorexia nervosa. *Neuroreport, 14,* 781-783.

Hu, X., Murphy, F., Karwautz, A., Li, T., Freeman, B., Franklin, D., Giotakis, O., Treasure, J., & Collier, D. A. (2002). Analysis of microsatellite markers at the UCP2/UCP3 locus on chromosome 11q13 in anorexia nervosa. *Molecular Psychiatry, 7,* 276-277.

Karwautz, A., Rabe-Hesketh, S., Hu, X., Zhao, J., Sham, P., Collier, D. A., & Treasure, J. L. (2001). Individual-specific risk factors for anorexia nervosa: a pilot study using a discordant sister-pair design. *Psychological Medicine, 31,* 317-329.

Kipman, A., Bruins-Slot, L., Boni, C., Hanoun, N., Ades, J., Blot, P., Hamon, M., Mouren-Simeoni, M., & Gorwood, P. (2002). 5-HT(2A) gene promoter polymorphism as a modifying rather than a vulnerability factor in anorexia nervosa. *European Psychiatry, 17,* 227-229.

Kiss, A., Hajek-Rosenmayr, A., Haubenstock, A., Wiesnagrotzki, S., & Moser, G. (1988). Lack of association between HLA antigens and anorexia nervosa. *American Journal of Psychiatry, 145,* 876-877.

Kiss, A., Hajek-Rosenmayr, A., Wiesnagrotzki, S., Abatzi, T. A., Sidl, R., Moser, G., & Haubenstock, A. (1989). Lack of association between HLA antigens and bulimia. *Biologic Psychiatry, 25,* 803-806.

Koizumi, H., Hashimoto, K., Itoh, K., Nakazato, M., Shimizu, E., Ohgake, S., Koike, K., Okamura, N., Matsushita, S., Suzuki, K., Murayama, M., Higuchi, S., & Iyo, M. (2004). Association between the brain-derived neurotrophic factor 196G/A polymorphism and eating disorders. *American Journal of Medical Genetics, 127B,* 125-127.

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. *Molecular Psychiatry, 7,* 82-85.

Koronyo-Hamaoui, M., Gak, E., Stein, D., Frisch, A., Danziger, Y., Leor, S., Michaelovsky, E., Laufer, N., Carel, C., Fennig, S., Mimouni, M., Apter, A., Goldman, B., Barkai, G., & Weizman, A. (2004). CAG repeat polymorphism within the KCNN3 gene is a significant contributor to susceptibility to anorexia nervosa: A case-control study of female patients and several ethnic groups in the Israeli Jewish population. *American Journal of Medical Genetics, 131B,* 76-80.

Lauzurica, N., Hurtado, A., Escarti, A., Delgado, M., Barrios, V., Morande, G., Soriano, J., Jauregui, I., Gonzalez-Valdemoro, M. I., Garcia-Camba, E., & Fuentes, J. A. (2003). Polymorphisms within the promoter and the intron 2 of the serotonin transporter gene in a population of bulimic patients. *Neuroscience Letters, 352,* 226-230.

Matsushita, S., Suzuki, K., Murayama, M., Nishiguchi, N., Hishimoto, A., Takeda, A., Shirakawa, O., & Higuchi, S. (2004). Serotonin transporter regulatory region polymorphism is associated with anorexia nervosa. *American Journal of Medical Genetics, 128B,* 114-117.

Nacmias, B., Ricca, V., Tedde, A., Mezzani, B., Rotella, C. M., & Sorbi, S. (1999). 5-HT2A receptor gene polymorphisms in anorexia nervosa and bulimia nervosa. *Neuroscience Letters, 277,* 134-136.

Nilsson, M., Naessen, S., Dahlman, I., Linden, H. A., Gustafsson, J. A., & Dahlman-Wright, K. (2004). Association of estrogen receptor beta gene polymorphisms with bulimic disease in women. *Molecular Psychiatry, 9,* 28-34.

Nishiguchi, N., 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. *Biologic Psychiatry, 50,* 123-128.

Quinton, N. D., Meechan, D. W., Brown, K., Eastwood, H., & Blakemore, A. I. (2004). Single nucleotide polymorphisms in the leptin receptor gene: studies in anorexia nervosa. *Psychiatric Genetics, 14,* 191-194.

Ribases, M., Gratacos, M., Armengol, L., de Cid, R., Badia, A., Jimenez, L., Solano, R., Vallejo, J., Fernandez, F., & Estivill, X. (2003). Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Molecular Psychiatry, 8,* 745-751.

Ribases, M., Gratacos, M., Badia, A., Jimenez, L., Solano, R., Vallejo, J., Fernandez-Aranda, F., & Estivill, X. (in press). Contribution of NTRK2 to the genetic susceptibility to anorexia nervosa, harm avoidance and minimum body mass index. *Molecular Psychiatry.*

Ricca, V., Nacmias, B., Boldrini, M., Cellini, E., Di Bernardo, M., Ravaldi, C., Tedde, A., Bagnoli, S., Placidi, G. F., Rotella, C. M., & Sorbi, S. (2004). Psychopathological traits and 5-HT(2A) receptor promoter polymorphism (-1438 G/A) in patients suffering from Anorexia Nervosa and Bulimia Nervosa. *Neuroscience Letters, 365,* 92-96.

Ricca, V., Nacmias, B., Cellini, E., Di Bernardo, M., Rotella, C. M., & Sorbi, S. (2002). 5-HT2A receptor gene polymorphism and eating disorders. *Neuroscience Letters, 323,* 105-108.

Rosenkranz, K., Hinney, A., Ziegler, A., Hermann, H., Fichter, M., Mayer, H., Siegfried, W., Young, J. K., Remschmidt, H., & Hebebrand, J. (1998). Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: Identification of several genetic variants. *The Journal of Clinical Endocrinology and Metabolism, 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. (1998). Screening for mutations in the neuropeptide Y Y5 receptor gene in cohorts belonging to different weight extremes. *International Journal of Obesity Related Metabolic Disorders, 22,* 157-163.

Rybakowski, F., Slopien, A., Dmitrzak-Weglarz, M., Czerski, P., Hauser, J., & Rajewski, A. (2004). Association study of 5-HT2A receptor gene polymorphism in anorexia nervosa in Polish population. *Archives of Psychiatry & Psychotherapy, 6,* 53-59.

Shinohara, M., Mizushima, H., Hirano, M., Shioe, K., Nakazawa, M., Hiejima, Y., Ono, Y., & Kanba, S. (2004). Eating disorders with binge-eating behaviour are associated with the s allele of the 3'-UTR VNTR polymorphism of the dopamine transporter gene. *Journal of Psychiatry & Neuroscience, 29,* 134-137.

Siegfried, Z., Kanyas, K., Latzer, Y., Karni, O., Bloch, M., Lerer, B., & Berry, E. M. (2004). Association study of cannabinoid receptor gene (CNR1) alleles and anorexia nervosa: differences between restricting and binging/purging subtypes. *American Journal of Medical Genetics, 125B,* 126-130.

Sorbi, S., Nacmias, B., Tedde, A., Ricca, V., Mezzani, B., & Rotella, C. M. (1998). 5-HT2A promoter polymorphism in anorexia nervosa. *Lancet, 351,* 1785.

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. *American Journal of Medical Genetics, 96,* 53-55.

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. *Molecular 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.

Urwin, R. E., Bennetts, B. H., Wilcken, B., Lampropoulos, B., Beumont, P. J., Russell, J. D., Tanner, S. L., & Nunn, K. P. (2003). Gene-gene interaction between the monoamine oxidase A gene and solute carrier family 6 (neurotransmitter transporter, noradrenalin) member 2 gene in anorexia nervosa (restrictive subtype). *European Journal of Human Genetics, 11*, 945-950.

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. (2001). Association between an agouti-related protein gene polymorphism and anorexia nervosa. *Molecular Psychiatry, 6*, 325-328.

Ziegler, A., Hebebrand, J., Gorg, 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. *Molecular Psychiatry, 4*, 410-412.

Publications

1. Slof, R., Mazzeo, S., and Bulik, C. M. (2003). Characteristics of women with persistent thinness. *Obesity Research, 11*, 971-977.
2. Bulik, C. M., Slof, M. C. T., and Sullivan, P. F. (2004). Comorbidity of eating disorders and substance-related disorders. In: Kranzler, H. R. and Tinsley, J. A., eds. *Dual diagnosis and psychiatric treatment: Substance abuse and comorbid disorders* (pp. 317-348). Marcel Dekker, New York.
3. Mazzeo, S. E., Slof, R. M., Tozzi, F., Kendler, K. S., and Bulik, C. M. (2004). Characteristics of men with persistent thinness. *Obesity Research, 12*, 1367-1369.
4. Slof-Op 't Landt, M. C., van Furth, E. F., Meulenbelt, I., Slagboom, P. E., Bartels, M., Boomsma, D. I., and Bulik, C. M. (2005). Eating disorders: from twin studies to candidate genes and beyond. *Twin Research and Human Genetics, 8*, 467-482.
5. Mazzeo, S. E., Slof-Op 't Landt, M. C. T., van Furth, E. F., and Bulik, C. M. (2006). Genetics of eating disorders. In: Wonderlich, S., Mitchell, J. E., de Zwaan, M., and Steiger, H., eds. *Annual Review of Eating Disorders Part 2* (pp. 17-34). Radcliffe publishing, Oxford.
6. Mazzeo, S. E., Slof-Op't Landt, M. C., Jones, I., Mitchell, K., Kendler, K. S., Neale, M. C., Aggen, S. H., and Bulik, C. M. (2006). Associations among postpartum depression, eating disorders, and perfectionism in a population-based sample of adult women. *International Journal of Eating Disorders, 39*, 202-211.
7. Bulik, C. M., Slof-Op 't Landt, M. C. T., van Furth, E. F., and Sullivan, P. F. (2007). The genetics of anorexia nervosa. *Annual Review of Nutrition, 27*, 263-275.
8. Slof-Op 't Landt, M. C. T., Bartels, M., van Furth, E. F., Van Beijsterveldt, C. E., Meulenbelt, I., Slagboom, P. E., and Boomsma, D. I. (2008). Genetic influences on disordered eating behaviour are largely independent of body mass index. *Acta Psychiatrica Scandinavica, 117*, 348-356.
9. Slof-Op 't Landt, M. C. T., Dolan, C. V., Rebollo-Mesa, I., Bartels, M., van Furth, E. F., Van Beijsterveldt, C. E., Meulenbelt, I., Slagboom, P. E., and Boomsma, D. I. (2009). Sex differences in sum scores may be hard to interpret: the importance of measurement invariance. *Assessment, 16*, 415-423.
10. Middeldorp, C. M., Slof-Op 't Landt, M. C. T., Medland, S. E., Van Beijsterveldt, C. E. M., Bartels, M., Willemsen, G., Hottenga, J. J., de Geus, E. J. C., Suchiman, H. E. D., Neale, M. C., Slagboom, P. E., and Boomsma, D. I. (2010).

Anxiety and depression in children and adults:influence of serotonergic and neurotrophic genes? *Genes, Brain and Behavior, 9*, 808-816.

11. Van Beijsterveldt, C. E., Middeldorp, C. M., Slof-Op't Landt, M. C., Bartels, M., Hottenga, J. J., Suchiman, H. E., Slagboom, P. E., and Boomsma, D. I. (2011). Influence of candidate genes on attention problems in children: a longitudinal study. *Behavior Genetics, 41*, 155-164.
12. Slof-Op 't Landt, M. C., Meulenbelt, I., Bartels, M., Suchiman, E., Middeldorp, C. M.,Houwing-Duistermaat, J. J., van Trier J., Onkenhout, E. J., Vink, J. M., Van Beijsterveldt, C. E., Brandys, M. K., Sanders, N., Zipfel, S., Herzog, W., Herpertz-Dahlmann, B., Klampfl, K., Fleischhaker, C., Zeeck, A., de, Z. M., Herpertz, S., Ehrlich, S., van Elburg, A. A., Adan, R. A., Scherag, S., Hinney, A., Hebebrand, J., Boomsma, D. I., van Furth, E. F., and Slagboom, P. E. (2011). Association study in eating disorders: TPH2 associates with anorexia nervosa and self-induced vomiting. *Genes, Brain and Behavior, 10*, 236-243.

Curriculum vitae

Margarita Cornelia Theodora Slof (-Op ‘t Landt) was born August 20th, 1980 in Ter Aar, the Netherlands. She attended secondary school in Alphen aan den Rijn at the Groene Hart Lyceum, where she passed her exams (atheneum) in 1998. In that same year she started her study Nutrition and Health at Wageningen University and Research centre (Netherlands). During this study she did an internship at the Virginia Institute for Psychiatric and Behavioral Genetics of the Virginia Commonwealth University (United States of America). She received her degree in September 2002. January 2003 she started working as a researcher at the Center for Eating Disorders Ursula on a project on the genetics of eating disorders. This was a PhD project in collaboration with the department of Molecular Epidemiology of the Leiden University Medical Center and the department of Biological Psychology of the VU University Amsterdam. She was supervised by Prof. dr. P.E. Slagboom and Dr. I. Meulenbelt at the department of Molecular Epidemiology (Leiden University Medical Center), by Prof. dr. D.I. Boomsma and Dr. M. Bartels at the department of Biological Psychology (VU University) and by Dr. E.F. van Furth at the Center for Eating Disorders Ursula. The results of the research performed during the project are described and discussed in this thesis. Rita will continue to work at the Center for Eating Disorders Ursula in Leidschendam.