Genetic determinants of eating disorders

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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.