Genetic determinants of eating disorders

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Chapter 2

Eating disorders: from twin studies to candidate genes and beyond

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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).

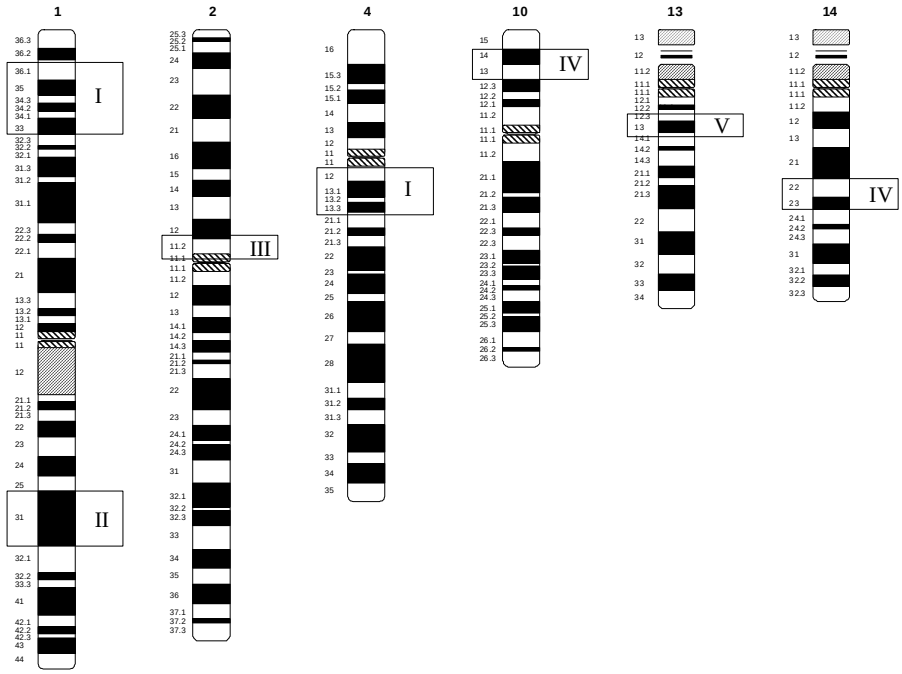

Figure 2.1. Linkage results of the genome-wide scans in eating disorders, with LOD-scores ≥ 1.97.

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.