Individual-specific risk factors for anorexia nervosa: a pilot study using a discordant sister-pair design

A. KARWAUTZ,[1] S. RABE-HESKETH, X. HU, J. ZHAO, P. SHAM, D. A. COLLIER AND J. L. TREASURE

From the Eating Disorder Unit, Department of Biostatistics and Computing, Department of Psychological Medicine, and the Department of Psychological Medicine and Neuropathology, Molecular Genetics Section, Institute of Psychiatry, Maudsley and Bethlem Trust Hospital, University of London

ABSTRACT

Background. The aim of this pilot study was to examine which unique factors (genetic and environmental) increase the risk for developing anorexia nervosa by using a case–control design of discordant sister pairs.

Methods. Forty-five sister-pairs, one of whom had anorexia nervosa and the other did not, were recruited. Both sisters completed the Oxford Risk Factor Interview for Eating Disorders and measures for eating disorder traits, and sib-pair differences. Blood or cheek cell samples were taken for genetic analysis. Statistical power of the genetic analysis of discordant same-sex siblings was calculated using a specially written program, DISCORD.

Results. The sisters with anorexia nervosa differed from their healthy sisters in terms of personal vulnerability traits and exposure to high parental expectations and sexual abuse. Factors within the dieting risk domain did not differ. However, there was evidence of poor feeding in childhood. No difference in the distribution of genotypes or alleles of the DRD4, COMT, the 5HT2A and 5HT2C receptor genes was detected. These results are preliminary because our calculations indicate that there is insufficient power to detect the expected effect on risk with this sample size.

Conclusions. A combination of intrinsic and extrinsic factors increases the risk of developing anorexia nervosa. It would, therefore, be informative to undertake a larger study to examine in more detail the unique genetic and environmental factors that are associated with various forms of eating disorders.

INTRODUCTION

Anorexia nervosa (AN) is a developmental disorder with an onset during the peripubertal period. The link between psychosocial stress and AN was recognized in the earliest case descriptions (Morton, 1694). With time, a broad group of environmental factors have been implicated, ranging from feminist to family issues. Most of the models that have been developed for anorexia nervosa are multifactorial (Garfinkel & Garner, 1982; Gillberg & Rastam, 1998) and include some specific aspects,

and more general risk factors shared with bulimia nervosa and other forms of psychopathology such as depression. Steiner and colleagues (1995) suggest that there may be a gradual accumulation of risk factors, which only manifest themselves during the stress of adolescence.

These risk factors appear to be both extrinsic (such as adverse experiences) and intrinsic (such as genetic vulnerability). They have been proposed to act either as fundamental aetiological risk factors (predisposing), as triggers for the illness (precipitating) or to prolong and exacerbate the illness (maintaining). However, it is unlikely that each risk factor can be simply classified in this way, as, for example, genes

[1] Address for correspondence: Dr Andreas Karwautz, Eating Disorders Unit, University Clinic of Neuropsychiatry of Childhood and Adolescence, Waehringer Guertel 18-20, A-1090 Vienna, Austria.

would be expected to act as both predisposing and maintaining factors, and some psychosocial factors may act in all three areas. Severe life events or difficulties trigger the onset of AN and may be true precipitating factors (Rastam & Gillberg, 1991, 1992; Schmidt *et al.* 1997).

An alternative way to structure these risk factors is to divide them into genes, environment and their interactions ($G \times E$) and correlations (G–E) (Plomin *et al.* 1997). In this model, the action of psychosocial risk factors on a genetically vulnerable individual is a key to the aetiology of the illness. However, a major problem in much of the research on aetiology is that it may not be possible to distinguish consequences rather than causes of the illness. Furthermore, many risk factors for eating disorders, such as personality, are themselves a consequence of a complex gene–environment interaction. Prospective studies, using a design sensitive to both genetic and environmental factors will be the only way to clarify these issues.

There is a general agreement that the results of twin and family studies suggest a genetic vulnerability to AN (Hebebrand & Remschmidt, 1995; Treasure & Holland, 1995; Walters & Kendler, 1995; Gorwood *et al.* 1998; Lilenfeld & Kaye, 1998; Wade *et al.* 1999; Bulik *et al.* 2000; Strober *et al.* 2000). Model-fitting of data from twin studies of eating disorders suggests that non-shared factors (i.e. individual specific factors, such as differential experiences and genes) are of greater importance than shared factors, in particular in AN (Treasure & Holland, 1995; Faith *et al.* 1997; Wade *et al.* 1998; Kendler *et al.* 1991; Hewitt, 1997; Bulik *et al.* 2000; Wade *et al.* 2000). However, these conclusions are uncertain as most twin studies to date have lacked statistical power and methodological rigour (Fairburn *et al.* 1999*a*; Bulik *et al.* 2000).

Several studies examining genetic risk factors in eating disorders have found that the 'A' allele and the 'AA' genotype of a polymorphism in the promoter region of the 5HT2A gene (-1438G/A) is more commonly found in the anorexia nervosa population than in the comparison group, particularly when considered to be recessive (Collier *et al.* 1997; Enoch *et al.* 1998; Sorbi *et al.* 1998). A meta-analysis found a significant odds ratio of 2·3 ($P = 5 \times 10^{-7}$,

Collier *et al.* 1999). However, the addition of two sets of new data from one group of investigators (Ziegler *et al.* 1999), both of which failed to show association, strongly reduced the significance of this finding.

Several developmental and early environment factors appear to increase the risk of developing an eating disorder (Connors, 1996). Cnattinigius *et al.* (1999) found the risk of pre-term birth was higher. Faddy eating in childhood has been linked to partial cases of anorexia nervosa (Marchi & Cohen, 1990). Dieting risk factors during development (such as parental and personal obesity, dieting in family members and adverse comments about weight/shape or eating) were not seen to be antecedents for anorexia nervosa (Fairburn *et al.* 1999*b*) in contrast to their established role in bulimia nervosa (Fairburn *et al.* 1997).

None of the risk factors that have been implicated so far appear to be necessary or sufficient to account for the development of anorexia nervosa and a multifactorial threshold model may best explain the data.

The aim of the present study is to establish whether differences in the environments before onset and/or genetic endowment could account for anorexia nervosa by investigating sisters who are discordant for AN. The issue of common (shared) and individual-specific (non-shared) environments has been relatively neglected in previous work in this area, which has generally involved interfamilial case–control studies.

The use of discordant siblings for genetic analysis is a potentially powerful method for locating genetic loci, particularly quantitative traits (Eaves & Meyer, 1994). For association analyses, a particular advantage is the avoidance of spurious associations because of poor case–control matching. Unlike the concordant sibling design, discordant siblings also retain the ability to look at non-shared environmental risk factors. The power to detect gene–environment effects is further increased by multivariate analysis (Schmitz *et al.* 1998). Extreme discordant sibpairs have considerable power across most plausible genetic models, an increase in power when there is residual correlation among sibs (as likely for multifactorial traits) and when the frequency of the risk allele is high (Risch & Zhang, 1995, 1996; Allison & Faith, 1997*a*). The present study should be regarded as ex-

ploratory, with the aim of establishing the feasibility of further research into the aetiology of eating disorders using discordant siblings, twins and 'high risk' groups.

METHOD

Design

A case–control design was used to compare 45 sister pairs, discordant for AN. The phenotype was described with a clinical interview and questionnaires administered to both sisters. The Oxford Risk Factor Interview for Eating Disorders was used to measure predisposing factors. DNA samples from both sisters were genotyped for a series of candidate genes.

Recruitment

In order to be included, the proband had to have a period of AN (according to DSM-IV, based on the information derived from the LIFE-interview) and a sister with no history of any form of eating disorder (AN, BN or Eating Disorder Not Otherwise Specified – EDNOS). The unaffected sisters were screened for eating disorder features using the LIFE interview and the EDI-2. The sister-pairs had to be less than 10 years apart in age and to have lived in the same family for a minimum of 8 years. If the patient had more than one sister, the sister closest in age was approached.

Research volunteers and current patients with sisters were screened. The research volunteer data base consists of over 500 subjects with a current or past eating disorder recruited for generic 'eating disorder research'. All subjects were sent a questionnaire to obtain an outline of their eating disorder history and pertinent family characteristics. Three sister pairs were excluded because on the initial screening their sister met the criteria for an eating disorder. Of the 50 pairs identified, two declined to ask their sister to participate. Of those who were interviewed, two pairs were excluded because the 'unaffected' sister had a covert eating disorder. One pair was excluded because it became apparent during the interview that they were not full biological siblings. Of the 45 sister pairs included in this study, 28 were from the research volunteer data base and 17 were current patients. The protocol for the study was approved by the Research Ethics Committee of the Bethlem and Maudsley

Trust. Patients and their sisters gave written informed consent for all procedures before inclusion in the study.

Assessment measures

Longitudinal Interval Follow-up Evaluation (LIFE) (Keller *et al.* 1987; LIFE II-BEI, Krämer, 1996)

A European adaptation of the LIFE, i.e. LIFE II-BEI was used to measure lifetime eating disorder history. This involved constructing anchor points and time lines for the development of eating disorder symptoms. The patients fulfilled the criteria of AN according to DSM-IV, their sisters did not fulfil criteria for any of the eating disorders at any time in their history.

Oxford Risk Factor Interview for Eating Disorders (ORFI) (Fairburn *et al.* 1997, 1998)

This is a semi-structured investigator-based interview designed to examine the specific risk factors associated with an eating disorder. The interview starts with establishing a time line with index age as the end point for the proband and sister to ensure that the variable of interest preceded the outcome (Kazdin *et al.* 1997; Kraemer *et al.* 1997). An inter-rater reliability study obtained a high level of agreement across the risk factors (main weighted kappa = 0·66, S.D. = 0·17; Fairburn *et al.* 1997). The areas covered by the ORFI and their combination within subdomains and domains can be found in Table 1. Five variables (parental eating disorders, obesity, parental depression, alcohol abuse and substance dependence) were – according to Fairburn *et al.* 1997 – assessed before and after the onset of the eating disorder because these factors seem to be highly heritable. In order to be comparable to the data of Fairburn *et al.* (1997, 1998, 1999*b*), we used all those variables, they had reported on in their studies on AN, BN, and BED. A few of them (see † in Table 1), such as 'parental obesity' or 'parental eating disorder history' were judged *a priori* as putatively common for both sisters and therefore not included into the main analysis.

Self-report measures

Eating Disorders Inventory-2 (*EDI*-2) (Garner, 1991)

This is a self-report measure of eating disorder

Table 1. *Distribution of risk factors in each domain and subdomain of vulnerability in the anorexia (AN) and the sister (S) group and the results of the statistics used McNemar tests, or t tests as appropriate (two-tailed levels of significance)*

Variable	AN (N = 45) N (%)	S (N = 45) N (%)	AN–S P statistic
Personal vulnerability domain			
Subdomain 1: Childhood characteristics			
Negative self-evaluation	33 (73)	8 (18)	***
Shyness	25 (56)	21 (47)	NS
Perfectionism	32 (71)	15 (33)	***
Extreme compliance	29 (64)	12 (27)	***
No close friends	13 (29)	2 (4)	**
School absence through anxiety	3 (7)	2 (4)	NS
Subdomain 2: Pre-morbid psychiatric disorder			
Major depression	13 (29)	3 (7)	NS
Drug abuse	0 (0)	0 (0)	—
Alcohol abuse	0 (0)	0 (0)	—
Subdomain 3: Behavioural problems			
Marked conduct problems	2 (4)	3 (7)	1·0
School absence through truancy	0 (0)	0 (0)	—
Deliberate self-harm	6 (13)	5 (11)	1·0
Subdomain 4: Parental psychiatric disorders (lifetime)			
Parental depression (lifetime)†	13 (29)	11 (24)	kappa
Parental alcoholism (lifetime)†	6 (13)	7 (16)	kappa
Parental drug abuse (lifetime)†	1 (2)	2 (4)	kappa
Environmental domain			
Subdomain 1: Parental problems			
Low parental contact	12 (27)	5 (11)	NS
Separation from parents	9 (20)	9 (20)	NS
Parental – arguments (between them)†	26 (58)	22 (49)	kappa
– criticism	14 (31)	6 (13)	NS
– high expectations	30 (67)	17 (38)	**
– overinvolvement	10 (22)	3 (7)	NS
– underinvolvement	20 (44)	13 (29)	NS
– minimal affection	20 (44)	13 (29)	NS
– control	20 (44)	12 (27)	NS
Subdomain 2: Disruptive events			
Parental death†	1 (2)	1 (2)	kappa
Change of parent figure†	15 (33)	15 (33)	kappa
Parental chronic illness†	4 (9)	6 (13)	kappa
Frequent house moves†	16 (33)	13 (29)	kappa
Severe personal health problems	9 (20)	3 (7)	NS
Subdomain 3: Parental psychiatric disorders			
Parental depression†	11 (24)	9 (20)	kappa
Parental alcoholism†	5 (11)	6 (13)	kappa
Parental drug abuse†	1 (2)	2 (4)	kappa
Subdomain 4: Teasing and bullying			
Teasing (not about shape, weight, eating, or appearance)	11 (24)	7 (16)	NS
Bullying	6 (13)	4 (9)	NS
Subdomain 5: Sexual or physical abuse			
Sexual abuse	16 (36)	5 (11)	**
Repeated severe sexual abuse	7 (16)	3 (7)	NS
Physical abuse	5 (11)	4 (9)	NS
Repeated physical abuse	1 (2)	0 (0)	NS
Repeated severe physical or severe sexual abuse	8 (18)	3 (7)	NS
Dieting vulnerability domain			
Subdomain 1: Dieting risk			
Family member dieting			
– for any reason†	19 (42)	15 (33)	kappa
– for shape or weight†	16 (36)	12 (27)	kappa
Repeated critical comments by family about shape, weight, or eating	13 (29)	11 (24)	NS
Repeated comments by others about shape, weight, eating, or appearance	10 (22)	7 (16)	NS
Teasing about shape, weight, eating, or appearance	20 (44)	13 (29)	NS

Table 1 (*cont.*)

Variable	AN (*N* = 45) *N* (%)	S (*N* = 45) *N* (%)	AN–S *P* statistic
Dieting at school common	12 (27)	11 (24)	NS
Parental obesity†	5 (11)	5 (11)	kappa
Childhood obesity	8 (18)	4 (9)	NS
Parental history of anorexia nervosa and bulimia nervosa†	3 (7)	4 (9)	kappa
Subdomain 2: Obesity risk			
Childhood obesity	8 (18)	4 (9)	NS
Parental obesity (lifetime)†	5 (11)	6 (13)	kappa
Subdomain 3: Parental eating disorder (lifetime)			
Parental history of anorexia nervosa or bulimia nervosa (lifetime)†	4 (9)	4 (9)	kappa
Pudicity domain			
Missing preparation for menarche	20 (44)	11 (24)	NS
Menarche mainly unpleasant	13 (29)	5 (11)	NS
Scared by early breast development	7 (16)	6 (13)	NS
Scared by late breast development	18 (40)	13 (29)	NS
Teasing about breasts at early age	8 (18)	4 (9)	NS
Breasts as source of embarrassment	15 (33)	9 (20)	NS
Additional risk factors			
Age at menarche, mean (S.D.)	13·7 (2·4)	13·2 (1·4)	NS
14–18 years	20 (44)	19 (42)	—
13	12 (27)	12 (27)	—
12	5 (11)	10 (22)	—
9–11	6 (13)	4 (9)	—
Primary amenorrhoea	2 (4)	0 (0)	—
Pregnancy	0 (0)	0 (0)	—
Parity	0 (0)	0 (0)	—
Abortion	0 (0)	0 (0)	—
Religion of marked importance	14 (31)	8 (18)	NS
Accidents affecting appearance	8 (18)	6 (13)	NS
Enuresis	2 (4)	1 (2)	NS
Feeding problems in childhood	25 (56)	11 (24)	*
Sister comparison variables			
Need to rival with sister	23 (51)	6 (13)	***
Sister as parents favourite	20 (44)	7 (16)	*
Negative self-evaluation comparing with sister	31 (69)	12 (27)	***
Sister's appearance better than subject's	21 (47)	1 (2)	***
Sister's shape better than subject's	24 (53)	2 (4)	***
Genetic vulnerability factors			
Serotonin receptor			
5HT2A			
G/G	17 (38·6)	11 (28·2)	
G/A	21 (47·7)	20 (51·3)	
A/A	6 (13·6)	8 (20·5)	
5HT2C			
CYS/CYS	31 (70·5)	27 (69·2)	
CYS/SER	11 (25)	11 (28·2)	
SER/SER	2 (4·5)	1 (2·6)	
Dopamine receptor			
DRD4			
23, 24, 33, 34, 44 repeats	31 (70·5)	25 (67·6)	
27, 37, 47 repeats	10 (22·7)	12 (32·4)	
77 repeats	3 (6·8)	0 (0)	
Catechol-*O*-methyltransferase			
COMT			
VAL/VAL	11 (25)	10 (26·3)	
VAL/MET	21 (47·7)	20 (52·6)	
MET/MET	12 (27·3)	8 (21·1)	

* $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$.
† Putatively shared risk factor (kappa statistics were performed which are reported in the text only).
— A comparison was not possible or appropriate.

symptoms and putative vulnerability factors with excellent internal consistency, content validity, criterion based validity, and construct validity and good test–retest reliability in eating disordered and healthy control subjects (Garner, 1991). The instrument was used to assess the current status of eating disorder related traits in patients and controls in order to get an additional estimate of discordance between the sisters.

Sibling Inventory of Differential Experiences (*Daniels & Plomin*, 1985*a*, *b*)

This is a 73-item self-report questionnaire that has been specifically designed to assess the non-shared environment within families (subdomains and domains see Table 3). The 2-week test–retest reliability ranges from 0·77 to 0·93 and acceptable. This instrument has been used to assess risk (referring to the time before index age) in the sister pairs.

Genotyping methods

Blood samples or cheek cell swabs were collected from both sisters and their parents to prepare genomic DNA by established procedures (for whole blood, Nucleon BACC3, Scotlab, UK, or for cheek swabs as described by Freeman *et al*. 1997). We examined the -1438 G/A polymorphism in the promoter region of the 5-HT2A gene (Spurlock *et al*. 1998), a Ser23Cys polymorphism in the 5-HT2C gene (Lappalainen *et al*. 1995), a variable number tandem repeat (VNTR) polymorphism in exon III of the dopamine D4 gene, DRD4 (Van Tol *et al*. 1992) and the functional Val158Met polymorphism of the catechol-*O*-methyltransferase (COMT) gene (Lachman *et al*. 1996). Genotyping was performed by PCR amplification of genomic DNA with taq DNA polymerase (Promega, UK), followed by cleavage with the appropriate restriction enzyme and/or sizing of the products next to a standard DNA ladder on an agarose gel by electrophoresis. The 5-HT2A -1438 G/A polymorphism was analysed by digestion of the 468-bp PCR product by *Hpa*II (New England Biolabs), which cuts the G allele only, giving 223 and 245 bp fragments (Spurlock *et al*. 1998). The 5-HT2C Cys23Ser polymorphism was analysed by digestion of the 104-bp PCR product by *Hin*fI (New England Biolabs), which cuts the Ser allele only, giving 86 and 18 bp fragments

(Lappalainen *et al*. 1995). The DRD4 VNTR was analysed as described (Shaikh *et al*. 1993) with sizing of the 2, 4, 6, and 7 repeat alleles by agarose gel electrophoresis next to DNA size standards (1 kb ladder, Gibco-BRL, UK). The COMT Val158Met was analysed by digestion of the 217 bp PCR product with N1aIII (Li *et al*. 1996), which gave fragments of 96, 80, and 40 bp for the Met158 allele.

Data analysis

We followed the analytical procedure used by Fairburn *et al*. (1997) and categorized the risk factors *a priori* into personal, environmental and dieting vulnerability domains with a few items in no specific domain. In addition, we included a domain of pudicity (sexual disgust) and sibling comparison variables (see Table 1). The clinical and demographic data from the two phenotypic extremes ($\pm$anorexia) was compared using paired *t* tests. The occurrence of individual risk factors (coded as categorical variables 1 = present, 0 = absent) was compared using univariate statistics by McNemar's tests. To investigate a dose–outcome relationship between the number of risk factors in a (sub)domain and case status conditional logistic regression analysis was used. Stepwise conditional logistic regression analysis was used to select subset of (sub)domains that jointly best predicted case status.

For the genetic analysis of the 5-HT2A -1438G/A, the 5-HT2C Cys23Ser, the COMT Val158Met, and the DRD4 polymorphisms the three possible genotypes at each locus were modeled by two dummy variables (Sham, 1998*a*, *b*). The first was a gene dosage effect (0, 1, 2) and the second a contrast between the heterozygous genotype and the two homozygous genotypes, representing an overdominance effect (i.e. one in which the heterozygous phenotype is more deviant than either of the homozygous genotypes). Conditional logistic regression was used for analysing the genetic data and to assess whether a dose–outcome relationship was present. Stepwise conditional logistic regression analysis was used to examine for confounding between the (sub)domains.

The sister pairs were compared regarding their EDI-2 scores using paired *t* tests. The mean scores on the Sibling Inventory of Differential Experiences were compared using conditional

logistic regression analysis over the four domains. The reliability in reporting between the sisters (for variables presumably common in both sisters) and between the raters was examined using kappa statistics. Subjects with a lifetime diagnosis of restricting and subjects with additional lifetime bingeing behaviour were compared using *t* tests, chi-square and Fisher's exact tests as appropriate.

All test results were considered as statistically significant if the *P* value was less than 0·01 except for the stepwise conditional regression analyses where a significance level of 0·05 was used. All reported probability values are two-tailed. Conditional logistic regression analyses and stepwise conditional logistic regression analysis were performed using the statistical program STATA (*Stata Reference Manual* 1985–1997). All the other analysis was carried out using SPSS-PC (Norušis, 1994).

Power analysis

Using data from previous studies (Fairburn *et al.* 1997) we estimated that a sample size of 31 pairs would have 80% power to detect a difference in proportions of 0·4 if half of the sisters are discordant for the variable and a McNemar's test of equality of paired proportions is used at a two-sided significance level of 1%. The numbers required to give comparable power for the detection of association with candidate gene polymorphisms were greater. Power calculations were applied to the discordant sib-pair design using a specially written SAS (© SAS Institute Inc., Carey, NC USA) program we called DISCORD, for power (or sample size) calculations of the sibling disequilibrium test method (SDT); (Horvath & Laird, 1998). Under a variety of assumptions of QTL variance and disease/QTL prevalence, we found that discordant pairs have good power to detect genetic effects when the risk ratio is high. For example, to detect a locus with a risk ratio of 6·8 (between the two homozygous genotypes), and which accounts for 10% of the genetic variance of a trait with heritability of 50%, 81 pairs would be required for a projected *P* value of 0·01 to be exceeded. If selection strategy is based on a QTL measure, then power is increased. For example a sibling pair in which one was in the upper 10% and the other in the bottom 25% of a QTL measure accounting for 10% of genetic variance would need only 45 pairs for a projected *P* value of 0·01. A sample of 50 discordant sister pairs is inadequate to detect genes with small affects on risk ratio; for example data from the 5-HT2A gene in anorexia indicate that this gene only has a modest homozygote risk ratio, of <2·5. Thus, the probability of detecting such an association with only 50 discordant pairs is small (Allison & Faith, 1997*b*). Unfortunately, restricted resources for this pilot study limited the size of the sample.

RESULTS

Clinical and demographic characteristics of the sister-pairs

The sisters were similar in age (affected subjects had a mean age of 27·7±8·5 years, the unaffected sisters 27·4±9·7 years). The mean age gap between the sister pairs was 3·2±1·9 years. The affected subject was the youngest of the pair in 49% of the cases. At the time of the interview the affected subjects had a mean body mass index (BMI) of 17·7±3·7 kg/m², whereas the unaffected sisters had a mean BMI of 22·4±3·8. The mean age of first established symptoms of an eating disorder (index age) was 15·3±3·2 years. The mean lowest lifetime BMI was 13·1±2·2 and the median duration of the illness was 3 years (interquartile range (IQR) 1·8–6·5). Fifty-seven per cent of the fathers had professional occupations. Eighteen per cent of the affected sisters were not working because of medical reasons in comparison to 2% of the unaffected sisters. Overall, the affected subjects had a poorer level of career adjustment than their sisters. The sisters with anorexia nervosa had significantly higher scores on all the 11 attitudinal behavioural subscales of the EDI-2.

There were no clinical differences between the cases in current treatment and the research volunteers apart from the expected difference in current weight (BMI 15·7±2·9 *v.* 18·9±3·6; *t* = 3·13; df = 43; *P* = 0·003) and an earlier onset of illness (13·9±2·0 years *v.* 16·1±3·6 years; *t* = 2·32; df = 43; *P* = 0·03). Two (7%) of the volunteers had never been treated for an eating disorder, 17 (61%) had treatment in the past and 9 (32%) had ongoing treatment.

Defining the subgroups

We followed the precedent set by the Price Foundation Collaborative Group (2000) and

Table 2. *Conditional logistic regression using the number of risk factors present and reporting odds ratios (OR), 95% confidence intervals (CI), and likelihood ratio probabilities* (P)

(Sub)domain (number of variables)	Odds ratio	CI	*P*
Personal vulnerability domain (12)	3·42	1·7–6·8	***
Subdomain 1 (6): Childhood characteristics	3·32	1·7–6·6	***
Subdomain 2 (3): Pre-morbid psychiatric problems	4·3	1·2–15·2	**
Subdomain 3 (3): Behavioural problems	1	0·4–2·9	NS
Environmental vulnerability domain (16)	1·77	1·3–2·5	***
Subdomain 1 (8): Parental problems	2·26	1·4–3·6	***
Subdomain 2 (1): Disruptive events	4	0·8–18·8	*
Subdomain 3 – not included (putatively shared)			
Subdomain 4 (2): Teasing and bullying	1·69	0·7–4·0	NS
Subdomain 5 (5): Sexual and physical abuse	2·58	1·1–5·8	*
Dieting vulnerability domain (7)	1·37	0·9–2·0	NS
Subdomain 1 (6): Dieting risk	1·37	0·9–2·0	NS
Subdomain 2 (1): Obesity risk	3	0·6–14·9	NS
Pudicity domain (6)			
Pudicity (6)	1·68	1·12–2·52	**
Genetic vulnerability domain			
Serotonin receptor subdomain			
5HT2A	0·70	0·35–1·4	NS
5HT2C	1	0·38–2·66	NS
Dopamine receptor subdomain DRD4	1	0·40–2·52	NS
COMT receptor sudomain	1·04	0·92–1·18	NS

Conditional logistic regression analysis over the genetic domains. Levels of significance: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

Table 3. *Sibling Inventory of Differential Experiences (SIDE): mean (S.D.) for the two groups, odds ratios, 95% confidence intervals (OR, CI), and likelihood ratio probabilities* (P) *for conditional logistic regression over the* 11 *subdomains with* χ^2, *degrees of freedom (df), and* P *value for conditional logistic regression analysis over the four domains*

	AN Mean (S.D.)	Sister Mean (S.D.)	OR (CI)	χ^2	df
Sibling interaction domain				17·43**	4
Sibling antagonism	3·08 (0·64)	3·22 (0·68)	0·78 (0·44–1·39)	NS	
Sibling caretaking	3·05 (0·66)	2·82 (0·77)	1·33 (0·81–2·19)	NS	
Sibling jealousy	2·39 (0·83)	3·25 (0·67)	0·41 (0·23–0·71)	***	
Sibling closeness	2·85 (0·69)	2·92 (0·62)	0·88 (0·49–1·58)	NS	
Maternal treatment domain				0·34	2
Maternal affection	3·25 (0·70)	3·17 (0·48)	1·20 (0·64–2·24)	NS	
Maternal control	2·88 (0·43)	2·89 (0·36)	0·94 (0·36–2·48)	NS	
Paternal treatment domain				1·38	2
Paternal affection	3·11 (0·69)	3·07 (0·59)	1·21 (0·66–2·22)	NS	
Paternal control	3·04 (1·24)	2·88 (0·37)	1·22 (0·69–2·15)	NS	
Peer group characteristics domain				8·36	3
Peer college orientation	2·88 (0·81)	3·11 (0·72)	0·74 (0·43–1·26)	NS	
Peer delinquency	3·06 (0·57)	2·87 (0·54)	1·52 (0·79–2·95)	NS	
Peer popularity	3·25 (0·69)	2·72 (0·62)	2·21 (1·15–4·25)	*	

A rating of 1 in each case means the statement is true for 'me much more', a rating of 5 means that it is true for 'her much more' (3 means 'both the same', 2 and 4 respectively mean 'me (she) a bit more'.
* $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$.

Kaye *et al.* (2000) and used a cut-off point of 3 years without any episodes of binge eating to define restricting anorexia nervosa. Twenty-nine subjects (64·4%) fulfilled this criterion. The restricting and the bingeing subgroup did not differ in age, age at first symptoms, birth weight, age at lowest maintained weight, and BMI at time of the interview. However, the restricting subgroup reached significantly lower BMI scores during the course of their disorder

(AN/RES − AN/BP; mean BMI (S.D.): 12·4 (2·1) − 14·4, $t = 3·22$, $P = 0·002$), and reported significantly lower scores on the bulimia subscale of the EDI-2.

Reliability of familial risk factors and inter-rater agreement

A few items in the interview (see † in Table 1) are factors that we would expect to be common to both sisters. The reliability was good (kappa between 1·0 and 0·61 according to Landis & Koch, 1977) for the majority of these items. The reliability of 'dieting behaviour in family members for any reason' ($\kappa = 0·34$) or 'dieting for weight and shape reasons in family members', or 'arguments between parents' ($\kappa = 0·47$) was less good. The reliability between the unblinded and blinded raters for the ORFI was good (mean κ (S.D.) = 0·87 (0·3)).

Comparison between the sisters regarding risk factors (Oxford Risk Factor Interview)

Individual risk factors

The sisters with AN had higher scores on the following personal vulnerability variables: negative self-evaluation, perfectionism, extreme compliance and 'no close friends' (Table 1). Within the environmental domain they reported greater levels of exposure to high parental expectations and sexual abuse. None of the risk factors in the dieting domain was higher. In the pudicity domain anorexic sisters reported more teasing about breast development. The sisters with AN reported higher levels of feeding difficulties in childhood. Comparing themselves directly with their sisters, the subjects with AN reported more pre-morbid rivalry with their sisters, and they were more likely to perceive their sister to be the parent's favourite. The sisters with AN had a lower self-evaluation comparing themselves to their sisters and specifically perceived their sister's appearance and shape to be superior.

Overall level of exposure to each sub-domain

The sisters with AN had a greater level of exposure to five domains shown in Table 2. In each case the greater the exposure the greater the risk of developing AN. Stepwise conditional logistic regression analysis (using the likelihood ratio) across the sub-domains had childhood characteristics ($\chi^2 = 30·3$; df = 1; $P < 0·0001$) entered first, followed by sexual and physical abuse ($\chi^2 = 4·8$; df = 1; $P = 0·03$), obesity risk ($\chi^2 = 4·5$; df = 1; $P = 0·03$), parental problems ($\chi^2 = 6·5$; df = 1; $P = 0·01$), and pre-morbid psychiatric disorders ($\chi^2 = 5·3$; df = 1; $P = 0·02$). After adjusting for these sub-domains, no other sub-domain entered the regression model.

Overall level of exposure to each domain

The sisters with AN had a greater level of exposure to three of the four (non-genetic) domains: stepwise conditional logistic regression analysis across the domains, had exposure to the personal vulnerability domain entered first ($\chi^2 = 33·3$; df = 1; $P < 0·0001$), followed by exposure to the environmental vulnerability domain ($\chi^2 = 3·8$; df = 1; $P = 0·05$). After adjusting for exposure to these two domains, exposure to the dieting vulnerability domain, and the pudicity domain did not enter the logistic regression model (Table 2).

Comparison between sisters on Sibling Inventory of Differential Experiences (SIDE)

The scores for the SIDE are shown in Table 3. There were significant differences in the sibling interaction domain and the peer group characteristics domain in that subjects with AN perceived themselves to have more pre-morbid jealousy of their sister than vice versa and considered that their sister was in a more popular peer group. Pre-morbid maternal and paternal treatment were perceived as similar by the sisters both on sub-domain level and on domain level.

A comparison of the genetic risk factors between the sisters

Tables 1 and 2 show the results of genotyping analysis for the 5-HT2A, 5HT2C, COMT, and DRD4 genes as odds ratios, 95% confidence intervals and P values. All overall comparisons between sisters, whether by allele or genotype, were negative. In view of the small sample size, these findings should be regarded as rather preliminary but worthy of further exploration. There was no evidence of either a gene dose effect or a dominance interaction at any locus.

A comparison of the risk factors for the restricting and binge/purging subtypes

Patients with additional bingeing behaviour were more likely to have been exposed to repeated critical comments by family members about

shape, weight, or eating and they reported more often parental obesity both before index age and lifetime. Using logistic regression analysis over the domains and subdomains of risk (environmental and genetic), respectively, no difference was found between those who restricted and those who additionally binged.

DISCUSSION

To our knowledge, this is the first study that has attempted to compare sisters regarding genetic and early risk factors for the development of AN. Complex disorders such as AN probably result from an interaction of genetic and environmental factors, and aetiological research should include both. However, large samples would be needed for such modelling. Both intrinsic factors (personal vulnerability, such as temperamental traits) and extrinsic factors (environment, such as sexual abuse and parental pressure) increased the risk for developing the disorder. The dieting risk domain was not significant. These findings replicate the work of Fairburn *et al.* (1999*b*). The only significant difference in risk factors was in the level of extreme compliance, which was twice as high in the present sample. AN patients compared themselves unfavourably with their sisters on several dimensions. There were no significant differences between affected and unaffected siblings on the genetic polymorphisms studied.

The restricting and binge/purging subtypes did not differ in terms of risk in domains and subdomains although there were differences in three environmental risk factors i.e. 'repeated critical comments by family members about shape, weight, or eating', and the frequency of 'parental obesity' reported (both before index age and regarding lifetime), being significantly more often reported by binge/purging subtype anorexics. Thus, this group resemble the other bulimic disorders (Fairburn *et al.* 1997, 1998).

The study has strengths and limitations. The use of healthy sisters as controls meant that many cultural and family factors were accurately matched so that individual-specific factors could be more clearly ascertained. The reliability of the instruments used was reasonable (kappa between 1·0 and 0·61 according to Landis & Koch, 1977) and similar to that found by Fairburn although the reliability of the dieting

vulnerability measures is questionable. Power limitations prevented modelling of the risk factors, for example into mediators or moderators (Baron & Kenny, 1986). Twin, family and adoption studies of considerable power will also be required to clarify these factors. Another limitation was that our sample was too small to allow definitive analysis of the interesting differences between the restricting and binge-eating subtypes of anorexia.

There are several potential sources of bias in the sample. First, not all of the probands had passed the age of risk of developing an eating disorder at the time of analysis, since 22 % of the healthy sisters ($N = 10$) were aged $\leqslant 19$. This source of bias would only serve to weaken the strength of any association we found. Secondly, there may be a bias in the ascertainment of the sample, since it was not systematically recruited from the general population using epidemiological methods. Unfortunately, because AN is uncommon, it is difficult to ascertain cases of AN from the community (Fairburn *et al.* 1999*b*; see Shoemaker, 1998 for review). The two sources of recruitment were from subjects who have attended the Bethlem and Maudsley hospital, and those from a national self-help organization group. The hospital provides the only eating disorder service in a catchment area of two million people. It also is a tertiary referral service and includes cases at the severe end of the spectrum. The volunteers had a broad range of severity and utilization of services, and 93 % had treatment for their eating disorder. Being or having been in treatment may cause a bias in recall of early environments.

A further limitation was that the interviewer was not blinded. We tried to minimize any bias by taping and re-coding the interviews by the senior author who was blind to case status. The present study is also retrospective in nature, and thus may be sensitive to recall bias (Brewin *et al.* 1993; Maughan & Rutter, 1997). However, Brewin *et al.* (1993) have given convincing evidence that behaviourally defined variables can largely reduce this bias.

It is possible that the factors within the personal vulnerability domain represent some of the personality traits linked to the disorder from other studies. Obsessional personality traits and perfectionism are present both in the acute (Rastam, 1992; Fairburn *et al.* 1999*b*; Halmi *et*

al. 2000) and the recovered phase of AN (Srinivasagam *et al.* 1995; Ward *et al.* 1998. It is possible that obsessive–compulsive personality disorder is the more general phenotype that is transmitted in families with AN, as this is elevated in probands and their relatives (Lilenfeld *et al.* 1998). Other personality traits noted in AN are those of anxiety and harm avoidance (Price Foundation Collaborative Group, 2000) but after recovery scores on this scale are within the normal range (Ward *et al.* 1998). These traits may contribute to psycho-sexual immaturity, conflict about sexuality and pudicity-related life events (Crisp, 1980; Schmidt *et al.* 1995, 1997).

Extrinsic risk factors were high parental expectations and sexual abuse. Gillberg & Rastam (1998) observed that their cases of AN came from families who were 'on the way up', i.e. were in the process of improving their socio-economic status. There appears to be a specific interaction between the parent and the child who goes on to develop AN, which might represent gene–environment correlation. Prior to the present study, several studies have found that sexual abuse is a risk factor for the later development of AN (Oppenheimer *et al.* 1985; Palmer *et al.* 1990; McClelland *et al.* 1991; Schmidt *et al.* 1993; Garfinkel *et al.* 1995). The risk is thought to be lower for restricting AN than for other eating disorder subtypes and strongest in bulimia nervosa (Steiger & Zanko, 1990; Waller, 1993; Fullerton *et al.* 1995). The pudicity (sexual disgust) dimension does not stand as a separate factor in the total model. It is possible that this also represents an interactive effect of the temperamental traits and the physiological changes in puberty.

The anorexic sisters had lower levels of all the risk factors within the dieting domain, apart from a family history of eating disorders. An interesting finding was that there was an as-sociation with poor feeding in childhood. This replicates the finding of early feeding difficulties in cases of AN (Rastam, 1992) and partial cases (Marchi & Cohen, 1990) and may represent a specific effect on appetite which could be genetically and or environmentally mediated, with scope for both gene–environment inter-action and correlation.

Items relying on a direct sister-pair com-parison were not included into modelling,

because they are unique to this subgroup of patients who have sisters. However, we found that those in the anorexic group were jealous of their sister whom they perceived to be more physically and socially attractive than they were. This domain may be coloured by dispositional judgements rather than objective truth.

These findings suggest that it would be informative to undertake a larger study to examine in more detail the non-shared genetic and environmental factors, which are associated with various forms of eating disorders. The best design would be a prospective study but a sample size of 20 000–100 000 would be needed to attain 100 cases. Alternative strategies would be to sample high risk groups, for example offspring with a family history of eating dis-orders. However, such approaches will require the preliminary identification of appropriate measures and risk factors from retrospective analysis, such as the present study.

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