Genes, Brain and Behavior
Official publication of the International Behavioural and Neural Genetics Society

Genes, Brain and Behavior (2011) **10:** 236–243 doi: 10.1111/j.1601-183X.2010.00660.x

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

M. C. T. Slof-Op 't Landt[*,†,‡,§], I. Meulenbelt[‡], M. Bartels[§], E. Suchiman[‡], C. M. Middeldorp[§,¶], J. J. Houwing-Duistermaat[**], J. van Trier[††], E. J. Onkenhout[‡‡], J. M. Vink[§], C. E. M. van Beijsterveldt[§], M. K. Brandys[§§,¶¶], N. Sanders[§§,¶¶], S. Zipfel[****], W. Herzog[†††], B. Herpertz-Dahlmann[‡‡‡], K. Klampfl[§§§], C. Fleischhaker[¶¶¶], A. Zeeck[******], M. de Zwaan[††††], S. Herpertz[‡‡‡‡], S. Ehrlich[§§§§,¶¶¶¶], A. A. van Elburg[¶¶,******], R. A. H. Adan[§§], S. Scherag[†††††], A. Hinney[†††††], J. Hebebrand[†††††], D. I. Boomsma[§], E. F. van Furth[†] and P. E. Slagboom[‡]**

[†]Center for Eating Disorders Ursula, Leidschendam, [‡]Department of Medical Statistics, Molecular Epidemiology Section, Leiden University Medical Centre, Leiden, [§]Department of Biological Psychology, VU University, Amsterdam, [¶]De Bascule, Academic Center for Child and Adolescent Psychiatry, Amsterdam, [**]Department of Medical Statistics and Bioinformatics, Leiden University Medical Centre, Leiden, [††]Department of Psychiatry and Psychology, St Antonius Hospital, Utrecht, [‡‡]Emergis, Department of Eating Disorders, Goes, [§§]Department of Neuroscience and Pharmacology, Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht, and [¶¶]Altrecht Mental Health Institute, Rintveld Centre for Eating Disorders, Zeist, The Netherlands, [****]Department of Psychosomatic Medicine and Psychotherapy, University Medical Hospital, Tübingen, [†††]Department of Psychosomatic and General Internal Medicine, University Medical Hospital, Heidelberg, [‡‡‡]Department of Child and Adolescent Psychiatry and Psychotherapy, Universitätsklinikum Aachen, Aachen, [§§§]Department of Child and Adolescent Psychiatry and Psychotherapy, Julius-Maximilians University of Würzburg, Würzburg, [¶¶¶]Clinic and Day Clinic of Child and Adolescent Psychiatry and Psychotherapy, Albert Ludwigs-University of Freiburg, Freiburg i. Br., [******]Department of Psychosomatic Medicine and Psychotherapy, University of Freiburg, Freiburg, [††††]Department for Psychosomatic Medicine and Psychotherapy, University Erlangen-Nuremberg, Erlangen, [‡‡‡‡]Clinic of Psychosomatic Medicine and Psychotherapy, LWL-University Clinic, Ruhr-University Bochum, and [§§§§]Department of Child and Adolescent Psychiatry, Charité – Universitätsmedizin Berlin, Berlin, Germany, [¶¶¶¶]Translational Developmental Neuroscience Section, Department of Child and Adolescent Psychiatry, University Hospital Carl Gustav Carus, Dresden University of Technology, Dresden, Germany, [********]Department of Child and Adolescent Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands, and [†††††]Department of Child and Adolescent Psychiatry and Psychotherapy, LVR Klinkum Essen, University of Duisburg-Essen, Essen, Germany

**Corresponding author: M. C. T. Slof-Op 't Landt, Center for Eating Disorders Ursula, PO Box 422, 2260 AK Leidschendam, The Netherlands. E-mail: r.optlandt@centrumeetstoornissen.nl*

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

Keywords: Anorexia nervosa, candidate genes, genetic association study, *TPH2* self-induced vomiting

Received 13 July 2010, revised 6 September 2010 and 27 September 2010, accepted for publication 29 September 2010

Eating disorders (EDs) are debilitating diseases with high chronicity and mortality rates (Crow *et al*. 2009; Steinhausen 2002; Steinhausen & Weber 2009). Genetic influences appear to be considerable for ED, with heritability estimates ranging from 28 to 83% in women (Bulik *et al*. 2006; Slof-Op 't Landt *et al*. 2005). A common and frequently occurring symptom in subjects with ED is self-induced vomiting (SV). This symptom was associated with greater clinical severity

(Dalle Grave *et al*. 2009; Reba *et al*. 2005) and also appears to be heritable (8–72%) (Sullivan *et al*. 1998; Wade *et al*. 2008).

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

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

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

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

In general, consistent associations in the ED field are lacking, possibly due to small sample sizes and the limited number of polymorphisms assessed (Scherag *et al*. 2010; Slof-Op 't Landt *et al*. 2005). In the current study, we selected 25 tagging SNPs across the four genes and tested them for association with AN ($N = 182$) and subjects ($N = 149$) with an ED characterized by SV. Replication occurred in a meta-analysis with two additional independent ED case–control samples from Germany and The Netherlands together providing 887 AN cases, 306 SV cases and 1914 controls.

Methods and materials

Subjects

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

A total of 389 female ED patients were recruited through 10 specialized ED units throughout The Netherlands (the GenED study). All subjects fulfilled the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for an ED, made by experienced clinicians based on a semi-structured interview at intake and via the self-report ED examination questionnaire (EDEQ) (Fairburn & Beglin 1994). For AN, criterion D – amenorrhea for three consecutive months – was discarded because some of the subjects despite having AN continued to menstruate (e.g. due to treatment with oral contraceptives). Of the 389 cases, 182 fulfilled the DSM-IV criteria (excluding criterion D) for AN. Based on the EDEQ (q14: Over the past 28 days, how many times have you made yourself sick (vomit) as a means of controlling your shape and weight?) and assessment interviews (current and past self-induced vomiting), we defined a subgroup of ED cases ($N = 149$) who reported regular SV. Frequencies of mean rates of SV were 30%, 2–8 times per month; 40%, 8–20 times per month and 30%, more than 20 times per month. Subjects with SV fulfilled the following DSM-IV diagnoses AN ($N = 64$), boulimia nervosa (BN) ($N = 74$) and ED not otherwise specified ($N = 11$) (Table 1). Thus, the two groups were partly overlapping, with 64 subjects belonging to both groups.

Random controls come from the population-based Netherlands twin registry (NTR), which was established in the late 1980s at the VU University in Amsterdam, The Netherlands. Data on the multiples (twins or triplets) and their families were collected every 2–3 years in the longitudinal survey studies (Boomsma *et al*. 2002). Subsamples of the multiples were invited to participate in experimental and laboratory studies and donate their DNA (Boomsma *et al*. 2006). For the current study, one woman per family served as control, yielding a control group of 607 unrelated women (Middeldorp *et al*. 2010).

For the meta-analysis, additional sample collections were used from Essen (The EDNET and Essen study, Germany) and Utrecht (The Netherlands) (Table 1). The EDNET and Essen samples consisted of 420 female subjects with AN according to the DSM-IV criteria and

Table 1: Cases and controls

				DSM-IV Eating disorder diagnosis				
Cases and controls	Total *N*	Mean age (SD)	Overlap AN and SV	Restricting AN	Binge-purge AN	Purging AN (without binging)	BN	EDNOS
GenED								
NTR Controls	607	25.4 (13.6)						
GenED AN	182	28.7 (9.9)	64	108	35	39	—	—
GenED SV	149	28.9 (9.9)	64	—	29	35	74	11
EDNET–Essen								
EDNET–Essen controls	189	24.6 (2.5)						
EDNET–Essen AN	420	21.4 (9.1)	—	152	NA	NA	—	—
Utrecht								
Gain GWA NTR controls	1118	44.0 (13.7)						
Utrecht AN	285	22.9 (4.8)	56	213	NA	NA	—	—
Utrecht SV	157	23.8 (5.7)	56	—	NA	NA	37	63

EDNOS, eating disorders not otherwise specified; NA, data not available.

Table 2: Selected SNPs per candidate gene

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

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

SNP selection and genotype measurements

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

HTR1D SNPs were selected based on previous association studies in AN (Bergen *et al*. 2003; Brown *et al*. 2006). For *BDNF*, *STMN1* and *TPH2* tagging SNPs were selected from HapMap Public Release #19 applying the efficient multimarker method with $r^2 > 0.8$ and minor allele frequency (MAF) > 0.05 as implemented in the HapMap web browsers (http://www.hapmap.org) (de Bakker *et al*. 2005). Two of the selected *HTR1D* SNPs (rs676643 and rs674386) were also present as tagging SNPs in the HapMap database. In Table 2, the selected SNPs and coverage rate per candidate gene are listed.

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

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

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

Statistical analyses

The χ^2 test for Hardy–Weinberg equilibrium (HWE) was calculated in the NTR controls using the HWE program of LINKUTIL (http://linkage.rockefeller.edu/ott/linkutil.htm).

To investigate the association of the 25 SNPs from four candidate genes, we applied a two-stepped approach. First, the allele frequencies for all the SNPs were compared between cases from the GenED study and controls from the NTR. SNPs that showed nominal significant association ($P < 0.05$) with either AN or SV in the first step were tested for replication in a meta-analysis with the two additional independent case–control samples (EDNET and Essen, and Utrecht).

Differences in the allele frequencies were compared and tested for significance by Pearson's χ^2 test with SPSS version 15 software (SPSS, Chicago, IL, USA). For the meta-analysis, the fixed- and random-effects model of DerSimonian and Laird (1986) was used to estimate the summary of ORs, as implemented in *R* (http://www.r-project.org/, package meta). The heterogeneity was quantified using the I^2 statistic for inconsistency (Higgins & Thompson 2002) and its statistical significance was tested with the χ^2 distributed Cochran Q statistic (Lau *et al*. 1997). I^2 describes the proportion of variation that is unlikely to be due to chance and is considered large for values more than 50% (Higgins & Thompson 2002). Two tailed *P*-values are reported for all analyses.

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

Results

SNP association analysis

In the NTR control group, none of the SNPs revealed a departure from HWE ($P > 0.01$). Depending on the MAF of the SNP, this initial study had adequate power (85% power at an alpha level of 0.05, log-additive or allelic model) to detect effect sizes ranging between 1.45 and 1.8 for AN and ranging between 1.48 and 1.85 for SV. The results of the association analysis in the initial study (GenED cases and NTR controls) are presented in Table 3. A nominal significant association ($P < 0.05$) was observed for *TPH2* rs1473473 in AN as well as SV. This SNP was followed-up in the meta-analysis.

Exploratory association analyses were performed in the restricting type AN subgroup ($N = 108$) of the GenED study and the NTR controls. The results of these analyses are

Table 3: MAF for each SNP in cases of the GenED study and NTR controls

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

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

presented in Table S1 (Supporting information). No significant association was observed for any of the 25 SNPs.

Meta-analysis

The *TPH2* SNP rs1473473 was genotyped in the EDNET and Essen and the Utrecht case–control samples. In the GAIN GWA control group, this SNP was imputed. The average maximum posterior probability, which represents how much uncertainty there is for the imputation of an SNP, was 0.99 for *TPH2* rs1437473. For the meta-analysis, genotype data were available for a total of 2987 individuals (887 AN cases, 306 SV cases and 1914 controls) which provide adequate power (85% power at an alpha level of 0.05, log-additive or allelic model, MAF of 0.16) to detect effect sizes higher than 1.25 for AN and higher than 1.4 for SV.

Table 4 shows ORs, their 95% CI and *P*-values within the individual case–control samples and the subsequent meta-analyses. For the minor C-allele (frequency 0.16) of *TPH2* SNP rs1473473, a significant association was observed in the meta-analyses with both AN and SV. We observed an OR of 1.25 (95% CI 1.06–1.47, $P < 0.009$) for AN and an OR of 1.34 (95% CI 1.06–1.69, $P < 0.013$) for SV. There was no significant evidence for heterogeneity of the effect in the AN or SV analyses ($P = 0.58$, $I^2 = 0\%$ and $P = 0.50$, $I^2 = 0\%$).

The OR for the combined group of AN and/or SV cases ($N = 1073$) was 1.24 (95% CI 1.06–1.44, $P < 0.006$). We could not observe significant evidence for heterogeneity of the effect ($P = 0.38$, $I^2 = 0\%$) between the different case–control samples. Based on the genotype frequencies of the *TPH2* SNP rs1473473 (Table 5), we expected a

Table 4: Meta-analysis of *TPH2* SNP rs1473473 in AN and EDs characterized by SV

		GenED				Utrecht				EDNET–Essen				Meta-analysis			
SNP	Pheno	OR	CIL	CIR	*P*	OR	CIL	CIR	*P*	OR	CIL	CIR	*P*	OR	CIL	CIR	*P*
rs1473473	AN	1.39	1.02	1.92	0.040	1.25	0.98	1.60	0.067	1.11	0.81	1.51	0.533	**1.25**	**1.06**	**1.47**	**0.009**
	SV	1.46	1.04	2.04	0.029	1.24	0.91	1.70	0.176	—	—	—	—	**1.34**	**1.06**	**1.69**	**0.013**

Number of AN and SV cases per study: GenED AN ($N = 182$), SV ($N = 149$); Utrecht AN ($N = 285$), SV ($N = 157$); EDNET–Essen AN ($N = 420$), SV ($N = 0$).
CIL, lower 95% CI, CIR, upper 95% CI.

Table 5: Genotype counts *TPH2* rs1473473 for the three case–control samples

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

dominant effect to be underlying the association. Therefore, we evaluated the association with this SNP in the combined case group under a dominant genotypic model. Figure 1 represents the results of this association. Homo- and/or heterozygous carriers of the minor allele of rs1473473 had an increased probability of either AN or SV (OR = 1.38, 95% CI 1.16–1.64, $P < 0.0003$). Again, no evidence for heterogeneity was observed ($P = 0.44$, $I^2 = 0\%$). As there is a general tendency for initial studies to overestimate effect sizes, we tested sensitivity of the association by excluding the discovery sample (GenED cases and NTR controls). Under the dominant genotypic model, carriers of the minor allele of rs1473473 had an OR of 1.29 (95% CI 1.05–1.59, $P < 0.018$) among the two replication case–control samples. In Fig. S1 (Supporting information), the LD plot between *TPH2* rs1473473 and the nine other selected *TPH2* tagging SNPs is depicted.

Because the LD structure among the SNPs was not completely independent, adjusting the *P*-value for the actual

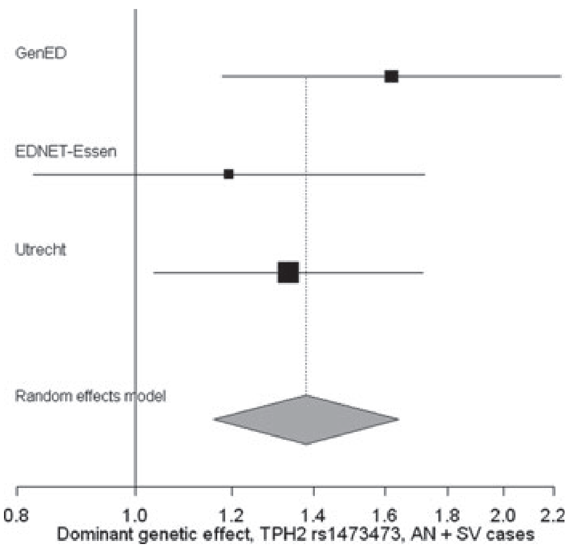

Figure 1: Random effect plot of the association between *TPH2* rs1473473 and the combined AN/SV cases under a dominant genotypic model. Results: GenED: OR = 1.62 (95% CI 1.18–2.23); EDNET–Essen: OR = 1.19 (95% CI 0.83–1.72); Utrecht: OR = 1.33 (95% CI 1.04–1.72); Random effect model total: OR = 1.38 (95% CI 1.16–1.64, $p < 0.0003$).

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

Discussion

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

To retain adequate statistical power, the current study only covered a selection of candidate genes for ED. To replicate previous results in ED, we selected genes for which the association was observed and confirmed in studies with an adequate sample size. Both *HTR1D* and *BDNF* fulfilled these criteria, although we acknowledge that inclusion of the gene encoding the opioid delta receptor (*OPRD1*) would also have been appropriate (Bergen *et al*.

2003; Brown *et al*. 2006; Ribases *et al*. 2004, 2005). Because of previous inconsistent results, the serotonin receptor 2a and the serotonin transporter genes were not included in our selection (for a review see Bulik *et al*. 2007; Slof-Op 't Landt *et al*. 2005). Besides replication of previous results, the current study also aimed to evaluate the involvement of two unexplored candidate genes for ED. Like *HTR1D* and *OPRD1*, *STMN1* was located under the linkage peak of restrictive AN (1p33-36) (Grice *et al*. 2002). Because the associations with *HTR1D* and *OPRD1* only explained part of the linkage, it was expected that additional candidate genes could underlie the linkage peak (Bergen *et al*. 2003). *TPH2* was selected because of the link between serotonin and ED. The role of *TPH2* in the synthesis of serotonin (Zill *et al*. 2007) makes it a plausible candidate gene for ED. Thus far, no other genes have been analysed in the GenED study.

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

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

The reported association between the functional *BDNF* Val-66-Met polymorphism (rs6265) and AN was not replicated in this study (Ribases *et al*. 2004, 2005). However, this result is in line with several other studies which also could not confirm this association (Dardennes *et al*. 2006; Dmitrzak-Weglarz *et al*. 2007; Friedel *et al*. 2005; Koizumi *et al*. 2004; de Krom *et al*. 2006; Mercader *et al*. 2007).

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

No consistent associations were observed for the other positional candidate gene, *STMN1*. Despite its position under the linkage peak for AN, it might not be involved in ED. However, because linkage was observed in the restrictive subtype of AN, it is also possible that an effect of this gene is only apparent in this specific ED subgroup. The exploratory analyses in restrictive AN ($N = 108$) of the GenED study and the NTR controls (Table S1) also did not reveal an association with *STMN1*. This exploratory study had adequate power (85% power at an alpha level of 0.05, log-additive or allelic model) to detect effects sizes around 1.6 for restricting AN. Thus, the association may have been missed due to limited statistical power.

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

Many genetic studies in AN have been performed, mainly in small populations measuring only one or a few SNPs (Bulik *et al*. 2007). In the current study, we used a large population of AN cases. We selected 25 SNPs to capture the majority of the common variation within four candidate genes (*STMN1*, *HTR1D*, *TPH2* and *BDNF*). Our two-step approach gave us the opportunity to explore association with all 25 SNPs in the first step and to evaluate the initial findings in two additional independent case–control samples. This approach has led to a robust association of the *TPH2* SNP rs1473473. The minor allele of this SNP was associated with a higher risk for AN, SV and for the combined group. It is interesting that the same SNP was associated with both types of ED. Although there was overlap between the two types of ED, 13% of the 887 AN cases also belonged to the SV group, the effect of rs1473473 is also present in the independent AN and SV groups. It has been hypothesized that AN, BN and also subthreshold forms of ED share at least some risk and liability factors (Kaye 2008; Strober *et al*. 2000). In a Swedish twin study, approximately half of the genetic factors contributed to liability to both AN and BN (Bulik *et al*. 2010). Our current finding is consistent with this hypothesis. For future studies, we aim to establish the effect of genetic variation at the *TPH2* gene on behaviors underlying different types of ED, like perfectionism, impulsivity or obsessive compulsiveness (Kaye 2008).

References

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

Barnett, J.H. & Smoller, J.W. (2009) The genetics of bipolar disorder. *Neuroscience* **164**, 331–343.
Ben-Tovim, D.I., Subbiah, N., Scheutz, B. & Morton, J. (1989) Bulimia: symptoms and syndromes in an urban population. *Aust N Z J Psychiatr* **23**, 73–80.
Bergen, A.W., van den Bree, M.B., Yeager, M., Welch, R., Ganjei, J.K., Haque, K., Bacanu, S., Berrettini, W.H., Grice, D.E., Goldman, D., Bulik, C.M., Klump, K., Fichter, M., Halmi, K., Kaplan, A., Strober, M., Treasure, J., Woodside, B. & Kaye, W.H. (2003) Candidate genes for anorexia nervosa in the 1p33-36 linkage region: serotonin 1D and delta opioid receptor loci exhibit significant association to anorexia nervosa. *Mol Psychiatr* **8**, 397–406.
Boomsma, D.I., Vink, J.M., van Beijsterveldt, T.C., de Geus, E.J., Beem, A.L., Mulder, E.J., Derks, E.M., Riese, H., Willemsen, G.A., Bartels, M., van den, B.M., Kupper, N.H., Polderman, T.J., Posthuma, D., Rietveld, M.J., Stubbe, J.H., Knol, L.I., Stroet, T. & van Baal, G.C. (2002) Netherlands twin register: a focus on longitudinal research. *Twin Res* **5**, 401–406.
Boomsma, D.I., de Geus, E.J., Vink, J.M., Stubbe, J.H., Distel, M.A., Hottenga, J.J., Posthuma, D., van Beijsterveldt, T.C., Hudziak, J.J., Bartels, M. & Willemsen, G. (2006) Netherlands twin register: from twins to twin families. *Twin Res Hum Genet* **9**, 849–857.
Boomsma, D.I., Willemsen, G., Sullivan, P.F., Heutink, P., Meijer, P., Sondervan, D., Kluft, C., Smit, G., Nolen, W.A., Zitman, F.G., Smit, J.H., Hoogendijk, W.J., van, D.R., de Geus, E.J. & Penninx, B.W. (2008) Genome-wide association of major depression: description of samples for the GAIN major depressive disorder study: NTR and NESDA biobank projects. *Eur J Hum Genet* **16**, 335–342.
Brocke, B., Lesch, K.P., Armbruster, D., Moser, D.A., Muller, A., Strobel, A. & Kirschbaum, C. (2010) Stathmin, a gene regulating neural plasticity, affects fear and anxiety processing in humans. *Am J Med Genet B Neuropsychiatr Genet* **153B**, 243–251.
Brown, K.M., Bujac, S.R., Mann, E.T., Campbell, D.A., Stubbins, M.J. & Blundell, J.E. (2006) Further evidence of association of OPRD1 & HTR1D polymorphisms with susceptibility to anorexia nervosa. *Biol Psychiatr* **61**, 367–373.
Bulik, C.M., Sullivan, P.F. & Kendler, K.S. (1998) Heritability of binge-eating and broadly defined bulimia nervosa. *Biol Psychiatr* **44**, 1210–1218.
Bulik, C.M., Sullivan, P.F., Tozzi, F., Furberg, H., Lichtenstein, P. & Pedersen, N.L. (2006) Prevalence, heritability, and prospective risk factors for anorexia nervosa. *Arch Gen Psychiatr* **63**, 305–312.
Bulik, C.M., Slof-Op 't Landt, M.C.T., van Furth, E.F. & Sullivan, P.F. (2007) The genetics of anorexia nervosa. *Annu Rev Nutr* **27**, 263–275.
Bulik, C.M., Thornton, L.M., Root, T.L., Pisetsky, E.M., Lichtenstein, P. & Pedersen, N.L. (2010) Understanding the relation between anorexia nervosa and bulimia nervosa in a Swedish national twin sample. *Biol Psychiatr* **67**, 71–77.
Crow, S.J., Peterson, C.B., Swanson, S.A., Raymond, N.C., Specker, S., Eckert, E.D. & Mitchell, J.E. (2009) Increased mortality in bulimia nervosa and other eating disorders. *Am J Psychiatr* **166**, 1342–1346.
Dalle Grave, R., Calugi, S. & Marchesini, G. (2009) Self-induced vomiting in eating disorders: associated features and treatment outcome. *Behav Res Ther* **47**, 680–684.
Dardennes, R.M., Zizzari, P., Tolle, V., Foulon, C., Kipman, A., Romo, L., Iancu-Gontard, D., Boni, C., Sinet, P.M., Therese, B.M., Estour, B., Mouren, M.C., Guelfi, J.D., Rouillon, F., Gorwood, P. & Epelbaum, J. (2006) Family trios analysis of common polymorphisms in the obestatin/ghrelin, BDNF and AGRP genes in patients with anorexia nervosa: association with subtype, body-mass index, severity and age of onset. *Psychoneuroendocrinol* **32**, 106–113.
DerSimonian, R. & Laird, N. (1986) Meta-analysis in clinical trials. *Control Clin Trials* **7**, 177–188.
Dmitrzak-Weglarz, M., Skibinska, M., Slopien, A., Szczepankiewicz, A., Rybakowski, F., Kramer, L., Hauser, J. & Rajewski, A. (2007) BDNF Met66 allele is associated with anorexia nervosa in the Polish population. *Psychiatr Genet* **17**, 245–246.
Ehrlich, S., Franke, L., Scherag, S., Burghardt, R., Schott, R., Schneider, N., Brockhaus, S., Hein, J., Uebelhack, R. & Lehmkuhl, U. (2010) The 5-HTTLPR polymorphism, platelet serotonin transporter activity and platelet serotonin content in underweight and weight-recovered females with anorexia nervosa. *Eur Arch Psychiatr Clin Neurosci* **260**, 483–490.
Fairburn, C.G. & Beglin, S.J. (1994) Assessment of eating disorders: interview or self-report questionnaire? *Int J Eat Disord* **16**, 363–370.
Friedel, S., Fontenla, H.F., Wermter, A.K., Geller, F., Dempfle, A., Reichwald, K., Smidt, J., Bronner, G., Konrad, K., Herpertz-Dahlmann, B., Warnke, A., Hemminger, U., Linder, M., Kiefl, H., Goldschmidt, H.P., Siegfried, W., Remschmidt, H., Hinney, A. & Hebebrand, J. (2005) Mutation screen of the brain derived neurotrophic factor gene (BDNF): identification of several genetic variants and association studies in patients with obesity, eating disorders, and attention-deficit/hyperactivity disorder. *Am J Med Genet* **132**, 96–99.
Garner, D.M., Garner, M.V. & Rosen, L.W. (1993) Anorexia nervosa 'restricters' who purge: implications for subtyping anorexia nervosa. *Int J Eat Disord* **13**, 171–185.
Grice, D.E., Halmi, K.A., Fichter, M.M., Strober, M., Woodside, D.B., Treasure, J.T., Kaplan, A.S., Magistretti, P.J., Goldman, D., Bulik, C.M., Kaye, W.H. & Berrettini, W.H. (2002) Evidence for a susceptibility gene for anorexia nervosa on chromosome 1. *Am J Hum Genet* **70**, 787–792.
Haavik, J., Blau, N. & Thony, B. (2008) Mutations in human monoamine-related neurotransmitter pathway genes. *Hum Mutat* **29**, 891–902.
Higgins, J.P. & Thompson, S.G. (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* **21**, 1539–1558.
Kaye, W. (2008) Neurobiology of anorexia and bulimia nervosa. *Physiol Behav* **94**, 121–135.
Kaye, W.H., Bailer, U.F., Frank, G.K., Wagner, A. & Henry, S.E. (2005a) Brain imaging of serotonin after recovery from anorexia and bulimia nervosa. *Physiol Behav* **86**, 15–17.
Kaye, W.H., Frank, G.K., Bailer, U.F., Henry, S.E., Meltzer, C.C., Price, J.C., Mathis, C.A. & Wagner, A. (2005b) Serotonin alterations in anorexia and bulimia nervosa: new insights from imaging studies. *Physiol Behav* **85**, 73–81.
Kim, Y.K., Lee, H.J., Yang, J.C., Hwang, J.A. & Yoon, H.K. (2009) A tryptophan hydroxylase 2 gene polymorphism is associated with panic disorder. *Behav Genet* **39**, 170–175.
Koizumi, H., Hashimoto, K., Itoh, K., Nakazato, M., Shimizu, E., Ohgake, S., Koike, K., Okamura, N., Matsushita, S., Suzuki, K., Murayama, M., Higuchi, S. & Iyo, M. (2004) Association between the brain-derived neurotrophic factor 196G/A polymorphism and eating disorders. *Am J Med Genet* **127B**, 125–127.
de Krom, M., Hendriks, J., Hillebrand, J., van Elburg, A. & Adan, R. (2006) A polymorphism in the 3′ untranslated region of the CCK gene is associated with anorexia nervosa in Dutch patients. *Psychiatr Genet* **16**, 239.
Lau, J., Ioannidis, J.P. & Schmid, C.H. (1997) Quantitative synthesis in systematic reviews. *Ann Intern Med* **127**, 820–826.
Li, J. & Ji, L. (2005) Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* **95**, 221–227.
Lim, J.E., Pinsonneault, J., Sadee, W. & Saffen, D. (2007) Tryptophan hydroxylase 2 (*TPH2*) haplotypes predict levels of *TPH2* mRNA expression in human pons. *Mol Psychiatr* **12**, 491–501.
Lucki, I. (1998) The spectrum of behaviors influenced by serotonin. *Biol Psychiatr* **44**, 151–162.
Mercader, J.M., Ribases, M., Gratacos, M., Gonzalez, J.R., Bayes, M., de Cid, R., Badia, A., Fernandez-Aranda, F. & Estivill, X. (2007) Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. *Genes Brain Behav* **6**, 706–716.

Middeldorp, C.M., Slof-Op 't Landt, M.C.T., Medland, S.E. *et al*. (2010) Anxiety and depression in children and adults:influence of serotonergic and neurotrophic genes? *Genes Brain Behav* **9**, 808–816.

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

Nyholt, D.R. (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* **74**, 765–769.

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

Pinheiro, A.P., Bulik, C.M., Thornton, L.M. *et al*. (2010) Association study of 182 candidate genes in anorexia nervosa. *Am J Med Genet B Neuropsychiatr Genet* **135B**, 1070–1080.

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

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

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

Ribases, M., Gratacos, M., Fernandez-Aranda, F. *et al*. (2004) Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations. *Hum Mol Genet* **13**, 1205–1212.

Ribases, M., Gratacos, M., Fernandez-Aranda, F. *et al*. (2005) Association of BDNF with restricting anorexia nervosa and minimum body mass index: a family-based association study of eight European populations. *Eur J Hum Genet* **13**, 428–434.

Scherag, S., Hebebrand, J. & Hinney, A. (2010) Eating disorders: the current status of molecular genetic research. *Eur Child Adolesc Psychiatr* **19**, 211–226.

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

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

Steinhausen, H.C. (2002) The outcome of anorexia nervosa in the 20th century. *Am J Psychiatr* **159**, 1284–1293.

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

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

Sullivan, P.F., Bulik, C.M. & Kendler, K.S. (1998) Genetic epidemiology of binging and vomiting. *Br J Psychiatr* **173**, 75–79.

Sullivan, P.F., de Geus, E.J., Willemsen, G. *et al*. (2009) Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. *Mol Psychiatr* **14**, 359–375.

Tsai, S.J., Hong, C.J., Liou, Y.J., Yu, Y.W., Chen, T.J., Hou, S.J. & Yen, F.C. (2009) Tryptophan hydroxylase 2 gene is associated with major depression and antidepressant treatment response. *Prog Neuropsychopharmacol Biol Psychiatr* **33**, 637–641.

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

Wade, T.D., Bulik, C.M. & Kendler, K.S. (2000) Reliability of lifetime history of bulimia nervosa. Comparison with major depression. *Br J Psychiatr* **177**, 72–76.

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

Walther, D.J. & Bader, M. (2003) A unique central tryptophan hydroxylase isoform. *Biochem Pharmacol* **66**, 1673–1680.

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

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

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

Acknowledgments

We thank the patients for their participation in this study. Other participating centers in The Netherlands were Amarum, Psychotherapy Practice H. van Agteren, Mentrum, PsyQ, GGZ Oost-Brabant, Breburggroep, GGZ Eindhoven. The study was supported by The Netherlands Organization for Scientific Research NWO/ZonMW (NWO 985-10-002, NWO/SPI 56-464-14192, NWO 480-04-004, ZonMW 911-03-016), the 'Bridge Award' (NIMH R56), the Marie Curie Research Training Network INTACT (Individually tailored stepped care for women with eating disorders; reference number: MRTN-CT-2006-035988), and the Geman Ministry of Education and Research (BMBF 01GV0602, 01GV0624, 01GV0905, 01GS0820). M. Bartels was financially supported by a senior fellowship of the EMGO+ institution. C. M. Middeldorp was financially supported by the Hersenstichting Nederland (13F05(2).47) and NWO-ZonMw (VENI grant 916-76-125).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1: Linkage disequilibrium (LD) plot for the 10 *TPH2* tagging SNPs based on HapMap. D′ values are presented, color scheme, bright red: $D' = 1$ and $LOD \geq 2$; shades of pink/red: $D' < 1$ and $LOD \geq 2$; blue: $D' = 1$ and $LOD < 2$; white: $D' < 1$ and $LOD < 2$.

Table S1: Minor allele frequencies (MAF) for each SNP in restricting AN cases of the GenED study and NTR controls.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.