Genetic determinants of eating disorders

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

Chapter 6

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

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

Abstract

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

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

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

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

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

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

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

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

Methods

Participants

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

The GenED study.

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

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

Controls

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

Netherlands Twin Registry (NTR) controls

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

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

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

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

Measures

Phenotypes

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

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

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

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

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

Genotype measurements

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

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

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

Study design

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

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

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

Statistical Analyses

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

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

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

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

Results

Phenotypic analyses

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

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

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

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

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

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

[1] Analyses in PC and PS corrected for age

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

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

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

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

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

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

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

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

Genetic analyses in twin-based controls

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

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

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

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

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

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

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

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

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

Genetic analysis in participants with an eating disorder

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

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

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

Discussion

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

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

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

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

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

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

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

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

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