Anorexia nervosa and the Val158Met polymorphism of the COMT gene: meta-analysis and new data

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Objectives **This study aimed to test the association between the Val158Met polymorphism (rs4680) of the catechol-*O*-methyl transferase gene and anorexia nervosa (AN).**

Methods **First, an association study on two cohorts (306 cases and 1009 controls from Utrecht, and 174 cases and 466 controls from Leiden/NTR) was performed. Subsequently, the results were integrated into a meta-analysis, together with all the case–control and family-based studies, which were testing the same hypothesis and were available in the literature. Altogether, eight studies (11 datasets) were included in this meta-analysis, with a total of 2021 cases, 2848 controls, and 89 informative (heterozygous) trios.**

Results **The present association studies found no association between AN and rs4680 when testing the allelic contrast [Utrecht odds ratio (OR)=1.14, *P*=0.14; Leiden OR=1.02, *P*=0.85]. There was an indication of an association under the dominant model of genetic effect in the Utrecht cohort (for the Met allele, OR=1.42, *P*=0.03). Nevertheless, the meta-analyses of both the allelic contrast and the dominant effect were nonsignificant (the allelic pooled OR=1.03, *P*=0.42 and the dominant pooled OR=1.1, *P*=0.18). The meta-analyses were performed under the fixed-effect model and there was no significant heterogeneity among the effect sizes.**

Conclusion **Meta-analytically combined evidence from the present genotypings and the literature search shows that the effect sizes are homogeneous across studies and that rs4680 is not associated with AN. *Psychiatr Genet* 22:130–136 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Anorexia nervosa (AN) is a debilitating disease, well known for its highest standardized mortality ratio among all psychiatric illnesses [with six to 10 times higher mortality rate, compared with the general population (Birmingham *et al.*, 2005; Papadopoulos *et al.*, 2009)]. Despite the seriousness of anorexia, its etiology remains elusive. Several twin and adoption studies have shown that genetic factors explain 46–78% of variance in AN (Wade *et al.*, 2000; Kortegaard *et al.*, 2001; Bulik *et al.*, 2010). A family study showed a 10-fold increase in the lifetime risk of AN for a first-degree female relative of a person affected with an eating disorder (ED) (compared with relatives of unaffected individuals) (Strober *et al.*, 2000). So far, studies on the genetic risk factors of AN have focused on candidate genes of neurotransmitter and neuropeptide pathways. The results of those studies have been inconclusive and many promising findings have not been replicated (Pinheiro *et al.*, 2010). One of the genes that were proposed to play a role in the development of AN susceptibility is catechol-*O*-methyl transferase (COMT). COMT is an enzyme responsible for the degradation of catecholamines, such as dopamine and noradrenaline (Chen *et al.*, 2004). COMT has been implicated in the pathogenesis of several mental disorders and in explaining variations in cognitive phenotypes (Mier *et al.*, 2010). In particular, rs4680 (Val/Met), a functional variant in the COMT locus, has been studied extensively. rs4680 is located in exon 3 of the gene and results in a valine-to-methionine substitution. The Met allele of this gene has been associated with a less stable

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product and, therefore, lower enzymatic activity (Lachman *et al.*, 1996), which in turn has been hypothesized to lead to higher dopamine availability (Shield *et al.*, 2004). rs4680 has been studied in mental disorders such as schizophrenia (Costas *et al.*, 2011), autism (Stergiakouli and Thapar, 2010), depression (Åberg *et al.*, 2011), and EDs (Frieling *et al.*, 2006; Mikolajczyk *et al.*, 2006). In the field of AN, initial encouraging findings (Frisch *et al.*, 2001) were not replicated (Gabrovsek *et al.*, 2004). However, the power to detect small effect sizes (ESs) in those studies was limited. The aim of the present study was to test the hypothesis of an association with more power and to provide a better estimation of the ES. To do so, we performed genotyping on two cohorts of patients with AN and controls from the Netherlands and combined the results in a meta-analysis of all the available case–control and family-based studies, which tested this same hypothesis. Not only is the statistical power of such an approach higher than that in a single-cohort study, but it also provides an insight into the heterogeneity of ESs across the studies.

Methods

Association study

Genotyping was performed on two sets of cases and controls from the Netherlands: the Utrecht cohort and the Leiden cohort.

The Utrecht study included 348 female patients with AN and 415 control individuals from the general population (obtained from the Immunogenetics and Transplantation Immunology Section of the Department of Immunohematology and Blood Transfusion, LUMC, Leiden, and referred to as control group A). In addition, the second control group for Utrecht cases (control group B) included 643 individuals (328 women), who were screened for not having psychiatric disorders and were whole-genome genotyped (Stefansson *et al.*, 2009). All cases and controls were of Dutch origin. Blood was collected from patients after referral to an ED treatment center (inpatients and outpatients, at various stages of the disease). Diagnoses, according to the Diagnostic and Statistical Manual of Mental Disorders-IV criteria, were established by experienced clinicians, using a semi-structured interview (Eating Disorder Examination; Cooper and Fairburn, 1987). Individuals for whom AN was not the primary diagnosis or those with physical illnesses, such as diabetes mellitus, were excluded.

The Leiden study included 174 cases, from 10 specialized ED centers in the Netherlands (the GenED study), and 607 unrelated female controls from the Netherlands Twin Registry (see Slof-Op't Landt *et al.*, 2011). Diagnoses of AN were established by experienced clinicians on the basis of a semi-structured interview at intake and using the self-report ED examination questionnaire (Fairburn and Beglin, 1994).

Genotyping of Utrecht and Leiden cases and controls was carried out by mass spectrometry (the homogeneous MassARRAY system; Sequenom, San Diego, California, USA) under standard conditions, except for the control group B, which was genotyped on an Illumina HumanHap 550k platform (Stefansson *et al.*, 2009).

The odds ratio (OR) of being a case was calculated at the level of alleles (allelic χ^2-test, with 1 df). The allelic contrast provides more statistical power than the genotype contrasts and indicates the effect of the allele in the population (Zintzaras and Lau, 2008).

Data were handled and analyzed using Plink (Purcell *et al.*, 2007). The study was approved by the ethical committee at UMC Utrecht (METC) and the committee for mental health institutions in the Netherlands (METiGG).

Meta-analysis

Search strategy and terms

A search for case–control and trio studies with genotype data for rs4680 was performed in Pubmed, Embase, and ICI Web of Knowledge search engines. The following terms were used (not restricted to any fields): (COMT OR Catechol-O-methyl OR Val158Met OR Val/Met OR rs4680 OR Val108/158Met OR G1947A) AND (anorexia OR eating disorders) AND (association OR gene-association OR genetic OR polymorphism) (see Supplementary Table 1, *http://links.lww.com/PG/A37* for a search flow diagram). In addition, references in the papers of interest were searched manually. The search was last updated on 11 April 2011. To be included, a study had to report genotype frequencies of rs4680 in cases with AN and controls (case–control design) or allele transmission (family-based design). Where datasets were overlapping, the largest one was selected.

Data extraction

In each study we extracted information about the author, year of publication, ethnicity of participants, sex of the participants, diagnostic status, sample size, genotype frequencies (case–control), and the Met allele transmission (trio design). Authors were contacted if the required data were not in the article.

Statistical analysis

A test for Hardy–Weinberg equilibrium was performed in each study and in the total sample [χ^2 goodness-of-fit test (1 df)].

A meta-analysis of the binary outcome was carried out with ORs as an ES. Confidence intervals (CI) of 95% were estimated. The weight of each study was determined in relation to its inverse variance.

Heterogeneity of ESs between studies was determined by the Cochran Q statistic (considered statistically significant for $P < 0.1$) (Munafo and Flint, 2004) and quantified with the I^2 metric ($I^2 = 100\% \times (Q - \mathrm{df})/Q$) (Higgins *et al.*, 2003). I^2 ranges from 0 to 100% (from low to high heterogeneity, respectively).

To determine whether the pooled ES or heterogeneity was strongly influenced by a single study, we performed an influence analysis, which recalculates the overall ES and I^2 with each study removed per calculation. To examine the stability of the pooled ES over time, the cumulative reversed analysis was performed. It recalculates the overall results each step, as the studies are added one by one in a reversed chronological order.

To examine a possibility of a publication bias, a funnel plot was included and the correlation between the sample size and the ES was calculated. These should be considered with caution due to the small number of included studies (Lau *et al.*, 2006).

Analyses were performed using R packages 'catmap' (Nicodemus, 2008) and 'meta' (Schwarzer, 2007). Package 'catmap' implements the algorithm for pooling of ESs from case–control and trio studies, as described in Kazeem and Farrall (2005).

Genetic power calculator was used for the calculation of power (Purcell *et al.*, 2003).

Results

Association study

The Utrecht cohort

In the case group and both control groups, individuals with more than 5% missing genotypes [genotyping was performed for multiple single nucleotide polymorphisms (SNPs)] were excluded. This resulted in the exclusion of 42 out of 348 cases, 49 out of 415 controls (control group A), and none out of 643 controls (control group B). In the remaining participants ($n_{cases} = 306$, $n_{controls} = 1009$), there were no missing genotypes for SNP rs4680.

The OR for association of the Met allele with a risk of AN in the allelic contrast was 1.14 (95% CI 0.95–1.37; $P = 0.14$). In addition, the dominant effect of the Met allele was tested. This produced a suggestive signal of association with an OR of 1.42 (95% CI 1.02–1.96; $P = 0.03$).

The Leiden cohort

None of the 174 cases and 80 of the 607 controls were excluded due to a threshold of 10% missed genotype calls (multiple SNPs were genotyped for these groups). Further 61 control participants were excluded due to a missed genotype call for rs4680 (88.4% genotyping success rate for rs4680), which resulted in 466 control genotypes being available for the analysis. The genotyping rate for rs4680 in the cases was 100%.

In the Leiden cohort, the OR for association of the Met allele in the allelic contrast was 1.02 (95% CI 0.8–1.31; $P = 0.85$). For the dominant effect of the Met allele, OR was 1 (95% CI 0.63–1.58; $P = 1$).

Genotypes were in Hardy–Weinberg equilibrium in all case and control groups (Table 2). To investigate the results from both cohorts further, we included them in a meta-analysis of all the studies testing this same association, which were available in the literature.

The data for two additional SNPs (rs174696 and rs165774) located in the COMT gene are available in Supplementary Table 2, *http://links.lww.com/PG/A38* (none was significantly associated).

Meta-analysis

Search results

The search and inclusion of studies are shown in the flow diagram (Supplementary Fig. S1, *http://links.lww.com/PG/A39*). Eight studies were identified as eligible for meta-analysis (including the present genotypings). A study by Gabrovsek *et al.* (2004) included four case–control cohorts and the trio part, which was not included due to an overlap with case–control groups. The samples were overlapping between Frisch *et al.* (2001) and Michaelovsky *et al.* (2005), and in Mikolajczyk *et al.* (2006) and Mikolajczyk *et al.* (2010). The larger datasets were selected. Overall, there were 10 case–control sets and one family-based set, amounting to a total of 2021 cases, 2848 controls, and 89 informative trios (i.e. with heterozygous parents). Studies' characteristics are presented in Table 1.

The test for Hardy–Weinberg equilibrium (Pearson's χ^2, df = 1) was significant for the control group in Dmitrzak-Węglarz *et al.* (2005) ($P = 0.001$; Table 2). Exclusion of this study did not materially affect the results (Supplementary Fig. S2, *http://links.lww.com/PG/A40* and Supplementary Fig. S7, *http://links.lww.com/PG/A45*).

Patients were diagnosed according to Diagnostic and Statistical Manual of Mental Disorders-IV in each study (American Psychiatric Association, 2000).

Publication bias

Visual inspection of the funnel plot revealed that one study (Michaelovsky *et al.*, 2005) was located outside of the pseudo-95% CIs, which indicates a possibility of a publication bias. Correlation between the weight and the ES was nonsignificant ($n = 11$, $r = 0.32$, $P = 0.33$; Fig. 1).

Heterogeneity and pooled effect size

A formal test for heterogeneity of ESs was nonsignificant, with a Cochrane Q statistic of 8.51 ($P = 0.58$; $I^2 = 0\%$). Therefore, meta-analysis was performed under the fixed-effect model (Mantel and Haenszel, 1959). This model assumes that differences in the ESs between studies are attributable to a sampling error and the true effect is homogeneous across populations.

The Met allele is considered the reference allele (an OR larger than 1 indicates that it is associated with an increased risk of being a case). Studies were weighed using the inverse variance method.

Meta-analysis of 11 cohorts (eight studies) in the allelic contrast resulted in a nonsignificant pooled OR of 1.03 (95% CI 0.95–1.13; $P = 0.42$) (Fig. 2).

Table 1 Characteristics of the included studies

Study	Study type	Ethnicity	Case definition	% Female cases	% Female cont.	Age in AN	Age in cont.	BMI in AN	BMI in cont.	*N* cases	*N* cont.
Karwautz *et al.* (2001)	c–c	British	DSM-IV	100	100	15.3 (3.2)[b]	27.4 (9.4)	13.1 (2.2)[c]	22.4 (3.8)	44	38[e]
Gabrovsek – Florence	c–c	Italian (Florence)	DSM-IV	99	–	18.3 (4.3)[b, d]	–	14.5 (0.3)[c, d]	–	89	83
Gabrovsek – Germany	c–c	German	DSM-IV	99	–	–	–	–	–	61	96
Gabrovsek – Milan	c–c	Italian (Milan)	DSM-IV	99	–	–	–	–	–	51	146
Gabrovsek – Spain	c–c	Spanish	DSM-IV	99	–	–	–	–	–	65	93
Dmitrzak-Węglarz *et al.* (2005)	c–c	Polish	DSM-IV ICD-10	100	100	18.5 (3.2)	34.9 (10)	–	–	91	135
Mikolajczyk *et al.* (2010)	c–c	Polish	DSM-IV ICD-10	100	100	22.07 (3.76)	22.85 (3.95)	15.05 (2.54)	20.65 (1.25)	61	105
Pinheiro *et al.* (2010)	c–c	European descent	DSM-IV	100	100	27.1 (8.8)	26.3 (8.3)	14.7 (2.5)4	22.1 (1.8)	1079	677
Leiden cohort 2011[a]	c–c	Dutch	DSM-IV	100	100	28 (10)	23.4 (12.1)	16.7 (2.9)	22.6 (14.1)	174	466
Utrecht cohort 2011[a]	c–c	Dutch	DSM-IV	100	33	24.13 (4.74)	–	14.65 (1.72)[c]	–	306	1009
										Inf. Trios	
Michaelovsky *et al.* (2005)	trio	Israeli	DSM-IV	100	–	15.96 (2.33)	–	14.71 (1.71)	–	89	–

AN, anorexia nervosa; BMI, body mass index; c–c, case–control design; cont., controls; DSM, Diagnostic and Statistical Manual of Mental Disorders; Inf. Trios, informative trios (i.e. with heterozygous parents).
[a]Present genotyping.
[b]Age at onset of AN.
[c]Lifetime minimum BMI.
[d]Age and BMI are the average of all the cohorts present in Gabrovsek *et al.* (2004).
[e]Controls were sisters of the probands.

Table 2 Genotypes (counts and frequencies) and Hardy–Weinberg equilibrium in each study

	Cases (%)		Cont. (%)		Cases (%)			Cont. (%)				
Study	Met allele (A)	Val allele (G)	Met allele (A)	Val allele (G)	Met/Met (A/A)	Val/Met (G/A)	Val/Val (G/G)	Met/Met (A/A)	Val/Met (G/A)	Val/Val (G/G)	HWE cases	HWE cont.
Karwautz *et al.* (2001)	45 (51.1)	43 (48.9)	36 (47.4)	40 (52.6)	12 (27.3)	21 (47.7)	11 (25)	8 (21.1)	20 (52.6)	10 (26.3)	0.77	0.73
Gabrovsek – Florence	80 (44.9)	98 (55.1)	78 (47)	88 (53)	19 (21.3)	42 (47.2)	28 (31.5)	17 (20.5)	44 (53)	22 (26.5)	0.66	0.56
Gabrovsek – Germany	70 (57.4)	52 (42.6)	107 (55.7)	85 (44.3)	22 (36.1)	26 (42.6)	13 (21.3)	31 (32.3)	45 (46.9)	20 (20.8)	0.32	0.62
Gabrovsek – Milan	50 (49)	52 (51)	143 (49)	149 (51)	15 (29.4)	20 (39.2)	16 (31.4)	40 (27.4)	63 (43.2)	43 (29.5)	0.12	0.1
Gabrovsek – Spain	66 (50.8)	64 (49.2)	90 (48.4)	96 (51.6)	18 (27.7)	30 (46.2)	17 (26.2)	23 (24.7)	44 (47.3)	26 (28)	0.54	0.61
Dmitrzak-Węglarz *et al.* (2005)	85 (46.7)	97 (53.3)	136 (50.4)	134 (49.6)	20 (22)	45 (49.5)	26 (28.6)	25 (18.5)	86 (63.7)	24 (17.8)	0.95	0.001
Mikolajczyk *et al.* (2010)	63 (51.6)	59 (48.4)	118 (56.2)	92 (43.8)	15 (24.6)	33 (54.1)	13 (21.3)	35 (33.3)	48 (45.7)	22 (21)	0.52	0.46
Pinheiro *et al.* (2010)	1100 (51)	1058 (49)	665 (49.1)	689 (50.9)	286 (26.5)	528 (48.9)	265 (24.6)	176 (26)	313 (46.2)	188 (27.8)	0.49	0.05
Leiden cohort 2011[a]	201 (57.8)	147 (42.2)	533 (57.2)	399 (42.8)	58 (33.3)	85 (48.9)	31 (17.8)	150 (32.2)	233 (50)	83 (17.8)	0.99	0.65
Utrecht cohort 2011[a]	336 (54.9)	276 (45.1)	1040 (51.5)	978 (48.5)	86 (28.1)	164 (53.6)	56 (18.3)	274 (27.2)	492 (48.8)	243 (24.1)	0.15	0.45
Total												
	Trans. Met	Untrans. Met										
Michaelovsky *et al.* (2005)	35 (39.3)	54 (60.7)										

Cont., controls; HWE, *P* for Hardy–Weinberg equilibrium test (χ^2 goodness-of-fit test, 1 df); (Un)trans, (un)transmitted allele.
[a]Present genotyping.

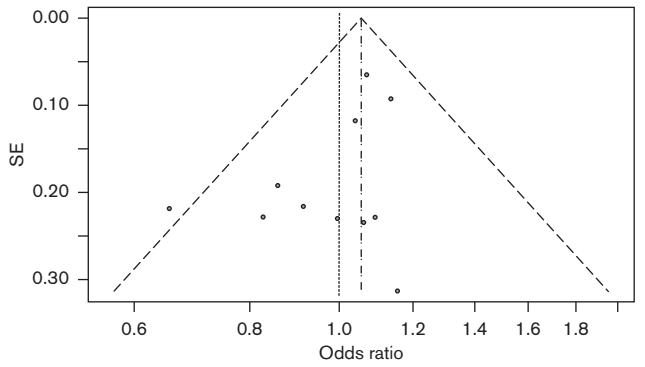
The reversed cumulative analysis and the influence analysis show that the pooled results were consistent over time and were not overly impacted by any of the single datasets (Supplementary Fig. S2, *http://links.lww.com/PG/A40* and Supplementary Fig. S3, *http://links.lww.com/PG/A41*).

As the ES in the present association study was larger when testing for the dominant effect of the Met allele, meta-analysis was also performed under this model of genetic association [without the family-based study (Michaelovsky *et al.*, 2005)].

The Cochran's Q statistic for heterogeneity was 9.21, which was nonsignificant ($P = 0.42$, $I^2 = 2.2\%$). The fixed-effect model of meta-analysis was used. The pooled OR for 10 case–control cohorts (seven studies) in the meta-analysis of the dominant effect of the Met allele was also nonsignificant and equalled 1.1 (95% CI 0.95–1.27; $P = 0.18$) (Supplementary Fig. S4, *http://links.lww.com/PG/A42*). The reversed cumulative and the influence plots are available in the supplementary materials, again showing that the pooled results were consistent over time and not overly impacted by any of the datasets (Supplementary Fig. S5, *http://links.lww.com/PG/A43* and Supplementary Fig. S6, *http://links.lww.com/PG/A44*).

With a total sample size of $n_{cases} = 2021$ and $n_{controls} = 2848$, the Met allele frequency of 48% in the European populations, and assuming the α-level at 0.05, we were able to detect an OR of 1.13 for the risk homozygote and an OR of 1.26 for the heterozygote, with 80% statistical power (for the allelic contrast). The true statistical power was larger, due to the contribution of the family-based studies (89 heterozygous trios).

Fig. 1

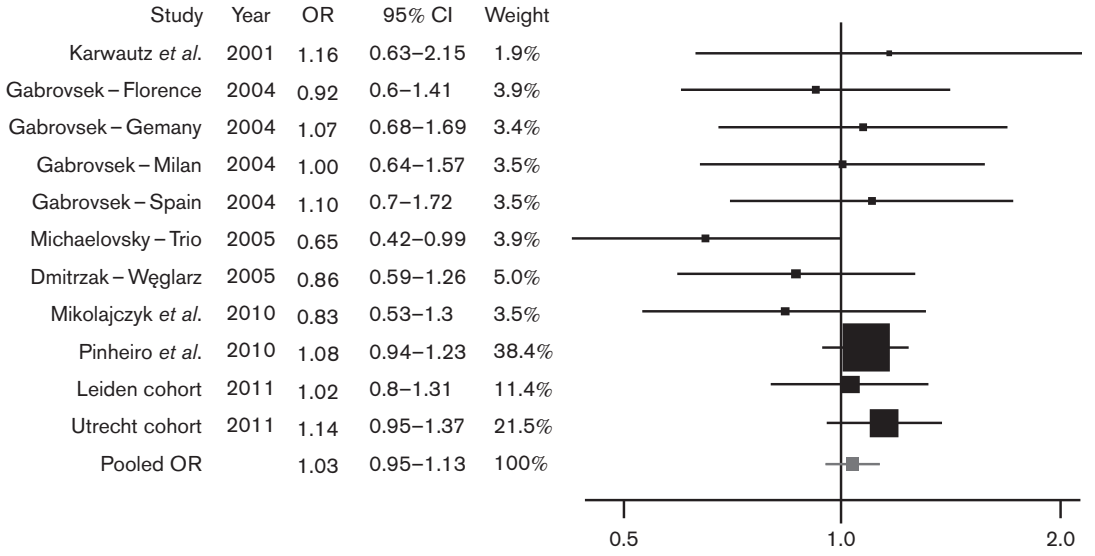


Funnel plot for 11 datasets (10 case–control, one family-based). Each dot represents a single cohort. Location outside the delineated triangle (pseudo-95% confidence intervals) suggests a publication bias.

Discussion

Since the early days of genetic association studies in psychiatry, a functional polymorphism rs4680 of the COMT gene was considered a candidate locus for association with AN (Gorwood *et al.*, 1998). So far, the results in the literature have not been conclusive and have been based on relatively small sample sizes. In the present paper, in order to test the hypothesis of association, we combined genotyping and association testing of cases and controls from the Netherlands with a meta-analysis of another six studies (nine datasets). The results of the association testing were

Fig. 2



Forest plot showing ORs in the allelic contrast (the Met allele as the risk variant). The weight of the studies is reflected by the size of squares and whiskers represent 95% CIs. The pooled OR under the fixed-effect model. I^2, as a measure of heterogeneity, was 0%. CI, confidence interval; OR, odds ratio.

nonsignificant for the allelic contrast in the Utrecht and Leiden cohorts, but an indication of an association was observed under the dominant model of a genetic effect (for the Met allele) in the Utrecht cohort. However, a meta-analysis, which in total included 2021 cases, 2848 controls, and 89 informative trios, revealed an absence of an association under both genetic models. There was little evidence of heterogeneity of ESs among the individual studies. Overall, the quality of the studies included was good. The location of one family-based study outside of the 95% CIs in the funnel plot (Michaelovsky *et al.*, 2005) might suggest a publication bias but it also could be reflective of the different ethnical composition of this sample. Whereas all the other studies included European participants, this one included Israeli participants. The weight of this study was 3.9% and it had little influence on the overall results.

Three studies in the literature have reported a significant association between rs4680 and AN. Two of them were based on an Israeli population (they used an overlapping sample) – this may explain why the results differ from the other studies, which used European populations (Frisch *et al.*, 2001; Michaelovsky *et al.*, 2005). Mikolajczyk *et al.* (2006) reported, in a small study, a significant association, but this was accompanied by violation of the Hardy–Weinberg equilibrium. The ES appeared to be unusually large (OR > 8). This calls the reliability of this finding into question, especially as in a later study, which used an overlapping sample, the association between rs4680 and AN was nonsignificant (Mikolajczyk *et al.*, 2010). Only the latter study was used in the meta-analysis.

By combining multiple studies, a meta-analysis is able to estimate the ES of interest with greater power and accuracy than any single study could do. It also enables the reader to observe the changes in ESs of single studies over time, drawing attention to a possible winner's curse [overestimation of the ES in the first study reporting a given association (Nakaoka and Inoue, 2009)]. A meta-analysis can utilize unpublished studies and expose possible publication biases. Providing that sufficient amounts of data are available, potential confounders and moderators of the association can be investigated. A meta-analysis is not a remedy for the methodological shortcomings of the studies included, but it provides tools for detection of such studies. Phenotypic heterogeneity (or misspecification) is a serious issue, especially in psychiatrics, where the definitions of phenotypes often vary across studies and the diagnoses are not unambiguous. By combining many studies, a meta-analysis can decrease the impact of misdefined phenotypes, but, for practical reasons, this may come at a price of losing some phenotypic specificity (e.g. analyzing patients with AN in total, rather than dividing into anorexia nervosa restricting type and anorexia nervosa bingeing-purging type). Meta-analyses of genetic data should be particularly vigilant for the problems of population stratification (ethnical differences between cases and controls may lead to a spurious association signal) and genetic heterogeneity (the effects of genetic variants may actually vary across populations).

The present design was relatively well powered for a single SNP analysis, although an association with a very small ES (OR < 1.1) cannot be ruled out. A possibility of more complex scenarios of associations, including gene × environment and epistatic interactions, should be acknowledged. Especially, a gene × environment interaction, if not detected and accounted for, can greatly reduce the overall power of the meta-analysis. The current design did not allow for testing of such interactions.

Because of limited data availability, the analysis did not include subtyping of the AN phenotype (AN restrictive and AN bingeing/purging). In addition, possible effect moderators, such as sex and ethnicity, were not accounted for in the analysis. However, given the fact that almost all cases were women and all studies except one (this study had a weight of 3.9%; Fig. 2) included European participants, the possibility of confounding was unlikely.

In conclusion, the present meta-analysis provides strong evidence that SNP rs4680 does not have a main effect on susceptibility to AN.

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Conflicts of interest

There are no conflicts of interest.

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