ORIGINAL INVESTIGATION

The Val66Met polymorphism of the BDNF gene in anorexia nervosa: New data and a meta-analysis

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Abstract

Objectives. The Val66Met polymorphism (rs6265) of the BDNF gene is a non-synonymous polymorphism, previously associated with anorexia nervosa (AN). *Methods.* We genotyped rs6265 in 235 patients with AN and 643 controls. Furthermore, we performed a systematic review of all case–control and family-based studies testing this SNP in AN, and combined the results in a meta-analysis. *Results.* The results of the case–control study were non-significant. For the meta-analysis, nine studies were identified ($n_{cases} = 2,767$; $n_{controls} = 3,322$, $n_{trios} = 53$) and included. Primarily, the analyses indicated an association with OR of 1.11 ($P = 0.024$) in the allelic contrast, and OR of 1.14 ($P = 0.025$) for the dominant effect of the Met allele. However, additional analyses revealed that the first published study (from those included in the meta-analysis) overly influenced the pooled effect size (possibly due to a phenomenon known as a winner's curse). When this case–control study was replaced by a trio study ($n_{trios} = 293$) performed on a largely overlapping sample, the effect size became smaller and non-significant, both for the allelic contrast (OR = 1.07, $P = 0.156$) and the dominant effect (OR = 1.07, $P = 0.319$). The quality of included studies was good and there was no significant heterogeneity across the effect sizes. *Conclusions.* Our analyses indicate that the BDNF Val66Met variant is not associated with AN at detectable levels.

Key words: *Anorexia nervosa, polymorphism, brain-derived neurotrophic factor (BDNF), eating disorders, genetics*

Introduction

Anorexia nervosa (AN) is a chronic and potentially lethal disorder. It is known for having the highest standardized mortality ratio of all psychiatric illnesses (mortality rate is 6–10 times higher than in a reference population (Birmingham et al. 2005; Papadopoulos et al. 2009)). Despite this seriousness, the etiology of AN remains unclear. Twin and adoption studies estimate that 46 to 78% of variance in AN is attributable to genetic factors (Wade et al. 2000; Kortegaard et al. 2001; Bulik et al. 2010) and family studies have determined a 10-fold increase in lifetime risk of developing AN for a first-degree female relative of a proband with AN (compared to relatives of unaffected individuals) (Strober et al. 2000). The main focus of genetic association studies in AN has

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been on candidate genes from neuropeptide and neurotransmitter pathways. The yield of those studies is very limited as many of the initially positive findings failed to replicate (Pinheiro et al. 2010). One way to deal with inconsistency of results is to perform a meta-analysis in which studies that test the same hypothesis are combined, thereby providing insight into potential sources of heterogeneity between them. The statistical power to detect an association is increased and conclusions are more solid, as compared to individual studies.

A gene that has been recurrently proposed as AN susceptibility locus is the brain-derived neurotrophic factor (*BDNF*) gene. BDNF is crucial for proliferation, differentiation and survival of neurons during development (Hyman et al. 1991), for neuronal plasticity and connectivity in adults (including activity-dependent forms of synaptic plasticity) (Waterhouse and Xu 2009), and it affects expression of many other genes (Berton et al. 2006). BDNF is involved in energy balance and food intake regulation (Lommatzsch et al. 2005), in peripheral regulation of metabolism (Pedersen et al. 2009), and it plays a substantial role in reward and stress processing (Narita et al. 2003; Cirulli and Alleva 2009). Patients with AN have decreased levels of BDNF in serum (Brandys et al. 2011). All these findings make the BDNF gene an interesting candidate for studies of AN.

The Val66Met (rs6265) is a non-synonymous polymorphism leading to a valine to methionine substitution in the proBDNF product (precursor of the mature BDNF protein). At the molecular level, the Met allele results in lowered intracellular trafficking and activity-dependent secretion of the BDNF protein (Egan et al. 2003; Chiaruttini et al. 2009), likely without changing its constitutive secretion (Chen et al. 2006).

The Val66Met has frequently been studied in the context of (neurodevelopmental) psychiatric disorders and other behavioral traits. This polymorphism has been implicated in structural variation in human brain at the level of prefrontal cortex (PFC) and hippocampus (with the Met allele carriers having lower volumes (Pezawas et al. 2004; Szeszko et al. 2005)). A meta-analysis of the variant's effects in major depressive disorder showed the Met allele to be associated with the condition (only in males, both in Asians and Europeans) (Verhagen et al. 2008). A meta-analysis of studies in ADHD refuted a putative involvement of the variant in the pathogenesis of that disorder (Sánchez-Mora et al. 2009). In schizophrenia, one meta-analysis rejected the association (Kawashima et al. 2009), whereas another one confirmed it (Gratacòs et al. 2007). Furthermore, a meta-analysis performed by Frustaci et al.

revealed the association of the Met allele with personality traits related to anxiety (the Met allele carriers displayed lower neuroticism than non-carriers) (Frustaci et al. 2008). On the other hand, a recent, large study found that Met allele homozygotes score higher on harm avoidance (a phenotype strongly correlated with neuroticism) (Montag et al. 2010).

Genetic variants associated with body mass index (BMI) are of particular interest for the AN phenotype, since extremely low body weight is the primary symptom of this illness (Brandys et al. 2009). Gunstad et al. (2006) reported that subjects from a healthy population who carry the Val allele had higher BMI than Met/Met homozygotes. Another study, however, found that the Met allele in women was associated with obesity (Beckers et al. 2008). Contrary to Beckers et al. and in line with Gunstad et al., a large study showed that the Met allele was associated with lower BMI in the general population (Shugart et al. 2009). Finally, a genome-wide study of over 30,000 subjects also found the Met allele to be associated with decreased BMI in the general population (Thorleifsson et al. 2009). Despite a very large number of published studies, there remains uncertainty regarding the strength and nature of any of these associations in relation to AN.

In 2007, a meta-analysis of case–control studies investigating the Val66Met polymorphism in four psychiatric diseases – substance-related disorders (SRD), EDs (AN and bulimia nervosa taken together), schizophrenia and mood disorders – implied that there is an association between the first three and rs6265 (but not with mood disorders) (Gratacòs et al. 2007). The direction of the association was opposite for EDs and substance related disorders. Whereas the Met allele increased susceptibility to ED (the fixed-effect pooled OR was 1.36 for a dominant model of genetic effect), it was also found to have a protective effect against SRD. With regards to EDs, the meta-analysis of Gratacos et al. (2007) was based on five datasets, with a total of 1733 cases and 1811 controls. A limitation of this study was that it did not include family-based association studies and that it considered all EDs together, whereas heterogeneity of this category is well-known (Wonderlich et al. 2007). Furthermore, since the time that the study was published, a substantial amount of data on the association between rs6265 and AN have become available. Therefore, we decided to study a more homogeneous phenotype within eating disorders – which, in our case, was AN – and include all the available data. Subsequently, a gene-association study on a sample of females with AN and healthy controls was performed and the results were combined with a meta-analysis of case–control and family based studies on the

Val66Met polymorphism in subjects with AN (in total, combining 9 datasets).

Subjects and methods

Association study

SNP rs6265 (Val66Met) of the BDNF gene was genotyped in a sample of female cases with AN ($n = 235$), all with ascertained Dutch descent (patients are asked whether all of their grandparents were of Dutch origin). Subjects were recruited for the study after referral to an ED treatment center (in- and outpatients, at various stages of the disease). Diagnoses were established by experienced clinicians according to DSM-IV criteria, using a semi-structured interview (Eating Disorder Examination (Cooper and Fairburn 1987)). Cases for whom AN was not the primary diagnosis or with physical illnesses such as diabetes mellitus were excluded. Eighty-one cases were overlapping with the sample used in de Krom et al. (de Krom et al. 2005) and they were excluded. Genotyping was performed by mass spectrometry (the homogeneous MassARRAY system; Sequenom, San Diego, CA) using standard conditions.

The control group consisted of 643 Dutch individuals (328 females) screened against psychiatric disorders (Stefansson et al. 2009). Healthy controls were genotyped on the Illumina HumanHap 550k platform.

Data were handled and analyzed with Plink (Purcell et al. 2007). The study has been approved by the Medical Ethical Committee at UMC Utrecht, The Netherlands.

Meta-analysis

Search strategy and terms. We searched for case–control and trio studies through Pubmed, Embase and ICI Web of Knowledge search engines, using the following terms (not restricted to any fields): (bdnf OR brain derived OR Val66Met OR Val/Met OR rs6265 OR 196G/A) AND (anorexia OR eating disorders) AND (association OR gene-association OR polymorphism). Additionally, the HuGE Navigator database was checked and a manual search through references in identified articles performed. The search was last updated on 21 October 2010. To be included, a study had to report genotype frequencies of rs6265 in cases with AN – be it the restricting or binging/purging type – and healthy controls (case–control design), or allele transmission (trio design). In case of overlapping datasets the larger one was selected. The meta-analysis was performed in compliance with the PRISMA statement (Moher et al. 2009).

Data extraction. The following information was extracted from each study: author, year of publication, ethnicity of participants, gender of participants, diagnostic status, sample size, genotype frequencies (case–control), the Met allele transmission (trio design). Authors were contacted if the required data were not in the article.

Statistical analysis. In the primary analyses, the odds ratios (ORs) were calculated at the level of alleles. Allele contrasts provide more statistical power than genotype contrasts and indicate the effect of the allele in the population (Zintzaras and Lau 2008). Since the dominant effect of the Met allele in EDs has also been suggested in the literature (Gratacòs et al. 2007), we tested for it in the additional analysis (discarding the trio studies).

Hardy–Weinberg equilibrium was tested in each study and in the total sample with a χ^2 goodness-of-fit test (1 df).

A meta-analysis of the binary outcome was performed with ORs as an effect size (ES). The Met allele was considered the risk allele. 95% confidence intervals (CI) were estimated where appropriate. Weight of each study was determined in relation to its inverse variance.

Three rounds of analyses were performed. First, using the allelic contrast with the complete dataset of nine studies (eight case–control, one trio); second, testing the dominant effect of the Met allele in eight case–control studies (the dominant effect in EDs was suggested in the literature (Gratacòs et al. 2007)); third, analysis in the allelic contrast with replacement of one case–control study (Ribases et al. 2004a) by the trio study on partially overlapping cases (Ribases et al. 2004b).

Heterogeneity of ESs between studies was estimated by Cochran Q-statistic (considered statistically significant for $P < 0.1$) (Munafo and Flint 2004) and quantified with I^2 metric ($I^2 = 100\%^*(Q - df)/Q$) (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 were strongly influenced by a single study we performed an influence analysis, which recalculates overall ES and I^2 with each study removed per calculation.

The ES in the first published report on a given association is often larger than in the later studies of the same hypothesis (Nakaoka and Inoue 2009). A reversed cumulative meta-analysis recalculates overall results as the studies are added one by one, in a reversed chronological order. It was used to investigate changes of the pooled ES, as particular ESs are reported over time.

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

Analyses were performed with 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 calculation of power (Purcell et al. 2003).

Results

Association study

Two hundred and thirty-five female cases (114 AN restricting type; 112 AN bingeing/purging type; nine AN with an undetermined subtype) were successfully genotyped (100% call rate). Healthy controls ($n = 643$), genotyped on the Illumina HumanHap 550k platform, also had a 100% call rate for this SNP. Case and control genotypes were in Hardy–Weinberg equilibrium (Table II).

In this sample, SNP rs6265 was not associated with AN under any model of genetic effect (OR = 1.058 in the allelic contrast, $P = 0.67$, 1df).

Meta-analysis

Search results. The flow diagram of the search is available in the supplementary materials (Supplementary Figure 1 available online).

The search identified eight eligible studies (seven case–control and one family-based). Additionally, the results of the current genotyping were added. One sample set was studied both in a case–control and a trio design (88% overlapping cases between Ribases et al. (2004b) and Ribases et al. (2004a)). Only the data from the case–control approach were used in the primary analysis (due to its larger sample size). In the second round of analysis, the trio study was included instead of the case–control one.

In all studies, patients were diagnosed according to DSM-IV (American Psychiatric Association 2000). The total sample size was 2,767 cases, 3,322 controls and 53 informative (with heterozygous parents) trios in the first analysis and 2,014 cases, 2,812 controls and 346 informative trios in the second analysis. All case and control groups were in Hardy–Weinberg equilibrium. Details of included studies are available in Tables I and II.

Table I. Characteristics of the studies included in the meta-analysis.

Study	Study type	Ethnicity	Case definition	ANR/ ANBP/AN	% female cases	% female cont.	Age in AN	Age in cont.	BMI in AN	BMI in cont.	N cases	N cont.
Ribases et al. 2004a	c-c	European, multicenter	DSM-IV	347/308/98	96	95.5	23.6 (8.9)	14.2 (2.3)[d]			753	510
Koizumi et al. 2004	c-c	Japanese	DSM-IV	36/36	100	100	25 (6)[c]	27 (6)			72	222
Friedel et al. 2005	c-c	German	DSM-IV		93.1	51	26.7 (11.5)	24.7 (2.6)	17.2 (3.7)	21.9 (1.1)	118	96
de Krom et al. 2005	c-c	Dutch Caucasian	DSM-IV		100	~50	26.7 (2.7)		15.9 (2.4)		195	580
Rybakowski et al. 2007	c-c	Polish	DSM-IV		100	100	17.6 (2.5)	20.7 (1.2)	15.4 (2.2)	20.1 (2.3)	144	86
Pinheiro et al. 2010	c-c	European descent	DSM-IV	415/664	100	100	27.1 (8.8)	26.3 (8.3)	14.7 (2.5)[d]	22.1 (1.8)	1079	677
Slof-Op't Landt et al. 2011	c-c	Dutch Caucasian	DSM-IV	102/49/20	100	100	28 (10)		16.7 (2.9)		171	508
Brandys et al. 2011a	c-c	Dutch Caucasian	DSM-IV	114/112/9	100	51	22.2 (4.9)		16.2 (1.8)		235	643
											Inf. trios	
Dardennes et al. 2007	trio	French	DSM-IV	30/23	100	–	N/A	–			53	
Ribases et al. 2004b[b]	trio	European, multicenter	DSM-IV	158/113/22	97.3[c]	–	20.8 (6.7)	–	13.66 (2.1)[d]		293	

[a]Present genotyping; [b]study used in the second round of analyses; [c]for all cases with eating disorders; [d]lifetime min BMI; c-c, case–control design; cont., controls; Inf. Trios, informative trios (i.e. with heterozygous parents); ANR/ANBP/AN, number of patients with AN restrictive, binge-purging or undetermined type

Table II. Counts and frequencies of genotypes and Hardy–Weinberg equilibrium in the included studies.

Study	Cases (%)		Cont. (%)		Cases (%)			Cont. (%)			HWE cases	HWE cont.
	Met (A)	Val (G)	Met (A)	Val (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)		
Ribases et al. 2004a	324 (21.5)	1182 (78.5)	175 (17.2)	845 (82.8)	28 (3.7)	268 (35.6)	457 (60.7)	15 (2.9)	145 (28.4)	350 (68.6)	0.14	0.99
Koizumi et al. 2004	62 (43.1)	82 (56.9)	184 (41.4)	260 (58.6)	10 (13.9)	42 (58.3)	20 (27.8)	42 (18.9)	100 (45)	80 (36)	0.11	0.28
Friedel et al. 2005	42 (17.8)	194 (82.2)	35 (18.2)	157 (81.8)	5 (4.2)	32 (27.1)	81 (68.6)	1 (1)	33 (34.4)	62 (64.6)	0.48	0.13
de Krom et al. 2005	82 (21)	308 (79)	226 (19.5)	934 (80.5)	12 (6.2)	58 (29.7)	125 (64.1)	23 (4)	180 (31)	377 (65)	0.15	0.79
Rybakowski et al. 2007	50 (17.4)	238 (82.6)	25 (14.5)	147 (85.5)	3 (2.1)	44 (30.6)	97 (67.4)	3 (3.5)	19 (22.1)	64 (74.4)	0.44	0.30
Pinheiro et al. 2010	445 (20.6)	1713 (79.4)	269 (19.9)	1085 (80.1)	42 (3.9)	361 (33.5)	676 (62.7)	26 (3.8)	217 (32.1)	434 (64.1)	0.47	0.86
Slof-Op't Landt et al. 2011	65 (19)	277 (81)	192 (18.9)	824 (81.1)	8 (4.7)	49 (28.7)	114 (66.7)	19 (3.7)	154 (30.3)	335 (65.9)	0.37	0.80
Brandys et al. 2011[a]	99 (21.1)	371 (78.9)	259 (20.1)	1027 (79.9)	11 (4.7)	77 (32.8)	147 (62.6)	29 (4.5)	201 (31.3)	413 (64.2)	0.82	0.47
Total	1169 (21.1)	4365 (78.9)	1365 (20.5)	5279 (79.5)	119 (4.3)	931 (33.6)	1717 (62.1)	158 (4.8)	1049 (31.6)	2115 (63.7)	0.61	0.06
	Trans. met	Untr. met										
Dardennes et al. 2007	28 (52.8)	25 (47.2)										
Ribases et al. 2004b[b]	154 (52.6)	139 (47.4)										

[a]Present genotyping; [b]study included in the second analyses; c-c, case–control design; cont., controls; (Un)trans., (un)transmitted allele; HWE, P for Hardy–Weinberg equilibrium test (χ^2 goodness-of-fit test, 1 df).

Heterogeneity. The hypothesis of no heterogeneity between ESs was not rejected (Cochrane Q–statistic = 4.31, $I^2 = 0\%$, $P = 0.83$; for all nine studies). Likewise, there was no significant heterogeneity in the analysis of the dominant effect of the Met allele in eight case–control studies ($Q = 7.2$, $I^2 = 2.8\%$, $P = 0.408$), nor in the second round of analysis (in which the case–control study (Ribases et al. 2004a) was replaced by the family-based study (Ribases et al. 2004b)). Therefore, the fixed-effect model of meta-analysis was applied (Mantel and Haenszel 1959). The fixed-effect model assumes that differences in the ESs between studies are attributable to a sampling error and the true effect is homogeneous across populations.

Publication bias. Visual inspection of the funnel plot does not suggest presence of a publication bias (Figure 1), which was confirmed by a non-significant result of the linear regression test of the funnel plot asymmetry ($P = 0.725$, for the case–control studies only). A correlation between the sample size and the effect size was non-significant ($r = 0.17$, $P = 0.688$ for cases and controls; $r = 0.26$, $P = 0.537$ for cases only).

Pooled effect size. The OR larger than 1 indicates that the Met allele is associated with increased risk of being a case. The inverse variance weighing method and the fixed-effect model of meta-analysis were used in all analyses.

First analysis: the allelic contrast and the dominant effect

In the analysis of one trio and eight case–control studies, the pooled OR was 1.11 (95%CI; 1.014–1.223; $P = 0.024$) (Figure 2).

To further investigate the nature of the association, the influence and the reversed cumulative meta-analyses were performed on the complete set of nine studies (using the allelic count contrast in the case–control studies).

In the reversed cumulative meta-analysis, studies are added from the most recent to the earliest one, and the pooled ES is recalculated per each iteration. It revealed that the association remains non-significant until the earliest study (Ribases et al. 2004a) is added (Figure 3). Also the influence analysis, which shows the overall results with one study removed per each calculation, confirmed this (Supplementary Table S1). Both analyses show that the overall association between rs6265 and AN is attributable predominantly to the first study by Ribases et al. (2004a).

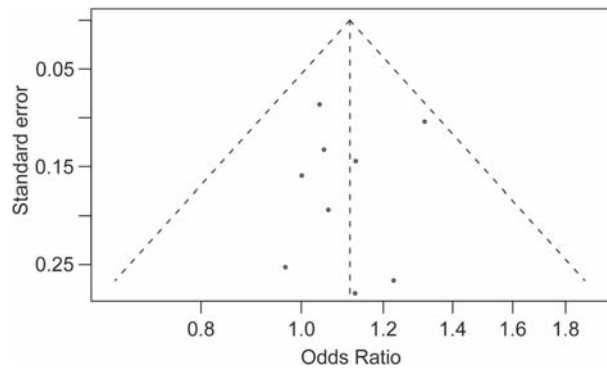The additional analysis of the dominant effect of the Met allele (Met/Met +Val/Met genotypes vs.

Figure 1. Funnel plot for nine studies (eight case–control and one family-based). Each dot represents one study. Location outside the delineated triangle (pseudo 95% confidence intervals) suggests a publication bias.

Val/Val), in eight case–control studies, resulted in the pooled OR of 1.138 (95%CI; 1.017–1.275; $P=0.025$) (Supplementary Figure S2). There, the first study was also largely responsible for driving the pooled association signal (Supplementary Figure S3; Supplementary Table S2).

Second analysis: the allelic contrast and the dominant effect

In view of the results from the influence and cumulative analyses we decided to run the meta-analysis again, this time replacing the case–control study from Ribases et al. (2004a) with a trio study by Ribases et al. (2004b) (cases between those studies are overlapping, therefore only one can be used at a time). This step was motivated by reasoning that a family-based study provides a better protection from effects of population stratification than a case–control design (at a slight loss of power, however).

In this analysis, a combined ES for nine studies was lower and it did not reach statistical significance (Figures 4 and 5), with the pooled OR of 1.071 ($P=0.156$; 95%CI; 0.974–1.179). Heterogeneity indicators were non-significant and lower than in the first analysis – Cochrane's Q was 1.809 ($I^2=0\%$; $P=0.986$).

Consistently, testing of the dominant effect without the case–control study from Ribases et al. (2004a) (seven studies included) resulted in a non-significant pooled OR of 1.068 ($P=0.319$; 95%CI; 0.9387; 1.2141). There was no significant heterogeneity ($Q=2.98$, $I^2=0\%$, $P=0.811$) (Supplementary Figures 4 and 5; Supplementary Table 3).

Given the total number of cases and controls ($n=2,676$ and $n=3,322$, respectively), a frequency of the Met allele in the European populations of 20%, and setting the alpha at 0.05, there was 80% power to detect an association with OR of 1.135 for the heterozygote and OR of 1.27 for the Met/Met homozygote (in the allelic contrast). For the dominant effect of the Met allele, with the same assumptions, there was 80% power for OR of 1.16 (for the Met allele carriers). Exclusion of the first published study on rs6265 in AN decreased power to 70%, under both scenarios. The real statistical power was slightly larger, since these calculations do not take into account the contribution from the family-based studies.

Discussion

The present study investigated a possible association between rs6265 polymorphism of the BDNF gene and AN. A meta-analytical framework was used to combine results from case–control and trio studies, with addition of new data from genotyping of the Utrecht cohort of patients with AN and healthy
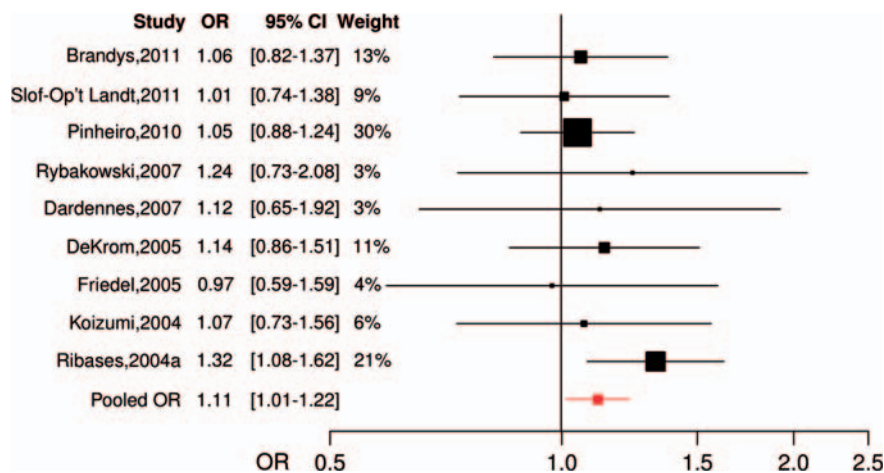

Figure 2. Forest plot presenting ORs for anorexia nervosa in the allelic contrast (the Met allele as the risk variant). Dardennes et al. 2007 is a family-based study, the remaining ones have a case–control design. The weight of each study is reflected by the size of squares, and whiskers represent 95% confidence intervals. The pooled OR is based on the fixed effect model. I^2, as a measure of heterogeneity, equals 0%.
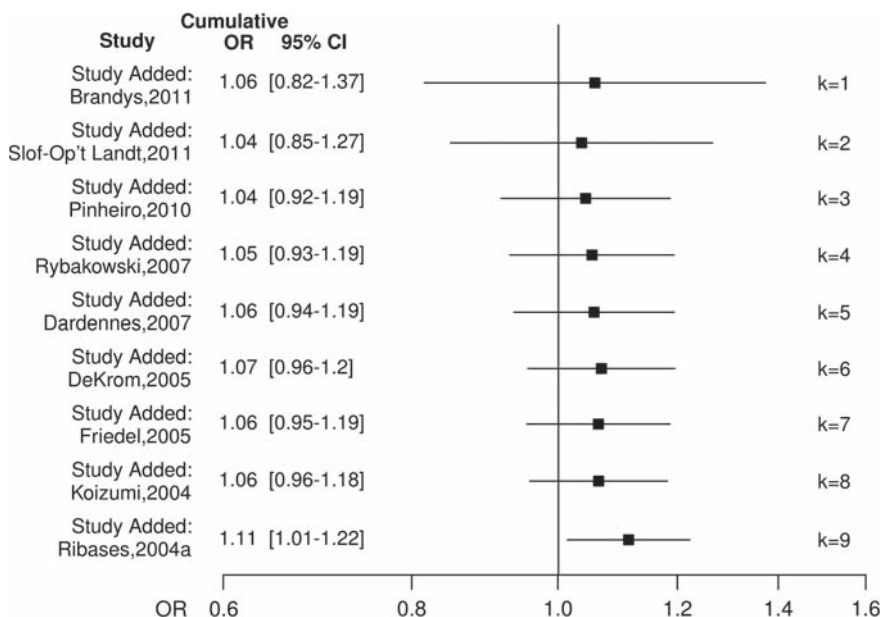
Figure 3. Cumulative meta-analysis. Studies are added in a reversed chronological order; each row represents the pooled OR for all studies added up to this point (based on the fixed-effect model and for the allelic contrast). The whiskers represent (cumulative) 95% confidence intervals. I^2, as a measure of heterogeneity, was 0% at every step.

controls. This meta-analysis included the largest number of cases tested for association with a single SNP in AN up to date.

Primary results showed that the association in question has an OR of 1.11 (a two-sided P of 0.024, in the allelic contrast). All ESs, except for one, were in the same direction (with the Met allele increasing the risk) and there was no significant heterogeneity among them. The OR became slightly more pronounced when the dominant effect of the Met allele was tested (without the trio study (Dardennes et al. 2007), in that case).

Nevertheless, the influential and cumulative analyses revealed that the pooled ES was strongly influenced by the earliest study (Ribases et al. 2004a). With the first study removed, the pooled OR became closer to unity and non-significant (in accordance with the so-called "winners curse", i.e. inflation of the ES in the first study in a group of studies investigating the same phenomenon (Nakaoka and Inoue 2009)). These observations suggest that the ES reported in the multicenter case–control study from Ribases et al. (2004a) might have been overestimated. By replacing this study with the trio study (Ribases
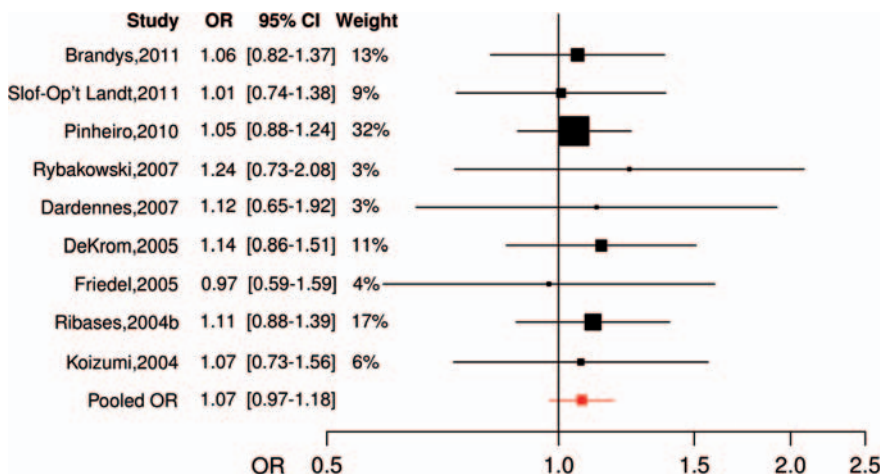

Figure 4. Forest plot presenting ORs for anorexia nervosa in the allelic contrast (the Met allele as the risk variant). Here, the case–control study from Ribases et al. (2004a) has been replaced with the trio study performed on a partially overlapping sample. Ribases et al. (2004b) and Dardennes et al. (2007) are family-based, the remaining studies have a case–control design. The weight of each study is reflected by the size of squares, and whiskers represent 95% confidence intervals. The pooled OR is based on the fixed effect model. I^2, as a measure of heterogeneity, equals 0%.
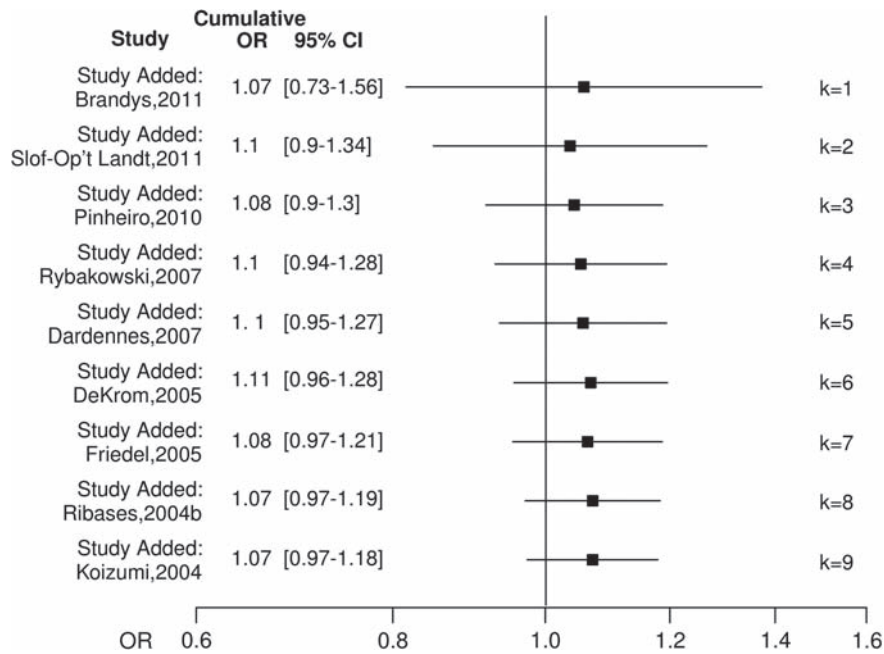
Figure 5. Cumulative meta-analysis. Studies are added in a reversed chronological order; each row represents the pooled OR for all studies added to this point (based on the fixed-effect model and for the allelic contrast). Here, the case–control study from Ribases et al. (2004a) has been replaced with the trio study performed on a partially overlapping sample. Ribases et al. (2004b) and Dardennes et al. (2007) are family-based, the remaining studies have a case–control design. The whiskers represent (cumulative) 95% confidence intervals. I^2, as a measure of heterogeneity, was 0% at every step.

et al. 2004b) of a largely overlapping set of subjects, the possibility of a cryptic population stratification was reduced. This step resulted in a shrinkage of the overall ES to a non-significant level and showed that the control group may be responsible for a slight overestimation of the ES in the case–control study (Ribases et al. 2004a) (regardless the fact that the controls were matched by ethnicity and sex). Frequencies of rs6265 polymorphism are very variable across populations (Petryshen et al. 2009) and a possibility of undetected population stratification is high. Similarly, when the meta-analysis was performed for the dominant effect of the Met allele, but with exclusion of the case–control study from Ribases et al. (2004a) (thus on seven case–control studies only), the association did not reach statistical significance. Very little heterogeneity in all scenarios of analysis suggests that the quality of evidence was good.

The current results for AN are different from the results reported for the whole category of EDs in the meta-analysis published in 2007 (Gratacòs et al. 2007). There, the ES was much higher and significant, with OR of 1.36 (95%CI; 1.18–1.57) (for the dominant model of the Met allele). This association signal might have been driven predominantly by EDs other than AN, i.e. bulimia nervosa, and ED not otherwise specified. A meta-analysis of those phenotypes – in separation from AN – is warranted. A promising (but practically challenging) approach to it is to use the classifications based on latent classes, latent profiles or taxometric analyses, rather than arbitrarily chosen sub- and intermediate phenotypes (Williamson et al. 2002; Wonderlich et al. 2007; Eddy et al. 2009). This strategy has already been employed with some success to the 5HTTLPR polymorphism in EDs (Steiger et al. 2009).

The present results do not exclude the possibility that genetic variation at the BDNF locus contributes to development of AN. It is still possible that the association lies within a range of very small effect sizes (OR < 1.1), and that it was not detectable with the present statistical power. A sample size of over 30,000 subjects would be necessary to achieve 80% power with OR of 1.071 (this ES was estimated in the second analysis). Furthermore, possibilities of more complex scenarios of association should be kept in mind. Epistatic and gene × environment interactions have not been addressed in the studies of AN and BDNF, and examples of such interactions in the different fields are abundant. For instance, two studies found no main effect of Val66-Met on neuroticism scores (NEO-PI-R (Costa and McCrae 1995)), but people with the Met allele and at least one copy of the DAT 9-repeat allele had lower neuroticism and harm avoidance (Hunnerkopf et al. 2007), and carriers of the Met allele in combination with 5-HTTLPR LL allele scored higher on neuroticism (Terracciano et al. 2010) (in the same study the Met allele had an increasing effect on introversion). These examples illustrate the difficulty

of finding a single SNP association, which may be obscured by the fact that the direction of the Met allele's effect might be modulated by other genetic variants. Moreover, gene × environment interactions are suspected to contribute substantially to the variance of mental illnesses (Uher 2009; Campbell et al. 2010). A gene × environment interaction that is not accounted for may greatly reduce the power to detect the association. Finally, rather than with a single SNP, the disease may be associated with certain haplotypes within the BDNF locus.

One of the weaknesses of meta-analyses is that it often has to sacrifice some phenotypic specificity of individual studies to be able to combine many of them. Due to insufficient information in 3 studies (and hence decreased sample size), we did not distinguish between AN subtypes (restricting and bingeing/purging) in the main analysis. The results of an exploratory analysis on AN subtypes are available in the Supplementary Table S5.

Another limitation is the fact that only nine studies were included in the meta-analysis. This number did not allow for analyses of potential moderators, such as ethnicity or sex. Nevertheless, almost all cases included in the meta-analysis were female, thus sex, as a confounder, should not play a major role. Furthermore, only one of the included studies was performed on Asian participants (weight of the study was 3.7%), whereas the rest included predominantly Caucasian subjects.

In conclusion, the present meta-analysis included eight studies from literature and new genotype data from patients with AN and healthy controls from Utrecht (The Netherlands). The quality of analyzed evidence was good and the study was relatively well-powered. The results showed that the supposed association between rs6265 and AN became non-significant when the first published study was excluded (or replaced by a trio study on a partially overlapping case set). This association has been considered as one of the more robust findings in the genetic association studies of AN, but we could not confirm it in the present meta-analysis.

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Statement of Interest

None to declare.

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Supplementary material available online

Supplementary materials