Brain Derived Neurotrophic Factor Gene Val66Met and –270C/T polymorphisms and personality traits predisposing to anorexia nervosa

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Abstract

OBJECTIVES: Polymorphisms in BDNF gene has been proposed, as susceptibility loci for stress-related psychiatric disorders. Several lines of molecular and biochemical evidence point to the role of BDNF in anorexia nervosa (AN). Presonality traits may constitute the intermediate phenotypes between genes and vulnerability to AN.

METHODS: BDNF Val66Met and –270C/T polymorphisms were genotyped in 149 patients with AN and 100 healthy control females. Temperamental traits in all subjects were measured with Temperament and Character Inventory. First in case-control analysis, we assessed, if analyzed genotypes confer risk for AN. Next, the association of BDNF gene variants with personality dimensions in patients and control subjects was analyzed.

RESULTS: No significant differences between patients with anorexia nervosa and controls in frequency of genotypes and alleles were observed. AN patients with Met allele showed higher Harm avoidance (ANOVA F=4.70; p=0.03) than Val/Val homozygotes. AN patients, who carried the T allele of BDNF –270C/T polymorphism showed higher Persistence (Anova F=4.04; p<0.05) and Harm avoidance (ANOVA F=7.93; p=0.006) than C/C homozygotes, however after correction for multiple testing only the latter association remained statistically significant. No significant relationship between Val/Met 66 genotype and –270C/T genotype with personality was observed in healthy females.

CONCLUSIONS: These results may suggest, that BDNF –270 C/T polymorphism may influence the personality trait associated with higher risk of AN.

ORIGINAL ARTICLE

INTRODUCTION

Anorexia nervosa (AN) is severe psychiatric disorder, affecting mainly young females. The outcome of the disorder might be poor in 50% of cases [36], and mortality rate reaches 5–10% [37]. The aetiology of AN has been regarded as multifactorial, with significant contribution of both genetic and environmental influences [12]. Twin studies suggest that heritability of AN might be higher than 50% [5].

Brain derived neurotrophic factor (BDNF) is widely expressed in the brain, and has been shown to regulate neuronal development and modulate synaptic plasticity [18]. Recently it was also established as an important factor in the energy homeostasis [23] and its role in stress-related psychiatric disorders is highly probable [10].

In several studies, the serum level of BDNF has been lower in patients with acute AN, than in healthy controls [26,25], and this result was not normalized, after partial weight recovery [27].

The BDNF gene has been mapped to chromosome 11p12-p14 [16]. In some [29,30,32], but not all studies [13], [8] several polymorphic sites within this gene has been associated with AN. A single nucleotide polymorphism (SNP) in the ‘5 prodomain region of the BDNF gene, resulting in valine to methionine substitution in codon 66 (BDNF Val66Met), has been associated with lower depolarization-induced production of BDNF and memory function [11]. Hariri *et al.* found that Met carriers exhibited diminished hippocampal engagement in comparison with Val homozygotes during verbal episodic memory task [17]. Recently, increased anxiety-related behaviors were reported in 66 Met/Met homozygous mice [5]. The data concerning the functional role of –270 C/T SNP polymorphism are not so plentiful, however some studies may suggest the association of this variant with neuroanatomical changes in the brain [1].

There is possiblity, that diagnostic criteria of DSM-IV and/or ICD-10 might not be the optimal object of genetic association studies [7]. The genetic background of complex disorders, such as AN might be better explained, when some simple dimensional constructs, called endophenotypes, or intermediate phenotypes are introduced [15]. It was proposed, that personality dimensions may become useful endophenotypes of some psychiatric disorders [4].

In many previous studies of personality in AN, the psychobiological model of Cloninger and collegues was used [6]. Results point to the significant differences between AN patients and healthy control females, especially with regard to temperamental dimensions of Harm avoidance and Persistence, which are elevated in patients with AN [20,33].

Previous reports also suggest an association between BDNF coding variants and some personality dimensions, mainly neuroticism [34] and trait-anxiety [22] in healthy subjects; however not all studies brought positive results [39,38].

To clarify whether two BDNF polymorphisms: Val66Met and –270 C/T increase risk of anorexia nervosa and influence personality traits associated with the susceptibility to develop the disorder, we compared frequency of genotypes in AN patients and controls and subsequently analyzed temperamental dimensions of TCI in AN patients and healthy controls with different genotypic variants of BDNF gene.

MATERIALS AND METHODS

One hundered and forty nine AN patiens and 100 healthy controls participated in the study. Written informed consent was obtained from all participants, and the study protocol was approved by the local ethical committee. In adolescent patients parental assent was also obtained. All experiments were conducted in accordance with the Declaration of Helsinki. The anorexia nervosa patients (mean age 17.6 years±2.9) were hospitalized in the Department of Child and Adolescent Psychiatry, Poznan University of Medical Sciences in Poland. Diagnoses were established according to DSM-IV [2] criteria with structured interview (SCID-P) and confirmed by senior researcher. The control females (mean age 20.8 years±1.2) came from the same geographical region of Poland, which shows high ethnic homogeneity. Control subjects have never been diagnosed with any psychiatric disorder, and have never received any psychiatric treatment. All controls have also negative result of the screening questionnaire for eating disorders (EAT-26) [14]. Because design of the study was to assess genetic variants in relation to personality, control group comprised only adult (>18 years) individuals, who are eligible to sign the informed consent themselves.

Assessments of personality traits with validated earlier [33] Polish version of Temperament and Character Inventory [6] (240 questions version) was performed in all subjects. The TCI is a self-report instrument of yes/no answers assessing the personality dimensions: Novelty Seeking (40 items), Harm Avoidance (35 items), Reward Dependence (24 items), Persistence (8 items), Self-Directedness (44 items), Cooperativeness (42 items), Self-Transcendence (33 items). All patients were assessed within three weeks from admission.

DNA was isolated from the peripheral blood leukocytes with salting out procedure [24]. The SNP polymorphism of BDNF gene is located in position 198 of the coding sequence, and is characterized by the substitution of adenine with guanine (A/G). Polymorphic variant has functional significance and results in the substitution of valine to methionine in aminoacid sequence. The amplification of the polymorphic site was performed, according to the methods described by Neves-Pereira *et al.* [28]. The PCR product of 197 base-pairs (bp) was digested with restrictive enzyme. The fragments were separated on 2% agarose gel, and were visualized with ethidium bromide. The uncut product size was 197 bp allele A (Met), and allele G

(Val) comprised the cut bands of 124 and 73 bp. The other SNP polymorphism of BDNF gene is located in position –270 of the promoter sequence, and is characterized by the substitution of cytosine with thymine (C/T). No functional significance of the variant has been established. The amplification of the polymorphic site was performed, according to the methods described by Kunugi *et al.* [21]. The PCR product (223 bp) was digested with restrictive enzyme. The fragments were separated on 3.5% agarose gel, and were visualized with ethidium bromide. The cut bands of 127, 78 and 18 bp indicated the presence of allele C, and allele T comprised the cut bands of 127, 63 and 33 bp.

Distribution of genotypes in patients and in healthy controls was checked for concordance with Hardy-Weinberg equilibrium. The frequency of genotypes in cases and controls were compared with Chi-square test with significance level of p=0.05. The analyses of the quantitative variables were performed with ANOVA test. Initially, age, height and weight were compared with diagnosis as independent variable. Secondly, the analysis of the relationship between genotypes and personality traits in AN patients and healthy controls was performed with genotypes as independent variables and dimensions of personality as dependent variables. Only temperamental traits, mainly influenced with genetic factors were analysed. The subjects were grouped according to the carrier status of Met allele (Val/Met 66 polymorphisms) and T allele (–270 C/T polymorphism). To control for multiple testing (comparison of genotypes with multiple personality traits) – the significance level was adjusted to p=0.0125. Analyses were performed with SPSS statistical package for Windows [35]. The power analysis was performed with internet-based on-line power calculator (www.stat.ubc.ca).

RESULTS

Patients with anorexia nervosa presented with significant differences from control subjects in all anthropometric characteristics, and showed lower age, weight, height and BMI than controls (Table 1).

The distribution of the BDNF Val/Met 66 polymorphism in both study groups and –270C/T polymorphism in control subjects were in Hardy-Weinberg equilibrium, however –270 C/T polymorphism in AN patients showed deviation from HWE. Power to detect a significant effect in the case-control study was 32% and 10%, respectively in analyses of Val/Met 66 and –270 C/T polymorphisms. We did not observe differences in the frequency of the two BDNF genotypes between AN patients and control females. The detailed results were presented in Table 2.

Examination of the relationship between analyzed genotypes and personality dimensions showed that BDNF Val/Met 66 polymorphism may be associated with personality traits in AN patients. Carriers of Met allele showed higher Harm avoidance (ANOVA p=0.03) than Val/Val homozygotes. No significant association between Val/Met 66 polymorphism and personality was observed in healthy control subjects. The detailed results are presented in Table 3. Similarily, no association between –270 C/T genotype and personality was observed in healthy controls. However, patients with anorexia nervosa, who carried the T allele showed significantly higher Persistence (ANOVA p<0.05) and Harm avoidance (ANOVA p=0.006) than C/C homozygotes. The detailed results are presented in Table 4. Finally, after the introduction of Bonferroni adjusted p-level, only the association between –270 C/T polymorphism and Harm avoidance, in AN patients met criteria of statistical significance.

Table 1. Antropometric characteristics of anorexia nervosa (AN) patients and healthy control subjects (HC).

Variable		Mean±SD	Range	ANOVA F	p-value
Age (yrs.)	**HC**	20.7±1.24	19.00–25.00	138.494	<0.01
	AN	17.58±2.50	11.00–25.00		
Height (cm)	**HC**	168.80±7.61	156.00–189.00	23.664	<0.01
	AN	163.99±6.30	142.00–179.00		
Weight (kg)	**HC**	59.98±9.73	45.00–95.00	227.267	<0.01
	AN	41.48±7.56	25.70–75.00		
BMI (kg/m^2)	**HC**	20.96±2.27	17.71–29.98	296.991	<0.01
	AN	15.37±2.24	10.75–24.49		

In columns means ± standard deviations, ranges, Anova F test results, and their significance were presented.

Table 2. Distribution of 66 Val/Met and –270 C/T BDNF genotypes in anorexia nervosa patients (AN) and healthy controls (HC).

		BDNF 66 Val/Met polymorphism			HWE
		Val/Val	Val/Met	Met/Met	
Study group	HC	64	19	3	Chi^2=1.06; p=0.30
		74.4%	22.1%	3.5%	
	AN	97	44	3	Chi^2=0.61; p=0.44
		67.4%	30.6%	2.1%	
		Chi2=2.20; p=0.33			
		BDNF –270 C/T polymorphism			
		C/C	C/T	T/T	
Study group	HC	85	5	0	Chi^2=0.07; p=0.79
		94.4%	5.6%	0%	
	AN	125	6	1	Chi^2=6.78; p<0.01
		94.7%	4.5%	0.8%	
		Chi^2=0.79; p=0.67			

In columns number (and percentage below) of subjects with different genotypes were reported. Right column shows test of concordance with Hardy-Weinberg Equilibrium (HWE). Below Chi-square test of association between each genotype and diagnosis of AN was presented.

DISCUSSION

In this study we did not observe the association between polymorphisms of BDNF gene and the diagnosis of anorexia nervosa in Polish population. However, given the typical effect size of genetic polymorphisms associated with psychiatric diagnoses, this result should be interpreted cautiously, and considered preliminary. Tentative relationship of BDNF gene variants with personality dimensions, which may predispose to the development of AN was observed.

Association studies of BDNF Val/Met 66 polymorphism in eating disorders brought conflicting results [13], however the largest case-control study of 1 142 participants showed that Met66 allele may increase the risk of different eating disorders, i.e. anorexia nervosa of both types, and bulimia nervosa [30]. This observation was later confirmed with family based design [32]. It was proposed, that, –270C allele may increase the risk of bulimic phenotype of eating disorders in some European populations [30], however these results were not confirmed in subsequent family-based design study [32]. Our preliminary results did not confirm the reported associations with the diagnosis of AN, however the study sample was underpowered to detect the significant effect.

In anorexia nervosa patients with T allele of –270C/T polymorphism higher Harm avoidance was observed, which is described as a trait associated with increased risk of AN [20]. In contrast to Val/Met 66 polymorphisms, this genetic variant has not been previously associated with personality dimensions. The –270C/T polymorphism was described as associated with late onset Alzheimer's disease [22], and our study may indicate it is also associated with personality trait predisposing to other psychiatric disorders. Although, there are some data, that –270C/T variant may have some functional role, we also can not exclude the possibility, that observed effect is caused by another polymorphic site, which is in linkage disequilibrium with the analyzed variant.

Association of Val/Met 66 polymorphism with higher Harm avoidance and –270 C/T polymorphism with higher Persistence in AN patients did not reach the level of statitstical significance. In previous studies of temperamental traits in eating disorders, Harm avoidance was considered an universal risk factor for all ED, and Persistence was probably more pronounced in patients with restrictive type pathology [21,33]. BDNF Met66

Table 3. Personality dimensions in healthy control subjects (HC) and anorexia nervosa patients (AN) with different genotypes of BDNF Val/Met 66 polymorphism.

Personality dimension	Genotype	HC			AN		
		Mean±SD	ANOVA F	p-value	Mean±SD	ANOVA F	p-value
Persistence	Val/Val	3.88±1.86	0.058	0.810	4.75±1.69	0.027	0.869
	Val/Met + Met/Met	3.75±2.21			4.81±1.92		
Novelty seeking	Val/Val	18.92±6.82	0.294	0.590	18.19±5.33	0.150	0.700
	Val/Met + Met/Met	19.94±6.33			17.76±5.39		
Harm avoidance	Val/Val	17.58±6.93	0.025	0.875	20.32±6.18	4.703	0.032
	Val/Met + Met/Met	17.29±4.63			23.06±5.99		
Reward dependence	Val/Val	15.33±3.69	0.531	0.469	15.82±3.34	1.314	0.254
	Val/Met + Met/Met	16.06±3.01			14.94±4.26		

Number of subjects in HC group: Val/Val (n=64) and Val/Met plus Met/Met (n=22); in AN group Val/Val (n=97) and Val/Met plus Met/Met (n=47). In columns means± standard deviations, Anova F test results, and their significance were presented.

Table 4. Personality dimensions in healthy control subjects (HC) and anorexia nervosa patients (AN) with different genotypes of BDNF –270 C/T polymorphism.

Personality dimension	Genotype	HC			AN		
		Mean±SD	ANOVA F	p-value	Mean±SD	ANOVA F	p-value
Persistence	C/C	3.73±1.81	3.711	0.058	4.72±1.74	4.037	0.047
	C/T + T/T	5.40±2.70			6.17±.75		
Novelty seeking	C/C	19.64±6.65	1.323	0.254	18.22±5.51	0.644	0.424
	C/T + T/T	15.75±4.35			16.20±4.49		
Harm avoidance	C/C	17.42±6.39	0.080	0.779	20.52±6.08	7.927	0.006
	C/T + T/T	16.50±4.51			27.67±5.05		
Reward dependence	C/C	15.38±3.43	2.361	0.129	15.51±3.72	0.409	0.524
	C/T + T/T	17.80±2.77			16.60±3.91		

Number of subjects in HC group: C/C (n=85) and C/T plus T/T (n=5); and in AN patients group: C/C (n=125) and C/T plus T/T (n=7). In columns means± standard deviations, Anova F test results, and their significance were presented.

allele was associated with increased HA and risk for anxiety and depression [20], however the association of Val66 with temperamental trait of neuroticism [5] and anxiety-related traits were also reported [23]. It was previously demonstrated, that anxious [3] and neurotic [10] personality traits may predispose to the development of AN. Indirect confirmation of the association between Met66 allele and anxiety-related traits comes also from animal model, in which mice homozygous for Met66 allele showed increased anxiety-related behaviors, when placed in stressful settings [6]. Moreover, it was recently reported, that polymorphism in BDNF-specific receptor gene (neurotrophic tyrosine kinase receptor type 2) show association both with anorexia nervosa and Harm avoidance [31]. Thus, Met66 allele of BDNF gene may transdiagnostically increase the risk of different internalizing disorders by influencing the temperamental dimensions, such as neuroticism and HA. Our results did not confirm the relationship between Met66 allele and Harm avoidance, however in larger sample of patients, the difference between genotypes may become significant.

This study has several limitations. We recruited only inpatients with AN, and the results may not be generalized to subjects with less severe symptoms. The study was underpowered to detect the significant differences in relation to diagnosis, however published data may be relevant in subsequent meta-analysis of BDNF polymorphisms in AN. Lack of association between 66Met polymorphism and –270T polymorphism with some personality traits in healthy subjects, may be related to higher heterogeneity of these traits in general population. Despite the fact, that patients and controls were recruited from the same geographical region, we can not exclude the possibility of population stratification. The significant difference in age between patients and control subjects resulted from design of the study, which required for healthy controls to be capable to sign the informed consent themselves.

In conclusion, these results suggest that in patients with AN, T allele of –270 C/T polymorphism may influence trait of Harm avoidance, which may predispose to the development of anorexia nervosa. The tentative associations between Val/Met 66 polymorphism with Harm avoidance, and –270 C/T polymorphism with Persistence do not reach the adjusted level of significance. Thus, the role of temperamental traits, as intermediate phenotype between genotype and clinical syndrome gathered partial support. Because of small study sample we were not able to confirm the association between BDNF polymorphisms and AN diagnosis in Polish population, however due to the lack of power no definitive conclusions could be made. Our results should be considered preliminary and require confirmation in a larger sample of patients.

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REFERENCES

1 Agartz I, Sedvall GC, Terenius L, Kulle B, Frigessi A, Hall H and Jonsson EG. BDNF gene variants and brain morphology in schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 2006; **141**(5): 513–23.
2 American Psychiatric Association., Diagnostic and statistical manual of mental disorders : DSM-IV,4th edn., American Psychiatric Association, 1994.
3 Anderluh MB, Tchanturia K, Rabe-Hesketh S and Treasure J. Childhood obsessive-compulsive personality traits in adult women with eating disorders: defining a broader eating disorder phenotype. Am J Psychiatry. 2003; **160**(2): 242–7.

4 Benjamin J, Ebstein RP and Belmaker RH. Genes for human personality traits: „endophenotypes" of psychiatric disorders? World J Biol Psychiatry. 2001; **2**(2): 54–7.
5 Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL and Lee FS. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science. 2006; **314**(5796): 140–3.
6 Cloninger CR, Svrakic DM and Przybeck TR. A psychobiological model of temperament and character. Arch Gen Psychiatry. 1993; **50**(12): 975–90.
7 Collier DA and Treasure JL. The aetiology of eating disorders. Br J Psychiatry. 2004; **185**: 363–5.
8 de Krom M, Bakker SC, Hendriks J, van Elburg A, Hoogendoorn M, Verduijn W, Sinke R, Kahn R and Adan RA. Polymorphisms in the brain-derived neurotrophic factor gene are not associated with either anorexia nervosa or schizophrenia in Dutch patients. Psychiatr Genet. 2005; **15**(2): 81.
9 Diaz-Marsa M, Carrasco JL and Saiz J. A study of temperament and personality in anorexia and bulimia nervosa. J Personal Disord. 2000; **14**(4): 352–9.
10 Duman RS and Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry. 2006; **59**(12): 1116–27.
11 Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B and Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003; **112**(2): 257–69.
12 Fairburn CG, Cooper Z, Doll HA and Welch SL. Risk factors for anorexia nervosa: three integrated case-control comparisons. Arch Gen Psychiatry. 1999; **56**(5): 468–76.
13 Friedel S, Horro FF, Wermter AK, Geller F, Dempfle A, Reichwald K, Smidt J, Bronner G, Konrad K, Herpertz-Dahlmann B, Warnke A, Hemminger U, Linder M, Kiefl H, Goldschmidt HP, Siegfried W, Remschmidt H, Hinney A and Hebebrand J. Mutation screen of the brain derived neurotrophic factor gene (BDNF): identification of several genetic variants and association studies in patients with obesity, eating disorders, and attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet. 2005; **132**(1): 96–9.
14 Garner DM, Olmsted MP, Bohr Y and Garfinkel PE. The eating attitudes test: psychometric features and clinical correlates. Psychol Med. 1982; **12**(4): 871–8.
15 Gottesman, II and Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry. 2003; **160**(4): 636–45.
16 Hanson IM, Seawright A and van Heyningen V. The human BDNF gene maps between FSHB and HVBS1 at the boundary of 11p13-p14. Genomics. 1992; **13**(4): 1331–3.
17 Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF and Weinberger DR. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci. 2003; **23**(17): 6690–4.
18 Huang EJ and Reichardt LF. Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem. 2003; **72**: 609–42.
19 Jiang X, Xu K, Hoberman J, Tian F, Marko AJ, Waheed JF, Harris CR, Marini AM, Enoch MA and Lipsky RH. BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. Neuropsychopharmacology. 2005; **30**(7): 1353–61.
20 Klump KL, Bulik CM, Pollice C, Halmi KA, Fichter MM, Berrettini WH, Devlin B, Strober M, Kaplan A, Woodside DB, Treasure J, Shabbout M, Lilenfeld LR, Plotnicov KH and Kaye WH. Temperament and character in women with anorexia nervosa. J Nerv Ment Dis. 2000; **188**(9): 559–67.
21 Kunugi H, Ueki A, Otsuka M, Isse K, Hirasawa H, Kato N, Nabika T, Kobayashi S and Nanko S. A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. Mol Psychiatry. 2001; **6**(1): 83–6.
22 Lang UE, Hellweg R, Kalus P, Bajbouj M, Lenzen KP, Sander T, Kunz D and Gallinat J. Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology (Berl). 2005; **180**(1): 95–9.
23 Lebrun B, Bariohay B, Moyse E and Jean A. Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. Auton Neurosci. 2006; **126–127**: 30–8.
24 Miller SA, Dykes DD and Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; **16**(3): 1215.
25 Monteleone P, Tortorella A, Martiadis V, Serritella C, Fuschino A and Maj M. Opposite changes in the serum brain-derived neurotrophic factor in anorexia nervosa and obesity. Psychosom Med. 2004; **66**(5): 744–8.
26 Nakazato M, Hashimoto K, Shimizu E, Kumakiri C, Koizumi H, Okamura N, Mitsumori M, Komatsu N and Iyo M. Decreased levels of serum brain-derived neurotrophic factor in female patients with eating disorders. Biol Psychiatry. 2003; **54**(4): 485–90.
27 Nakazato M, Hashimoto K, Yoshimura K, Hashimoto T, Shimizu E and Iyo M. No change between the serum brain-derived neurotrophic factor in female patients with anorexia nervosa before and after partial weight recovery. Prog Neuropsychopharmacol Biol Psychiatry. 2006; **30**(6): 1117–21.
28 Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F and Kennedy JL. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. Am J Hum Genet. 2002; **71**(3): 651–5.
29 Ribases M, Gratacos M, Armengol L, de Cid R, Badia A, Jimenez L, Solano R, Vallejo J, Fernandez F and Estivill X. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. Mol Psychiatry. 2003; **8**(8): 745–51.
30 Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, Cavallini MC, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Wagner G, Treasure J, Collier DA and Estivill X. Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations. Hum Mol Genet. 2004; **13**(12): 1205–12.
31 Ribases M, Gratacos M, Badia A, Jimenez L, Solano R, Vallejo J, Fernandez-Aranda F and Estivill X. Contribution of NTRK2 to the genetic susceptibility to anorexia nervosa, harm avoidance and minimum body mass index. Mol Psychiatry. 2005; **10**(9): 851–60.
32 Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, Cristina Cavallini M, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Tomori M, Wagner G, Treasure J, Collier DA and Estivill X. Association of BDNF with restricting anorexia nervosa and minimum body mass index: a family-based association study of eight European populations. Eur J Hum Genet. 2005; **13**(4): 428–34.
33 Rybakowski F, Slopien A, Zakrzewska M, Hornowska E and Rajewski A. Temperament and Character Inventory (TCI) in adolescents with anorexia nervosa. Acta Neuropsychiatrica. 2004; **16**(3): 169–174.
34 Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB and Burmeister M. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology. 2003; **28**(2): 397–401.
35 SPSS, Statistical Package for Social Sciences, 2001.
36 Steinhausen HC. The outcome of anorexia nervosa in the 20th century. Am J Psychiatry. 2002; **159**(8): 1284–93.
37 Sullivan PF. Mortality in anorexia nervosa. Am J Psychiatry. 1995; **152**(7): 1073–4.
38 Tochigi M, Otowa T, Suga M, Rogers M, Minato T, Yamasue H, Kasai K, Kato N and Sasaki T. No evidence for an association between the BDNF Val66Met polymorphism and schizophrenia or personality traits. Schizophr Res. 2006; **87**(1–3): 45–7.
39 Willis-Owen SA, Fullerton J, Surtees PG, Wainwright NW, Miller S and Flint J. The Val66Met coding variant of the brain-derived neurotrophic factor (BDNF) gene does not contribute toward variation in the personality trait neuroticism. Biol Psychiatry. 2005; **58**(9): 738–42.