Association between brain-derived neurotrophic factor Val66Met and obesity in children and adolescents

Marijana Skledar[a1], Matea Nikolac[b1], Katarina Dodig-Curkovic[c], Mario Curkovic[d], Fran Borovecki [e], Nela Pivac [b*]

[a]National Public Health Institute for Zagreb County, Ulica Grada Vukovara 72/V, HR-1000 Zagreb, Croatia
[b]Division of Molecular Medicine, Rudjer Boskovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia
[c]Department of Child and Adolescent Psychiatry, University Health Centre Osijek, Josipa Huttlera 4, 31000 Osijek, Croatia
[d]Family Health Centre, School of Medicine, Park Kralja Petra Kresimira 4/6, 31000 Osijek, Croatia
[e]Department for Functional Genomics, Center for Translational and Clinical Research, University of Zagreb School of Medicine, Salata 2, and University Hospital Center Zagreb, Kispaticeva 6, HR-10000 Zagreb, Croatia

*Corresponding author: Tel: +385 1 4571 207; Fax: + 385 1 456 1010; E-mail address: npivac@irb.hr (N. Pivac)
[1]These authors contributed equally to this work.

Abstract
Obesity in children and adolescents is a worldwide health problem, characterized by various somatic, psychosocial and psychiatric complications, and is often associated with adult obesity and related complications. Brain-derived neurotrophic factor (BDNF) is a neurotrophin with important roles in feeding behavior, food intake regulation, energy metabolism and weight control. A common polymorphism of the BDNF genotype (Val66Met) has been associated with various forms of eating disorders, alterations in body mass index (BMI) values and obesity in adult populations. The aim of this study was to determine the association between the gene variants of the BDNF Val66Met polymorphism and obesity in 300 healthy Caucasian children and adolescents of the same ethnic background of Croatian origin, subdivided according to the BMI percentile, but without any form of eating disorders. The frequency of the Met/Met, Met/Val and Val/Val genotypes, Met and Val alleles, and Met carriers (the combined Met/Met and Met/Val genotypes versus the homozygous Val/Val genotype) differed significantly between underweight, normal weight, overweight and obese children, and the presence of one or two Met alleles contributed to this significant effect. These results showed for the first time the significant association between the presence of one or two Met alleles and obesity in ethnically homogenous groups of healthy Caucasian children and adolescents. These data confirmed the major role of BDNF in energy metabolism, food regulation and BMI.

Key words: brain-derived neurotrophic factor (BDNF Val66Met) polymorphism; Body mass index; Caucasians; healthy children; obesity

Abbreviations[1]

[1] **Abbreviations:** BDNF; Brain-derived neurotrophic factor; BMI; Body mass index; GWAS; genome wide association study; HWE; Hardy-Weinberg equilibrium; Met: methonine; Met carriers: the combined Val/Met + Met/Met genotyopes; Val: valine; power = power of calculation; R = absolute value of the residual; TrkB; tropomyosin-related kinase B.

1 Introduction

Obesity in children and adolescents is a worldwide health problem, characterized by somatic complications such as hypertension, dyslipidemia, inflammation, atherosclerosis, chronic inflammation, elevated insulin levels, endothelial dysfunction, heart disease, increased tendency for blood clotting, kidney and liver dysfunction, neurological complications, type 2 diabetes (Ebbeling et al., 2002), but also psychosocial complications such as engagement in high-risk behaviors, decline in self-esteem, loneliness, nervousness, sadness and negative self-image (Swallen et al., 2005), eating disorders (Babio et al., 2008; Sancho et al., 2007), and adult obesity (Bouchard, 1997). Standard definition and cut off points for overweight and obesity in children (Cole et al., 2000) is determined by Body mass index (BMI) with underweight children defined as below the 5^{th} percentile, normal weight between 5^{th} and 85^{th} percentile, overweight between the 85^{th} and 95^{th} percentile, and obese as above the 95^{th} percentile.

Brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in the proliferation, differentiation, activity-dependent plasticity and survival of neurons in the central nervous system (Noble et al., 2011). BDNF affects the noradrenergic, dopaminergic, serotonergic, glutamatergic, and cholinergic neurotransmitters (Gratacos et al., 2007; Russo-Neustadt, 2003; Tapia-Arancibia et al., 2004), and is therefore implicated in the etiology of different mental disorders and altered behaviors (Russo-Neustadt, 2003). Its location in the hypothalamic regions such as the paraventricular, arcuate and ventromedial nuclei points to its role in feeding behavior, food intake regulation, energy expenditure, energy homeostasis, glucose homeostasis and weight control (Lebrun et al., 2006; Noble et al., 2011). There are reports of a positive (Bus et al., 2011; Iughetti et al., 2011), as well as inverse relationship (Araya et al., 2008; Gray et al., 2006; Han et al., 2008) and lack of association (Saito et al., 2009), between serum and/or plasma BDNF levels and obesity or BMI. The secretion of the precursor and the mature BDNF protein is affected by the single nucleotide polymorphism (SNP), dbSNP reference number rs6265, in the coding region of exon V of the BDNF gene, located on chromosome 11p13-11p14. This SNP (BDNF Val66Met), results in the substitution of valine (Val) for methionine (Speliotes et al., 2010) in the 66 position of the amino-acid sequence of the protein (Egan et al., 2003). The Met variant is thought to be responsible for the abnormal intracellular packaging of the precursor of BDNF (proBDNF) and decreased production of the mature BDNF (Chen et al., 2004).

Studies examining the possible association between the BDNF Val66Met variants and obesity in healthy children and adolescents are still rare, and one genome wide association study (GWAS) reported significant association between BDNF gene and BMI in healthy children (Zhao et al., 2009), while other studies failed to find any significant association between BDNF Val66Met and obesity (Arija et al., 2010; Friedel et al., 2005). Most of the studies evaluated the role of BDNF Val66Met in eating disorders, and found either a significant association (Akkermann et al., 2011; Gratacos et al., 2007), or no association (Arija et al., 2010; Friedel et al., 2005) between the Met allele and eating disorders. The ethnic (Pivac et al., 2009), as well as population-related genetic differences (Petryshen et al., 2010) might explain the conflicting association results

showing either that BDNF Val66Met Met/Met genotype was more frequently found in adult women with higher (Beckers et al., 2008) or with lower (Shugart et al., 2009) BMI. Therefore, the aim of this study was to determine the association between BDNF Val66Met variants and obesity in healthy Caucasian children and adolescents of the same ethnic background of Croatian origin, subdivided according to the BMI measures.

2 Materials and Methods

2.2 Study participants

The study included 300 healthy children and adolescents (134 boys and 166 girls), recruited consecutively during 2010-2011. All participants were unrelated Caucasians of Croatian origin, medication-free with no personal or family history of psychopathology, especially eating disorders or substance abuse. BMI measures from all participants were calculated from weight and standing height. Measures were taken by clinicians from University Hospital Osijek, Child and Adolescent Psychiatry, Health Center Osijek, Family Medicine, Osijek, Croatia, and National Public Health Institute for Zagreb County, Zagreb, Croatia, during regular check-ups. Screening was done by family medicine physicians during a medical exam to exclude the existence of any disorders that require medical intervention or therapy. In addition, an interview (to assess their willingness to participate in the study) was done with parents and their children. None of the subjects received any medication, and none of them were on a diet; none were attempting to reduce their weight with extreme measures such as extreme physical activity. The mean age ± SD was 10.78 ± 4.06 (range 5-18) years, the average weight was 43.24 ± 18.68 kg (range 15.5-99.0), the average height was 146.77 ± 23.09 cm (range 96-190) and the average BMI was 19.02 ± 3.92 kg/m^2 (range 11.0-35.3), respectively. Before starting the study, we obtained permission from the local Ethics Committees. The procedure was fully explained to children and their parents. Written informed consent was obtained from the children's parents and adolescents.

2.2 Genotyping

Blood samples (4 ml) were drawn using plastic syringes with 1 ml of acid citrate dextrose anticoagulant at 08.00 h during usual standard laboratory procedures. The DNA was isolated from blood using the 'salting-out' method (Miller et al., 1988). The dbSNP ID for Val66Met is rs6265. The BDNF Val66Met polymorphism was genotyped in a total volume of 10 μL, containing 30–100 ng of DNA, with the ABI Prism 7300 Sequencing Detection System apparatus (ABI, Foster City, USA). Genotyping was carried out using the Taqman-based allele-specific polymerase chain reaction assay, according to manufacturer's instructions. The primers and probes were purchased from Applied Biosystems as TaqMan® Drug Metabolism Genotyping Assay (assay ID: C_11592758_10).

2.3 Statistical analyses

Statistical evaluation of the data (with Sigma Stat 3.5, Jandell Scientific Corp. San Raphael, California, USA) was done using Pearson's χ^2 test, multiple regression (stepwise procedure) and linear regression with BMI as the dependent variable and sex, age and BDNF Val66Met variants as independent variables. A logistic regression model was used to examine the association of BDNF genotype and the risk of developing

obesity in children, with age and sex included in the model as covariates. The power of calculation (which should be ≥ 0.800), and sample size was also calculated using Sigma Stat 3.5. Hardy-Weinberg equilibrium (Saleh et al., 2006) and Standardized residuals (R) to determine which genotype was the major influence on the significant chi-square test statistic (http://www.acastat.com/Statbook/chisqresid.htm) were evaluated using Microsoft Excel. Since the frequency of the homozygous Met/Met genotype is 3-4% in the Croatian population (Pivac et al., 2009; Pivac et al., 2011), to gain more statistical power, the combined Met/Val and Met/Met genotypes were grouped into Met carriers and compared with the homozygous Val/Val genotype. The study had adequate power (≥0.800) and sufficiently large sample size (n=248 required for this power; and the study included n=300) to detect a significant association. The level of significance was set to $\alpha = 0.05$, with 2-tailed *p* values.

3 Results

Out of the 300 children and adolescents in the study, 4.3% were underweight, 71.0 % had normal weight, 12.0% were overweight and 12.7% were obese. Multiple regression analysis (stepwise procedure) revealed a significant model ($F_{2,297}=42.282$, $p<0.0005$) with age (Beta=.465, $p<0.0005$) and BDNF genotype (Beta=.131, $p=0.011$) accounting for 21.6% of the variance in the children's BMI scores. Since gender was not a significant predictor in this model, in the further analyses (χ^2 tests) the data from boys and girls were collapsed (Table 1).

The genotype distribution in all children, or when children were subdivided according to BMI percentile groups into underweight, normal weight, overweight, and obese children, or when children were subdivided into normal (including underweight and normal weight) and obese (including overweight and obese) groups, was in the expected HWE (data not shown).

Linear regression model was used to examine the effect size of BDNF genotype on BMI, adjusted for age and sex. The result of the linear regression model showed that each BDNF Met allele was significantly associated ($p=0.017$) with 0.123 (95% CI=0.156-1.610) kg/m^2 increase in BMI.

Table 1 shows that the frequency of the Met/Met, Met/Val and Val/Val genotypes, the Met and Val alleles, and Met carriers (the combined Met/Met and Met/Val genotypes versus the homozygous Val/Val genotype) differed significantly ($p=0.011$; $p=0.004$ and $p=0.002$, respectively) between underweight, normal weight, overweight and obese children. These differences were the result of the significantly higher frequency of the Met/Val genotype (58%), the Met allele (34%) and Met carriers (63%) in obese children compared to other groups. The Met allele (R=3.00) and the combined Met/Met and Met/Val genotypes (R=2.98) contributed to this significant effect. To further confirm these results, our participants were additionally categorized into two groups: a normal weight group (including underweight and normal weight group), and an obese group (including overweight and obese group), Table 2. There were significant differences in the frequencies of the BDNF genotypes, alleles and Met carriers ($p=0.034$; $p=0.050$; $p=0.019$, respectively) versus the homozygous Val/Val genotype between normal weight

children and obese children. Although the absolute R values were not above 2.0, the highest R values were again detected for the Met/Val genotype (R=1.85), Met allele (R=1.62) and Met carriers (R=1.73), indicating a significant association of one or two Met alleles with obesity. A logistic regression model adjusted for sex and age was used to examine the association of BDNF genotype and obesity in these groups of normal weight and obese children. This model revealed a significant association of BDNF Val/Met genotype (OR=1.86, 95% CI=1.05-3.29, p=0.032) with the risk for obesity in children.

To evaluate whether the children and adolescents in the present study have a similar distribution of the gene variants of the BDNF Val66Met polymorphism with other healthy groups of the same ethnic origin, we compared the distribution of BDNF variants between the young participants of the present study (10.8 ± 4.1 years old) with the distribution in 556 healthy adult subjects (33.8 ± 9.1 years old) and 402 healthy aging subjects (75.7 ± 7.4 years old) included in our previous studies (Pivac et al., 2009; Pivac et al., 2011). No significant differences were found in the frequency of the genotypes (χ^2 = 1.506; d.f. = 4; p = 0.826), alleles (χ^2 = 0.271; d.f. = 2; p = 0.873) or Met carriers versus Val/Val homozygotes (χ^2 = 0.605; d.f. = 2; p = 0.739) between these three different age groups of healthy Caucasian control subjects of Croatian origin.

4 Discussion

To the best of our knowledge, this is the first study evaluating the association between BDNF Val66Met and BMI in healthy Caucasian children and adolescents of the same ethnic background, who were free of somatic or psychiatric disorders including eating disorders. In line with the results of the GWAS in a large pediatric cohort of European ancestry (Zhao et al., 2009), we have found a significant association between obesity, evaluated using BMI percentile (Cole et al., 2000), and one or two Met alleles of the BDNF Val66Met polymorphism. In our study obese children and adolescents were more likely to be carriers of the Met/Val genotype, the Met allele, and the combined Met/Met and Met/Val genotypes compared to normal weight groups. Each BDNF Met allele was significantly associated with an increase in BMI, and the BDNF Val/Met genotype significantly increased the risk for obesity in children. This finding partially replicates a recent report showing that girls who were Met carriers had a tendency for higher BMI (Akkermann et al., 2011), however in contrast to our participants, these Caucasian girls of Estonian origin practiced extreme weight restriction behaviors. On the other hand, our results diverge from a study of young participants of German origin that failed to detect significant differences in the distribution of the BDNF Val66Met variants between extremely obese children and adolescents, underweight students, patients with attention deficit hyperactivity disorder, anorexia nervosa, bulimia nervosa and normal weight controls (Friedel et al., 2005), and from the data showing no association between BDNF Val66Met and susceptibility to develop eating disorders in Spanish schoolchildren of Caucasian background (Arija et al., 2010). The discrepancies between our and the aforementioned results might be explained by the fact that we included only healthy young participants, while other studies (Akkermann et al., 2011; Arija et al., 2010; Friedel et al., 2005) included either extremely obese or underweight participants, as well as subjects with eating disorders or risk for eating disorders. Aria et al. (2010) hypothesized that the lack of association between the BDNF Val66Met polymorphism

and risk for eating disorders might be due to an effect of age. The multiple regression analysis (stepwise procedure) showed that age was significantly associated with BMI in our study. Therefore, in addition to differences in study groups, the possible effect of age on BMI values might explain the differences between our study and Aria et al. (2010) study. Since our children and adolescents were 10.8 years old on average, with an age range of 5-18 years, and the effect of age was significant, to elucidate the effect of age on BMI and BDNF Val66Met variants, we additionally evaluated the association between BMI and BDNF Val66Met variants in a subgroup of 97 children 6-7 years old. The results were the same, i.e. obesity was significantly associated with one or two Met alleles of the BDNF genotype (data not shown). Gender has been proposed to affect the association between BDNF Val66Met and BMI (Arija et al., 2010; Beckers et al., 2008), however multiple regression analysis in our study excluded the possible effect of gender, at least in our sample, presumably due to the fact that most of them were pre-pubertal and did not reach neurodevelopmental maturity, as emphasized in previous studies (Arija et al., 2010). Since we detected significant differences in the distribution of the BDNF Val66Met variants between Caucasian (Croatian) and Asian (South Korean) healthy adult subjects (Pivac et al., 2009), and there is substantial global population diversity in the BDNF Val66Met polymorphism (Petryshen et al., 2010), to exclude population genetic differences in BDNF, we selected only Caucasian children and adolescents of Croatian origin. Furthermore, although the present study included a relatively small group of children and adolescents (N=300), this group had an analogous distribution of the BDNF Val66Met variants to larger groups of 556 and 402 subjects from our previous studies (Pivac et al., 2009; Pivac et al., 2011).

Our finding obtained in young participants is in line with data showing over-expression of the Met allele in a large cohort of obese adult women from Belgium (Beckers et al., 2008), but differs from the results obtained in carriers of the Met/Met genotype in obese populations of British women (Shugart et al., 2009), and healthy Caucasian adults without significant medical or psychiatric history (Gunstad et al., 2006) which had lower mean BMI than subjects with Met/Val or Val/Val genotypes. The divergent results might be attributed to different study designs, different study groups (obese versus healthy), or the population genetic differences in BDNF Val66Met (Petryshen et al., 2010), that may lead to the conflicting association results between studies, evaluating the role of BDNF Val66Met in various psychiatric disorders and altered behaviors.

Our results have confirmed the important role of the BDNF Val66Met polymorphism in obesity, especially the contribution of the Met allele. Since the Met allele of the BDNF Val66Met genotype is associated with intracellular packaging and secretion of the proBDNF precursor, and sorting of BDNF into the nerve terminals, this "hypofunctional" Met allele reduces the activity-dependent BDNF secretion and therefore the production and amount of the mature BDNF (Chen et al., 2004; Egan et al., 2003). Animal studies support the anorectic and satiating properties of BDNF, which, when administered centrally, induces appetite suppression and weight loss (Lebrun et al., 2006; Noble et al., 2011; Rask-Andersen et al., 2010; Tapia-Arancibia et al., 2004). BDNF binds to its tropomyosin-related kinase B (TrkB) receptor (Noble et al., 2011) and modulates energy metabolism, food regulation and BMI by central as well as peripheral action, and

regulates physical activity, hyperactivity, anxiety, and hyperphagia (Lebrun et al., 2006; Noble et al., 2011; Rask-Andersen et al., 2010; Tapia-Arancibia et al., 2004). Likewise, mutations in the genes coding for BDNF and TrkB are responsible for obesity and eating disorders (Lebrun et al., 2006; Noble et al., 2011). Since lower serum or plasma BDNF levels are found in obesity (Araya et al., 2008; El-Gharbawy et al., 2006; Gray et al., 2006; Krabbe et al., 2007; Lommatzsch et al., 2005), compared to normal weight subjects, although opposite results also exist (Bus et al., 2011; Iughetti et al., 2011), our data suggest that carriers of one or two Met alleles in our study had decreased amount of the mature BDNF, possibly lower plasma BDNF levels, and therefore they had higher values of BMI and were more frequently obese. This speculation might be confirmed by the fact that weight gain, induced by antipsychotic drugs, is associated with BDNF serum levels in female schizophrenic patients, while carriers of the Met/Met genotype have lower BDNF levels than carriers of the Val allele (Zhang et al., 2008). Our finding of the significant association between BDNF Val66Met genotype and obesity in children and adolescents is supported by GWAS data, which found a significant association between BDNF Val66Met and obesity in children (Zhao et al., 2009) and adults (Croteau-Chonka et al., 2011; Speliotes et al., 2010).

The limitation of the study is that only one polymorphism (rs6265) of the *BDNF* gene was determined. However, it is a functional variant reported to influence BDNF expression (Chen et al., 2004; Egan et al., 2003). The advantage of the study is in a reasonably large ethnically homogenous group of healthy Caucasian children and adolescents free of psychopathology, especially eating disorders or substance abuse, and in adequate statistical power ($\geq$0.800) and sufficiently large sample size (n=248 required for this power; and the study included n=300) to detect a significant associations. Nevertheless, these findings should be regarded as preliminary because of the relatively modest sample size, and should be replicated in a larger cohort.

5 Conclusions

These results show for the first time a significant association between having one or two Met alleles and obesity in an ethnically homogenous group of healthy Caucasian children and adolescents. These data support the major role that BDNF plays in energy metabolism, food regulation and BMI.

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Conflict of interest

None of the authors have an actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of commencement of the submitted work that could inappropriately influence, or be perceived to influence, their work.

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Table 1 The BDNF genotype and allele count and frequencies (percentages) in children subdivided according to the BMI percentile into groups of underweight (below 5), normal weight (between 5-85), overweight (between 85-95), and obese (above 95) children.

BDNF Val66Met	**underweight**	**normal weight**	**overweight**	**obese**
	n (%)	n (%)	n (%)	n (%)
Val/Val genotype	10 (76.9)	145 (68.1)	25 (69.4)	14 (36.8)
Val/Met genotype	3 (23.1)	61 (28.6)	11 (30.6)	22 (57.9)
Met/Met genotype	0 (0.0)	7 (2.3)	0 (0.0)	2 (5.3)
$\chi^2 = 16.496$; d.f. = 6; $p = 0.011$				
R could not be calculated since one of the cells is 0				
Val allele	23 (88.5)	351 (82.4)	61 (84.7)	50 (65.8)
Met allele	3 (11.5)	75 (17.6)	11 (15.3)	26 (34.2)
$\chi^2 = 13.451$; d.f. = 3; $p = 0.004$				
$R = 3.00$ for Met allele in obese children				
Val homozygotes	10 (76.9)	145 (68.1)	25 (69.4)	14 (36.8)
Met carriers	3 (23.1)	68 (31.9)	11 (30.6)	24 (63.2)
$\chi^2 = 15.173$; d.f. = 3; $p = 0.002$				
$R = 2.98$ for Met carriers in obese children				

n is the number of subjects. Frequencies (%) are shown in parentheses. BDNF: brain derived neurotrophic factor*; Met*: methionine; Met carriers: the combined Val/Met + Met/Met genotypes; Val: valine; *power* = power of calculation; *R* = absolute value of the residual.

Table 2 The BDNF genotype and allele count and frequencies (percentages) in children subdivided into normal weight group (including underweight and normal weight) and obese children (overweight and obese group).

BDNF Val66Met	**normal weight**	**obese**
	n (%)	n (%)
Val/Val genotype	155 (68.6)	39 (52.7)
Val/Met genotype	64 (28.3)	33 (44.6)
Met/Met genotype	7 (3.1)	2 (2.7)
$\chi^2 = 6.771$; d.f. = 2; $p = 0.034$		
R = 1.85 for Val/Met genotype in obese children		
Val allele	374 (82.7)	111 (75.0)
Met allele	78 (17.2)	37 (25.0)
$\chi^2 = 3.830$; d.f. = 1; $p = 0.050$		
R = 1.62 for Met allele in obese children		
Val homozygotes	155 (68.6)	39 (52.7)
Met carriers	71 (31.4)	35 (47.3)
$\chi^2 = 5.478$; d.f. = 1; $p = 0.019$		
R = 1.73 for Met carriers in obese children		

n is the number of subjects. Frequencies (%) are shown in parentheses. BDNF: brain derived neurotrophic factor*; Met*: methonine; Met carriers: the combined Val/Met + Met/Met genotyopes; Val: valine; *power* = power of calculation; *R* = absolute value of the residual.