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ASSOCIATION OF THE BRAIN-DERIVED NEUROTROPHIC FACTOR Val66Met POLYMORPHISM WITH BODY MASS INDEX, FASTING GLUCOSE LEVELS AND LIPID STATUS IN ADOLESCENTS

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) has an important role in energy balance. It suppresses food intake, reduces hepatic glucose production and converts white fat into brown fat in adipose tissue, leading to energy dissipation, lowered blood glucose and a lean phenotype. Studies have shown that the single nucleotide polymorphism (SNP) Val66Met within BDNF may be associated with obesity, insulin sensitivity, type 2 diabetes mellitus (T2DM) and dyslipidemia. The objective of the study was to investigate the association of the Val66Met polymorphism with body mass index (BMI), fasting glucose levels and lipid profile in Serbian adolescents. The study included 308 randomly selected healthy adolescents, 153 (49.68%) boys and 155 girls (50.32%), 15 years of age. Data including age, gender, height, weight, lipid profile and fasting glucose were recorded. Genotyping was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. No association of this polymorphism was found with BMI and lipid profile. However, significant association was observed between this polymorphism and fasting blood glucose (FBG). Carriers of a Val/Val genotype had significantly higher mean values of fasting glucose level compared to carriers of Val/Met and Met/Met genotypes ($p = 0.01$). To confirm these results multiple linear regression analysis was performed. Body mass index and gender were taken as covariates. Carriers of the Val/Val genotype had significantly higher levels of FBG ($\beta = –0.152$, $p = 0.02$). A statistically significant association between BMI and glucose level was also observed ($\beta = 0.124$, $p = 0.033$). This polymorphism could be associated with fasting glucose level in Serbian adolescents, thus further research would be of great interest to validate these results.

Keywords: Body mass index (BMI); Brain-derived neurotrophic factor (BDNF); Fasting glucose level; Gene polymorphism; Lipid profile.

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INTRODUCTION

Obesity is becoming a major health problem of modern society and affects all socio-economic categories, regardless of age, gender and ethnicity. Adolescent obesity is associated with metabolic, cardiovascular, psychological, orthopedic, neurological, respiratory and other disorders [1,2]. Of these, the most common is metabolic syndrome and type 2 diabetes mellitus (T2DM) [3]. Regarding the research of children and adolescent obesity and metabolic diseases, brain-derived neurotrophic factor (BDNF) is gaining increased interest. Brain-derived neurotrophic factor belongs to the neurotrophine family and plays an important role in neurodevelopment, neuronal survival, differentiation, synaptic plasticity and connectivity in the brain. Its function is reflected on memory formation, learning, mood, cognition and stress response [4]. Brain-derived neurotrophic factor is mainly expressed in the central nervous system, but can pass the blood-brain barrier in both directions, and it circulates systemically [5,6]. Through its receptors, BDNF can exert various effects on non-neuronal cells such as testes, thymus, skin, salivary glands and mammary ducts. Besides these functions,

BDNF has an important role in food intake regulation, weight control, glucose homeostasis, energy homeostasis, blood pressure and lipid status [3,5]. Through central pathways involved in appetite regulation and energy expenditure, BDNF suppresses food intake, facilitates glucose uptake in the brain, reduces hepatic glucose production and converts white fat into brown fat in adipose tissue, leading to energy dissipation, lowered blood glucose and a lean phenotype [5].

Studies on rodents reported that peripheral injection of BDNF had hypoglycemic and hypophagic effects on obese hyperglycemic animals but not on normal animals [7]. These findings are refereeing to anti diabetic and anti obesity effects. The human *BDNF* gene is located on chromosome 11p13-11p14 and it contains 11 exons [8]. Within the *BDNF* gene sequence, several single nucleotide poly-morphisms (SNPs) have so far been investigated and the most commonly analyzed SNP is rs6265 (c.196G>A), which causes the change of valine to methionine at amino acid residue 66 in the pro region of the BDNF protein. The methionine allele is associated with the abnormal intracellular trafficking and packaging of pro-BDNF, and consequently, has an impact on secretion of mature peptide [9]. However, Lang *et al.* [10] found a decreased concentration of serum BDNF in healthy Val/Val subjects compared to Val/Met individuals. It has been observed that in healthy subjects defective intracellular protein signaling might be compensated by constitutive upregulation of peripheral BDNF concentration [10].

Association studies of BDNF Val66Met polymorphism and body mass index (BMI), of healthy children and adolescents have shown contradictory results [3,11,12], while studies of association between the Val66Met polymorphism and glucose level as well as lipid status of children and adolescents are still rare [12]. The aim of this study was to investigate the association of the Val66Met (rs6265) polymorphism with BMI, fasting glucose levels and lipid profiles of healthy adolescents.

MATERIAL AND METHODS

Study Design and Study Population. Subjects enrolled in the study ($n = 308$) were randomly selected school children of both genders, 15 years of age, who participated in the Yugoslav Study of the Precursors of Atherosclerosis in School Children (YUSAD), a prospective multicentric study that lasted from 1989 to 2008. Cross-sectional data used in this study were collected during 2003. Age, gender, height, weight, lipid profile [total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) cholesterol, triglycerides (TG)] and fasting glucose level were recorded during an annual general medical examination performed by pediatricians at 11 pediatric departments of primary health care centers (Čukarica, Palilula, Požarevac, Užice, Kraljevo, Knjaževac, Bor, Niš, Subotica, Arilje and Despotovac) in Serbia. The children had normal growth and development, without any morbidities (acute inflammatory disease or chronic disease) at the time of examination. Data were collected using standardized questionnaires and clinical examinations (height, body mass). Body mass index was calculated as the participant's weight in kilograms divided by the square of their height in meters. Height was measured without shoes to the nearest of 1 cm. Stadiometer and stretch stature methods were used. Weight was measured wearing light clothes and without shoes to the nearest 0.1 kg using scales precalibrated by the research team. The children were classified in normal weight, overweight (above 85th percentile) and obese (above 97th percentile) groups according to BMI charts published for Serbian children 15 years of age [13]. Type 1 or type 2 diabetes mellitus, genetic syndromes, cerebral palsy, chronic immobility, generalized inflammation, cardiovascular and malignant diseases were criteria for exclusion. Signed informed consent was obtained from each participant's parent or guardian. Siblings and relatives were not included in the study. Study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Belgrade, Serbia.

Genotyping. Molecular-genetic analyses were performed at the Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Belgrade, Serbia. Genomic DNA was extracted from 5 mL of peripheral blood according to Miller *et al.* [14]. Genotypes of SNP Val66 Met (rs6265) were detected by classic polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. The PCR conditions were: initial denaturation for 5 min. at 95 °C, followed by 35 cycles of 1 min. denaturation at 95 °C, primer annealing for 30 seconds at 55 °C, extension and polymerization for 1 min. at 72 °C, and final extension for 7 min. at 72 °C. The forward primer used in genotyping was F: 5'-ACT CTG GAG AGC GTG AAT GG-3', and reverse primer was R: 5' ACT ACT GAG CAT CAC CCT GGA-3'. Amplified products of 171 bp were digested with *Eco*72I restriction enzyme for 2 hours at 37 °C. The amplified fragments were analyzed on 8.0% polyacrylamide gel electrophoresis (PAGE). After electrophoretic separation, depending on the genotype, the different fragment lengths were notable. Fragments of 171 bp corresponded to genotype AA, while fragments of 99 and 72 bp were seen in genotype GG [15].

Biochemical Analysis. Each participant fasted for 12 hours before blood sample collection. Levels of serum glucose, total cholesterol, HDL-C and TG were measured as described previously, LDL-C concentrations in samples were calculated using Friedewald's equation [13].

Statistical Analyses. For statistical analyses, the Statistical Package for Social Science (SPSS) version 17 (SPSS Inc., Chicago, IL, USA) was used. Quantitative

variables were expressed as mean ± SD. The association of genotypes and BMI, fasting glucose level and serum lipid levels was tested by Student's *t*-test or Mann-Whitney test depending on the variable distribution. In order to evaluate the association of fasting blood glucose (FBG) levels and *BDNF* genotypes with gender and BMI as covariates, mul-tivariate linear regression analysis was performed.

RESULTS

Of 308 adolescents included in the study, 153 (49.68%) were boys and 155 were girls (50.32%). Of the boys, 26 (17.8%) were overweight and 20 (13.7%) were obese. In the group of girls, 29 (18.7%) were overweight and 17 were obese (11.0%). The mean values and ranges of analyzed parameters depending on the gender are presented in Table 1. Based on the guidelines for cardiovascular health and risk reduction in children and adolescents [16] we observed that in the adolescents included in our study there were 48 (15.58%) and 16 (10.46%) boys and 32 (20.64%) girls with high levels of total cholesterol (TC) and LDL-C (TC level higher than 5.2 mmol/L and LDL-C level higher than 3.35 mmol/L), while 13 adolescents (4.22%), eight boys (5.23%) and five girls (3.22R) had HDL-C lower than 0.9 mmol/L. Moreover, 25 adolescents (8.12%), 13 boys (8.50%) and 12 girls (7.74%) had high levels of TG (higher than 1.7 mmol/L). There was statistically significant difference in total cholesterol levels between the groups of boys and girls ($p = 0.001$), meaning that girls had significantly higher mean values of total cholesterol. Statistically significant difference was not observed in the other analyzed parameters.

Frequencies of Genotypes and Alleles of rs6265 (Table 2). The distribution of genotypes were in Hardy-Weinberg equilibrium. As only three samples were genotyped as AA, GA and AA genotypes were grouped for further analyses.

The mean values of analyzed parameters depending of the *BDNF* genotypes are presented in Table 3. Our analysis

Table 1. Mean values and range of analyzed parameters depending on gender.

Parameters	Total (*n* = 308) (range)	Boys (*n* = 153)	Girls (*n* = 155)	*p* Value
BMI (kg/m^2)	22.03±4.25 (13.8-43.72)	21.77±4.22	22.29±4.29	0.405
FBG (mmol/L)	4.68±0.63 (2.43-6.30)	4.75±0.59	4.61±0.66	0.660
TG (mmol/L)	0.98±0.55 (0.32-2.54)	0.97±0.62	0.99±0.47	0.891
TC (mmol/L)	4.35±0.86 (2.64-6.70)	4.18±0.76	4.51±0.93	**0.001**
HDL-C (mmol/L)	1.41±0.40 (0.28-2.63)	1.37±0.42	1.45±0.38	0.159
LDL-C (mmol/L)	2.47±0.86 (0.53-4.82)	2.39±0.81	2.55±0.90	0.057

BMI: body mass index; FBG: fasting blood glucose; TG: triglycerides; TC: statistically significant total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 2. Frequencies of *BDNF* genotypes and alleles.

BDNF rs6265 Genotypes	Frequency *n* (%)	Alleles	Frequencies (%)
GG	202 (65.6)	G	0.82
GA	103 (33.4)	–	–
AA	3 (1.0)	A	0.18

BDNF: brain-derived neurotrophic factor.

Table 3. Mean values of analyzed parameters by *BDNF* rs6265 genotype (mean±SD).

Parameters	Genotype	Total	*p* Value	Boys	*p* Value	Girls	*p* Value
BMI (kg/m^2)	GG GA+AA	22.17±4.37 21.76±3.82	0.378	21.79±4.14 21.74±4.40	0.715	22.54±4.57 21.80±3.67	0.367
FBG (mmol/L)	GG GA+AA	4.74±0.66 4.56±0.56	**0.010**	4.79±0.62 4.68±0.55	0.302	4.70±0.70 4.43±0.55	**0.023**
TG (mmol/L)	GG GA+AA	0.99±0.52 0.97±0.59	0.599	0.96±0.54 0.99±0.75	0.679	1.02±0.52 0.95±0.36	0.624
TC (mmol/L)	GG GA+AA	4.36±0.86 4.34±0.88	0.931	4.18±0.74 4.20±0.82	0.848	4.53±0.93 4.48±0.94	0.756
HDL-C (mmol/L)	GG GA+AA	1.42±0.42 1.44±0.44	0.484	1.34±0.34 1.42±0.53	0.580	1.45±0.41 1.48±0.33	0.659
LDL-C (mmol/L)	GG GA+AA	2.47±0.87 2.48±0.84	0.911	2.38±0.85 2.40±0.80	0.879	2.55±0.93 2.57±0.84	0.873

BDNF: brain-derived neurotrophic factor; BMI: body mass index; FBG: Statistically significant fasting blood glucose; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

showed that mean values of FBG were significantly higher in carriers of the GG genotype compared to carriers of the GA and AA genotypes ($p = 0.01$). To confirm these results, multiple linear regression analysis was performed. Body mass index and gender were taken as covariates. Carriers of the GG genotype had significantly higher levels of FBG ($\beta = –0.152$, $p = 0.02$). Statistically significant association between BMI and glucose level was also observed ($\beta = 0.124$, $p = 0.033$). There was no statistically significant difference in BMI and lipid profile of adolescents depending on their rs6265 polymorphism genotype.

DISCUSSION

The aim of this study was to investigate the association between the Val66Met polymorphism of *BDNF* and BMI, FBG, TG, TC, HDL-C and LDL-C in 308 15-year-old school children. In previous studies, this polymorphism was linked to obesity and metabolic syndrome [3,17,18]. Some studies demonstrated that BDNF may suppress food intake, insulin resistance and maintain glucose homeostasis [5,19]. In our study, association of the Val66Met polymorphism with BMI was not found. A previous study conducted by Friedel *et al.* [20] also did not find significant association between the analyzed polymorphism and obesity in a group of adolescents who were carriers of the methionine variant. However, a study conducted on Chinese children [21] refers to the valine allele as a risk allele associated with higher BMI and obesity in children, which is in agreement with the results obtained in a group of 2131 children, age 6-18, who reported that methionine allele carriers had significantly lower BMI [12]. Another study also revealed that, in healthy Caucasians, the Met/Met genotype had a protective effect against obesity [4].

However, there are studies with contradictory results. Healthy Croatian children and adolescents carrying one or two methionine alleles tend to have higher BMI and are prone to obesity [3]. Furthermore, a Mexican study in a pediatric population showed a significant association between the Met/Met genotype and obesity [11].

In our study, we did not observe a statistically significant association between the *BDNF* polymorphism and lipid profile of adolescents. Association of the Val66Met polymorphism and lipid parameters [12] in their group of children and adolescent was also not found. However, Peng *et al.* [5], noted a correlation between *BDNF* Val66 Met and lipid profiles where the methionine variant was linked to higher triglyceride levels, but lower HDL-C levels in the group of long-lived individuals.

The major finding of our study is the association of the Val66Met polymorphism and levels of FBG. Statistical analysis revealed that carriers of the Val/Val genotype had significantly higher mean values of FBG compared to carriers of the Val/Met or Met/Met genotypes ($p = 0.01$). Kalenda *et al.* [12] found that FBG levels were not associated with the Val66Met genotype, but they did find lower postprandial glucose levels in a post-pubertal group of children who were carriers of the methionine allele. It has been observed that BDNF levels are related to glucose concentrations [22]. Furthermore, normal weight, regular exercise, nonstressed, and nonsmoking middle aged individuals with Val/Met and Met/Met alleles were less likely to develop glucose intolerance and T2DM [23]. Minelli *et al.* [24], and Lang *et al.* [10] have shown the association of the Val/Val genotype with low levels of serum BDNF in healthy subjects. This could explain the observed association between the Val/Val genotype and higher glucose level in our study. However, the association of the Val66 Met genotype with BDNF serum or plasma levels in children, adolescents or adults, was not confirmed in other studies [12,25,26]. Peripheral level of BDNF depends on numerous factors such as age, gender, hormonal status, circadian variation, platelet count, diet and exercise [12,27,28]. This complex mechanism of regulation of BDNF levels could be the reason for the contradictory results regarding the association of the Val66Met polymorphism with peripheral BDNF levels.

In conclusion, we did find that carriers of the Val/Val genotype had statistically significant higher mean values of FBG compared to carriers of the Val/Met or Met/Met genotype. However, the results are still contradictory and it would be of great interest to conduct a study on a larger sample size and to include other *BDNF* gene polymorphisms.

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