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A protective effect of the *BDNF* Met/Met genotype in obesity in healthy Caucasian subjects but not in patients with coronary heart disease

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Abstract. – OBJECTIVE: Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor with an important role in the regulation of body weight, body mass index (BMI) and obesity. Increased BMI that leads to obesity is a substantial risk factor for coronary heart disease (CHD). The functional *BDNF* Val66Met polymorphism (rs6265) has been associated with CHD, obesity and BMI. The aim of the study was to determine the association between *BDNF* rs6265 polymorphism and CHD and/or BMI in patients with CHD and healthy control subjects.

PATIENTS AND METHODS: The study included 704 Caucasian subjects: 206 subjects with CHD and 498 healthy control subjects. The *BDNF* rs6265 genotype frequency was similar in male and female subjects, and there were no differences in the frequency of the *BDNF* rs6265 genotypes in 206 patients with CHD and in 498 healthy subjects. When study participants were subdivided according to the BMI categories into normal weight, overweight and obese subjects, significantly different *BDNF* rs6265 genotype frequency was found within healthy subjects, but not within patients with CHD. Healthy subjects, but not patients with CHD, subdivided into carriers of the Met/Met, Met/Val and Val/Val genotype, had different BMI scores.

RESULTS: The BDNF rs6265 genotype frequency was similar in male and female subjects, and there were no differences in the frequency of the *BDNF* rs6265 genotypes in 206 patients with CHD and in 498 healthy subjects. When study participants were subdivided according to the BMI categories into normal weight, overweight and obese subjects, significantly different *BDNF* rs6265 genotype frequency was found within healthy subjects, but not within patients with CHD. Healthy subjects, but not patients with CHD, subdivided into carriers of the Met/Met, Met/Val and Val/Val genotype, had different BMI scores. *BDNF* rs6265 polymorphism was not associated with a diagnosis of CHD or with BMI categories among patients with CHD. In contrast, healthy Caucasians, carriers of the *BDNF* Met/Met genotype, had more frequently normal weight compared to carriers of other BDNF genotypes*BDNF* rs6265 polymorphism was not associated with a diagnosis of CHD or with BMI categories among patients with CHD. In contrast, healthy Caucasians, carriers of the *BDNF* Met/Met genotype, had more frequently normal weight compared to carriers of other *BDNF* genotypes.

CONCLUSIONS: *BDNF* rs6265 polymorphism is associated with BMI categories, and the *BDNF* Met/Met genotype has a protective role in obesity in healthy subjects, while this effect was not present in patients with CHD.

Key Words:
Brain-derived neurotrophic factor, *BDNF* Val66Met polymorphism, Body mass index, Coronary heart disease, Caucasian subjects.

Abbreviations

BDNF = brain-derived neurotrophic factor; BMI = body mass index; CHD = coronary heart disease; CNS = central nervous system; SNP = single nucleotide polymorphism; Met = methionine; Val = valine.

Introduction

Increased body mass index (BMI) might lead to obesity, as well as to coronary heart disease

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(CHD). These conditions are two most common human health problems worldwide. Defined as an excess of body fat, both increased BMI and obesity are risk factors for CHD[1]. Overweight and obesity are characterized by the abnormal or excessive fat accumulation that disturbs well-being and impairs health[2]. Atherosclerosis is an inflammatory disorder, associated with adipose tissue dysfunction[3], that frequently contributes to the initiation and progression of obesity-induced metabolic and cardiovascular complications such as cardiovascular disease and CHD[4]. Obesity, cardiovascular diseases and CHD are multifactorial disorders influenced by the complex interaction between various genetic and environmental factors[5].

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophine family, has major roles in neurodevelopment, neuronal survival and differentiation, neuronal function, synaptic plasticity and connectivity in the brain, as well as in the memory formation and processing, learning, mood, cognition, and stress response[6,7]. It is widely distributed in the central nervous system (CNS), especially in the hippocampus, amygdala, cerebral cortex and hypothalamus[8], where it modulates serotonergic, glutamatergic, cholinergic, and dopaminergic neurotransmission[6,9]. BDNF, localized in the hippocampus and the hypothalamus, modulates both energy metabolism and synaptic plasticity and, therefore, affects cognition and metabolic regulation[10]. Besides CNS, where it regulates energy homeostasis[11], BDNF is also localized at the periphery in different cell types associated with different pathologies occurring in CHD and coronary artery disease[12]. The cardiovascular and metabolic effects of BDNF suggest its role in human coronary atherosclerosis and metabolic syndrome[13,14]. BDNF can be stored and released from platelets into plasma[15], but it is also found in developing heart cells[16], atherosclerotic vessels[17], macrophages[18], endothelial cells[16] and vascular smooth muscle cells[17,19]. All these cell types may synthesize BDNF, therefore providing an explanation for its association with atherosclerotic and cardiometabolic disease[20]. Among various other polymorphisms of the *BDNF* gene, there is one functional single nucleotide polymorphism (SNP) which produces amino acid substitution (Valine/Val/to Methionine/Met/) at position 66 (G196A or Val66Met) in the pro-BDNF sequence and results in the impaired intracellular trafficking[21,22]. *In vitro* studies demonstrated that the Met variant of the *BDNF* Val66Met polymorphism is related to the lower activity-dependent BDNF secretion from the cultured hippocampal neurons[21]. Due to the multiple functions of this neurotrophic factor, *BDNF* has been evaluated as a candidate gene in various neuropsychiatric disorders including eating disorders[7,9,23]. In addition, BDNF and *BDNF* Val66Met polymorphism have been reported to be associated with body weight regulation, BMI, obesity[11,24-29], and cardiometabolic disease[3,17,30]. However, these associations are far from clear, and the results regarding *BDNF* Val66Met and BMI[25,29,31,32] and/or CHD[12,33] are inconsistent.

The aim of the study was to evaluate the association between *BDNF* Val66Met polymorphism and CHD and/or BMI in patients with CHD and in healthy controls. The hypothesis was that different *BDNF* Val66Met genotypes will be associated with CHD or with increased BMI in CHD patients and healthy control subjects that were all Caucasians of the Croatian origin.

Patients and Methods

Participants

The study included 704 subjects who were divided into 206 subjects with coronary heart disease (CHD), and 498 healthy control subjects, all Caucasians of the Croatian origin. Diagnosis of CHD was done by a cardiologist using ICD-10 criteria. Ischemic heart disease is reported in ICD-10 with categories I20-I25. These categories include angina pectoris, myocardial infarction, current complications following myocardial infarction and chronic ischemic heart disease. The criteria for diagnosing CHD were: > 50% stenotic lesions in at least one major coronary vessel determined by coronary angiography or multi-slice computed tomography (MSCT), myocardial infarction, coronary stent implantation and coronary artery bypass surgery. Exclusion criteria for CHD subjects were: neurodegenerative and neuropsychiatric disorders, use of antidepressants and anxiolytic medication, acute infections and inflammations. The control group consisted of 498 healthy subjects, screened during the routine physical check-ups. Exclusion criteria were: CHD, neurodegenerative and neuropsychiatric disorders, current use of antidepressants, anxiolytic medication, acute infections and inflammations. These study methods were approved by the local Ethics Committee of the School of Medicine, University of Zagreb. All participants provided written informed consent prior to participation.

Body mass index (BMI)

Height was measured to the nearest 0.5 cm. Body weight was measured with a digital scale to the nearest 0.1 kg. BMI was calculated as weight (kg) over height (m^2). According to the BMI categories, subjects were classified as normal-weight (BMI = 18.5-24.9), overweight (BMI = 25.0-29.9), or obese (BMI ≥ 30.0) subjects.

Genotyping

Blood samples were obtained between 7:30 am and 8:00 am. Blood samples were drawn into 8.5 ml yellow-top Vacutainer tubes with 1.5 ml of acid citrate dextrose anticoagulant. Genomic DNA was isolated from blood using the "salting out" method[34]. The *BDNF* Val66Met polymorphism (dbSNP ID rs6265) was genotyped in a total volume of 10 µl, containing 20 ng of DNA, using ABI Prism 7300 real-time PCR System apparatus (Applied Biosystems, Foster City, CA, 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® SNP Genotyping Assay (assay ID: C_11592758_10). The possibility of genotyping errors was excluded by randomly choosing 5% of samples for repeated genotyping with 100% concordance.

Statistical Analysis

The results were evaluated with Sigma Stat 3.5 (Jandel Scientific Corp., San Jose, CA, USA) and presented as numbers and percentages, or median and 25th (Q1) and 75th (Q3) percentile. The *BDNF* Val66Met (rs6265) genotype frequencies and deviation from Hardy-Weinberg equilibrium were compared using χ^2-tests and Yates correction for continuity. To evaluate what category contributed to rejecting the null hypothesis, standardized residual (R)[35] was calculated. Normality of distribution was assessed with the Kolmogorov-Smirnov test. A multiple linear regression analysis was used to check for the influence of diagnosis, age, and gender on BMI. Age, height, weight and BMI values were evaluated with Kruskal-Wallis ANOVA on ranks or Mann-Whitney test, as normality of the data failed. In the case of normal distribution of the data, BMI values between two groups were compared with Student *t*-test. The level of significance was corrected to 0.025, and tests were two-tailed. G*Power 3 Software[36] was used for conducting power analyses, i.e. to determine a priori sample size and actual power. For multiple linear regression analysis (with $\alpha = 0.025$; power = 0.800; a small effect size = 0.30; number of predictors = 3), total desired sample size was 49, and the actual sample size was 704. For genetic analyses with a χ^2-test (with $\alpha = 0.025$; power $1 - \beta$) = 0.800 and a small effect size ($\omega = 0.30$; df = 4), total desired sample size was 157; if df = 2, total desired sample size was 128; and if df = 1, total desired sample size was 106, while the actual total sample size was 206 for CHD patients and 498 for healthy controls. Therefore, the study included appropriate sample size and statistical power to de-

Table I. Demographic data (age, gender, weight, height and BMI) of study participants subdivided according to diagnosis.

	Patients with CHD n = 206		Healthy subjects n = 498
Gender (n; %)			
Men	140; 68.0		203; 40.8
Women	66; 32.0		295; 59.2
χ^2-test	$\chi^2 = 42.06$; d f = 1;	$p < 0.001$*	
Age (years)	57; 56-58		52; 51-53
Mann-Whitney test	U =31845.50;	***p* < 0.001***	
Height (cm)	175; 173-176		167; 166-168
Mann-Whitney test	U = 31743.00;	***p* < 0.001***	
Weight (kg)	86; 84-88		77; 75-79
Mann-Whitney test	U=33831.50;	***p* < 0.001***	
BMI (kg/m^2)	27.7; 27.3-30.0		27.1; 26.7-27.8
Mann-Whitney test	U = 44063.00;	***p* = 0.003***	

BMI, body mass index; CHD, coronary heart disease; age, height, weight and BMI are shown as median and 95% CI for the median.

tect significant differences in the studied groups. $p < 0.05$ was considered statistically significant.

Results

Demographic data (gender, age, height, weight and BMI) for study participants are shown in Table I. The frequency (χ^2 test) of male and female subjects differed significantly ($p < 0.001$), as there were significantly (R=3.96) more male (68%) than female (32%) patients in CHD group, compared to the group of healthy subjects (Table I). Patients with CHD were significantly (Mann-Whitney test) older ($p < 0.001$), significantly taller ($p < 0.001$), significantly heavier ($p < 0.001$) and had significantly higher BMI ($p < 0.001$) than healthy subjects (Table I).

The distribution of the *BDNF* Val66Met (rs6265) genotypes was in the Hardy-Weinberg equilibrium in patients with CHD ($\chi^2 = 0.85$; df = 1; $p = 0.358$) and in healthy subjects ($\chi^2 = 0.72$; df = 1; $p = 0.190$). The frequency of *BDNF* rs6265 genotypes did not differ significantly between male and female subjects in healthy group ($\chi^2 = 0.76$; df = 2; $p = 0.685$) or in patients with CHD ($\chi^2 = 0.36$; df = 2; $p = 0.834$). Therefore, in the further evaluations subjects were not subdivided according to gender.

The *BDNF* rs6265 genotype frequency (Met/Met, Val/Met and Val/Val genotypes) did not differ significantly ($\chi^2 = 0.210$; df = 2; $p = 0.900$) between patients with CHD and healthy subjects. These results revealed that *BDNF* Val66Met polymorphism was not significantly associated with diagnosis of CHD in our sample.

To evaluate the possible association between *BDNF* rs6265 polymorphism and BMI, previously found in a group of adolescents[24] but not in adult[29] subjects from the same origin, patients with CHD and healthy subjects were subdivided according to the BMI categories into normal-weight, overweight and obese subjects (Table II). The frequency of the *BDNF* rs6265 Met/Met, Val/Met and Val/Val genotypes did not differ significantly between normal-weight, overweight and obese patients with CHD ($p = 0.441$). In contrast, the distribution of the *BDNF* Val66Met genotypes differed significantly ($p = 0.017$) between normal-weight, overweight and obese healthy subjects (Table II). Results showed that the Met/Met genotype in the group of healthy subjects with normal weight contributed to this significant result (R = 2.15), since the most prominent difference was found in the frequency of Met/Met genotype between normal weight (52.4%), overweight (38.1%) and obese (9.5%) healthy subjects.

As the finding of the significant association between *BDNF* rs6265 polymorphism and BMI disagreed with our previous results in adults[29], to confirm this data, patients with CHD and healthy subjects were subdivided into carriers of the Met/Met, Val/Met and Val/Val genotypes, and their BMI values were evaluated. Kruskal-Wallis ANOVA on Ranks showed no significant (H =

Table II. The distribution of the *BDNF* rs6265 genotypes in 206 patients with coronary heart disease (CHD) and in 498 healthy participants, subdivided according to the body mass index (BMI) category.

	Normal weight		Overweight		Obese	
	n	%	n	%	n	%
Patients with CHD						
BDNF Val66Met genotype						
Met/Met	3	7.3	1	1.5	4	4.0
Val/Met	14	34.2	16	24.6	25	25.3
Val/Val	25	58.5	48	73.9	70	70.7
χ^2-test	$\chi^2 = 3.75$; df = 4; $p = 0.441$					
Healthy subjects						
BDNF Val66Met genotype						
Met/Met	11*	8.0	8	3.8	2	1.3
Val/Met	40	29.0	50	23.7	50	33.6
Val/Val	87	63.0	153	72.5	97	65.1
χ^2-test	$\chi^2 = 12.01$; df = 4; $p = 0.017$*; R = 2.15					

BDNF, brain derived neurotrophic factor, n is the number of subjects. Frequencies are expressed as percentages (%).

0.69; df = 2; p = 0.709) differences in the BMI values in patients with CHD, subdivided according to their *BDNF* rs6265 genotypes. Namely, the Met/Met homozygotes had median BMI value of 28.9 (Q1 = 23.5; Q3 = 34.2), which was not significantly different from the median BMI value of Val/Met carriers (27.5; Q1 = 24.8; Q3 = 30.5) or the Val/Val carriers (27.7; Q1 = 26.3; Q3 = 31.1). On the other hand, there was a nominally significant (H = 6.02; df = 2; p = 0.049) difference in the BMI values within healthy control subjects subdivided into carriers of the Met/Met, Val/Met and Val/Val genotypes (Figure 1) since the lowest BMI values were found in healthy carriers of the Met/Met genotype compared to carriers of other *BDNF* genotypes. Dunn's method showed nominally significant difference (p = 0.046) in BMI values between healthy subjects, carriers of the Met/Met and Met/Val genotypes (Figure 1).

To evaluate the possible association between BMI and age, gender and diagnosis (CHD vs. healthy group), multiple linear regression analysis was used. This analysis used BMI as the dependent variable, and age, gender and diagnosis as independent variables, and revealed a significant model ($F_{3,700}$ = 4.56, p = 0.004, R_{adj}^2 = 0.015). However, there was no significant effect of age (p = 0.066), gender (p = 0.131) or diagnosis (p = 0.072) on BMI values. These results suggested that these independent variables did not affect BMI values.

Discussion

The main findings from the present study are: (1) similar *BDNF* rs6265 genotype frequency between patients with CHD and healthy controls and a lack of gender-related differences in the frequency of the *BDNF* rs6265 genotype; (2) significant differences in *BDNF* rs6265 genotype frequency between normal-weight, overweight and obese healthy subjects, but not within normal-weight, overweight and obese patients with CHD; (3) overrepresentation of the Met/Met genotype in healthy subjects with normal weight; (4) nominally significant difference in BMI values within healthy control subjects, but not within patients with CHD, subdivided into carriers of the *BDNF* rs6265 Met/Met, Val/Met and Val/Val genotypes; (5) the lowest BMI values in healthy carriers of the Met/Met genotype compared to carriers of other *BDNF* genotypes.

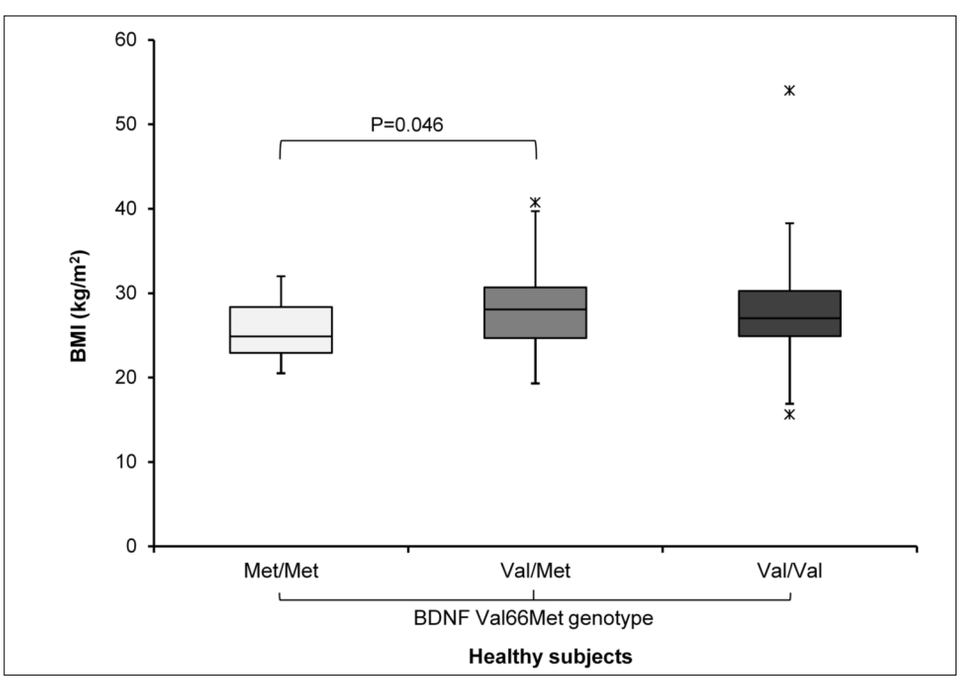

Figure 1. Values of the body mass index (BMI) in 498 healthy participants, subdivided according to the *BDNF* rs6265 polymorphism into carriers of the Met/Met, Val/Met and Val/Val genotypes. Central box represents the values from the lower to upper quartile and the middle line represents the median. The horizontal line extends from the minimum to the maximum value and the far out" values (outliers) are displayed as separate asterisks. *p = 0.046 for BMI values between the Met/Met and Val/Met genotype carriers (Dunn's test).

In our work, the *BDNF* rs6265 genotype frequency did not differ between Caucasian patients with CHD and healthy controls. These results are in line with the findings obtained on Caucasian Italian subjects[33] where the allelic and genotypic frequencies of *BDNF* rs6265 polymorphism were similar in patients with coronary artery disease and healthy controls. That study showed a gender-specific association[33] since there was a higher frequency of the Met allele in female patients with coronary artery disease compared to female control subjects, suggesting a higher cardiovascular risk. However, the cited work was underpowered, as it included only 12 patients with coronary artery disease[33], and hence the reported significance might be due to the type 1 error. In contrast to these data, in our study gender was not significantly associated with *BDNF* rs6265 polymorphism either in patients with CHD, or in healthy subjects. Therefore, we collapsed all participants (CHD and controls) and subdivided them according to gender, and found no significant ($\chi^2 = 0.196$; df = 2; $p = 0.906$) differences in the *BDNF* rs6265 genotype frequency between 343 men and 361 women. The lack of any gender-related differences in the frequency of the *BDNF* rs6265 genotypes is in line with the previous data[7,24,29,37].

Divergent to our results, a significant association between the *BDNF* rs6265 polymorphism and coronary artery disease was reported in Chinese patients[12]. That study, which included a sufficient number of participants, found a protective effect of the *BDNF* Met/Met genotype on the occurrence of the unstable angina pectoris. The differences might be due to the ethnic differences in the distribution of the *BDNF* rs6265 genotypes between Caucasian and Asian participants, especially in the frequency of the Met/Met genotype[9,37]. To evaluate this ethnic difference in all subjects included in our present and the cited report[12], we evaluated the distribution of the *BDNF* rs6265 genotypes, and confirmed significant ($\chi^2 = 302.867$; df = 2; $p < 0.001$) differences between these two ethnic groups, as the *BDNF* Met/Met genotype was found in 4.5% of Croatian participants compared to 21.2% of Chinese participants. Our research, performed on the homogenous sample of Caucasian subjects of the Croatian origin, did not find a significant association between *BDNF* rs6265 polymorphism and CHD. This result was not confirmed in a recent meta-analysis[38] that included Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) consortium with > 22 000 coronary artery disease cases and > 60 000 controls. This investigations found that *BDNF* rs6265 polymorphism was significantly associated with coronary artery disease, although with a modest effect[38]. The discrepancies between studies lay in the number of subjects involved, but also in the fact that CARDIoGRAM included more heterogeneous sample. However, this work provided evidence that smoking, a variable that was not controlled in our study, did not significantly affect the association between *BDNF* rs6265 polymorphism and CHD[38]. Therefore, we might conclude that a larger sample of CHD cases might help reveal a significant association, which was not detected in our present sample.

Although unexpected[24,29], our next major finding was a significant association between *BDNF* rs6265 polymorphism and BMI categories in healthy adults, but not in CHD subjects, since Met/Met genotype was more frequently found in healthy control subjects with normal weight. This result is in line with other literature data showing that this polymorphism was associated with BMI[25,31,32,39], and it was confirmed when healthy subjects were subdivided into carriers of different *BDNF* rs6265 genotypes, and their BMI values were compared. Namely, lower BMI values were detected in carriers of the Met/Met genotype, suggesting a protective role of this genotype in obesity in healthy, but not in CHD subjects. The result agrees with the significant association between the Met/Met genotype and lower BMI[25,31,32]. Our results agree with the reported association between *BDNF* rs6265 polymorphism and obesity in a large number of children of European origin[40], large groups of Filipino women[41], and in a large number of European[42] and Korean[31] subjects. As *BDNF* rs6265 is a functional polymorphism[21,22], the protective effect of the *BDNF* 66Met allele in obesity, found in our study in healthy subjects, might be explained by the higher *BDNF* concentration associated with the *BDNF* 66Met allele[38,43] in normal weight subjects. If *BDNF* Val carriers have reduced *BDNF* mRNA expression and, therefore, decreased BDNF concentration in the brain regions that regulate feeding and energy expenditure, this might result in excessive energy intake and increased weight gain. Similar findings were shown for other *BDNF* polymorphisms, as rs12291063 was associated with obesity[44], while other polymorphisms in the *BDNF* gene were also associated with BMI in a multi-ethnic meta-analyses[40,45]. The association between *BDNF*

rs6265 polymorphism and serum BDNF concentration was recently confirmed in large cohort[38], but contradictory data also exist[12,46.]

Our data do not agree with findings showing a lack of association between *BDNF* rs6265 polymorphism and BMI[29,47-49], or with data showing increased BMI related to more frequent presence of the *BDNF* 66Met allele in adult women[26] or adolescents[24]. As compared to a lack of significant association between BMI and *BDNF* rs6265 polymorphism in our previous study[29], the differences might be explained by the fact that the present research included a larger healthy control group that was sampled in the same center. Also, this previous study sampled subjects longitudinally at three-time points (1972, 1982 and 2006), while the present study sampled subjects during the year 2014. Based on the WHO estimates, 57.7% of the adult population (> 20 years old) in Croatia are overweight and 24.2% are obese. This increase in obesity in Croatia follows trends in EU[50], which is confirmed by the BMI values (27.4 ± 4.5) reported article previously including 1553 healthy control volunteers[51]. These BMI values are similar to average BMI values (27.6 ± 4.2) in the present study. However, in our previous article[29] marginally lower BMI values could be observed in carriers of the Met/Met genotype, but, due to the rare (3.5%) frequency of the Met/Met genotype, subjects were classified into Met carriers and Val/Val homozygotes.

The present paper found no significant association between *BDNF* rs6265 polymorphism and BMI values or BMI categories in patients with CHD. There was a similar trend towards the higher Met/Met genotype frequency in normal weight patients with CHD, that followed the results in healthy subjects, but this trend was not significant. As the Met/Met genotype is rare in Croatian subjects, this result might be explained by the smaller group of patients included, and larger groups are needed to confirm this result. Similarly, no significant association between *BDNF* rs6265 polymorphism and metabolic syndrome, which is characterized also by the increased BMI, was found in Thai subjects[48].

Therefore, as recently discussed[52], our results suggest a major role of BDNF and its tropomyosin receptor kinase B (TrkB) in adaptive responses of the brain and body to metabolic challenges, response to metabolic, oxidative, and excitotoxic stress, and in the control of energy metabolism via divergent effects achieved in the CNS but also in pancreas, liver, skeletal muscle, and heart. Its beneficial effects are seen in the suppression of appetite, increase in insulin sensitivity and parasympathetic cardiovascular tone, while detrimental effects are associated with reduced BDNF signaling, lower BDNF concentrations and development of the metabolic syndrome and obesity, leading to CHD[52].

Although the study had sufficiently large sample size (n = 704) to detect a significant associations and adequate statistical power (≥ 0.800) with the level of significance corrected to 0.025, possible limitation was a relatively small sample size of CHD group (n = 206) which is why we might have missed to detect a possible association of *BDNF* rs6265 polymorphism and BMI in this group. Only one SNP of the *BDNF* gene (rs6265) was assessed. However, this is a functional polymorphism[21,22] reported to be associated with plasma/serum BDNF concentration[39], BMI[25] and CHD[38]. As the frequency of the *BDNF* Met/Met genotype is very rare in Caucasians of Croatian origin[29,37,53,54], the frequency of the Met/Met genotype was less than 5% in overweight and obese subjects included in this study. Hence, these results should be taken into consideration with caution, and these investigations should be confirmed in larger groups. Advantages of the study were the inclusion of ethnically homogenous non-related Caucasian subjects of the European ancestry.

Conclusions

BDNF rs6265 polymorphism was not associated with diagnosis of CHD or with BMI in patients with CHD. However, *BDNF* rs6265 polymorphism was associated with BMI among healthy control subjects due to the most frequent presence of the *BDNF* Met/Met genotype in healthy subjects with normal weight.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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