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Association study of the *BDNF* gene polymorphism (G196A) with overweight/ obesity among women from Northwest of Iran

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Abstract

Background: Obesity is a health problem defined by surplus body fat accumulation and is one of the leading causes of morbidity and mortality. Earlier studies indicated the influence of brain-derived neurotrophic factor (*BDNF*) molecular alterations in the development of obesity. One of these variations is the G196A single nucleotide polymorphism (Val66Met; SNP rs6265), which impairs intracellular trafficking and reduces the secretion of BDNF. In this study, we evaluated the possible association of G196A polymorphism of the *BDNF* gene with body mass index (BMI) among women from the Iranian Azeri Turkish ethnic group. Four hundred eighty-four women including 343 women with obesity or overweight and 141 age-sex and ethnically matched healthy controls were genotyped for G196A SNP of *BDNF* gene by applying polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method. The association of this polymorphism with BMI was evaluated using analysis of covariance (ANCOVA), and the comparison of alleles and genotypes frequencies between patients (obese and/or overweight participants) and healthy controls was carried out using logistic regression models.

Results: Individuals carrying Met-Met genotype have a significantly lower mean of BMI in comparison to those carrying non-Met/Met polymorphisms ($P = 0.0138$).

Conclusions: In this study, the association of the Val66Met polymorphism of the *BDNF* gene with BMI as an obesity trait has been confirmed among the women from the Northwest of Iran.

Keywords: Brain-derived neurotrophic factor, Obesity, Polymorphism

Background

Obesity is one of the important health problems in which excess body fat accumulation can lead to increased mortality and risk of disease, such as metabolic syndrome, type 2 diabetes, stroke, coronary heart disease, liver and gallbladder disease, cancer, osteoarthritis, and sleep disorders [1]. In 1997, obesity was formally recognized as a global epidemic by the World Health Organization (WHO) [2]. According to the WHO, more

than 1.9 billion adults (39% of men and 40% of women) were overweight in 2016. Of these, over 650 million adults (11% of men and 15% of women) had obesity and approximately 41 million children under 5 years of age were overweight or obese [3].

The rate of obesity is increasing in both developed and developing countries over the past two decades [4]. In the Middle East, including the Arabian Peninsula, Eastern Mediterranean, Turkey, and Iran, obesity is a notable health issue [5]. In Iran, the prevalence of obesity has reached epidemic proportions and according to a recent study, it was 26.3% in 2008 [6].

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Table 1 Baseline phenotypes and rs6265 (Val66Met) genotype distribution of participant

		SD
Phenotypes		
Individual (*n*)	484	
Age mean (years)	35.85	9.18
BMI mean (kg/m^2)	30.26	6.72
Weight mean (kg)	78.45	16.64
Height mean (cm)	159.77	6.075
Additional phenotypes		
Fat percent	37.62	7.25
Waist mean (cm)	98.005	15.56

SD standard deviation

As a multifactorial and complex disease, obesity can be influenced by genetic and environmental factors [7]. Several studies have reported some variants in multiple genes that may contribute to gaining weight and distribution of body fat [8]. The contribution of genetic factors in obesity development is estimated to be 40–70% [9].

It has been documented that BDNF belongs to the neurotrophin family of growth factors and supports the survival and differentiation of existing neuronal populations in the peripheral and central nervous systems and synapses through its receptor, tropomyosin-related kinase B (TrkB) [10]. It is abundant in the central nervous system (CNS), especially in the hypothalamus, basal forebrain, cortex, hippocampus, and amygdala, areas that are critical in memory and learning [11]. Recent evidence suggests the effect of BDNF in weight regulation and its association with obesity in multiple types of knockout mouse models [12, 13].

The role of BDNF in the regulation of food absorption [14] and its dose-dependent appetite inhibition and weight loss were already described in previous studies [15].

Earlier studies indicated the involvement of *BDNF* molecular alterations in the development of obesity and eating disorders (EDs). One of these variations is the G196A single nucleotide polymorphism (Val66-Met; SNP rs6265) [16]. rs6265 is a common SNP in the *BDNF* gene that has been associated with several clinical traits such as anxiety disorders, learning and memory disorders, neurodegenerative disorders including Parkinson's and Alzheimer's, obesity, and BMI [17, 18]. This exchange of valine to methionine at codon 66 located at the prodomain of BDNF interferes with the intracellular processing, trafficking, and secretion of BDNF [19].

The goal of this study was to evaluate the possible association of *BDNF* Val66Met polymorphism with susceptibility to obesity and BMI among women from the Turkish Azeri ethnic group. To the best of our knowledge, this would be the first investigation of the Val66Met polymorphism of *BDNF* gene in this population.

Methods
Study subjects
Our study included 484 women from the northwest region of Iran, who were divided into three groups: obesity (82 subjects), overweight (261 subjects), and control (141 subjects) groups from 17 to 59 years of age (mean ± SD age, 35.85 ± 9.18). This study was carried out in Tabriz city, the capital of East Azerbaijan Province, located in the Northwest of Iran, from December 2016 to August 2017. Participants were recruited through announcements and flyer distribution in public areas of the city. The inclusion criteria were between the ages of 17 and 60 years and living in Tabriz for at least 5 consecutive years. Also, all contributors in this study were premenopausal and not pregnant or lactating at the time of the study. Subjects who had been participated in weight loss programs during the last 6 months and those suffering from alcoholism, substance addiction, psychotic disorders, or a serious disease such as cardiovascular and endocrine diseases were excluded from the study.

Biological parameters
The patients with light clothing and no shoes were weighed and measured after a 12-h fasting period. Body weight was measured using an electronic scale that was calibrated periodically (SECA Birmingham, UK) with an accuracy of 0.1 kg. Height was measured using a fixed stadiometer to the nearest 0.1 cm. BMI was calculated as a person's weight in kilograms divided by height in square meter (kg/m^2). According to the BMI categories, determined by the World Health Organization in 1997, subjects were classified as obese (BMI ≥ 30.0), overweight (BMI = 25.0–29.9), and normal weight (BMI = 18.5–24.9) [20].

Genotyping
Four milliliters of venous blood samples was collected from all participants in EDTA anticoagulant polypropylene tubes. Written consent to inclusion was obtained from all participants and they were informed of the study. Our protocol was reviewed and approved by the Tabriz University of Medical Science Ethical Committee and assigned with the registry number 1395.1.133 (IR.TBZMED.REC.1397.347).

Genomic DNA from subjects was extracted from peripheral blood leukocytes by the use of simple salting-out procedure. The *BDNF* Val66Met polymorphism (dbSNP ID rs6265) at position G196A was identified by a polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) technique as reported previously [21]. In brief, the 272-bp region of the *BDNF*

Table 2 Comparison of allele and genotype frequencies between patients (obese and/or overweight participants) and healthy controls

	Control BMI < 25	Obesity + overweight BMI ≥ 25	OR	95% CI Lower	Upper	P value
Genotype						0.465
G/G	102 (72.34%)	240 (69.97%)	Ref			
A/A	6 (4.3 %)	9 (2.62%)	0.637	0.221	1.837	0.311
A/G	33 (23.40%)	94 (27.40%)	1.211	0.765	1.916	0.217
Allele						
G	237 (84%)	574 (83.7%)	Ref			
A	45 (16%)	112 (16.3%)	1.028	0.704	1.499	0.887
	Control BMI < 25	**Obese BMI ≥ 30**	**OR**	**95% CI Lower**	**Upper**	**P value**
Genotype						0.5149
G/G	102 (72.34%)	188 (72%)	Ref			
A/A	6 (4.3 %)	6 (2.3%)	0.543	0.171	1.725	0.2636
A/G	33 (23.40%)	67 (25.7%)	1.102	0.681	1.783	0.2686
Allele						
G	237 (84%)	443 (84.9%)	Ref			
A	45 (16%)	79 (15.1%)	0.939	0.630	1.399	0.758
	Control BMI < 25	**Overweight 25 ≤ BMI < 30**	**OR**	**95% CI Lower**	**Upper**	**P value**
Genotype						0.3054
G/G	102 (72.34%)	52 (63.4%)	Ref			
A/A	6 (4.3 %)	3 (3.7%)	0.981	0.236	4.080	0.7237
A/G	33 (23.40%)	27 (32.9%)	1.605	0.873	2.949	0.2799
Allele						
G	237 (845%)	131 (79.9%)	Ref			
A	45 (16%)	33 (20.1%)	1.327	0.807	2.181	0.264
	Overweight 25 ≤ BMI < 30	**Obese BMI ≥ 30**	**OR**	**95% CI Lower**	**Upper**	**P value**
Genotype						0.3194
G/G	52 (63.4%)	188 (72%)	Ref			
A/A	3 (3.7%)	6 (2.3%)	0.553	0.134	2.286	0.5747
A/G	27 (32.9%)	67 (25.7%)	0.686	0.399	1.180	0.8514
Allele						
G	131 (79.9%)	443 (84.9%)	Ref			
A	33 (20.1%)	79 (15.1%)	0.708	0.451	1.111	0.132

OR odds ratio, *CI* confidence interval

gene, including the Val66Met polymorphism site, was amplified by using PCR. The PCR product was overnight digested by the restriction enzyme NlaIII at 37 °C. The genotype was identified by electrophoresis on 8% polyacrylamide gel and stained with ethidium bromide and visualized under ultraviolet light.

Statistical analysis

Statistical analysis was carried out using SAS 9.2 online software and SPSS. The association between Val66Met polymorphism and BMI (comparison of BMI means

between genotypes) was evaluated using analysis of covariance (ANCOVA), and the comparison of allele and genotype frequencies between patients (obese or overweight participants or both of them) and healthy controls was carried out using logistic regression models. In this study, we regarded probability values of 0.05 or less as statistically significant. All analyses were adjusted for age.

Results

The baseline phenotypes and Val66Met genotypes of the participants in our study are demonstrated in Table 1.

Table 3 BMI comparison by genotype and study group

BDNF rs6265	Number	BMI (kg/m^2), mean	SD	P value
AA	15	26.85	5.2	0.0470
AG	127	31.1	6.2	
GG	342	30.74	6.8	
GG	342	30.74	6.8	0.0474
AG + AA	142	30.37	6.29	
AA	15	26.85	5.2	0.0138
AG + GG	469	30.50	6.74	

The distribution of BDNF Val66Met (rs6265) genotypes conformed to the Hardy-Weinberg equilibrium (P = 0.5). To characterize the risk of BDNF variant on obesity in this population, we evaluated the risk of being overweight and/or obese according to BDNF genotypes, by use of logistic regression models. Our participants were initially divided into two groups: the control group (BMI < 25) and patients with BMI ≥ 25. A comparison of the allele and genotype frequencies of Val66Met polymorphism between these two groups showed no significant differences (P = 0.465) (Table 2).

Afterward, we performed several analyses and compared allele and genotype frequencies between the following groups: obese participants (BMI ≥ 30) and controls (P = 0.5149), overweight (25 ≤ BMI < 30) participants and controls (P = 0.3054), and obese and overweight individuals (P = 0.3194) (Table 2).

In this cohort, we classified participants into three groups based on their genotypes (A/A, A/G, G/G). A comparison of BMI means among these groups revealed statistically significant differences (P = 0.0470). Individuals carrying AA genotypes in comparison to those carrying non-AA genotypes have statistically significant lower BMI (P = 0.0138) (Table 3).

Discussion

In the present study, we analyzed the possible association between the Val66Met (rs6265) polymorphism of BDNF gene and BMI among 484 women from the Iranian Azeri Turkish ethnic group, and to the best of our knowledge, this is the first survey of this association in Iran. Val66Met polymorphism was detected to be significantly linked with BMI in this study and women with AA (Met-Met) genotype had lower BMI than those with AG (Val-Met) and GG (Val-Val) genotypes. No significant association was observed between genotype and allele frequencies of overweights and controls and between obese and overweight participants.

The prevalence of obesity and correlated morbidities such as coronary heart disease, certain cancers, and type 2 diabetes are ascending, and obesity has become a serious public health issue [1]. Some environmental and lifestyle factors such as lack of physical activity and eating high-fat foods certainly increase the prevalence of obesity worldwide. However, differences in genetic makeup may lead to diversities of body weight in a population sharing the same environment [22]. Brain-derived neurotrophic factor (BDNF), encoded by the BDNF gene, is a member of the neurotrophin family of growth factors that bind to the tyrosine kinase receptor tropomyosin-related kinase B (TrkB) and activate signaling [10]. Haploinsufficient mice in which the BDNF gene has been eliminated demonstrate obesity with hyperactivity and hyperphagia [23].

It has also been reported that conditional deletion of BDNF in mice leads to stimulated longitudinal growth, 80–150% body weight gain, and increased serum of insulin, leptin, cholesterol, and glucose in the blood [13]. Val66Met polymorphism is a common single nucleotide polymorphism in the BDNF gene, which interferes with the intracellular trafficking of BDNF, resulting in the reduced secretion of this protein [19].

Our findings are broadly consistent with the studies carried out on British women populations by Gunstad et al. and Shugart et al. and also with the study on the Korean population reported by Hong et al. [18, 24, 25]. However, in other studies, individuals carrying Met-Met (A/A) or Val-Met (A/G) genotypes had higher mean BMI and also they were more likely obese than those carriers of Val-Val (G/G) genotypes [26–28]. These contradictory outcomes could be due to differences in lifestyle, environmental factors, and genetic backgrounds. The current study showing a significant association of Val66Met with BMI is restricted to the female participants from the Iranian Azeri Turkish ethnic group. So, we recommend a larger study including both men and women to characterize the gender differences in this association.

Conclusions

In our study, the association of the Val66Met polymorphism of the BDNF gene with BMI as an obesity trait has been confirmed in the Northwest of Iran women population. Mean BMI was significantly lower in women carrying AA (Met-Met) genotype compared to those with AG (Val-Met) and GG (Val-Val) genotypes (P = 0.0138).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The authors give thanks to all the study participants. Written consent to inclusion was obtained from all participants and they were informed of the study. Our protocol was reviewed and approved by Tabriz University of Medical Science Ethical Committee and assigned with the registry number 1395.1.133 (IR.TBZMED.REC.1397.347).

Consent for publication
Not applicable.

Competing interests
The authors confirm that they have no competing interests.

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