Original article

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The relationship of Bradykinin B_2 receptor gene variation with obesity, hypertension and lipid variables in obese patients

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Objective. This study examined the association of C-58T genotypes with obesity/hypertension related parameters and serum lipids in obese (n=108) and non-obese (n=80) patients. **Materials and methods.** Bradykinin receptor (B_2R) C-58T genotypes were determined by PCR–RFLP. **Results**. B_2R gene C-58T frequencies for T/T (homozygous wild type), T/C (heterozygous) and C/C (homozygous polymorphic) genotypes for obese and non-obese patients were respectively: 36.1%, 37.5%; 45.4%, 52.5% and 18.5%, 10%. Obese patients using diuretic medication had lower C/C genotype frequency compared to T/T and T/C genotypes. Total cholesterol (T-Chol) (p=0.035) levels were found to be associated with B_2R C-58T polymorphism, where the T/T genotype had higher total cholesterol levels compared to the T/C genotype in obese patients. Non-obese patients using oral antidiabetic medication had higher C/C genotype frequency than that of T/T and T/C genotypes. Waist circumference (p=0.016) and diastolic blood pressure (p=0.01) levels were elevated in the non-obese subjects with the C/C genotype compared to T/C and T/T. **Conclusion.** Although B_2R C-58T gene polymorphism was not found to be effective on obesity with logistic regression analysis in the whole study population in obese subjects, the T-Chol decreasing effect of the B_2R gene C allele and the higher waist circumference measurements in the non-obese subjects may indicate there may be a link between B_2R gene C-58T polymorphism and obesity in study populations of higher numbers.

Key words: B_2R C-58T polymorphism, PCR, Obesity, Serum lipids, Oral antidiabetic.

Introduction

Obesity is a multifactorial disease influenced by genetic and environmental factors (1, 2). The kallikrein-kinin system (KKS) has important regulatory roles in peripheral glucose utilization (3-5), insulin action, blood pressure and sodium regulation in the renal tubulus (6-9). It is hypothesized that the Renal-Bradykinin-System plays an important role in the development of hypertension (10). Infusion of bradykinin in the renal artery mediates the release of prostoglandins and nitric oxide (NO), following increased blood flow to the kidney, thus leading to di-

uresis and natriuresis (11, 12). Bradykinin and kallidin (Lys-bradykinin) are enzymatically cut and released via kallikreins during inflammation and related states (13-16). Moreover, the kinins have attendant increasing insulin sensitivity obtained with angiotensin converting enzyme (ACE) inhibitors, in both animal models and in humans with an insulin resistant condition (17, 18).

A study performed on different tissues of B_2R gene knockout mice, corresponds to a state similar to insulin resistance (19). B_2R acts by potentiating insulin-induction and bradykinin enhanced insulin- stimulated GLUT4 translocation from intracellular fraction, insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1, and B_2R enhances dephosphorylation of the insulin receptor (20). The bradykinin receptor has two subtypes, namely; bradykinin B1 (B_1R) and bradykinin B2 (B_2R) (9, 21-24). The bradykinin subtypes are categorized under G protein coupled receptor superfamily. B_2R is known to play predominant role in the KKS (25). B_2R is constitutively expressed in most tissues (14). B_2R exerts a protective role in hypertension and cardiovascular disease (25). Human B_2R has been proved to be candidate gene for essential hypertension and cardiovascular disease, whereas its exact role in obesity and type 2 diabetes mellitus (T2DM) still remains to be elucidated. Human B_2R genomic structure has been characterized (25). Four polymorphisms located in each of the 3 exons and 1 polymorphism located in the promoter region have been identified within the B_2R gene (26, 27). C-58T polymorphism in the B_2R gene is known to have contradictory effects against hypertension in different races, with a protective effect in Asians and Afro-Americans, but not in Caucasians (28).

The aim of the present study was to determine and compare the genotypic frequencies of B_2R gene C-58T polymorphism in obese and non-obese patients. The associations of C-58T polymorphism with obesity related phenotypes, blood pressure levels and medication were also studied.

Materials and methods

Study subjects

The study was performed between April 2012 and February 2014. Blood samples were collected from Istanbul University Cerrahpasa Medical Faculty, Department of General Surgery (Istanbul, Turkey) from obese and non-obese patients. A total of 108 obese (BMI ≥25) and 80 non-obese control individuals were included in the study. Subjects with secondary hypertension (renal artery stenosis, glomerulonephritis), diabetic nephropathy (Kimmelstiel-Wilson syndrome), hypertension with endocrinopathies (phoechromocytoma, Cushing syndrome, hyper and hypothyroidism), patients with pseudohypertension, neoplasia and those who were taking oral contraceptives and illicit drugs were not included in the study. All disease diagnoses were made by an expert endocrinologist from Istanbul University Cerrahpasa Medical Faculty, and medication usage information was taken from the hospital files. Height was measured in meters with a stadiometer, by measurement of the maximum distance from the floor to the highest point on the head, when the subject was facing directly ahead. The individual's shoes were removed, their feet were together, and arms by their sides. Heels, buttocks and upper back were also allowed to be in contact with the wall during height measurement. Weight measurement was performed using a calibrated scale while the individual was standing with minimal movement, with hands by their sides. Shoes and excess clothing were removed during weight measurement.

Obesity, T2DM and hypertension were diagnosed according to the International Diabetes Federation (IDF) guidelines (29). Body mass index is defined as the individual's body mass divided by the square of their height (kg/m^2). Body fat quantification:, first lean body mass (LBM) was calculated by the formula given by Hume (30): for males, LBM (kg): 0.32810 X weight (in kilograms) +0.33929 X height (in cm) -29.5336; for females (kg): 0.29569 X weight (in kg) +0.41893 height (in centimeters) -43.2933. Body fat was calculated by subtracting the lean body mass from the present body weight. For evaluation of arterial blood pressure, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines were used (31). Metabolic syndrome (MS) patients met all the criteria defined by the American Heart Association's National Heart, Lung, and Blood Institute (NHLBI) (32). The MS criterion was a cluster of three or more of the following abnormalities: waist circumference >102 cm in men and >88 cm in women, serum triglycerides ≥1.7 mmol/L; high-density lipoprotein cholesterol (HDL-Chol) <1.03mmol/L in males and <1.29 mmol/L in females or specific treatment for this lipid abnormality (fibrates and nicotinic acid); blood pressure ≥130 / ≥85 mmHg or fasting serum glucose ≥5.6 mmol/L or drug treatment for hypertension or type 2 diabetes, respectively (32).

Biochemical measurements

Serum glucose was detected by the enzymatic reference method with glucose oxidase. HDL-Cholesterol and low-density lipoprotein-cholesterol (LDL-Chol) were directly determined by enzymatic colorimetric assay; serum total cholesterol was measured using the enzymatic, colorimetric method by cholesterol esterase; triglycerides were determined by the enzymatic colorimetric method (GPO/PAP) with cholesterol phosphate oxidase and 4-aminophenazone on an opeRA analyzer.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a salting out method (33). DNA concentration was measured spectrophotometrically. Absorbance ratios at 260nm and 280nm were used to assess the purity of DNA. Ratios over 1.8 were subjected to PCR analysis. Purified DNA (concentration of 50 ng) was stored at -20°C. The B_2R gene C-58T polymorphism was determined by the polymerase chain reaction (PCR) method, followed by restriction fragment length polymorphism (RFLP). The B_2R polymorphism studied was characterized by substitution of a thymine for cytosine at nucleotide position -58 in the promoter region (28). Since C-58T substitution does not change the recognition sequence for Mae III, a partial recognition site for Mae III was added as a single mismatched base in the sense primer for PCR amplification. The Mae III site was then completed in the presence of the -58C allele. The PCR primers were chosen to specifically target the human bradykinin gene covering B_2R polymorphism in the promoter/exon1 region. The PCR conditions were 30–50 ng genomic DNA, 0.2 units of Taq Polymerase (Fermentas), 0.5µl of 100 mol/l dNTPs, and 0.15 µl of 50 µmol/l primers in a 25µl reaction. The PCR cycling conditions were: 95°C 7 min; 35 x (94°C 20 sec, 55°C 20 sec, 72°C 20 sec), 72°C 10 min (28). The PCR products were restriction digested for 4 hr at 37°C. The C-58T primer sequences were as follows: left primer, 5'-GCCCAGGAGGCTGATGACGTCA-3'; right primer, 5'-TCACCAACCCTCCGGACCC-3'. Digestion was overnight with 5 units of Mae III (*Fermentas*). The PCR products were 110 bp

in length, producing 92 bp and 18 bp fragments after Mae III digestion. The digested products were evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for genotype analysis.

Ethics statement

The study was approved by the local Ethics Committee of Marmara University. All the subjects who contributed to the study gave informed consent prior to participating in the study.

Statistical analysis

Statistical analyses were performed using the SPSS 17.0 software program. Data were expressed as Median (Min-Max) IQR for numeric data. Data distribution testing was performed using the Shapiro-Wilks test. In the case of normal data distribution, one-way ANOVA was used for genotype comparisons (Table 4), followed by the Bonferroni test for statistically significant results for pairwise comparison. In the case of discrete data (Table 5) the Kruskal-Wallis test was used for comparison of genotypes, and afterwards for statistically significant parameters, the Bonferronni corrected Mann Whitney- U test was used for pairwise comparison. For the Bonferronni corrected Mann Whitney-U test, the limit for statistical significance was p=0.016. The categorical variables were expressed as a sample number (%). For categorical variables, c2 testing was used to assess differences in proportions (or Fisher's exact test when cell frequencies were small). The general significance level was $p<0.05$.

Results

The B_2R gene C-58T genotype frequencies in obese and non-obese study groups are presented in Table 1. The B_2R gene C-58T polymorphism frequencies for wild type homozygous (T/T), heterozygous (T/C) and homozygous polymorphic (C/C) genotypes were respectively: 36.1%, 45.4%, 18.5%, in the obese group, and 37.5%; 52.5% 10% in the non-obese group. The B_2R gene C-58T genotype frequencies did not differ significantly between the study groups (χ^2=2.749, p=0.253) and genotype frequency distributions did not obey the Hardy-Weinberg equilibrium (Table 1).

The disease data and the characteristics of the study population is given respectively in Table 2 and Table 3.

LDL-cholesterol, TG, T-cholesterol, glucose, systolic blood pressure, diastolic blood pressure, waist circumference, BMI, fat mass were significantly higher in obese compared to non-obese patients (Table 3).

The associations of B_2R gene C-58T genotypes in the obese group, with the analyzed biochemical and clinical parameters are presented in Table 4. The B_2R gene C-58T genotypes were not found to be associated with the analyzed phenotypes such as: weight, height, waist circumference, body mass in-

Table 1 Bradykinin C-58T polymorphism genotype frequencies in obese and non-obese subjects

Subjects	Bradykinin C-58T genotypes		
	Homozygous wild type n (%)	Heterozygous n (%)	Homozygous polymorphic n (%)
Obese	39 (36.1)	49 (45.4)	20 (18.5)
Non-Obese	30 (37.5)	42 (52.5)	8 (10)

There were no significant differences between the groups (χ^2=2.749, p=0.253).

Table 2 Disease data of the study population

Diseases	Subjects	
	Obese, n (%)	Non-Obese, n (%)
Hypertension	51 (47.2)	12 (15)
Type 2 Diabetes Mellitus	54 (50)	12 (15)
Dyslipidemia	26 (24.1)	13 (16.2)

Table 3 Characteristics of the study population

Characteristics	Subjects						p
	Obese (n=108)			Non-Obese (n=80)			
	Median	Min-Max	IQR	Median	Min-Max	IQR	
Age (years)	61.0	44.0-82.0	14.0	56.0	25.0-90.0	13.5	0.031
Weight (kg)	80.0	50.0-120.0	17.0	68.0	50.0-108.0	16.0	0.001
Height (m)	1.6	1.4-1.8	0.1	1.6	1.5-1.9	0.1	0.004
LBM (kg)	47.0	34.3-69.2	9.9	47.1	34.3-66.6	7.9	0.723
FM (kg)	30.3	15.7-50.8	9.4	20.0	10.2-44.8	12.1	0.0001
BMI (kg/m^2)	31.1	22.2-42.3	5.7	24.6	20.0-42.9	6.8	0.0001
Waist (cm)	101.5	72.0-125.0	12.7	86.0	67.0-130.0	28.2	0.0001
T-Chol (mmol/l)	5.5	3.2-7.6	75.0	4.6	1.3-7.9	53.9	0.040
TG (mmol/l)	1.5	0.6-4.3	65.1	1.2	0.6-3.4	42.5	0.018
HDL-Chol (mmol/l)	1.2	0.6-2.0	18.3	1.2	0.6-2.1	18.6	0.518
LDL-Chol (mmol/l)	3.1	0.6-5.7	81.0	2.3	0.7-9.5	72.8	0.007
Glucose (mmol/l)	7.4	4.3-21.2	116.0	4.5	2.2-16.5	55.2	0.0001
SBP (mmHg)	150.0	120.0-220.0	20.0	125.0	100.0-180.0	25.0	0.0001
HbA1c (%)	8.2	4.9-13.8	4.6	6.2	4.9-11.3	2.4	0.181
DBP (mm Hg)	90.0	65.0-110.0	10.0	75.0	60.0-120.0	10.0	0.0001

BMI=Body mass index; LBM=Lean body mass; FM=Fat mass; T-Chol=Total cholesterol; HDL-Chol=High-density lipoprotein; LDL-Chol=Low-density lipoprotein; TG=Triglyceride; SBP=Systolic blood presure; DBP=Diastolic blood presure.

Table 4 Association of bradykinin C-58T genotypes with various phenotypes in the obese group

Characteristics	Bradykinin genotypes									p
	Homozygous wild type (n=39)			Heterozygous (n=49)			Homozygous polymorphic (n=20)			
	Median	Min-Max	IQR	Median	Min-Max	IQR	Median	Min-Max	IQR	
Weight (kg)	80.0	60.0-105.0	19.0	80.0	60.0-110.0	19.0	79.0	(65.0-120.0)	17.0	0.903
Height (m)	1.6	1.4-1.8	0.1	1.6	1.4-1.8	0.2	1.6	(1.5-1.8)	0.1	0.466
Waist (cm)	102.0	74.0-130.0	18.0	102.0	71.0-125.0	19.0	100.0	(72.0-120.0)	16.0	0.814
BMI(kg/m^2)	29.5	25.1-42.9	6.8	30.0	22.0-42.3	6.4	30.4	(25.4-39.2)	5.6	0.953
LBM (kg)	48.8	25.8-61.1	9.6	47.0	38.6-69.3	11.0	49.5	(40.2-69.2)	11.9	0.415
FM (kg)	29.0	20.6-51.1	13.6	30.3	13.9-48.9	9.9	29.7	(21.6-50.8)	11.0	0.923
T-Chol (mmol/l)	5.8	3.8-8.8	2.1	4.6	3.2-7.1	1.7	5.4	(3.7-7.0)	1.9	0.035
TG (mmol/l)	1.5	0.8-3.4	1.2	1.4	0.6-4.3	0.8	1.4	(0.6-2.6)	0.9	0.514
HDL-Chol (mmol/l)	1.1	0.9-2.1	0.6	1.2	0.6-1.9	0.4	1.1	(0.6-1.6)	0.6	0.521
LDL-Chol (mmol/l)	2.1	0.8-7.2	2.3	2.7	0.6-4.6	2.0	3.3	2.1-4.7	1.5	0.229
Glucose (mmol/l)	4.5	3.0-16.6	4.5	6.1	3.2-9.6	5.3	5.6	4-2.17.0	4.0	0.194
SBP (mmHg)	150.0	100.0-180.0	38.0	140.0	110.0-220.0	34.0	140.0	(120.0-170.0)	30.0	0.433
DBP (mmHg)	85.0	60.0-120.0	10.0	82.5	65.0-110.0	18.0	90.0	(70.0-100.0)	10.0	0.867

IQR=Inter quartal range; BMI=Body mass index; LBM=Lean body mass; FM=Fat mass; T-Chol=Total cholesterol; HDL-Chol=High-density lipoprotein; LDL-Chol=Low-density lipoprotein; TG=Triglyceride; SBP=Systolic blood presure; DBP=Diastolic blood presure.

dex (BMI), lean body mass (LBM), fat mass (FM), triglycerides (TG), high density lipoprotein-cholesterol (HDL-chol), low density lipoprotein-cholesterol (LDL-chol), systolic blood pressure (SBP), diastolic blood pressure (DBP) in obese patients (Table 4).

B_2R gene C-58T polymorphism was found to be associated with T-Chol (p=0.035) in

the obese patients (Table 4). The paired comparison of bradykinin C-58T genotypes for T-chol levels in the obese group showed that higher T-Chol levels in the T/T genotype existed in comparison to the T/C genotype (p=0.01) by the Bonferronni test (data not included, data normally distributed). The differences for T-Chol in the obese group and systolic blood pressure and waist measurement in the non-obese group between bradykinin C-58T genotypes occur mostly between the CC genotype and the others (TT and TC). Especially in the non-obese group the small number of CC genotype carriers is a limitation and may influence the magnitude of the significant association detected.

The asssociations of B_2R gene C-58T genotypes in the analyzed biochemical and clinical parameters in the non-obese group are presented in Table 5. B_2R gene C-58T polymorphism was not found to have any significant relation to serum lipids in the non-obese study group by the Kruskal Wallis test, since the data were discrete. The waist circumference (p=0.016) and diastolic blood pressure (DBP) (p=0.010) measurements were significantly higher in the C/C genotype carrying non-obese patients in comparison to T/T and T/C genotype carriers (Table 5). The paired comparisons of B2R gene C-58T genotypes for SBP levels in the non-obese group showed them to be significantly higher in the C/C genotype carrying non-obese patients than those of T/T (p=0.002) and T/C genotype (p=0.003) using the Bonferronni corrected Mann-Whitney U test (data not given). The paired comparison of bradykinin C-58T genotypes for DBP levels showed them to be significantly higher in the C/C genotype carrying non-obese patients in comparison to those with the T/C genotype with Bonferronni corrected Mann-Whitney U test (p=0.002) (data not included). The paired comparison of bradykinin C-58T genotypes for the waist circumference measurements in non-obese group showed them to be higher in the C/C genotype carrying non-obese patients in comparison to T/T (p=0.011) and T/C genotypes (p=0.009) using the Bonferronni corrected Mann-Whitney U test (data not in-

Table 5 Association of bradykinin C-58T genotypes with various phenotypes in the non-obese group

Characteristics	Bradykinin genotypes									p
	Homozygous wild type (n=39)			Heterozygous (n=49)			Homozygous polymorphic (n=20)			
	Median	Min-Max	IQR	Median	Min-Max	IQR	Median	Min-Max	IQR	
Weight (kg)	65.0	45.0 -80.0	8.0	62.5	41.0-86.0	15.0	64.0	50.0-67.0	5.0	0.495
Height (m)	1.7	1.5-1.8	0.1	1.6	1.5-1.9	0.2	1.6	1.5-1.7	0.1	0.291
Waist (cm)	72.0	67.0-100.0	15.0	74.5	67.0-94.0	11.0	87.0	78.0-106.0	12.0	0.016
BMI (kg/m^2)	22.8	18.7-45.0	1.9	22.7	17.7-45	2.3	23.6	22.2-25.0	2.0	0.356
LBM (kg)	47.4	37.8-58.5	5.1	44.6	32.5-57.8	12.3	46.4	34.3-48.1	8.7	0.410
FM (kg)	17.3	7.2-25.0	4.9	17.3	7.5-31.4	5.0	16.9	15.2-20.6	3.2	0.999
T-Chol (mmol/l)	4.8	3.0-5.9	1.7	4.5	1.3-8.0	1.3	ISN	-	-	
TG (mmol/l)	1.1	0.8-1.4	0.5	1.1	0.9-3.0	0.3	ISN	-	-	
HDL-Chol (mmol/l)	1.3	1.0-1.7	0.3	1.3	0.7-1.8	0.5	1.3	1.1-1.7	0.5	0.885
LDL-Chol (mmol/l)	1.1	0.7-4.1	2.4	1.8	0.8-9.5	1.7	3.2	1.4-3.4	1.1	0.216
SBP (mmHg)	120.0	100.0-140.0	13.0	120.0	100.0-160.0	15.0	145.0	130.0-170.0	25.0	0.007
DBP (mmHg)	70.0	60.0-90.0	15.0	70.0	60.0-90.0	10.0	85.0	80.0-100.0	13.0	0.010

IQR=Inter quartal range; BMI=Body mass index; LBM=Lean body mass; FM=Fat mass; T-Chol= Total cholesterol; HDL-Chol=High-density lipoprotein; LDL-Chol=Low-density lipoprotein; TG=Triglyceride; SBP=Systolic blood presure; DBP=Diastolic blood presure; ISN=Inadequate sample number.

cluded). The mean ± SE; median (min-max) values are not given for T-chol and TG due to the absence of the data (Table 5).

The frequency of Bradykinin C-58T genotypes for different medications in obese patients are represented in Table 6. Obese patients using diuretic medication were found to have T/T genotype in higher freqency than those with T/C and C/C genotypes (Table 6). There was no signifcant difference between genotype groups for any other medication used by the obese patients (Table 6).

The frequencies of Bradykinin C-58T genotypes for different medications in

Table 6 The frequencies of Bradykinin C-58T genotypes for different medications in obese patients.

Medication	Bradykinin C-58T Genotypes			
	T/T	T/C	C/C	p
	n (%)	n (%)	n (%)	
Diuretic	13 (61.9)	14 (38.9)	3 (20)	0.038
ACE	4 (20)	10 (30.3)	4 (26.7)	0.720
BB	12 (57.1)	18 (50)	8 (53.3)	0.872
Nitrit	2 (9.5)	7 (19.4)	0 (0)	0.180
ASA	9 (42.9)	18 (50)	7 (46.7)	0.872
ARB	1 (4.8)	3 (8.3)	2 (13.3)	0.730
CCB	2 (9.5)	2 (5.6)	4 (26.7)	0.110
Oral antidiabetic	10 (47.6)	20 (55.6)	9 (56.3)	0.818
Sulphonylurea	1 (4.8)	5 (13.9)	5 (33.3)	0.073
Glinide	5 (23.8)	14 (38.9)	6 (40)	0.458
Metformin	8 (38.1)	13 (36.1)	1 (6.7)	0.067
Insulin	4 (19)	10 (27.8)	17 (23.6)	0.076
Statin	8 (38.1)	16 (44.4)	3 (20)	0.259

T/T= Homozygous wild type; T/C= Heterozygous; C/C= Homozygous polymorphic; ACE=Angiotensin converting enzyme inhibitor; BB=Beta blocker; ASA=Acetyl salicylic acid; ARB=Angiotensin II receptor blocker; CCB=Calcium Channel Blocker.

Table 7 The frequencies of Bradykinin C-58T genotypes for different medications in non-obese patients

Medication	B2R C-58T genotypes			p
	T/T n (%)	T/C n (%)	C/C n (%)	
Beta blocker	1 (7.1)	4 (16)	1 (14.3)	0.840
Oral antidiabetic	3 (21.4)	5 (20)	3 (42.9)	0.045
Statin	1 (7.1)	1 (4)	3 (42.9)	0.032

T/T=Homozygous wild type; T/C=Heterozygous; C/C=Homozygous polymorphic.

Table 8 Identification of risk factors for their association with obesity by multiple logistic regression analysis

Risk factor	All Subjects			
	B	SE	OR	p
B2R C-58T T/T Genotype	-	-	-	0.226
B2R C-58T T/C Genotype	-1.098	0.647	0.333	0.089
B2R C-58T C/C Genotype	-0.933	0.841	0.394	0.268
Dyslipidemia	-1.416	0.738	0.243	0.055
Type 2 Diabetes Mellitus	0.653	0.601	1.920	0.278
Hypertension	0.415	0.629	1.514	0.510

B=indicates estimated coefficient; SE=standard error; OR=adjusted odds ratio.

non-obese patients are given in Table 7. Non-obese subjects using oral antidiabetics (p=0.045) and statin (p=0.032) were found to have the C/C genotype in higher frequencies than T/T and T/C genotypes (Table 7).

Risk factors associated with obesity such as: T2DM, dyslipidemia, hypertension and bradykinin C-58T polymorphism were evaluated using logistic regression analysis (Table 8). The bradykinin C-58T genotypes were not found to be independent progressive or regressive factors related to obesity (Table 8).

Discussion

According to our knowledge, this is the first study evaluating the relationship between B2R gene C-58T variation and obesity in Turkish subjects. Fallo et al. (9) reported B_2R C-58T polymorphism with 21.7% C/C, 51.1% C/T and 27.2% T/T genotype frequency distributions in obese patients. Other studies evaluating the effects of the same polymorphism have mostly been performed in essential hypertension patients. In detail, Mulatero et al. (28) found the C/C, C/T and T/T genotype frequencies respectively to be: 32.3%, 49.1%, 18.6% in hypertensive primary aldosteronism patients. A study performed on 200 Japanese individuals (100 hypertensive, 100 normotensive) reported B_2R C-58T genotype frequencies, where Mukae et al. (34) found the hypertensive and normotensive frequencies to be respectively: 28% and 18% for C/C, 59% and 57% for C/T, 13% and 25% for T/T. Fu et al. (35) analyzed 275 hypertensive and 441 normotensive patients for the effects of B_2R C-58T variation on essential hypertension. The hypertensive and normotensive genotype distributions were found to be respectively: 24% and 22% for C/C, 51% and 52% for C/T, 25% and 26% for T/T (35). Fu et al. (35) were not able to find any association between B_2R C-58T variation and essential hypertension. C-58T polymorphism is located at position -58 of the B_2R gene promoter.

The presence of -58C allele results in a decrease in gene transcription (27). B_2R is a candidate gene in the pathogenesis of insulin resistance and is often related to other diseases in metabolic syndrome (36, 37). C-58T polymorphism has been found to be related to bradykinin activity as a vasodilator in a limited number of studies (24, 38). In our study group, the frequencies of hypertensive patients were respectively 46% and 15% within the obese and non-obese groups. The B_2R gene C-58T frequencies observed in our study were similar to the results of Fallo et al. (9) and Fu et al. (35), where the polymorphic genotype frequencies were higher in obese versus non-obese subjects (9, 35). Despite a trend in our non-obese patients towards increased diastolic ($p<0.01$) and systolic ($p>0.05$) blood pressure values across genotypes, with the highest values in C/C and lowest in T/T, the lack of significant differences in obese patients does not allow the confirmation of our data. Insulin resistance is a predominant factor leading to T2DM, dyslipidemia and hypertension (39). As previously mentioned, insulin resistance may not necessarily be associated with an increase in LDL-cholesterol levels, but rather with a combination of elevated levels of other serum lipids (32, 40, 41). A close relationship between insulin resistance and hypertension has also been established in some studies (39, 41, 42, 43). Approximately half of all patients with essential hypertension are known to be insulin-resistant (44). Barros et al. (45) showed that genetically obese mice (ob/ob) lacking the B_2R gene (obB_2KO) showed increased fasting glycemia, hyperinsulinemia and impaired glucose tolerance compared to ob/ob control mice (obWT) which indicates

the presence of insulin resistance and impaired glucose homeostasis (45).

Researchers have shown that mutant mice lacking B2Rs display a moderate rise in basal blood pressure, but under a heavy sodium diet they showed heavy hypertension and end-organ damage (46, 47). A recent meta-analysis of B_2R gene C-58T polymorphism with hypertension suggested that the T allele exhibits a protective effect on hypertension in Asians and Afro-Americans, but not in Caucasians. Mulatero et al. (28) analyzed the effects of B_2R gene C-58T genotypes on BMI, and found insignificantly lower levels in T/T carriers, than those with variant and heterozygous genotypes (28). In our study, we detected non-significantly higher BMI measurements in those with T/T genotypes only in the non-obese group. Additionally we also detected lower measurements of waist circumference in non-obese patients with the T/T genotype of the B_2R gene. Two study groups investigated the B_2R gene C-58T variant C allele that increased both systolic and diastolic blood pressures in hypertensive patients in comparison to the wild type allele (28, 34). We observed that C/C genotype carriers have higher diastolic blood pressure levels, and the decreasing effect of T/T genotypes over diastolic blood pressure in non-obese groups, in accordance to the results of Mulatero et al. (28), and Mukae et al. (34), which was reported in hypertension study groups.

The higher frequency of B_2R gene C-58T T/T genotype frequency in obese diuretic users may be due to the relatively high frequency (46%) of hypertension in the obese group. Additionally, polymorphic C/C genotype frequency was observed to be higher in the non-obese patients using oral antidiabetics compared to the T/T and C/T genotypes. None of the risk factors such as: hypertension, type 2 diabetes, dyslipidemia and B_2R gene C-58T genotypes were found as independent risk factors for obesity when tested by logistic regression analysis. Among the analyzed serum lipids, in the obese group only T-Chol levels were found to be associated with B_2R C-58T polymorphism, where T/T genotype patients had higher T-Chol measurements than those of the T/C genotype.

The relatively small number of the study size, together with the low number of CC genotype carriers in the non-obese group limit us by rather low statistical power to determine any association of C-58T polymorphism with obesity or to detect any significant difference or interactions between other parameters.

Conclusion

In conclusion, while our results need to be confirmed in a more representative, large scale population, B_2R C-58T gene polymorphism was not found to be effective on obesity with logistic regression analysis in the whole study population. In the obese subjects, the T-Chol decreasing effect of the B_2R gene C allele and the higher waist circumference measurements in the non-obese subjects may indicate there may be a link between B_2R gene C-58T polymorphism and obesity in study populations with higher numbers.

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