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Association between B3-Adrenergic receptor *(ADRB3)* gene polymorphism with body mass index and bone mineral density in Turkish postmenopausal women

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Abstract: Previous studies have suggested that β3-adrenergic receptor *(ADRB3)* gene is associated with body mass index (BMI), which is an important predictor of bone mineral density (BMD). However, little is known concerning the effect of the *ADRB3* gene on BMD. The present study investigated the relationship between *ADRB3* Trp64Arg polymorphism, BMI and BMD in Turkish postmenopausal women. 133 postmenopausal women (81 osteoporotic and 52 healthy control) were recruited. For the detection of *ADRB3* Trp64Arg polymorphism, polymerase chain reaction-restriction fragment lenght polymorphism (PCR-RFLP) techniques have been used. BMD was measured at the lumbar spine and hip by dual-energy X-ray absorptiometry. Distribution of *ADRB3* Trp64Arg genotypes was similar in the study groups ($p>0.05$). "Arg/Arg" genotype was not observed. In the osteoporotic group, subjects with "Trp/Trp" genotype tended to have lower BMI values compared to those with "Trp/Arg" genotype, the difference was closely tied to statistical significance ($p=0.052$). Subjects with "Trp/Trp" genotype had also lower BMD values of femoral neck ($p=0.044$) and total hip ($p=0.043$) than those with "Trp/Arg" genotype. No significant effects of the *ADRB3* Trp64Arg genotypes on BMI and BMD values were found in the control group. Our results suggested that *ADRB3* Trp64Arg "Trp/Trp" genotype might be associated with osteoporosis risk by affecting body mass index and bone mineral density values in Turkish postmenopausal women.

Key words: β3-adrenergic receptor, gene polymorphism, body mass index, bone mineral density, postmenopausal women

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Introduction

β3-adrenergic receptor is coupled to guanine-nucleotide-binding (G) proteins and is primarily expressed in adipose tissue. Stimulation of the receptor by β-adrenergic agonists activates adenylate cyclase, which increases intracellular concentrations of cyclic adenosine monophosphate and results in increased lipolysis and thermogenesis (Emorine et al., 1989; Krief et al., 1993; La Fontan et al., 1993). β3-adrenergic stimulation can also induce expression of osteoclast differentiation factors in osteoblastic cells, leading to a stimulation of osteoclastogenesis (Takeuchi et al., 2001). The activation of beta-adrenergic receptors on two osteoblast-like cells can stimulate bone resorption in intact mouse calvariae (Moore et al., 1993).

Osteoporosis and obesity are diseases that may be inversely associated, with obese individuals having higher bone mineral density (BMD) than non-obese individuals (Wang et al., 2006). Several studies have shown positive correlations between BMD, body weight and body mass index (BMI) (Shiraki et al., 1991; Trevisan et al., 1991; Edelstein et al., 1993; Franceschi et al., 1996; Nguyen et al., 2005) which are the predictors of the two diseases. BMD is under strong genetic control with heritability estimates that range between 0.5 and 0.9 (Pocock et al., 1987; Slemenda et al., 1991; Arden et al., 1996) although many environmental factors, such as dietary intakes, phsycial activities play important roles in BMD (Wang et al., 2007). Genetic factors may also account for up to 90% of the BMI variance (Allison et al., 1996; Elks et al., 2012). The body weight and BMI were found to be the determinant of BMD and BMD variability was explained by the variation in these parameters (Orozco & Nolla, 1997; Martínez Díaz-Guerra et al., 2001).

A mutation identified in the β3-adrenergic receptor gene that results in the replacement of tryptophan by arginine (Trp64Arg) in the first intracellular loop of the receptor at position 64 (Waltson et al., 1995), has been associated with BMI and obesity in several studies (Clement et al., 1995; Fujisawa et al., 1998; Corella et al., 2001; Oizumi et al., 2001; Kurokawa et al., 2001; Marti et al., 2002; Park et al., 2005; de Luis et al., 2008a, b; Yamakita et al., 2010; Malik et al., 2011; Mirrakhimov et al., 2011), while there were no relations found in some (Gagnon et al., 1996; Büettner et al., 1998; Matsushita et al., 2003; Terra et al., 2005; Lwow

et al., 2007; Gjesing et al., 2008). Studies regarding the association of *ADRB3* gene variants with BMD values are very scarce (Wang et al., 2006; Ogawa et al., 1998; Katsumata et al., 2002; Lee et al., 2014). β-adrenergic blockers were found to reduce the risk of fracture partly by increasing bone mineral density in a Geelong osteoporosis study (Pasco et al. 2004). However, the effect of *ADRB3* gene on BMD was not examined in Turkish population so far. Therefore, we aimed to investigate the association between the *ADRB3* Trp64Arg polymorphism, BMI and BMD in healthy and osteoporotic Turkish postmenopausal women.

Materials and methods

Subjects

The cohort of this study comprised 133 Turkish postmenopausal women (81 osteoporotic and 52 healthy control, 59.06±7.31 mean aged), attending the Uskudar State Hospital in Istanbul. During ascertainment the WHO definitions and criteria for osteoporosis were used (World Health Organization Study Group, 1994). The patients received a detailed, standardized questionnaire including questions regarding the osteoporosis risk factors, such as family history of osteoporosis, menopausal status and age, smoking habit, alcohol consumption, medication use and other medical conditions. Only patients with a clinical diagnosis of osteoporosis were recruited. The control group contained individuals with normal BMD. Exclusion criteria included conditions, diseases and/or treatments known to interfere with bone metabolism, such as malignancies, endocrinologic disorders (hypo- and hyperparathyroidism, hyperthyroidism, Cushing's syndrome), severe liver or gastroenteral diseases, skeletal diseases (Paget's disease, osteogenesis imperfecta, osteomalacia and rheumatoid arthritis) and current pharmacological treatment with corticosteroids, anabolic androgenic steroids, estrogens or estrogen-related molecules, anticonvulsants before enrollment. Menopause was defined as amenorrhoea of at least one year duration. The study was approved by the Local Ethical Committee of Istanbul University, Istanbul Medical Faculty (Protocol No: 2006/2145, 20/12/2006) and written, informed consent was obtained from each participant prior to giving their blood sample.

BMD measurement

BMD for lumbar spine (L1-L4) and hip (femoral neck and total hip) was measured by GE-Lunar DPX Pro (GE Healthcare, Madison, WI, USA) Pencil Beam DXA densitometer. All DEXA scans were performed by the same technician and analyzed according to software (Encore 2005 version, 9.30.044, GE Healthcare) provided by the manufacturer. Briefly, subjects were positioned in the scanner according to standard procedures and remained motionless for approximately 10 minutes during scanning. The instrument was calibrated daily according to the manufacturer's instructions. BMD was expressed as grams per centimeter square (g/cm^2) and T scores which indicate the standard deviations of individiaul BMD determinations compared to those of young.

Genotype study

Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with salting out procedure (Miller et al., 1998). Trp64Arg polymorphism of the *ADRB3* gene was determined in duplicate using polymerase chain reaction-restriction fragment lenght polymorphism (PCR-RFLP) methods (Clement et al., 1995; Waltson et al., 1995). The primers (MBI Fermentas, Lithuania) used for PCR to amplify *ADRB3* gene fragments were as follows; forward primer 5'-CCAGTGGGCTGCCAGGGG-3' and reverse primer 5'-GCCAGTGGCGCCCAACGG-3'. PCR reactions were carried out in a final volume at 25 µL containing 10X reaction buffer (KCl), 1 mM of each nucleotide (dATP, dCTP, dGTP and dTTP) (MBI Fermentas, Lithuania), 1.5 mM $MgCl_2$, 25 picomolar of each primer, 0.3 U Taq DNA polymerase (MBI Fermentas, Lithuania) and 50 ng of DNA. Thermal profiles for amplification of *ADRB3* gene fragments consisted of an initial denaturating step of 3 min at 95ºC followed by 35 cycles of denaturation at 95ºC for 45 sec, annealing at 65ºC for 45 sec and extension at 72ºC for 45 sec with a final extension step for 5 min at 72ºC. *ADRB3* Trp64Arg genotypes were amplified specific polymerase chain reaction product in a DNA thermal cycler (GeneAmp 9700 PCR System; Applied Biosystems, CA, USA). PCR products were then digested with restriction endonuclease MvaI (BstNI; MBI Fermentas, Lithuania). Digestion products were seperated by

agarose gel electrophoresis and stained with ethidium bromide to visualise the fragmented DNA. Genotypes for Trp64Arg polymorphism of the *ADRB3* gene were classified as Trp/Trp, Trp/Arg and Arg/Arg.

Statistical analysis

The analyses were performed using SPSS software for Windows, version 13.0. Continuous variables are presented as means (± standard deviation-S.D.), while categorical variables are presented as frequencies. Chi-square test was used for genotype and allele frequencies comparison and Hardy-Weinberg Equilibrium (HWE). BMD values of different genotypes and alleles were compared by Student's t-test. A p-value of less than 0.05 was considered to be statistically significant.

Results

Demographic characteristics and BMD status

The demographic characteristics and BMD status of the study groups were presented in Table 1. There were significant differences in age, BMI, lumbar spine, femoral neck and total hip BMD values between osteoporotic and control subjects ($p<0.001$), whereas no significant differences were detected in age of menopause, smoking habit and family history of osteoporosis ($p>0.05$).

Table 1. Characteristics and BMD values of the study population

	Control	Osteoporotic
Number	52	81
Age	55.53 ± 7.04	61.20 ± 7.58 *
Age at menopause	46.47 ± 5.17	46.11 ± 5.48
BMI (kg/m^2)	33.22 ± 5.11	27.82 ± 4.21*
Smoking (%)	5 (9.6 %)	7 (8.6 %)
Family history of osteoporosis (%)	24 (46.2 %)	31 (38.3 %)
Lumbar spine (L_1-L_4) BMD (g/cm^2)	1.183 ± 0.110	0.827 ± 0.068*
Femoral neck BMD (g/cm^2)	0.966 ± 0.101	0.767 ± 0.095*
Total hip BMD (g/cm^2)	1.044 ± 0.099	0.814 ± 0.095*

Values are means ± SD except where noted. BMI: Body mass index, BMD: Bone mineral density

* $p<0.001$ vs. control group.

ADRB3 genotype and allele distribution

The genotype and allele frequencies of the *ADRB3* Trp64Arg polymorphism in the study groups were shown in Table 2. "Arg/Arg" genotype was not observed in the study population. The distribution of *ADRB3* Trp64Arg genotypes was consistent with the Hardy-Weinberg Equilibrium ($p>0.05$). No statistically significant differences were observed in the *ADRB3* Trp64Arg genotype and allele frequencies between the study groups ($p>0.05$).

Table 2. The distribution of *ADRB3* Trp64Arg genotype and allele frequencies in the study groups

Genotypes	Control n, (%)	Osteoporotic n, (%)	Total group n, (%)
Trp/Trp	44 (84.6 %)	65 (80.2 %)	109 (82 %)
Trp/Arg	8 (15.4 %)	16 (19.8 %)	24 (18 %)
Arg/Arg	-	-	-
Alleles			
Trp	96 (92.3 %)	146 (90.1 %)	242 (91 %)
Arg	8 (7.7 %)	16 (9.9 %)	24 (9 %)

The association of ADRB3 Trp64Arg polymorphism with BMI and BMD

The association of *ADRB3* Trp64Arg genotypes with the BMI and BMD values were shown in Table 3. In the osteoporotic group, subjects with "Trp/Trp" genotype tended to have lower BMI values compared to those with "Trp/Arg" genotype ($p=0.052$), the difference was closely tied to statistical significance. Subjects with "Trp/Trp" genotype had also lower BMD values of femoral neck ($p=0.044$) and total hip ($p=0.043$) than those with "Trp/Arg" genotype. No significant effects of the *ADRB3* Trp64Arg genotypes on BMI and BMD values were found in the control group.

Table 3. The association of *ADRB3* Trp64Arg polymorphism with BMI and BMD values in the study groups

	***ADRB3* Trp64Arg Genotypes**			
	Control		**Osteoporotic**	
	Trp/Trp n=44	**Trp/Arg n=8**	**Trp/Trp n=65**	**Trp/Arg n=16**
BMI	33.43± 5.21	32.45± 5.04	27.37± 4.19	29.65± 3.88
Lumbar spine BMD	1.19±0.11	1.13±0.06	0.82±0.07	0.85±0.05
Femoral neck BMD	0.97±0.11	0.95±0.05	0.76±0.10*	0.81±0.08
Total hip BMD	1.05±0.10	1.01±0.07	0.80±0.10*	0.86±0.07

* $p<0.05$ vs. Trp/Arg genotype in the osteoporotic group.

Discussion

The *ADRB3* Trp64Arg gene polymorphism have largely been investigated for an association with BMI in several populations. However, only few studies have been performed regarding a relation between BMD and *ADRB3* genotypes (Wang et al., 2006; Ogawa et al., 1998; Katsumata et al., 2002; Lee et al., 2014). In this study population-based, case-control study of postmenopausal Turkish women, we found that the *ADRB3* Trp64Arg polymorphism affected BMI and BMD values in osteoporotic women while this polymorphism did not have any significant effect in control subjects.

In most of the previous studies, subjects with "Arg" allele were found to have higher BMI values than non-carriers (Oizumi et al., 2001; de Luis et al., 2008a, b; Malik et al., 2011; Mirrakhimov et al., 2011) whereas no significant relation was found in some (Büettner et al., 1998; Matsushita et al., 2003; Terra et al., 2005; Lwow et al., 2007; Gjesing et al., 2008; Dunajska et al., 2008). It was also indicated that "Arg" allele carriers had a higher BMI than non-carriers in two meta-analysis studies with Japanese individuals (Fujisawa et al., 1998; Kurokawa et al., 2001). In accordance with these studies, the present study suggested that "Arg" allele carriers ("Trp/Arg" genotype in this study) tended to have higher BMI compared to the subjects with "Trp/Trp" genotype in the osteoporotic postmenopausal women.

We also found an association between "Trp/Trp" genotype and decreased BMD values of femoral neck and total hip. However, our results are inconsistent with the findings of Lee *et al.* (2014), Wang *et al.* (2006) and Katsumata *et al.* (2002) in Korean, Caucasion and Japanese populations, respectively, showing no significant associations between BMD and *ADRB3* genotypes. Ogawa *et al.* (1998) did not find any significant difference for lumbar spine BMD between the subjects with or without "Trp" allele, either, but identified differences in total body BMD suggesting "Arg/Arg" homozygote group were associated with lower values than women carrying at least one "Trp" allele. However, they compared Z scores between genotype groups instead of BMD values. In contrast, in our study, we found that "Trp/Trp" genotype was associated with low BMD values of femoral neck and total hip. But we did not evaluate the BMD values for the total body. We are unable to give a precise explanation for this result, but we presume that this may stem from the differences in geographic background, sample size and environmental factors between the study populations.

In the present study, the distribution of *ADRB3* Trp64Arg genotype frequencies were in accordance with previous Turkish population studies (Ozata, 2003; Çakır et al., 2007) and also some other population studies (Wang et al., 2006; Ogawa et al., 1998; Katsumata et al., 2002; Lee et al., 2014). We could not observe "Arg/Arg" genotype in our study population. Likewise, Çakır *et al.* (47) did not found "Arg/Arg" genotype in their control group, found in two cases of patient's group, while Ozata (2003) found the "Arg/Arg" genotype just in one case in Turkish population.

The main limitation in this report is relatively small study population. Furthermore, the frequency of "Arg/Arg" genotype of *ADRB3* Trp64Arg polymorphism was not observed in this study. Therefore, we couldn't use ANOVA statistical test for comparison of genotypes and BMD parameters. We believe that further multi-center studies with a higher number of case and control subjects with the contributions of previous studies may be necessary to conclude with greater certainty of the association between Trp64Arg polymorphism of the *ADRB3* gene and predisposition to osteoporosis and low BMD status.

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