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Original Article

PPARγ Pro12Ala and C161T polymorphisms, but not *PPARα* L162V, are associated with osteoporosis risk in Turkish postmenopausal women

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ABSTRACT

Stimulation of peroxisome proliferator-activated receptors (*PPARs*) causes mesenchymal stem cells of the human bone marrow differentiate into adipocytes instead of osteoblasts leading to a decreased number of osteoblasts and a decrease in bone mineral density (BMD). Thus, *PPARs* may have impacts on bone metabolism. 224 postmenopausal women (171 osteoporotic and osteopenic, 53 healthy control) were included in this study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and agarose gel electrophoresis techniques were performed to detect *PPARα* L162V and *PPARγ* Pro12Ala/C161T polymorphisms. The distribution of *PPARγ* Pro12Ala genotype and allele frequencies was not statistically different in control and patient (osteopenic+osteoporotic) groups ($p>0.05$). However, in the patient group, subjects with "Pro12Pro" genotype had lower lumbar spine (L1-L4) BMD values than those with "Ala" allele ($p<0.05$). The frequency of *PPARγ* C161T "CC" genotype was higher in the patient group when compared with that in the control group ($p<0.05$). There were no significant associations between the genotype and allele frequencies of *PPARγ* C161T/ *PPARα* L162V and BMD values ($p>0.05$). We suggested that *PPARγ* Pro12Ala and C161T gene variants might be contributing factors in the development of osteoporosis.

Keywords: Peroxisome proliferator-activated receptor, bone mineral density, osteoporosis, polymorphism

INTRODUCTION

Peroxisome proliferator-activated receptors (*PPARs*) belong to the superfamily of nuclear receptors which are are ligand-activated transcription factors. Three different *PPAR* subtypes have been identified; *PPARα*, *PPARβ* (also called *PPARδ*), and *PPARγ* (Abbot 2009). *PPARα* is found in tissues such as heart, muscle, liver and kidney where fatty acid catabolism is important and so regulates genes involved in lipid metabolism. *PPARα* is activated by natural ligands (polyunsaturated fatty acids, lipolytic products of lipoproteins, oxidized phospholipids) and by synthetic ligands (gemfibrozil and fenofibrate) (Touyz and Schiffrin 2006). Fenofibrate which is currently used for the treatment of hypercholesterolemia and hypertriglyceridemia, also maintains bone mass. Whole body and femoral bone mineral density (BMD) values were higher in ovariectomized rats given fenofibrate, compared to controls (Stunes et al. 2011).

PPARγ is heavily expressed in adipose tissue and controls adipocyte differentiation and lipid storage. *PPARγ* regulates the action of insulin through its effects on adipose tissue and skeletal muscle (Touyz and Schiffrin 2006). Natural agonists (eicosanoids and oxidized, polyunsaturated fatty acids) and synthetic agonists (thiazolidinediones; a family of antidiabetic drugs-rosiglitazone and

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pioglitazone) for *PPARγ* decrease peripheral insulin resistance and thereby reduce blood glucose levels in type 2 diabetic patients (Touyz and Schiffrin 2006; Harsløf et al. 2011). After activation of *PPARγ* by rosiglitazone, mesenchymal stem cells differentiate into adipocytes instead of osteoblasts leading to increased number of adipocytes and decreased number of osteoblasts and decreased BMD in mouse bone marrow (Rzonca et al. 2004; Ali et al. 2005).

To date, several polymorphisms within the human *PPARα* gene have been identified. Of these, a C→G transversion at position 484 in exon 5, leading to a substitution of valine for leucine (L162V) at codon 162, has functional effects on *PPARα* activity (Flavell et al. 2002; Do et al. 2009). Some studies have found associations between *PPARα* L162V polymorphism and plasma lipids and atherosclerosis development, however, the effects of this polymorphism on bone metabolism haven't been investigated so far.

Cytosine-guanine exchange in exon B (codon12) is the most common gene mutation in human *PPARγ* gene resulting proline (Pro) to alanine (Ala) substitution in the protein (Temelkova-Kurktschiev et al. 2004). C161T substitution in exon 6 of the *PPARγ* gene was also described (Meirhaeghe et al. 1998). *PPARγ* was found to be related with cardiovascular diseases (Takano and Komuro, 2009), diabetes mellitus (Cho et al. 2008), carcinogenesis (Elrod and Sun 2008) and inflammation (Kapoor et al. 2007a; 2007b; Szanto and Nagy 2008) in some studies. *PPARγ* was also associated with bone mineral density, osteoporosis, osteoarthritis and non-traumatic hip fracture risk in various populations (Ogawa et al. 1999; Harslof et al. 2010; Tamaki et al. 2010; Fahmi et al. 2011; Dragojevič et al. 2011; Wang et al. 2013). However, no association was found between *PPARγ* and bone mineral density variation in Chinese nuclear families (Yue at al. 2010) and in Japanese postmenopausal women (Wang et al. 2013).

To the best of our knowledge, there is no study regarding the association of *PPARα* polymorphisms with BMD and osteoporosis. Little is known about the association of the *PPARγ* polymorphism with the osteoporosis risk and also the results are controversial. Therefore, we aimed to investigate the relation between *PPARα* and *PPARγ* gene variants and osteoporosis in Turkish postmenopausal women.

MATERIALS AND METHODS

Subjects

224 Turkish postmenopausal women (171 osteoporotic and osteopenic, 53 healthy control), attending the Uskudar State Hospital in Istanbul were recruited in this study. World Health Organization (WHO) definitions and criteria for osteopenia and osteoporosis were used during ascertainment (World Health Organization Study Group, 1994). The patients received a standardized questionnaire including questions regarding the osteoporosis risk factors (age, menopausal status, smoking, family history of osteoporosis), medication use and other medical conditions. Demographic and morphometric characteristics were also recorded. Subjects with a clinical diagnosis of osteopenia/osteoporosis and those with normal BMD values were included in the study group. Exclusion criteria were conditions, diseases, and/or treatments known to interfere with bone metabolism, such as malignancies, severe liver or gastroenteral diseases, endocrinologic disorders (hypo-hyperparathyroidism, hyperthyroidism, Cushing's syndrome), skeletal diseases (rheumatoid arthritis, osteomalacia, osteogenesis imperfecta and Paget's disease) and current pharmacological treatment with anabolic androgenic steroids, estrogens or estrogen-related molecules, corticosteroids and anticonvulsants before enrollment. Menopause was defined as amenorrhoea of at least one year duration. The study protocol was approved by the Local Ethical Commitee of Istanbul University, Istanbul Medical Faculty (Protocol No: 2006/2145). All participants signed written, informed consent forms prior to giving their blood sample.

BMD measurement

Dual energy X-ray absorptiometry (DXA; Lunar DPX (GE Lunar Corporation, Madison, WI, USA) was used to determine BMD of the lumbar spine (L1-L4) and hip (femoral neck and total hip). All DEXA scans were analyzed according to software (encore version 2005, 9.30.044) provided by the manufacturer. BMD was expressed as grams per centimeter square (g/cm^2).

Genotype study

Genomic DNA samples were extracted from whole blood with salting out procedure (Miller et al. 1988). Polymerase chain reaction-restriction fragment lenght polymorphism (PCR-RFLP) analysis were used to detect *PPARα* L162V and *PPARγ* Pro12Ala/C161T polymorphisms as previously reported (Yen et al. 1997; Flavell et al. 2002).

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software package programme version 20.0 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as frequencies, while continous variables are presented as means (± standard deviation-S.D.). Chi-square (χ^2) 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. Allele frequencies were calculated by gene counting method. Values of $p<0.05$ were considered statistically significant.

RESULTS

Demographic characteristics and BMD status

The baseline characteristics of the study population were presented in Table 1. As expected, the body mass index (BMI), BMD values of lumbar spine (L1-L4), femoral neck and total hip showed significant differences between control and patient (osteopenic+osteoporotic) groups ($p<0.001$), whereas no significant differences were detected in age, age of menopause, smoking and family history of osteoporosis ($p>0.05$).

PPARγ Pro12Ala/C161T and *PPARα* L162V genotypes and allele distribution

The genotypic and allelic frequencies of *PPARγ* Pro12Ala/C161T and *PPARα* L162V polymorphisms were shown in Table 2. *PPARγ* C161T "CC" genotype frequency was found to be higher in the patient group when compared to that in the control group ($p=0.028$). No significant differences were found in the frequencies of *PPARγ* Pro12Ala and *PPARα* L162V

genotypes and alleles within two groups (p>0.05). "Ala12Ala", "TT" and "VV" genotypes were not observed in the control group.

The association of *PPARγ* and *PPARα* polymorphisms with BMD values

The association of *PPARγ* Pro12Ala/C161T and *PPARα* L162V genotypes with BMD values were presented in Table 3. In the patient group, subjects with "Pro12Pro" genotype had lower lumbar spine (L1-L4) BMD values than those with "Ala" allele (p<0.05). No significant association was found between *PPARγ* C161T and *PPARα* L162V genotypes and BMD values in the study groups (p>0.05).

DISCUSSION

The present study is the first one in Turkish population showing an association between *PPARγ* gene and the risk of the development of osteoporosis. Subjects with *PPARγ* "Pro12Pro" genotype had lower lumbar spine BMD values than those with "Ala" allele in our patient group. Similarly, in a study with β-thalassemia major patients, the risk of osteopenia was significantly higher in subjects with "Pro12Pro" genotype than the carriers of the rare alleles (Sahmani et al. 2013). In contrast to our results, postmenopausal women with "Pro12Pro" genotype had higher BMD of lumbar spine than that of subjects with "Pro12Ala" genotype (Yue et al. 2010). *PPARγ* Pro12Ala gene variants were not associated with BMD in elderly and young Swedish women (Herlin et al. 2015). Also, *PPARγ* Pro12Ala was not independently associated with BMD values in postmenopausal Japanese women, either (Wang et al. 2013). We think

Table 1. The baseline characteristics of the study population

	Control n=53	Patient n=171
Age	55.55±6.99	57.24±5.99
Age of menopause	46.47±5.17	46.50±5.13
BMI (kg/m^2)	33.22±5.11	29.52±4.83 *
Smoking (n, %)	5 (9.4 %)	17 (9.9 %)
Family history of osteoporosis (n, %)	24 (47.1 %)	74 (43.3 %)
Lumbar spine (L1-L4) BMD (g/cm^2)	1.183±0.110	0.940±0.113 *
Femoral neck BMD (g/cm^2)	0.966±0.101	0.827±0.088 *
Total hip BMD (g/cm^2)	1.044±0.099	0.882±0.093 *

n: number of subjects, BMI: Body mass index, BMD: Bone mineral density.
Values are means±SD except where noted.
* p<0.001 vs. control group.

Table 2. The distribution of *PPARγ* Pro12Ala/ C161T and *PPARα* L162V genotypes and allele frequencies in the study groups

Genotypes / Alleles	Control n, (%)	Patient n, (%)
***PPARγ* Pro12Ala**		
Pro12Pro	42 (79.2%)	149 (87.1%)
Pro12Ala	11 (20.8%)	20 (11.7%)
Ala12Ala	0 (0 %)	2 (1.2%)
Pro	95 (89.6%)	318 (93%)
Ala	11 (10.4%)	24 (7%)
***PPARγ* C161T**		
CC	37 (69.8%)	142 (83%)*
CT	16 (30.2%)	24 (14%)
TT	0 (0 %)	5 (3%)
C	90 (84.9%)	308 (90.1%)
T	16 (15.1%)	34 (9.9%)
***PPARα* L162V**		
LL	39 (73.6%)	116 (67.8%)
LV	14 (26.4%)	50 (29.2%)
VV	0 (0%)	5 (2.9%)
L	92 (86.8%)	282 (82.5%)
V	14 (13.2%)	60 (17.5%)

n: number of subjects, *PPARγ*: Peroxisome proliferator activated receptor gamma, *PPARα*: Peroxisome proliferator activated receptor alpha
* p< 0.05 vs. control group.

Table 3. Association of *PPARγ* and *PPARα* genotypes with BMD values in study population

	PPARγ Pro12Ala		*PPARγ* C161T		*PPARα* L162V	
Groups/BMD	**Pro12Pro**	**Pro12Ala +Ala12Ala**	**CC**	**CT+TT**	**LL**	**LV+VV**
Control						
Lumbar spine	1.190±0.115	1.160±0.087	1.190±0.119	1.170±0.089	1.170±0.075	1.230±0.171
Femoral neck	0.974±0.110	0.934±0.049	0.976±0.116	0.943±0.053	0.968±0.103	0.960±0.101
Total hip	1.064±0.101	0.990±0.048	1.062±0.107	1.006±0.069	1.038±0.091	1.061±0.124
Patient						
Lumbar spine	0.940±0.114*	0.990±0.082	0.940±0.114	0.950±0.103	0.940±0.111	0.940±0.115
Femoral neck	0.825±0.084	0.850±0.091	0.828±0.084	0.833±0.093	0.822±0.088	0.841±0.079
Total hip	0.883±0.090	0.892±0.094	0.882±0.089	0.895±0.095	0.881±0.094	0.890±0.082

BMD: Bone mineral density, *PPARγ*: Peroxisome proliferator activated receptor gamma, *PPARα*: Peroxisome proliferator activated receptor alpha
*p<0.05 vs. ProAla+Ala12Ala genotypes

that these results differ depending on geographic background and number of subjects in the studies.

In our study, *PPARγ* C161T "CC" genotype frequency was found to be higher in the patient group when compared with the control group, however, no association was found between the C161T genotypes and BMD values. In contrast, Z scores of the lumbar and total body BMD was found to be higher in Japanese postmenopausal women with *PPARγ* C161T "CC" genotype than those in the subjects with "CT+TT" genotype. It was suggested that there is an association between *PPARγ* gene and BMD and the possible involvement of C161T polymorphism in the cause of postmenopausal osteoporosis in Japanese women (Ogawa et al. 1999). Femoral neck and total hip BMD values were significantly higher in Japanese premenopausal women with "CC" genotype than the values in subjects with "CT/TT" genotypes (Tamaki et al. 2010). Similar to our results, no association was found between *PPARγ* C161T genotypes and BMD of lumbar spine/femoral neck in healthy Korean pre- and postmenopausal women (Rhee et al. 2005).

An association was found between polymorphisms in *PPARγ*, BMD and fracture risk in Danish population indicating that the effect may be modified by environmental factors (Harsløf et al. 2011). Besides, *PPARγ* Pro12Ala and C161T polymorphisms did not have any significant relation with the non-traumatic hip fracture risk in the elderly Slovenian population (Dragojevič et al. 2011).

In the present study, the frequencies of "Ala" allele were 10.4% and 7% in the control and in the patient groups, respectively. C161T "rare allele (T)" frequency was 15.1% in the control group versus 9.9% of the patient group. "Ala12Ala" and "TT" genotypes were not observed in the control group (0%) whereas the frequencies of them were 1.2% and 3% in the patient group, respectively. The genotype populations of *PPARγ* Pro12Ala/C161T were in accordance with those in Turkish patients with inflammatory bowel disease, coronary heart disease and gastric cancer (Atug et al. 2008; Yilmaz-Aydogan et al. 2011; Canbay et al. 2012). Similar to our results, Erdogan et al. (2007) reported the frequency as 0% for "Ala12Ala" genotype in the control group and in diabetic patients with and without diabetic nefropathy. However, in their study, the frequency for "Ala" allele was found as 0% and 0.5% in control group and in diabetic group, respectively. A remarkable difference was observed in their study in terms of "Ala" allele distribution. This observed difference may be based on the different number of healthy controls and patients in these two studies. Also the different patient group of Erdogan's study consisting diabetic subjects may affect the allelic differences between the two studies. Similar to our results, "T" allele frequencies were found as 11.5 and 9.3 in control and patient group in Erdogan's study.

Pro12Ala and C161T frequencies in our study are similar to those in Slovenian population (Dragojevič et al. 2011) and those in Chinese postmenopausal women (Yue at al. 2010). The genotype and allelic frequencies of Pro12Ala was also found to be similar to our results in a meta-analysis study with European Caucasion population (Zhang et al. 2012) and in studies with Japanese postmenopausal women (Ogawa et al. 1999; Wang et al. 2013). Pro12Ala genotype and allelic frequencies in two studies with Iranian population are similar to the frequencies in the present study, however, they could not find any subjects with "Ala12Ala" genotype in their study (Namvaran et al. 2011; Sahmani et al. 2013). C161T frequencies in Japanese women (Tamaki et al. 2010) and Korean women (Rhee et al. 2005) are in accordance with our results.

As of now, there isn't any study showing a relation between *PPARα* L162V polymorphism, BMD and the risk of osteoporosis. However, no significant association was found between *PPARα* L162V genotype and allele frequencies and BMD values in our study population. The genotype and allele frequencies of *PPARα* L162V are similar to those in a previous Turkish study with coronary heart disease based on the presence of diabetes (Yilmaz-Aydogan et al. 2013) and to those in another study with Turkish subjects (Koytak et al. 2008). Besides, our frequencies differ from those found in Spanish Mediterranean, Brazilian and Croatian populations (Francès et al. 2008; Chen et al. 2010; Nadalin et al. 2014). "VV" genotype was not found in Croatian population (Nadalin et al. 2014) and in multi-ethnic Malaysian population (Chia et al. 2015). Similarly we found no subjects with "VV" genotype in our control group whereas we found only five subjects in our patient group.

The frequencies of Pro12Ala-Ala12Ala genotype, C161T-TT genotype and L162V-VV genotype are very rarely observed in the present study. Therefore, ANOVA statistical test couldn't be used for the comparison of genotypes and BMD values. Furthermore, this report was comprised of a relatively small study population. These represents the limitations of the study. We think that further studies with higher number of subjects may be necessary to conclude with greater certainty of the relation between *PPARγ* Pro12Ala/C161T polymorphisms and decreased BMD status.

CONCLUSION

The present study suggests that *PPARγ* Pro12Ala and C161T polymorphisms may contribute to the development of osteoporosis in Turkish postmenopausal women.

Ethics Committee Approval: The study protocol was approved by the Local Ethical Commitee of Istanbul University, Istanbul Medical Faculty (Protocol No: 2006/2145).

Informed Consent: All participants signed written, informed consent forms prior to giving their blood sample.

Peer-review: Externally peer-reviewed.

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