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Association of PPAR-α Gene Polymorphism (rs4253778) with Osteoporosis in Postmenopausal Turkish Women

Postmenopozal Türk Kadınlarında PPAR-α Gen Polimorfizminin (rs4253778) Osteoporoz ile İlişkisi

Şengül Tural, Amir Saghafian, Gamze Alaylı*, Ercan Tural, Esra Tekcan*****

Ondokuz Mayıs University Faculty of Medicine, Department of Medical Biology, Samsun, Turkey
*Ondokuz Mayıs University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Samsun, Turkey
**Ondokuz Mayıs University Faculty of Health Sciences, Samsun, Turkey
***Ondokuz Mayıs University Faculty of Medicine, Central Laboratory, Samsun, Turkey

Abstract

Objective: Osteoporosis is a multifactorial disease characterized by decreased bone mineral density and deterioration of the microstructure of bone tissue. The existence of various candidate genes affecting bone mass has been reported. In this study, we examined the relationship between *PPAR-α* gene intron 7 G>C (rs4253778) polymorphism and osteoporosis.
Materials and Methods: The stud included 250 postmenopausal women (150 cases with osteoporosis and 100 postmenopausal healthy controls) mostly from the Central Black Sea Region. Peripheral blood samples were taken from the patient and control groups, and deoxyribonucleic acid isolation was performed using the kit methodology. Genotyping was performed by polymerase chain reaction - restriction fragment length polymorphism method. Statistical Package for the Social Sciences 20 program and chi-square analyzes were performed for statistical analysis.
Results: Because of the study, while there was no statistical significant association between genotype distribution and osteoporosis, the C allele frequency was higher in the patient group and it was statistically significant (p=0.017).
Conclusion: To our knowledge, this is the first study conducted in the Central Black Sea Region, Turkey. By expanding the study population and combining the results, more descriptive results regarding the susceptibility of polymorphic regions to osteoporosis can be obtained.
Keywords: Osteoporosis, gene polymorphism, postmenopausal woman

Öz

Amaç: Osteoporoz, kemik mineral yoğunluğunun azalması ve kemik dokusunun mikro yapısının bozulması ile karakterize multifaktöriyel bir hastalıktır. Kemik kütlesini etkileyen çeşitli aday genlerin varlığı bildirilmiştir. Bu çalışmada *PPAR-α* geni intron 7 G>C (rs4253778) polimorfizmi ile osteoporoz arasındaki ilişkiyi incelemeyi amaçladık.
Gereç ve Yöntem: Çalışmaya çoğunlukla Orta Karadeniz Bölgesi'nden 250 postmenopozal kadın (osteoporozlu 150 olgu ve 100 postmenopozal sağlıklı kontrol) dahil edildi. Hasta ve kontrol gruplarından periferik kan örnekleri alındı ve kit metodolojisi kullanılarak DNA izolasyonu yapıldı. Genotiplendirme polimeraz zincir reaksiyonu-kısıtlama parçası uzunluk polimorfizmi yöntemi ile gerçekleştirildi. İstatistiksel analiz için Statistical Package for the Social Sciences 20 programı ve ki-kare analizi yapıldı.
Bulgular: Çalışma sonucunda genotip dağılımı ile osteoporoz arasında istatistiksel olarak anlamlı bir ilişki bulunmazken, C allel sıklığı hasta grubunda daha yüksekti ve istatistiksel olarak anlamlıydı (p=0,017).
Sonuç: Bildiğimiz kadarıyla bu, Türkiye'de Orta Karadeniz Bölgesi'nde yapılan ilk çalışmadır. Çalışma popülasyonunun genişletilmesi ve sonuçların birleştirilmesiyle polimorfik bölgelerin osteoporoza duyarlılığı ile ilgili daha açıklayıcı sonuçlar elde edilebilir.
Anahtar kelimeler: Osteoporoz, gen polimorfizmi, postmenopozal kadın

Address for Correspondence/Yazışma Adresi: Şengül Tural Assoc. Prof., Ondokuz Mayıs University Faculty of Medicine, Department of Medical Biology, Samsun, Turkey
Phone: +90 362 312 19 19-2846 **E-mail:** stural@omu.edu.tr **ORCID ID:** orcid.org/0000-0002-8946-8165
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Introduction

Osteoporosis (OP) (Online Mendelian Inheritance in Man: OMIM 166710) is a skeletal disorder in which the risk of fracture increases due to the decrease in bone strength, which reflects the composition of bone quality and quantity (1,2). The most important health problem of OP is fracture formation. With the prolongation of human life and the aging of the world population, OP and OP-related fractures have become an important health problem due to their negative effects on morbidity and quality of life (3). OP is included in the group of multifactorial diseases in which genetic and environmental factors play important roles together (4). Therefore, we aimed to examine *PPAR-α* gene that may cause OP development. The *PPAR-α* gene is a transcription factor and coactivator gene that controls lipid, glucose and energy homeostasis, *PPAR-α* is particularly highly expressed in tissues that catabolize fatty acids such as liver, skeletal and cardiac muscle, and its association with OP and bone loss has been reported. PPARs are members of the nuclear receptor family and have three isoforms as PPARα, PPARβ/δ and PPARγ. PPARs play an important role in lipid, glucose homeostasis, metabolic control and related to osteoblasts and adipocytes (5-8). Especially PPARγ important for bone and bone related diseases interms of effects of activation on bone turnover, fat-bone connection, adipocyte differentiation and effects on osteoblasts. PPAR-α and PPAR-β/γ has been reported to promote osteoblastogenesis. Regarding their effects on osteoclast formation and activity in PPARα and PPARβ/δ, both PPARα and PPARβ/δ agonists inhibited osteoclast formation in differentiated cells, whereas the PPARα agonist failed to reduce resorption (9-12). There are many polymorphic regions, the (G>C rs4253778) polymorphic region is located in intron 7 and its relationship with physical activity has been reported in previous studies. In the light of this information, our study aimed to examine the relationship between *PPAR-α* gene polymorphisms and OP in postmenopausal Turkish women.

Materials and Methods

Subjects

This study included 250 postmenopausal women mostly from the Central Black Sea Region from Turkey. Among them, a total of 150 had osteoporotic bone mineral density (BMD) (T-score <-2.5) and 100 had normal BMD (T-score >-1). The mean age of the patients was 62.8±8.485 (standard deviation) (the minimum was 47, the maximum was 87), and the mean age of the controls was 58.91±7.910 (the minimum was 44, the maximum was 78). Participants were asked to fill in the information form asking for information such as body mass index (BMI), height, weight, age at menarche, age of menopause, number of children and births, smoking/alcohol use, and an informed consent form was signed. In addition, Ondokuz Mayıs University Clinical Research Ethics Committee approved the study (decision no: OMÜ-KAEK 2022/17, date: 26.01.2022). The Power Analysis was made with the Open Epi program, and the minimum number of individuals required in each group was calculated as n=56 in the power analysis performed by taking power =80% and it was decided to include at least n=100 individuals in each group. We do not have financial support from any source for his research.

Bone Mineral Density Measurements

The dual-energy X-ray absorptiometry (DEXA) method is the most widely used method for bone density measurement. We used DEXA (Norland EXCELL, USA) to define OP according to the World Health Organization. In this method, while the patient is lying still on a table, measurements are made with a moving camera and a beam source, and the results are evaluated with the help of a computer. Processing time is about 10 minutes.

DNA Isolation and Genotyping *PPAR-α* gene Polymorphism

Peripheral blood samples were taken from the patient and control groups and DNA isolation was performed using the kit methodology (Gene Jet, Lithuanian). Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods. The polymorphic region of the *PPAR-α* gene (rs4253778) identified according to the PCR-RFLP method of Eynon et al. (8). The 266-bp segment containing the C/T substitution of the *PPAR-α* gene was amplified by PCR method. Primers were F - 5′-TAAAGATGTCTCCTCTGATT -3′ and (R) 5′-GGGACACATTGAACAATGAATAGGATTG -3′. Amplification reaction in 50 µL total volume by adding 100-200 ng genomic DNA, 200 µM dNTP, 1x reaction buffer (1.5 mM $MgCl_2$), 1.5 mM $MgCl_2$, 0.25 units Taq polymerase and 2-6 pm of each primer for each reaction carried out. Then, in the (Techne Gradient) thermal cycling device, 30 cycles of initial denaturation at 94 °C for 8 minutes, 30 cycles at 94 °C for 30 seconds, at 59 °C for 30 seconds, and at 72 °C for 60 seconds applied. Final elongation applied at 72 °C for 10 minutes. PCR products cut with Taq E restriction enzyme for 3 hours at 37 °C. In case of a recognition site when cut with Taq E restriction enzyme, 216 bp and 50 bp fragments obtained. The resulting products run on a 3% agarose gel. 266 bp fragments considered as AA genotype; Fragments of 253 bp, 216 bp, and 50 bp considered as CA genotype and fragments of 232bc and 21bp considered as CC genotype.

Statistical Analysis

For the statistical analysis we used SPSS15 (SPSS, Chicago, IL, US) program and OpenEpi Info Software program. Chi-square analysis was used to calculate genotype distribution and allele frequencies.

Results

The mean age was 62.87±8.48 years in patients and 59.37±7.91 years in control group. Table 1 represents demographic and clinical findings of patients and controls. Table 2 represents the *PPAR-α* gene genotype distribution and allele frequency findings of the patients and controls. As a result of the study a statistically significant difference found between the

groups with respect to *PPAR-α* allelic frequency (p=0.017). As a result of the study, while there was no statistical significant association between genotype distribution and OP, C-allele frequency was higher in patient group and it was statistically significant (p=0.017). *PPAR-α* gene C-allele frequency was indicated as 58.57% in the patients and 32.85% in the control group (Table 2). *PPAR-α* gene genotype correlation with fracture history and BMI presented in Table 3. There was no statistically significant difference between fracture history and genoype correlation (p=0.69). There is also no statistically significant difference between BMI and genoype correlation (p=0.41). There was also no statistical significant difference between the study and control groups in terms of fracture history and BMI (Table 4).

Discussion

OP is a complex disease that occurs with the interaction of many genetic and environmental factors. Twin and family studies have shown that genetic factors have a significant effect on bone formation. In diseases with complex pathophysiology such as OP, it is important to investigate whether there is a relationship between a polymorphic genetic marker and the phenotype of the disease in the identification of related genes. It is hoped that understanding the complex interactions between genetic

Table 1. Clinical and laboratory Findings

Characteristics	Patients (n=150) (mean ± SD)	Median (min-max)	Controls (n=100)	Median (min-max)
BMI (kg/m^2)	27.52±4.777	28.00 (12-45)	29.38±4.177	29 (27-41)
Age at menapause (yr)	48.24±5.525	48.60 (34-60)	45.66±4.7243	46 (33-59)
Age at menarche (yr)	13.32±1.481	13.0 (9-18)	13.26±1.41	13 (11-17)
Number of birth (n)	3.66±1.938	3.0 (0-10)	3.0±1.528	3 (0-6)
Serum calcium (mg/dL)	9.75±0.550	9.55 (8.30-11.5)	9.65±0.4992	9.85 (8.6-11)
Serum phosphorus (mg/dL)	3.83±2.65	3.73 (2.2-26.30)	3.84±0.67825	4 (2.05-5.29)
Serum PTH (pg/mL)	78.02±52.94	78.2 (11.80-453)	74.22±28.553	73.6 (31-178)
Serum ALP (U/L)	188.03±62.93	189.325 (2.32-420)	188.55±46.156	187.7 (97-301)

SD: Standard deviation, BMI: Body mass index, yr: Year, PTH: Parathyroid hormone, ALP: Alkaline phosphatase, min-max: Minimum-maximum

Table 2. Genotype and allele frequencies of *PPAR-α* gene intron 7 G>C (rs4253778) polymorphism

Genotype/allele	Patients (n=150)		Controls (n=100)		χ^2	p-value	OR (95 %CI)
Genotype	n	%	n	%			
CC	97	60.6	71	71			
CG	11	6.9	4	4	χ^2=3.076	p=0.215	
GG	52	32.5	25	25			
Total	150		100				
Allele	n	%	n	%			
C	205	58.57	146	47.71	χ^2=4.481	p=0.017*	0.65 (0.45-0.97)
G	115	32.85	54	15.42			

*p<0.05 is statistically significant, OR: Odds ratio, CI: Confidence interval

Table 3. *PPAR-α* gene genotype correlation with fracture history and BMI

	Patients, n (%)			c^2	p-value	Controls/n (%)			c^2	p-value
	G/G	G/C	C/C			G/G	G/C	C/C		
Fracture history										
+	12 (8)	2 (1.3)	11 (7.3)	0.422	0.83	2 (2)	1 (1)	2 (2)	0.723	0.69
-	81 (54)	9 (6)	35 (23.4)	5.191	0.27	65 (65)	6 (6)	24 (24)		
BMI										
<25	12 (8)	3 (2)	13 (8.6)	7.341	0.12	10 (10)	2 (2)	4 (4)	1.402	0.41
≥25	81 (54)	6 (4)	35 (23.4)			56 (56)	5 (5)	23 (23)		

*p<0.05 is statistically significant, BMI: Body mass index, +: Patients have fracture, -: Patients have no fracture

Table 4. PPAR-α gene genotype correlation with fracture history and BMI between groups

	Patients (n=150)		Controls (n=100)		c^2	p-value
	G-allele	C-allele	G-allele	C-allele		
Fracture history						
+	26 (17)	24 (16)	5 (5)	5 (5)	0.013	0.90
-	171 (114)	79 (53)	136 (136)	54 (54)	0.517	0.47
BMI						
<25	27 (18)	29 (20)	22 (22)	10 (10)	3.48	0.06
≥25	168 (112)	76 (51)	117 (117)	51 (51)	0.02	0.08

*p<0.05 is statistically significant, BMI: Body mass index, +: Patients have fracture, -: Patients have no fracture

factors and environmental factors will contribute to the clarification of disease pathogenesis and to the development of future genetic-based risk regulation and disease prevention and treatment. In order to contribute to the studies showing the relationship between the *PPAR-α* gene and OP, we aimed to examine *PPAR-α* gene in postmenopausal Turkish women. Our study showed that, the C-allele of the *PPAR-α* gene may be a risk factor for the development of OP. In terms of fracture history and BMI there was no statistically significant difference between genotype correlations in the present study. There was also no statistical significant difference between the study and control groups in terms of fracture history and BMI. Our study revealed that *PPAR-α* gene is not associated with fracture risk in our study population. Harsløf et al. (13) examined *PPARγ* gene in Danish osteopotic patients and they suggested an association with *PPARγ* gene and fracture risk. They also indicated that the effect may be modifiable by environmental factors (13). In a study conducted on postmenopausal Turkish women, the *PPARα L162V* gene polymorphism was examined and it was found that the CC genotype was at a higher frequency in the patient group and was statistically associated with the development of OP (p<0.05) (14). In a study conducted on Turkish elite athletes, the effect of *PPAR-α* gene polymorphism on performance was examined and it was determined that this gene, which affects the skeletal and muscular system, is highly correlated with performance (8). An animal study examined the positive effects of *PAR-α* activation on the skeleton (5). Studies on the relationship between the *PPAR-α* gene and OP or bone-related diseases are very limited. In a recent review published in 2019, it was reported that *PPAR-α* gene polymorphism is associated with atherosclerosis and metabolic diseases (15). The relationship of *PPAR-α* gene with metabolic diseases has been revealed (16). It is known that OP is also considered as a metabolic disease (16,17), so we wanted to reveal its relationship with *PPAR-α* in our study. And our results confirmed these data, with the *P* gene C-allele being higher in the patient group. In our previous study we examined relationship between OP and *BGLAP, ESR1, COL1-A1* and *CALCR* genes in Turkish postmenopausal women and our results showed that, while there was no statistical significant difference in terms of allelic and genotype frequency, *ER1* gene CC genotype was detected 2-fold increased risk for OP (p=0.039) (18). In our different previous study we examined *IL-10* and *TGF-beta* genes in postmenopausal osteoporotic Turkish women and there was a statistical significant difference in terms of *IL-10* genotype distribution (p=0.001) and allele frequencies (p<0.0002) (19).

Conclusion

In conclusion, polimorphic C-allele of *PPAR-α* gene is found to be associated with development of OP in postmenopausal osteoporotic Turkish women. However, to confirmation our results larger and further studies should be done.

Ethics

Ethics Committee Approval: In addition, Ondokuz Mayıs University Clinical Research Ethics Committee approved the study (decision no: OMÜ-KAEK 2022/17, date: 26.01.2022).

Informed Consent: Participants were asked to fill in the information form asking for information such as body mass index, height, weight, age at menarche, age of menopause, number of children and births, smoking/alcohol use, and an informed consent form was signed.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.S., E.T., Design: Ş.T., Data Collection or Processing: A.S., G.A., Analysis or Interpretation: G.A., Es.T., Literature Search: Ş.T., E.T., Es.T., Writing: Ş.T.

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