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The Relationship Between Postmenopausal Osteoporosis and Autophagic Gene Polymorphisms

Postmenopozal Osteoporoz ve Otofajik Gen Polimorfizmleri Arasındaki İlişki

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Abstract

Objective: This study investigates the role of *ATG16L1* rs2241880, *ATG10* rs1864183 and *ATG5* rs2245214 gene polymorphisms, which are involved in autophagosome formation, in the susceptibility to postmenopausal osteoporosis (PMO) disease.
Materials and Methods: Hundred PMO patients and 100 healthy controls without PMO were included into the study. The distribution of the genotypes of *ATG16L1* rs2241880, *ATG10* rs1864183 and *ATG5* rs2245214 polymorphisms in these subjects were analyzed using the TaqMan 5´-exonuclease allelic discrimination assay.
Results: The T allele was detected more frequent among patients with osteoporosis (53%) of the *ATG10* rs1864183 polymorphism. C allele in *ATG16L1* rs2241880 polymorphism in the group of patients with osteoporosis was observed more frequent (56.5%). Besides, the G allele of the *ATG5* rs2245214 polymorphism was identified more common in PMO (34%) than in control group. However, no significant difference were detected in genotype and allele frequencies in terms of these polymorphisms between the patient and the control groups (p>0.05).
Conclusion: In summary, the results of our study do not support the hypothesis that *ATG16L1* rs2241880, *ATG10* rs1864183 and *ATG5* rs2245214 polymorphisms influence the predisposition for osteoporosis in postmenopausal women.
Keywords: Autophagy, gene polymorphism, postmenopausal osteoporosis

Öz

Amaç: Bu çalışmada otofajik mekanizmada otofagozom oluşumunda yer alan *ATG16L1* rs2241880, *ATG10* rs1864183 ve *ATG5* rs2245214 gen polimorfizmlerinin postmenopozal osteoporoz (PMO) hastalığına yatkınlıktaki rolü incelenmiştir.
Gereç ve Yöntem: Bu çalışmaya 50 yaş ve üzeri PMO'su olan 100 hasta ve PMO gelişimi olmayan 100 kontrol grubu alınmıştır. *ATG16L1* rs2241880, *ATG10* rs1864183 ve *ATG5* rs2245214 polimorfizmlerinin genotiplendirmesi amacıyla polimorfik dizileri çoğaltmak için diziye özgü primerler ve her polimorfizmin her iki alelini saptamak için TaqMan 5´-ekzonükleaz alelik diskriminasyon yöntemi kullanılmıştır.
Bulgular: *ATG10* rs1864183 polimorfizminin osteoporozlu (%53) hastalarda T aleli daha sık saptanmıştır. Osteoporozlu hasta grubunda *ATG16L1* rs2241880 polimorfizmindeki C aleli daha sık görülmüştür (%56,5). Ayrıca, *ATG5* rs2245214 polimorfizminin G aleli, PMO'da (%34) kontrol grubuna göre daha yüksek olarak tanımlanmıştır. Ancak genotip ve alel frekanslarında hasta ve kontrol grupları arasındaki bu polimorfizmler açısından anlamlı fark saptanmamıştır (p>0,05).
Sonuç: Özetle, çalışmamızdaki sonuçlar *ATG16L1* rs2241880, *ATG10* rs1864183 ve *ATG5* rs2245214 polimorfizmlerinin postmenopozal kadınlarda osteoporoza yatkınlık sağlayabileceği hipotezini desteklememektedir.
Anahtar kelimeler: Gen polimorfizmi, postmenopozal osteoporoz, otofaji

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Introduction

According to the World Health Organization, Osteoporosis is a systemic skeletal disease that is characterized by low bone mass, decreased bone strength, leading to increased microarchitectural structure and quality of bone tissue together with an increase in the risk of fractures (1). Osteoporosis is a metabolic bone disease that causes an important public health problem in terms of the incidence of osteoporosis fractures associated with morbidity and mortality with the increase of the aging population in our country as well as all over the world (2). The disease progresses silently until a secondary disease like cardiovascular disease or fractures resulting in mortality (3). In the FRACTURK study, which examined the epidemiology of osteoporosis in Turkey in recent years, the prevalence of osteoporosis in the femoral neck was determined to be 7.5% in men over the age of 50, while the women was 33.3% (4). Hip fractures are the ones that have the highest economic burden and are mortal among other osteoporotic fractures. In the Turkish population, the probability of hip fracture in individuals aged 50 and over is 3.5% in men and 14.6% in women for the rest of their life (5).

Osteoporosis is a multifactorial disease that is affected by genetic, hormonal, nutritional factors and lifestyle (6,7). Peak bone mass is reached in adult life, and both men and women begin to lose more or less bone mass at this point depending on the combination of internal and external factors (8,9). This process can become worse with the presence of other chronic diseases like immobilization (10), long-term corticosteroid therapy (11), estrogen deficiency (12), aging (13) and diabetes, especially for postmenopausal women.

Although estrogen deficiency in postmenopausal women is considered to be the main reason for bone loss and osteoporosis, studies showed that it is one of the most important factors contributing to bone loss caused by aging and increased oxidative stress in bone tissue (14,15).

Autophagy is a catabolic process that degradation of the cellular content occurs lysomally, which is stored by a double membrane vesicle called autophagosome; thus, cells maintain homeostatic functions such as protein breakdown and organelle turnover process. Under physiological conditions, although autophagy is responsible for the removal of damaged or unnecessary organelles, under pathological conditions, it helps to redistribute the intracellular nutrients to cover the energy requirement. In this way, it controls the energy and chemical homeostasis of each cell and various types of tissues, including bones (16). Recent studies have shown that autophagy plays an important role in remodelling and differentiation of the stem cells, so that the relation between autophagy and bone metabolic disease pathogenesis has attracted a lot of interest (17).

In the literature, studies on the relation between autophagy-related gene (*ATG*) polymorphisms and osteoporosis are very limited. In this study, our purpose was to examine the effect of polymorphisms in genes involved in the formation of autophagosome in the autophagic mechanism on the risk of developing postmenopausal osteoporosis (PMO). In this context, the role of *ATG16L1* rs2241880, *ATG10* rs1864183 and *ATG5* rs2245214 gene polymorphisms in the susceptibility to PMO disease was investigated.

Materials and Methods

Subjects

A hundred postmenopausal osteoporosis patients aged 50 years and over, and 100 control individuals without the development of postmenopausal osteoporosis, who referred to SANKO University, Sani Konukoğlu Practice and Research Hospital, Clinic of Physical Medicine and Rehabilitation in the last 1 year, were included in the study. Individuals who had early menopause, malabsorption, major gastrointestinal operation, metabolic bone diseases, hyper and hypothyroidism, hormone replacement therapy, and also, individuals using antiosteoporosis and active vitamin D3 medications that could affect bone and calcium metabolism were excluded from the study.

The risk assessment of the participants for fracture risks was examined with the dual energy X-ray absorptiometry device (GE-Lunar DPX) and standard protocol was used as bone mineral density (BMD) (g/cm^2) in the femur neck and lumbar spinal area (18). SANKO University Clinical Research Ethics Committee of approved the study protocol (decision no: 05, date: 24.07.2018). Informed consents were obtained from all participants.

DNA Isolation and Polymorphism Genotyping

Genomic DNA was extracted from 5 peripheral blood samples of 200 individuals according to the protocol recommended by the manufacturer. Genotypings of *ATG2B* rs3759601, *ATG16L1* rs2241880, *ATG10* rs1864183 and *ATG5* rs2245214 polymorphisms were performed using TaqMan 5´-exonuclease allelic discrimination assays that contain sequence-specific forward and reverse primers to amplify the polymorphic sequences and two probes labeled with VIC and FAM dyes to detect both alleles of each polymorphism (19). Polymerase chain reaction (PCR) reactions were carried out using TaqMan universal PCR Master Mix following instructions in a Step-One Plus Real-time PCR system. In the application of this method, Real-time PCR device in the Department of Biology of Gaziantep University Faculty of Science was used. The autophagic gene polymorphisms and locations that were examined are given in Table 1.

Table 1. Autophagy polymorphisms analyzed in the study

SNP	SNP ID	Base change	SNP	Chromosomal location
ATG16L1	rs2241880	T>C	T300A	2
ATG10	rs1864183	C>T	T212M	5
ATG5	rs2245214	C>G	Intronic	10

SNP: Single nucleotide polymorphism

Statistical Analysis

Statistical analysis was made using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY). Categorical data were analyzed by Pearson chi-square test and Fisher's exact test. The normality assumptions were controlled by the Shapiro-Wilk test. The differences between the two groups were evaluated with Student's t-test for normally distributed data, or with Mann-Whitney U test for non-normally distributed data. Data are expressed as n (%), mean ± standard deviation (range) or median (range), as appropriate. P values <0.05 were considered statistically significant.

Results

Demographic Characteristics and Bone Mineral Density Status of Participants

The baseline characteristics of the study population are presented in Table 2. As expected, the body mass index, BMD values of the lumbar spine (L1-L4), femoral neck and total hip showed significant differences between patient and control groups.

Table 2. Comparison of clinical characteristics between groups

	Control (n=100)	Patients (n=100)
Age	61.08±7.13 (50-76)	64.62±7.16 (50-78)*
BMI	32.46 (21.94-346.81)	28.5 (17.75-41.12)*
Habitual smoking		
Yes	5 (8.8)	14 (14.1)
No	52 (91.2)	85 (85.9)
Total L1-L4	-0.4 (-2.3-2.9)	-2.5 (-4.7-0.5)*
Total L2-l4T	-0.4 (-2.3-3.6)	-2.5 (-5.1-3.2)*
Total femur T	-0.2 (-2.4-2.8)	-1.75 (-6.9-0.7)*
Total neck T	-0.7 (-2.3-10)	-1.8 (-5-2)*
Presence of fractures		
Yes	6 (11.5)	37 (38.9)*
No	46 (88.5)	58 (61.1)

Data are presented as n (%), mean ± standard deviation (range), median (range). *Significant p-values are represented
BMI: Body mass index

ATG10, *ATG16L1* and *ATG5* Genotypes and Allele Distributions

The genotypic frequencies and the result of the association analysis of *ATG10* rs1864183, *ATG16L1* rs2241880 and *ATG5* rs2245214 polymorphisms in PMO and controls are summarized in Table 3. The distribution of allelic frequencies for *ATG10*, *ATG16L1* and *ATG5* polymorphisms are shown in Table 4.

The T allele of the *ATG10* polymorphism was more common among patients with osteoporosis (53%) than in normal controls (51%). C allele in *ATG16L1* rs2241880 polymorphism in the group of patients with osteoporosis was observed more frequent (56.5%) than in control group (55%). Besides, the G allele of the *ATG5* rs2245214 polymorphism was identified more common in PMO (34%) than in control group (29.5%). However, no significant difference was detected in genotype and allele frequencies in terms of these polymorphisms between the patient and control groups (p>0.05) (Table 4).

Moreover, among the studied groups, the effect of autophagy gene polymorphisms on the risk of developing PMO was

Table 3. Genotypic frequencies of autophagy genes polymorphisms among cases and controls

	Control	Patients	p
ATG5			
CC	42 (42%)	48 (48%)	0.599
CG	48 (48%)	45 (45)%	-
GG	10 (10%)	7 (7%)	-
ATG10			
CC	23 (23%)	25 (25%)	0.926
CT	48 (48%)	48 (48%)	-
TT	29 (29%)	27 (27%)	-
ATG16L1			
TT	22 (22%)	20 (20%)	0.604
TC	43 (43%)	50 (50%)	-
CC	35 (35%)	30 (30%)	-

Data are presented as n (%). Pearson chi-square test.

Table 4. Allele frequencies of autophagy gene polymorphisms among cases and controls and the association with postmenopausal osteoporosis

SNP	Allele	Patients	Controls	p
ATG10 rs1864183	C	94 (47%)	98 (49%)	0.764
	T	106 (53%)	102 (51%)	
ATG16L1 rs2241880	T	87 (43.5%)	90 (45%)	0.840
	C	113 (56.5%)	110 (55%)	
ATG5 rs2245214	C	132 (66%)	141 (70.5%)	0.390
	G	68 (34%)	59 (29.5%)	

Data are presented as n (%). Pearson chi-square test

evaluated and correlated with bone parameters. No significant differences were found in the analysis of the different clinical forms and the genotypic distributions of the polymorphisms included in our study.

Discussion

Autophagy is a catabolic process that is responsible for the fragmentation and recycling of cellular components like unnecessary organelles and proteins. The process begins with the formation of "autophagosome" merged with lysosomes and hydrolase that is responsible for disrupting the content of the target (20,21). At least 18 *ATG* genes (related to autophagy) were identified in the formation of autophagosome (22). It was shown in recent years that proteins that are involved in bone destruction and construction of autophagy play important roles in regulating osteoclastogenesis (23,24) but the effect of autophagic genes in osteoporosis is not yet unexplained completely. It has also been shown that autophagy plays critical roles in the onset and progression of pathological conditions that are characterized by many metabolic disorders, including both physiological process and metabolic disease (25), cancer (26), neurodegenerative diseases (27), aging (28) and bone-related diseases (29).

In vitro studies demonstrated that autophagy increases oxidative stress in osteoblast-like cells and stimulates apoptosis in these cells with pharmacological inhibition of autophagy (30). Unlike this, in osteoblast-like cell cultures, the induction of autophagy reduces oxidative stress and inhibits apoptosis (31). It was reported that estrogen inhibits osteoblast apoptosis *in vitro* and induces autophagy in these cells (32).

Studies revealed that autophagy plays an important role in osteoclast-mediated bone resorption. Gene deletions (*ATG5*, *ATG7, ATG4B* and LC3) that encode key proteins in the formation of autophagosome reduced bone resorption and increased bone volume in mice after ovariectomy (in the osteoclast brush border) (33,34). Some authors suggested that autophagy inhibition in osteoclasts might serve as a possible therapeutic mechanism against bone diseases, which means an excessive increase in bone resorption. It was observed that pharmacological and genetic inhibition of autophagy prevented bone loss in mice caused by ovariectomy or glucocorticoid treatment, reducing osteoclast genesis and bone resorption (35).

Aging is among the most closely related factors with the onset of osteoporosis with the changes in hormones and increased oxidative stress (36). Parallel to this, the autophagic activity level in many cells decreases during aging, more pronounced in osteocytes and osteoclasts. This hypothesis was supported by studies conducted on many animal models (37,38).

It was shown in previous studies that autophagy modulation has therapeutic potential in the prevention and treatment of bone-related diseases. However, the modulation of autophagic activity may not be enough alone to affect the overall remodeling process of the skeletal system. And, larger and randomized controlled clinical studies are needed to be conducted on humans to validate the possible relation between autophagic dysfunction and osteoporosis and develop potential pharmacological treatments for bone diseases.

In the literature, there are several studies in which *ATG16L1* was investigated for the effect of polymorphism on the predisposition to autoimmune diseases, and it was found that Crohn's disease, ulcerative colitis, palmoplantar pustulosis play roles in this respect (39-41). High *ATG5* levels were detected in autoimmune demyelination and multiple sclerosis in mice model and humans (42), *ATG* expression in synovial tissue, between disease activity and severity in patients with active rheumatoid arthritis relations were found to be significant (43).

In another study, it was reported that GC genotype has protective effects on the development of small-cell lung cancer in *ATG5* rs2245214 gene polymorphism (44). In a study that was conducted in Spain a relation was detected between *ATG10* rs1864183 and laryngeal cancer, *ATG16L1* rs241880 and oral carcinoma development. Also, in Spain, a study examined the effects of the same autophagic gene polymorphism in Paget's disease, which is the most common metabolic bone disease after osteoporosis. It was shown that carrying *ATG16L1* rs2241880 and *ATG5* rs2245214 polymorphisms were associated with increased risk of developing Paget's disease, and *ATG10* rs1864183 polymorphism was associated with increased Paget's disease risk, and carrying T allele of *ATG10* rs1864183 polymorphism was associated with reduced risk (45).

This study is the first that examined *ATG16L1* rs2241880, *ATG10* rs1864183 and *ATG5* rs245214 gene polymorphisms in PMO women. No significant relations were detected between PMO patients and control groups in terms of these polymorphisms. As a result, the data of this study showed that the polymorphisms might not contribute to the predisposition of PMO. A future study is planned to be conducted in different populations and larger sampling sizes.

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Ethics

Ethics Committee Approval: SANKO University Clinical Research Ethics Committee of approved the study protocol (decision no: 05, date: 24.07.2018).

Informed Consent: Informed consents were obtained from all paticipants.

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