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Interleukin-23 receptor gene polymorphisms in osteoporosis

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ABSTRACT

Objectives: Osteoporosis (OP) is a usual disease with a possible genetic predisposition. IL-23 plays a role in physiological bone remodeling and regulates the activity of cells of the bone either directly or indirectly on bone-resorbing osteoclasts as well as on bone-forming osteoblasts. Recent animal and human trials have revealed the main pro-osteoclastogenic activities for the IL-23 pathway. We examined nine single nucleotide polymorphisms (SNPs) in the interleukin-23 receptor (IL-23R) in 100 OP patients and gender- and age-matched 96 healthy volunteers. The most analyzed SNPs in the recent rheumatology literature were selected.

Methods: In addition to gene polymorphisms several laboratory parameters (osteocalcin, parathormone, vitamine D) were investigated. Independent Samples t-test and Mann-Whitney-U test were used to compare several demographic and clinical parameters between the groups. P - value < 0.05 was accepted to be statistically significant.

Results: Having the heterozygous GA genotype of IL-23R rs1004819 and the heterozygous CT genotype of Il-23R rs7530511 significantly increase the risk of developing OP (adjusted OR: 3.51, $p = 0.031$ and OR: 2.41, $p = 0.027$, respectively). The wild homozygous GG genotype of Il-23R rs11209032 had higher osteocalcin levels compared with the mutant homozygous AA genotype (18.75 ± 9.76, $p = 0.009$).

Conclusions: Our findings suggest that several IL-23R gene polymorphisms are seen more often in osteoporosis patients than in healthy volunteers. In addition, some SNPs were related to higher serum osteocalcin levels.

Keywords: Osteoporosis, interleukin 23 receptor, gene polymorphisms, osteocalcin

Osteoporosis (OP) is a widespread health problem that leads to significant morbidity and mortality, especially related to fractures. In healthy individuals, both osteoblasts and osteoclasts sustain bone homeostasis owing to controlled balanced activity including bone-forming or bone-resorbing, respectively [1]. Interleukin-23 (IL-23) is a heterodimeric proinflammatory cytokine, and includes a p40 and a p19 subunits. The p19 subunit has specific and increased attention for the interleukin-23 receptor (IL-23R). IL-23 regulates the activity of an immune system and promotes inflammation through the arrangement of IL-23R. IL-23R also acts a part in signal transduction in the IL-23/IL-17 pathway [2]. IL-23 can play a role in physiological bone remodeling and regulates the activity of cells of the bone either directly or indirectly

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on bone-resorbing osteoclasts as well as on bone-forming osteoblasts [3]. Recent animal and human trials have revealed the main pro-osteoclastogenic activities for the IL-23 pathway. The mice who had bone marrow cells without IL-23p19-/- expression, had a lower differentiating capacity of osteoclasts, reduced osteoclast formation, and subsequently less bone resorptive activity. On the contrary, overexpression of IL-23 causes systemic bone loss in mice [4]. IL-23 has both direct and indirect effects on osteoclasts through T cells, and synovial fibroblasts in addition to osteoclast precursor cells [5]. IL-23 can induce osteoclastogenesis in a few different ways: 1) overexpression of receptor activator of nuclear factor-kB (RANK) on osteoclast precursor cells [6]; 2) overexpression of receptor activator of nuclear factor-kB ligand (RANKL) on fibroblasts or T-helper cells; 3) activation of DNAX activating protein of 12kDa-(DAP12) ITAMs through independent of RANKL; 4) stimulation of tartrate-resistant acid phosphatase (TRAP) activity of osteoclasts [7]. By supporting the whole pathophysiological mechanism, preservative effects of anti-IL-23 treatment on bone loss have been emphasized by recent studies [8].

In addition to pro-osteoclastogenic capacity, IL-23 has also pleiotropic roles on bone-forming osteoblasts. IL-23 may directly regulate osteoblast formation as its binding to the IL-23R on human mesenchymal stem cells leads to highly-expressed osteoblast-related genes. IL-23 may affect bone formation through the induction of IL-17 and IL-22. Increased expression of IL-22 leads to the new periosteal bone formation through ignal transducer and activator of transcription 3 (STAT3) activation and increased expression of genes that regulate the bone formation, including the Wnt family members. In vitro studies, IL-23R-/- mice had lower cortical bone mass related to indirect effects of IL-23R on osteoblasts [9]. Also, IL-23 can reduce osteoclastogenesis via the induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) in murine T cells, which can reduce osteoclast formation [10].

Recently, polymorphisms of the IL-23R were investigated in many inflammatory rheumatic diseases. A potential relationship with susceptibility to rheumatoid arthritis was emphasized by authors [11]. The variants of the IL-23R were related to psoriatic skin disease whereas were not related to a joint disease such as psoriatic arthritis (PsA) [12]. Also, the SNPs in the IL-23R or the IL-23 cytokine are well-known genetic factors in spondyloarthritis related to human enthesitis. So blockade of IL-23 pathway obtained therapeutic improvement in PsA with enthesitis [13]. In addition to the above-mentioned inflammatory diseases, Kasamatsu T *et al* revealed the association between the IL-23R HH genotype and plasmacytoma and bone lesions in Multiple Myeloma [14]. Also, recent studies revealed that polymorphisms of the IL-23R gene (rs1569922, rs7539625, and rs4655686) were significantly associated with an increased risk of osteonecrosis of the femoral head [15]. In the presence of all this knowledge, we aim to investigate IL-23R gene polymorphisms in OP patients, hypothesizing that it may be associated with metabolic bone diseases. The most analyzed nine single nucleotide polymorphisms (SNPs) in recent literature and in the above-mentioned studies were selected. These gene polymorphisms in the IL-23R gene were rs11805303, rs7530511, rs1004819, rs11209026, rs10489629, rs11209032, rs2201841, rs7517847 and rs10889677 subsequently.

METHODS

Patient Selection

We included 196 participants in the present study (96 participants for the control group and 100 participants for the OP group). All OP patients had no other systemic diseases. All of the patients were initially diagnosed in the rheumatology outpatient clinics between 2014-2015. Sex-, gender-, body mass index (BMI)-matched healthy individuals were enrolled voluntarily among subjects who did not have any inflammatory and/or autoinflammatory disease and/or drug use. BMI is equalized initially to form homogeneous patient groups. It was not used as a covariate factor in any of the gene examinations. BMIs of the whole study group were calculated by taking a person's weight, in kilograms, divided by their height, in meters squared [16]. This gene study was a single-center experience that was conducted in the selected regional health center where many patients have been evaluated daily from various provinces around the South Aegean region.

DNA Extraction and Sequence Analysis

All included individuals signed up consent forms for further investigation. 2 ml of venous blood from each subject was collected in ethylenediaminetetraacetic acid (EDTA) tubes for obtaining deoxyribonucleic acid (DNA) isolation, and stored at -80 °C. By using 1 microL of the tissue digest solution, a polymerase chain reaction (PCR) was used for the amplification of the target DNA. A "PCR-Restriction Fragment Length Polymorphism' (RFLP) method was applied for the examination of the IL-23R gene-related polymorphisms. Genotyping and allele identification was made after acquiring polymerase chain reaction (PCR) products using a 2.0% agarose gel electrophoresis. PCR products were restricted by specific enzymes. A total of nine candidate polymorphisms were examined. The SNP numbers of the polymorphisms and the enzymes used were as follows: rs11805303 (MnlI enzyme), rs7530511 (HphI enzyme), rs1004819 (Taal enzyme), rs11209026 (Hpy188I enzyme), rs10489629 (SspI enzyme), rs11209032 (BseMI enzyme), rs2201841 (HpyF3I enzyme), rs7517847 (BseMII enzyme) and rs10889677 (MnlI enzyme). Successfully genotyping was made for all subjects. All patients were also analyzed for serum osteocalcin (OC), parathormone (PTH), and vitamin D levels.

This study was conducted after the approval of the local Ethics Committee of our tertiary health center (Pamukkale University Ethics Committee approved by a number and date with June 206/2016), by following the Helsinki declaration.

Statistical Analysis

Initially, we determined an index study. According to the reference study results; they had a medium effect size (O.R=0.55) [17]. For this effect size, if we include 93 participants we can achieve power at %80 level with %95 confidence level. Since our study was planned to be in 2 groups, we included 196 participants in the present study (96 participants for the control group and 100 participants for the OP group). Descriptive statistics for the continuous variables were given as median, minimum, and maximum according to the distribution of the data. Categorical variables were reported as frequency and percentage. The normality of quantitative variables was checked with the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests. To compare several demographic and clinical parameters between the groups the Independent Samples t-test was used when the numerical data were normally distributed and the Mann-Whitney U test was used otherwise. To compare groups according to categorical variables, the Pearson Chi-Square test was used when the expected value for each cell was above 5 and the Fisher-Freeman-Halton test was used when the expected value was below five. The risk factors for osteoporosis were evaluated with univariate and multiple logistic regression analyses. Both clinical and statistical significance was considered for the variables which were included in the multivariate model. Statistical analyses were performed with the Jamovi project (2020), Jamovi (Version 1.8.1) [Computer Software] (Retrieved from https://www.jamovi.org), and JASP (Version 0.14.1.0) (Retrieved from https://jasp-stats.org) programs and statistical significance (p - value) was accepted to be 0.05.

RESULTS

Patient's Characteristics

The mean age was 53.0 ± 9.9 years in the OP group, and 52.6 ± 12.8 years in the healthy control group. The groups were statistically similar in terms of age, gender, and BMI. Seventy-six (76) % of the control group and 78 % of the osteoporosis group were females (p = 0.876) (Table 1). The laboratory levels of serum PTH, OC, and vitamin D were shown in Table 1. Only, osteocalcin levels were higher in OP patients than healthy controls.

Distributions of Alleles

Genotypes and allele distribution for each SNPs of 100 OP patients and 96 healthy controls are shown in Table 2. The genotypes with rs11805303(TT), rs10889677(AA), and rs7530511(CT) polymorphisms in the IL-23R gene were seen more often in OP patients than healthy controls. In addition, the TT genotype with rs2201841, GG genotype with rs11209032, AA genotype with rs10489629, and TT genotype with rs7517847 of the IL-23R gene were detected more frequently in OP patients (Tables 2 and 3).

Having the GA genotype of IL-23R rs1004819 and the CT genotype of IL-23R rs7530511 increase the risk of developing OP with a statistical significance (adjusted OR: 3.51, p = 0.031 and OR: 2.41, p = 0.027,

Table 1. Patient characteristics of the whole study group

Variables	Osteoporosis (n = 100)	Healthy controls (n = 96)	*p* value
Age	52.98 ± 9.87	52.55 ± 12.7	0.802
Gender (F/M)	78/22	73/23	0.876
BMI (kg/m2)	26.73 ± 4.46	27.18 ± 5.40	0.525
Lomber total 1-4 (T score)	-3.13 ± 1.02	-0.77 ± 1.1	**0.0001**
Femur neck (Tscore)	-1.56 ± 1.07	-0.40 ± 1.0	**0.0001**
PTH (mg/dl)	58.81 ± 30.4	52.5 ± 25.04	0.577
OCN (ng/ml)	16.32 ± 9.07	13.06 ± 11.07	**0.005**
Vitamin D (ng/ml)	24.05 ± 13.7	21.09 ± 12.62	0.321

Data are given as mean ± standard deviation. F = female, M = male, BMI = body mass index, PTH = parathormone, OCN = osteocalcin. Independent Samples t-test was used.

respectively) (Table 3).

Having the subsequent genotypes with IL-23R gene polymorphisms with rs2201841(TC, CC), rs7517847 (TG, GG), rs11209032 (GA, AA), and rs10489629 (GA, GG) had protective roles from OP with a statistical significance for each one (Table 3).

There was a statistical significance among rs11209032 different alleles for OC levels ($p = 0.009$). The GG genotype of Il-23R rs11209032 had higher OC levels than the AA genotype (18.75 ± 9.76, $p = 0.009$). No other difference was detected for PTH or Vitamin D levels (Table 4).

DISCUSSION

To the best of our knowledge, the study firstly investigates the association between IL-23R gene polymorphisms and OP in our population. Our findings suggest that several SNPs of the IL-23R gene could play a role in the predisposition to OP. The surprising conclusion was unusual high Odds ratios (ODs) for each susceptible SNPs when compared with usual genome-wide association studies. This condition may be explained by the that the study includes a relatively small patient size compared with population-based cohort studies. Also, patient selection bias could be aforementioned due to being a single-center experience. Although this conclusion needs multi-center studies including a large population to confirm the clinical significance of this association, literature involves a few trials about IL-23R gene polymorphisms in different bone diseases. As mentioned above, recently, IL-23R H3Q was investigated for susceptibility to multiple myeloma. Although the result was negative, this study showed the association between IL-23R gene polymorphism and bone disease. According to the study, having IL-23R HH genotype was related to bone lesions and poor prognosis [14].

Many trials in the literature investigate the potential effects of variable cytokine gene polymorphisms other than the IL-23R gene in OP patients. In the recent literature, the carriers of the IL-6 GG genotype had lower bone mineral density (BMD) values among postmenopausal women who were involved in the study designed to investigate OP-related cytokine gene polymorphisms including IL-1beta, IL-2, IL-6 [18]. In addition to IL-6, polymorphisms of the IL-17F gene were significantly associated with bone mineral density in a Japanese cohort [19]. On the contrary, there was no association between BMD and glutathione S-transferase (GST) and progesterone receptor gene (PROGINS) polymorphisms in OP patients [20]. This study is the first one that reveals the association between IL-23R gene polymorphism and OP.

Serum OC levels were compared between postmenopausal cases and controls in a comprehensive meta-analysis, and the analysis revealed no significant difference in serum OC levels. This condition was attributed to heterogeneous OC molecules in the circulation that can be influenced by glucose metabolism [21]. In another study, serum OC concentration was

Table 2. IL-23R gene genotype and allele distribution in OP patients and control group

IL-23R polymorphism	Osteoporosis (n = 100)	Healthy controls (n = 96)	p value
rs11805303			
(mutant) TT, n (%)	22 (22)	(-)	**< 0.001**
(heterozygous) CT, n (%)	46 (46)	61 (63.5)	
(wild) CC n, (%)	32 (32)	35 (36.5)	
C allele	110 (55)	131 (68.3)	**0.007**
T allele	90 (45)	61 (31.7)	
rs10889677			
(mutant) AA, n (%)	16 (16)	-	**< 0.001**
(heterozygous) CA, n (%)	49 (49)	60 (62.5)	
(wild) CC, n (%)	35 (35)	36 (37.5)	
C allele	119 (59.5)	132 (68.8)	0.056
A allele	81 (40.5)	60 (31.2)	
rs1004819			
(mutant) AA, n (%)	14 (14)	11 (11.5)	0.686
(heterozygous) GA, n (%)	48 (48)	43 (44.8)	
(wild) GG, n (%)	38 (38)	42 (43.8)	
G allele	124 (62)	127 (66.1)	0.393
A allele	76 (38)	65 (33.9)	
rs2201841			
(mutant) CC, n (%)	8 (8)	10 (10.4)	**< 0.001**
(heterozygous) TC, n (%)	41 (41)	82 (85.4)	
(wild) TT, n (%)	51 (51)	4 (4.2)	
T allele	143 (71.5)	90 (46.9)	**< 0.001**
C allele	57 (28.5)	102 (53.1)	
rs11209032			
(mutant) AA, n (%)	20 (20)	53 (55.2)	**< 0.001**
(heterozygous) GA, n (%)	43 (43)	37 (38.5)	
(wild) GG, n (%)	37 (37)	6 (6.3)	
G allele	117 (58.5)	49 (25.5)	**< 0.001**
A allele	83 (41.5)	143 (74.5)	
rs7530511			
(mutant) TT, n (%)	-	1 (1)	**0.046**
(heterozygous) CT, n (%)	24 (24)	11 (11.5)	
(wild) CC, n (%)	76 (76)	84 (87.5	
C allele	176 (88)	179 (93.3)	**< 0.001**
T allele	24 (12)	13 (6.7)	
rs11209026			
(mutant) AA, n (%)	-	1 (1.04)	0.066
(heterozygous) GA, n (%)	11 (11)	21 (21.8)	
(wild) GG, n (%)	89 (89)	74 (77.08)	
G allele	189 (94.5)	169 (88.1)	**0.036**
A allele	11 (5.5)	23 (11.9)	
rs10489629			
(mutant) GG, n (%)	31 (31)	45 (46.9)	**< 0.001**
(heterozygous) AG, n (%)	33 (33)	46 (47.9)	
(wild) AA, n (%)	36 (36)	5 (5.2)	
A allele	105 (52.5)	56 (29.2)	**< 0.001**
G allele	95 (47.5)	136 (70.8)	
rs7517847			
(mutant) GG, n (%)	9 (9)	16 (16.7)	**0.005**
(heterozygous) TG, n (%)	42 (42)	54 (56.3)	
(wild) TT, n (%)	49 (49)	26 (27.1)	
T allele	140 (70)	106 (55.2)	**< 0.002**
G allele	60 (30)	86 (44.8)	

IL-23R = interleukin-23 receptor

Table 3. The comparison of the control and the osteoporosis groups according to the presence of several polymorphisms

		Group			Crude		Adjusted	
	Genotype	Control (n = 96)	Osteoporosis (n = 100)	*p* value*	OR [95%CI]	*p* value	OR [95%CI]	*p* value
rs11805303 (%)	TT	0 (0.0)	22 (22.0)	**< 0.001**	46533388.5 [0-Inf]	0.983	-	-
	CC	35 (36.5)	32 (32.0)		**Reference**		-	-
	CT	61 (63.5)	46 (46.0)		0.82 [0.45-1.52]	0.538	-	-
rs10889677 (%)	AA	0 (0.0)	16 (16.0)	**< 0.001**	43760378.41 [0-Inf]	0.986		
	CC	36 (37.5)	35 (35.0)		**Reference**		-	-
	CA	60 (62.5)	49 (49.0)		0.84 [0.46-1.53]	0.568	-	-
rs1004819 (%)	AA	11 (11.5)	14 (14.0)	0.686	1.41 [0.57-3.47]	0.459	1.48 [0.36-6.1]	0.591
	GG	42 (43.8)	38 (38.0)		**Reference**		**Reference**	
	GA	43 (44.8)	48 (48.0)		1.23 [0.68-2.25]	0.494	3.51 [1.12-10.93]	**0.031**
rs2201841 (%)	CC	10 (10.4)	8 (8.0)	**< 0.001**	0.06 [0.02-0.25]	**< 0.001**	0.04 [0.01-0.25]	**< 0.001**
	TT	4 (4.2)	51 (51.0)		**Reference**		**Reference**	
	TC	82 (85.4)	41 (41.0)		0.04 [0.01-0.12]	**< 0.001**	0.03 [0.01-0.11]	**< 0.001**
rs11209032 (%)	AA	53 (55.2)	20 (20.0)	**< 0.001**	0.06 [0.02-0.17]	**< 0.001**	0.01 [0.01-0.05]	**< 0.001**
	GG	6 (6.2)	37 (37.0)		**Reference**		**Reference**	
	GA	37 (38.5)	43 (43.0)		0.19 [0.07-0.50]	**< 0.001**	0.08 [0.02-0.32]	**< 0.001**
rs7530511 (%)	TT	1 (1.0)	0 (0.0)	**0.034**	0 [0-Inf]	0.987		
	CC	84 (87.5)	76 (76.0)		**Reference**			
	CT	11 (11.5)	24 (24.0)		2.41 [1.11-5.25]	**0.027**		
rs11209026 (%)	GG	75 (78.1)	89 (89.0)	0.062	0.44 [0.20-0.97]	**0.043**	0.32 [0.08-1.28]	0.106
	GA	21 (21.9)	11 (11.0)					
rs10489629 (%)	GG	45 (46.9)	31 (31.0)	**< 0.001**	0.1 [0.03-0.27]	**< 0.001**	0.03 [0.01-0.17]	**< 0.001**
	AA	5 (5.2)	36 (36.0)		**Reference**		**Reference**	
	GA	46 (47.9)	33 (33.0)		0.1 [0.04-0.28]	**< 0.001**	0.04 [0.01-0.17]	**< 0.001**
rs7517847 (%)	GG	16 (16.7)	9 (9.0)	**0.005**	0.3 [0.12-0.77]	**0.012**	0.19 [0.04-0.89]	**0.035**
	TT	26 (27.1)	49 (49.0)		**Reference**		**Reference**	
	TG	54 (56.2)	42 (42.0)		0.41 [0.22-0.77]	**0.005**	0.21 [0.07-0.6]	**0.004**

Data are given as frequency (%). *Pearson Chi-square test or Fisher Freeman Halton test was used. OR = Odds Ratio, CI = Confidence Interval

Table 4. Laboratory values in osteoporosis patients according to polymorphisms

	PTH	*p* value	Osteocalcin	*p* value	Vitamin D	*p* value
rs11805303						
TT (n = 22)	66.45 ± 32.38	0.252**	16.78 ± 7.41	0.310**	21.88 ± 13.58	0.248**
CC (n = 67)	54.28 ± 25.74		15.29 ± 10.77		20.61 ± 12.25	
CT (n = 107)	54.30 ± 27.57		13.95 ± 10.34		23.99 ± 13.78	
rs10889677						
AA (n = 16)	60.36 ± 31.17	0.516**	18.59 ± 9.54	0.150**	21.83 ± 17.17	0.573**
CC (n = 71)	57.76 ± 33.83		15.42 ± 10.23		21.37 ± 14.28	
CA (n = 109)	53.60 ± 22.23		13.71 ± 10.20		23.51 ± 11.97	
rs1004819						
AA (n = 25)	56.23 ± 26.56	0.463**	15.88 ± 10.72	0.687**	21.91 ± 11.96	0.716**
GG (n = 80)	52.79 ± 25.05		15.10 ± 10.19		21.85 ± 12.57	
GA (n = 91)	58.02 ± 30.09		14.08 ± 10.15		23.45 ± 14.28	
rs2201841						
CC (n=18)	68.42 ± 29.76	0.093**	17.25 ± 10.33	0.152**	21.23 ± 14.81	0.362**
TT (n = 55)	57.93 ± 31.87		16.36 ± 10.03		24.85 ± 13.75	
TC (n = 123)	52.78 ± 24.80		13.62 ± 10.19		21.79 ± 12.82	
rs11209032						
AA (n = 73)	53.83 ± 29.09	0.454**	**12.67 ± 10.80**	**0.009****	22.02 ± 11.80	0.613**
GG (n = 43)	60.93 ± 32.14		**18.75 ± 9.76**		21.43 ± 12.81	
GA (n = 80)	54.49 ± 23.42		14.44 ± 9.35		23.75 ± 14.79	
rs7530511						
CC (n = 160)	56.62 ± 29.32	0.302*	14.44 ± 10.14	0.322*	22.28 ± 13.34	0.644*
CT (n = 35)	51.26 ± 18.52		16.33 ± 10.51		23.42 ± 12.80	
rs11209026						
GG (n = 164)	56.39 ± 28.96	0.404*	15.19 ± 10.43	0.146*	22.94 ± 13.59	0.413*
GA (n = 32)	51.91 ± 19.62		12.32 ± 8.73		20.83 ± 11.58	
rs10489629						
GG (n = 76)	56.28 ± 32.94	0.550**	14.73 ± 10.83	0.946**	22.53 ± 13.26	0.629**
AA (n = 41)	58.58 ± 25.44		15.12 ± 8.98		24.25 ± 13.07	
GA (n=79)	53.54 ± 22.97		14.52 ± 10.31		21.80 ± 13.50	
rs7517847						
GG (n = 25)	49.17 ± 20.75	0.248**	15.38 ± 9.86	0.477**	22.13 ± 11.40	0.920**
TT (n = 75)	54.95 ± 26.31		15.68 ± 10.55		23.09 ± 14.34	
TG (n = 96)	57.90 ± 30.10		13.81 ± 10.05		22.33 ± 12.99	

Data are given as mean ± standard deviation. *Independent Samples t-test was used. **One-Way ANOVA was used.

negatively correlated with fasting plasma glucose and predicted an increased risk for diabetes in postmenopausal women [22]. Although having comorbidities such as diabetes mellitus and insulin resistance were excluded in this study, the correlation between serum glucose and OC levels was not analyzed. Despite matching patient groups according to BMIs and absence of diabetes mellitus, OP patients had higher OC levels than healthy controls. However, it should be kept in mind, the mean BMIs of the two groups are high that may be related to insuline resistance. Recently, some authors from India investigated serum levels of OC in patients with osteopenia and osteoporosis with enzyme-linked immunosorbent assay (ELISA). They reported that having higher levels of OC upper than 14.9 ng/mL may be sensitive and cost-effective screening for low bone density. The cut-off value was higher in osteoporotic patients than in osteopenic patients [23]. In support of the previous study, high OC levels may be explained by lower bone mineral densities. In addition, other studies showed that serum levels of phosphorus and alkaline phosphatase were positively correlated with serum OC levels rather than common genetic variants of the OC gene [24]. However, the studies related to OC polymorphism have inconsistent results. The polymorphism of the OC gene did not show any effect on bone quantity in the Hungarian population [25]. However, the rs1800247 polymorphism in the OC gene was related to fracture and serum total OC levels in Chinese patients [26]. When we analyzed the laboratory values according to the different genotypes, the only significant result was detected in rs11209032 of the Il-23R gene. Having the GG genotype of rs11209032 was related to higher OC levels than having the AA genotype.

Limitations

There are a few limitations of our study. Firstly, we did not analyze the potential confounder effects of other alleles. Secondly, more homogeneous and large patient groups may be obtained by matching based on other situations as smoking, ethnic differences, age at menarche, and numbers of parity. Ethnical differences were not classified due to features of the regional health center in where many patients from various provinces around the South Aegean have been evaluated daily. Also, we did not have an opportunity to determine serum levels of sIL-23R and IL-23. We could not look for the correlation between expression levels of IL-23 and IL-23R gene polymorphisms.

CONCLUSION

Our findings suggest that several analyzed IL-23R gene polymorphisms are seen more often in OP patients than in healthy individuals. So, the IL-23/Th17 pathway can be one of the underlying pathogenetic mechanisms in OP. We need multi-center large population studies to certify the clinical significance of this relationship between gene polymorphisms and susceptibility to the OP.

Authors' Contribution

Study Conception: FU, GOÇ, VÇ; Study Design: FU, GOÇ, VÇ; Supervision: FU, GOÇ; Funding: FU, GOÇ; Materials: GOÇ, VÇ; Data Collection and/or Processing: GOÇ, VÇ; Statistical Analysis and/or Data Interpretation: GOÇ, VÇ; Literature Review: FU, GOÇ, VÇ; Manuscript Preparation: FU, GOÇ, VÇ and Critical Review: FU, GOÇ, VÇ.

Conflict of interest

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REFERENCES

1. Lane JM, Russell L, Khan SN. Osteoporosis. Clin Orthop Relat Res 2000;372:139-50.
2. Floss DM, Schroder J, Franke M, Scheller J. Insights into IL-23 biology: from structure to function. Cytokine Growth Factor Rev 2015;26:569-78.
3. Razawy W, van Driel M, Lubberts E. The role of IL-23 receptor signaling in inflammation-mediated erosive autoimmune arthritis and bone remodeling. Eur J Immunol 2018;48:220-9.
4. Shin HS, Sarin R, Dixit N, Wu J, Gershwin E, Bowman EP, et al. Crosstalk among IL-23 and DNAX activating protein of 12 kDa-dependent pathways promotes osteoclastogenesis. J Immunol 2015;194:316-24.
5. Ju JH, Cho ML, Moon YM, Oh HJ, Park JS, Jhun JY, et al. IL-23 induces receptor activator of NF-kappa B ligand expression on CD4+ T cells and promotes osteoclastogenesis in an autoim-

mune arthritis model. J Immunol 2008;181:1507-18.
6. Chen L, Wei XQ, Evans B, Jiang W, Aeschlimann D. IL-23 promotes osteoclast formation by up-regulation of receptor activator of NF-kappa B (RANK) expression in myeloid precursor cells. Eur J Immunol 2008;38:2845-54.
7. Gravallese EM, Schett G. Effects of the IL-23-IL-17 pathway on bone in spondyloarthritis. Nat Rev Rheumatol 2018;14:631-40.
8. Shukla P, Mansoori MN, Singh D. Efficacy of anti-IL-23 monotherapy versus combination therapy with anti-IL-17 in estrogen deficiency-induced bone loss conditions. Bone 2018;110:84-95.
9. Razawy W, Alves CH, Koedam M, Asmawidjaja PS, Mus AMC, Oukka M, et al. IL-23 receptor deficiency results in lower bone mass via indirect regulation of bone formation. Sci Rep 2021;11:10244.
10. Quinn JM, Sims NA, Saleh H, Mirosa D, Thompson K, Bouralexis S, et al. IL-23 inhibits osteoclastogenesis indirectly through lymphocytes and is required for the maintenance of bone mass in mice. J Immunol 2008;181:5720-9.
11. Song GG, Bae SC, Choi SJ, Ji JD, Lee YH. Associations between interleukin-23 receptor polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. Mol Biol Rep 2012;39:10655-63.
12. Huffmeier U, Lascorz J, Bohm B, Lohmann J, Wendler J, Mossner R, et al. Genetic variants of the IL-23R pathway: association with psoriatic arthritis and psoriasis vulgaris, but no specific risk factor for arthritis. J Invest Dermatol 2009;129:355-8.
13. Bridgewood C, Sharif K, Sherlock J, Watad A, McGonagle D. Interleukin-23 pathway at the enthesis: The emerging story of enthesitis in spondyloarthropathy. Immunol Rev 2020;294:27-47.
14. Kasamatsu T, Kimoto M, Takahashi N, Minato Y, Gotoh N, Takizawa M, et al. IL17A and IL23R gene polymorphisms affect the clinical features and prognosis of patients with multiple myeloma. Hematol Oncol 2018;36:196-201.
15. Kim TH, Hong JM, Oh B, Cho YS, Lee JY, Kim HL, et al. Association of polymorphisms in the Interleukin 23 receptor gene with osteonecrosis of the femoral head in the Korean population. Exp Mol Med 2008;40:418-26.
16. Peterson CM, Thomas DM, Blackburn GL, Heymsfield SB. The universal equation for estimating ideal body weight and body weight at any BMI. Am J Clin Nutr 2016;103:1197-203.
17. Cakmak B, Inanir A, Karakus N, Ates O, Yigit S. Association between the ACE gene I/D polymorphism and osteoporosis in a Turkish population. Z Rheumatol 2015;74:346-50.
18. Czerny B, Kaminski A, Kurzawski M, Kotrych D, Safranow K, Dziedziejko V, et al. The association of IL-1beta, IL-2, and IL-6 gene polymorphisms with bone mineral density and osteoporosis in postmenopausal women. Eur J Obstet Gynecol Reprod Biol 2010;149:82-5.
19. Oishi Y, Watanabe Y, Shinoda S, Naka M, Ozawa Y, Matsuyama T, et al. The IL6 gene polymorphism -634C>G and IL17F gene polymorphism 7488T>C influence bone mineral density in young and elderly Japanese women. Gene 2012;504:75-83.
20. Moura KF, Haidar M, Bonduki C, Feldner PC Jr, Silva I, Soares JM Jr, et al. Frequencies of interleukin-6, GST, and progesterone receptor gene polymorphisms in postmenopausal women with low bone mineral density. Sao Paulo Med J 2014;132:36-40.
21. Liu Z, Chen R, Jiang Y, Yang Y, He L, Luo C, et al. A meta-analysis of serum osteocalcin level in postmenopausal osteoporotic women compared to controls. BMC Musculoskelet Disord 2019;20:532.
22. Garcia-Martin A, Cortes-Berdonces M, Luque-Fernandez I, Rozas-Moreno P, Quesada-Charneco M, Munoz-Torres M. Osteocalcin as a marker of metabolic risk in healthy postmenopausal women. Menopause 2011;18:537-41.
23. Bhadricha H, Khatkhatay MI, Desai M. Development of an in-house ELISA for human intact osteocalcin and its utility in diagnosis and management of osteoporosis. Clin Chim Acta 2019;489:117-23.
24. Zhang XY, He JW, Fu WZ, Liu YJ, Zhang ZL. Associations of serum osteocalcin and polymorphisms of the osteocalcin gene with bone mineral density in postmenopausal and elderly Chinese women. J Nutrigenet Nutrigenomics 2016;9:231-42.
25. Bosnyak E, Trager E, Protzner A, Komka Z, Gyore I, Szmodis M, et al. Osteocalcin gene polymorphism and bone density in Hungarian athletes. Anthropol Anz 2016;73:2.
26. Ling Y, Gao X, Lin H, Ma H, Pan B, Gao J. A common polymorphism rs1800247 in the osteocalcin gene was associated with serum osteocalcin levels, bone mineral density, and fracture: the Shanghai Changfeng Study. Osteoporos Int 2016;27:769-79.