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TNF-α and *IL10* gene polymorphisms in women with postmenopausal osteoporosis

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ABSTRACT

Objective: Postmenopausal osteoporosis is a common disorder characterized by decreased bone mineral density (BMD). Proinflammatory cytokines are among the significant factors involved in bone turnover. They are the stimulants of bone resorption, acting directly on osteoclasts and osteoclast precursors. In this study, we examined the *TNF-α* (−308G>A) (rs1800629) and *IL10* (−1082G>A) (rs1800896), (−592C>A) (rs1800872) polymorphisms in postmenopausal women with BMD *T*-scores less than and greater than or equal to −2.5 SD.

Study design: This study included 224 postmenopausal women with BMD *T*-scores lower than −2.5 SD (mean: −3.02 ± 0.53) and 238 postmenopausal women with BMD *T*-scores −2.5 SD and greater (mean: −1.33 ± 0.51).

Results: There was a decrease in the frequency of *IL10* 1082 G allele carriers (GG and GA genotypes) in women with *T*-scores below −2.5 SD (GG + GA vs AA: OR = 0.65, 95% CI = 0.44–0.97, $p = 0.037$). With regard to the *TNF-α* −308 G>A polymorphism, in the women with *T*-scores below −2.5 SD, the increased frequency of GG homozygotes and G allele carriers was detected (AA + GA vs GG: OR = 0.54, 95% CI = 0.35–0.82, $p = 0.004$).

Conclusions: The results of our study suggest an association between *TNF-α* −308G>A and *IL10* −1082G>A polymorphisms and postmenopausal osteoporosis.

Introduction

Postmenopausal osteoporosis is a common disorder characterized by decreased bone mineral density (BMD), changes in bone microarchitecture and increased fracture risk. Osteoporosis is a disease caused by the interaction of genetic and environmental factors. The environmental factors can control gene expression and accordingly, the process of the disease [1]. The risk factors among women include race, lower height, body mass index, low-calcium diet, use of corticosteroids for over six months and menopausal status.

Earlier studies showed that environmental effects and genetic control influenced bone turnover [2]. Proinflammatory cytokines are among the significant factors involved in bone turnover. They are the stimulants of bone resorption, acting directly on osteoclasts and osteoclast precursors. Recent studies suggest that immune cells and osteoclasts share a number of regulatory molecules, including cytokines, receptors, signalling pathways, growth factors and transcription factors [3]. Therefore, cytokines that are produced by immune cells and regulate adaptive responses may also affect osteoclast function through common receptors, signalling pathways, and transcription factors. This hypothesis is supported by evidence gathered from experimental animal models and human observations with autoimmune diseases [4,5].

One of such responsible cytokines is TNF-α, a cytokine that increases osteoclast formation by stimulating bone marrow stromal cell production of RANKL and M-CSF, and by promoting the responsiveness of osteoclast precursors to RANKL [6,7]. IL-10 is an anti-inflammatory cytokine with significant inhibitory effects

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on osteoclastogenesis [8]. Numerous studies have investigated the associations between cytokine gene polymorphisms and susceptibility to osteoporosis and BMD in various populations. In this study, we examined the *TNF-α* (−308G>A) (rs 1800629) and *IL10* (−1082G>A) (rs1800896) and (−592C>A) (rs1800872) gene polymorphisms in postmenopausal women with BMD *T*-scores less than and greater than or equal to −2.5 SD.

Materials and methods

This study included 224 postmenopausal women (at least three years after the last menstruation; mean age, 63.3 ± 5.1 years; age of first menarche, 13.2 ± 2.3 years; age of last menarche, 47.2 ± 5.4 years; BMI, 26.2 ± 3.5) who visited the Department of Orthopaedics for bone mass examination with a diagnosed BMD *T*-score lower than −2.5 SD (mean: −3.02 ± 0.53) and 238 postmenopausal women (at least three years after the last menstruation; mean age, 64.8 ± 6.3 years; age of first menarche, 13.5 ± 2.7 years; age of last menarche, 47.7 ± 5.9 years; BMI, 26.7 ± 3.8) with a BMD *T*-score of −2.5 SD and greater (mean: −1.33 ± 0.51). All subjects underwent careful physical examination and a medical history review. Blood glucose and hepatic and renal functions were determined. Women who had undergone ovariectomy or who had hepatic or renal diseases, diabetes mellitus or other endocrine diseases were excluded from this study. None of the subjects had received any medication known to affect bone metabolism, such as glucocorticoids, thyroxin, antiepileptics, bisphosphonates, calcitonin or hormone replacement therapy, for more than three months. BMD was measured by DXA (Lunar Densitometer). The measurements were performed at the lumbar spine and femoral neck. Normative values were used for the determination of *T*-scores (comparison with a young, gender-matched, non-diseased reference Polish population). The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Genotyping

Genomic DNA was extracted from leukocytes in 200 μl whole blood samples using a GeneMATRIX Quick Blood DNA Purification Kit (EURx, Poland). Approximately 300 ng/μl of DNA was obtained from each sample. Then, the DNA was standardized with a Nanodrop ND-1000 spectrophotometer and concentrations were equalized to 20 ng/μl. This material was used as a template for amplification in a real-time polymerase chain reaction (RT-PCR). The polymorphisms within the *IL10* gene (*IL10* −1082G>A and *IL10* −592C>A) and *TNF-α* gene (*TNF-α* −308G>A) were genotyped with TaqMan genotyping assays using the 7500 FAST Real-Time PCR System (Applied Biosystems, USA). The final step was the documentation of fluorescence data by taking a photograph using a DS-34 Direct Screen Camera (Polaroid), under UV light (Transilluminator 4000, Stratagene). Photographs were scanned and saved in the form of graphic files with the extension jpeg.

Plasma concentrations of bone alkaline phosphatase were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) Quantikine kit (IBL International GMBH, Germany) according to the manufacturer's protocols.

Statistical analysis

The distributions of the cytokine genotypes and alleles between women with *T*-scores greater than or equal to and less than −2.5 SD were compared using Fisher's exact test. The odds ratio (OR) and its 95% confidence interval (95% CI) were calculated for the group with a *T*-score less than −2.5 SD vs. the group with a *T*-score greater than or equal to −2.5 SD. The association of BMD values with cytokine genotypes was statistically evaluated using the Kruskal–Wallis test followed by the Mann–Whitney *U*-test. $p < 0.05$ was considered to indicate a statistically significant result. The Statistica 7 program was used for the calculations.

The statistical power of the study with $n = 224$ and $n = 238$ subjects in the compared groups was sufficient to detect with 80% probability the true effect size measured as the OR in the range 1.46–1.75 or 0.48–0.67 (depending on the minor allele frequency) for differences in allele frequencies of the three polymorphisms.

Results

As shown in Table 1, there was the decreased frequency of *IL10* −1082 G allele carriers (GG and GA genotypes) among the women with *T*-scores below −2.5 SD (GG + GA vs AA: OR = 0.65, 95% CI = 0.44–0.97, $p = 0.037$).

Table 1
Distribution of *IL10* and *TNF-α* genotypes and alleles in women with BMD *T*-score ≥−2.5 SD and <−2.5SD.

	Women with BMD *T*-score ≥−2.5SD		Women with BMD *T*-score <−2.5SD		Comparison	*p* value[a]	OR (95% CI)
	n	%	*n*	%			
IL10 −1082G>A genotype							
AA	62	27.68%	88	36.97%	GG + GA vs AA	0.037	0.65 (0.44–0.97)
GA	114	50.89%	111	46.64%	GG vs GA + AA	0.19	0.72 (0.45–1.15)
GG	48	21.43%	39	16.39%	GG vs AA	0.044	0.57 (0.34–0.98)
IL10 −1082G>A allele							
A	238	53.12%	287	60.29%			
G	210	46.88%	189	39.71%	G vs A	0.029	0.75 (0.57–0.97)
IL10 −592C>A genotype							
CC	137	61.16%	133	55.88%	AA + CA vs CC	0.25	1.24 (0.86–1.80)
CA	80	35.71%	90	37.82%	AA vs CA + CC	0.12	2.09 (0.83–5.21)
AA	7	3.13%	15	6.30%	AA vs CC	0.12	2.21 (0.87–5.59)
IL10 −592C>A allele							
C	354	79.02%	356	74.79%			
A	94	20.98%	120	25.21%	A vs C	0.13	1.27 (0.93–1.73)
TNF-α −308G>A genotype							
GG	151	67.41%	189	79.41%	AA + GA vs GG	0.004	0.54 (0.35–0.82)
GA	66	29.46%	47	19.75%	AA vs GA + GG	0.097	0.26 (0.05–1.28)
AA	7	3.13%	2	0.84%	AA vs GG	0.085	0.23 (0.05–1.11)
TNF-α −308G>A allele							
G	368	82.14%	425	89.29%			
A	80	17.86%	51	10.71%	A vs G	0.002	0.55 (0.38–0.81)

[a] Fisher exact test for comparison of genotypes or alleles specified in "Comparison" column.