European Journal of Obstetrics & Gynecology and Reproductive Biology 164 (2012) 98–101

Contents lists available at SciVerse ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Is there any relation between IL-6 gene −174 G>C polymorphism and postmenopausal osteoporosis?

Derya Deveci [a], Zehra Sema Ozkan [b,*], Huseyin Yuce [a]

[a] Firat University School of Medicine, Department of Medical Biology and Genetics, Elazig, Turkey
[b] Kecioren Education and Research Hospital, Department of Obstetric and Gynecology, Ankara, Turkey

ARTICLE INFO

Article history:
Received 9 October 2011
Received in revised form 19 April 2012
Accepted 13 May 2012

Keywords:
Bone mineral density
IL-6
Polymorphism
Postmenopausal osteoporosis

ABSTRACT

Objective: IL-6 gene single nucleotide polymorphisms (SNPs) have been reported to have a protective effect against bone resorption. We aimed to investigate the association between bone mineral density and IL-6 promoter region −174 G>C SNP.

Study design: This study included 356 postmenopausal Turkish women, of whom 201 were osteoporotic (lumbar spine *T* score < −2.5 SD) and 155 non-osteoporotic (lumbar spine *T* score > −1.5 SD). Bone mineral density (BMD) measures were obtained using dual-energy X-ray absorptiometry. SNP of the IL-6 gene (−174 G>C) was examined by polymerase chain reaction-restriction fragment length polymorphism.

Results: The frequencies of the variant C allele (24% vs. 30%, $p = 0.074$) and mutant CC genotype (12% vs. 20%, $p = 0.094$) were higher in non-osteoporotic women. Lumbar spine and total hip BMD values were lowest among women with the G/G genotype, intermediate in the heterozygotes, and highest in women with the C/C genotype. The GG ($p = 0.022$) and GC ($p = 0.037$) genotypes were covariates which approached statistical significance in the regression model fitting of BMD.

Conclusion: IL-6 promoter region SNP showed an association with BMD in this postmenopausal Turkish population and these data suggest that the wild GG genotype influences the phenotype.

1. Introduction

Osteoporosis is a multifactorial and polygenic disease characterized by decreased bone mineral density (BMD) and deterioration of the micro-architecture of bone tissue which result in increased risk of bone fractures [1]. Age and gender are two factors that affect the fracture risk independently of BMD values [2,3]. BMD has strong genetic determination with a high heritability of 50–80% [4–6]. Many gene polymorphisms, especially the cytokines, hormones, vitamins and their receptors which influence bone turn-over, have been identified [7–10]. Interleukin-6 (IL-6) is a multifunctional cytokine that is involved in osteoclast differentiation [11]. IL-6 was found to mediate estrogen-deficiency-related bone loss in rodents [12] and its expression is up-regulated in the bone tissue of osteoporotic patients [13]. Dinucleotide repeat polymorphisms at the IL-6 locus have been associated with BMD in postmenopausal women, suggesting that allelic variants of IL-6 may influence osteoporosis susceptibility [11,14,15]. The promoter region of the IL-6 gene contains a common G−174C polymorphism, where the C allele has been associated with decreased promoter activity and plasma IL-6 levels [16,17]. This promoter variant has been reported in association with bone metabolism and BMD in recent studies [18–21]. We therefore decided to investigate the potential relationship between apparently functional IL-6 gene promoter −174 G>C polymorphism and BMD in a cohort of postmenopausal osteoporotic and non-osteoporotic Turkish women.

2. Materials and methods

2.1. Subjects

This study was conducted with 356 postmenopausal Turkish women who attended the Gynecology Department of Kecioren Education and Research Hospital, Ankara and the Department of Nuclear Medicine, Firat University Medicine School, Elazig, Turkey, between May 2009 and November 2009. The mean age, postmenopausal period, height, weight and body mass index of the study population were 57 ± 7 years, 9 ± 6 years, 155 ± 6 cm, 71 ± 13 kg and 29 ± 5 kg/m^2 respectively. We divided this population into two groups according to their lumbar spine *T* score of BMD. The first group ($n = 201$) was osteoporotic women whose lumbar spine *T* score was lower than −2.5 SD and the second group ($n = 155$)

* Corresponding author at: Firat Universitesi Tip Fakultesi Kadin Hastaliklari ve Dogum Anabilim Dali, 23119 Elazig, Turkey. Tel.: +90 5053983219; fax: +90 4242388096.
E-mail address: zehrasema@yahoo.com (Z.S. Ozkan).

0301-2115/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ejogrb.2012.05.026

was non-osteoporotic women whose lumbar spine T score was greater than −1.5 SD. All subjects underwent careful physical examination and a medical history review. Demographic and lifestyle factors including postmenopausal period, smoking history, average weekly alcohol consumption and dietary calcium intake were ascertained by lifestyle and food frequency questionnaires completed at baseline. Fasting blood glucose, hepatic and renal functions were determined. Women who had undergone ovariectomy or who had hepatic or renal disease, diabetes mellitus or other endocrine diseases were excluded from this study. The ethnicity of all the women was the same. None of the subjects had received any medication known to affect bone metabolism (such as glucocorticoids, thyroxin, anti-epileptics, bisphosphonates, calcitonin or hormone replacement therapy) for more than 3 months. Written informed consent was obtained from all subjects.

2.2. BMD measurement

Area BMD (g/cm^2) at the lumbar spine L2–L4 and total hip was measured by dual energy X-ray absorptiometry (DEXA). Densitometers were calibrated daily. The coefficient of variation for the BMD was 0.52%.

2.3. SNP genotyping

DNA for genetic analysis was extracted from peripheral venous blood by standard methods. A PCR product 431 bp fragment, which includes the site of a G to C polymorphism at position −174 in the promoter region of the IL-6 gene, was amplified. Primers for IL-6 −174 G>C were: forward 5′-CAG AAG AAC TCA GAT GAC TGG-3′ and reverse 5′-GTG GGG CTG ATT GGA AAC C-3′ (Sigma). The PCR reactions were run initial denaturation at 95 °C for 5 min followed by five cycles of 95 °C for 30 s, 57.3 °C for 60 s, followed by 72 °C for 60 s, followed by 25 cycles of 95 °C for 30 s, 56 °C for 60 s, followed by 72 °C for 60 s, followed by five cycles of 95 °C for 30 s, 54 °C for 60 s, followed by 72 °C for 60 s and a final extension at 72 °C for 7 min. PCR products was cleaved using NlaIII restriction endonucleases. Following amplification, 15 μl of the PCR products were digested overnight with 1 μl of restriction endonuclease NlaIII (Hsp92I) (Promega) enzyme at 37 °C. Digestive samples were maintained on 2% agarose gels. After digestion alleles: G/G 229 bp, 173 bp, and 29 bp; G/C 229 bp, 173 bp, and 122 bp; C/C 229 bp, 122 bp, 51 bp, and 29 bp [22].

2.4. Statistical analysis

Statistical analysis was performed by using the Statistical Package for Social Science (SPSS) 17.0 (Inc., Chicago, IL, USA) program. Results were expressed as mean and standard deviation or number and percentage, as appropriate. Differences between the means were analyzed by Student's t-test and Mann–Whitney U-test according to the distribution of data. The significance of differences between the two groups was assessed using χ^2 test or Fisher's exact test for categorical variables, where applicable. For detecting lumbar spine and total hip BMD of each SNP genotype, analysis of variance (ANOVA) was performed. Hardy–Weinberg equilibrium was tested for each genotyped SNP using χ^2 statistics. Binary logistic regression analysis was employed on BMD using BMI, age, height and weight, smoking, alcohol and calcium consumption, GG, GC and CC genotypes to identify significant covariates with BMD. In all examinations, a p value of <0.05 was considered statistically significant. Power analysis of the study was performed with program of G Power 3 and the power of our study was 82%.

Table 1
Demographic characteristics of women.

Characteristics	T score <-2.5 (no = 201)	T score >-1.5 (no = 155)	p-Value
Age (years)	57 ± 7	57 ± 6	0.89
Menopausal period (years)	9 ± 7	8 ± 6	0.12
Height (cm)	155 ± 6	156 ± 7	0.11
Weight (kg)	70 ± 13	71 ± 14	0.39
Body mass index (kg/m^2)	28 ± 5	30 ± 5	<0.05
Lumbar spine BMD (g/cm^2)	0.865 ± 0.105	1.102 ± 0.271	<0.01
Total hip BMD (g/cm^2)	0.896 ± 0.079	1.072 ± 0.201	<0.01
Lumbar spine Z score	−1.9 ± 0.7	1.1 ± 1.1	<0.01
Total hip Z score	−1.2 ± 0.6	1.1 ± 0.9	<0.01
Smoking (%)	23	28	0.57
Alcohol consumption (%)	0.1	0.1	0.78
Daily calcium intake (mg)	1100 ± 203	1150 ± 312	0.84

Values are expressed as mean ± SD and percent. BMD: bone mineral density.

3. Results

The demographic characteristics of the study population can be seen in Table 1 Lumbar spine BMD of osteoporotic and non-osteoporotic women were 0.865 ± 0.105 and 1.102 ± 0.271 g/cm^2; total hip BMD of osteoporotic and non-osteoporotic women were 0.896 ± 0.079 and 1.072 ± 0.201 g/cm^2 respectively (Table 1). The BMI of the non-osteoporotic women was significantly higher than that of the osteoporotic women. Lumbar spine and total hip BMDs were significantly different between groups.

The −174 G>C SNP genotype and allele frequencies are presented in Table 2 The difference of frequencies of genotypes and alleles between the groups was not significant, but the frequency of variant allele (C) and mutant genotype (CC) among non-osteoporotic women was higher than among osteoporotic women. The frequency of alleles was similar with Hardy–Weinberg equilibrium ($\chi^2 = 2.3$; $p = 0.3$). Variance analyses, performed for each genotype to determine the difference for the mean height, weight, BMI, lumbar spine and total hip BMD, revealed no significant difference between genotypes, but lumbar spine and total hip BMD increased sequentially from wild to mutant genotypes (Table 3). Binary logistic regression analyses

Table 2
IL-6 −174 G>C SNP genotype and allele frequencies.

	T score <-2.5 no (%)	T score >-1.5 no (%)	p-Value	OR	95% CI
Genotype					
GG	127 (63)	93 (60)	0.094	0.9	0.086–0.097
GC	50 (25)	31 (20)			
CC	24 (12)	31 (20)			
Total	201 (100)	155 (100)			
Allele					
G	304 (76)	217 (70)	0.093	0.75	0.53–1.06
C	98 (24)	93 (30)			
Total	402	310			