J Bone Miner Metab (2006) 24:53–57
DOI 10.1007/s00774-005-0646-z

ORIGINAL ARTICLE

Jung Gu Kim · Kyung Sil Lim · Seung-Yup Ku
Seok Hyun Kim · Young Min Choi · Shin Yong Moon

Relations between interleukin-1, its receptor antagonist gene polymorphism, and bone mineral density in postmenopausal Korean women

Received: May 20, 2005 / Accepted: June 30, 2005

Abstract We investigated the relation between polymorphisms in the interleukin-1(IL-1) and IL-1 receptor antagonist (IL-1ra) gene, and bone mineral density (BMD) in postmenopausal Korean women. The IL-1α $C^{-889}T$ polymorphism, and IL-1β $C^{-511}T$ polymorphism were examined by restriction fragment length polymorphism, and 86-bp variable number tandem repeat polymorphism in the IL-1ra gene was analyzed by the polymerase chain reaction and electrophoresis in 202 postmenopausal Korean women. Serum osteocalcin, and C-telopeptide of type I collagen were measured using a radioimmunoassay and enzyme-linked immunosorbent assay, respectively. BMD at the lumbar spine and proximal femur was measured by dual-energy X-ray absorptiometer. No significant differences in BMD or in serum bone markers levels were noted across the IL-1α or IL-1β genotype. There were no significant differences in the distribution of IL-1α or IL-1β genotype according to the status of bone mass. BMD in women carrying the A2 allele of the IL-1ra gene was significantly lower than those without this allele, and the A2 allele was more frequent in osteoporotic women than in normal women. These data suggest that IL-1ra gene VNTR polymorphism is a genetic factor that may affect BMD in Korean women.

Key words bone mineral density · interleukin (IL)-1 system gene · polymorphism

J.G. Kim (✉) · K.S. Lim · S.-Y. Ku · S.H. Kim · Y.M. Choi · S.Y. Moon
Department of Obstetrics and Gynecology, College of Medicine, Seoul National University Hospital, 28 Yeunkeun-dong, Chongno-gu, Seoul 110-744, Korea
Tel. +82-2-2072-3256; Fax +82-2-762-3599
e-mail: kimjg@plaza.snu.ac.kr

J.G. Kim · S.-Y. Ku · S.H. Kim · Y.M. Choi · S.Y. Moon
Clinical Research Institute, Seoul National University Hospital, Seoul, Korea

Introduction

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture [1]. Bone mineral density (BMD) accounts for approximately 70% of bone strength. It has been estimated that genetic influences account for 70% of individual variances in BMD, the major determinant of fracture risk [2]. However, studies of several candidate genes in various populations have reached different conclusions [3]. The relation between candidate gene polymorphisms and bone mass is not universal among different ethnic populations, and osteoporosis genes have not yet been defined. We have recently demonstrated that estrogen receptor (ER) PvuII and XbaI polymorphisms [4], insulin-like growth factor (IGF-I) (CA) polymorphism [5], and the vitamin D receptor (VDR) baTL haplotype [6] are possible candidate genetic factors in Korean women. Moreover, it has been suggested that osteoporosis has a polygenic trait; therefore, further studies on other candidate genes are required.

The interleukin-1 (IL-1) system is composed of IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra). IL-1α, and IL-1β are known to be potent stimulators of bone resorption [7]. They stimulate the proliferation and differentiation of osteoclast precursors and activate mature osteoclasts. IL-1ra is a competitive inhibitor of IL-1, as it binds to the same receptor sites as IL-1. IL-1 system genes are located on chromosomes 2q14-21 [8]. The $C^{-511}T$ polymorphism in the promoter region of *IL-1β* gene [9], and the 86-bp variable number tandem repeat (VNTR) in intron 2 of the *IL-1ra* gene [10] have been reported. A few studies [11–14] have recently examined the possible relations between these polymorphisms and BMD, but results remain controversial. Furthermore, the association between the $C^{-889}T$ polymorphism in the *IL-1α* gene [15] and bone mass has not been studied.

In the present study we investigated the relation between polymorphisms in the *IL-1α*, *IL-1β*, and *IL-1ra* genes and the BMD in postmenopausal Korean women.

Materials and methods

Subjects

The study included 202 postmenopausal women, between the ages of 48 and 70 years, who visited the Menopause Clinic of Seoul National University Hospital for bone mass examination and who agreed to participate in the study. All of the subjects had been without spontaneous menses for at least 1 year. They underwent a careful physical examination and a medical history review. Blood glucose and hepatic and renal functions were determined. Women who had undergone bilateral oophorectomy or women with current hepatic disease, renal disease, or diabetes mellitus were excluded. None of the subjects had received any medication known to affect bone metabolism before the study. All subjects gave their informed consent, and the study protocol was approved by the Institutional Review Board of Seoul National University Hospital.

Measurements of BMD and body mass index

The BMD was measured in grams per square centimeter at the lumbar spine (L2–L4), femoral neck, trochanter, and Ward's triangle using a Lunar DPX-L dual-energy X-ray absorptiometer (Lunar Radiation, Madison, WI, USA). It was categorized into three groups according to the World Health Organization's criteria [16]: normal, osteopenic, or osteoporotic, relative to the means and standard deviation of young adult Korean women. The in vivo coefficient of variation was 1.4% for the lumbar spine, 2.1% for the femoral neck, 1.1% for the trochanter, and 2.1% for Ward's triangle. The body mass index (BMI) was calculated by dividing body weight (kg) by the square of body height (m^2).

Measurements of serum osteocalcin and carboxy-terminal C-telopeptide of type I collagen (CrossLaps)

Blood samples were collected from all subjects in accordance with the guidelines of the Declaration of Helsinki. Serum osteocalcin (OST) was measured using a competitive radioimmunoassay kit (Techno Genetics, Milan, Italy), with a minimum detection limit of 0.1 nM/l. The intra- and interassay variations for OST were 4.0% and 5.1%, respectively. Serum carboxy-terminal C-telopeptide of type I collagen (CTX) was measured using a serum CTX one-step enzyme-linked immunosorbent assay kit (Osteometer Biotech, Herlev, Denmark), with a minimum detection limit of 94 pM/l; its intra- and interassay variations were 5.4% and 5.1%, respectively.

Determination of IL-1 system gene polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes using a QiaAmp blood kit (Qiagen, Hilden, Germany). The 86-bp VNTR polymorphism of the IL-1ra was analyzed as previously described [10]. A1 allele (four repeats) was 410 bp, A2 allele (two repeats) 240 bp, A3 allele (five repeats) 495 bp, and A4 allele (three repeats) 325 bp. The 304-bp fragment of genomic DNA containing the AvaI polymorphic portion at position –511 ($C^{-511}T$) relative to the start site of transcription of the *IL-1β* gene was amplified by PCR, as described by Chen et al. [14]. To analyze the NcoI polymorphic site at position –889 ($C^{-889}T$) of the *IL-1α* gene, the 99 base fragment was amplified by PCR using the same oligonucleotide primers and PCR reaction steps described by McDowell et al. [15]. Absence of the AvaI and NcoI sites was designated A or N allele, respectively, and the presence of these restriction cutting sites was designated a or n allele, respectively.

Statistical analysis

All data are expressed as the mean ± SE. Statistical analysis was performed using the SAS statistical program (SAS Institute, Cary, NC, USA). The genotype frequencies for each of the three polymorphisms against Hardy-Weinberg ratios and linkage disequilibria resulting from the nonrandom associations of the genotypes at the three polymorphic sites were assessed by the χ^2 test. The distributions of IL genotypes in normal, osteopenic, and osteoporotic postmenopausal women were also assessed by the χ^2 test or by Fisher's exact test. Differences in anthropometric characteristics between the various IL genotypes were tested using one-way analysis of variance (ANOVA) followed by Tukey's test or using Student's *t*-test. Similar comparisons were made for BMD or the serum levels of bone turnover markers using Student's *t*-test, or after adjusting potential confounding factors such as age, years since menopause, or BMI by analysis of covariance (ANCOVA). $P < 0.05$ was considered significant for all analyses.

Results

Four alleles in the IL-1ra VNTR polymorphism were observed: A1 96.0%, A2 2.6%, A3 0.7%, and A4 0.7%. When study subjects were grouped according to carriage of the A2 allele, two genotypes were noted: the heterozygotes A1A2 and A3A2 (5%) and the noncarriage group, A1A1, A1A4, and A3A3 (95%). The distributions of the IL-1α NcoI and IL-1β AvaI polymorphisms were as follows: nn 84.6%, Nn 14.9%, NN 0.5%, aa 31.7%, Aa 51.0%, and AA 17.3%, respectively. These IL-1 system allele frequencies followed the Hardy-Weinberg equilibrium. Linkage disequilibrium analysis did not reveal any significant relations between the IL-1ra VNTR polymorphism or the IL-1α NcoI and IL-1β AvaI polymorphisms.

The frequency of A2 allele positivity was significantly higher in osteoporotic women than in women with normal BMD (10.3% vs 2.0%, $P < 0.05$), but there were no differences in the prevalence of IL-1α NcoI and IL-1β AvaI genotypes between the former and the latter group (Table

Table 1. Distribution of the inteleukin system genotypes in normal, osteopenic, and osteoporotic postmenopausal women

Genotype	Postmenopausal women			
	Normal (n = 51)	Osteopenia (n = 83)	Osteoporosis (n = 68)	Total (n = 202)
Il-1α NcoI				
nn	42 (82.3%)	72 (86.7%)	57 (83.8%)	171 (84.6%)
Nn	8 (15.7%)	11 (13.3%)	11 (16.2%)	30 (14.9%)
NN	1 (2.0%)	0	0	1 (0.5%)
	P = 0.51			
Il-1β AvaI				
aa	17 (33.3%)	33 (39.7%)	14 (20.6%)	64 (31.7%)
Aa	27 (53.0%)	35 (42.2%)	41 (60.3%)	103 (51.0%)
AA	7 (13.7%)	15 (18.1%)	13 (19.1%)	35 (17.3%)
	P = 0.29			
IL-1ra A2 allele				
Negative	50 (98.0%)	81 (97.6%)	61 (89.7%)	192 (95.0%)
Positive	1 (2.0%)	2 (2.4%)	7 (10.3%)	10 (5.0%)
	P = 0.04			

A2 allele-negative: A1A1, A1A4, A3A3; A2 allele-positive: A1A2, A3A2

1). Mean age, years since menopause, and BMI were not statistically different among the IL system genotypes. BMD at the lumbar spine ($P < 0.05$), femoral neck ($P < 0.05$), Ward's triangle ($P < 0.05$), or trochanter ($P < 0.005$) in women carrying the A2 allele was found to be significantly lower than in those not carrying the corresponding allele (Table 2). Serum OST levels in women carrying the A2 allele was significantly higher than in those not having the A2 allele ($P < 0.005$). No significant differences in adjusted BMD of the lumbar spine or proximal femur or the adjusted serum levels of bone turnover markers were noted across the IL-1α or IL-1β genotype (Table 2).

Discussion

Many genes are known to be involved in the regulation of bone mass, and genotypic effects on bone mass may be ethnically dependent. We previously reported that genetic variation in the ER, IGF-I, and VDR loci may affect BMD in ethnically homogeneous postmenopausal Korean women [4–6]. In the present study we examined several polymorphisms of the IL-1 system genes and found that among these polymorphisms the IL-1ra A2 allele is related to BMD.

Genotype frequencies of the IL-1 system genes in our subjects differ from those reported in Caucasians. The frequency of the aa genotype of the *IL-1β* gene was lower in Koreans (17.3%) than in Caucasians (43.4%) [11], and the NN genotype of the *IL-1α* gene in Koreans was rare compared to that in Caucasians (0.5% vs. 12.3%) [15]. However, the frequency of the A1 allele of the *IL-1ra* gene was higher in Koreans (93.1%) than in Caucasians (72%–74%), whereas the A2 allele was more common in Caucasians (21%–26%) than in Koreans (5.0%) [10,12]. These IL-1ra allele frequencies in Koreans are similar to those previously reported in the Taiwanese [14].

To our knowledge, this is the first report on the relation between IL-1α polymorphism and bone mass; and no relationship was found. In addition, BMD at the lumbar spine or proximal femur in postmenopausal Korean women were not dependent on the IL-1β genotype. These findings are similar to those reported by Langdahl et al. [11], and Chen et al. [14], who did not find any evidence for an association between the IL-1β genotype and BMD in postmenopausal Danish or Taiwanese women. However, these findings are contrary to those of Nemetz et al. [13], who observed that the IL-1β genotype is associated with decreased bone mass in patients with inflammatory bowel disease. Unlike other investigators [11], who observed that BMD of the lumbar spine is reduced in the A1A1/A3 genotypes of the *IL-1ra* gene, compared with other genotypes in postmenopausal Danish women, we found that BMD at the lumbar spine and proximal femur in women carrying the A2 allele of the *IL-1ra* gene was significantly lower than in those not carrying this allele. In addition, we found that the A2 allele is more frequent in osteoporotic women than in normal women. This finding is inconsistent with that of Bajnok et al. [12], who observed a lack of association between the *IL-1ra* gene polymorphism and BMD in postmenopausal Hungarian women. It is similar, however, to the increased frequency of the A2 allele in several diseases of autoimmune or inflammatory nature, such as ulcerative colitis [17], multiple sclerosis [18], and diabetes mellitus [19].

The background of the association between BMD and IL-1ra VNTR is unknown. This polymorphic site of intron 2 of the *IL-1ra* gene contains three protein binding sites: an α-interferon silencer A; a β-interferon silencer A; and an acute-phase response element [10]. These sites with potential regulating effects may affect the production of the interleukin system, which plays an important role in bone metabolism. The A2 allele is associated with increased production of both IL-1ra and IL-1β in vitro [20]. Taking into consideration that IL-1β is a potent stimulator of bone resorption and that IL-1ra is a competitive inhibitor of IL-1,

Table 2. Bone mineral density and bone turnover markers in relation to IL-1α NcoI, IL-1β AvaI, and IL-1ra polymorphisms

Parameter	IL-1α NcoI			IL-1β AvaI				IL-1ra A2 allele		
	nn (n = 171)	Nn (n = 30)	P^a	aa (n = 64)	Aa (n = 103)	AA (n = 35)	P	Negative (n = 192)	Positive (n = 10)	P^a
Age (years)	58.0 ± 0.5	55.3 ± 1.6	0.07	57.7 ± 1.0	57.9 ± 0.7	56.4 ± 1.6	0.62	57.9 ± 0.5	58.2 ± 2.1	0.91
Years since menopause	9.1 ± 0.5	7.7 ± 1.5	0.29	8.6 ± 0.8	9.3 ± 0.7	8.3 ± 1.1	0.69	8.9 ± 0.5	9.3 ± 2.3	0.87
BMI (kg/m^2)	24.4 ± 0.2	24.2 ± 0.5	0.82	24.7 ± 0.4	24.2 ± 0.3	24.2 ± 0.5	0.45	24.5 ± 0.2	22.1 ± 0.7	0.02
CTX (pM/l)	3025.58 ± 304.4	3028.3 ± 550.3	0.99	3052.1 ± 388.5	3261.3 ± 449.3	2298.8 ± 483.5	0.45	3042.2 ± 290.3	2448.5 ± 989.7	0.75
OST (ng/ml)	20.5 ± 2.7	22.3 ± 6.4	0.79	22.8 ± 5.2	21.7 ± 3.5	14.2 ± 1.3	0.48	17.3 ± 1.8	72.1 ± 26.6	0.001
LS BMD (g/cm^2)	0.990 ± 0.014	1.004 ± 0.041	0.70	1.024 ± 0.023	0.970 ± 0.020	1.000 ± 0.025	0.19	0.998 ± 0.013	0.872 ± 0.086	0.04
FN BMD (g/cm^2)	0.805 ± 0.009	0.807 ± 0.023	0.95	0.821 ± 0.015	0.795 ± 0.012	0.807 ± 0.021	0.43	0.811 ± 0.008	0.704 ± 0.045	0.01
WT BMD (g/cm^2)	0.644 ± 0.012	0.647 ± 0.027	0.91	0.662 ± 0.017	0.634 ± 0.016	0.645 ± 0.024	0.49	0.650 ± 0.011	0.545 ± 0.054	0.03
TR BMD (g/cm^2)	0.716 ± 0.009	0.693 ± 0.025	0.35	0.723 ± 0.013	0.707 ± 0.012	0.710 ± 0.020	0.71	0.719 ± 0.008	0.596 ± 0.039	0.001

Values are means ± SE
P, ANCOVA; P^a, Student's t-test
BMI, body mass index; CTX, CrossLaps; OST, osteocalcin; LS, lumbar spine; FN, femoral neck; WT, Ward's triangle; TR, trochanter
A2 allele-negative: A1A1, A1A4, A3A3; A2 allele-positive: A1A2, A3A2

an increased capacity to produce IL-1β might be more critical than increased IL-1ra production in developing low bone mass in a woman with the A2 allele. In this study, women carrying the A2 allele showed higher serum osteocalcin levels, indicative of a high bone turnover rate, than women not carrying the A2 allele. Interestingly, no significant difference in CTX levels between A2 allele positive and negative groups. However, it is possible that all the bone turnover markers do not necessarily have to show a significant change because each marker has a different metabolic pathway (e.g., renal or hepatic), a different range of diurnal variation, and different coefficients of variation as for their assay [21,22].

The present study has potential limitations. First, ethnically homogenous Korean women only were studied, and present findings are valid only for Korean women. Second, only 10 of the study subjects carried the A2 allele that related to BMD of the lumbar spine and proximal femur. Therefore, further studies on the relation between IL-1ra VNTR polymorphism and BMD are necessary in larger populations and other ethnic groups.

In conclusion, the IL-1ra VNTR polymorphism is a genetic factor that may affect the BMD of the lumbar spine and proximal femur in Korean women. The cause of the association between the IL-1ra A2 allele and bone mass is at present unclear. A study of the relation between IL-1 system polymorphisms and IL production by whole blood cells is underway in our laboratory.

Acknowledgments This study was supported by a grant from the Korea Health 21 R & D Project, Ministry of Health and Welfare, Republic of Korea (02-PJ1-PG3-21001-0005).

References

1. NIH Consensus Statement (2004) Osteoporosis prevention, diagnosis, and therapy JAMA 285:785–795
2. Jouanny P, Guillemin F, Kuntz C, Jaendel C, Pourel J (1995) Environmental and genetic factors affecting bone mass: similarity of bone density among members of healthy families. Arthritis Rheum 38:61–67
3. Liu YZ, Liu YJ, Recker RR, Deng HW (2003) Molecular studies of identification of genes for osteoporosis: the 2002 update. J Endocrinol 177:147–196
4. Kim JG, Lim KS, Kim EK, Choi YM, Lee JY (2001) Association of vitamin D receptor and estrogen receptor gene polymorphisms with bone mass in postmenopausal Korean women. Menopause 8:222–228
5. Kim JG, Roh KR, Lee JY (2002) The relationship among serum insulin-like growth factor-I, insulin-like growth factor-I gene polymorphism and bone mineral density in postmenopausal women in Korea. Am J Obstet Gynecol 186:345–350
6. Kim JG, Kwon JH, Kim SH, Choi YM, Moon SY, Lee JY (2003) Association between vitamin D receptor gene haplotypes and bone mass in postmenopausal Korean women. Am J Obstet Gynecol 189:1234–1240
7. Romas E, Martin TJ (1997) Cytokines in the pathogenesis of osteoporosis. Osteoporos Int 7 (Suppl 3):S47–S53
8. Steinkasserer A, Spurr NK, Cox S, Jeggo P, Sim RB (1992) The human IL-1 receptor antagonist gene (IL1RN) maps to chromosome 2q14-q21, in the region of the IL-1 alpha and IL-1 beta loci. Genomics 13:654–657

9. Di Giovine FS, Takhsh E, Blakemore AI, Duff GW (1992) Single base polymorphism at –511 in the human interleukin-1 beta gene (IL1 beta). Hum Mol Genet 1:450
10. Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A, Duff GW (1993) Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 91:403–404
11. Langdahl BL, Lokke E, Carsyens M, Stenker L, Eriksen EF (2000) Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1-receptor antagonist gene but not with polymorphism in the interleukin-1β gene. J Bone Miner Res 15:402–414
12. Bajnok E, Takacs I, Vargha P, Speer G, Nagy Z, Lakatos P (2000) Lack of association between interleukin-1 receptor antagonist protein gene polymorphism and bone mineral density in Hungarian postmenopausal women. Bone 27:559–562
13. Nemetz A, Toth M, Garcia-Gonzalez MA, Zagoni T, Fecher J, Pena AS, Tulassay Z (2001) Allelic variation at the interleukin 1 beta gene is associated with decreased bone mass in patients with inflammatory bowel diseases. Gut 49:644–649
14. Chen HY, Chen WC, Wu MC, Tsai FJ, Lin CC (2003) Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphism in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis. Maturitas 44:49–54
15. McDowell TL, Symons JA, Ploski R, Forre O, Duff GW (1995) A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. Arthritis Rheum 38:221–228
16. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141
17. Mansfield JC, Holden H, Tarlow JK, Di Giovine FS, McDowell TL, Wilson AG, Holdsworth CD, Duff GW (1994) Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 106:637–642
18. Crusius JB, Pena AS, Van Oosten BW, Bioque G, Garcia A, Dijkstra CD, Polman CH (1995) Interleukin-1 receptor antagonist gene polymorphism and multiple sclerosis. Lancet 346:979–980
19. Blakemore AI, Cox A, Gonzalez AM, Maskil JK, Hughes ME, Wilson RM, Ward JD, Duff GW (1996) Interleukin-1 receptor antagonist allele (IL1RN*2) associated with nephropathy in diabetes mellitus. Hum Genet 97:369–374
20. Santtila S, Savinainen K, Hurme M (1998) Presence of the IL-1RA allele (IL1RN*2) is associated with enhanced IL-1 beta production in vitro. Scand J Immunol 47:195–198
21. Watts NB (1999) Clinical utility of biochemical markers of bone remodeling. Clin Chem 45:1359–1368
22. Eyre DR (1997) Bone biomarkers as tools in osteoporosis management. Spine 22(Suppl):17S–24S