Original Article

Polymorphisms of the human IL-1 receptor antagonist gene and forearm bone mineral density in postmenopausal women

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ABSTRACT

Context: Studies on the human interleukin 1 receptor antagonist (IL-1RA) gene polymorphism have provided conflicting data regarding the bone mass and quality. **Aim and Design:** The objective of this case-control study was to investigate the association between the forearm bone mineral density (BMD) and the IL1RA gene polymorphisms. **Materials and Methods:** A total of 400 postmenopausal Bulgarian women participated in this study. BMD was measured at the forearm by X-ray absorptiometry on a DTX-100 device (Osteometer Meditech, USA). A PCR product was isolated. The alleles were scored according to their length: A1 – 410 bp – 4 repeats; A2 – 240 bp – 2 repeats; A3 – 500 bp – 5 repeats; A4 – 325 bp – 3 repeats; A5 – 595 bp – 6 repeats. All analyses were evaluated for statistical significance (χ^2-test and T-test). **Results:** Four alleles were observed – A1, A2, A3, and A4. The A1A1 genotype was more common in cases with low BMD than in controls with normal BMD (95% vs. 90%, χ^2 $P < 0.01$). The A2A2 genotype was equally distributed among cases and controls (both 5%). The other two genotypes (A3A3 and A4A4) as well as A1A3 were present only in controls with normal BMD. The A2A2 genotype was associated with higher BMD and the A1A1 – with lower BMD at both forearm sites. The odds ratio for low BMD in the presence of the A1A1 genotype was 2.11. The etiological factor reflecting the association between the polymorphism and the disease was 0.50. In our study sample the IL1RA genetic polymorphisms were associated with the forearm BMD. **Conclusion:** This genetic polymorphism may become a useful genetic marker for the study of osteoporosis.

Key words: Bone mineral density, IL-1 receptor antagonist, osteoporosis, polymorphisms

INTRODUCTION

The IL-1RA is structurally related to and competes with the IL-1 for the occupancy of the IL-1 cell receptors. It acts as a competitive inhibitor and does not trigger signal transduction. The IL-1RA gene is localized on the long arm of the chromosome 2 (2q13). The polymorphism in intron 2 of the IL-1RA gene is caused by the variable copy number of an 86 bp sequence which is supposed to have some functional significance. The published gene sequence contained three copies of the 86 bp sequence of the IL- 1RA gene.[1] Later a four-allelic variable polymorphism has been reported by Lennard *et al.*[2] Tarlow *et al.* investigated the IL-1RA gene to determine whether the length variation of the A2 allele was due to different copy numbers of this 86 bp sequence and found allelic association with several chronic inflammatory and degenerative diseases.[3] Recently, the IL-1RA gene was proposed as a major factor in predisposing to gastritis and malignant transformation.[4,5] In a study in 108 postmenopausal women Keen *et al.* found that the IL-1RA genotype was significantly associated with the early postmenopausal bone loss at the spine for a 5-year period.[6] Langdahl *et al.* proved that the 86 bp repeat polymorphism of the IL-lRA gene is associated with an increased risk of osteoporotic fractures.[7] In a study in postmenopausal Mediterranean women Fontova *et al.* found that those carrying the A2 allele (A2+) had higher

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lumbar spine and hip BMD than noncarriers (A2−).[8] They concluded that the IL-1RA and tumor necrosis factor-alpha can both be candidate loci defining susceptibility for postmenopausal osteoporosis. Han *et al.* investigated the IL-1RA polymorphism in Korean postmenopausal women.[9] The women had received hormone replacement therapy for 1 year and the authors found no difference in spine and femoral neck BMD among the different IL-1RA genotypes. Kim *et al.* studied 202 postmenopausal Korean women and found significantly lower BMD in women carrying the A2 allele, with the A2 allele being more frequent in women with osteoporosis.[10] These data led to the conclusion that the IL-1RA gene polymorphism might be a genetic factor affecting BMD in Korean women.

The aim of this pilot study was to determine whether the length variation and different copy numbers of the 86 bp sequence are associated with differences in bone mineral density (BMD) at the forearm measurement sites in Bulgarian women.

Materials and Methods

Subjects

The study includes 400 women aged 36-77 years of age. Informed consent was obtained from each subject. All studied individuals are from Bulgarian ethnical origin (white Caucasians) and have no ties of relationship. About one-third of them were referrals to our Osteoporosis Centre by GPs or other practicing medical specialists. The second-third were self-referrals and were measured because of their concern about possible osteoporosis based mainly on maternal history of low-trauma fractures and known risk factors. The remaining one-third came from a population-based screening program of apparently healthy women living in an urban area. All studied women were postmenopausal. They had no diseases causing secondary osteoporosis (endocrine, gastro-intestinal, liver diseases, kidney diseases, genetic or other etiologies). None of them received inhibitors of bone resorption or medications, causing osteoporosis (glucocorticoids, immune suppressors, heparin, anticonvulsants, thyroid hormones, and others), calcium or vitamin D. Physical activity was minimal in all participants.

Two biochemical markers were used for the assessment of bone turnover. Serum osteocalcin was measured as a marker of bone formation and urinary deoxypyridinoline - as a marker of bone resorption. Both markers were measured by enzyme-linked immunosorbent assay. The respective reference ranges were 3.7-10.0 ng/ml for osteocalcin and 3.0-7.4 nmol/nmol creatinine for deoxypiridinoline.

Bone densitometry

BMD was measured at the distal forearm by single-energy X-ray absorptiometry (SXA) on a DTX-100 unit (Osteometer Meditech, USA). On the DTX-100 the distal region of interest begins at the 8 mm separation point between the radius and ulna and then continues proximally for a distance of 24 mm. The ultradistal site extends from the radial endplate proximally to the 8 mm point. BMD was measured according to the manufacturer's instructions in g/ cm^2 separately for the distal (including radius and ulna and consisting primarily of cortical bone) and the ultradistal site (including only the radius and containing more trabecular bone). T-scores were calculated automatically based on the manufacturer's Danish database (issued 1994). [11] The standardization was performed daily by scanning a manufacturer-supplied forearm phantom (for SXA).

The participants were grouped according to their BMD: 220 participants had low BMD or osteoporosis (labeled as cases) and 180 had normal BMD (controls).

Genotyping

The DNA was isolated from whole blood. The primers and PCR conditions for amplifying the intron 2 of the IL-1RA gene were designed.[3] dATP, dCTP, dTTP, dGTP - 1.25 mM were each used to amplify intron 2 with Taq DNA polymerase. A total of 100 ng of the DNA were used as template in the PCR reactions. Primers flanking the repeat region were used to amplify the PCR product. The PCR products were electrophoresed through a 2% agarose gel. The length of the repeats was compared with a marker of 100 bp (ladder). The alleles were scored in correspondence to their reported length: A1 – 410 bp – 4 repeats; A2 – 240 bp – 2 repeats; A3 – 500 bp – 5 repeats; A4 – 325 bp – 3 repeats; A5 – 595 bp – 6 repeats.

Statistical analysis

The data were evaluated by Pearson's χ^2 and Student's T-tests and presented as means ± SD. The significance level was set as $P < 0.05$.

The odds ratio (OR) for low BMD in the presence of a specific allele was calculated as:

$$OR = (a \times d)/(b \times c)$$

where a is the number of carriers among the cases, b is the number of not carriers among the cases, c is the number of carriers among the controls, d is the number of not carriers among the controls.

The etiological factor (EF) showing what part of the condition (low BMD) might be attributable to the associated

factor (the IL-1RA polymorphism) on a population level was calculated as:
EF = [(OR – 1) / OR] [a / (a + b)].

The coefficient of variation (CV) of the bone turnover markers (osteocalcin and deoxypyridinoline) was calculated separately in each subgroup. The correlation between the markers and the IL-1RA marker was described by Pearson's coefficient (CC).

Results

Four alleles were observed – A1, A2, A3, and A4. These alleles corresponded to the presence of 4, 2, 5, and 3 copies of the 86 bp sequence. The distribution of the alleles and genotypes by length polymorphism in the subgroups of cases (with low BMD or osteoporosis, T-scores below −1.0 at either site) and controls (with normal BMD, T-scores above −1.0 at both sites) is shown in Table 1. The polymorphism information content (PIC) of this particular polymorphism was 0.09 and the heterozygoity at this locus −0.10.

The A1A1 genotype was more common in cases with low BMD or osteoporosis than in controls with normal BMD (95% vs. 90%, χ^2 $P < 0.01$). The A2A2 genotype was equally distributed among cases and controls (both 5%). The other two genotypes (A3A3 and A4A4) were present only in controls with normal BMD. The heterozygotes A1A3 were also found only among controls (0.5%) [Table 1].

The BMD values at the distal and ultradistal forearm sites in the presence of the different IL-1RA genotypes are shown separately for cases (T-scores ≤ –1.0) and controls (T-scores > −1.0) in Table 2. Figure 1 summarizes the mean BMD values in carriers of the A1A1 and A2A2 genotypes in the whole study sample. Higher forearm BMD was found in A2A2 carriers and lower BMD in A1A1 carriers whether or not subdivided to their BMD values.

The OR for low BMD or osteoporosis in the presence of the A2A2 genotype was 2.11. The etiological factor (EF) showing what part of the illness might be attributed to the genetic factor under study on a population level was found to be equal to 0.50.

Discussion

The number of repeats in the IL1RA gene is supposed to have functional significance. The sequence contains three potential protein binding sites – an α-interferon silencer A, a β-interferon silencer B, and an acute phase response element. It is known that the α-interferon silencer A is contained in the viral response element and acts as a repressor when four tandem copies are positioned between an enhancer and a promoter. Tetramers of the sequence mediate inducibility by viruses. Dimers are inactive or very weekly active.[12] The β-interferon silencer B and the acute phase response element are contained within the viral response element in the human β-interferon gene.[13] Individuals with different copy numbers of the 86-bp repeat sequence in the IL-1RA gene also express different numbers of the protein-binding sites.

The IL-1RA polymorphism had been studied in Caucasian populations where the frequency of the A1 allele (associated with lower BMD) was about 74%. The corresponding frequency in the Bulgarian population is 92.9%. Therefore the Bulgarian population differs by the frequency of the A1 allele from other European populations and is among these with the highest allele frequencies. Since the A1 allele is associated with lower BMD, the other European populations might be at lower risk for osteoporosis and osteopenia than ours.

In our study sample the A1A1 genotype was also associated with lower BMD. The A2A2 genotype which was equally distributed among cases and controls (5%) was associated with higher BMD. The A3A3 and A4A4 genotypes, as well as the A1A3 heterozygotes were found only among controls. Other heterozygotes were absent both among cases and controls. We compared the IL-1RA allelic frequencies with published data for European menopausal women - 37.9%

Table 1: The distributions of the IL-1RA genotypes and alleles are shown

Genotype	Cases (BMD T-score ≤ -1.0)					Controls (BMD T-scores > -1.0)				
	A1A1	A2A2	A3A3	A4A4	A1A3	A1A1	A2A2	A3A3	A4A4	A1A3
Number	209	11	0	0	0	162	9	5	3	1
Frequency	0.95	0.05	0	0	0	0.90	0.05	0.028	0.017	0.005
H_0 / PIC	0.10 / 0.09									
P	$P < 0.05$ df=2									

Alleles	A1	A2	A3	A4	A1	A2	A3	A4
Number	418	22	0	0	325	18	11	6
Frequency	0.95	0.05	0	0	0.90	0.05	0.03	0.02
P	$P < 0.05$ df=1							

Table 2: The bone turnover markers and the forearm bone mineral density are shown in carriers of the different IL-1RA genotypes as means ± SD

IL-1RA genotype		Cases (T-score ≤−1.0)		Controls (T-score > −1.0)				
		A1A1	A2A2	A1A1	A2A2	A3A3	A4A4	A1A3
Age (years)		52.9 ± 7.8	54.7 ± 7.8	54.8 ± 7.3	49.2 ± 1.7	51.0 ± 2.5	51.0 ± 1.0	50.0
Years since menopause		5.0 ± 4.7	4.0 ± 4.7	4.3 ± 5.3	0.6 ± 0.7	1.0 ± 1.0	0.7 ± 0.6	0.5
BMI (kg/m²)		22.2 ± 2.4	22.1 ± 1.5	26.4 ± 1.3	25.3 ± 1.8	26.2 ± 2.7	21.0 ± 2.2	23.7
Osteocalcin (ng/ml)		15.1 ± 2.9	9.6 ± 1.8*	9.2 ± 1.0	7.7 ± 1.2	6.4 ± 0.8	8.1 ± 1.6	5.3
CV (%)		19.2	18.7	10.9	15.6	12.5	19.8	-
Pearson's correlation coefficient		0.16						
Deoxypyridinoline nmol/nmol Creat.		9.3 ± 1.6	8.3 ± 0.9**	4.9 ± 1.4	6.1 ± 1.4	5.5 ± 0.9	5.7 ± 0.2	6.2
CV (%)		17.2	10.8	28.6	22.9	16.4	3.5	-
Pearson's coeff.		0.13						
Forearm BMD (g/cm²)	Distal site	0.431 ± 0.034	0.434 ± 0.036	0.466 ± 0.029	0.527 ± 0.039****	0.494 ± 0.049	0.469 ± 0.017	0.486
	Ultradistal site	0.307 ± 0.029T	0.333 ± 0.040***	0.368 ± 0.017	0.413 ± 0.040*****	0.383 ± 0.048	0.364 ± 0.011	0.368

*P = 0.003 for the osteocalcin in A2A2 versus A1A1 (in cases). **P = 0.05 for the deoxypyridinoline in A2A2 versus A1A1 (in cases). ***P = 0.013 for the BMD in A2A2 versus A1A1 (in cases). ****P < 0.001 for the BMD in A2A2 versus A1A1 (in healthy controls). *****P = 0.003 for the BMD in A2A2 versus A1A1 (in healthy controls).

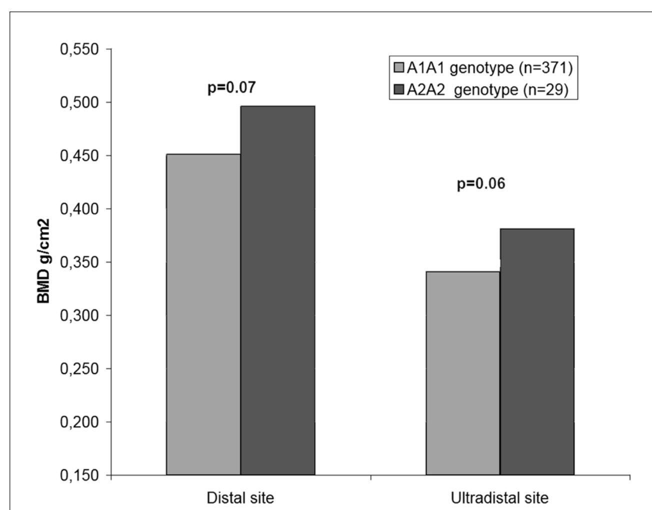

Figure 1: Bone mineral density (in g/cm²) at the distal and ultradistal forearm sites according to the different genotypes in the whole study sample

for A1A1, 5.8% for A2A2, and 40.8% for A1A2.[6,14] The observed differences in our study were highest for the A1A2 genotype which was not found in our population sample. The A1A1 genotype was more commonly found in our sample.

Our results are corroborated by findings in other European populations. Fontova *et al.* found higher central site BMD in A2 allele carriers among 104 postmenopausal women.[8] Langdahl *et al.* found an elevated relative risk of osteoporotic fractures in individuals with the A1A1/A3 genotypes.[7] BMD of the lumbar spine was reduced in individuals with the A1A1/A3 genotypes and the differences in bone mass between A1A1/A3 and A2A1/A2 tended to increase with increasing age. These authors found no differences in the biochemical markers of bone turnover as did also Kim *et al.*[7,10] Our results show significant differences for both biochemical markers. The A1A1 genotype is associated with lower BMD and a higher value of urine deoxypyridinoline than the A2A2 genotype (P = 0.03). The A1A1 genotype is associated with an elevated serum osteocalcin level compared with the A2A2 (P < 0.01).

The genetic variance of the IL-1RA locus may influence the acquisition and maintenance of the peak bone mass and predispose individuals to bone loss and osteoporosis. The observed association between the A1 allele and low BMD demonstrates that the IL-1RA gene might be related to osteoporosis. The higher frequency of the A1A3 heterozygotes in the controls shows that the heterozygous state is probably not associated with a predisposition to low BMD.

When considering our data several study limitations should be kept in mind. First, our study population was of moderate size and quite heterogeneous which might be a source of bias. It was composed of patients referred by other physicians, self-referrals or women screened in a population-based program. Controls were 2-5 years younger and had spent 1-4 years lesser time after menopause when compared to cases (A1A1 and A2A2) which could have influenced partially BMD although the observed differences among the subgroups are large enough. The small sample number did not allow analysis for confounding variables such as BMI or years since menopause. Our study had not sufficient power to analyze these populations separately. This heterogeneous population is, however, representative of the typical population receiving DXA scans at our hospital-based osteoporosis center. In addition, forearm

BMD was measured at the distal and ultradistal sites, which are clearly different from the recommended by the International Society for Clinical Densitometry 33% radius site.

In conclusion, this is a pilot study examining the prevalence of IL-1RA genotype polymorphism and its association with the forearm, lumbar spine, and femoral neck BMD. It reveals differences in the allele frequencies specific for this Bulgarian cohort. Nevertheless these preliminary results suggest a potential substantial impact of the different IL1-RA genotypes on the BMD at the lumbar spine, the forearm, and the femoral neck.

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