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Lack of Relationship between Vitamin D Receptor Polymorphism and Bone Erosion in Rheumatoid Arthritis

We performed this study to investigate the possible association between vitamin D receptor (VDR) gene polymorphism and the focal bone erosion in rheumatoid arthritis (RA) patients in Korea. One hundred and fifty-seven RA patients were enrolled and two control groups were selected. The focal bone erosion score was assessed by modified Sharp's method. Genotyping of VDR polymorphisms was performed by polymerase chain reaction and restriction fragment length polymorphism analysis using two restriction enzyme *Taq* I and *Bsm* I. Notably, the distribution of VDR genotype in Korean population was different from Caucasians. The frequencies of "tt" and "BB" genotypes were very rare both in RA patients and in control groups. The frequency distribution of the *Taq* I and *Bsm* I genotype was not different between RA patients (TT, 93.6%; Tt, 6.4%; tt, 0%; BB, 0.6%; Bb, 5.1%; bb, 94.3%) and control groups (TT, 90.8%; Tt, 7.5%; tt, 1.7%; BB, 1.4%; Bb, 8.1%; bb, 90.5%). There was no significant difference in the focal bone erosion score (mean±SD) according to the VDR genotypes of RA patients (TT, 0.92±1.79; Tt, 0.4±0.79; Bb, 0.43±0.80; bb, 0.92±1.79; $p>0.05$). In conclusion, these results suggest that VDR gene polymorphisms are not associated with the focal bone erosion in RA patients in Korea.

Key Words: *Receptors, Calcitriol; Polymorphism (Genetics); Arthritis, Rheumatoid; Bone and Bones*

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INTRODUCTION

Radiographic changes in rheumatoid arthritis (RA) include the presence of juxta-articular osteopenia and focal bone erosion in subchondral bone and at the joint margins in areas of direct pannus invasion (1). In addition, there has been an increasing awareness that patients with RA are also at greater risk for the development of a generalized form of bone loss that affects the entire axial and appendicular skeleton. Recently, associations between polymorphisms of the vitamin D receptor (VDR) gene and bone mineral density (BMD) or intestinal calcium absorption have been reported (2-6). With respect to the focal erosive bone changes, several studies have shown that these erosions tend to progress throughout the course of the disease and that, in general, there is a correlation between the presence of focal bone destruction and disease severity (7, 8). Genes that may influence the skeleton's response to systemic disease like RA could have important effects on both local and generalized bone loss in RA. Morrison et al. have reported that polymorphisms of the VDR gene account for 75% of the primary genetic factor for BMD (2). We examined the relationship between the polymorphisms of the VDR and focal erosive bone changes in RA, which allowed us to explore the hypothesis that common variations in this gene might, in part, determine susceptibility to increased focal bone loss in patients with RA.

MATERIALS AND METHODS

Patients

One hundred and fifty-seven unrelated patients with RA (19 men and 138 women, age range 16-82 yr) were studied. These patients met the 1987 American College of Rheumatology criteria (9) and had disease duration of at least two years and had been followed for more than a year at the rheumatology outpatient clinic of Asan Medical Center in Seoul, Korea. All patients had been treated with various combinations of disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate, hydroxychloroquine, and sulfasalazine during the course of the disease. Patients were excluded if they received hormone replacement therapy (HRT) or any other medi-

cations known to affect bone such as bisphosphonates. Two control groups (211 for *Bsm* I and 120 for *Taq* I) were selected from healthy blood donor for determining the VDR genotype. They were 46 men and 284 women with age 16-82 yr.

Medical records of the patients were reviewed for age at onset, duration of disease and the presence of rheumatoid factor (RF; patients were considered seronegative if the nephelometric RF determination was <20 IU/mL on two occasions at least three months apart).

Radiologic measurements

The radiographs of patients' hands were evaluated for the presence of erosion by two bone radiologists without any information of the identity of the patients. An erosion score was estimated by Sharp's method with minor modification (10). In the modified Sharp's method, erosion (grades 0-5) was counted in 8 proximal interphalangeal joints, 2 interphalangeal joints of the thumbs, and 10 metacarpophalangeal joints. In each wrist, joint erosions were assessed in 6 joint areas. The interobserver coefficiency was 0.80 and intraobserver coefficiencies of two radiologists were 0.962 and 0.965, respectively. The time-corrected erosion score was obtained by dividing the erosion score by the duration of disease.

DNA analyses

DNA was extracted from leukocytes. The VDR genotype was determined by polymerase chain reaction (PCR) amplification and restriction length fragment polymorphisms (RFLP) as previously described (2,11). *Bsm* I polymorphisms were determined in a single PCR fragment of 870 base pairs (bp) from genomic DNA using a forward primer in exon 7 (5'-CAACCAAGACTCAAGTACCGCGTCAGTGA-3') and a reverse primer in intron 8 (5'-AACCAGCGGAAGAGGTCAAGGG-3'). For the detection of *Taq* I polymorphism, a forward primer in intron 8 (5'-CAGAGCATGGACAGGGAGCAAG-3') and a reverse primer in exon 9 (5'-GCAACTCCTCATGGCTGAGGTCTC-3') were used. The PCR products for the *Taq* I polymorphism was 740 bp long. PCR was performed under standard conditions. After amplification, the PCR products were digested with *Bsm* I (New England Biolabs, Beverly, MA, U.S.A.) and *Taq* I (New England Biolabs) endonuclease. Following restriction endonuclease digestion, genotyping was determined by ethidium bromide-UVB illumination of the fragments separated on gels of 1% (*Bsm* I) or 2% (*Taq* I) agarose. The presence of the *Bsm* I or *Taq* I restriction site was defined as the lower-case "b" and "t", respectively, and the absence of the site was defined as the upper-case "B" or "T". Homozygous presence of *Bsm* I restriction site (bb) results in fragments of 700 bp and 170 bp while homozygous absence (BB) leaves the 870 bp. Heterozygotes (Bb) exhibit all bands (870, 700, and 170 bp). Homozygous absence of the *Taq* I restriction site (TT) results in 2 fragments (245 and 495 bp), while homozygous presence of the restriction site (tt) results in 3 fragments (205, 245, and 290 bp). Heterozygotes (Tt) exhibit all 4 fragments (Fig. 1).

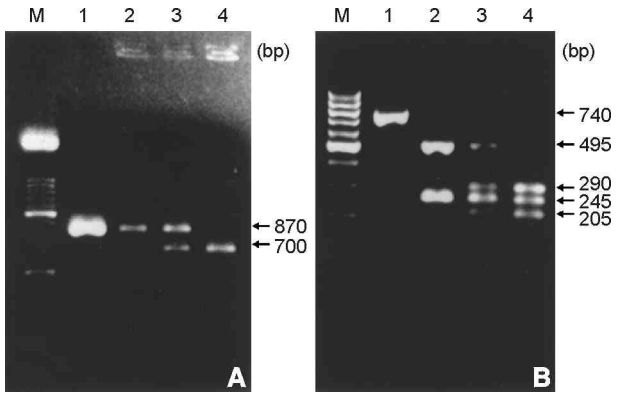

Fig. 1. Analysis of vitamin D receptor genotypes as defined by an restriction fragment length polymorphism. **A:** Genotyping of VDR using *Bsm I* (M, 100 bp size marker; lane 1, PCR product; lane 2, BB type; lane 3, Bb type; lane 4, bb type) (170 base pair bands are so dim that the bands are not shown on this figure). **B:** Genotyping of VDR using *Taq I* (M, 50 bp size marker; lane 1, PCR product; lane 2, TT type; lane 3, Tt type; lane 4, tt type).

Statistical analysis

Statistical analysis was done using the SAS 6.12 for Windows program. Fisher's exact test was used to compare the frequency distribution of VDR genotypes. Wilcoxon rank sum test was used to compare the bone erosion score according to VDR genotype.

RESULTS

The distribution of genotypes as detected by *Bsm* I and *Taq* I is presented in Table 1. The frequency of *Bsm* I and *Taq* I of VDR gene was similar to those previously described in Korean population (12-13). There was no difference between control groups (TT, 90.8%; Tt, 7.5%; tt, 1.7%; BB, 1.4%; Bb, 8.1%; bb, 90.5%) and RA patients (TT, 93.6%; Tt, 6.4%; tt, 0%; BB, 0.6%; Bb, 5.1%; bb, 94.3%) with respect to the genotypic distribution. The frequencies of "tt" and "BB" genotypes were very rare both in RA patients and in control groups.

The correlations between *Bsm* I and *Taq* I genotypes were noted (bb with TT, Bb with Tt). The presence (t)

Table 1. The frequency of vitamin D receptor genotypes in rheumatoid arthritis and control groups

Genotype	Control		RA		*p**
Taq I					
TT	109	90.8%	147	93.6%	
Tt	9	7.5%	10	6.4%	0.301
tt	2	1.7%	0	0%	
Total	120	100%	157	100%	
Bsm I					
BB	3	1.4%	1	0.6%	
Bb	17	8.1%	8	5.1%	0.488
bb	191	90.5%	148	94.3%	
Total	211	100%	157	100%	

Values are number of subjects and %
* Fisher's exact test

or absence (T) of the polymorphic *Taq* I restriction site are in linkage disequilibrium with the absence (B) or presence (b), respectively, of the polymorphic *Bsm* I site. The concordant rate is 95% in our study population (kappa analysis, kappa=0.565).

The effect of genotypes on bone erosion was examined. Time-corrected bone erosion score was calculated by modified Sharp's method divided by the duration of disease. Due to the rarity of "tt" and "BB" alleles, we only compared bone erosion score between two genotypes ("TT" and "Tt", "bb" and "Bb"). There was no significant difference between two genotypic groups with respect to bone erosion score (Table 2).

The RF was confirmed as a severity marker. When patients were divided into two groups based on the presence of RF, no significant differences in the frequency of genotype and bone erosion score were found between those with RF and those without (Table 3).

DISCUSSION

Considering the inter-racial differences in the frequency of VDR gene polymorphism, it can be assumed that the distribution of VDR genotypes in Korean might be different from that in Caucasians. According to the previous reports (2-4, 14), the frequencies of VDR genotype in Caucasians are as follows: BB (12.0-26%), Bb (34.4-53.0%), bb (28.7-48.0%), TT (11.7-41.0%), Tt (41.0-56.0%), tt (12.0-39.2%). In our data, the frequencies of "tt" and "BB" alleles were very rare (Table 1). These results are compatible with the previous reports in Koreans (12, 13) or in Japanese and Chinese populations (15, 16). There was no difference between control groups and RA patients with respect to genotypic distribution in our data (Table 1).

Although the exact mechanisms by which the VDR genotypes are linked to bone density have yet to be

Table 2. Effect of genotypes on bone erosion in rheumatoid arthritis patients

Genotype	Bone erosion score*	*p*†
Taq I		
TT	0.92±1.79	
Tt	0.4±0.79	0.256
Bsm I		
Bb	0.43±0.80	
bb	0.92±1.79	0.284

* Mean±Standard deviation
† Wilcoxon rank sum test

Table 3. Vitamin D receptor genotypes and bone erosion score according to rheumatoid factor status

	RF (+) Positive (n=126)	RF (–) Negative (n=31)	*p*
Taq I			
TT	117	30	0.688*
Tt	9	1	
Bsm I			
BB	1	1	
Bb	7	30	0.765*
bb	118		
Bone erosion	0.93±1.88	0.71±1.04	0.945†

* Fisher's exact test
† Wilcoxon rank sum test

determined, they might be related to the established actions of vitamin D. The function of $1,25(OH)_2$ vitamin D_3 in synovial tissue remains to be explored. The presence of specific receptors for $1,25(OH)_2$ vitamin D_3, not only in normal monocytes but also in peripheral lymphocytes and synovial tissue-derived fibroblast from patients with RA, suggests a role for the hormone in joint disease (17, 18). The effect via VDR on bone loss in RA is of interest. Previous studies showed that $1,25(OH)_2$ vitamin D_3 has several important VDR-mediated immunological effect such as potentiating defense mechanisms mediated by monocytes-macrophages probably involving γ-interferon or interleukin-1; the latter cytokine is a potent stimulator of bone resorption. $1,25(OH)_2$ vitamin D_3 also inhibits the proliferation of active B and T lymphocytes and the production of interleukin-2 (17, 19, 20). Other reports described the effect of $1,25(OH)_2$ vitamin D_3 in promoting bone resorption. The fusion of macrophages to multinucleated giant cells that can resorb bone is promoted by $1,25(OH)_2$ vitamin D_3 (21). $1,25(OH)_2$ vitamin D_3 also inhibits collagen synthesis by osteoblast (22).

Other reports showed that extrarenal synthesis of $1,25(OH)_2$ vitamin D_3 occurs in RA, and that there are differences in calcium homeostasis in response to stimulation with $1,25(OH)_2$ vitamin D_3 according to VDR

genotype (23, 24). The extrarenal synthesis of $1,25(OH)_2$ vitamin D_3 is a characteristic of activated macrophages and has been demonstrated to occur in vitro in synovial fluid macrophages from patients with inflammatory arthritis. Such synthesis may be of significance in the immunoregulatory system in the affected joint and may be proven to have relevance to the development of periarticular osteoporosis in RA (23). After the administration of oral calcitriol, pre-menopausal women with the "bb" genotype have a greater decrease in the serum parathyroid hormone concentration and greater increase in serum osteocalcin than women with the "BB" genotype (24). These data indicate that the VDR alleles are associated with differences in the vitamin D endocrine system and may have significant implications in relation to the pathophysiology of osteoporosis.

VDR genotypes have also been linked to the regulation of the intestinal absorption of calcium: when the dietary intake of calcium is low, women with "BB" alleles may absorb calcium less efficiently than those with "bb" alleles (6). Thus, differences in allelic status of VDR may be related to bone mineralization through the different intestinal absorption of calcium. As the VDR has a direct effect on the calcium/vitamin D endocrine system, polymorphisms at this site can be suitable candidates for a role in determining progression of a disease characterized by bone and joint destruction such as RA.

Changes in the VDR may alter disease expression in RA by affecting patient's susceptibility to secondary osteoporosis, or locally in the joints themselves, by altering cellular interactions. To evaluate the relationship between VDR alleles and bone erosion in RA patients, they were categorized according to the alleles at each polymorphic site. Because "BB" and "tt" alleles were very rare, only two genotypic groups ("bb" and "Bb", "TT" and "tt") were compared regarding to bone erosion. There were no significant differences in bone erosion between two genotypic groups (Table 2).

There are several possible explanations for this result. First, we enrolled patients having disease duration of at least two years and calculated bone erosion in a cross-sectional manner. Gough et al. reported that "tt" allele in early RA patients was associated with accelerated bone loss (25). It is possible that the above association could be missed due to different study population and study design. Second, "BB" and "tt" alleles, which are linked with low BMD in some (2-5, 11, 24) but not all studies (26, 27), were very rare and were excluded from the comparative study. If the frequencies of these two alleles were high enough for comparison, it would be possible that VDR genotype might be associated with bone erosion in RA patients. To include these rare VDR alleles, large-scale analysis is needed. Third, in the absence of knowledge on the functional sequence variations in the VDR gene in linkage with the polymorphism described so far, neither allelic heterogeneity nor linkage disequilibrium with another bone-metabolism related gene can be excluded as an explanation for the different results of genetic effect of VDR genotype on bone density between different studies (28, 29).

We studied the association between VDR polymorphism and bone erosion corrected for RF status, since RF status is a confirmed predictor of severity (30). However, the conclusion was not affected by the RF status. Bone erosion was not different between RF positive and negative groups (Table 3). Possible explanations for this lack of association between RF positivity and bone erosion can be a variation in patient selection or a different definition of seropositivity.

In conclusion, VDR gene alleles seem to be not associated with bone erosion in Korean RA patients. However, "tt" or "BB" alleles known to be responsible for bone loss are so rare in our study population, a longitudinal large-scale study is needed to confirm this result.

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