RA is a systemic inflammatory arthritis that leads to local and systemic bone loss. Osteoporosis or the systemic bone loss associated with RA increases the risk for fragility fractures, which can affect quality of life dramatically in RA patients. Although traditional and RA-related risk factors have been defined and studied for osteoporosis associated with RA, genetic factors such as polymorphic variants in the traditional candidate genes for osteoporosis, such as the vitamin D receptor (VDR), type 1 collagen A1 (COLIA1) and oestrogen receptor-α (ESR1), have not been well elucidated in RA patients. This review summarizes the currently available literature on the association of VDR polymorphisms with local and systemic bone loss in RA. It also discusses potential targets for genetic research in this area, such as polymorphisms in genes, such as IL-6 (IL6) and TNF receptor type 2 (TNFRSF1B), which control the inflammatory response in RA and may influence bone loss in RA. Defining such genetic factors, in addition to traditional and RA-related risk factors for osteoporosis in RA, may facilitate early identification of patients at high risk for fractures who can then be targeted for treatment.

**KEY WORDS:** Bone loss, Rheumatoid arthritis, Vitamin D receptor, Polymorphism.

### Introduction

RA is defined by its ability to induce bone and cartilage destruction. Erosive RA is associated with pain, disability, deformity and early mortality [1]. In addition, bone destruction in RA is associated with systemic osteoporosis and susceptibility to fragility fractures, because both phenomena fundamentally reflect high inflammatory disease activity [2]. Recent data support that even minimal, subclinical inflammation can lead to bone loss and increased fracture risk [3]. Osteoporosis and related fragility fractures are one of the most common complications in patients with RA and affect quality of life dramatically in these patients. Several factors such as race, BMI, dietary calcium and vitamin D intake, duration and activity of RA, grade of disability, use of corticosteroids and DMARDs and menopausal status have been studied for their influence on bone loss in RA. The role of genetic factors, especially allelic variants in candidate genes for osteoporosis is less well defined in RA-associated osteoporosis. In this regard, the role of vitamin D and its receptor in bone loss in RA is intriguing. In addition to its central role in calcium homeostasis, vitamin D has an immunoregulatory role, and polymorphisms in the vitamin D receptor (VDR) gene appear to influence the susceptibility to bone loss in RA. This article will review the current state of knowledge on the impact of VDR polymorphisms on bone loss in RA. It will explore the utility of screening for these and other polymorphisms in osteoporosis candidate genes and inflammatory cytokine genes as potential genetic markers for the early identification of osteoporosis and fracture risk in RA patients.

### Vitamin D, its receptor, and RA

Vitamin D exists as vitamin D₂ (ergocalciferol) or vitamin D₃ (cholecalciferol). Ergocalciferol is found in irradiated fungi or yeast whereas cholecalciferol is produced in the skin after exposure to sunlight or found in certain fish such as salmon and mackerel. Vitamin D₂ or D₃ after it enters the circulation is bound to the vitamin D binding protein. This complex is transported to the liver where vitamin D undergoes hydroxylation at position 25 to form 25-hydroxyvitamin D (25(OH)D). This is then carried to the kidney where it undergoes hydroxylation by the enzyme 1α-hydroxylase to form 1,25 dihydroxyvitamin D (1,25(OH)₂D), the hormonal form of vitamin D. 1,25(OH)₂D circulates, bound to the vitamin D binding protein, and reaches the target cell. After entering the cell, it binds to VDR in the cytoplasm and this complex then enters the nucleus and heterodimerizes with the retinoic acid X receptor to increase the transcription of vitamin D-dependent genes important in calcium and bone metabolism [4].

The importance of vitamin D as a hormone regulating calcium homeostasis and maintaining skeletal health cannot be overstated. In recent years, an immunomodulatory role for vitamin D and the vitamin D receptor (VDR) has been described. Activated but not resting lymphocytes, and monocytes express VDR [5, 6]. It has been shown that lymphocytes from RA patients express VDR [7]. 1,25(OH)₂D inhibits T-cell proliferation and the release of Th1 cytokines such as IL-2, IFN-γ and TNF-α [8]. In vivo studies demonstrate that vitamin D supplementation prevents the onset and delays progression of CIA in rodents [9]. In addition, VDR has been demonstrated in the rheumatoid synovium and areas [10] of cartilage erosion in human RA. Extrarenal synthesis of vitamin D in vitro in SF macrophages from patients with inflammatory arthritis has been demonstrated suggesting that vitamin D may be a mediator of inflammation [11].

Clinical data also support a role for vitamin D in immune regulation and inflammation. Vitamin D intake was inversely associated with the risk of developing RA in a prospective cohort study [12]. This study of 29 368 women aged 55–69 yrs without a history of RA at baseline measured vitamin D intake and estimated the relative risk (RR) of RA during 11 yrs of follow-up. Higher intake of vitamin D was associated with a lower risk for RA (RR 0.67; 95% CI 0.44, 1.00; P = 0.05). Another study of 206 patients with early inflammatory polyarthritis examined the association between serum levels of 25(OH)D and 1,25(OH)₂D and swollen and tender joint counts, HAQ (a standardized measure of disability), CRP levels (a marker of inflammation) and the disease activity score 28-joint assessment (DAS28) scores. There was an inverse relationship between 25(OH)D levels and the tender joint...
count, DAS 28 and HAQ scores, and between the 1,25(OH)_{2}D level and the HAQ score [13]. Results of this and the previous study support a role for vitamin D in immune regulation and suggest that vitamin D supplementation may prevent the onset and ameliorate the course of inflammatory arthritis.

### VDR gene polymorphisms

The VDR gene is located on chromosome 12 (12q13.11). It has 11 exons and contains four polymorphic regions. Three of these polymorphic regions are located at the 3'-end of the gene and these restriction fragment length polymorphisms (RFLPs) are detected by the restriction enzymes BsmI, Apal (intron 8) and TaqI (exon 9) [14, 15] (Fig. 1). The other polymorphic region is located in the start codon and is detected by restriction enzyme FokI. The B allele (of the BsmI polymorphism) is in tight linkage with the t allele of the TaqI polymorphism. The function of these VDR alleles is not fully understood; they have been associated with autoimmune diseases such as multiple sclerosis [16] and type I diabetes mellitus [17, 18]. Not surprisingly, VDR polymorphisms have also been associated with bone turnover [14] and bone density [19, 20]. The effects of these polymorphisms on VDR function or gene transcription are unclear suggesting that these polymorphisms may occur in linkage disequilibrium with other functional polymorphisms in the VDR gene. A meta-analysis of 75 association studies of these polymorphisms with BMD concluded that these polymorphisms were significantly associated with BMD and that the genotype effect was more pronounced in pre-menopausal than post-menopausal women [21]. Another meta-analysis examined association studies of the BsmI polymorphism with BMD and concluded that the BsmI genotype was associated with lower lumbar spine BMD, but not femoral neck BMD [22]. The FokI polymorphism introduces an alternative translational start site, resulting in a shorter isoform of the VDR gene, but its effects on BMD are unclear [23].

### VDR genotypes and bone loss in RA

The BsmI, Apal, TaqI and FokI polymorphisms have been studied for their effects on bone loss in RA patients and the results of these studies are summarized in the next few sections (Table 1).

In a study of 232 Caucasian patients (160 females, 72 males) with early RA, BMD measurements were obtained at the lumbar spine and hip at baseline and every 12 months for 3 yrs. Tender and swollen joint counts, a joint tenderness score index (Ritchie index), functional status and disability (HAQ) and CRP levels were assessed at baseline, 3, 6, 12 months and then yearly in all patients who were genotyped for the VDR TaqI polymorphism. Seventy healthy controls were also genotyped for the polymorphism. There was no difference in the frequency of the TaqI genotype between the RA patients and controls. RA patients carrying the VDR TT and tt groups were similar with respect to age, gender, height, weight and disease scores, such as the joint counts, Ritchie index, HAQ and CRP. The VDR genotype (TT vs tt) influenced bone loss in females, but not in males. The mean rate of bone loss at the lumbar spine in women with RA with the TT genotype was 4.9% compared with 0.1% in those with the TT genotype (P < 0.05). Similarly at the hip, women with RA with the TT genotype had bone loss of 9.6% while the TT group had 3.9% (P < 0.01). Interestingly, both the HAQ and CRP were significant factors in determining bone loss at the spine and hip in both men and women with RA. Thus, this study showed that women with RA with the VDR TaqI tt genotype had accelerated bone loss during the 3 yrs of study compared with women with the VDR TaqI TT genotype and that inflammation influenced bone loss in RA [24].

In another study, 62 Caucasian RA patients and 40 controls were genotyped for the VDR BsmI, TaqI and FokI polymorphisms. In addition, several markers of bone turnover, such as serum intact OC, PTH, bone-specific alkaline phosphatase (B-ALP), the carboxyterminal extension peptide of type I procollagen, 25[OH]D and urinary deoxypyridinoline (DPD) excretion were measured. Markers of inflammation, such as CRP, ESR and RF were also measured. Bone density was not measured. The bB and tT genotypes (heterozygotes; for BsmI, P = 0.026, for TaqI, P = 0.017) were more frequent in RA patients than in controls. There was a significant association between the FokI polymorphism and family history of RA (P = 0.0012). There was linkage between the BsmI and TaqI polymorphisms, specifically between the b and T, and B and t alleles (P = 0.0001). There was no association between the VDR genotypes and markers of bone turnover. CRP correlated with DPD excretion in RA patients (P < 0.01, r = 0.4). These results suggest that the VDR FokI polymorphism may be a marker for familial RA and that inflammation in RA is associated with increased bone turnover.

### Table 1. Summary of VDR polymorphisms with clinical effects in RA

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Location and postulated function of polymorphism</th>
<th>Clinical effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FokI C/T</td>
<td>Start codon; Introduces an alternative translational start site, resulting in a shorter isoform of the VDR gene</td>
<td>No effect on bone turnover markers</td>
<td>Marker for familial RA</td>
</tr>
<tr>
<td>BsmI A/G</td>
<td>Intron 8 near 3' regulatory region; Function unknown</td>
<td>No effect on bone turnover markers</td>
<td>Lower BMD and higher bone turnover markers</td>
</tr>
<tr>
<td>Apal G/T</td>
<td>Intron 8 near 3' regulatory region; Function unknown</td>
<td>No effect on focal bone erosion</td>
<td>No effect on disease susceptibility in RA</td>
</tr>
<tr>
<td>TaqI T/C</td>
<td>Exon 9 in 3' regulatory region; Function unknown</td>
<td>Accelerated bone loss in women with RA</td>
<td>No effect on bone turnover markers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No effect on focal bone erosion</td>
<td>Early onset RA</td>
</tr>
</tbody>
</table>
The former finding should be considered in light of the small sample size and consequent underpowering of this study [25].

Rass et al. [26] genotyped 64 Caucasian RA patients and 40 healthy controls for the VDR BsmI polymorphisms. Clinical (swollen and tender joint counts, visual analogue scale (VAS) for pain and patient and physician assessment of disease activity) and laboratory (CRP, RF) measures of disease activity were measured. BMD of the lumbar spine was measured. Markers of bone turnover, such as urinary DPD, urinary calcium and serum OC, were measured in RA patients and controls. The frequency of the BsmI bb genotype was higher among RA patients than among controls, but this was not statistically significant. BMD was lower among RA patients with the BB and Bb genotypes (0.84 ± 0.11 g/cm²; 0.82 ± 0.07 g/cm²) compared with those with the bb genotype (0.91 ± 0.17 g/cm²; P < 0.05). Urinary DPD excretion was higher in RA patients carrying the BB and Bb genotypes (9.34 ± 1.53 and 11.74 ± 1.26 nM/mM, respectively) compared with those with the bb genotype (6.81 ± 0.83 nM/mM; P < 0.05). Urinary calcium excretion was higher in patients with the BB genotype (0.61 ± 0.10 mM/Mm) than those with the Bb genotype (0.37 ± 0.06 mM/Mm) or bb (0.38 ± 0.03 mM/Mm; P < 0.05). Serum OC levels were higher in patients with the BB genotype compared with the other two genotypes, but this was not statistically significant. The control population displayed normal BMD and markers of bone turnover. The BsmI genotype was not associated with any markers of disease activity; the b allele was associated with higher titres of the RF. These results suggest that the B allele may be a marker for increased osteoclastic activity in RA patients and thereby increased bone resorption and bone loss [26].

One study examined the association of the VDR TaqI and BsmI alleles on focal bone erosions in RA in contrast to generalized bone loss (as measured by BMD) in the previous studies described. One hundred and fifty-seven Korean patients with RA and two healthy control groups (211 for BsmI and 120 for TaqI) were genotyped for the TaqI and BsmI polymorphisms. Hand radiographs of the patients were scored for erosions using the modified Sharp’s score, a standardized, validated method for scoring erosions in RA. The distribution of the TaqI and BsmI genotypes were similar between RA and control groups, although the tt and BB genotypes were rare. Neither of the VDR genotypes had a significant effect on the focal bone erosion scores. It is noteworthy that this was a relatively underpowered study because of the combination of the small sample size and the low frequency of the TaqI and BsmI alleles in this population [27].

VDR genotypes and susceptibility to RA

Specific HLA DRB1 alleles [also called the shared epitope (SE) alleles] are associated with susceptibility to RA and its severity [28]. The combined influence of the VDR and SE alleles on disease onset in RA was examined. One hundred and twenty-two Spanish RA patients and 200 healthy controls were genotyped for the VDR BsmI, ApaI and TaqI polymorphisms and SE alleles. The distribution of the VDR genotypes were similar in RA patients and controls, and hence these polymorphisms were not associated with susceptibility to RA. As in previous studies, the BsmI B allele was in linkage disequilibrium with the TaqI t allele. RA patients carrying the TaqI tt genotype had an earlier onset of RA compared with the TT and Tt genotype groups combined (38.5 yrs ± 13.36 vs 45.90 ± 13.55; P = 0.04). This association was observed in males and females with RA, although statistically significant only in the female group. When the female group was stratified based on SE status, patients carrying the SE alleles and the tt genotype had the earliest disease onset (28.80 ± 9.88 vs 44.06 ± 13.26 yrs; P = 0.01). These results suggest a possible role for the VDR BsmI and TaqI alleles in disease susceptibility in RA [29]. Interestingly, in a genome-wide association study of susceptibility to seven common diseases that included 2000 patients with RA and 3000 shared controls, the VDR region of chromosome 12 was not associated with RA susceptibility, although other regions on chromosome 12 showed associations [30].

Conclusions

Osteoporosis is a complex disease with a strong genetic component. BMD variation is predominantly determined by genetic factors, with heritability estimates ranging from 50 to 90% after adjusting for covariates, such as age and gender [31]. Polymorphisms in classical candidate genes, such as the vitamin D receptor gene, have been examined in RA for their effects on bone loss. The results of these studies support an association between specific VDR alleles and bone loss in RA. The TaqI t and BsmI B alleles (in tight linkage) were associated with accelerated generalized bone loss in RA [24, 26], but surprisingly not with focal bone loss [27]. In addition, inflammation as measured by CRP was associated with increased bone loss and bone turnover [24, 25]. The FokI and TaqI polymorphisms were linked to familial RA and early onset RA, respectively [25, 29]. These findings taken together support an immunoregulatory role for vitamin D mediated through VDR with effects on disease susceptibility and bone loss in RA. Further, lending credence to this are findings from epidemiological studies in RA which show that vitamin D supplementation may be associated with lower risk for the disease and lower disease activity [12, 13]. These effects of VDR genotypes and vitamin D supplementation are not surprising, given that the central pathological feature in RA is bone and joint destruction. Polymorphisms in genes, such as VDR whose products directly impact calcium and vitamin D metabolism are thus likely to influence bone loss in RA.

Findings from the studies reviewed herein should be interpreted with caution for several reasons. Only four polymorphisms in the VDR gene have been studied extensively so far, although there exist several other functional VDR SNPs as evident in databases, such as the International HapMap Consortium (www.hapmap.org) and dbSNP, which may potentially have effects on bone loss in RA. There has been no attempt yet to test for associations within the gene systematically by incorporating tag SNPs across the gene to investigate associations with bone loss. All the studies performed to date have been underpowered, which may have resulted in spurious genotype–phenotype associations. The results of the smaller studies described above need to be replicated in prospective multi-centre trials with large numbers of patients before any valid conclusions can be drawn about such associations.

Future directions

Genetic risk factors play an important role in the pathogenesis of osteoporosis. This is evident from the fact that a positive family history of hip fracture is a strong risk factor for low BMD and osteoporotic fracture [32, 33]. Although fracture is the most important clinical complication of osteoporosis, most genetic studies on osteoporosis have focused on BMD as this is a highly heritable trait [34, 35] and a strong clinical predictor of osteoporotic fracture risk [36].

Other polymorphisms in VDR, such as the Cdx 2 promoter polymorphism, a G to A polymorphism in a binding site for an inflammatory transcription factor called Cdx 2, may influence bone loss and deserve to be explored in RA patients [37, 38]. Polymorphisms in classical candidate genes other than the VDR gene, such as type I collagen A1 (COL1A1) [39–43] and oestrogen receptor a (ESR1) [44, 47] genes have been linked to susceptibility to osteoporosis; however, these have not been studied in patients with RA. The increased risk of bone loss due to the inflammatory state in RA supported by some of the studies reviewed here [24, 25] may be further exacerbated by a genetic susceptibility conferred by these polymorphisms. However, polymorphisms in
inflammatory response genes, such as TNF receptor type 2 (TNFRSF1B) [48, 49] and IL-6 (IL6) [50–53], which have been associated with susceptibility to osteoporosis may be more relevant in RA where inflammation is the driving force for bone loss. A genome-wide association study with large sample sizes may be the best way to comprehensively search for such genes that may influence bone loss in RA. Characterization of such genetic risk factors, in addition to RA-related and traditional risk factors, may facilitate identification of RA patients with early bone loss prior to clinically detectable osteoporosis who can then be treated preventively to avoid fractures. Future research in this area, with genome-wide association studies to identify the effects of gene regions, such as IL6 and TNFRSF1B and variations in these regions on bone loss, may also clarify the role of inflammation in bone loss and potentially yield new therapeutic targets for bone loss in inflammatory diseases such as RA.

Rheumatology key messages

- Polymorphic alleles in candidate genes for osteoporosis, such as the VDR TaqI t and BsmI B, are associated with accelerated generalized bone loss in RA.
- Polymorphisms in VDR and inflammatory response genes (IL6, TNFRSF1B) may serve as genetic markers of systemic bone loss in RA.

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