Vitamin D receptor gene (VDR) polymorphisms: effect on bone mass, bone loss and parathyroid hormone regulation

Carlos Gómez Alonso, Manuel L. Naves Díaz, Carmen Díaz-Corte, Jose L. Fernández Martín and Jorge B. Cannata Andía

Bone and Mineral Research Unit, Instituto Reina Sofía de Investigación, Hospital Central de Asturias, Oviedo, Spain

Key words: alleles; bone mass; calcitriol; polymorphisms; receptors; vitamin D receptor gene

Introduction

On the basis of studies performed in families and twins, genetic influence can account for up to 80% of the variance in bone mineral density [1,2]. The final bone mass is a result of all the mechanisms involved in bone metabolism. In consequence, environmental factors would have less effect on bone, and this would depend on genetic conditioning. Taking into account the effect of a great number of systemic and local hormones on the regulation of bone metabolism, it is highly possible to have a polygenic influence. In many studies, the influence of the genes encoding the vitamin D receptor (VDR) [3], the oestrogen receptor [4], the synthesis of transforming growth factor (TGF)-β [5] or the synthesis of collagen type Iα-1 [6] has been shown.

The VDR gene has been most widely studied among all these. The publication of the study of Morrison et al. [3] aroused great interest in the subject. Using the BsmI restriction enzyme in a wide population of adult twins, they found variations of ± 1 SD for the mean values of bone mass among the three possible genotypes. In their population-based study, they also found that the most favourable genotype passes the fracture threshold (−2 SD from the peak bone mass) 10 years later than the less favourable genotype.

Although the molecular mechanism of action is not entirely clear, we know that the effect of vitamin D on the tissues is influenced by the type of metabolite as well as by its levels, by the receptors’ density (up-regulated by substrate levels) and probably also by qualitative aspects of the receptor. The receptor’s consistency and quality could both also be modified by the different polymorphisms of the VDR gene.

Genomic typing of VDR alleles

The VDR gene, located on chromosome 12, is made up of 5.6 kb (Figure 1). Most of the gene comprises non-coding regions where frequent modifications and/or deletions of the base sequence are found and, although this does not modify the resulting amino acids of the receptor structure, we know it can change the transcription of the coding region or the stability of the mRNA.

The changes in the sequence of bases in the coding region are less frequent, and they are usually located in intronic portions (‘mute sequences’). On the other hand, the substitution of bases in the exon fragment does not always alter the resulting amino acid because we can obtain a synonym codon for the same amino acid. There is only one modification described, in the second exon, which modifies the initial codon (ATG) and whose presence adds three extra amino acids to the vitamin D receptor structure. Although there are four sites at which base substitutions have been described, there is only one which can impose structural changes on the amino acid sequence. Those mutations are related to changes in the non-coding region (known as the ‘linkage imbalance’ phenomenon) and can be used as their indirect markers.

The resulting new base sequences are recognized by restriction enzymes which split the DNA at the site where the mutation is located. If the restriction site is present, we obtain a fragment of DNA containing the new sequence and type the allele using the lower case initial letter of the restriction enzyme used. When fragmentation does not occur, the allele is typed using the upper case initial letter of the enzyme. So, taking into account the chromosomal duplicity, we have two alleles and three different genotypes for each variation. Thus, a large number of polymorphisms can be obtained if we combine different restriction enzymes (Table 1).

In practice, the alleles are typed by using primers...
Fig. 1. Scheme of the VDR morphology. Restriction sites with the electrophoretic bands of the different alleles are shown.

Table 1. Alleles and genotypes corresponding to each of the restriction enzymes used. Although there is a great association among *BsmI*, *TaqI*, and *ApaI* enzymes (69–90%) we can find genotypes combining all of them (BBttAAFF, BbTTAaFf, etc.)

<table>
<thead>
<tr>
<th>Restriction enzyme</th>
<th>Alleles</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BsmI</td>
<td>B</td>
<td>BB</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>Bb, bb</td>
</tr>
<tr>
<td>TaqI</td>
<td>T</td>
<td>TT, Tt</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>Tt</td>
</tr>
<tr>
<td>ApaI</td>
<td>A</td>
<td>AA, aA</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>aA, aa</td>
</tr>
<tr>
<td>FokI</td>
<td>F</td>
<td>FF, Ff</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>Ff</td>
</tr>
</tbody>
</table>

and polymerase chain reaction (PCR) cycles that amplify the DNA fragment with the restriction site recognized by the enzyme. The amplification product is incubated with the restriction enzyme and the digestion products are resolved by electrophoresis, giving rise to different fragments according to their size. For example, if we use the *BsmI* enzyme, we obtain a single band of 188 bp in subjects in which both genes have no changes and are not attacked by the enzyme (genotype BB). On the other hand, if one of the genes has its sequence altered, this will give us two bands of 77 and 111 bp and another band of 188 bp from the normal gene (genotype Bb). Finally, if both genes are altered, we obtain only two bands of 77 and 111 bp (genotype bb).

Once we have typed the different VDR alleles, we can study their influence on bone metabolism. So, we can consider the effects on hormone regulation [7,8], biochemical markers of bone turnover [7,9–11], bone mass and bone mineral density changes with time [3,10–16], effects on the incidence and prevalence of fractures and also effects on the different therapeutic responses [17–18].

VDR polymorphisms and bone density

The influence of VDR polymorphisms on bone mineral density (BMD) and its changes has been widely studied, and has led to divergent results. The differences in BMD among the three genotypes obtained initially with the *BsmI* enzyme would condition a genotype associated with high values of BMD (bb) and a genotype with low values of BMD and faster velocity of bone loss (BB) [3]. Later studies, using the same restriction enzyme, have led to concordant but modest results [12], discordant results [10,11] and also opposite results, due to interaction with the age of the subjects in the study, i.e. positive in pre-menopausal patients and negative in elderly patients [13].

Preliminary studies in our own population were also non-homogeneous [19]. We studied a population-based random sample of 326 people of both sexes (50% of each sex) and older than 54 years old (67 ± 8) (EPOS study). We measured BMD in the lumbar spine and in the femoral neck on two occasions with a 4-year interval using a DXA densitometer. We also performed radiological studies of the thoracic and lumbar spine. These results were analysed with the VDR genotype, using the *BsmI* restriction enzyme.

We did not find differences in the basal BMD, in the rate of BMD changes or in the prevalence of spinal fractures in the male population. On the other hand, in women, we obtained higher values in the lumbar BMD in those patients with genotype bb compared with genotypes Bb and BB ($P<0.05$); similar but non-significant results were observed in the femoral neck BMD ($P=0.07$) (Figure 2). Significant differences were only found in the evolution of BMD in the femoral neck, where women with genotype bb showed a positive
VDR gene polymorphisms and bone metabolism

Fig. 2. Bone mineral density values and the percentage of annual changes in lumbar spine and femoral neck of women depending on the presence or absence of the B allele in the genotype. Mean values ± standard error are shown.

Figures: Diagrams showing bone mineral density (BMD) values and percentage of annual changes in lumbar spine and femoral neck. * p<0.05, # p=0.07.

Table 2. Factors affecting the heterogeneity of the results on the influence of VDR gene polymorphisms in variables related to bone metabolism

<table>
<thead>
<tr>
<th>Factor</th>
<th>influence of VDR gene polymorphisms in variables related to bone metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of the sample</td>
<td></td>
</tr>
<tr>
<td>Population bias</td>
<td></td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td></td>
</tr>
<tr>
<td>Exclusion criteria</td>
<td></td>
</tr>
<tr>
<td>Randomization</td>
<td></td>
</tr>
<tr>
<td>Precision of the measurement of the control variable</td>
<td></td>
</tr>
<tr>
<td>Binding imbalance</td>
<td></td>
</tr>
<tr>
<td>Allelic heterogeneity</td>
<td></td>
</tr>
<tr>
<td>Pathogenic interferences</td>
<td></td>
</tr>
<tr>
<td>Variability of environmental factors</td>
<td></td>
</tr>
</tbody>
</table>

VDR polymorphisms and PTH regulation

The possible influence of the different VDR polymorphisms on the regulation of the calcium–parathyroid hormone (PTH)–vitamin D axis is particularly important in patients with severe chronic renal failure, where other factors related to renal osteodystrophy make this interaction more complex.

In population-based samples, differences in the basal values of PTH with respect to the VDR polymorphisms have not been described [7,14,19]. However, in the study of Howard et al. [7], basal calcitriol values were...
that the bb genotype is more sensitive to the vitamin. The BMD in women with the Bb or BB genotype may be
observed that PTH was only influenced by 25-OH vitamin D (0.11–0.82; P < 0.05), whereas in the Bb and BB genotype groups, PTH was significantly and independently predicted by 25-OH vitamin D (b = −0.77), age (b = 0.34) and serum creatinine (b = 22.7) (r = −0.40; P < 0.05). These results suggest that the bb genotype is more sensitive to the vitamin D substrate, and this fact may explain the influence of other factors, such as age and the renal function, in the regulation of PTH secretion.

However, there are also controversial results in this area. In the study performed by Fernández et al. [8] on patients with chronic renal failure undergoing chronic haemodialysis, the results were different. Patients with the bb genotype had a lower risk of developing hypoparathyroidism (odds ratio = 0.3; 0.11–0.82; P < 0.01) than the general population on dialysis. Conversely, the BB genotype was overrepresented in the low PTH group (32.3%) compared with patients with the bb genotype (12.5%; P < 0.05). These results suggest that the BB allele appears to be more sensitive to changes able to influence the down-regulation of PTH. These results, contrary to those in the population with normal renal function, agree with the findings obtained in patients with primary hyperparathyroidism where bb alleles and bb genotypes were more frequent [21].

Practical implications of knowledge of VDR polymorphisms

Knowledge of VDR alleles will give important information related to the epidemiological distribution of the different genotypes, which could partly explain the known differences in bone mass among different populations. As an example, the prevalence of the BB genotype (associated with a low bone mass) is higher in the Caucasian population (17.2%) compared with black (4.9%) or Asiatic populations (2.3%) [22]. This knowledge will also provide the possibility of choosing patients who may respond better to calcium or vitamin D treatment, such as the group of patients who have the combination of the more favourable alleles [17,18].

The selection of subjects with special risk of bone loss who could benefit from prophylactic interventions is another important area of interest. A good example would be patients who have undergone renal transplantation who have limitations on their quality of life due to different bone problems including bone fractures. The findings of Torres et al. have special importance in this respect. In a population of 34 non-diabetic patients who had undergone renal transplantation, they measured bone density using quantitative computerized tomography (QCT), and showed that bb genotype patients had a moderate loss of trabecular bone (basal Z-score 0.25 ± 1.39; final Z-score −0.37 ± 1.16; P < 0.05), whereas Bb and BB genotype patients had a more significant trabecular bone loss (basal Z-score 0.26 ± 2.04; final Z-score −1.10 ± 1.20; P < 0.05). The differences between the groups were significant after the first year of transplantation, but they had not been evident immediately following transplantation [16].

Knowledge of VDR polymorphisms could also condition the setting up of other prophylactic interventions such as long-term corticosteroid therapy. It seems that the BMD in women with the Bb or BB genotype may be affected more by corticosteroids than in women with the bb genotype (Z-score −1.39 for Bb and −2.19 for BB genotypes with respect to a Z-score of −0.84 for bb; P < 0.05) [23].

To summarize, there is a great deal of evidence showing the influence of the different VDR polymorphisms in the regulation of bone metabolism. The polygenic nature of this phenomenon and the great variety of other factors involved [24,25,26] represent a challenge for future research in this field.

Acknowledgements. We thank Dr A. Torres and the technicians of his lab (from the Hospital Universitario of Canary Islands) for the measurement of VDR polymorphisms in the EPOS study and Dr Coto (Biochemical Department of the Hospital Central de Asturias) for the measurement of calcium-related hormones in our studies. The EPOS study was supported by a grant from FIS 94/1901-E and BIOMED 93-94 BMH-CT092-0182.

References

5. Eriksen EF, Langdahl B. Genetics of osteoporosis. Bone 1997; 20 [Suppl]: 1s


19. Gómez C, Rodríguez-Rebollar A, Naves ML et al. Effect of the different alleles of vitamin D in bone mass and in other biochemical parameters in people older than 54 years with normal renal function. Bone 1997; 20 [Suppl]: 27s


