Vitamin D receptor polymorphisms as a determinant of bone mass and PTH secretion: from facts to controversies

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Introduction

During recent years, powerful molecular biology techniques such as restriction enzymes and positional cloning have been used to identify genetic diseases even before the responsible genes were characterized. However, clinical studies have shown that bone mineral density (BMD) is under genetic control, probably polygenic in origin, and several candidate genes, namely oestrogen and vitamin D receptors (VDR), as well as collagen type I z 1 among others, may mediate important differences in bone mass and bone metabolism. Since the first description by Morrison et al. [1], several groups have shown that genetic polymorphisms at the 3'-untranslated region of the VDR gene may account for at least some of the genetic variation in bone mass. These polymorphisms are defined by the presence or absence of a restriction site for the enzymes BsmI, ApaI and TaqI. Since that initial report, VDR gene polymorphisms have been associated with BMD, peak bone density, bone turnover and the serum levels of some biochemical bone markers, as well as the rate of bone loss, risk of osteoporotic fracture and the relative response to several treatments of osteoporosis such as vitamin D or calcium.

VDR polymorphisms in patients without renal failure

According to the most widely analysed BsmI restriction site—B absence, b presence of a cleavage site—several studies have documented that in patients without chronic renal failure (CRF), the presence of two copies of the allele b (genotype bb) is associated with a greater BMD than the heterozygous genotype Bb, whereas the genotype BB is associated with the lowest BMD [1]. Generally speaking, the BBAATT genotype has usually been related to a lower bone mass. However, there is not general agreement and several reports have failed to confirm such a relationship. A recent meta-analysis provided evidence for an effect of the VDR polymorphisms on BMD, but it was quantitatively modest [2]. It has also been shown that environmental factors may influence the effect of genetically determined BMD. Thus, VDR genetic polymorphisms have been linked to differences in intestinal fractional calcium absorption. As such, individuals with the bbbaTT haplotype showed a higher rate of radiocalcium absorption [3]. Conversely, individuals with the BB genotype had a lower efficiency of calcium absorption after dietary calcium restriction and had a lower BMD than those with the bb genotype [4]. This finding would be consistent with the presence of functional differences in the intestinal VDR among different VDR genotypes. However, the mean increase of BMD after treatment with vitamin D was significantly higher in individuals with the BB and Bb genotypes compared with the bb genotype [5]. A more pronounced suppression of PTH concentration by calcitriol has also been described in individuals with the bb genotype. Furthermore, VDR polymorphisms have been associated with urinary calcium excretion, but in this specific study they were not related to BMD [6]. Consequently, VDR polymorphisms seem to represent one of the genetic factors affecting BMD, but they account only partially for the overall genetic effect on bone mass and this effect is not observed in all the screened populations.

VDR polymorphisms in primary and secondary hyperparathyroidism

The VDR genetic polymorphisms have also been linked to the development of primary and secondary hyperparathyroidism.
rathyroid disorders. Whereas the \textit{BBAA}tt genotype has usually been related to lower BMD in non-renal populations, an increased prevalence of the polymorphic VDR alleles \textit{a}, \textit{b}, and \textit{T} has been demonstrated in sporadic primary hyperparathyroidism (HPT). The VDR haplotype \textit{ba}T seems to be a risk factor for parathyroid adenomas, possibly by interfering with the inhibitory action of calcitriol [7,8]. Thus, in patients who were homozygous the \textit{ba}T allele parathyroid tumours exhibited lower VDR mRNA and higher parathyroid hormone (PTH) mRNA levels than those harbouring the \textit{BB}, \textit{AA} or \textit{tt} genotypes [7]. In contrast, the \textit{BA}t haplotype has recently been shown to be under-represented in primary HPT but is related to larger parathyroid lesions, as well as a less deranged calcium sensor protein expression and parathyroid cell function. In these patients, primary HPT may be associated with genetic determinants, which may act mainly by altering the regulation of cell proliferation, rather than the calcium-sensing mechanism of the parathyroid cells [8].

However, data correlating VDR polymorphisms with secondary HPT and renal bone disease are sparse. Higher PTH levels in individuals with the \textit{bb} genotype and lower PTH levels in the \textit{BB} genotype have been reported in patients undergoing dialysis [9,10]. In a large haemodialysis population, Tsukamoto et al. [9] found that the \textit{bb} genotype correlated with higher PTH levels than did the \textit{BB} genotype. This finding has been confirmed by others. In addition, Fernandez et al. [10] have independently described the presence of a higher frequency of the \textit{BB} genotype and the \textit{B} allele in their low PTH group. Both PTH and osteocalcin levels have also been reported to be higher in the \textit{aa} and \textit{bb} genotype, and preliminary data showed that the \textit{aa} genotype may be linked to an acute higher PTH increase when serum calcium was lowered during dialysis. However, many other groups have been unable to relate the VDR genotype with the severity of secondary HPT. VDR mRNA levels or the pattern of renal osteodystrophy. All these inconsistencies, and the poor reproducibility of results among different populations (either with or without CRF), may be caused by sampling bias, ethnicity (the prevalence of the suspected high-risk genotypes is very low in some populations and this factor would limit the statistical power of analysis), confounding environmental and dietary influences, age, obesity, physical activity, sex, menopausal status or other yet unidentified factors.

\textbf{VDR polymorphisms in renal transplantation}

The genetic expression of VDR alleles has also been studied in renal transplant patients to analyse whether these alleles may predict post-transplant loss of bone mass [11]. In this context, the \textit{bb} genotype was linked to a better rate of bone recovery between 3 and 12 months after grafting, independent of the prevailing PTH levels [11]. Thus, patients with the \textit{bb} genotype are, to some extent, protected against the common bone loss occurring after renal transplantation, since those exhibiting the \textit{B} allele had lower BMD from the third month after grafting. These results are in agreement with those initially presented by Morrison et al. [1] in osteoporotic populations, as well as some preliminary data described in orthotopic hepatic transplantation (Guardiola et al., unpublished data). Therefore, it seems likely that the effect of the VDR genotype on BMD may become more evident under challenging conditions (such as calcium restriction or following corticosteroid treatment). Nevertheless, in CRF patients, there are so many interrelated confounding variables, affecting both bone and parathyroid gland function, that the relative effect of a specific genetic background may be easily masked by other environmental or physiopathological factors with a stronger direct influence on those tissues. As a result, it seems clear that VDR polymorphisms are not one of the main determinants of BMD in patients undergoing dialysis, although it may affect bone mass in some subgroups of patients or in certain populations.

\textbf{Physiological consequences of VDR polymorphisms}

It is worth mentioning that the previously stated restriction enzymes act in an untranslated region of the DNA, and so none of the restrictive polymorphisms change the translated protein. Consequently, it is difficult to establish a link between the presence of the different alleles and differences in VDR expression or functionality. It was previously thought that the \textit{b} allele was linked to a decreased transcriptional activity or VDR mRNA stability, and that such reduction of VDR expression in the parathyroids of \textit{bb} patients could lead to decreased vitamin D action (calcitriol resistance) and contribute to parathyroid cell proliferation. On the contrary, it was possible that the \textit{B} allele could be associated with an increased VDR mRNA expression or stability. Although the \textit{ba}T alleles have been shown to be linked to lower VDR and higher PTH mRNA levels in primary HPT [7], VDR polymorphisms do not seem to affect the abundance of the VDR mRNA in other studies and recent data do not confirm allele-specific differences in mRNA [12,13]. As a consequence, the mechanistic association between VDR polymorphisms and their phenotypic consequences is not yet clear. A recently described polymorphism at the first of the two potential translation initiation sites (ATG) in the promoter region of the VDR gene (defined as starting codon polymorphism by the FokI restriction enzyme) may provide more helpful information [14]. The T/C polymorphism defines two distinct VDR protein lengths with apparently distinct affinity for its ligand and therefore different biological activity. However, only preliminary and inconsistent information is currently available on FokI polymorphisms in patients with CRF. Moreover, inheritance of bone mass is probably under polygenic control and a linkage disequilibrium effect between the
VDR gene and any other disease-causing gene loci nearby seems likely. The fact that to date no differences in the quantity, properties or cellular responsiveness to calcitriol have been found, which significantly correlate with VDR genotypes argues in favour of such a hypothesis.

**Conclusion**

In summary, the relevance of VDR polymorphisms are still a matter of debate since correlations are poorly reproducible. Classic VDR polymorphisms seem to have a modest impact on BMD, but their role in determining calcitriol resistance and PTH levels in patients with CRF is inconsistent. In any case, in this context known VDR polymorphisms do not seem to be the main determinants of BMD, although they might have an effect in some subpopulations. A better characterization of encoding DNA polymorphisms and the regulatory regions of the gene, as well as their intrinsic relationship with new polymorphisms, may help to resolve these controversies. Currently no clear-cut genetic parameter is available that could allow us to manage patients with osteopenia or renal osteodystrophy.

**References**

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**How to identify the haemodialysis access at risk of thrombosis? Are flow measurements the answer?**

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**Introduction**

It is clear to most clinicians and even recognized by the NKF-DQOI task force that the primary choice of vascular access is an arteriovenous fistula (AVF), typically connecting the radial artery with the cephalic vein system [1]. However, many patients depend on an implanted graft for their access to the blood stream. Usually the graft is manufactured from polytetrafluoroethylene (PTFE). In some populations in the United States PTFE dialysis grafts account for as many as 83% of access placements [1]. The European Dialysis and Transplantation Association (EDTA) does not collect data on this issue. A survey on 1 January 1997, which included the majority of the approximately 3000 haemodialysis patients of The Netherlands found that 30% of this population was dialysed using a graft, mostly PTFE grafts.