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Vitamin D Receptor Alleles and Bone Physiology

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Abstract The vitamin D endocrine system is central to the control of bone and calcium homeostasis. The active hormonal form of vitamin D, 1,25 dihydroxyvitamin D (calcitriol), the circulating level of which is tightly regulated, acts through a specific receptor to mediate its genomic actions on almost every aspect of calcium homeostasis. Because of its transactivation function, it is possible that a small difference in vitamin D receptor level could be amplified into a biologically significant alteration in physiological setpoint. The recent finding that polymorphisms in the vitamin D receptor gene are predictive of bone density (Morrison et al., Nature 367:284–287, 1994) is the first example of an allelic effect in such a homeostatically controlled system. This raises the possibility that such central operators may exist in other regulatory pathways, and could explain a large part of the observed "normal" population distribution that exists for all physiological parameters. © 1994 Wiley-Liss, Inc.

Key words: vitamin D, calcitriol, bone, genetics, steroid hormone receptor, vitamin D receptor, retinoic acid receptor, calcium, homeostasis, calcitonin, parathyroid hormone

The vitamin D endocrine system is central to the control of bone and calcium homeostasis. The active hormonal form of vitamin D is 1,25 dihydroxyvitamin D (calcitriol), the circulating level of which is tightly regulated and acts through a specific receptor to mediate its genomic actions on almost every aspect of calcium homeostasis (Fig. 1). The aim of this perspective is to consider how minor modifications of that transactivation pathway could be translated into biologically significant alterations in physiological set points. While this model will focus on newly identified polymorphisms in the vitamin D receptor gene and its effect on bone biology, it is possible that such a model may operate in all regulatory pathways and could explain a large part of the observed "normal" population distribution that exists for all physiological parameters.

The complexity of physiological endocrine feedback loops implies that regulated parameters, e.g., serum calcium, are counter-regulated to a specific optimal value for biological survival and well-being. While there is a *normal range* for any physiological parameter, no single *normal value* exists, yet each individual tends to maintain *a particular value* over extended periods of time. This is seen clearly in such variables as weight, blood pressure, or serum sodium and in the bone and calcium area, serum calcium, and bone density. While acute counter-regulatory fluctuations around a physiological setpoint for each individual are easily seen for parameters such as serum calcium, they may not be so obvious in a parameter such as bone density or bone mass. This difficulty led to the common misconception that bone is static over time. To the contrary, bone is in a permanent state of flux, albeit with long time constants, and is always being remodeled, that is removed (resorption) and replaced (formation).

The coupling of these two opposing processes is such that bone mass remains constant in the face of many physiological threats. Part of this coupling depends upon close communication between the osteoblast, bone-forming cell, and the osteoclast, bone-resorbing cell. Three potent hormones, parathyroid hormone (PTH), calcitonin, and calcitriol, are powerful endocrine regulators of both bone formation/mineralization and bone resorption. Endocrine, autocrine, and paracrine regulators of bone and calcium homeostasis act on both cell types [1–4] modulating function upon a baseline set by these endocrine calciotropic hormones.

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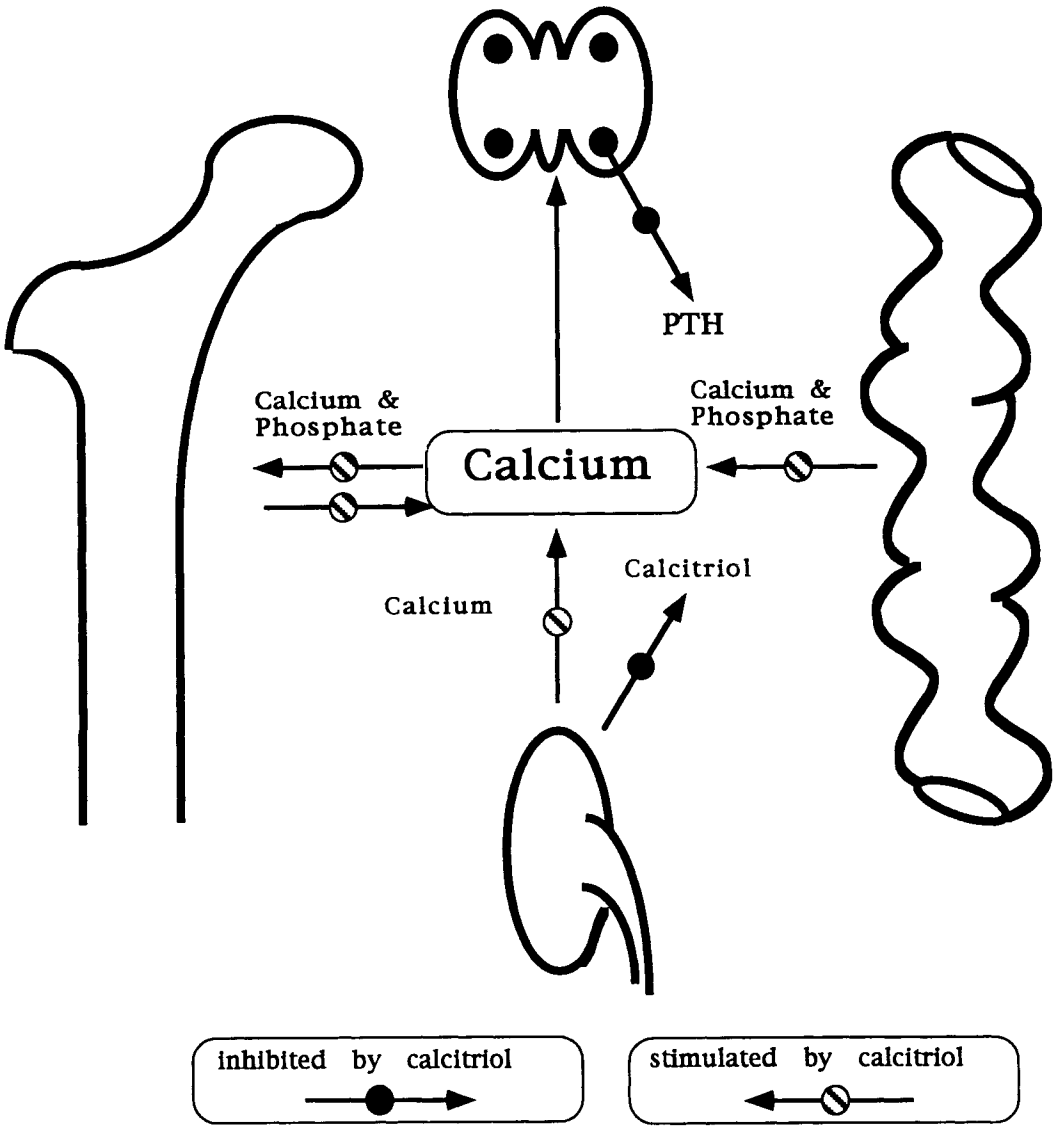

Fig. 1. Central role of calcitriol in the maintenance of calcium homeostasis.

Calcitonin is a potentially powerful acute regulator of bone resorption acting directly on the osteoclast. In pathophysiological states of calcitonin deficiency or marked excess, however, there are no sustained alterations in bone and calcium homeostasis. This may relate to the well recognized tachyphylaxis to calcitonin action and does not support a major role for calcitonin in chronic bone and calcium homeostasis.

PTH actions in bone appear to be mediated through the osteoblast cell lineage. Indeed many hormonal regulators of bone and calcium homeostasis from outside bone act exclusively on cells of the osteoblast lineage even when their end effect is to modulate osteoclast activity [5]. At this time, the exact mechanism of this linkage and the biochemical messengers employed are unknown.

Calcitriol acts on cells of both osteoblast and osteoclast lineages. It plays a critical role in regulating osteoclast numbers by contributing to the control of the differentiation of cells of the monocyte/macrophage lineage into osteoclast precursors. Interestingly, it subsequently regulates osteoclast activity only through its actions on osteoblastic cells.

While bone is structurally vital with the capacity to exert its own selective pressure, from a survival viewpoint maintenance of serum calcium could be expected to exert a greater selective effect. Therefore, in conjunction with its potent biological effects on bone, it is important

to consider the various target organs of calcium homeostasis affected by calcitriol (see Fig. 1). The intestine is the only portal for calcium entry, and calcitriol is the only known endocrine regulator of active calcium absorption. Dietary factors such as total dietary calcium intake and other dietary constituents will modify absorption, however, calcitriol is the only potent physiological regulator of dietary calcium utilization. Similarly, apart from some losses through the skin as sweat and desquamated cells and normal intestinal cellular sloughing, which are not subject to counter-regulation, the kidney is the only physiological site for calcium excretion. The renal excretion of calcium is tonically regulated by calcitriol and acutely by PTH. Serum calcium levels rise in response to both hormones, while simultaneously inhibiting their release in a classical endocrine feedback loop. These two hormones are also tightly counter-regulated, with PTH being the most powerful stimulator of renal calcitriol synthesis while calcitriol downregulates PTH synthesis and secretion. Importantly, calcitriol has a further potent regulatory loop in that it inhibits its own production in the kidney.

The importance of the interaction between calcitriol and the vitamin D receptor has been clarified through molecular studies of inherited receptor defects [6] which lead to profound abnormalities in bone and calcium metabolism. However, while these gross defects manifest as distinct clinical aberrations, the general population exhibits a physiological balance between bone formation and resorption that presents a normal range of bone densities varying by as much as 20% [7]. In the lower part of that range the risk of sustaining an osteoporotic fracture of the hip and spine is increased. Some studies support the concept that the vitamin D endocrine system is integrally involved in the establishment of osteoporosis, citing lower serum calcitriol levels [8], lower intestinal calcium absorption, and impaired calcitriol response to infused PTH [9]. Others contest a central role for calcitriol in osteoporosis, demonstrating no relationship between calcitriol and early postmenopausal bone loss [10]. The relative risk of developing osteoporotic fractures has been considered dependent on environmental factors and a polygenic pattern of inheritance, analogous to other population-based studies of hypertension or hyperlipidaemia and relative risk of cardiovascular events. Recent studies have highlighted the possibility that allelic differences in the vitamin D receptor gene may account for the inherited variability in parameters of bone turnover and bone density [11], both within a given population and potentially between different ethnic groups. These findings provide a model by which a single gene with pleiotropic actions could exert wide-ranging effects in target tissue.

GENETIC EFFECTS GOVERNING BONE DENSITY AND BONE TURNOVER

Evidence that there was a strong genetic influence on bone density first became apparent in twin studies [12–15] and was confirmed by studies of mother–daughter pairs [16]. Using the twin model, Kelly et al. [17] observed that serum osteocalcin, a useful marker of osteoblast function and bone formation [18], was under strong genetic influence. Genetic factors explained up to 80% of the variance in osteocalcin levels. Perhaps most importantly the difference in osteocalcin levels between monozygotic (MZ) and dizygotic (DZ) twin pairs predicted the difference in bone density within the twin pairs, indicating that genetic factors controlling bone turnover determined bone density. The finding that serum levels of type 1 procollagen C-terminal propeptide (a bone formation marker) and to a lesser extent type 1 collagen telopeptide (a bone resorption marker) are also under genetic control supports the concept of a stronger genetic influence on osteoblastic bone formation than bone resorption [19]. The mechanism of this genetic effect has been uncertain. Recently, however, restriction fragment length polymorphisms (RFLP) of the human vitamin D receptor gene for the endonucleases *Bsm*1 and *Apa*1 have been identified that predict circulating osteocalcin levels independent of age and menopausal state [11]. As the genes for osteocalcin and the vitamin D receptor are located on chromosome 1 and 12, respectively, this association is not due to a linkage between the two genes. Furthermore, the vitamin D receptor with its ligand, calcitriol, is a potent regulator in trans of the osteocalcin gene. These data indicate that the RFLP markers of the human vitamin D receptor gene identify functionally different alleles of a potent transactivating factor with the potential for even small differences in receptor number or function to be amplified into a widely distributed physiological effect that could determine the normal physiological range of bone mineral density. The twin model confirmed this strong genetic link between vitamin D receptor gene

RFLP and bone density, with 75% of the difference in lumbar spine bone density between MZ and DZ twin pairs explained by discordance for the *Bsm*1 RFLP. Those DZ twins discordant for their vitamin D receptor gene alleles exhibited a threefold greater difference in lumbar spine bone mineral density than those DZ twins with shared alleles [20]. In keeping with the previously demonstrated codominant effect of the *Bsm*1 allele on serum osteocalcin [11], a linear relationship was observed between the degree of difference in genotype and the difference in bone density within twin pairs.

POTENTIAL FOR ALTERED VITAMIN D RECEPTOR GENE EXPRESSION TO EXPLAIN DIFFERENCES IN BONE DENSITY

Given the central role of vitamin D in the control of bone and calcium homeostasis, natural allelic variations in the vitamin D receptor gene have the potential to determine a large part of the normal physiological diversity in bone cell biology, though the exact molecular mechanism remains unclear. It is possible, however, that the vitamin D receptor gene RFLPs are markers for linked genes that exert a dominant effect on bone turnover and bone density. Candidate genes on chromosome 12 include the retinoic acid receptor γ gene [21] and a gene linked to the Type 1 vitamin D–resistant rickets phenotype [22]. The retinoic acid receptor γ gene is expressed primarily in skin and the developing bone [23], making it an attractive candidate for linkage with the vitamin D endocrine system. The need, however, for supraphysiological doses of retinoic acid to exert even a modest biological effect on osteocalcin gene transcription compared to that seen with physiological doses of calcitriol challenges the hypothesis that retinoic acid receptor γ gene linkage is responsible for a physiological effect of the magnitude reported for the vitamin D receptor alleles.

The most plausible hypothesis is that the polymorphic sites define, or are in linkage with, a region of the vitamin D receptor gene that is critical for receptor number, ligand affinity, or binding of the receptor to its DNA response element (VDRE). Small changes in receptor number or function could amplify any transactivating events on target genes in sites where the constitutive levels of receptor gene expression are low. In comparison to other target tissues central to the regulation of calcium homeostasis, such as the intestine and kidney, the levels of vitamin D receptor in the osteoblast are relatively low [24]. Thus small increases in receptor levels at this site have the potential to exert a significant biological response. Some authors view the principal role of calcitriol in bone cell biology as coordinating bone resorption [25]. The presence of a vitamin D receptor in the intestine that is more responsive to calcitriol in stimulating calcium absorption would provide a powerful selective force in geographical areas of calcium restriction or low ultraviolet radiation. Conservation of the genotype throughout the population would be expected at the expense of accelerated bone turnover and lower bone mineral density. Furthermore, in studies examining the role of calcitriol in the pathogenesis and treatment of osteoporosis, ethnic differences [26] in response to active forms of vitamin D may be due to differences in the allelic forms of the vitamin D receptor gene within the study populations.

MECHANISMS WHEREBY VITAMIN D RECEPTOR ALLELES MAY EXERT THEIR EFFECT ON BONE TURNOVER AND DENSITY

The potent transactivation of the vitamin D receptor on osteocalcin gene transcription in normal diploid osteoblasts is cell and stage specific, the protein only being expressed during the late postproliferative phase of the evolving osteoblast phenotype [27]. Furthermore, calcitriol enhancement of osteocalcin synthesis in vitro is reciprocally related to the basal level of osteocalcin gene transcription [27]. This suggests that a number of factors, some identified as necessary for the in vitro binding of the vitamin D receptor to the osteocalcin VDRE [28,29], interact with proximal basal elements [30] to coordinate developmental and calcitriol-enhanced transactivation of the osteocalcin gene. At a molecular level it has been shown that calcitriol increases osteocalcin gene transcription via complex DNA–protein interactions between the intracellular vitamin D receptor and well characterized consensus sequences in the promoters of the human and rat osteocalcin gene [31–34]. While classical steroid hormone receptor–DNA interactions involved homodimers binding their palindromic response elements [35,36], the subfamily of vitamin D, retinoic acid, and thyroid hormone nuclear receptors has demonstrated greater diversity, with potent transactivation of target genes in bone via heterodimeric [28,29] and homodimeric [37] complexes of subfamily

members, with the phosphorylation and ligand-bound state of the vitamin D receptor complex important for DNA binding and transcriptional activation [38–40]. The defined vitamin D response elements predominantly consist of direct repeats of hexameric half-sites with significant differences between the human and rodent consensus sequences. The hexameric sequence and nucleotide spacing between the half-sites may contribute to the degree of specificity for vitamin D and retinoid receptors [41,42]. Binding of these nuclear receptors to their cognate response elements is considered to be preferentially in the form of heterodimeric complexes with 9-*cis* retinoic acid receptors (RXR) [43,44]. However, potential RXR-independent pathways also exist for vitamin D regulation of bone-specific genes [37]. Furthermore, in the human osteocalcin gene, vitamin D and retinoic acid receptor heterodimers have been shown to bind to the VDRE [45] and to confer combined calcitriol and *all-trans* retinoic acid responsiveness to this osteocalcin promoter element. A dominant negative effect of the vitamin D receptor on gene transcription has not been demonstrated in osteoblasts, though examples of such an effect have been shown within this subfamily of nuclear receptors [46]. Strom et al. [47] noted the possibility that such an effect of the vitamin D receptor might explain their results in the vitamin D–deficient rat intestine. These studies in concert suggest that various receptor dimers, ligand affinities, and response element configurations expand the possible diversity for regulation of vitamin D–responsive genes in bone.

The concept must also now be entertained that quantitative or functional differences in vitamin D receptor alleles as identified by RFLPs can contribute further to this diversity, by altering nuclear receptor–receptor and receptor–DNA interactions. Alterations in osteoblast receptor number could be a powerful way to modify the transactivation potential of calcitriol. Calcitriol upregulates expression of the vitamin D receptor gene in osteoblasts [24] and other tissues [47]. This effect has been shown in rat osteosarcoma cells to be modulated by PTH [48] and in intestine and kidney cells by calcium [49,50]. Vitamin D receptor levels in osteoblast-like cells are influenced also by growth factors such as TGFβ [51] and retinoic acid [52], glucocorticoids [53], and estradiol [54] to extents that significantly alter osteoblast production of calcitriol-inducible proteins such as osteocalcin. Vitamin D receptor transactivation of course is not limited to osteocalcin gene transcription, but has the potential to vary a wide range of developmentally and functionally important genes within the osteoblast. Altered expression of these genes could conceivably modify cell phenotype and the balance between bone formation and resorption, thereby altering the physiological setpoint and manifesting clinically as altered bone density. Much evidence also points to the hormone's role in facilitating bone resorption through osteoblast-mediated osteoclast recruitment, actions that are dependent on the interplay with other growth factors [55,56]. It is therefore clear that subtle changes in vitamin D receptor levels or activity would be expected to have pleiotropic effects on the wide range of target organs involved in the maintenance of the vitamin D endocrine system.

SUMMARY

Calcitriol influences every phase of bone and calcium homeostasis through the vitamin D receptor regulating gene transcription. Although nongenomic actions of calcitriol may exist, their ligand specificity indicates that the vitamin D receptor or another transduction pathway with comparable ligand requirements would be operational. Thus the vitamin D receptor occupies a central position in bone and calcium homeostasis and is a key part of a mechanistic pathway whereby a wide range of bone and calcium homeostatic functions lead to the maintenance of "normal" serum calcium and structurally sound bone. The concept that within a population of normal individuals, minor variations in receptor activity or number could have major effects on a wide range of physiological variables is a critical new insight. Subtle variations in receptor activity (qualitative and/or quantitative) could be functionally equivalent to alterations in any of its target genes. Importantly, such effects are likely to differ widely depending on the binding affinity or amount of vitamin D receptor present, the direction of target gene regulation (up or down), and the complement of co-associating proteins in tissues such as intestine, kidney, parathyroid gland, and bone, where the receptor mediates its principal biological effects. Clearly, there is potential for great phenotypic diversity due to varying combinations of alleles of the vitamin D receptor gene and its target genes. This makes the magnitude of the allelic effect on bone density all the more impressive.

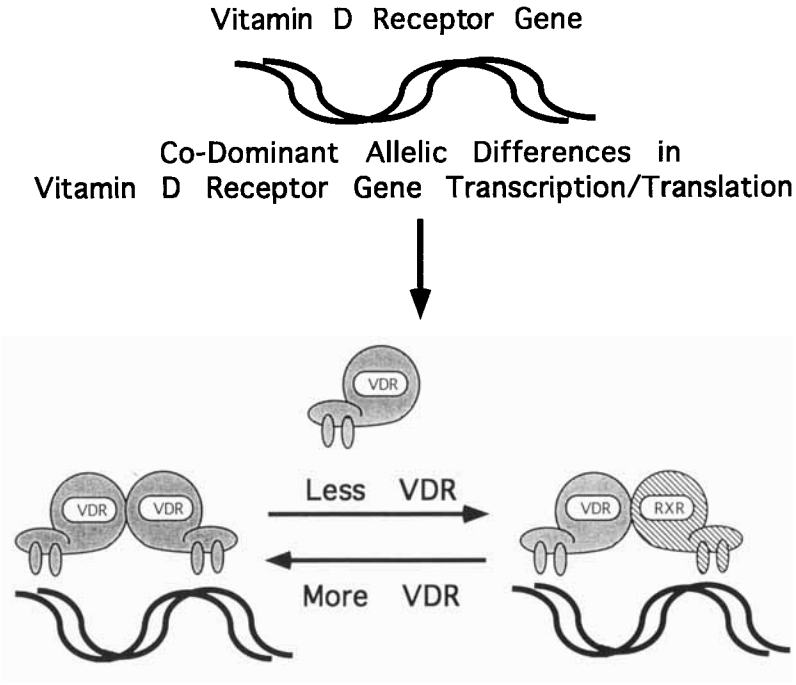

Fig. 2. The balance between hetero- and homodimer formation could be altered by simple changes in vitamin D receptor number (VDR). Changes in ligand and/or DNA affinity of the VDR or its dimerization domains could amplify such effects. Importantly, these effects must differ between genes depending upon their VDR transactivation mechanism.

The question arises as to how variations in one transduction mechanism, which affects multiple pathways, could lead to a different setpoint rather than achieve that setpoint with greater or lesser activity of that regulator's ligand. It could be argued that counter-regulatory mechanisms should reset each individual to the same setpoint. However, it is clear that counter-regulation brings the individual "towards" but not "to" the ideal setpoint. Deviation from ideal may be determined to a large extent by natural allelic variants in the signal transduction regulatory pathways. The vitamin D endocrine system also regulates in both a positive and negative sense. It is clear that for some DNA regions, the effect of the receptor is mediated through heterodimer formation of the vitamin D receptor with nuclear accessory factors, which in some tissues are RXRs (Fig. 2). This has been well studied in relation to the positive regulators. However the gene downregulatory DNA regions are less well defined and it is clear that the DNA consensus regions for inhibition are quite different from those implicated in upregulatory pathways [57]. It is not clear whether vitamin D regulates these regions as heterodimers or homodimers. Receptor monomers or homodimers could influence regulation of these genes, even if receptor heterodimers are optimal. If heterodimerization and homodimerization are involved, simple changes in steady state receptor level could favor formation of homo- or heterodimers and thus alter the biological response of each tissue. Similarly with distinct DNA sites involved for positive or negative actions, subtle receptor variations in quantity or quality could materially alter the final biological effect.

Since calcitriol regulates its target genes in both a positive and negative fashion, variations in receptor gene alleles leading to qualitative and/or quantitative variation of receptor within the "normal range" could be expected to result in differences in biological setpoints as demonstrated by our studies of bone turnover and steady state bone mass and density. Although vitamin D receptor gene alleles and bone turnover/bone mass which we have explored is the first such example, it seems likely that many other examples will be identified in equivalently complex regulatory systems. Our recent finding that normal allelic variations in the vitamin D receptor affect the setpoint of several bone and calcium parameters opens the possibility that normal variations in transactivating genes may

determine much of the normal variation in physiological parameters usually expressed as a "reference range." Understanding these mechanisms opens new prospects for improved targeted approaches to therapy in those biological situations where "disease" affects individuals at one or the other extreme of the reference range.

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