

# The vitamin D receptor: biological and molecular properties

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## Summary

Vitamin D/Vitamin D Receptor (VDR) endocrine system plays an essential role in regulation of calcium homeostasis and bone metabolism together with other calcitropic hormones. However recent studies evidenced further unexpected roles for this complex system ranging from control of cell proliferation and differentiation to modulation of the immune system. Similarly to other proteins, belonging to the family of nuclear hormone receptors, VDR shows different molecular mechanisms supporting the wide range of its biological actions. Among them the regulation of transcription of vitamin D-dependent genes is the most important one. This paper is a comprehensive review of the biological actions of VDR but for each of them an attempt to give details of the molecular mechanism has been made. Special attention has been paid in evidencing the important role of genomic and proteomic approaches in the study of such a complex system where different signal pathways are involved.

The second part of the paper is dedicated to polymorphic variants of VDR gene with special care for functional implications, thus functional studies rather than genetics ones have been reviewed in this section.

**KEY WORDS:** vitamin D receptor, functionality, genetics, polymorphisms.

## Introduction

Vitamin D receptor (VDR) (1) is the responsible for much of the vitamin D [1,25-(OH)<sub>2</sub>D<sub>3</sub>] signalling. It is a direct regulator of gene transcription, belonging to the nuclear receptor family. Within this family, it has sequence and structure resemblance with the sub-family that includes retinoic acid, thyroid hormone and peroxisome proliferator activator receptor (PPAR) receptors (2). VDR is a protein of 427 amino acids, with a molecular mass of approximately 48 kDa. Similarly to the other nuclear receptors VDR consists of several distinct functional domains, namely

the NH2 terminus A/B domain whose function is still unclear, a DNA binding domain (DBD), termed C domain, spanning from amino acid 20 to 90, a hinge or D domain (amino acids 90 to 130) and the ligand binding domain (LBD), or E domain, spanning from amino acid 130 to 423 (3). The last one is the most complex domain of the protein as it is responsible for the specific binding of VDR ligands, for heterodimerization with retinoid X receptor (RXR) and for interactions with transcription factors (Figure 1) (2).

VDR cDNA was firstly cloned from chicken (4) and shortly thereafter from human (1). Genomic organization of the human VDR gene, which locates on chromosome 12q13-14, is similar to other nuclear receptor genes. The gene is made up of 11 exons spanning approximately 75 kb (Figure 2). The non coding 5' end of the gene includes several exon 1 isoforms (from 1A to 1E) while exons 2-9 encode the structural portion of the VDR gene product. Alternative splicing of exon 1 provides at least five different VDR mRNA transcripts, while the presence of a polymorphic sequence in exon 2 determines the presence or absence of an alternative start translation site (5). Most of the promoter sequence is upstream exon 1 and has high GC content but does not contain an apparent TATA box. The promoter

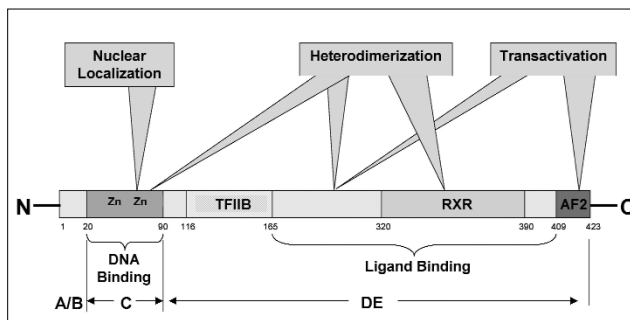


Figure 1 - Schematized linear amino acid sequence illustration of VDR protein showing the functional domains of protein that mediate ligand and DNA binding, nuclear localization, heterodimerization with retinoid X receptor (RXR) and transactivation. COOH-terminal ligand activation function 2 (AF2) is shown in anthracite-grey.

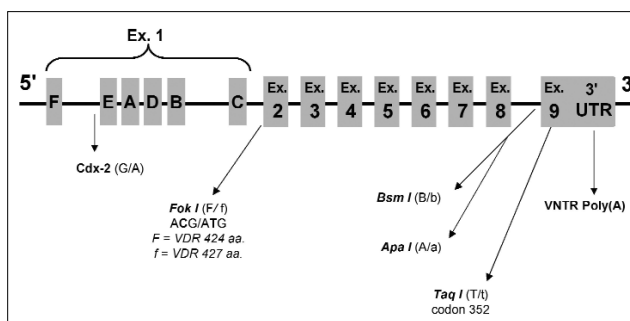


Figure 2 - Schematic view of the genomic sequence of VDR gene containing Exon (Ex.) – Intron structure and position of known polymorphic variants.

is capable to generate multiple tissue-specific transcripts (6) and is positioned just downstream from collagen type II alpha 1 gene (7, 8). A unique feature for this gene is the presence of an additional exon that is not found in other nuclear hormone receptors family members. It encodes for an insertion peptide of about 40 amino acid that locates in the LBD of the receptor (5). VDR binds its physiological ligand 1,25-(OH)<sub>2</sub>D<sub>3</sub> with high affinity (about 10<sup>-10</sup> M) (9, 10) and both hydroxyl groups seem to be relevant in determining it as the absence of either results in approximately a 500-fold decrease in affinity (11).

Although widely expressed, VDR protein levels are usually low and reach relatively high values only in targets tissues such as bone, kidney and intestine (3,000-6,000 fmol/mg protein) (12). In contrast to other nuclear receptors which are usually bound to cytoplasmic proteins before hormone binding (13), VDR is predominantly nuclear (14).

Similarly to other nuclear receptors VDR is active as a heterodimer with members of the RXR family of receptors and the binding with RXR LBD stabilizes both heterodimerization and high affinity interaction with DNA (15). VDR regulates target genes transcription in part by binding specific DNA sequences known as vitamin D responsive elements (VDREs), located in the 5'-flanking region of target genes and composed of tandem hexameric motifs with the consensus PuG(G/T)TCA which are often arranged as direct repeats separated by 3 bp (DR3-type) but that occur also as inverted repeats separated by 6 or 9 bp (16-19).

VDR regulates gene transcription by ligand-dependent recruitment of coregulators such as SRC family proteins (20-23) and transcriptional "integrators" like Calcium binding protein (CBP) and p300 which, in addition to other functions, have been demonstrated to act as histone acetylases (24-26). This series of events leads to DNA structure remodelling through acetylation of histones and their subsequent release from DNA and consequently to the opening of the promoter to the transcriptional machinery with the final event of an increased rate of transcription of that gene. Although not speculative, the proposed mechanism is far from being well characterized, a number of details and the exact sequence of events remaining unclear at this point.

Vitamin D signalling has numerous biological effects affecting the physiology of a broad range of human tissues (3, 27). First of all it controls calcium transport in the intestinal epithelia affecting the physiology of bone metabolism; in several studies vitamin D effects on cellular proliferation and differentiation have been demonstrated, as well as an antiproliferative action on several kinds of cancer such as myeloid leukaemia, melanoma and different carcinomas (reviewed in 3). Moreover vitamin D analogues have proved to have chemopreventive action in animal models of colon, hamster cheek pouch, hepatocellular, gastrointestinal and skin carcinogenesis (28). Consistently with the broad expression of VDR in cells of the immune system and with its effect on cell differentiation, an important role as modulator of the immune response has been demonstrated (29).

Polymorphic sequence variations in VDR gene occur frequently in the population, but they are less analysed and their effects on gene function are poorly understood. Most of them consist of anonymous variations that do not modify the coding region with unknown functional effects. Several studies using candidate gene approach demonstrate that supplementation of 1,25-(OH)<sub>2</sub>D<sub>3</sub> increases bone mineral density (BMD) and decreases the risk of osteoporotic fracture (30, 31). Thus, to understand the mechanisms underlying these associations we need to analyze genomic organisation of VDR locus (polymorphisms, linkage disequilibrium and haplotypes). Literature regarding VDR polymorphisms in relation to bone metabolism diseases, and osteoporosis in particular, is well represented, while studies on

VDR polymorphisms and other cancer and immune response diseases are restricted and started later on.

### Transcriptional regulation by VDR

Ligand binding, involving the AF2 domain, imparts a conformational change in the secondary structure of the VDR which triggers the recruitment of motor proteins (32) responsible for cytoplasmic VDR transition to the nucleus along microtubules (33). VDR heterodimerization with its protein partner RXR confers a conformational structure to the receptor which is critical for transactivation function (34) by high affinity interaction with VDREs in the promoter region of vitamin D-responsive genes (Figure 3). During VDR-RXR interaction with the most common VDRE type, DR3, RXR binds the 5' half-site and the VDR occupies the 3' one (35). According to mutagenesis experiments in the VDRE of avian parathyroid hormone (*PTH*) gene promoter, the switch between VDR-RXR-activated or -repressed gene transcription depends on the polarity of the VDR/RXR-VDRE complex (35). However recent experimental evidences regarding VDR interactions with nuclear coregulator molecules and their action on VDR-mediated regulation of gene transcription have provided further complexity to the entire mechanism. In fact RXR heterodimerization and AF2 domains are both involved in interactions with nuclear proteins which serves as VDR-coregulators (36). In details the ligand-induced conformational change in AF2 domain is critical for the receptor to interact with components of the transcription initiation complex, RNA polymerase II and nuclear transcription coactivators which promote chromatin remodelling necessary to gene transcription. Some of them in fact, such as SRC-1 and CBP/p300 are histone acetylases which determines the recruitment of a second complement of transcription coactivators, the DRIP-TRAP complex. This is a 15 proteins-complex which facilitates the assembly of the preinitiation complex thus strengthening VDR-induced gene transcription. Binding of VDR-RXR complex

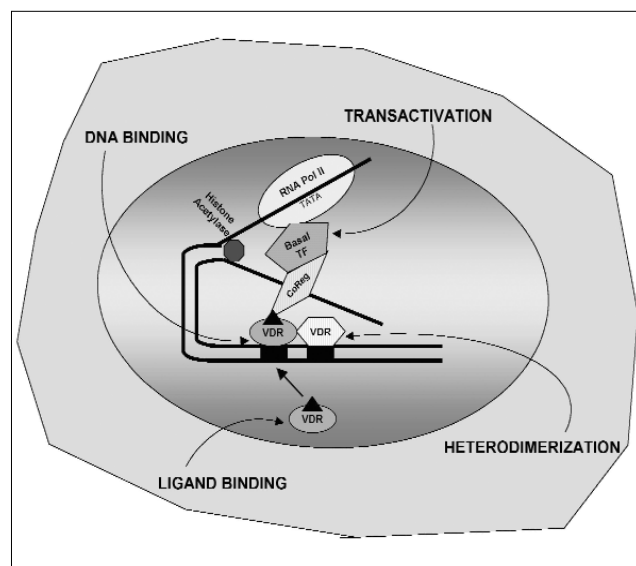


Figure 3 - Proposed model for the control of VDR-mediated transactivation. Vitamin D (represented by black triangle) - VDR complex heterodimerizes with RXR. The resultant heterodimer binds specific sequences in the promoter region of target genes. The DNA - bound heterodimer recruits components of the RNA polymerase II (Pol II) preinitiation complex and nuclear transcription regulators, thereby altering the rate of gene transcription.

to a negative VDRE promotes recruitment of corepressors which have histone deacetylase activity and then inhibit binding of proteins promoting the assembly of the transcription machinery (37, 38). The situation is further complicated by comodulator proteins, such as NCoA62/Skip which can act as corepressor or coactivator depending on the specific cell context of coregulator molecules (39). The NH<sub>2</sub>-terminal region of Skip protein is targeted by both nuclear corepressors, such as NCoR, and coactivators, like CBP/p300, and more importantly some of them are under VDR-mediated transcriptional regulation (39, 40). Recently an ATP-dependent chromatin remodeling complex has been demonstrated to amplify VDR activation and repression of transcription (41).

### Calcium homeostasis

Vitamin D metabolism is a classic endocrine system that responds to changes in serum calcium concentrations. A decrease in serum calcium concentration, due to low dietary calcium intake, causes an increased production and release of PTH which, among many other functions, stimulates renal 1 $\alpha$ -hydroxylase activity and leads to increased conversion of 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] to its active metabolite 1,25(OH)<sub>2</sub>D<sub>3</sub>. Increased level of serum 1,25(OH)<sub>2</sub>D<sub>3</sub> activates VDR in a tissue specific manner and stimulates the expression of vitamin D-responsive genes in districts that control calcium homeostasis, for example TRPV6 and calbindin D9k in intestine, osteocalcin and RANKL in bone, TRPV5 and calbindin D28k in kidney (42). The traditional reductionist approach used in the past decades, to understand biological processes, has been undoubtedly useful for the elucidation of signal transduction pathways and will certainly continue to be so but it has the fault to deal only with proteins and functions we already know, testing single hy-

pothesis of their involvement in the biological process, while the newly born genomic and proteomic approach let us gather the global changing (at the mRNA or protein levels) accompanying a biological process.

Calcium is critical for a number of life's essential functions and probably its employment in so important biological process (neural transmission, muscle contraction and relaxation, exocrine secretion, blood clotting and cell adhesion) occurs because of the constancy and abundance of calcium in seawater which is the medium where higher animals arose (Figure 4). The high abundance of calcium in the seawater also explains its use in the construction of structural elements such as the skeleton. The important calcium involvement in the biology of higher animals is the reason for being one of the most tightly regulated substance in their plasma (43) and make it reasonable that the evolution of the calcium homeostatic system took place as animals emerged from the sea into fresh water and further onto land. This is a very complex system that involves many hormones with the vitamin D endocrine system being the basic one in managing plasmatic calcium levels, with equally important roles for PTH and calcitonin (3).

### Calcitropic hormones

#### Role of PTH

The parathyroid gland is the calcium-sensing organ in the body (43, 44) and in a few seconds it responds to even slight hypocalcaemia by secreting the 84-amino acid peptide hormone PTH (45). Its receptor is expressed in the nephron and in osteoblasts but not in intestine and osteoclasts for example (46). In the kidney PTH acts as a phosphate antireabsorptive agent causing a phosphate diuresis (47), activates 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase and inhibits 24-hydroxyvitamin D-1 $\alpha$ -hydroxylases

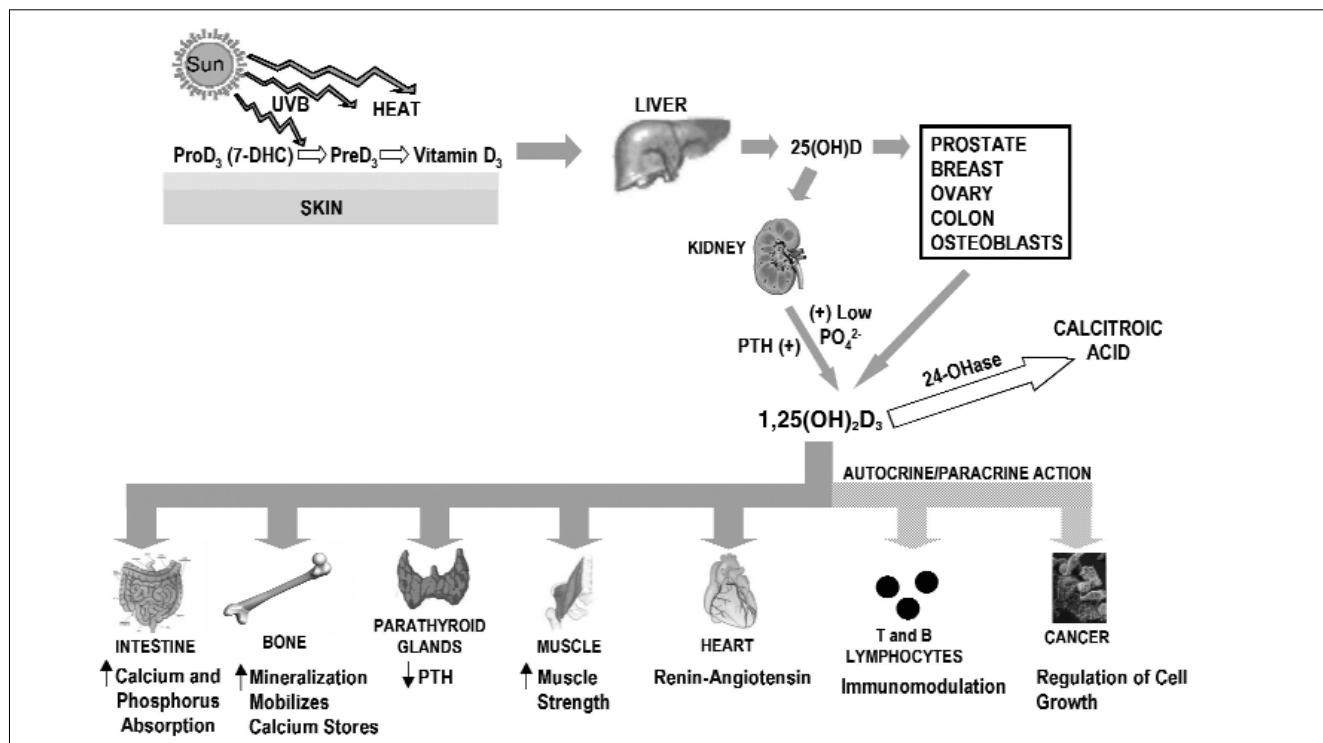


Figure 4 - Biological actions of vitamin D in calcium and phosphorus homeostasis in mammals. Vitamin D is mostly synthesized in the dermis under UV B radiation from its precursor (7-dehydrocholesterol). It is hydroxylated to 25(OH)D in the liver and then activated to 1,25(OH)<sub>2</sub>D<sub>3</sub> (calcitriol) in the kidneys. Calcitriol induces intestinal absorption, controls bone remodelling, suppresses parathyroid function, and renal calcium reabsorption to maintain calcium on limit levels for normal cell physiology and skeletal integrity. Renal vitamin D production also serves autocrine and paracrine functions.

through cAMP increase (48) thus causing an increment in plasma levels of active  $1,25(\text{OH})_2\text{D}_3$  (49) (Figure 4).

#### Role of vitamin D

Vitamin D initiates an active intestinal calcium transport in the small intestine (50). This action has the longer lifetime, measurable in days, while the other actions are shorter (51). The results of the action of PTH and  $1,25(\text{OH})_2\text{D}_3$  is the mobilization of calcium by the skeleton into the plasma compartment and this is obtained by either osteoclast stimulation to resorb bone and to reverse calcium transport from bone fluid compartment to plasma (52-54) (Figure 4). Even in the distal renal tubule PTH and  $1,25(\text{OH})_2\text{D}_3$  act synergistically to reabsorb the last 1% of the filtered load of calcium into the plasma compartment (55, 56). Interestingly, through the study of animal models, defective for either  $1,25(\text{OH})_2\text{D}_3$  or PTH, it has become evident that the presence of both hormones is required for this system to operate *in vivo* although the precise mechanism of the interaction between them is still poorly understood (57). Finally the rise in serum calcium level resets the sensing point of the calcium receptor and shuts down the secretion of PTH (3).

#### Role of calcitonin

To guard against the calcification effects of hypercalcaemia (dangerous for kidney, heart, aorta and intestine) there is also the response of parafollicular and C cells of the thyroid which respond to hypercalcaemia with calcitonin secretion, a 34-amino acid peptide hormone that causes lowering in serum calcium level by its action on the skeleton through inhibition of osteoclasts and osteocytes activities (58). There have been reports of calcitonin effects on kidney and intestine but these are derivative respect to those on the skeleton (3) and a sort of regulation on  $1,25(\text{OH})_2\text{D}_3$  metabolism has been described but this has been shown to be largely secondary to changes in parathyroid secretion (59, 60).

### Physiological action of VDR

#### Effects on intestinal calcium

The role of vitamin D in intestinal absorption of calcium is well known and the vitamin D endocrine system has been finally identified as the agent that stimulates intestinal calcium absorption to meet the needs of the skeleton (61) (Figure 4). Epithelial calcium channel TRPV6 (CaT1 or EcaC2) is necessary for calcium uptake together with TRPV5 (EcaC1). Then calbindin D shuttles the ion across the cell and finally the plasma membrane  $\text{Ca}^{2+}$  ATPase (PMCA1b), and the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX1) deliver it into the bloodstream (62). TRPV5 and TRPV6 expression levels depend on VDR regulation as demonstrated by their reduction in VDR-null mice and their induction by calcitriol supplementation in wild-type mice (62-64). These two channels confer high  $\text{Ca}^{2+}$  selectivity and negative feedback regulation to intestinal  $\text{Ca}^{2+}$  influx, strictly resembling that in native distal renal cells (65). Vitamin D has a clear role even in stimulating intestinal absorption of phosphate which is another active calcium transport mechanism but which appears to be completely independent from the direct one (66-68). VDR-mediated mechanism to maintain the  $\text{Ca}\cdot\text{PO}_4$  ion product seems to primary involve induction of phosphate translocating proteins in kidney and perhaps in intestine. Indeed the renal sodium-phosphate cotransporter-2 (NPT2) is a likely vitamin D-induced protein containing a VDRE in the promoter region (69, 70). Another role of VDR in phosphate homeostasis is due to

$1,25(\text{OH})_2\text{D}_3$  induction of PEX gene expression. PEX gene, which harbours a VDRE in its promoter region (71), is a phosphate-regulating gene, showing high homologies to endopeptidases located on the X-chromosome and postulated to be the proteolytic agent for inactivation of phosphatonins (72) which in turn have phosphaturic action through potent inhibitory effects on NPT2 and  $1\alpha$ -hydroxylase (73, 74). From a mechanistic point of view, intestinal active phosphate transport is stimulated by a vitamin D-induced increase of  $\text{Na}\cdot\text{PO}_4$  cotransporter and plasma membrane fluidity of enterocytes (75, 76). On the contrary the mechanism that mediates phosphate transport through basolateral membrane is still unknown.

#### Effects on bone calcium mobilization

A vitamin D deficient animal on a zero-calcium diet will adjust serum calcium level at the expense of skeleton when given  $1,25(\text{OH})_2\text{D}_3$  in the presence of PTH (57). A clear stimulatory effect on osteoclastic bone resorption has been widely demonstrated for vitamin D (77, 78) although there is no expression of PTH receptor nor of VDR in osteoclast (79). On the contrary PTH and vitamin D interact with osteoblast arising a paracrine signal which facilitates osteoclast differentiation (80-82) and calcium mobilization from bone fluid compartment to the plasma one (83). Stimulation of osteoclastic bone resorption is, however, rarely finalized to provide calcium for plasma but more likely it is coupled to formation in completing the bone-remodelling process. Thus  $1,25(\text{OH})_2\text{D}_3$  results to be involved in important processes which strengthen bone and repair microfractures (78, 84).

From a molecular point of view vitamin D/VDR system permits a regular coupling in bone turnover by controlling the interaction between receptor activator of NF- $\kappa$ B ligand (RANKL) and receptor activator of NF- $\kappa$ B (RANK). In fact RANKL, expressed on osteoblast surface, can bind either RANK, inducing a signalling cascade leading to differentiation and maturation of osteoclasts, or to an osteoblast-produced decoy receptor, osteoprotegerin (OPG), which in turn blocks this signalling (85, 86). Vitamin D, PTH and prostaglandins stimulate RANKL expression but the first one also inhibits OPG synthesis (87,88) (Figure 5).

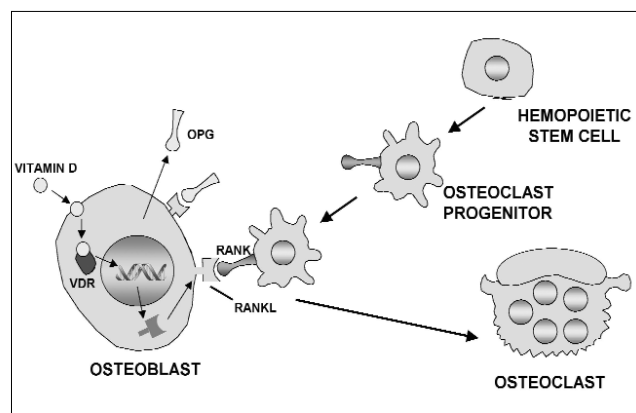


Figure 5 - Schematized representation of Vitamin D-regulated osteoclastogenesis. Vitamin D regulates this process by transcriptional control of both receptor activator of NF- $\kappa$ B (RANK) ligand (RANKL) and osteoprotegerin (OPG). Vitamin D - VDR complex increases the expression of RANKL on the surfaces of osteoblast, where it interacts with RANK promoting maturation of osteoclast progenitor cells to mature osteoclasts. Vitamin D - VDR complex also represses the expression of OPG (a bait receptor that binds RANKL and prevents RANK-mediated osteoclastogenesis).

Moreover data obtained from VDR-null mice together with the ability of a lactose-, calcium- and phosphate-rich diet to reverse their bone abnormalities, stress the concept that vitamin D plays its important physiologic effects on intestinal absorption of calcium and phosphate although some calcium regulating devices, such as depressed renal calbindin  $D_{9k}$  mRNA expression in kidney, are not restored under the above diet (89). A VDRE has in fact been demonstrated to be present in the calbindin  $D_{9k}$  promoter in mammals (90), thus accounting for a direct effect of VDR on its expression.

#### *Effects on parathyroid glands*

Vitamin D acts in parathyroid tissue through its binding to VDR and consequent regulation of gene transcription (91, 92) and a VDRE was demonstrated in the promoter region of the *PTH* gene (93, 94). Thus  $1,25(\text{OH})_2\text{D}_3$  exerts a negative feedback on PTH production in response to PTH-dependent activation of calcium mobilization from kidney and bone, through a VDR-mediated silencing of *PTH* gene transcription (95-97). Moreover  $1,25(\text{OH})_2\text{D}_3$  regulates parathyroid level of VDR and its response to calcium. The first effect is due to an increase in VDR mRNA level, possibly secondary to serum calcium increase (98), as well as to a ligand-dependent VDR protection from proteosomal degradation (99). The second one directly involves VDR-mediated regulation of calcium sensing receptor (CaSR) gene transcription as evoked by the presence of two VDREs in its promoter region (98, 100). The other important effect of  $1,25(\text{OH})_2\text{D}_3$  on parathyroid glands involves cell growth regulation and is described in other chapter.

#### *Effects on the kidney*

One of the major effect of vitamin D in the kidney is its own homeostasis through inhibition of  $1\alpha$ -hydroxylase and stimulation of  $24$ -hydroxylase expression as well as through induction of megalin expression in the proximal tubule (101). Moreover it stimulates renal calcium reabsorption and enhances calbindin expression, accelerating PTH-dependent calcium transport in the distal tubule (102) (Figure 4). Finally vitamin D/VDR acts on TRPV5 promoter to increase its mRNA and protein levels which is an important actor in vitamin D-mediated calcium reabsorption (103).

### **Regulation of cell proliferation/differentiation and chemopreventive actions of vitamin D**

Vitamin D has been shown to have pro-differentiation action on preadipocyte cell lines (104), on immature basal layer skin cells into keratinocytes (105) and on haematopoietic cell lines along the macrophage/monocyte pathway (106-108). Finally a potential use of  $1,25(\text{OH})_2\text{D}_3$  in the treatment of leukaemia and other myeloproliferative disorders is suggested by observations on vitamin D ability to inhibit clonal proliferation and promote a more differentiated and less aggressive phenotype in a variety of human leukaemia cell lines (109) (Figure 4).

#### *Suppression of cell growth*

A common aspect to different mechanisms through which vitamin D suppresses cell growth is the arrest at  $G_1$ - $G_0$  transition. 1) Vitamin D induces gene transcription of p21, a cyclin-dependent kinases inhibitor, inducing growth arrest and promoting cell differentiation of monocyte-macrophage lineage (110). 2)

Vitamin D induces p27 synthesis through VDR-Sp1 interaction at the p27 promoter and inhibits p27 degradation rate through reduction of CDK2 activity and Skip2 protein level (111). 3) In TGF- $\alpha$ /EGFR-driven tumorigenesis vitamin D sequesters ligand-activated EGFR thus reducing growth signal at the cell membrane and EGFR-mediated activation of cyclin D1 gene transcription (112). Vitamin D efficacy in inhibiting mitogenic signals from the TGF- $\alpha$ /EGFR growth loop is also fundamental in mediating its efficacy in treating psoriasis and scleroderma. 4) Vitamin D is also able to induce C/EBP $\beta$  expression with the consequent suppression of the oncogenic-cyclin D1 signature in human epithelial tumours (113). Moreover the dominant negative isoform of C/EBP $\beta$ , LIP, lacking the transactivation domain, strengthens cyclin D1 induction of cell growth and the ratio C/EBP $\beta$ :LIP has been indicated as a major mechanism for EGFR-induced proliferative action (114). Vitamin D-mediated induction of C/EBP $\beta$  expression should contribute to higher C/EBP $\beta$ :LIP ratio and, consequently, to reduce proliferation rates (115).

#### *Regulation of apoptosis*

Vitamin D exerts proapoptotic as well as antiapoptotic effects which affect both normal tissues growth and function and cancerous as well as noncancerous hyperproliferative tissues. Evidences regarding certain VDR alleles association with cancer-prone phenotypes suggest the involving of VDR in these effects (116, 117). For instance, in breast cancer cells vitamin D induces apoptosis through reciprocal modulation of Bcl2 and Bax content (118). Moreover it causes the calcium-dependent proapoptotic proteases microcalpain and caspase 12 activation through intracellular calcium increase (119, 120). Vitamin D proapoptotic actions have been demonstrated even in glioma (121) and in melanoma (117), while they are absent in normal astrocytes, melanocytes and mammary cells (122). Rather, vitamin D protects keratinocytes from UV radiation- or chemotherapy-initiated apoptosis (123) and melanocytes from TNF- $\alpha$ - and UV irradiation-dependent apoptosis through induction of sphingosine 1-phosphate (122). Other mechanisms through which vitamin D affects apoptosis seem to be tissue specific: for instance in colorectal cancer VDR expression is negatively influenced by transcription factor Snail, which is recruited to the VDR promoter, with the consequent lowering of E-cadherin expression which in turn influences cell fate during colon cancer progression. This mainly influences the efficacy of vitamin D adjuvant therapy in colon cancer (124). On the other hand, in prostate cancer, defective nuclear vitamin D localization and SMRT corepressor altered expression levels, but not reduced VDR levels, are responsible for resistance to vitamin D therapy (125).

### **Regulation of the immune response**

Vitamin D endocrine system positively affects infection, autoimmune diseases, tolerance in transplantation and this mainly derives from prodifferentiating effects on monocyte-macrophages, antigen-presenting cells, dendritic cells (DC) and lymphocytes (126) as evidenced from *in vivo* and *in vitro* studies (Figure 4).

One of the mechanisms mediating vitamin D function in resistance to infections is the already mentioned induction of C/EBP $\beta$  which enhances monocyte differentiation to macrophage, immune function, host defence against bacterial infection and tumour cell growth and production of IL-12, the cytokine inducing Th1 response (115, 127). On the other hand  $\gamma$ -interferon is a potent inducer of  $1\alpha$ -hydroxylase in

macrophage thus increasing vitamin D local production through a C/EBP $\beta$ -mediated mechanism (128). Local macrophage-produced vitamin D is also an inducer of T cell response to cutaneous antigens *in vivo*, including CD4-Th2 cell-mediated and mucosal antibody responses (129). In contrast to these stimulatory effects on monocyte-macrophages, vitamin D is an immunosuppressor for lymphocytes (130). This effect is due to a vitamin D/VDR-mediated inhibition of expression of cytokines involved in T cell functions, including IL-2 (131). Vitamin D plays also an important role in the establishment and maintenance of immunological self-tolerance as observed in studies on animal models demonstrating a vitamin D-induced inhibition of disease induction in experimental autoimmune encephalomyelitis, thyroiditis, insulin-dependent diabetes mellitus, inflammatory bowel disease, systemic lupus erythematosus and both collagen-induced arthritis and Lyme arthritis (129, 132). Finally vitamin D inhibits rejection of transplanted tissue probably through a VDR-mediated mechanism involving TGF $\beta$ /Smad3 interactions (133). In conclusion vitamin D seems to be a modulator of the immune response mainly acting through a paracrine loop which may block inflammation and/or modulate the differentiation of activated CD4 T cells as well as the suppressor T cell function (126).

### VDR polymorphisms and VDR function

VDR gene polymorphisms are one of the more intriguing and controversial questions, in terms of genetics and functional understanding about genetics of bone. Nevertheless, in the last years the interest about these polymorphisms on other diseases, such as breast, prostate, colon cancer and immune response, is growing. The majority of VDR polymorphisms are in the regulatory areas, such as 5' promoter and 3'UTR regions, rather than in coding exons. The reason for this hot-spot location is that the variation in the protein sequence could result in drastic functional effects, such as alterations on ligand and DNA binding. Therefore, polymorphic variation, that can explain population variance, exist in areas of gene that mainly affect VDR expression level.

#### Genetics of VDR polymorphisms

Several polymorphisms have been identified in human VDR gene locus using various approaches that include the following: a) screening with different restriction enzymes for polymorphic banding patterns in Southern blot hybridisation experiments (RFLPs); b) VDR sequencing in a number of different individuals; and c) *in silico* polymorphism identification through bio-informatics approaches (Figure 2). Among numerous osteoporosis candidate genes that harbour polymorphic sites, the gene encoding for VDR was the first to be described (134) and, according to its functional role, it was proposed as a major locus for genetic effect on osteoporosis (135-137).

The restriction endonucleases *TaqI*, *Apal*, *BsmI* and *EcoRV* allow to recognise the allelic variants due to single nucleotide polymorphisms (SNPs) at the 3' region of human VDR gene. Another polymorphic variant, recognized by *FokI* endonuclease, is located in a putative initiation transcription codon of exon 2. The alleles are named T-t, A-a, B-b, E-e and F-f respectively, where the lowercase letter means presence of restriction site and the uppercase letter indicates absence of restriction site. PolyA variable number of tandem repeats (VNTR) is present in the 3' UTR (138). This polymorphism determines at least 12 different alleles, with a bimodal distribution. Subjects can be classified as short or long PolyA carriers. Finally, caudal-related homeodomain (Cdx2) polymorphism was found through sequence analysis (139). This new VDR polymorphism

(G to A) is located in Cdx2 binding site at 5' VDR promoter area.

#### VDR polymorphisms and association studies

Morrison and co-workers analysed an Australian population sample and evidenced the presence of association between allelic variants in the 3' region (for B allele) and osteocalcin bone turnover marker levels in serum (135). Since 1992, several studies about correlation of VDR polymorphisms with BMD and bone turnover markers have been published, generating conflicting data (140-163). In order to elucidate these pitfalls, a more recent meta-analysis study confirmed the contribution of *BsmI* allelic variants on variation of BMD values, even if this analysis showed weaker association than originally claimed (165). This observation is also supported by another meta-analysis study, where allele B significant association with spine BMD was demonstrated, following a supposed recessive model of transmission, with the *BB* genotype having lower BMD than *Bb* and *bb* genotypes at the baseline (166). Consequently, BMD resulted to be associated to VDR gene polymorphisms with high levels of confidence. Moreover, it is not possible to completely exclude a presence of linkage disequilibrium between VDR polymorphisms and other important genes for bone metabolism, such as *ER $\alpha$* . In fact *ER $\alpha$*  exerts a modulation effect on VDR in BMD determination, suggesting the existence of gene-gene interaction (167, 168).

Several studies on population of Mexican-American (169), Japanese (170), North American (171) and Italian (172) postmenopausal women, showed an association between low lumbar BMD and *ff* genotype, while no significant association was found in French (173) and Swiss women (143). These contrasting results may be due to ethnic and age differences. Moreover environmental factors, such as calcium intake, may conceal *FokI* genotype effects on BMD. A recent study on a Finnish population indicates that the *Ff* genotype is associated with higher forearm BMD and calcaneal ultrasound values in adolescent boys (174).

Cdx2 polymorphism may modulate BMD in postmenopausal Japanese women (139). In fact, Japanese women who carry the A allele have higher BMD. Nevertheless, this result was not confirmed for Caucasian women where this allele resulted to be associated only with a decrease of fracture risk (175).

It is largely demonstrated that VDR polymorphisms may influence bone metabolism. Nevertheless, some recent isolated studies showed the association between VDR polymorphisms and other diseases risk. It was found a role for VDR polymorphisms (*Apal* and *TaqI*) increasing the risk of developing multiple sclerosis (176). Variants of *BsmI* VDR polymorphism were associated with increased risk of developing hypercalcemia in peritoneal dialysis patients (177). Lower VDR mRNA levels associated with *b*, *a*, and *T* alleles may affect the calcitriol-mediated control of parathyroid function and thereby contribute to the development of sporadic primary hyperparathyroidism (178). *FokI* VDR polymorphism may influence parathyroid response in chronic renal failure (179). *FokI* C allele was also found to be associated to high risk for colorectal cancer (180). Another study found sun exposure and VDR polymorphisms act synergistically in the aetiology of prostate cancer (181). And finally, *BsmI* VDR genotypes in combination with low Vitamin D circulating levels, may increase risk of breast cancer in Caucasian population (182). However, these observations need to be confirmed by further independent studies.

#### VDR polymorphisms and haplotyping

Linkage disequilibrium measures the association of alleles of adjacent polymorphisms (183). Besides, many studies have

addressed on the relationship between multiple individual polymorphisms in *VDR* gene and bone health, but only few have analysed *VDR* gene data in terms of haplotypes. Haplotypes are blocks of linked alleles of neighbour polymorphisms, whereby the length of such a block coincides with the strength of linkage disequilibrium across the area. When a haplotype is identified as the risk allele it is possible to determinate which variants on that haplotype allele truly causes this effect.

A meta-analysis study with data on 3' *VDR* gene polymorphisms was performed in order to estimate haplotype frequencies, determine linkage disequilibrium and estimate the magnitude of the association between haplotypes and osteoporosis/ BMD (184). Results show that the most common haplotype for *VDR* gene, regardless of ethnicity, is the *baT*, followed by *BAt* and *bAT* in Caucasians, and *bAT* followed by *BaT* in Asians, indicating strong linkage disequilibrium between *BsmI* and *TaqI* polymorphisms. These observations demonstrate a gain in the power considering the haplotypes rather than single polymorphism. In fact, in this study, *VDR* gene polymorphisms were not significantly associated with osteoporosis risk on their own, but *Bat* and *BAt* haplotypes were significantly associated.

PolyA polymorphism showed strong linkage with *BsmI* polymorphism (142), where *b* allele was associated with long PolyA allele and *B* allele with the short one. Combining the results the following can be deduced: *baT* haplotype is linked to long PolyA allele and *BAt* haplotype to short one.

#### *VDR polymorphisms and its ethnic distribution*

Similarly to other genes polymorphisms, significant differences exist in *VDR* polymorphisms distribution among different ethnic groups. A possible explanation to polymorphism generation phenomena is DNA damage events in an ancient small population that grow up in frequency becoming polymorphisms in modern populations. *VDR* haplotypes distribution reflects out-of-Africa evolution theory that describe human populations origin and dispersion around the world, where gene-environment interactions favours the survival of some allelic variants. Old polymorphisms might show large population/ethnic variability (*FokI*), while new ones are likely characterized by small population variability (*Cdx2*). Assuming that a polymorphism should have the same functional effect in different ethnic groups, different allele frequencies across these groups may explain differences in the incidence of pathologies and variability on drug response among them (i.e. Asians seem to be more vitamin D-sensitive while Caucasians appear more oestrogen-sensitive than other ethnic groups). In the case of non-functional polymorphisms, frequencies of these markers are very different among ethnic groups. It is also difficult to understand consequences of ethnic allele variation in this case, because of environmental factors interference, such as diet and physical exercise. This emphasises that an haplotypes map of *VDR* polymorphisms in different ethnic groups is necessary (116).

#### *Functionality of VDR polymorphisms*

Functional studies are needed to determinate the way certain haplotypes in a candidate gene affects protein function. Compared to the larger number of genetic association studies, there has been little published on the mechanism through which *VDR* gene polymorphisms might influence *VDR* receptor functionality.

Functional studies include *in vitro* cell biological and molecular studies and *in vivo* measurements of biological markers, and response to treatments (vitamin D, calcium, hormone replace-

ment therapy and bisphosphonates). Whereas the functionality of *Cdx2* and *FokI* alleles are well known, there are less certainty and some controversies regarding 3' region RFLPs and VN-TR polymorphisms effects. As these ones do not involve amino acid substitutions in the protein, these allelic variants may be related to regulative differences. The 3'UTR of genes is known to be involved in regulation of expression and in mRNA stability. Some studies show that the 3'UTR *VDR* gene polymorphisms do not affect abundance of *VDR* mRNA, nor its stability (185-189). These findings suggest that these polymorphisms do not affect *VDR* function, but rather may be a marker for a nearby gene that is responsible for the genotype associated variation.

The case of *FokI* polymorphic variant (ATG/ACG) of *VDR* gene (169) is something different. It generates a length difference of 3 amino acids in the protein. *In vitro* studies on HeLa cells showed that *FF* genotype (short form) gave an approximately 1.7-fold increase in transcription activation (170). Whereas, no difference was found using GMK-Cos7 and human fibroblasts (190). More recent data confirmed Arai results using Cos7, HeLa and ROS 2/3 cell lines (191), and peripheral mononuclear cells (192). Jurutka et al. demonstrated the short form interacts more efficiently with transcription factor TFIIIB, and Col-in found that it also had a lower dose effect and thus determined a more active *VDR* molecule in inhibiting the (Phytohemagglutinin-stimulating) cell growth. In conclusion this polymorphism seems to be functional in terms of *VDR* transactivation function.

Functional studies showed that *Cdx2* polymorphism affects *VDR* expression in the small intestine (139). As intestine is the predominant area for calcium absorption, it is possible that *Cdx2* influences vitamin D-mediated regulation of calcium absorption. The allele *A*, which shows a more efficient binding to *Cdx2* transcription factor, is thought to cause increased *VDR* expression in intestine with a consequent increase in the transcription of calcium transporting proteins. This process can enhance calcium absorption resulting in higher BMD values. However, this increase was only demonstrated for Japanese women (139), and was not found in Caucasian ones (174), where *A* allele correlate with lower fracture risk independently of BMD values. Despite this controversial results, the functionality of this polymorphism has been demonstrated but this last issue (fracture risk) requires further studies. In fact, a recent study (193) regarding haplotype alleles of the 5' promoter region and of the 3' UTR region, strongly associated with increased fracture risk, was performed. This study demonstrates lower *VDR* mRNA levels in an osteoblast cell line harbouring the fracture risk haplotype. Low *VDR* mRNA levels impact on vitamin D signalling efficiency and might contribute to the increased fracture risk observed for these risk haplotype alleles.

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## References

1. Baker AR, McDonnell DP, Hughes M et al. Cloning and expression of full-length cDNA encoding human vitamin D receptor. *Proc Natl Acad Sci USA*. 1988;85:3294-3298.
2. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science*. 1988;240:889-895.
3. Jones G, Strugnelli SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. *Physiol Rev*. 1998;78:1193-1231.
4. McDonnell DP, Mangelsdorf DJ, Pike JW et al. Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. *Science*. 1987;235:1214-1217.
5. Miyamoto K, Kesterson RA, Yamamoto H et al. Structural organization of the human vitamin D receptor chromosomal gene and its promoter. *Mol Endocrinol*. 1997;11:1165-1179.
6. Crofts LA, Hancock MS, Morrison NA et al. Multiple promoters direct the tissue-specific expression of novel N-terminal variant human vitamin D receptor gene transcripts. *Proc Natl Acad Sci USA*. 1998;95:10529-10534.
7. Huang MC, Seyer JM, Thompson JP et al. Genomic organization of the human procollagen alpha 1(II) collagen gene. *Eur J Biochem*. 1991;195:593-600.
8. Takahashi E, Hori T, Sutherland GR. Mapping of the human type II collagen gene (COL2A1) proximal to fra(12) (q13.1) by nonisotopic in situ hybridization. *Cytogenet Cell Genet*. 1990;54:84-85.
9. Makin G, Lohnes D, Byford V et al. Target cell metabolism of 1,25-dihydroxyvitamin D3 to calcitric acid. Evidence for a pathway in kidney and bone involving 24-oxidation. *Biochem J*. 1989;262:173-180.
10. Ross TK, Prah J, DeLuca HF. Overproduction of rat 1,25-dihydroxyvitamin D3 receptor in insect cells using the baculovirus expression system. *Proc Natl Acad Sci USA*. 1991;88:6555-6559.
11. DeLuca HF, Schnoes HK. Metabolism and mechanism of action of vitamin D. *Ann Rev Biochem*. 1976;45:631-666.
12. Dame MC, Pierce EA, DeLuca HF. Identification of the porcine intestinal 1,25-dihydroxyvitamin D3 receptor on sodium dodecyl sulfate/polyacrylamide gels by renaturation and immunoblotting. *Proc Natl Acad Sci USA*. 1985;82:7825-7829.
13. Hutchinson KA, Scherrer RC, Czar MJ et al. Regulation of glucocorticoid receptor function through assembly of a receptor-heat shock protein complex. *Ann NY Acad Sci*. 1993;684:35-48.
14. Stumpf WE, Sar M, Reid FA et al. Target cells for 1,25-dihydroxyvitamin D3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. *Science*. 1979;206:1188-1190.
15. Aranda A, Pascual A. Nuclear hormone receptors and gene expression. *Physiol Rev*. 2001;81:1269-1304.
16. Schrader M, Nayeri S, Kahlen JP et al. Natural vitamin D3 response elements formed by inverted palindromes: polarity-directed ligand sensitivity of vitamin D3 receptor-retinoid X receptor heterodimer-mediated transactivation. *Mol Cell Biol*. 1995;15:1154-1161.
17. Thummel KE, Brimer C, Yasuda K et al. Transcriptional control of intestinal cytochrome P-4503A by 1alpha,25-dihydroxy vitamin D3. *Mol Pharmacol*. 2001;60:1399-1406.
18. Drocourt L, Ourlin JC, Pascussi JM et al. Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J Biol Chem*. 2002;277:25125-25132.
19. Thompson PD, Jurutka PW, Whitfield GK et al. Liganded VDR induces CYP3A4 in small intestinal and colon cancer cells via DR3 and ER6 vitamin D responsive elements. *Biochem Biophys Res Commun*. 2002;299:730-738.
20. Masuyama H, Brownfield CM, St-Arnaud R et al. Evidence for ligand-dependent intramolecular folding of the AF-2 domain in vitamin D receptor-activated transcription and coactivator interaction. *Mol Endocrinol*. 1997;11:1507-1517.
21. Onate SA, Tsai SY, Tsai MJ et al. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science*. 1995;270:1354-1357.
22. Renaud JP, Rochel N, Ruff M et al. Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. *Nature*. 1995;378:681-689.
23. Wagner RL, Apriletti JW, McGrath ME et al. A structural role for hormone in the thyroid hormone receptor. *Nature*. 1995;378:690-697.
24. Chen H, Lin RJ, Schiltz RL et al. Nuclear receptor coactivator AC-TR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell*. 1997;90:569-580.
25. Jenster G, Spencer TE, Burcin MM et al. Steroid receptor induction of gene transcription: a two-step model. *Proc Natl Acad Sci USA*. 1997;94:7879-7884.
26. Kamei Y, Xu L, Heinzl T et al. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*. 1996;85:403-414.
27. Slominski A, Wortsman J. Neuroendocrinology of the skin. *Endocr Rev*. 2000;21:457-487.
28. Guyton KZ, Kensler TW, Posner GH. Cancer chemoprevention using natural vitamin D and synthetic analogs. *Annu Rev Pharmacol Toxicol*. 2001;41:421-442.
29. O'Kelly J, Hisatake J, Hisatake Y et al. Normal myelopoiesis but abnormal T lymphocyte responses in vitamin D receptor knockout mice. *J Clin Invest*. 2002;109:1091-1099.
30. Buckley LM, Hillner BE. A cost effectiveness analysis of calcium and vitamin D supplementation, etidronate, and alendronate in the prevention of vertebral fractures in women treated with glucocorticoids. *J Rheumatol*. 2003;30:132-138.
31. Chapuy MC, Arlot ME, Duboeuf et al. Vitamin D3 and calcium to prevent hip fractures in elderly women. *New Engl J Med*. 1992;327:1637-1642.
32. Racz A, Barsony J. Hormone-dependent translocation of vitamin D receptors is linked to transactivation. *J Biol Chem*. 1999;274:19352-19360.
33. Barsony J, McKoy W. Molybdate increases intracellular 3',5'-guanosine cyclic monophosphate and stabilizes vitamin D receptor association with tubulin-containing filaments. *J Biol Chem*. 1992;267:24457-24465.
34. Haussler MR, Haussler CA, Jurutka PW et al. The vitamin D hormone and its nuclear receptor: molecular actions and disease states. *J Endocrinol*. 1997;154 Suppl:S57-S73.
35. Haussler MR, Whitfield GK, Haussler CA et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res*. 1998;13:325-349.
36. Carlberg C. Ligand-mediated conformational changes of the VDR are required for gene transactivation. *J Steroid Biochem Mol Biol*. 2004;89-90:227-232.
37. Jurutka PW, Whitfield GK, Hsieh JC et al. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. *Rev Endocr Metab Disord*. 2001;2:203-216.
38. Rachez C, Freedman LP. Mechanisms of gene regulation by vitamin D(3) receptor: a network of coactivator interactions. *Gene*. 2000;246:9-21.
39. Leong GM, Subramaniam N, Issa LL et al. Ski-interacting protein, a bifunctional nuclear receptor coregulator that interacts with N-CoR/SMRT and p300. *Biochem Biophys Res Commun*. 2004;315:1070-1076.
40. Dunlop TW, Vaisanen S, Frank C et al. The genes of the coactivator TIF2 and the corepressor SMRT are primary 1alpha,25(OH)2 D3 targets. *J Steroid Biochem Mol Biol*. 2004;89-90:257-260.
41. Kato S, Fujiki R, Kitagawa H. Vitamin D receptor (VDR) promoter targeting through a novel chromatin remodeling complex. *J Steroid Biochem Mol Biol*. 2004;89-90:173-8.
42. Fleet JC. Genomic and proteomic approaches for probing the role of vitamin D in health. *Am J Clin Nutr*. 2004;80:1730S-1734S.
43. Rasmussen H, DeLuca HF. Calcium homeostasis. *Ergeb Physiol*. 1963;53:108-173.
44. Rubin RP, Weiss GB, Putney JW Jr. Calcium in biological systems. (Editors) New York: Plenum, 1985, 1-737.
45. Siegel N, Wongsurawat N, Armbrrecht HJ. Parathyroid hormone stimulates dephosphorylation of the renoredoxin component of the 25-hydroxyvitamin D3-1 alpha-hydroxylase from rat renal cortex. *J Biol Chem*. 1986;261:16998-17003.
46. Abou-Samra AB, Juppner H, Kong XF et al. Structure, function,



- and expression of the receptor for parathyroid hormone and parathyroid hormone-related peptide. *Adv Nephrol Necker Hosp.* 1994;23:247-264.
47. Brown EM, Gamba G, Riccardi D et al. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature.* 1993;366:575-580.
  48. Horiuchi N, Suda T, Takahashi H et al. In vivo evidence for the intermediary role of 3',5'-cyclic AMP in parathyroid hormone-induced stimulation of 1 $\alpha$ ,25-dihydroxyvitamin D3 synthesis in rats. *Endocrinology.* 1977;101:969-974.
  49. Thakker RV, Fraher LJ, Adami S et al. Circulating concentrations of 1,25-dihydroxyvitamin D3 in patients with primary hyperparathyroidism. *Bone Miner.* 1986;1:137-144.
  50. Boyle IT, Miravet L, Gray RW et al. The response of intestinal calcium transport to 25-hydroxy and 1,25-dihydroxy vitamin D in nephrectomized rats. *Endocrinology.* 1972;90:605-608.
  51. Halloran BP, De Luca HF. Intestinal calcium transport: evidence for two distinct mechanisms of action of 1,25-dihydroxyvitamin D3. *Arch Biochem Biophys.* 1981;208:477-486.
  52. Holick MF, Garabedian M, DeLuca HF. 1,25-dihydroxycholecalciferol: metabolite of vitamin D3 active on bone in anephric rats. *Science.* 1972;176:1146-1147.
  53. Suda T, Takahashi N, Martin TJ Modulation of osteoclast differentiation: update 1995. *Endocr Rev.* 1995;4:266-270.
  54. Tanaka Y, Frank H, DeLuca HF. Biological activity of 1,25-dihydroxyvitamin D3 in the rat. *Endocrinology.* 1973;92:417-422.
  55. Kumar R Vitamin D and the kidney. In: ed. D. Feldman, FH Glorieux, and JW Pike Vitamin D. San Diego, CA: Academic, 1997, chapt 17:275-292.
  56. Yamamoto M, Kawanobe Y, Takahashi H et al. Vitamin D deficiency and renal calcium transport in the rat. *J Clin Invest.* 1984;74:507-513.
  57. Garabedian M, Tanaka Y, Holick MF et al. Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D3 in thyroparathyroidectomized rats. *Endocrinology.* 1974;94:1022-1027.
  58. Chambers TJ, Magnus CJ. Calcitonin alters behaviour of isolated osteoclasts. *J Pathol.* 1982;136:27-39.
  59. Beckman MJ, Goff JP, Reinhardt TA et al. In vivo regulation of rat intestinal 24-hydroxylase: potential new role of calcitonin. *Endocrinology.* 1994;135:1951-1955.
  60. Galante L, Colston KW, MacAuley SJ et al. Effect of calcitonin on vitamin D metabolism. *Nature.* 1972;238:271-273.
  61. Boyle IT, Gray RW, Omdahl JL et al. Calcium control of an in vivo biosynthesis of 1,25-dihydroxyvitamin D3: Nicolaysen's endogenous factor. In: ed. S. Taylor. *Endocrinology* 1971, London: Heinemann; 1972:468-476.
  62. Bouillon R, Van Cromphaut S, Carmeliet G. Intestinal calcium absorption: Molecular vitamin D mediated mechanisms. *J Cell Biochem.* 2003;88:332-339.
  63. Van Cromphaut SJ, Dewerchin M, Hoenderop JG et al. Duodenal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects. *Proc Natl Acad Sci USA.* 2001;98:13324-13329.
  64. van Abel M, Hoenderop JG, van der Kemp AW et al. Regulation of the epithelial Ca2+ channels in small intestine as studied by quantitative mRNA detection. *Am J Physiol Gastrointest Liver Physiol.* 2003;285:G78-G85.
  65. Hoenderop JG, Chon H, Gkika D et al. Regulation of gene expression by dietary Ca2+ in kidneys of 25-hydroxyvitamin D3-1  $\alpha$ -hydroxylase knockout mice. *Kidney Int.* 2004;65:531-9.
  66. Chen TC, Castillo L, Korycka-Dahl M et al. Role of vitamin D metabolites in phosphate transport of rat intestine. *J Nutr.* 1974; 104:1056-1060.
  67. Harrison HE, Harrison HC. Intestinal transport of phosphate: action of vitamin D, calcium, and potassium. *Am J Physiol.* 1961; 201:1007-1012.
  68. Kowarski S, Schachter D. Effects of vitamin D on phosphate transport and incorporation into mucosal constituents of rat intestinal mucosa. *J Biol Chem.* 1969;244:211-217.
  69. Tenenhouse HS. Cellular and molecular mechanisms of renal phosphate transport. *J Bone Miner Res.* 1997;12:159-164.
  70. Taketani Y, Miyamoto K, Tanaka K et al. Gene structure and functional analysis of the human Na+/phosphate co-transporter. *Biochem J.* 1997;324:927-934.
  71. Rowe PS, Goulding JN, Francis F et al. The gene for X-linked hypophosphataemic rickets maps to a 200-300kb region in Xp22.1, and is located on a single YAC containing a putative vitamin D response element (VDRE). *Hum Genet.* 1996;97:345-352.
  72. Francis F, Hennig S, Korn B et al. A gene (PEX) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. *Nat Genet.* 1995;11:130-136.
  73. Econs MJ, Drezner MK. Tumor-induced osteomalacia--unveiling a new hormone. *N Engl J Med.* 1994;330:1679-1681.
  74. Cai Q, Hodgson SF, Kao PC et al. Brief report: inhibition of renal phosphate transport by a tumor product in a patient with oncogenic osteomalacia. *N Engl J Med.* 1994;330:1645-1649.
  75. Yagci A, Werner A, Murer H et al. Effect of rabbit duodenal mRNA on phosphate transport in *Xenopus laevis* oocytes: dependence on 1,25-dihydroxy-vitamin-D3. *Pflugers Arch.* 1992;422:211-216.
  76. Kurnik BR, Hruska KA. Mechanism of stimulation of renal phosphate transport by 1,25-dihydroxycholecalciferol. *Biochim Biophys Acta.* 1985;817:42-50.
  77. Raisz LG, Trummel CL, Holick MF et al. 1,25-dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. *Science.* 1972;175:768-769.
  78. Stern PH 1,25-DihydroxyvitaminD3 interactions with local factors in bone remodelling. In: ed. D. Feldman, FH Glorieux, and JW Pike. *Vitamin D* San Diego, CA: Academic, 1997, chapt 22:341-352.
  79. Merke J, Klaus G, Hugel U et al. No 1,25-dihydroxyvitamin D3 receptors on osteoclasts of calcium-deficient chicken despite demonstrable receptors on circulating monocytes. *J Clin Invest.* 1986;77:312-314.
  80. McSheehy PM, Chambers TJ. Osteoblast-like cells in the presence of parathyroid hormone release soluble factor that stimulates osteoclastic bone resorption. *Endocrinology.* 1986;119:1654-1659.
  81. McSheehy PM, Chambers TJ. 1,25-Dihydroxyvitamin D3 stimulates rat osteoblastic cells to release a soluble factor that increases osteoclastic bone resorption. *J Clin Invest.* 1987;80:425-429.
  82. Suda T, Takahashi N. Vitamin D and osteoclastogenesis. In: ed. D. Feldman, FH Glorieux, and JW Pike. *Vitamin D* San Diego, CA: Academic, 1997, chapt 21:329-340.
  83. Talmage RV. Morphological and physiological considerations in a new concept of calcium transport in bone. *Am J Anat.* 1970;129: 467-476.
  84. Gallagher JC, Riggs BL. Action of 1,25-dihydroxyvitamin D3 on calcium balance and bone turnover and its effect on vertebral fracture rate. *Metabolism.* 1990;39(4 Suppl 1):30-34.
  85. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature.* 2003;423:337-342.
  86. Khosla S. Minireview: the OPG/RANKL/RANK system. *Endocrinology.* 2001;142:5050-5055.
  87. Kitazawa S, Kajimoto K, Kondo T et al. Vitamin D3 supports osteoclastogenesis via functional vitamin D response element of human RANKL gene promoter. *J Cell Biochem.* 2003;89:771-777.
  88. Kondo T, Kitazawa R, Maeda S et al. 1  $\alpha$ ,25 dihydroxyvitamin D3 rapidly regulates the mouse osteoprotegerin gene through dual pathways. *J Bone Miner Res.* 2004;19:1411-1419.
  89. Li YC, Pirro AE, Demay MB Analysis of vitamin D-dependent calcium-binding protein messenger ribonucleic acid expression in mice lacking the vitamin D receptor. *Endocrinology.* 1998;139:847-51.
  90. Darwish HM, Krisinger J, Strom M et al. Molecular cloning of the cDNA and chromosomal gene for vitamin D-dependent calcium-binding protein of rat intestine. *Proc Natl Acad Sci USA.* 1987; 84:6108-6011.
  91. Henry HL, Norman AW. Studies on the mechanism of action of calciferol VII. Localization of 1,25-dihydroxy-vitamin D3 in chick parathyroid glands. *Biochem Biophys Res Commun.* 1975;62:781-788.
  92. Hughes MR, Haussler MR. 1,25-Dihydroxyvitamin D3 receptors in

- parathyroid glands. Preliminary characterization of cytoplasmic and nuclear binding components. *J Biol Chem.* 1978;253:1065-1073.
93. Demay MB, Kiernan MS, DeLuca HF et al. Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D3 receptor and mediate transcriptional repression in response to 1,25-dihydroxyvitamin D3. *Proc Natl Acad Sci USA.* 1992;89:8097-8101.
  94. Kronenberg HM, Igarashi T, Freeman MW et al. Structure and expression of the human parathyroid hormone gene. *Recent Prog Horm Res.* 1986;42:641-663.
  95. Silver J, Russell J, Sherwood LM. Regulation by vitamin D metabolites of messenger ribonucleic acid for preproparathyroid hormone in isolated bovine parathyroid cells. *Proc Natl Acad Sci USA.* 1985;82:4270-4273.
  96. Sugimoto T, Brown AJ, Ritter C et al. Combined effects of dexamethasone and 1,25-dihydroxyvitamin D3 on parathyroid hormone secretion in cultured bovine parathyroid cells. *Endocrinology.* 1989;125:638-641.
  97. Mackey SL, Heymont JL, Kronenberg HM et al. Vitamin D receptor binding to the negative human parathyroid hormone vitamin D response element does not require the retinoid X receptor. *Mol Endocrinol.* 1996;10:298-305.
  98. Brown AJ, Zhong M, Finch J et al. Rat calcium-sensing receptor is regulated by vitamin D but not by calcium. *Am J Physiol.* 1996;270:F454-F460.
  99. Wiese RJ, Uhland-Smith A, Ross TK et al. Up-regulation of the vitamin D receptor in response to 1,25-dihydroxyvitamin D3 results from ligand-induced stabilization. *J Biol Chem.* 1992;267:20082-20086.
  100. Canaff L, Hendy GN. Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. *J Biol Chem.* 2002;277:30337-30350.
  101. Liu W, Yu WR, Carling T et al. Regulation of gp330/megalyn expression by vitamins A and D. *Eur J Clin Invest.* 1998;28:100-107.
  102. Friedman PA, Gesek FA. Vitamin D3 accelerates PTH-dependent calcium transport in distal convoluted tubule cells. *Am J Physiol.* 1993;265:F300-F308.
  103. Hoenderop JG, Muller D, Van Der Kemp AW et al. Calcitriol controls the epithelial calcium channel in kidney. *J Am Soc Nephrol.* 2001;12:1342-1349.
  104. Dace A, Martin-el Yazidi C, Bonne J et al. Calcitriol is a positive effector of adipose differentiation in the OB 17 cell line: relationship with the adipogenic action of triiodothyronine. *Biochem Biophys Res Commun.* 1997;232:771-776.
  105. Hosomi J, Hosoi J, Abe E et al. Regulation of terminal differentiation of cultured mouse epidermal cells by 1 alpha,25-dihydroxyvitamin D3. *Endocrinology.* 1983;113:1950-1957.
  106. Botling J, Oberg F, Torma H et al. Vitamin D3- and retinoic acid-induced monocytic differentiation: interactions between the endogenous vitamin D3 receptor, retinoic acid receptors, and retinoid X receptors in U-937 cells. *Cell Growth Differ.* 1996;7:1239-1249.
  107. Iwata K, Koultab N, Ogata H et al. Differential regulation of vitamin D receptors in clonal populations of a chronic myelogenous leukemia cell line. *Exp Cell Res.* 1996;225:143-150.
  108. Nakajima H, Kizaki M, Ueno H et al. All-trans and 9-cis retinoic acid enhance 1,25-dihydroxyvitamin D3-induced monocytic differentiation of U937 cells. *Leuk Res.* 1996;20:665-676.
  109. Abe E, Miyaura C, Sakagami H et al. Differentiation of mouse myeloid leukemia cells induced by 1 alpha,25-dihydroxyvitamin D3. *Proc Natl Acad Sci USA.* 1981;78:4990-4994.
  110. Liu M, Lee MH, Cohen M et al. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. *Genes Dev.* 1996;10:142-153.
  111. Li P, Li C, Zhao X et al. p27(Kip1) stabilization and G(1) arrest by 1,25-dihydroxyvitamin D(3) in ovarian cancer cells mediated through down-regulation of cyclin E/cyclin-dependent kinase 2 and Skp1-Cullin-F-box protein/Skp2 ubiquitin ligase. *J Biol Chem.* 2004;279:25260-25267.
  112. Cordero JB, Cozzolino M, Lu Y et al. 1,25-Dihydroxyvitamin D down-regulates cell membrane growth- and nuclear growth-promoting signals by the epidermal growth factor receptor. *J Biol Chem.* 2002;277:38965-38971.
  113. Lamb J, Ramaswamy S, Ford HL et al. A mechanism of cyclin D1 action encoded in the patterns of gene expression in human cancer. *Cell.* 2003;114:323-334.
  114. Baldwin BR, Timchenko NA, Zahnow CA. Epidermal growth factor receptor stimulation activates the RNA binding protein CUG-BP1 and increases expression of C/EBPbeta-LIP in mammary epithelial cells. *Mol Cell Biol.* 2004;24:3682-3691.
  115. Ji Y, Studzinski GP. Retinoblastoma protein and CCAAT/enhancer-binding protein beta are required for 1,25-dihydroxyvitamin D3-induced monocytic differentiation of HL60 cells. *Cancer Res.* 2004;64:370-377.
  116. Uitterlinden AG, Fang Y, van Meurs JB et al. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. *J Steroid Biochem Mol Biol.* 2004;89-90:187-193.
  117. Valrance ME, Welsh J. Breast cancer cell regulation by high-dose Vitamin D compounds in the absence of nuclear vitamin D receptor. *J Steroid Biochem Mol Biol.* 2004;89-90:221-225.
  118. Wagner N, Wagner KD, Schley G et al. 1,25-dihydroxyvitamin D3-induced apoptosis of retinoblastoma cells is associated with reciprocal changes of Bcl-2 and bax. *Exp Eye Res.* 2003;77:1-9.
  119. Mathiasen IS, Sergeev IN, Bastholm L et al. Calcium and calpain as key mediators of apoptosis-like death induced by vitamin D compounds in breast cancer cells. *J Biol Chem.* 2002;277:30738-30745.
  120. Sergeev IN Calcium as a mediator of 1,25-dihydroxyvitamin D3-induced apoptosis. *J Steroid Biochem Mol Biol.* 2004;89-90:419-425.
  121. Elias J, Marian B, Edling C et al. Induction of apoptosis by vitamin D metabolites and analogs in a glioma cell line. *Recent Results Cancer Res.* 2003;164:319-332.
  122. Sauer B, Ruwisch L, Kleuser B. Antiapoptotic action of 1alpha,25-dihydroxyvitamin D3 in primary human melanocytes. *Melanoma Res.* 2003;13:339-347.
  123. Diker-Cohen T, Koren R, Liberman UA et al. Vitamin D protects keratinocytes from apoptosis induced by osmotic shock, oxidative stress, and tumor necrosis factor. *Ann N Y Acad Sci.* 2003;1010:350-353.
  124. Palmer HG, Larriba MJ, Garcia JM et al. The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. *Nat Med.* 2004;10:917-919.
  125. Khanim FL, Gommersall LM, Wood VH et al. Altered SMRT levels disrupt vitamin D3 receptor signalling in prostate cancer cells. *Oncogene.* 2004;23:6712-6725.
  126. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol.* 2005;289:F8-F28.
  127. Gorgoni B, Maritano D, Marthyn P et al. C/EBP beta gene inactivation causes both impaired and enhanced gene expression and inverse regulation of IL-12 p40 and p35 mRNAs in macrophages. *J Immunol.* 2002;168:4055-4062.
  128. Esteban L, Vidal M, Dusso A. 1alpha-Hydroxylase transactivation by gamma-interferon in murine macrophages requires enhanced C/EBPbeta expression and activation. *J Steroid Biochem Mol Biol.* 2004;89-90:131-137.
  129. Hayes CE, Nashold FE, Spach KM et al. The immunological functions of the vitamin D endocrine system. *Cell Mol Biol. (Noisy-le-grand).* 2003;49:277-300.
  130. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. *Trends Mol Med.* 2002;8:174-179.
  131. Alroy I, Towers TL, Freedman LP. Transcriptional repression of the interleukin-2 gene by vitamin D3: direct inhibition of NFATp/AP-1 complex formation by a nuclear hormone receptor. *Mol Cell Biol.* 1995;15:5789-5799.

132. Adorini L, Penna G, Giarratana N et al. Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. *J Steroid Biochem Mol Biol.* 2004;89-90:437-441.
133. Yanagisawa J, Yanagi Y, Masuhiro Y et al. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science.* 1999;283:1317-1321.
134. Faraco JH, Morrison NA, Baker A et al. Apal dimorphism at the human vitamin D receptor gene locus. *Nucleic Acids Res.* 1989; 17:2150.
135. Morrison NA, Yeoman R, Kelly PJ et al. Contribution of trans-acting factors alleles to normal physiological variability: Vitamin D receptor gene polymorphisms and circulating osteocalcin. *Proc Natl Acad Sci USA.* 1992;89:6665-6669.
136. Arai H, Miyamoto KI, Yoshida M et al. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. *J Bone Miner Res.* 2001;16: 1256-1264.
137. Gunnes M, Berg JP, Halse J. et al. Lack of relationship between Vitamin D receptor genotype and forearm bone gain in healthy children, adolescents and young adults. *J Clin Endocrinol Metab.* 1997;82:851-855.
138. Morrison NA, Cheng JQI, Akifumi T et al. Prediction of bone density from Vitamin D receptor alleles. *Nature.* 1994;367:284-287.
139. Suarez F, Zeghoud F, Rossignol C et al. Association between Vitamin D receptor gene polymorphism and sex-dependent growth during the first 2 years of life. *J Clin Endocrinol Metab.* 1997; 82:2966-2970.
140. Ye WZ, Reis AF, Velho G. Identification of a novel Tru9 I polymorphism in the human vitamin D receptor gene. *J Hum Genet.* 2000;45:56-57.
141. Ingles SA, Ross RK, Yu MC et al. Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. *J Natl Cancer Inst.* 1997;89:166-170.
142. Sainz J, Van Tornout JM, Loro ML et al. Vitamin D receptor gene polymorphisms and bone density in pre-pubertal American girls of Mexican descent. *New Engl J Med.* 1997;337:77-82.
143. Ferrari SL, Rizzoli R, Slosman DO et al. Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? *J Bone Miner Res.* 1998;13:363-370.
144. Tao C, Yu T, Garnett et al. Vitamin D receptor alleles predict growth and bone density in girls. *Arch Dis Child.* 1998;79:488-493.
145. Lorentzon M, Lorentzon R, Nordstrom P. Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy Caucasian men: a cross-sectional and longitudinal study. *J Clin Endocrinol Metab.* 2000;85: 1666-1670.
146. Garnero P, Borel O, Sornay-Rendu E. et al. Vitamin D receptor gene polymorphisms do not predict bone turnover and bone mass in healthy pre-menopausal women. *J Bone Miner Res.* 1995;10:1283-1288.
147. Garnero P, Borel O, Sornay-Rendu E. et al. Vitamin D receptor gene polymorphisms are not related to bone turnover, rate of bone loss and bone mass in post-menopausal women: the OFELY study. *J Bone Miner Res.* 1996;11:827-834.
148. Zmuda JM, Cauley JA, Danielson ME et al. Vitamin D receptor gene polymorphisms, bone turnover and rates of bone loss in older African-American women. *J Bone Miner Res.* 1997;12:1446-1452.
149. Howard G, Nguyen T, Morrison N et al. Genetic influences on bone density: physiological correlates of Vitamin D receptor gene alleles in pre-menopausal women. *J Clin Endocrinol Metab.* 1995; 80:2800-2805.
150. Keen RW, Major PJ, Lanchbury JS et al. Vitamin D receptor gene polymorphisms and bone loss. *Lancet.* 1995;345:990.
151. Kikuchi R, Uemura T, Gorai I et al. Early and late post-menopausal bone loss is associated with Bsm I Vitamin D receptor gene polymorphism in Japanese women. *Calcif Tissue Int.* 1999;64:102-106.
152. Gomez C, Naves ML, Barrios Y et al. Vitamin D receptor gene polymorphisms, bone mass, bone loss and prevalence of vertebral fracture: difference in post-menopausal women and men. *Osteoporos Int.* 1999;10:175-182.
153. Houston LA, Grant SFA, Reid DM et al. Vitamin D receptor polymorphism, bone mineral density and osteoporotic vertebral fracture: studies in a UK population. *Bone.* 1996;18:249-252.
154. Langdahl BL, Gravholt CH, Brixen K et al. Polymorphisms in the Vitamin D receptor gene and bone mass, bone turnover and osteoporotic fractures. *Eur J Clin Invest.* 2000;30:608-617.
155. Berg JP, Falch JA, Haug E. Fracture rate, pre- and post-menopausal bone mass and early and late post-menopausal bone loss are not associated with Vitamin D receptor genotype in a high endemic area of osteoporosis. *Eur J Endocrinol.* 1996;135:96-100.
156. Yanagy H, Tomura H, Kawanami K et al. Vitamin D receptor gene polymorphisms are associated with osteoporosis in Japanese women. *J Clin Endocrinol Metab.* 1996;81:4179-4180.
157. Aerssens J, Dequeker J, Peeters J et al. Polymorphisms of the VDR, ER and COLIA1 genes and osteoporotic hip fracture in elderly post-menopausal women. *Osteoporos Int.* 2000;11:583-591.
158. Kinyamu UK, Gallagher JC, Knezetic JA et al. Effect of vitamin D receptor genotypes on calcium absorption, duodenal vitamin D receptor concentration, and serum 1,25 dihydroxyvitamin D levels in normal women. *Calcif Tissue Int.* 1997;60:491-495.
159. Uitterlinden AG, Weel AE, Burger H et al. Interaction between the Vitamin D receptor gene and collagen type I 1 gene in susceptibility for fracture. *J Bone Miner Res.* 2001;16:379-385.
160. Wishart JM, Horowitz M, Need AG et al. Relations between calcium intake, calcitriol, polymorphisms of vitamin D receptor gene, and calcium absorption in premenopausal women. *Am J Clin Nutr.* 1997;65:798-802.
161. Ferrari S, Rizzoli R, Chevallery T et al. Vitamin D receptor gene polymorphisms and change in lumbar spine bone mineral density. *Lancet.* 1995;345:423-424.
162. Salamone LM, Glynn NW, Black DM et al. Determinants of post-menopausal bone mineral density: the interplay of genetic and lifestyle factors. *J Bone Miner Res.* 1996;11:1557-1565.
163. Kiel DP, Myers RH, Cupples LA et al. The BsmI vitamin D receptor restriction fragment length polymorphism (bb) influences the effect of calcium intake on bone mineral density. *J Bone Miner Res.* 1997;12:1049-1057.
164. Gong G, Stern HS, Cheng SC et al. The association of bone mineral density with vitamin D receptor gene polymorphisms. *Osteoporos Int.* 1999;9:55-64.
165. Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. *J Bone Miner Res.* 1996;11:1841-1849.
166. Thakkestian A, D'Este C, Eisman J et al. Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. *J Bone Miner Res.* 2004;19: 419- 428.
167. Gennari L, Becherini L, Masi et al. Vitamin D and estrogen receptor allelic variants in postmenopausal women: evidence of multiple gene contribution on bone mineral density. *J Clin Endocrinol Metab.* 1998;83:939-944.
168. Willing M, Sowers M, Aron D et al. Bone mineral density and its change in white women: estrogen and vitamin D receptor genotypes and their interaction. *J Bone Miner Res.* 1998;13:695-705.
169. Gross C, Eccleshall TR, Malloy PJ et al. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in post-menopausal Mexican-American women. *J Bone Miner Res.* 1996; 11:1850-1855.
170. Arai H, Miyamoto K, Taketani Y et al. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *J Bone Miner Res.* 1997;12:915-921.
171. Harris SS, Eccleshall TR, Gross C et al. The vitamin D receptor

- start codon polymorphism (Fok I) and bone mineral density in premenopausal American black and white women. *J Bone Miner Res.* 1997;12:1043-1048.
172. Gennari L, Becherini L, Mansani R et al. Fok I polymorphism at translation initiation site of the vitamin D receptor gene predicts bone mineral density and vertebral fractures in postmenopausal Italian women. *J Bone Miner Res.* 1999;14:1379-86.
173. Eccleshall TR, Garnero P, Gross C et al. Lack of correlation between start codon polymorphism of the vitamin D receptor gene and bone mineral density in pre-menopausal French women: the OFELY study. *J Bone Miner Res.* 1998;13:31-35.
174. Laaksonen MM, Karkkainen MU, Outila TA et al. Vitamin D receptor gene start codon polymorphism (FokI) is associated with forearm bone mineral density and calcaneal ultrasound in Finnish adolescent boys but not in girls. *J Bone Miner Metab.* 2004;22:479-485.
175. Fang Y, van Meurs JB, Bergink AP et al. Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. *J Bone Miner Res.* 2003;18:1632-1641.
176. Tajouri L, Ovcaric M, Curtain R, et al. Variation in the vitamin D receptor gene is associated with multiple sclerosis in an Australian population. *J Neurogenet.* 2005;19:25-38.
177. Akcay A, Ozdemir FN, Sezer S et al. Association of vitamin D receptor gene polymorphisms with hypercalcemia in peritoneal dialysis patients. *Perit Dial Int.* 2005;25 Suppl 3:S52-55.
178. Carling T, Rastad J, Akerstrom G et al. Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors. *J Clin Endocrinol Metab.* 1998;83:2255-2259.
179. Vigo Gago E, Cadarso-Suarez C, Perez-Fernandez R et al. Association between vitamin D receptor FokI. Polymorphism and serum parathyroid hormone level in patients with chronic renal failure. *J Endocrinol Invest.* 2005;28:117-121.
180. Park K, Woo M, Nam J et al. Start codon polymorphisms in the vitamin D receptor and colorectal cancer risk. *Cancer Lett.* 2005 Jul 11; [Epub ahead of print]
181. John EM, Schwartz GG, Koo J et al. Sun exposure, vitamin D receptor gene polymorphisms, and risk of advanced prostate cancer. *Cancer Res.* 2005;65:5470-5479.
182. Lowe LC, Guy M, Mansi JL et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. *Eur J Cancer.* 2005;41:1164-1169.
183. Wall JD, Pritchard JK. Haplotype blocks and linkage disequilibrium in the human genome. *Nat Rev Genet.* 2003;4:587-597.
184. Thakkestian A, D'Este C, Attia J. Haplotype analysis of VDR gene polymorphisms: a meta-analysis. *Osteoporos Int.* 2004;15:729-734.
185. Mocharla H, Butch AW, Pappas AA, Flick JT, Weistein RS, De Togni P, Jilka RL, Roberson PK, Parfitt AM, Manolagas SC. Quantification of vitamin D receptor mRNA by competitive polymerase chain reaction in PBMC: lack of correspondence with common allelic variants. *J Bone Miner Res.* 1997;12:726-733.
186. Verbeeck W, Gombart AF, Shiohara M et al. Vitamin D receptor: no evidence for allele-specific mRNA stability in cells that are heterozygous for the Taq I restriction enzyme polymorphism. *Biochem Biophys Res Commun.* 1997;238:77-80.
187. Gross C, Musiol IM, Eccleshall TR et al. Vitamin D receptor gene polymorphisms: analysis of ligand binding and hormone responsiveness in cultured skin fibroblasts. *Biochem Biophys Res Commun.* 1998;242:467-473.
188. Durrin LK, Haile RW, Ingles SA et al. Vitamin D receptor 3'-untranslated region polymorphisms: lack of effect on mRNA stability. *Biochim Biophys Acta.* 1999;1453:311-320.
189. Whitfield GK, Remus LS, Jurutka PW et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Mol Cell Endocrinol.* 2001;177:145-159.
190. Gross C, Krishnan AV, Malloy PJ et al. The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. *J Bone Miner Res.* 1998;13:1691-1699.
191. Jurutka PW, Remus LS, Whitfield GK et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. *Mol Endocrinol.* 2000;14:401-420.
192. Colin EM, Weel AE, Uitterlinden AG et al. Consequences of vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1, 25-dihydroxyvitamin D3. *Clin Endocrinol.* 2000;52:211-216.
193. Fang Y, van Meurs JB, D'Alessio A et al. Promoter and 3'-untranslated region haplotypes in the vitamin D receptor gene predispose to osteoporotic fracture: the Rotterdam study. *Am J Hum Genet.* 2005;76:807-823.