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Significance of the Vitamin D Receptor on Crosstalk with Nuclear Receptors and Regulation of Enzymes and Transporters

Keumhan Noh^{1,2} · Edwin C. Y. Chow^{1,3} · Holly P. Quach¹ · Geny M. M. Groothuis⁴ · Rommel G. Tirona⁵ · K. Sandy Pang¹

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Abstract

The vitamin D receptor (VDR), in addition to other nuclear receptors, the pregnane X receptor (PXR) and constitutive androstane receptor (CAR), is involved in the regulation of enzymes, transporters and receptors, and therefore intimately affects drug disposition, tissue health, and the handling of endogenous and exogenous compounds. This review examines the role of $1\alpha,25$ -dihydroxyvitamin D_3 or calcitriol, the natural VDR ligand, on activation of the VDR and its crosstalk with other nuclear receptors towards the regulation of enzymes and transporters, notably many of the cytochrome P450s including CYP3A4 and sulfotransferase 2A1 (SULT2A1) as well as cholesterol 7α -hydroxylase (CYP7A1). Moreover, the VDR upregulates the intestinal channel, TRPV6, for calcium absorption, LDL receptor-related protein 1 (LRP1) and receptor for advanced glycation end products (RAGE) in brain for β -amyloid peptide efflux and influx, the sodium phosphate transporters (NaP_i), the apical sodium-dependent bile acid transporter (ASBT) and organic solute transporters (OST α -OST β) for bile acid absorption and efflux, respectively, the renal organic anion transporter 3 (OAT3) and several of the ATP-binding cassette protein transporters—the multidrug resistance protein 1 (MDR1) and the multidrug resistance-associated proteins (MRPs). Hence, the role of the VDR is increasingly being recognized for its therapeutic potential and pharmacologic activity, giving rise to drug-drug interactions (DDI). Therapeutically, ligand-activated VDR shows anti-inflammatory effects towards the suppression of inflammatory mediators, improves cognition by upregulating amyloid-beta ($A\beta$) peptide clearance in brain, and maintains phosphate, calcium, and parathyroid hormone (PTH) balance and kidney function and bone health, demonstrating the crucial roles of the VDR in disease progression and treatment of diseases.

KEY WORDS $1\alpha,25$ -dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] · crosstalk · drug-metabolizing enzymes · transporters · vitamin D receptor (VDR)

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Abbreviations

$1\alpha(OH)D_2$	1α -Hydroxyvitamin D_2
$1\alpha(OH)D_3$	1α -Hydroxyvitamin D_3 or Alfacalcidol®
$1,24(OH)_2D_3$	$1\alpha,24$ -Dihydroxyvitamin D_3 or Tacalcitol®
$1,24,25(OH)_3D_3$	$1\alpha,24,25$ -Trihydroxyvitamin D_3
$1,25(OH)_2D_2$	$1\alpha,25$ -Dihydroxyvitamin D_2
$1,25(OH)_2D_3$	$1\alpha,25$ -Dihydroxyvitamin D_3 or calcitriol (active VDR ligand)
$24,25(OH)_2D_3$	$24,25$ -Dihydroxyvitamin D_3
$25(OH)D_3$	25 -Hydroxyvitamin D_3
$A\beta$	β -Amyloid peptide
ABC	ATP-binding cassette
ADL	Alzheimer's disease
ASBT	Apical sodium-dependent bile acid transporter
BBB	Blood–brain barrier

BCRP	Breast cancer resistance protein
CAR	Constitutive androstane receptor
CDCA	Chenodeoxycholic acid
CKD	Chronic kidney disease
CYP2R1	Cytochrome P450 isoform 2R1, microsomal vitamin D 25-hydroxylase
CYP3A4	Cytochrome P450 isoform 3A4
CYP7A1	Cytochrome P450 isoform 7A1, cholesterol 7 α -hydroxylase
CYP24A1	Cytochrome P450 isoform 24A1, 24-hydroxylase
CYP27A1	Cytochrome P450 isoform 27A1, mitochondrial vitamin D 25-hydroxylase
CYP27B11	Cytochrome P450 isoform 27B1, mitochondrial 1 α -hydroxylase
DBD	DNA-binding domain
DBP	Vitamin D-binding protein
Fgf15	Fibroblast growth factor 15
FGF19	Fibroblast growth factor 19
FGF23	Fibroblast growth factor 23
FGFR4	Fibroblast growth factor receptor 4
FR α	Folate receptor- α
FXR	Farnesoid X receptor
LBD	Ligand-binding domain
LBP	Ligand-binding pocket
LCA	Lithocholic acid
LRH1	Liver receptor homolog 1
LRP1	LDL receptor-related protein 1
LXR α	Liver X receptor- α
MDR1	Multidrug resistance protein 1
MRP2	Multidrug resistance-associated protein 2
MRP3	Multidrug resistance-associated protein 3
MRP4	Multidrug resistance-associated protein 4
NaP _i	Sodium-dependent phosphate cotransporter
NR	Nuclear receptor
OAT1	Organic anion transporter 1
OAT3	Organic anion transporter 3
OST α -OST β	Organic solute transporter- α - β
PCFT	Proton-coupled folate transporter
PEPT1	Oligopeptide transporter 1
P-gp	P-Glycoprotein
PPAR α	Peroxisome proliferator-activated receptor- α
PPAR γ	Peroxisome proliferator-activated receptor- γ
PTH	Parathyroid hormone
PXR	Pregnane X-receptor
RAGE	Receptor for advanced glycation end products

RFC	Reduced folate carrier
RXR	Retinoid X receptor
SHP	Small heterodimer partner
SHPT	Secondary hyperparathyroidism
SLC	Solute carrier
SULT2A1	Sulfotransferase 2A1
TRPV5	Transient receptor potential cation channel, subfamily V, member 5
TRPV6	Transient receptor potential cation channel, subfamily V, member 6
UGT	UDP glucuronosyltransferase
VDR	Vitamin D receptor
VDRE	Vitamin D response element

Introduction

Undeniably, many of the nuclear receptors (NRs) strongly influence the fate of xenobiotics by regulating transporters (1–3) and enzymes (4–8), and therefore drug disposition. NRs are multifunctional proteins that transduce signals of their cognate ligands. As a component of the nuclear receptor class 1 (NR1) superfamily, the vitamin D receptor (VDR) is known for its role in the regulation of transporters and enzymes, affecting the balance of not only endogenous molecules such as cholesterol, bile acids, active peptides, and ions but also the disposition of xenobiotics. Like many nuclear receptors, the VDR heterodimerizes with the retinoid X receptor- α (RXR α), and, upon activation by ligand, initiates gene transcription. Common structures of the NRs include an amino terminal ligand-independent activation function domain (AF-1) for interaction with cofactors, a central DNA-binding domain (DBD) consisting of two zinc finger motifs that target the nuclear receptor to highly specific DNA sequences or DNA response elements, a carboxy-terminal ligand-binding domain (LBD) that differs for each NR and allows for specific hormonal and non-hormonal ligand binding, and a less conserved flexible hinge region that connects the DBD and LBD, governing conformational changes, receptor dimerization, and coregulator interactions at the activation function-2 region (AF-2) for biological response (9, 10). The VDR forms a protein complex as a homodimer or heterodimer with RXR when bound to ligands. The active VDR transcription factor complexes by binding to a specific response element sequences (vitamin D response elements (VDREs)) on target genes that are typically comprised of half-sites of direct or inverted hexanucleotide repeats separated by different nucleotide lengths (11).

The Vitamin D Receptor, VDR

Being an important nuclear receptor for calcium, phosphate, and bone homeostasis, the VDR is present ubiquitously in

tissues such as the intestine, kidney, and skin, although less in the liver and bone (12–16). The VDR protein is almost undetectable in mouse and human livers and is present at low but perceptible levels in specific regions of the brains of mouse (17), rat (18), and human (19). The protein homology and alignment of rodent and human VDR have been examined (20), revealing that the amino acids in the DNA-binding domain are highly conserved across mouse, rat, and human receptors (100%) (Fig. 1). The mouse VDR ligand-binding domain amino acid sequence is 89% identical to that in human and 96% to that in rat. Although the mouse internal region includes a portion of the ligand-binding domain (aa 159 to 206) that differs from that of man and rat, being only 55% and 78% identical to that in human and rat, respectively (20), the actual hinge region (aa 88 to 125) is highly conserved among mice, rats, and humans (97%) as well as

VDR hinge mutations do not affect the ligand-binding affinity (21).

The binding of ligands to the VDR results in a conformational change of the receptor that promotes its translocation from the cytosol to the nucleus and heterodimerization with the RXR α (22). This VDR-RXR α complex then binds to VDREs in the promoter region of VDR-responsive genes, recruiting nuclear proteins/coregulators into the transcriptional pre-invitation complex to initiate gene transcription. There is currently no crystal structure of the unoccupied ligand-binding domain of the VDR. Our understanding of the molecular mechanisms of VDR activation has been advanced with crystal structures of VDR bound to its various ligands, both native and synthetic ones. The crystal structure of VDR, in complex with 1 α ,25-dihydroxyvitamin D₃, active, endogenous VDR ligand (or calcitriol), has revealed

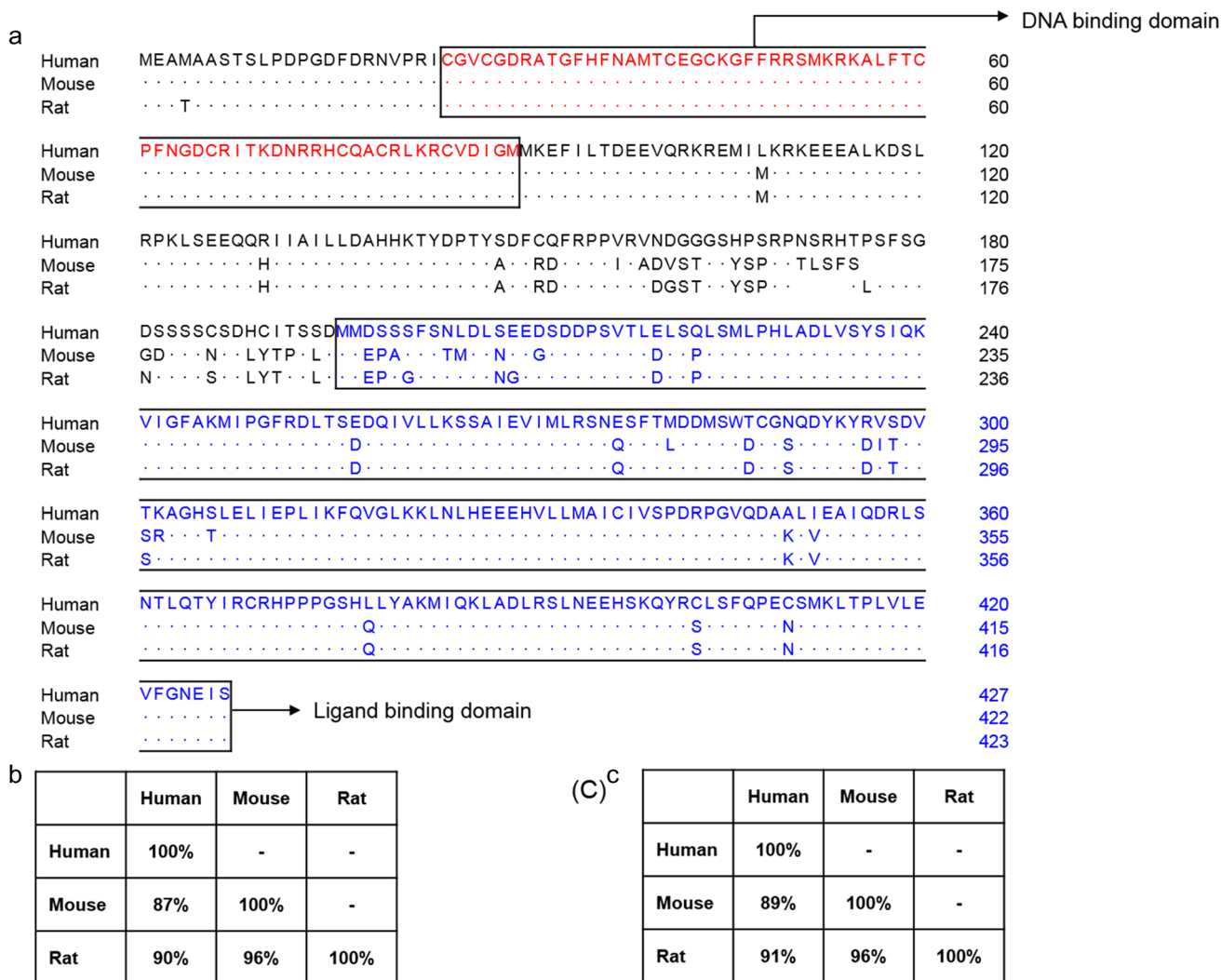


Fig. 1 a Amino acid sequences of VDRs in mouse (GenBank: EDL04218.1), rat (GenBank: EDL87095.1), and human (GenBank: BAA83389.1). Identical amino acids are shown as dots. **b** The

homology for the entire amino acid sequences and **c** for the ligand-binding domain (LBD) of VDRs among mouse, rat, and human

the presence of several water molecules near the A-ring linking the ligand C-2 position to the protein surface (23). In rat and human VDR, the 6 amino residues in the ligand-binding pocket form hydrogen bonds with the three hydroxyl groups on the $1\alpha,25$ -dihydroxyvitamin D_3 molecule (23, 24). Ser237 and Arg274 interact with the 1α -hydroxyl group, and Arg274 of the human VDR forms the strongest hydrogen bond with calcitriol. Mutation of this residue is associated with type II rickets (25). Tyr143 and Ser278 interact with the 3-hydroxyl group; His305 and His397 interact with the 25-hydroxyl group. The VDR LBD has a structure of a three-layered, antiparallel α -helical sandwich, and the ligand-binding pocket (LBP) is located in the lower part of the LBD (26, 27). Wan *et al.* showed that there are 10 and 6 amino acid residues, which stabilize the sandwich structure and form hydrogen bonds with the VDR ligand, respectively, that have identical sequences across mice, rats, and humans (28). Moreover, the amino acid sequences forming LBP are almost the same, except for one amino acid, and this is not important for folding (28, 29). However, there is absence of structural information about the mouse VDR ligand-binding pocket although it is speculated that the amino acids that reside in the ligand-binding pocket would be similar to those for human and rat due to the similarity in the amino acid-binding sequence of the VDR protein (20).

While considerable debate exists regarding the subcellular localization of the VDR and events that transpire after activation (30, 31), there is a general consensus that VDR activation occurs in a similar manner to that for other NRs to regulate channels and transporters, classical targets of the involved in calcium, phosphate, and bone homeostasis (32–34). The VDR exerts its effects on its targets both genomically and non-genomically (35), exemplified by the numerous regulatory studies on enzymes and transporters within the last decade. Increasingly, there is documentation on the VDR as a regulator of drug disposition since calcitriol aptly increases CYP3A4 and MDR1 activities, important enzyme and efflux transporter, respectively, which demonstrate many of the functional aspects of the Caco-2 cell line (36–40). Indeed, specific VDREs have been found in the the CYP3A4 and ABCB1 (MDR1) gene promoters (5, 39).

Crosstalk of VDR with Other NRs on Cholesterol and Bile Acid Homeostasis

From *in vitro* DNA-binding studies, the preferred response elements for PXR, CAR, and VDR are hexanucleotide direct repeats (DR3 or DR4) that are 3- or 4-nucleotide spaced (41–45). Much crosstalk exists between the VDR and other nuclear receptors (NRs). In particular, the VDR shares similar homology with PXR and CAR, and is activated by its cognate ligands such as calcitriol or the alternate VDR ligand, lithocholic acid (LCA) (46). Due to their similar

homology, PXR and CAR are able to transactivate CYP24A by binding to VDREs of the CYP24A promoter region (47, 48). The VDR inhibits the farnesoid X receptor (FXR), the bile acid sensor (49), and the liver X receptor- α (LXR α) (50), both important regulators of bile acid and cholesterol homeostasis. The crosstalk between the VDR and FXR in the regulation of cholesterol and bile acid homeostasis in the intestine and liver has been previously examined *in vivo* in the rat (51). Additionally, VDREs were found in the mouse and human SHP, the small heterodimer partner (17), and the fibroblast growth factor 15 (Fgf15 in rodent or FGF19 in human) (52–54). Calcitriol administration to *fxr*(+/+) and *fxr*(-/-) mice *in vivo* increased the activities of liver Cyp7a1, the rate limiting enzyme that metabolizes cholesterol to bile acids in a FXR-independent manner through inhibition of the repressive nuclear receptor, SHP (17). Consequently, VDR inhibition of Shp/SHP led to induction of Cyp7a1/CYP7A1 in murine and human hepatocytes (17), enhancing cholesterol metabolism to bile acids in mice *in vivo* (17). However, the VDR was found to stimulate Fgf15 (rodent) and FGF19 (human), hormonal signaling molecules that activate the liver fibroblast growth factor receptor 4 (Fgfr4/FGFR4) (52), which in turn downregulate Cyp7a1/CYP7A1 in liver (54).

Species differences have been found. First, differences exist in the regulation of the apical sodium-dependent transporter (ASBT), an important bile acid uptake transporter in the intestinal ileum (45, 55) that absorbs bile acid in a sodium-dependent fashion (56). Increased bile acid levels trigger FXR activation in the intestine, which in turn represses liver receptor homolog-1 (LRH-1), the competence factor needed for ASBT expression, and activates liver SHP (55, 57). For the rat, there is absence of the *cis*-element in rat Lrh-1 (45), hence resulting in induction of rat ileal Asbt with the VDR (45) but not mouse Asbt (17). Mouse Asbt expression is aptly repressed by bile acids (45) or remains unchanged with VDR activation (17). Other reports show that the VDR positively impacts the expression of peroxisome proliferator-activated receptor- α and peroxisome proliferator-activated receptor- γ (PPAR α and PPAR γ) (58, 59). PPAR γ binds to VDR and inhibits $1\alpha,25$ -dihydroxyvitamin D_3 -mediated transactivation of CYP24A1, the VDR signature gene responsible for the degradation of its ligand (60).

Species difference in VDR activity on rodent Oat3 has been noted. Murine Oat3 expression was upregulated by VDR activation (61) but downregulated in rats (62). We had speculated that the difference could be due to a species difference in protein kinase C (PKC) localization in the kidney: VDR increases PKC activity (63, 64) that could lead to Oat3 inhibition (65). PKC is not expressed in the murine proximal tubules (66), while it is highly expressed in the brush border of the proximal tubes in rats (67). Collectively, the species difference in the regulation of enzymes or transporters by

VDR is attributed to differences downstream to VDR, not to VDR itself.

As lithocholic acid, the secondary, toxic bile acid, is another activator of VDR, FXR, and PXR, crosstalk between nuclear receptors in the regulation of bile acid transporters has been investigated. In the intestine, the major bile acid transporters include ASBT and MRP2 at the apical and OST α -OST β and MRP3 at the basolateral enterocyte membranes. VDR activation has resulted in rat Asbt induction (45) but no change or slight inhibition in mouse (68), showing that there are species differences (see later, Table III). Similarly, the observations for OST α -OST β are heterogeneous with respect to the regulation by VDR (see later, Table III). MRP3 induction by VDR occurs by direct regulation of the *ABCC3* gene (69). On the other hand, FXR induces OST α -OST β (70) while downregulating ASBT (71) in the intestine. There is some information regarding regulation of intestinal bile acid transporters by PXR or the constitutive androstane receptor (CAR). Indeed, the expression of human OST β is induced (72) while mouse Mrp2 and Mrp3 are induced by by CAR activation (73). With respect to the regulation of hepatocyte bile acid transporters, the effects of VDR activation appear modest or inconsequential (Table III). The key hepatocyte bile acid transporters are NTCP, OATP, BSEP, MRP3, and MRP4. Downregulation of BSEP by 1,25(OH) $_2$ D $_3$ occurs indirectly through VDR-mediated inhibition of FXR transactivation of the *ABCC11* gene (49). However, FXR activation is well known to regulate hepatic bile acid transporter expression including downregulation of NTCP (74) together with induction of BSEP (75) and OST β (76). Induction of Mrp3 and Mrp4 in mouse liver by bile acid treatment appears to

be independent of FXR activation (77). Moreover, Mrp2, Mrp3, and Mrp4 are upregulated in liver by PXR/CAR activation (78).

1 α ,25-Dihydroxyvitamin D $_3$ (1,25(OH) $_2$ D $_3$ or Calcitriol), Active VDR Ligand

1 α ,25-Dihydroxyvitamin D $_3$ or calcitriol is the natural, active ligand of the VDR (79) that is formed from inert precursor forms of vitamin D $_3$ or vitamin D $_2$, derived from animals and plants, respectively (80). Vitamin D $_3$ may be obtained from exogenous sources such as milk and cod liver oil, or via exposure to UV rays from sunlight, converting 7-dehydrocholesterol to vitamin D $_3$ (80, 81). Vitamin D $_3$ is lipophilic and is highly bound to the vitamin D-binding protein (DBP) (81, 82) and stored mainly in the adipose tissue rather than circulating in blood (83). The first bioactivation step of vitamin D $_3$ is via hydroxylation of carbon 25 primarily in the liver by 25-hydroxylases, CYP27A1 (84), and CYP2R1 (85) to the relatively inactive metabolite, 25-hydroxyvitamin D $_3$ [25(OH)D $_3$], in higher abundance (nM range) (Fig. 2a). Both 25(OH)D $_3$ and 1,25(OH) $_2$ D $_3$ are metabolized to inactive metabolites, 24,25-(OH) $_2$ D $_3$ and 1,24,25(OH) $_3$ D $_3$, respectively, by the 24-hydroxylase (CYP24A1), the degradation enzyme that is upregulated by the VDR (86, 87). Bioactivation of 25(OH)D $_3$ occurs in the kidney with CYP27B1, the mitochondrial 1 α -hydroxylase, to form 1,25(OH) $_2$ D $_3$ in a rate-limiting fashion (80). The expression of CYP27B1 is downregulated by the VDR when excess 1,25(OH) $_2$ D $_3$ is present. Thus, levels of 1,25(OH) $_2$ D $_3$ are tightly regulated, CYP27B1 is inhibited whereas CYP24A1 is induced, at high calcitriol concentrations by the VDR (Fig. 2b) (80,

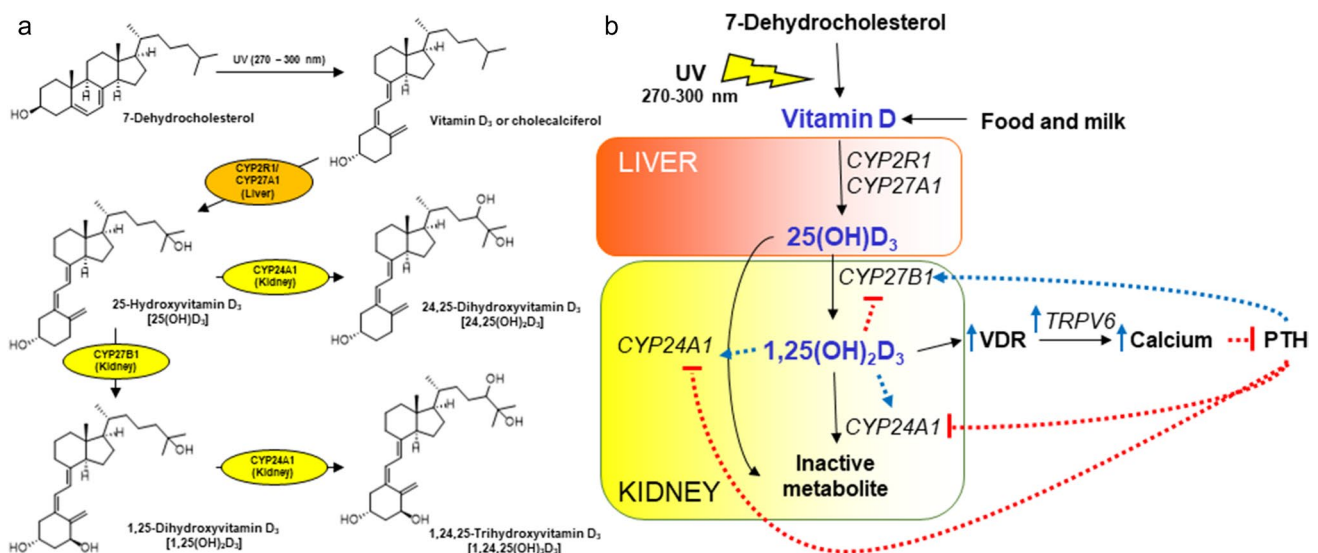


Fig. 2 a, b Metabolism of vitamin D to 25(OH)D $_3$ with CYP2R1 and CYP27A1 in liver and to 1,25(OH) $_2$ D $_3$ via CYP27B1 in kidney. The enzymes CYP27B1 and CYP24A1 are subject to inhibition (red

arrow) and induction (blue arrow), respectively, by calcitriol and PTH; the intestinal calcium channel, TRPV6, for calcium absorption is under VDR activation

88). Plasma calcium is a significant contributing factor for concerted regulation of calcitriol levels via CYP27B1 and CYP24A1 controls via the parathyroid hormone (PTH) feedback mechanism, stimulating the calcium-sensing receptor (CaSR) and triggering PTH production in the parathyroid gland that leads to upregulation of renal CYP27B1 and downregulation of CYP24A1, respectively, at low plasma calcium concentrations (80, 89). For vitamin D, it is further known that phase 2 metabolism also occurs (90–92), albeit most of the formed metabolites are inactive and the pathways are less important.

Over 99% of vitamin D metabolites are bound to plasma proteins, mostly to the vitamin D binding protein (DBP) and to albumin or lipoproteins to lesser extents (93–95). In plasma, 25(OH)D₃, which exhibits the strongest binding affinity towards DBP and whose concentration is 20 times higher than other vitamin D metabolites, is highly bound (82). The lipophilic nature of vitamin D and the high binding of vitamin D metabolite 25(OH)D₃ in plasma contribute to reduced metabolism in tissues and a longer, circulating half-life (28 h). The 25(OH)D₃-bound DBP complex is filtered through the glomerulus in the kidney and taken up by endocytic receptor, megalin, present on the brush border of the renal proximal tubule cells (96). Once in tubular cells in the kidney, DBP is degraded by legumain (97), and free 25(OH)D₃ is metabolized by the mitochondrial 1 α -hydroxylase or CYP27B1 to form the active 1,25(OH)₂D₃ (98, 99). There is evidence that this rate-limiting enzyme is also present in the brain and skin, albeit playing a much lesser role in the formation of calcitriol (19, 100).

Due to the importance of the VDR, the clinical status of vitamin D sufficiency or deficiency is a health concern. Clinically, the vitamin D status is determined by circulating levels of 25(OH)D₃ which is more readily determined as it is present at higher nM range (~75 nM), unlike calcitriol, which is present at much low pM levels (101, 102). It is usually considered that a patient is vitamin D deficient when plasma 25(OH)D₃ levels fall below 20 ng/ml or 50 nM (103, 104), and vitamin D insufficient when the 25(OH)D₃ plasma level is between 20 and 30 ng/ml (105–108). Due to the tight regulation of calcitriol on its production and degradation, excess 1,25(OH)₂D₃ in plasma inhibits renal Cyp27B1 to curtail calcitriol production and enhance CYP24A1-mediated degradation (88). At low doses of calcitriol, when CYP24A1 is not induced, the half-life is the longest, whereas at high levels or with escalating calcitriol doses, CYP24A1 is greatly induced and CYP27B1 completely inhibited, reducing the half-life and increasing calcitriol clearance (88).

Vitamin D Analogs

Many vitamin D analogs have been developed as therapeutic agents to improve the function and activity of the VDR and overcome the well-known hypercalcemic toxicity of

calcitriol (see later). These targets include diseases such as secondary hyperparathyroidism (SHPT), psoriasis, cancer, and osteoporosis (109, 110). Structural modification of the vitamin D moiety not only affects ligand-specific conformational change in VDR, influencing pharmacological action by VDR activation, but also affects the binding affinity to DBP in plasma, altering tissue distribution (109). Among the vitamin D analogs, 22-oxa-1,25(OH)₂D₃ or Oxacalcitriol®, 19-nor-1,25(OH)₂D₂, 1 α (OH)D₂, Hectero® or doxercalciferol, and 1,25(OH)₂-26,27-F₆-D₃ or Falcitriol® have been developed to treat SHPT in patients with chronic kidney disease due to their lower hypercalcemic side effects (110). Notably, doxercalciferol (1 α (OH)D₂), the precursor of 1,25(OH)₂D₂, requires activation in the liver and is less toxic than 1 α (OH)D₃, Alfacalcidol®, an active precursor that converts to 1,25(OH)₂D₃ in the liver (111). Furthermore, several vitamin D analogs have been developed to treat psoriasis and cancers as well as suppress the immune system. Calcipotriol®, possessing a similar binding affinity for VDR as 1,25(OH)₂D₃ but is 200 times less potent, is designed to treat psoriasis. Tacalcitol® or 1,24(OH)₂D₃ showed therapeutic potential in psoriasis by inhibiting DNA synthesis of epidermal keratinocytes, having similar activity to that of 1,25(OH)₂D₃ but less toxic towards hypercalcemia than 1,25(OH)₂D₃. Not all VDR analogs retain chemical structural similarity to that of 1,25(OH)₂D₃. As mentioned earlier, lithocholic acid (LCA), the toxic, secondary bile acid, is also a VDR ligand (4). It is structurally dissimilar to 1,25(OH)₂D₃ but activates the VDR at μ M concentrations rather than at nM concentrations (4, 112).

Pharmacologic or Therapeutic Effects of the VDR

The vitamin D receptor, upon binding to its active ligand, acts as biological sensor that is vital to calcium and phosphorus homeostasis and controls many other genes in the regulation of other hormonal actions, immune responses, cell proliferation and differentiation, and tissue health (113). The VDR exerts its pharmacologic action through transcriptional control of various transporters and enzymes. As discussed previously, it tightly regulates plasma calcitriol concentrations via CYP27B1 and CYP24A1. Moreover, the VDR is important for the maintenance of calcium and phosphate homeostasis via the calcium channel, transient receptor potential cation channel, subfamily V, member 6 (TRPV6) in intestine primarily, as well as the sodium-dependent phosphate cotransporter (NaP_i) in the intestine and kidney, and osteoblasts and osteoclasts in bone.

Calcium and Phosphate Homeostasis The presence of the VDR in multiple tissues suggests its definitive and important role in the body (114, 115). Although 1,25(OH)₂D₃ or calcitriol could be synthesized in many tissues such as skin

and brain (19, 100), it is produced specifically for direct cellular use and not purely for mineral requirements. The VDR is best known for its role in calcium and phosphate homeostases and preserving bone health (Fig. 3). The function of the vitamin D endocrine system ensures that calcium and phosphate are kept in a delicate balance for proper bodily functions, requiring adequate communication among tissues such as the kidney, bone, parathyroid gland, and intestine in order to maintain appropriate plasma levels of calcitriol. Calcitriol controls the absorption of calcium from the intestine or reabsorption via the kidney and bone, and regulates PTH in the parathyroid gland (34, 116–119) as well as phosphate balance via the NaP_i in both intestine and kidney. The TRPV6 calcium channel, together with NaP_i and PTH, regulates calcium and phosphate levels (Fig. 3) (120–124). In small intestine enterocytes, calcitriol stimulates the VDR to increase calcium absorption from the gut lumen by TRPV6, the epithelial calcium channel (15, 34, 114, 125), increases calcium transport across the cell by inducing calbindin D9K, a cytosolic calcium-binding protein, and elevates basolateral plasma membrane ATPase (PMCA1) that transports calcium ion into the bloodstream (22). Calcitriol upregulates renal TRPV5, though of minor importance in the overall calcium absorption, and calbindin D28K to increase calcium reabsorption (22, 126). VDR activation also increases active phosphate transport through the induction of the apical NaP_i cotransporters in the kidney and intestine (127–129). In bone, calcitriol activates osteoblasts and stimulates the maturation of osteoclasts to resorb calcium from bone and reverse transport calcium from bone compartment to plasma (80). For control of plasma calcium concentrations, feedback mechanisms exist to curtail calcitriol production and calcium reabsorption. First, the calcium-sensing receptor (CaSR) is

present in the parathyroid gland for the detection of plasma calcium (80). When the plasma calcium concentration is high, calcitonin, a 32-amino acid polypeptide produced in the parafollicular cells of the thyroid gland, increases to inhibit intestinal calcium absorption and osteoclast activity in bones (80). These events act as feedback controls to decrease the plasma levels of calcitriol and calcium (Fig. 3).

Secondary Hyperparathyroidism (SHPT) in Chronic Kidney Disease Vitamin D analogs are used as therapeutic agents for various diseases such as SHPT in chronic kidney disease (CKD) (117, 127, 130, 131). CKD is a progressive disease characterized by a decline in kidney function that is classified from mild (stage 1) to severe (stage 5) stages according to the glomerular filtration rate (GFR). According to the Kidney Foundation of Canada in 2016 (<http://www.cihr-irscgc.ca/e/50872.html>), CKD afflicts over one in ten Canadians. In stage 5 CKD, kidney function is minimal or absent, and patients require regular dialysis or kidney transplant for survival. With increasing renal dysfunction, the activity of 1α -hydroxylase or CYP27B1 that rate-limits the formation of calcitriol from $25(\text{OH})\text{D}_3$ is much curtailed. The interaction of calcitriol, calcium, phosphate, PTH, and CYP27B1 is complex (Fig. 3), and the delicate equilibrium is upended by renal diseases. With CKD, the function of the kidney to synthesize the active ligand, $1,25$ -dihydroxyvitamin D_3 , by CYP27B1 is reduced. This leads to a rise in circulating levels of PTH, a condition known as SHPT, which occurs when calcium and calcitriol levels are low. With elevated PTH acting directly on bone cells, the bone hormone, fibroblast growth factor 23 (FGF23) is increased (129) to maintain phosphate balance by increasing urinary phosphate excretion and reducing phosphate

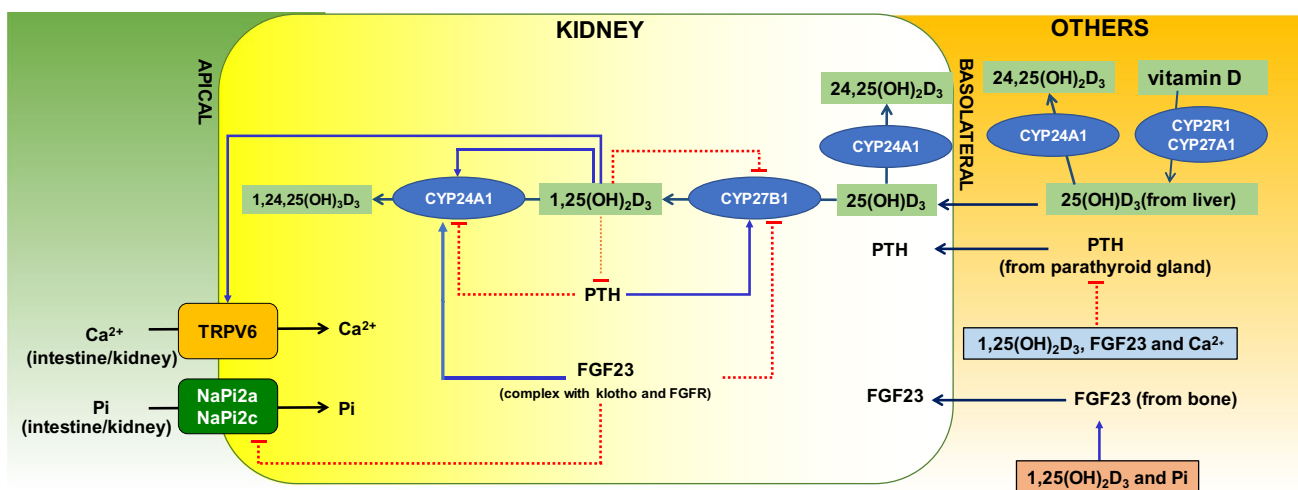


Fig. 3 Interplay of calcitriol, PTH, and FGF23 on calcium and phosphate homeostasis in kidney, and changes in CKD with alterations in calcium and PTH homeostasis and bone health. Klotho is a mem-

brane protein that enables the function of FGF23, the bone hormone that regulates phosphate homeostasis

VDR Targets Enzymes, Transporters, and Receptors

Levels of calcitriol are tightly regulated by the synthetic mitochondrial enzyme, CYP27B1, in the kidney and the catabolic or degradative enzyme, CYP24A1, which has a more ubiquitous distribution (15). The VDR also targets other noteworthy enzymes and transporters. Western blotting has verified the presence of VDR protein expression in the intestine, liver, kidney, brain, cultured cells *in vitro* in immortalized Caco2 and HepG2 cells, and intestinal and liver slices. In our laboratory, we identified changes of in the expression of enzymes and transporters upon induction/inhibition via the VDR with four intraperitoneal pharmacologic albeit slightly toxic doses of 2.5 µg/kg of calcitriol, given every day or every other day to rodents. This dose is hypercalcemic and pharmacologically active (15, 17, 51, 142). Pursuant to dose administration, we were able to identify the pharmacokinetics and pharmacodynamics of calcitriol. Upon continued administration of calcitriol to mice, the half-lives of calcitriol become shortened due to induction of Cyp24a1 and inhibition of synthesis via Cyp27b1 in the mouse (15, 17, 51, 88).

Phase I Enzymes The intestine and liver are both endowed with CYPs for the metabolism of drugs (143, 144). The VDR strongly modulates the expression of Cyp3a isoforms (4, 6, 51, 69, 142, 145–149) in rodents and upregulates CYP3A4 in the Caco-2 cell line (34, 40, 148, 150, 151). Fan *et al.* (40) further compared the inductive effects of other vitamin D analogs— $1\alpha(\text{OH})\text{D}_2$ and $1\alpha(\text{OH})\text{D}_3$ —against calcitriol for the intestinal CYP3A4 activity in Caco-2 cells. VDR induction is due to the presence of a VDRE in the human CYP3A4 gene (4, 5). Other studies (Table I) documented that calcitriol treatment of human intestinal cell lines such as Caco-2, LS180, HT-29, and SW480 (5, 36–38, 40, 148, 150–153) as well as human HepG2 (148, 154), HEK293 (4), and HPAC (36) cell lines and human slices induced CYP3A4 expression *in vitro*. Later studies revealed that the inductive role of VDR is not limited to human CYP3A4 only; the expression of human CYP2B6 and CYP2C9 in primary human hepatocytes (155) is also increased with calcitriol treatment.

In rodents *in vivo*, rat Cyp3a9 (146, 147) and Cyp3a1/3a23 (6, 51, 147), orthologs of human CYP3A4, are induced in the intestine. Chow *et al.* (142) showed that calcitriol treatment to rats *in vivo* induced Cyp3a species differentially in different organs: Cyp3a1 in the intestine and Cyp3a9 in the kidney. Kutuzova and DeLuca (147) demonstrated that calcitriol upregulated genes for detoxification in the rat intestine, such as inducing the expression of the catabolic enzyme, Cyp24a1, as well as Cyp3a1 and Cyp1a1. Rat and human intestinal slices that were incubated with calcitriol led to increased expression of rat Cyp3a1 in

jejunum, ileum, and colon and Cyp3a2 in ileum (6, 51, 145) and human CYP3A4 (145).

Phase II Enzymes In contrast to P450 studies, only a few studies showed that the VDR plays an important role in the regulation of phase II enzymes. $1,25(\text{OH})_2\text{D}_3$ treatment elicited an increase in human sulfotransferases SULT1C2 and SULT2A1 *in vitro* (7, 159) and in mouse (68, 160) as well as mouse Sult2a2 *in vivo* (69), and rat UDP glucuronosyltransferase, Ugt1a (147) (Table II). However, no significant change in SULT2A1 mRNA levels was observed following $1,25(\text{OH})_2\text{D}_3$, $1\alpha(\text{OH})\text{D}_2$, and LCA treatment for 3 consecutive days in Caco-2 cells, despite that other VDR target genes CYP24A1 and TRPV6 were also induced (40). This discrepancy, according to Song *et al.* (159), may be explained by different cell types and the abundance of SULT2A1 expression among Caco-2 cells (40) vs. liver cells. There is some evidence to suggest that glutathione S-transferase may also be induced by the VDR (147) (Table II).

Regulation of Solute Carrier Family Several solute carrier (SLC) absorptive transporters may be under the regulation of VDR (Table III). While many of the transporters are tested, only the transporters prone to change with the VDR are reported. Our laboratory noted that a VDRE existed in the rat *Asbt* gene, and that induction of rat *Asbt* with calcitriol treatment was due to the absence of the *cis*-element in rat liver receptor homolog-1, Lrh-1 (45). Hence with calcitriol treatment, *Asbt* protein was upregulated in the rat intestine (45, 51, 149) and rat kidney (142, 149) (Table III). Without a proper functioning Lrh-1 in rats, VDR-mediated induction of rat *Asbt* is expected to be absent. Instead, inhibition of mouse *Asbt* was found with the proper functioning intestinal Lrh-1 (68).

The VDR is involved in the regulation of the folate transporters: there are at least three—the folate receptor- α (FR α), the proton-coupled folate transporter (PCFT), and the reduced folate carrier (RFC). *In vitro* studies first established that the mRNA expression of the PCFT, which is present abundantly in the small intestine, is increased by calcitriol in a dose-dependent fashion in Caco-2 cell line and rat duodenal segments due to the presence of a VDRE and folate transport function in Caco-2 cells (161). A later study, however, showed that VDR(-/-) mice or vitamin D status ($25(\text{OH})\text{D}_3$ or $1,25(\text{OH})_2\text{D}_3$ plasma concentrations) in rats are not related to the plasma and liver folate status or intestinal PCFT mRNA expression (163). In brain, where all three folate transporters—PCFT, FR α , and RFC—are expressed (162), FR α is suggested to play a critical role in the maintenance of CNS folate transport across the choroid plexus epithelium. The co-existence of the FR α and PCFT, in HeLa sublines, traps folate into vesicles and enhances folate transport into the cytosol (168). Despite that Eloranta

Table 1 Regulation of Phase I Enzymes by the VDR

Ligand	Target genes	Cell lines/species	References
1,25(OH) ₂ D ₃	↑Cyp1a1	Rat intestine (<i>in vivo</i>)	(147)
	↑Cyp2b2	Rat intestine (<i>in vivo</i>)	(147)
	↑CYP2B6	Human primary hepatocytes (<i>in vitro</i>)	(155)
	↑Cyp2b15	Rat intestine (<i>in vivo</i>)	(147)
	↑CYP2C9	Human primary hepatocytes (<i>in vitro</i>)	(155)
	↑Cyp3a1	Rat, transfected in HEK293 cells	(4)
	↑Cyp3a1	Rat intestinal slices (<i>in vitro</i>)	(145)
	↑Cyp3a1	Rat intestine (<i>in vivo</i>)	(6, 51, 147)
	↑Cyp3a2	Rat intestinal slices (<i>in vitro</i>)	(145)
	↑Cyp3a9	Rat intestine (<i>in vivo</i>)	(146, 147)
	↑Cyp3a23	Rat intestine (<i>in vivo</i>)	(6, 51, 147)
	↑Cyp3a2	Rat liver (<i>in vivo</i>)	(51)
	↓Cyp3a2	Rat kidney (<i>in vivo</i>)	(142)
	↑Cyp3a9	Rat kidney (<i>in vivo</i>)	(142, 149)
	↑Cyp3a11	Mouse colon (<i>in vivo</i>)	(69)
	↑Cyp3a11	Mouse primary hepatocytes (<i>in vitro</i>)	(148)
	↓Cyp3a11	Mouse liver (<i>in vivo</i>)	(68)
	↑Cyp7a1	Mouse primary hepatocytes Mouse liver on Western diet (<i>in vivo</i>)	(17)
	↑CYP2B6	Human primary hepatocytes (<i>in vitro</i>)	(155)
	↑CYP2C9	Human primary hepatocytes (<i>in vitro</i>)	(155)
	↑CYP3A4	Caco-2 cells (<i>in vitro</i>)	(36–38, 40, 148, 150, 151)
	↑CYP3A4	Human intestinal slices (<i>in vitro</i>)	(145)
	↑CYP3A4	LS180 cells (<i>in vitro</i>)	(37, 38, 150, 151)
	↑CYP3A4	SW480 cells (<i>in vitro</i>)	(152)
	↑CYP3A4	HT-29 cells (<i>in vitro</i>)	(5, 153)
	↑CYP3A4	HPAC cells (<i>in vitro</i>)	(36)
	↑CYP3A4	HepG2 cells (<i>in vitro</i>)	(148, 154)
↑CYP3A4	HEK293 cells (<i>in vitro</i>)	(4)	
↑CYP3A4	Human primary hepatocytes (<i>in vitro</i>)	(36, 155)	
↑CYP3A4	Human liver slices (<i>in vitro</i>)	(145)	
↑CYP7A1	Human primary hepatocytes	(17)	
1α(OH)D ₂	↑Cyp3a9	Rat kidney (<i>in vivo</i>) Caco-2 cells (<i>in vitro</i>)	(149)
	↑CYP3A4	Caco-2 cells (<i>in vitro</i>)	(40)
1α(OH)D ₃	↑CYP3A4	Caco-2 cells (<i>in vitro</i>)	(36, 40)
	↑Cyp3a11	Mouse intestine and liver (<i>in vivo</i>)	(4, 68)
	↑Cyp7a1	Mouse liver (<i>in vivo</i>)	(68, 156, 157)
19-nor 1,25(OH) ₂ D ₂	↑Cyp3a9	Rat intestine (<i>in vivo</i>)	(146)
	↑CYP3A4	Caco-2 cells (<i>in vitro</i>)	(151)

et al. (161) failed to observe changes in RFC mRNA expression, Alam *et al.* demonstrated that RFC is increased by calcitriol treatment and is capable towards folate uptake in the brain microvessel endothelial cell line, hCMEC/D3, and isolated mouse brain capillaries, and increased protein and mRNA expression of RFC (164). That RFC enhances folate uptake in mice was demonstrated in Folr-1 KO (162).

The relative protein expressions of the organic anion transporters 1 and 3 (OAT1 and OAT3), which play a

major role on organic anion uptake from blood circulation to renal proximal tubular cells. The expression levels of these anionic transporters are reduced following treatment with calcitriol in rats, leading to reduced clearance of cefdinir and cefadroxil, organic anion transporter substrates (62, 142, 149, 165). Although rat Oat1 protein expression was decreased (62, 149), murine Oat1 protein in kidney is unchanged with calcitriol treatment (61). Murine Oat3 protein is decreased in vitamin D deficiency, and cholecalciferol

Table I (continued)

Ligand	Target genes	Cell lines/species	References
LCA	↑Cyp3a	Mouse intestine (<i>in vivo</i>)	(154)
	↑Cyp3a1	HEK293 cells transfected with rat Cyp3a1 (<i>in vitro</i>)	(4)
	↑Cyp3a1	Rat intestinal slice (<i>in vitro</i>)	(158)
	↑Cyp3a2	Rat intestinal slice (<i>in vitro</i>)	(158)
	↑Cyp3a9	Rat intestinal and liver slices (<i>in vitro</i>)	(158)
	↑Cyp3a11	Mouse Cyp3a11 transfected in HEK293 cells (<i>in vitro</i>)	(4, 152)
	↑Cyp3a11	Mouse colon (<i>in vivo</i>)	(4)
	↑Cyp3a11	Mouse intestine (<i>in vivo</i>)	(4, 152)
	↑Cyp3a11	Mouse liver (<i>in vivo</i>)	(4)
	↑CYP3A4	Transfected in HEK293 cells (<i>in vitro</i>)	(4, 152)
	↑CYP3A4	HT-29 cells (<i>in vitro</i>)	(153)
	↑CYP3A4	HepG2 and LS174T cells (<i>in vitro</i>)	(154)
	↑CYP3A4	SW480 cells (<i>in vitro</i>)	(152)
	↑CYP3A4	Human intestinal slice (<i>in vitro</i>)	(158)
LCA acetate	↑Cyp3a11	Mouse intestine (<i>in vivo</i>)	(152)

or calcitriol treatment restored murine Oat3 expression (61). Moreover, Alam *et al.* reported an elevated mRNA expression of murine Oat3 after calcitriol treatment (162). The VDR regulatory changes appear to be opposite in the rat and mouse, and there has been some debate that it is attributed to the co-presence of the inhibitor, protein kinase C (PKC) (61).

The efflux of bile acids is handled specifically by the protein complex formed by the organic solute transporters α and β (OST α and OST β) that are expressed mostly in the colon, liver, small intestine, and kidney (169), whose major role is towards the net efflux or uptake of bile acids at the basolateral membrane of enterocytes (56), thus contributing an important role in the enterohepatic circulation of bile acids.

The expression of the half-transporters, OST α and OST β that must coexist for the transport of bile acids, has been shown to be induced by chenodeoxycholic acid (CDCA), a FXR ligand (70). Khan *et al.* found that this heteromeric transporter pair consisting of the gene products of OST α and OST β is under the negative regulation of the VDR *in vitro* (166). Both calcitriol and LCA treatment decreased the mRNA expression of Ost α and Ost β in rat ileum slices, while calcitriol had no effect on human ileum, and rat and human liver slices. But LCA, which is a ligand for both FXR and VDR (152), increased OST α and OST β mRNA expression in rat liver slices, and human ileum and liver slices, likely because of FXR regulation (166). Treatment of mice with 1 α (OH)D₃ reduced renal Ost α mRNA levels by 71% and Ost β levels by 17%, and this effect was shown to be independent of FXR, as a reduction in mRNA of both genes still occurred in *fxr(-/-)* mice (68).

The VDR inductive pattern for the oligopeptide transporter 1 (PEPT1) is, however, not clear. Rat intestinal Pept1 function is induced following calcitriol treatment *in vivo* (142), as evidenced by an increase in apical-to-basolateral (A \rightarrow B) transport of glycylsarcosine, a PepT1 substrate, in the everted rat intestinal sac (167). However, no induction was found in Caco-2 cells (40), and Pept1 protein expression in rat kidney (142, 149) and mouse Pept1 (61) were decreased and unchanged, respectively.

Receptors The VDR is known to be involved in the important function of calcium homeostasis that is vital to bone health (34). The promoters of the genes encoding the two

Table II Regulation of Phase II Enzymes by the VDR

Target enzymes	System studied	References
↑Sult2a1	Mouse or rat Sult2a1 transfected in HepG2 and Caco-2 cells	(7)
↑Sult2a1	Mouse liver (<i>in vivo</i>)	(68, 160)
↑SULT2A1	Transfected in HepG2 and Caco-2 cells Not detected in Caco-2 cells for mRNA or protein Mouse <i>in vivo</i>	(7, 40, 69)
↑Sult2a2	Mouse colon (<i>in vitro</i>)	(150)
↑Ugt1a	Rat intestine (<i>in vivo</i>)	(147)
↑Gst4 α	Rat intestine (<i>in vivo</i>)	(147)

Table III Regulation of SLC Transporters by the VDR

SLC transporters	System studied	Changes	References
↔ ASBT	Caco-2	No change in mRNA and protein expression	(40)
↑Asbt	Rat intestine (<i>in vivo</i>)	↑Protein in intestine after calcitriol and doxercalciferol treatment	(45, 51, 149)
↑Asbt	Rat kidney (<i>in vivo</i>)	↑Protein in kidney by calcitriol and doxercalciferol	(142, 149)
↔ or ↓Asbt not significantly	Mouse intestine and kidney (<i>in vivo</i>)	No change after 1,25(OH) ₂ D ₃ and 1α(OH)D ₃	(68)
↑Asbt	Mouse intestine (<i>in vivo</i>)	↑mRNA after 1α(OH)D ₃ treatment	(156)
↑PCFT	Caco-2 cells and rat duodenum (<i>ex vivo</i>)	↑mRNA after 1,25(OH) ₂ D ₃ treatment	(161)
↑Pcft	Isolated mouse brain capillaries; mice (<i>in vivo</i>)	↑mRNA in isolated brain capillaries and liver after 1,25(OH) ₂ D ₃ treatment	(162)
↔ Pcft	<i>Vdr</i> ^{-/-} mice (<i>in vivo</i>); rats (<i>in vivo</i>)	No change in mRNA between WT and <i>Vdr</i> ^{-/-} mice No change in folate status	(163)
↑Rfc	Isolated mouse brain capillaries; mice (<i>in vivo</i>)	↑mRNA in isolated brain capillaries, liver, kidney, and duodenum after 1,25(OH) ₂ D ₃ treatment	(162, 164)
↔ RFC	Caco-2 cells	No change in mRNA after 1,25(OH) ₂ D ₃ treatment	(161)
↑RFC	hCMEC/D3 cells	↑mRNA and protein after 1,25(OH) ₂ D ₃ treatment	(164)
↓Oat1	Rat intestine (<i>in vivo</i>)	↓mRNA expression after calcitriol treatment	(142)
↓Oat1	Rat kidney (<i>in vivo</i>)	↓mRNA and protein expressions after calcitriol and 1α(OH)D ₂ treatment	(62, 142, 149, 165)
↔ Oat1	Protein, mouse unchanged	No change in mRNA and protein expression	(61)
↓Oat3	Rat kidney (<i>in vivo</i>)	↓mRNA expression after calcitriol treatment	(62, 142, 165)
↑Oat3	Mouse kidney (<i>in vivo</i>)	Vitamin D-deficient mice—decreased Oat3 protein and mRNA, increased back with replenishment ↑mRNA and protein expressions after calcitriol treatment	(61)
↑Oat3	Mouse kidney (<i>in vivo</i>)	↑mRNA in kidney after 1,25(OH) ₂ D ₃ treatment	(162)
↔ OSTα	Human intestinal and liver slices (<i>in vitro</i>)	No change after 1,25(OH) ₂ D ₃ treatment	(166)
↑OSTα	Human intestine and liver slices (<i>in vitro</i>)	↑mRNA after treatment with CDCA and LCA, as substrates of both FXR and VDR	(166)
↓Ostα	Rat intestinal slice (<i>in vitro</i>)	↓mRNA after 1,25(OH) ₂ D ₃ treatment	(166)
↔ OSTα	Rat liver slice (<i>in vitro</i>)	No change after 1,25(OH) ₂ D ₃ treatment	(166)
↓Ostα	Rat kidney (<i>in vivo</i>)	↓mRNA expression after 1,25(OH) ₂ D ₃ treatment	(142)
↓Ostα	Mouse liver and kidney (<i>in vivo</i>)	↓mRNA expression after 1(OH)D ₃ treatment, but no change after 1,25(OH) ₂ D ₃ treatment	(68)
↔ OSTβ	Human intestinal and liver slices (<i>in vitro</i>)	No change after 1,25(OH) ₂ D ₃ treatment	(166)
↑OSTβ	Human intestine and liver slices (<i>in vitro</i>)	↑mRNA after treatment with CDCA and LCA, as substrates of both FXR and VDR	(166)
↓Ostβ	Rat intestinal slice (<i>in vitro</i>)	↓mRNA after 1,25(OH) ₂ D ₃ treatment	(166)
↑Ostβ	Rat liver slice (<i>in vitro</i>)	↑mRNA after treatment with CDCA and LCA, as substrates of both FXR and VDR	(166)
↓Ostβ	Mouse kidney (<i>in vivo</i>)	↓mRNA expression after 1(OH)D ₃ treatment, but no change after 1,25(OH) ₂ D ₃ treatment	(68)
↔ PEPT1	Caco-2	↔ mRNA and protein expression after calcitriol treatment	(40)
↑Pept1	Rat intestine (<i>in vivo</i>)	↑Protein expression after calcitriol treatment	(142, 167)
↓Pept1	Rat kidney (<i>in vivo</i>)	↓mRNA and protein expression after calcitriol treatment and 1α(OH)D ₂	(142, 149)

Table III (continued)

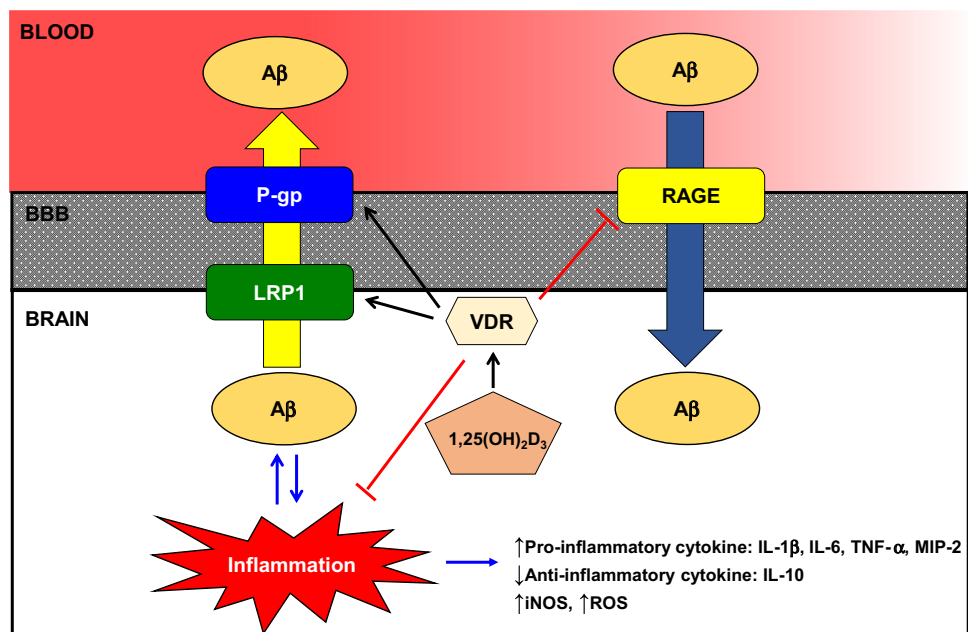
SLC transporters	System studied	Changes	References
↔ Pept1	Vit D-deficient mice (<i>in vivo</i>)	↔ Pept1 protein in intestine and kidney	(61)

calcium channels, the transient receptor potential vanilloid subfamily member 6 (TRPV6) of the intestine and members 5 and 6 (TRPV5 and TRPV6) of the kidney, contain VDREs that regulate their expressions (170). Among these channels, the intestinal TRPV6 is the one that is most induced by the VDR and is found responsible for the toxic side effect of hypercalcemia (125). Unfortunately, excess calcitriol will elicit the untoward effect of hypercalcemia that deters the use of vitamin D analogs as therapeutic agents.

The possible role of the VDR that targets the clearing receptors of the β -amyloid peptides or $A\beta$ has sparked interests as a mechanism to attenuate the accumulation of $A\beta$ monomers in Alzheimer’s disease (171). The receptors involved are RAGE, a receptor for advanced glycation end products, that allows $A\beta$ entry into the brain, and LRP1, the low-density receptor-related protein 1, that effluxes β -amyloid peptides out of the blood–brain barrier (BBB) (172) (Fig. 5). Guo *et al.* (173) reported the regulatory effect of calcitriol on the expression of LRP1 and RAGE, favoring the efflux and inhibiting the influx of $A\beta$ following calcitriol treatment in mice. Studies in rats and mice in our laboratory failed to produce any substantial changes in Lrp1 or Rage (141, 174, 175), likely due to the lower pharmacologic doses used. Our recent studies in guinea pigs that are maintained on the same calcitriol dosing regimen led to increased LRP1 and reduced RAGE (unpublished data of H.B. Peng and K.S. Pang).

Regulation of ABC Transporters: P-gp, MRPs, and BCRP One of the first discoveries of the VDR on regulation of drug transporters is its ability to increase the multidrug resistance protein (*MDR1*) and its gene product, P-glycoprotein, which transports many lipophilic substrates (176). The observation has sparked much interests since this ATP-binding cassette (ABC) transporter, P-gp, poses a major obstacle in cancer chemotherapy and other pharmacotherapy (177) by promoting the cellular efflux of anticancer drugs—anthracyclines, steroids, vinca alkyloids, peptides, epipodophylotoxins, HIV protease inhibitors, taxanes, and others via efflux transporters. The mRNA expression of *MDR1*, present ubiquitously in major eliminating organs, the liver, kidney (178), and BBB (179), was induced by the VDR in intestinal Caco-2 cells (150) and LS180 cells (38). Tachibana *et al.* (180) reported that the mRNA expression of *MDR1* was induced in intestinal LS174T cells by calcitriol as well as LCA treatment, and the increased mRNA level of *MDR1* by calcitriol was higher than that following treatment with LCA (22- vs. 2.5-fold). The mechanism of induction involves direct gene regulation through a response element for VDR (VDRE) on the human *MDR1* gene (39). It was further shown that an increase in P-gp function was accompanied by increased P-gp protein in the Caco-2 system; basolateral to apical (B \rightarrow A) transport of the P-gp substrate digoxin increased in cells treated with 1,25(OH)₂D₃ (40). Both *MDR1* mRNA and P-gp protein expressions were increased

Fig. 5 VDR regulation of receptors governing the influx (RAGE) and efflux (LRP1 and P-gp) of $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides in brain; other benefits of the VDR include anti-inflammatory activities and lowering of reactive oxygen species



following treatment with other vitamin D analogs, $1\alpha(\text{OH})\text{D}_3$ and $1\alpha(\text{OH})\text{D}_2$ (40).

The above *in vitro* results have been verified *in vivo* in both rats and mice, although *in vivo* results are highly tissue-specific. Chow *et al.* demonstrated that treatment with calcitriol in mice resulted in induction of *Mdr1a* mRNA and P-gp protein in the rat liver (51) and kidney but not in the intestine, despite the relatively high VDR protein expression levels there (142). The lack of intestinal induction was further verified by a lack of increase in P-gp function following the same treatment (167), although P-gp protein was induced following treatment with $1\alpha(\text{OH})\text{D}_2$ (149). Treatment with calcitriol increased *Mdr1* mRNA and P-gp protein in the mouse *in vivo* as well, in both the kidney and brain, resulting in increased clearance of digoxin from these two tissues compared to vehicle-treated mice (181). Similar to $1,25(\text{OH})_2\text{D}_3$, $1\alpha(\text{OH})\text{D}_3$ induced *Mdr1a*/P-gp in the kidney even to a greater extent (eightfold vs. threefold), due to higher circulating $1,25(\text{OH})_2\text{D}_3$ levels elicited by $1\alpha(\text{OH})\text{D}_3$ treatment, compared to direct treatment with $1,25(\text{OH})_2\text{D}_3$ (68).

At the BBB, calcitriol induction of murine brain P-gp by calcitriol is found to enhance the clearance of $\text{A}\beta_{1-40}$, the amyloid peptide of 40-amino acid length (182). The increased P-gp expression as well as function in the brain was also observed *in vivo*; brain efflux of quinidine, a P-gp substrate, was enhanced following $1,25(\text{OH})_2\text{D}_3$ treatment in rats due to P-gp induction in the brain (175). Because of the special interests towards brain accumulation and brain transport, induction of MDR1/P-gp in the brain was studied in the rat (RBE4) and human (hCMEC/D3) brain microvessel endothelial cell lines treated with $1,25(\text{OH})_2\text{D}_3$ (174). In the RBE4 cells, *Mdr1b* mRNA and P-gp protein were increased following $1,25(\text{OH})_2\text{D}_3$ treatment, accompanied by a decrease in the cellular accumulation of the P-gp substrates, rhodamine 6G (R6G) and HiLyte Fluor-labeled human $\text{A}\beta_{1-42}$ (h $\text{A}\beta_{1-42}$). Similar results were seen in the hCMEC/D3 cell line, an increase in *MDR1* mRNA and P-gp protein, followed by a decrease in R6G and HiLyte Fluor h $\text{A}\beta_{1-42}$ accumulation (174). The same study further demonstrated that in isolated rat brain capillaries, treated with $1,25(\text{OH})_2\text{D}_3$, P-gp protein was induced and intraluminal efflux of the fluorescent P-gp substrate [N- ϵ (4-nitrobenzofurazan-7-yl)-d-Lys(8)]-cyclosporin A (NBD-CSA) was increased compared to those treated with vehicle (174).

The VDR is involved in the regulation of all of multidrug resistance-associated protein (MRP) transporters. Like P-gp, the MRPs are also ATP cassette efflux transporters that mediate the transport of a wide range of substrates. The isoforms are expressed in numerous organs. MRP1 transports organic anions and is ubiquitously expressed in the body (183), while MRP2, which transports bilirubin,

conjugates, glucuronidated bile acids, and glutathione adducts (184, 185), is abundantly expressed in the liver, kidney, and intestine. MRP3 is found in the colon, kidney, and liver (186, 187). MRP4 is present in the intestine, liver, kidney (188, 189), and BBB (190), and transports nucleoside analogs (191) as well as glucuronide (192) and glutathione conjugates (189, 193). Fan *et al.* (40) found that calcitriol treatment of Caco-2 cells induces human MRP2 and MRP4, though post-transcriptionally for the latter. Higher expression of MRP2, which excretes drug/conjugates and endobiotics at the apical membrane, is found in numerous systems following calcitriol treatment. In Caco-2 cells, increased MRP2 expression and B \rightarrow A transport activity of the MRP2 substrate 5-(and-6)-carboxy-2',7'-dichlorofluorescein ensues following $1,25(\text{OH})_2\text{D}_3$ treatment (40). This effect was mirrored following treatment with other vitamin D analogs, $1\alpha(\text{OH})\text{D}_3$ and $1\alpha(\text{OH})\text{D}_2$ but not LCAa (40). In the rat intestine (142), but not the kidney and liver (51, 142), calcitriol treatment induced protein expression of *Mrp2* and was accompanied by a change in function, evidenced by increased B \rightarrow A efflux of 5-(and-6)-carboxy-2',7'-dichlorofluorescein using the everted sac model (167). It was further shown that in rat and human intestine slices, LCA-induced *MRP2* mRNA levels (158), and that $1\alpha(\text{OH})\text{D}_3$ induced *Mrp2* in the mouse kidney (156, 157).

The inductive changes for MRP3 with VDR are not that consistent. In Caco-2 cells, no significant change in *MRP3* mRNA expression was observed following treatment with $1,25(\text{OH})_2\text{D}_3$, $1\alpha(\text{OH})\text{D}_2$, and LCAa. In contrast, *MRP3* mRNA level was increased in human intestine but not in liver slices following treatment with LCA (158). Rat *Mrp3* mRNA expression was increased in liver (51) and intestine (142) following calcitriol treatment, as was mouse *Mrp3* expression in kidney with $1\alpha(\text{OH})\text{D}_3$ (156); the change was once again greater for $1,25(\text{OH})_2\text{D}_3$ compared to $1\alpha(\text{OH})\text{D}_3$ (68). *Mrp3* expression was shown to be increased in the mouse colon following treatment with LCA, the VDR, and FXR ligands (69) in mouse *in vivo*. It is surmised that the discrepancy in the extent of VDR induction could be due to the relative abundances of the VDR present in tissues.

MRP4 protein induction was observed in the Caco-2 cell line in response to treatment with calcitriol without alteration in *MRP4* mRNA expression (40), a change attributed to post-translational regulation. Treatment of cells with cycloheximide, an inhibitor of protein synthesis, revealed that the VDR increases the stability of MRP4 protein rather than increasing it at the transcriptional level (40). The MRP4 substrate, adefovir dipivoxil, is transported into Caco-2 cells (194). An increase in MRP4 function was observed in the Caco-2 system; B \rightarrow A transport of the MRP4 substrate, adefovir, was greater across the cell monolayer when cells were treated with calcitriol (167). Similar results were seen for *Mrp4* in rat intestine following

calcitriol treatment; Mrp4 protein increased (142) with concomitant increase in Mrp4 function—enhanced B → A transport of adefovir in the everted gut sac model (167). Vitamin D analogs elicited similar effects as calcitriol; 1α(OH)D₃ induced Mrp4 in the mouse intestine (156), kidney (68, 156, 157), and liver (68); the renal induction was verified to be greater for 1α(OH)D₃ compared to 1,25(OH)₂D₃ (68). No significant changes in *MRP4* mRNA

levels occurred following treatment with 1α(OH)D₂ and LCAa in the Caco-2 system. Induction of mouse Mrp2, Mrp3, and Mrp4 *in vivo* in rat and human intestinal slices was further demonstrated to occur with vitamin D analogs, LCA, and 1α(OH)D₃ (156–158).

The breast cancer resistance protein (BCRP) is also one of important ABC efflux transporters, and numerous compounds are substrates of BCRP. Although PPARγ-mediated

Table IV Effects of VDR Ligands on ATP Transporters

VDR ligands	Target genes	Cell lines/species	References
1,25(OH) ₂ D ₃	↑MDR1/P-gp	Caco-2 cells (<i>in vitro</i>)	(39, 40, 150, 194)
	↑MDR1	LS174T cells (<i>in vitro</i>)	(180)
	↑MDR1/P-gp	LS180 cells (<i>in vitro</i>)	(37, 38, 151)
	↑Mdr1/P-gp	Rat kidney (<i>in vivo</i>)	(142, 149, 175)
	↑Mdr1/P-gp	Mouse kidney (<i>in vivo</i>)	(68, 181)
	↑Mdr1/P-gp	Mouse brain (<i>in vivo</i>)	(141, 181)
	↑Mdr1/P-gp	Induction mouse brain BBB	(182)
	↑MDR1/P-gp	RBE4 and hCMEC/D3 cells (<i>in vitro</i>)	(174)
	↑Mdr1a/P-gp	Rat liver (<i>in vivo</i>)	(51)
	↑Mdr1a/P-gp	Rat brain (<i>in vivo</i>)	(175)
	↑MRP2	Caco-2 cells (<i>in vitro</i>) Increase in mRNA and protein, and function	(40)
	↑Mrp2	Rat intestine (<i>in vivo</i>)	(142, 167)
	↑MRP3	HT-29 cells (<i>in vitro</i>)	(69)
	↔MRP3	Caco2	(39)
	↑Mrp3	MCA-38 cells (<i>in vitro</i>)	(69)
	↑Mrp3	Mouse colon (<i>in vivo</i>)	(69)
	↑Mrp3	Rat liver (<i>in vivo</i>)	(51)
	↑Mrp3	Rat intestine (<i>in vivo</i>)	(142)
	↑MRP4	Increase in protein; post-translational regulation and increased stability of MRP4 in Caco-2 cells (<i>in vitro</i>)	(40, 194)
	↑Mrp4	Mouse kidney (<i>in vivo</i>)	(68)
	↑Mrp4	Rat intestine (<i>in vivo</i>)	(142, 167)
	↓Mrp4	Rat kidney (<i>in vivo</i>)	(142)
1α(OH)D ₂	↑MRP2	Caco-2 cells (<i>in vitro</i>)	(40)
	↑MRP4	Caco-2 cells (<i>in vitro</i>)	(40)
	↑Mdr1/P-gp	Rat kidney (<i>in vivo</i>)	(149)
1α(OH)D ₃	↓Bsep	Mouse liver (<i>in vivo</i>)	(157)
	↑Mdr1/P-gp	Mouse kidney (<i>in vivo</i>)	(68)
	↑Mrp2	Mouse kidney (<i>in vivo</i>)	(156, 157)
	↑Mrp2	Mouse liver (<i>in vivo</i>)	(157)
	↓Mrp3	Mouse intestine (<i>in vivo</i>)	(68)
	↑Mrp3	Mouse kidney (<i>in vivo</i>)	(68, 156)
	↑Mrp4	Mouse intestine (<i>in vivo</i>)	(156)
	↑Mrp4	Mouse kidney (<i>in vivo</i>)	(68, 156, 157)
	↑MRP4	Caco-2 cells (<i>in vitro</i>)	(40)
	LCA	↑MDR1	LS174T cells (<i>in vitro</i>)
↑MRP2		Human intestinal slice (<i>in vitro</i>)	(158)
↑Mrp2		Rat intestinal slice (<i>in vitro</i>)	(158)
↑MRP3		Human intestinal slice (<i>in vitro</i>)	(158)
↑Mrp3		Mouse colon (<i>in vivo</i>)	(69)

regulatory mechanism has been reported (195), the role of VDR in BCRP regulation has not been reviewed thoroughly. Durk *et al.* (175) and Fan *et al.* (40) reported that brain *Bcrp/BCRP* mRNA and protein expression was not altered in rats following repeated injections of 1,25(OH)₂D₃ (q2d × 4). Moreover, vitamin D-deficiency in mice did not result in significant changes in *Bcrp* mRNA in liver, kidney, and intestine tissue, with or without 20 µg/kg cholecalciferol treatment (q2d × 4) (61). A review by Gorczyca and Aleksunes (196) also revealed that VDRE is not found in the promoter region of ABCG2. These studies clearly suggest that the VDR is not involved in BCRP regulation.

Concluding Remarks on Significance of the Regulatory Function of the VDR

From this short communication, we learn to appreciate that the role of the VDR is not limited to its own synthesis and degradation, but is associated with other therapeutic and regulatory functions. These noteworthy functions of the VDR on NR crosstalk and drug disposition should be recognized, in particular on the maintenance of bone health, proper kidney and bone functions, and brain fitness. The VDR strongly exerts tight control on circulating plasma concentrations of the active ligand, calcitriol (Fig. 2), and is paramount in maintaining calcium, phosphate, and bone homeostasis (Fig. 3). VDR-mediated alterations in enzymes, transporters, and receptors (Tables I, II, III, and IV) showing the changes in CYPs, SULT2A1, SLCs, and ABCs such as P-gp and MRPs will affect the disposition of xenobiotics. VDR suppresses SHP in upregulating CYP7A1 expression in the liver (17) (Fig. 4). Increased P-gp activity in brain (Fig. 5) suggests a therapeutic potential in Alzheimer's disease (AD) due to the anti-inflammatory properties of the VDR (197, 198). The reduction in Aβ plaque (142) and suppression of proinflammatory cytokines in the brain with the VDR are important sequelae towards the preservation of brain health. Of note is that VDR enhances Aβ efflux via LRP1 and P-gp and suppresses influx via RAGE to reduce Aβ accumulation (Fig. 5). Accumulation of Aβ in the brain and siRNA to VDR significantly downregulated the nerve growth factor, showing the importance of the VDR (199). Among rats replenished with low to high doses of vitamin D, dietary vitamin D deficiency increased iNOS expression and oxidative stress in the brain and contributed to significant nitrosative stress in brain and may promote cognitive decline in middle-aged and elderly adults (200). It is surmised that vitamin D deficiency or lowered serum 25(OH) D₃ levels increase the prevalence of dementia including AD (201). Vitamin D supplementation with memantine showed a better effect on cognition in AD patients (202). The use of calcitriol treatment has been implicated via clinical studies,

indicating a significant correlation between VDR gene polymorphism and AD susceptibility (203, 204). Lastly, the vitamin D receptor is of vital importance in the maintenance of kidney health and offers benefits towards preservation of calcium and phosphate homeostasis and tissue repair, reducing renal fibrosis and systemic and renal inflammation (205). Moreover, antiproliferative properties of calcitriol are appreciated by many cell types, particularly epithelial cells, providing the rationale for adjunct therapy to treat cancer (206, 207).

Given these beneficial effects of the VDR, it would be unreasonable for us to ignore calcitriol as a therapeutic modality. Unfortunately, it is well known that pharmacologic doses of calcitriol cause hypercalcemia (15, 17, 45, 51, 61, 68, 142, 149) due to overstimulation of intestinal TRPV6. Because of its lipophilic nature, calcitriol is able to induce TRPV6 rapidly after intravenous, intraportal, or oral administration, and all routes are equally toxic. Hypercalcemia leads to dehydration, diarrhea, and death, and curtails the use of 1,25(OH)₂D₃. The need for vitamin D receptor analogs for the treatment of CKD is undisputable (208–210). Indeed, the less hypercalcemic paricalcitol (211, 212) and doxercalciferol (213–216) are used therapeutically in CKD. It is expected that the development of safer, non-calcemic vitamin D analog drugs will broaden the therapeutic landscape for medications that act on the VDR.

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Declarations

Conflict of Interest The authors declare no conflict of interest.

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