Transcriptional activation of the wild-type and mutant vitamin D receptors by vitamin D₃ analogs

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Abstract

The active form of vitamin D₃ (1α,25(OH)₂D₃, also known as calcitriol) controls the expression of target genes via the vitamin D receptor (VDR). Vitamin D-dependent rickets type II (VDDR-II) is a congenital disease caused by inactivating mutations in the VDR. The condition is treated with high doses of calcitriol, but the therapeutic effects of other synthetic VD₃ analogs have not yet been investigated. In the present study, we analyzed the transcriptional activity of seven different VD₃ analogs with VDRs carrying ligand-binding domain mutations identified in VDDR-II patients. Wild-type VDR (WT-VDR) and seven mutant VDRs were expressed in TSA201 human embryonic kidney cells, HepG2 human liver cancer cells, and MC3T3-E1 mouse calvaria cells, and their transcriptional activation with VD₃ analogs were analyzed by performing transient expression assays, western blotting, and quantitative real-time PCR. The results demonstrated that falecalcitriol stimulated significantly higher transcriptional activation of the WT-VDR and some mutant VDRs than did calcitriol. Calcitriol showed almost no transcriptional activation of the VDR with the I268T mutation identified in a severe case of VDDR-II, whereas falecalcitriol caused a dose-dependent increase in the activation of this mutant VDR. Our findings demonstrate that falecalcitriol stimulated significantly higher transcriptional activation of the WT-VDR and some mutant VDRs than did calcitriol. Calcitriol showed almost no transcriptional activation of the VDR with the I268T mutation identified in a severe case of VDDR-II, whereas falecalcitriol caused a dose-dependent increase in the activation of this mutant VDR. Our findings demonstrate that falecalcitriol has a VDR activation profile distinct from that of calcitriol and may exhibit therapeutic effects even on difficult-to-treat VDDR-II cases resistant to calcitriol. It is also possible that VDDR-II patients responding to high doses of calcitriol could be appropriately treated with low doses of falecalcitriol.

Key Words
- vitamin D receptor
- calcitriol
- falecalcitriol
- vitamin D-dependent rickets type II
- transcription

Introduction

The active form of Vitamin D₃ (VD₃) is a fat-soluble vitamin that performs transcriptional control of target genes by binding to and activating the VD receptor (VDR). In the liver, the proactive form of VD₃ is hydroxylated at position 25, mainly by microsomal vitamin D 25-hydroxylase, CYP2R1, resulting in the formation of...
25-hydroxyvitamin D₃ (25(OH)D₃) (Cheng et al. 2004), which undergoes another hydroxylation at position 1 by 25-hydroxyvitamin D₃ 1-alpha-hydroxylase (CYP27B1) in the kidneys yielding the active form of VD₃, i.e. 1α,25(OH)₂D₃ (Takeyama et al. 1997).

Synthetic VD₃ analogs are important therapeutic agents for treating renal osteodystrophy, psoriasis vulgaris, osteoporosis, and other diseases (Matsumoto et al. 1990, Brown & Coyne 2002, Morii et al. 2004, Tsukamoto 2004, O’Neill & Feldman 2010). Among the VD₃ analogs, calcitriol is the physiologically active form of VD₃, and alfalcaldiol is a prodrug that requires hydroxylation in the liver. Maxacalcitol and falecalcitriol are used as therapeutic drugs for secondary hyperparathyroidism. Eldecalcitol is used for the treatment of osteoporosis. Calcipotriol and tacalcitol have been used as topical agents for psoriasis in Japan.

The VDR is a ligand-inducible transcription factor of the nuclear receptor superfamily class II, which exerts transcriptional control of target genes in the nucleus (Mangelsdorf et al. 1995). The VDR is widely expressed in bones, kidneys, small intestine, liver, heart muscle, blood vessels, immune cells, and nervous system, where it binds to 1α,25(OH)₂D₃ to exert various physiological effects (Haussler et al. 1998). The structure of the VDR can be subdivided into two functional domains: an N-terminal DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD).

A well-known disease resulting from the VDR gene mutations is vitamin D-dependent rickets type II (VDDRRII) (Scriver et al. 1998). Nearly all cases of this disease develop within a few weeks after birth, and signs such as hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, and alopecia are noted (Malloy et al. 1999). The underlying mechanism is the reduction in the VDR transcriptional activity because the mutated VDR either cannot interact with 1α,25(OH)₂D₃ and is not translocated to the nucleus or is deficient in DNA-binding affinity; thus, the mutant receptors cannot activate the transcription of the target genes.

VDDRRII resulting from DBD mutations in VDR cause the ligand-binding positive phenotype because the VDR can still interact with 1α,25(OH)₂D₃ (calcitriol), but cannot bind DNA and activate the transcription of VDR-target genes. If the LBD is mutated, VDR binding to 1α,25(OH)₂D₃ is either attenuated or absent, likewise reducing transcriptional activation of the target genes. A nonsense mutation identified in the hinge region eliminated the LBD altogether (Kristjansson et al. 1993), whereas a mutation in intron 4 caused exon skipping during RNA processing and a frameshift in translation, resulting in receptor truncation (Hawa et al. 1996).

Therapeutic approaches to treating VDDRRII include the administration of high calcitriol doses and/or a high dose of calcium. While almost all patients with DBD mutations are highly VD₃-resistant and do not respond well even to very high doses of VD₃, many patients with LBD mutations can be treated with high doses of calcitriol, a synthetic VD₃ analog (Malloy et al. 2014). However, responses vary widely in different cases depending on the extent of reduction in the VDR transcriptional activity; therefore, the required amount of calcitriol depends on the response of an individual patient, and there are cases where calcitriol treatment alone is not effective.

Currently, there are six VD₃ analogs other than calcitriol that have been approved for clinical use; however, none of them has been established as a drug of choice for the treatment of VDDRRII. Furthermore, no molecular biological study has been yet conducted to examine their comparative transcriptional activity with the mutant VDRs (mVDRs). The present study is the first to investigate the transcriptional activation of seven mVDRs carrying LBD mutations reported in VDDRRII patients using seven clinically approved VD₃ analogs, with the aim to evaluate their therapeutic potentials.

Materials and methods

Cell culture

TSA201 cells, a clone of human embryonic kidney 293 cells (Tagami et al. 1999), were grown in DMEM (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; HyClone laboratories Inc, Logan, UT, USA), penicillin (100 U/mL), streptomycin (100 μg/mL), and 1-glutamine (2 mM). HepG2 human liver cancer cells and MC3T3-E1 mouse osteoblast-like cells were purchased from RIKEN BioResource Center (Wako City, Japan). HepG2 cells were grown in DMEM with the same supplements used for TSA201 cells. MC3T3-E1 cells were grown in MEMa (Wako Pure Chemical Industries) with 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL), and 1-glutamine (2 mM). For cell differentiation experiments, MC3T3-E1 cells were seeded in six-well plates (5.0×10⁴ cells/well) and incubated overnight. Subsequently, the medium was changed to osteoblast differentiation medium, using the Osteoblast-Inducer Reagent (Takara Bio). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂.
Materials

Eldecalcitol and falecalcitriol were kindly provided by Chugai Pharmaceutical Co. (Tokyo, Japan) and Shiono-finesse Co. (Osaka, Japan), respectively. Calcitriol, alfalcacidol, and calcipotriol were purchased from Cayman Chemical, LKT Laboratories, Inc (St Paul, MN, USA), and Toronto Research Chemicals Co. (Toronto, ON, Canada), respectively. Tacalcitol and maxacalcitol were purchased from Santa Cruz Biotechnology. Ethanol used as VD3 solvent was purchased from Wako Pure Chemical Industries.

Plasmid construction

The pCMX-VDR expression vector was provided by K Umesono and R M Evans (Salk Institute, San Diego, CA, USA) (Umesono et al. 1991, Chen et al. 1996). Seven mutant VDRs were generated by oligonucleotide-directed mutagenesis based on previously reported VDDRII cases. Mutation positions are indicated in Fig. 1A: Q259E on helix 4 (Macedo et al. 2008), I268T on helix 5 (Malloy et al. 2004), H305Q on the loop connecting two α-helices (Malloy et al. 1997), I314S and G319V on helix 7 (Whitfield et al. 1996, Macedo et al. 2008), R391C on helix 10 (Whitfield et al. 1996), and E420K on helix 12 (Malloy et al. 2002).

The WT and mutant receptors were subcloned into the pCMX, and the WT receptor was pCMX-VP16 vectors described previously (Umesono et al. 1991, Tagami et al. 1999) for in vitro transcription/translation and transient expression in transfected cells. The Gal4-NCoR, Gal4-SMRT, Gal4-SRC1, Gal4-CBP, Gal4-GRIP1, and Gal4-p300 plasmids containing nuclear receptor corepressors and coactivators were described previously (Tagami et al. 1998a,b). These plasmids were used for mammalian two-hybrid assay for protein–protein interaction analysis. The VD response element (VDRE)-tk-Luc plasmid contains three copies of the mouse osteopontin VDRE (Veenstra et al. 1998) inserted between the SacI and HindIII sites, and the Cyp27b1 promoter-Luc plasmid contains the mouse Cyp27b1 promoter inserted between the EcoRI and XbaI sites in the pGL-3 vector (Promega) (Kozai et al. 2013). The Gal4 reporter plasmid with the upstream activation site UAS-E1b-TATA-Luc contains five copies of the UAS element upstream of E1b-TATA in pA3-Luc (Tagami et al. 1998b).

Transient expression assays

TSA201 cells were transfected with VDR plasmids using the calcium phosphate method (Tagami et al. 1997), and HepG2 cells were transfected using the Lipofectamine3000 Reagent (Thermo Fisher Scientific). The total amount of expression plasmids was kept constant in different experimental groups by adding corresponding amounts of the empty vehicle plasmids. After transfection by calcium phosphate-DNA precipitation for 6h, phenol red-free DMEM (Wako Pure Chemical Industries) with 10% charcoal-stripped FBS was added. Cells were harvested after 20h for the measurement of luciferase activity according to the manufacturer’s instructions (Dual-Luciferase Reporter Assay System; Promega). Transfection efficiency was monitored using the internal control.
Western blotting

Nuclear extracts or cytosol fractions from transfected TSA201 cells prepared using the Nuclear Extract Kit (Active Motif, Carlsbad, CA, USA) were analyzed by SDS-PAGE in 10% acrylamide gels. Proteins were transferred onto nitrocellulose membranes and reacted with a polyclonal antibody against the N-terminal VDR domain (Santa Cruz Biotechnology).

Expression analysis by quantitative real-time PCR

Messenger RNAs from cells were isolated using the RiboZol RNA Extraction Reagent (AMRESCO, Solon, OH, USA) and cDNA was synthesized from 1 μg RNA by reverse transcription. Real-time PCR was performed in the CFX Connect Real-Time PCR Detection System (Bio-Rad) using iTaq Universal SYBR Green Supermix and gene-specific primer pairs: human CYP24A1 sense 5′-GAAGGCTATCGCGACTACC-3′ and antisense 5′-GCTTCATCATTTCCCCCTTGTT-3′; mouse alkaline phosphatase (Alp) sense 5′-CGTGGGATTGTGACTACCA-3′ and antisense 5′-CATGTATTTCCGGCCGCGA-3′; mouse osteopontin (Opn) sense 5′-CTGACCCATTGAGAAGCAGA-3′ and antisense 5′-TCATCCGAGTCCACAGAATCC-3′; mouse collagen type I (Col1) sense 5′-TGGTTTGGAGAGAGCATGACGAGA-3′ and antisense 5′-TGGTCCATGTAGGCTACGCTGTT-3′; mouse receptor activator of nuclear factor kappa-B ligand (Rankl) sense 5′-CAGCATCGCTCTGTTCCTGTA-3′ and antisense 5′-CTGCGTTTTCATGGAGTCTC-3′. The quality of PCR products was monitored by post-PCR melting-curve analysis.

Statistical analysis

The data are presented as the mean ± s.d. from at least three independent transfections performed in triplicate. Statistical analysis of the data was performed by one-way ANOVA followed by Bonferroni’s correction. Differences were considered to be statistically significant at P < 0.05.

Results

Expression of the mVDRs

To observe the expression and subcellular localization of the mVDRs, we analyzed cytoplasmic and nuclear fractions of mVDR-transfected TSA201 cells for mVDR expression by western blotting. The results confirmed that all VDRs were translocated into the nucleus (Fig. 1B).

Transcriptional activation of the wild-type (WT) VDR and mVDRs was compared after stimulation with 10 nM calcitriol. The results indicated that the I314S and G319V mutants had higher transcriptional activity, while other mVDRs had significantly lower transcriptional activity compared with the WT-VDR (Fig. 1C).

The effect of VD₃ analogs on the transcriptional activity of the WT-VDR

In TSA201 cells, transcriptional activation of the WT-VDR by 10.0 nM treatment with tacalcitol, falecalcitriol, and maxacalcitol was significantly higher (by 1.2 ± 0.2, 1.4 ± 0.02 and 1.2 ± 0.03-fold, respectively) than that of calcitriol (10.0 nM). By contrast, in HepG2 cells, WT-VDR transcriptional activity was significantly higher in only falecalcitriol-treated cells (10.0 nM; 1.70 ± 0.10-fold increase; Fig. 2B). However, WT-VDR transcriptional activation in both cell types following alfalcacidol and eldecalcitol treatment was significantly lower when
compared with that observed following calcitriol stimulation (Fig. 2A and B).

The effect of VD₃ analogs on the transcriptional activity of the mVDRs

Comparison of the interaction of VD₃ analogs with the mVDRs showed that falecalcitriol and maxacalcitol exerted more significant transcriptional activation of five mVDRs (Q259E, I268T, H305Q, R391C, and E420K) than did calcitriol at the same dose (Fig. 3). These mutant receptors were then examined for their responses to different doses of the VD₃ analogs (Fig. 4). Maxacalcitol demonstrated higher transcription activation than calcitriol for all mVDRs except R391C. Falecalcitriol increased transcriptional activity of the WT-VDR and all five mVDRs in a dose-dependent manner.

Effects of falecalcitriol and maxacalcitol on the interaction between the VDR and cofactors

In the presence of falecalcitriol and maxacalcitol as well as calcitriol (used as control), the VDR recruited coactivators in a dose-dependent manner. In addition, calcitriol and falecalcitriol inhibited the recruitment of a corepressor in a dose-dependent manner. Compared with calcitriol, falecalcitriol significantly promoted the recruitment of SRC1 (Fig. 5A, 1.0 nM), GRIP1 (Fig. 5B, 1.0–10.0 nM), and CBP (Fig. 5C, 10.0 nM), while significantly inhibiting the recruitment of SMRT (Fig. 5D, 10.0 nM). No VD₃ analogs had any effect on the recruitment of p300 and NCoR (data not shown).

Effects of calcitriol and falecalcitriol on the activity of the Cyp27b1 promoter via the WT-VDR

The Cyp27b1 gene is negatively regulated by the activated VDR. In our experiments, both calcitriol and falecalcitriol significantly repressed Cyp27b1 promoter activity via interaction with the WT-VDR, but no difference was observed in their repressive effects (Fig. 6A). However, in the case of the falecalcitriol-sensitive mVDR (I268T), falecalcitriol demonstrated significantly stronger repression of the Cyp27b1 promoter activity than calcitriol (Fig. 6B, 10.0 nM).

Effects of calcitriol and falecalcitriol on mRNA expression

For functional analysis of transcription regulation exerted by falecalcitriol on the VDR-target genes, real-time quantitative PCR experiments were performed to analyze CYP24A1 mRNA expression in TSA201 cells, as well as Alp,
Figure 4
The effects of different doses of falecalcitriol and maxacalcitriol on the transcriptional activity of VDRs (A–F). TSA201 cells were co-transfected with the expression plasmids (20 ng) carrying the wild-type (WT) or indicated mutant VDRs (mVDR), VDRE-tk-Luc (50 ng), and pGL4.70 (5 ng) in the absence or presence of increasing concentrations of VD3 analogs (0.1–10 nM). The data are presented as the mean ± s.d. from three transfections performed in triplicate. **P < 0.01, ***P < 0.001.

Figure 5
Interactions of cofactors with the wild-type VDR in the mammalian two-hybrid assay. TSA201 cells were co-transfected with the VP16-VDR (WT) expression plasmid (50 ng), UAS-E1b-TATA-Luc (100 ng), and Gal4-cofactor (50 ng) in the absence or presence of VD3 analogs and analyzed for the recruitment of coactivators (A, B and C) and corepressor (D). The data are presented as the mean ± s.d. from three transfections performed in triplicate. *P < 0.05, **P < 0.01.
Opn, Col1, and Rankl1 mRNA expression in MC3T3-E1 cells. The results showed that falecalcitriol stimulated CYP24A1, Alp, Opn, Col1, and Rankl1 mRNA expression levels by 2.3±0.1, 2.6±0.1, 1.5±0.1, 1.9±0.2, and 3.5±0.7-fold compared with those observed following calcitriol stimulation, respectively, suggesting that falecalcitriol exerts a higher transcription-promoting effect (Fig. 6C).

Discussion

In this study, we investigated seven VD$_3$ analogs and compared their transcriptional activity with WT-VDR and mVDRs containing mutations in the LBD, excluding nonsense mutations, identified in clinical VDDRII cases. To the best of our knowledge, this is the first comparative study on the activity of VD$_3$ analogs with the mutant VDRs relevant to rickets type II, conducted with the aim to develop therapeutic approaches to treat VDDRII caused by LBD mutations.

The results of this study demonstrate that calcipotriol, tacalcitol, falecalcitriol, and maxacalcitol exhibit transcriptional activation either equivalent to or greater than that of the natural calcitriol. However, alfacalcidol and eldecalcitol demonstrated weak transcriptional activation of the WT-VDR (1.2±0.1 and 3.2±0.5-fold compared with the unstimulated receptor in TSA201 cells). One reason for this difference in activity is that alfacalcidol is a prodrug that is converted to calcitriol via hydroxylation in the liver (Orimo & Schacht 2005), but not in human embryonic kidney TSA201 cells. The binding affinity of eldecalcitol to the VDR is about 50% of that of calcitriol (Hatakeyama et al. 2010), which partly accounts for its very weak transcriptional activation of the VDR.

The treatment for VDDRII caused by LBD mutations has been the administration of high doses of calcitriol and/or oral calcium; however, the therapeutic efficiency varies depending on the mutation site in the LBD (Malloy et al. 1999). Among VDDRII patients with LBD mutations, I314S carriers could be treated with pharmacological doses of VD$_2$, and Q259E, H305Q, G319V, and R391C carriers responded to high doses of calcitriol (Macedo et al. 2008). However, patients with I268T and E420K showed no improvement in symptoms even when high calcitriol doses were administered (Malloy et al. 2002, 2004).

Our results showing transcriptional activation of the I314S mVDR with 10.0 nM calcitriol (by 31.6±2.5-fold compared with unstimulated, control TSA201 cells)
are consistent with the data on the therapeutic efficacy of high calcitriol doses. Furthermore, none of the tested VD₃ analogs exerted significantly higher mVDR (I314S) activation than calcitriol.

According to previous reports, four patients identified with Q259E, H305Q, G319V, and R391C mVDRs presented various VDREI symptoms such as leg deformation and alopecia, which were mitigated by administering high doses of calcitriol (Whitfield et al. 1996, Malloy et al. 2004, Macedo et al. 2008). The results of this study show that for G319V, calcitriol stimulation at the high dose of 10.0 nM increased receptor transcriptional activity by 29.5 ± 0.5-fold, demonstrating almost the same efficacy as with the WT-VDR. The transcriptional activity of the Q259E, H305Q, and R391C mVDRs was significantly increased by calcitriol only at high doses; however, falecalcitriol activated these receptors at all tested doses, starting as low as 0.1 nM.

It has been shown that the I268T and E420K mutations result in the development of severe rickets in infancy, and the disease was resistant to high doses of calcitriol (Malloy et al. 2002, 2004). Our results indicated that stimulation with a high dose (10.0 nM) of calcitriol did not activate these mVDRs (I268T, 4.2 ± 2.8-fold; E420K, 2.7 ± 0.2-fold; WT, 26.3 ± 2.2-fold, compared with vehicle in TSA201 cells); however, 10.0 nM falecalcitriol caused considerably higher activation, especially of I268T (I268T, 37.4 ± 2.1-fold; E420K, 3.5 ± 0.1-fold; WT, 32.1 ± 0.5-fold, compared with vehicle in TSA201 cells). Furthermore, 1.0 and 10.0 nM falecalcitriol caused significantly higher mVDR activation than calcitriol.

Regardless of whether they are characterized or unknown, many transcriptional factors and cofactors can potentially interact with VDR and their target genes to exert the pharmacological effects by VD₃ analogs. Thus, we employed reporter assay systems and real-time quantitative PCR to evaluate overall transcriptional processes in this study. To elucidate the mechanism underlying the increase in receptor transcriptional activation by falecalcitriol, the interaction between cofactors and the WT-VDR was analyzed using the mammalian two-hybrid system. The results showed that the recruitment-promoting activity of falecalcitriol toward SRC1, GRIP1, and CBP was significantly higher than that of calcitriol. Similarly, falecalcitriol inhibited the recruitment of SMRT in a dose-dependent manner, and the effect was significantly stronger than that of calcitriol. Thus, the increase in transcriptional activation of the VDR by falecalcitriol can be explained, at least partly, by promoting the recruitment of coactivators and inhibiting that of corepressors.

The I268T mutation has been reported to inhibit the recruitment of coactivators SRC1 and DRIP205 (Malloy et al. 2004), but falecalcitriol did not alleviate this inhibition, although it promoted cofactor recruitment to the WT-VDR and stimulated its transcriptional activity. Still, falecalcitriol demonstrated significantly higher activation of the mVDR (I268T) than calcitriol, suggesting an unknown mechanism of transcriptional activation unrelated to the recruitment of coactivators.

The transcriptional control exerted by falecalcitriol via the VDR was analyzed with the VDR target genes. Cyp27b1 encodes VD₃ 1α-hydroxylase involved in the biosynthesis of 1α,25(OH)₂D₃. It has been shown that 25-hydroxyvitamin D₃ synthesized in the liver undergoes hydroxylation at position 1α by CYP27B1 in the kidneys, where it becomes 1α,25(OH)₂D₃ (Takeyama et al. 1997). Cyp27b1 expression is repressed when the VDR-RXR heterodimer formed by calcitriol binds to a negative VDR response element on the Cyp27b1 promoter (Turunen et al. 2007). CYP24A1 is an enzyme that initiates the degradation of 1α,25(OH)₂D₃ by hydroxylation of the side chain at position 24 of 1α,25(OH)₂D₃; the expression of this enzyme is positively regulated by VDR-RXR heterodimer binding to VDRE on the CYP24A1 promoter (Ohyama et al. 1994). Alp, Opn and Col1 are known as differentiation markers of osteoblast cell lines, and Rankl is an osteoclast differentiation factor. Their mRNA expression levels are increased by 1α,25(OH)₂D₃ (Bikle 2012). Expression analysis indicated that 10.0 nM falecalcitriol increased mRNA levels more significantly than calcitriol. With the WT-VDR, there was no significant difference between calcitriol and falecalcitriol in the repression of the Cyp27b1 promoter; however, with mVDR (I268T), falecalcitriol demonstrated stronger Cyp27b1 promoter repression than calcitriol.

The affinities of eldecalcitol and falecalcitriol for the WT-VDR are approximately 50% (Hatakeyama et al. 2010) and 33% (Inaba et al. 1989, Katsumata et al. 1996) compared with that of calcitriol. For maxacalcitol, the affinity is slightly weaker than that of calcitriol (Valaja et al. 1990) and is almost the same as that of calcipotriol (Binderup & Bramm 1988). The affinity of tacalcitol is comparable with that of calcitriol (Matsumoto et al. 1990). Alfacalcidol does not directly bind to the WT-VDR in vitro because it is a produg converted to calcitriol via hydroxylation in the liver (Orimo & Schacht 2005). Although the binding affinity of falecalcitriol to the VDR is approximately 25% of that
of calcitriol (Inaba et al. 1989, Katsumata et al. 1996), its activity in increasing the expression level of osteopontin, a protein involved in bone formation, has been reported to be stronger than that of calcitriol (Miyamoto et al. 1995). Falecalcitriol may exert its potent pharmacological effects synergistically with ST-232, a main metabolite of falecalcitriol in cells (Katsumata et al. 1996). The binding affinity of eldecalcitrol to the VDR is about 50% of that of calcitriol (Hatakeyama et al. 2010), and the molecular mechanism underlying its superior therapeutic effect for treating osteoporosis is currently unknown. One characteristic feature of eldecalcitrol is its high binding affinity for vitamin D-binding protein in serum (Kikuta et al. 2013), suggesting that free eldecalcitrol can be present in vivo without binding to the VDR. These observations suggest that although VD₃ analogs act through the VDR, other factors may be involved in their transcriptional activities (Carlberg 2014).

In summary, the present study shows the possibility that in the treatment of certain types of VDDRlI, falecalcitriol may have therapeutic effect on the cases resistant even to high doses of calcitriol. It is also conceivable that VDDRlI patients responding to high doses of calcitriol could be appropriately treated with low doses of falecalcitriol. Our findings suggest that the selection of an appropriate VD₃ analog, including falecalcitriol, according to the mutation site in VDDRlI cases could be expected to produce an improvement in therapeutic effect.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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