The Vitamin D Receptor and the Syndrome of Hereditary 1,25-Dihydroxyvitamin D-Resistant Rickets*

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The Syndrome of Hereditary 1,25-Dihydroxyvitamin D-Resistant Rickets (HVDRR)

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Vitamin D, the primary regulator of calcium homeostasis in the body, is particularly important in skeletal development and in bone mineralization. The active form of vitamin D, 1α,25-dihydroxyvitamin D, [1,25(OH)2D] (this notation will be used to signify either D3 or D2), mediates its actions by binding with high affinity to specific vitamin D receptors (VDRs) located in the nucleus of target cells. Hereditary vitamin D-resistant rickets (HVDRR) is a rare genetic disorder caused by a generalized resistance to 1,25(OH)2D action (1–3). Heterogeneous mutations in the VDR that alter the function of the receptor are the molecular basis of HVDRR. A variety of mutations have been identified, some of which render the VDR nonfunctional, imparting a complete hormone-resistant state, while other mutations reduce VDR activity, causing a hyporesponsive state. In this review, we will describe the clinical manifestations of HVDRR, the VDR and its gene, the mechanism of vitamin D action, and the genetic defects in the VDR that result in this hormone-resistant syndrome. Other recent reviews of these subjects can be found in the recently published volume entitled “Vitamin D” and the references therein (4).

A. Historical

In 1978, Brooks et al. (5) described a patient with osteomalacia who exhibited hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism. Interestingly, the patient had markedly increased serum levels of 1,25(OH)2D. Brooks et al. (5) suggested that the rickets was due to impaired responsiveness of target organs to 1,25(OH)2D. They termed this disease vitamin D-dependent rickets type II (VDDR-II) to distinguish it from a closely related syndrome known as vitamin D-dependent rickets type I (VDDR-I),
which is due to a defect in an enzyme leading to the synthesis of 1,25(OH)_2D (see below, Section II.B). Later the same year, Marx et al. (6) reported similar findings in two children, and the authors again suggested that the disease was due to end-organ resistance to 1,25(OH)_2D. Since these initial studies, there have been many reports of patients with apparent target organ resistance to 1,25(OH)_2D. Over the years a number of different terms have been used to describe this syndrome. As noted, the original reports referred to this entity as vitamin D-resistant rickets type II. The disease also has been called pseudo-vitamin D deficiency rickets type II (PDDR-II), calcitriol-resistant rickets, vitamin D-resistant rickets, and hereditary hypocalcemic vitamin D-resistant rickets. We prefer the designation hereditary vitamin D-resistant rickets (HVDRR) as a simple and accurate description of this syndrome caused by genetic resistance to vitamin D.

B. Clinical features of HVDRR

The major clinical findings in patients with HVDRR, hypocalcemia and rickets, are due to defective intestinal calcium absorption leading to impaired mineralization of newly forming bone and preosseous cartilage. The rickets is often severe and is usually exhibited within months of birth. Patients suffer from bone pain, muscle weakness, hypotonia, and occasionally convulsions from hypocalcemia. Children are often growth retarded, and in some cases they develop severe dental caries or exhibit hypoplasia of the teeth (7–13). Some infants have died from pneumonia caused by poor respiratory movement due to severe rickets of the chest wall (8, 11, 14). Many children with HVDRR have sparse body hair, and some have total scalp and body alopecia, including eyebrows and in some cases eyelashes (Fig. 1). Alopecia will be discussed in more detail below (Section I.D).

An example of the typical serum biochemistry levels found in HVDRR cases is shown in Table 1. The abnormalities include low concentrations of calcium and phosphate and elevated serum alkaline phosphatase activity. The hypocalcemia leads to secondary hyperparathyroidism with elevated PTH levels and hypophosphatemia. The 25(OH)D values are normal and, importantly, the 1,25-(OH)_2D levels are elevated. This clinical feature distinguishes HVDRR from 1α-hydroxylase deficiency (VDDR-I or PDDR discussed below, Section II.B) since the serum 1,25-(OH)_2D values in the latter syndrome are depressed. In the cases in which it has been measured, 24,25(OH)_2D levels have been normal or low (8, 11, 15–21). Unlike patients with 1α-hydroxylase deficiency, most HVDRR individuals are resistant to supraphysiological doses of all forms of vitamin D therapy (Table 1).

HVDRR follows an autosomal recessive pattern of inheritance. The recessive nature of the disease is evident from the parents who are heterozygous for the genetic trait but show a normal phenotype with no symptoms of the disease and normal bone development. In many, if not all cases, parental consanguinity is associated with the disease. Males and females are equally affected and often a family has several affected children. An extensive pedigree of seven related families with HVDRR is illustrated in Fig. 2 (22).

Fig. 1. Children with HVDRR and alopecia. [Reprinted with permission from J. F. Rosen et al.: J Pediatr 94:729–735, 1979 (7)].

C. Pathophysiology

Among the many biological processes attributed to vitamin D, maintenance of calcium and bone homeostasis is most apparent. 1,25-(OH)_2D is essential for promoting calcium and phosphate transport across the small intestine and into the circulation, which is necessary for the normal mineralization of bone. Approximately 50% of the total intestinal calcium absorption is attributed to 1,25-(OH)_2D action, while the remaining 50% is due to passive absorption (23, 24). It is now well established that the biological actions of 1,25-(OH)_2D are mediated by the VDR, a nuclear transcription factor that regulates gene expression in 1,25-(OH)_2D-responsive cells. Since vitamin D regulates the translocation of calcium and phosphate, interference with the 1,25-(OH)_2D action pathway causes decreased mineral transport and hypocalcemia. The hypocalcemia, in turn, results in secondary hyperparathyroidism, which induces hypophosphatemia. The calcium and phosphate deficiencies interfere with normal bone mineralization, leading to rickets in children and osteomalacia in adults. In HVDRR, 1,25-(OH)_2D target organs such as the intestine are resistant to hormone action, and therefore the intestine is less...
efficient in promoting calcium and phosphate absorption into the circulation. The vitamin D resistance is due to mutations in the VDR that render the receptor nonfunctional or less functional than the wild-type VDR.

D. Alopecia

A clinical feature that is generally found in patients with HVDRR is alopecia totalis (Fig. 1). The majority of HVDRR patients have sparse body hair, and some exhibit total scalp and body alopecia (17, 25, 26). Children with extreme alopecia often lack eyebrows and in some cases eyelashes. Hair loss may be evident at birth or it occurs during the first few months of life. An analysis of HVDRR patients shows that there is some correlation between the severity of rickets and the presence of alopecia (26). Patients with alopecia generally have more severe resistance to calcitriol than those without alopecia. In families with a prior history of the disease, the absence of scalp hair in newborns provides initial diagnostic evidence for HVDRR. The mechanism causing alopecia is unknown, but VDRs are present in the hair follicle (27, 28). Skin biopsy has revealed apparently normal follicles with no hair shaft present. The lack of 1,25-(OH)2D action during a critical stage of hair follicle development is the suspected cause of alopecia.

E. 1,25-(OH)2D action and HVDRR

The biological actions of 1,25-(OH)2D in tissues and cells are orchestrated through complex changes in gene expression (29, 30). These changes lead to cell-specific alterations in the level of proteins directly responsible for a myriad of differentiated cell functions, as well as in proteins that act as transcription factors or as signaling molecules to regulate secondary and tertiary levels of gene expression (31). In the latter case, these molecules may function directly within the cell or indirectly via additional cellular signaling pathways in either autocrine or paracrine fashion. As indicated earlier,

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**Table 1. Biochemical profile of a typical patient with HVDRR on therapy**

<table>
<thead>
<tr>
<th>Biochemical marker</th>
<th>Normal values</th>
<th>Referral values</th>
<th>40 days&lt;sup&gt;a&lt;/sup&gt;</th>
<th>80 days&lt;sup&gt;b&lt;/sup&gt;</th>
<th>100 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/liter)</td>
<td>2.2–2.6</td>
<td>1.86</td>
<td>1.77</td>
<td>1.80</td>
<td>1.71</td>
</tr>
<tr>
<td>Phosphate (mmol/liter)</td>
<td>1.4–2.2</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>145–320</td>
<td>3056</td>
<td>3991</td>
<td>3800</td>
<td>3609</td>
</tr>
<tr>
<td>25(OH)D (nmol/liter)</td>
<td>25–85</td>
<td>30</td>
<td>37.4</td>
<td>250</td>
<td>211</td>
</tr>
<tr>
<td>1,25(OH)2D (pmol/liter)</td>
<td>40–105</td>
<td>521</td>
<td>953</td>
<td>1830</td>
<td>1560</td>
</tr>
<tr>
<td>PTH (pmol/liter)</td>
<td>&lt;8</td>
<td>—</td>
<td>34.2</td>
<td>69.9</td>
<td>64.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatment: 250 mg elemental calcium 4 times per day and 0.5 µg calcitriol (Rocaltril) twice per day and 20,000 IU vitamin D3 daily.

<sup>b</sup> Treatment: 250 mg elemental calcium 4 times per day and 5 µg calcitriol twice per day. ALP, Alkaline phosphatase. [Adapted from W. J. Zhu et al.: J Bone Miner Res 13:259–264, 1998 (248) with permission of The American Society for Bone and Mineral Research.]
most, if not all, of the molecular actions of 1,25-(OH)₂D in the nucleus are mediated by the VDR. The classic role of 1,25-(OH)₂D is to regulate mineral homeostasis, achieved through its coordinated actions on intestine, kidney, bone, and parathyroid gland (32, 33). It is not surprising, therefore, that initial evidence for the existence of the VDR derived from early investigations in these tissues (34-42). Interestingly, the VDR is expressed in a wide variety of tissues, including kidney, skin, liver, pancreas, muscle, breast, prostate, adrenal, thyroid, and cells of mesenchymal or hematopoietic origin (27, 28, 43-47). Although the VDR in these tissues appears to arise from the same chromosomal gene, its role in cellular function is not homeostatic in nature but rather pleiotropic. Whereas the classic actions of vitamin D are to regulate calcium homeostasis, the expanded scope of vitamin D pleiotropic actions include stimulation of differentiation, inhibition of cell proliferation, and suppression of the immune response (45-48). In addition, the regulation of cellular proliferation and differentiation by 1,25-(OH)₂D appears to be a common feature in many tissues examined, and it is likely that this regulatory feature is a fundamental component of all biological responses to 1,25-(OH)₂D. Notwithstanding the complexity and diversity of biological responses elicited by 1,25-(OH)₂D, the profound skeletal abnormalities demonstrated in patients with HVDRR emphasizes the fundamental and essential role of 1,25-(OH)₂D in calcium homeostasis.

Although there are multiple pleiotropic tissue responses regulated by 1,25-(OH)₂D, children with HVDRR appear relatively normal except for the constellation of features that relate to their calcium deficiency, rickets, and alopecia. VDRs have been found in endocrine glands such as pituitary, pancreas, parathyroid, gonads, and placenta, and 1,25-(OH)₂D₃ regulates hormone synthesis and secretion from these glands (29, 45, 46, 49-51). VDRs have also been found in hematolymphopoietic cells, and 1,25-(OH)₂D₃ regulates cell differentiation and the production of interleukins and cytokines (52). Hochberg et al. (53) examined hormone secretion in patients with HVDRR and found no abnormalities in insulin, TSH, PRL, GH, and testosterone levels in serum. Even et al. (54) showed that urinary cAMP and renal excretion of potassium, phosphorous, and bicarbonate were normal in HVDRR patients treated with PTH. However, PTH failed to decrease urinary calcium and sodium excretion in these patients to the extent found in controls. This suggests that 1,25-(OH)₂D may selectively modulate the renal response to PTH and facilitate the PTH-induced reabsorption of calcium and sodium (54). Although minor aberrations have been noted in the fungicidal activity of neutrophils from HVDRR patients (55), the patients do not exhibit any clinically apparent immunological defects. In the light of the diverse actions of 1,25-(OH)₂D demonstrated in many nonosteogenic tissues, the absence of related findings in children with HVDRR suggests that the pleiotropic responses regulated by 1,25-(OH)₂D in these nonosteogenic tissues are redundant and that other factors or compensatory mechanisms subsume the role of vitamin D in such a way that abnormalities are not clinically manifested. Similarly, the VDR knockout mouse displays the same phenotypic and physiological patterns as patients with HVDRR (56, 57). The VDR knockout mouse model can be used to analyze the abnormalities caused by the loss of VDR action in detail not possible in the HVDRR patients (see Section IX).

II. Vitamin D Physiology

A. Metabolism

Vitamin D is a fat-soluble secosteroid that exists in two forms, vitamin D₃ (cholecalciferol) from animal sources and vitamin D₂ (ergocalciferol) from plant sources. Since vitamin D is synthesized in the skin, it is a hormone and not a true vitamin. In animals, the precursor (provitamin) molecule, 7-dehydrocholesterol, is cleaved between carbon-9 and -10 in the B ring, using the energy derived from UV B rays of sunlight, which opens the ring and creates the secosteroid structure (Fig. 3) (58). Vitamins D₂ and D₃ are essentially biologically inactive and must be converted to hydroxylated metabolites to gain hormonal activity. Upon entering the circulation, vitamin D (D₃ or D₂) binds to the vitamin D-binding protein, a 58-kDa plasma α-globulin (59, 60). In the liver, vitamin D is hydroxylated at the carbon-25 position to form 25-hydroxyvitamin D [25(OH)D] by the enzyme 25-hydroxylase (61). This enzyme, also known as CYP27, is a multifunctional cytochrome P450 oxidase that also hydroxylates cholesterol and bile acids. In the kidney the enzyme 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase), also a P450 oxidase, adds a second hydroxyl to form 25(OH)D to form 1,25-(OH)₂D, the hormonally active form of vitamin D.

The level of renal 1α-hydroxylase activity is tightly regulated by PTH (62). Patients with hyperparathyroidism have elevated serum levels of 1,25-(OH)₂D, whereas patients with hypoparathyroidism show reduced levels of the hormone (62, 63). Other factors, including phosphate and 1,25-(OH)₂D and possibly phosphatonin, also regulate 1α-hydroxylase activity. High phosphate levels in the serum lead to a suppression of 1α-hydroxylase activity while low phosphate levels tend to increase the amount of enzyme activity. 1,25-(OH)₂D regulates its own production, both by suppressing PTH secretion and feedback inhibition of renal 1α-hydroxylase activity (62). In the feedback inhibition loop, low 1,25-(OH)₂D levels lead to increased 1α-hydroxylase activity and high 1,25-(OH)₂D levels inhibit the enzyme activity in the kidney. 1α-Hydroxylase activity is also influenced by the concentration of calcium in the serum. The calcium acts indirectly by regulating PTH levels via the calcium-sensing receptor, thereby determining 1α-hydroxylase activity.

The catabolism of 1,25-(OH)₂D involves a series of enzymatic steps, the first of which is catalyzed by the enzyme 25-hydroxyvitamin D-24-hydroxylase (24-hydroxylase) (64). 24-Hydroxylation generates the trihydroxy form of vitamin D₃, 1,24,25(OH)₃D, a biologically less active form of 1,25-(OH)₂D, that is further subjected to additional hydroxylations and oxidations leading to the production of calcitropic acids, which is excreted in the urine (65, 66). Interestingly, 1,25-(OH)₂D induces 24-hydroxylase activity in a number of cells and, therefore, regulates the rate of its own metabolism. The genes encoding the rat and human 24-hydroxylase
CYP24) have been cloned (67–69), and a vitamin D-response element (VDRE) (see below, Section III.C) has been identified in the regulatory region of these genes (70, 71). Regulation of 24-hydroxylase gene expression or enzyme activity is a very useful marker of 1,25-(OH)2D responsiveness in the evaluation of HVDRR (16). In humans, the 24-hydroxylase gene is found on chromosome 20 at 20q13 (69).

In addition to hydroxylating 1,25-(OH)2D, 24-hydroxylase can convert 25(OH)D to 24,25(OH)2D. The biological role of 24,25(OH)2D has not been established with certainty. However, recent studies with a 24-hydroxylase knockout mouse model strongly suggest that 24,25(OH)2D may have some biological activity distinct from 1,25-(OH)2D, especially on cartilage cells (72).

**B. 1α-Hydroxylase deficiency**

We will discuss this disease entity briefly since it has some elements in common with HVDRR and must be distinguished from it (Table 2). Decreased production of 1,25-(OH)2D is found in patients with 1α-hydroxylase deficiency. In 1961, Prader et al. (73) described a patient with a genetic form of vitamin D-resistant rickets that ultimately was due to a deficiency of renal 1α-hydroxylase. This disease has been known as vitamin D-dependent rickets type I (VDDR-I) and also as pseudo vitamin D deficiency rickets (PDDR) (74).

Genetic linkage studies indicate that the mutation causing 1α-hydroxylase deficiency is linked to chromosome 12 at 12q14 (75–77). Recently, several groups have cloned the 1α-hydroxylase gene (78–83), and it has been confirmed that the
locus on chromosome 12 is the 1α-hydroxylase gene and that
VDDR-I (or PDDR) is due to mutations in the gene encoding
the 1α-hydroxylase. In several cases, mutations in the 1α-
hydroxylase gene have been elucidated (78, 83).

1α-Hydroxylase deficiency is an autosomal recessive dis-
 ease that is manifested at an early age, presenting with hy-
potonia, muscle weakness, growth failure, and rickets. Hy-
pocalcemia, elevated PTH levels, increased alkaline
phosphatase activity, and low urine calcium excretion are
also found. Tetany and convulsions may occur with severe
hypocalcemia. These symptoms are also characteristic of
VDDR patients with this condition are treated with physiological
doses of 1,25-(OH)2D3 that bypass the
defective enzyme and restore serum calcium concentrations
to normal. The low serum levels of 1,25-(OH)2D and the
therapeutic response to physiological doses of
1,25(OH)2D3 distinguish 1α-hydroxylase deficiency from
HVDRR (Table 2).

III. 1,25-Dihydroxyvitamin D Action Mediated by the
Vitamin D Receptor (VDR)

A. Historical aspects of VDR structure and function

A number of observations have established the central role
played by the VDR in the biological activities of 1,25-
(OH)2D3. Initial support evolved from early studies which
revealed that the vitamin D ligand (or its precursor) inter-
acted with a protein found in the nucleus (34 –36, 84, 85). This
protein was expressed in low copy number exclusively in
nuclear cell types. Recent crystallographic studies for several of
the receptors support the predicted structure-function rela-
tionships (see below).

With respect to the nuclear receptor gene family, the
common structural domains are designated A–F (Fig. 4) (96).

1. Overview. The VDR exhibits a modular domain structure
generally similar to that of other members of the nuclear
receptor gene family (see Fig. 4). Since the mutations within
the VDR gene, which will be detailed subsequently in this
review, are distributed across several of its domains, we will
describe the structural organization of the functional protein.
While the structural domains were initially deduced from the
gene sequence, it is important to note that functional corre-
lations with these domains have evolved from extensive
examination of receptor activities of natural mutations in
patients with HVDRR as well as site-directed mutagenesis of
the cDNA and recombinant expression in host mammalian
cell types. Recent crystallographic studies for several of the
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ships (see below).

With respect to the nuclear receptor gene family, the
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While a considerable degree of sequence and structural ho-

mology exists across the superfamily for several of the re-
ceptor domains, others exhibit little or no sequence homol-
gy and in some instances domains may be abbreviated or
absent (92). This diversity is manifest most strongly in re-

gions A/B, D, and F. Segment A/B includes all residues that
extend amino terminal to the DNA-binding domain (DBD) of
the receptor. Its size is highly variable, ranging from hun-
dreds of amino acids (aa) in the progesterone receptor (PR),
for example, to approximately 24 aa in the VDR. Region C
comprises the highly conserved DBD of the receptor gene
family and represents the hallmark feature of this group of
proteins, which will be described in detail below. The D
domain, which is least conserved among the nuclear recep-
tors, appears to serve as a hinge between the DBD and the
E region. The D region within the VDR is 50 residues longer
than that found in the other steroid receptors and is likely the
result of an additional exon within the VDR chromosomal
gene (97). Consistent with other receptors, this region in the
VDR exhibits very limited species conservation, particularly
at the level of the inserted exon. The hinge region is also a site
of serine phosphorylation (98, 99) and may play additional
functional roles. The E region encodes the ligand-binding
domain (LBD) of the hormone-activated receptors and ex-
hibits several important functional activities, which will be

### Table 2. A comparison of 1α-hydroxylase deficiency and HVDRR

<table>
<thead>
<tr>
<th>Feature</th>
<th>1α-Hydroxylase deficiency*</th>
<th>HVDRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene mutated</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Autosomal recessive</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Manifested at early age</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Rickets</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Alopecia</td>
<td>No</td>
<td>Sometimes</td>
</tr>
<tr>
<td>PTH</td>
<td>Elevated</td>
<td>Elevated</td>
</tr>
<tr>
<td>25(OH)D levels</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1,25(OH)2D levels</td>
<td>Low</td>
<td>Elevated</td>
</tr>
<tr>
<td>Response to physiological doses of 1,25(OH)2D3</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

* 1α-Hydroxylase deficiency is also known as VDDR I or PDDR.

The VDR cDNA revealed that it is structurally and function-
ally homologous to an emerging family of genes that en-
coded receptors for the sex and adrenal steroids, thyroid
hormone, and retinoic acid (92, 93). This superfamily of nu-
clear receptor and transcription factor genes, derived from
both vertebrate and invertebrate sources, presently includes
more than 60 members (94). It is comprised of the known
nuclear ligand-activated receptors, as well as an ever grow-
ing number of receptors for which ligands have not yet been
identified. While many of these orphan receptors may not
require ligands for activation, investigation of several of
these receptors led to the identification of at least four new
lipophilic ligands, which include 9-cis retinoic acid, prosta-
glandin J2, and certain farnesol intermediates (94), as well as
several derivatives of cholesterol (95).

B. The domain structure of the VDR

1. Overview. The VDR exhibits a modular domain structure
generally similar to that of other members of the nuclear
receptor gene family (see Fig. 4). Since the mutations within
the VDR gene, which will be detailed subsequently in this
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hibits several important functional activities, which will be
The small F region is not conserved within the nuclear receptor family and is, in fact, absent in the VDR as well as in several other members of the family.

2. DBD. The DBD of the VDR (aa residues 24–90) contains nine highly conserved cysteine residues that function to coordinate a single zinc atom. Elucidation of the three-dimensional structure of several related receptors followed by molecular modeling of the VDR reveals two α-helices (helix A and B) shaded in the diagram and located on the carboxy-terminal side of each zinc module. Amino acid residues essential to functional interaction of these α-helices with either DNA or the VDR protein partner are boxed and designated the P box and D box, respectively. A third region comprised of two short α-helices (α-helix C) is also shaded and designated T/A. The functional activities of each of these three conserved regions of the DBDs, as documented both by mutagenesis and through molecular modeling studies, are indicated below the structure.

discussed below. The small F region is not conserved within the nuclear receptor family and is, in fact, absent in the VDR as well as in several other members of the family.

2. DBD. The DBD of the VDR (aa residues 24–90) contains nine highly conserved cysteine residues and consists of two similar motifs each comprised of a zinc-coordinated finger structure (Fig. 5). Each zinc atom is tetrahedrally coordinated through four of the highly conserved cysteine residues that serve to stabilize the finger structure itself. These finger modules are structurally unrelated to the multiple zinc fingers found in transcription factor IIIA wherein the zinc atom is coordinated through two cysteines and two histidines (100, 101). Interestingly, although the two zinc modules of the VDR appear highly related structurally, they are not topologically equivalent (100). This lack of equivalency is consistent with the fact that each module serves a different function within the protein. The amino-terminal module, comprised of an α-helix known as the P box (aa residues 41–46), functions to direct specific DNA-binding in the major groove of the DNA-binding site. The carboxy-terminal module, on the other hand, which also contains an α-helix known as the D box (aa residue 61–65), serves as a dimerization interface for interaction with a partner protein (see below) (102, 103). This region comprised of two short α-helices (α-helix C) is also shaded and designated T/A. The functional activities of each of these three conserved regions of the DBDs, as documented both by mutagenesis and through molecular modeling studies, are indicated below the structure.
nucleotides located between the two DNA half-sites, thus strengthening the interaction of the VDR with its DNA-binding elements. Posttranslational modification of VDR by phosphorylation of Ser51 inhibits its ability to complex with the VDRE and may serve as a negative regulator of VDR activity (104, 105). When the three-dimensional structure of the VDR DBD emerges, either as a result of nuclear magnetic resonance spectroscopy or x-ray crystallography studies, our understanding of the structural organization of these modules, as well as the mechanisms through which they function to interact with DNA, will be enhanced. Recent successes using these techniques with the estrogen receptor (ER), glucocorticoid receptor (GR), retinoic acid receptor (RAR), and RXR DBDs have provided significant insights into the structure of this domain (106–111). As will be discussed below, mutations in critical amino acids within both zinc finger modules have rendered the VDR nonfunctional and have caused HVDRR presumably by interfering with VDR binding to DNA.

3. LBD. The E region of the VDR represents a complex multifunctional domain that retains strong dimerization and transactivation potential, induced and manifested through the binding of 1,25-(OH)2D3. Despite the fact that mutations have been identified that compromise hormone binding, little is known regarding the overall structure of the ligand-binding pocket of the VDR. It is hypothesized that binding leads to conformational changes that expose, enhance, or reduce affinity for 1,25-(OH)2D3. Alternatively, mutations in the VDR gene prevented 1,25-(OH)2D3 induction of target genes such as 24-hydroxylase (16, 18, 134). An understanding of the molecular mechanisms through which 1,25-(OH)2D3 and its receptor modulate gene expression is outlined in Fig. 3 and emerged initially through examination of the promoters for genes known to be regulated by this hormone. A major focus of these studies was to demonstrate that 1,25-(OH)2D3 could regulate promoter activity, that specific DNA sequences within the promoter were required, and that a functional VDR was an essential component of the transacting machinery (121, 122). It is likely that these latter interactions occur in both gene promoter- and cell-specific ways to produce the observed tissue-selective actions of 1,25-(OH)2D3 that are well documented. As with other nuclear receptors, these tissue-selective actions are particularly manifested by the VDR when associated with synthetic vitamin D analogs, suggesting that subtle differences in receptor conformation are capable of producing profoundly different biological consequences (114, 123). Mutations throughout the LBD may cause HVDRR by a number of mechanisms. They may completely prevent ligand binding or reduce affinity for 1,25-(OH)2D3. Alternatively, mutations may alter VDR conformation, compromising its ability to dimerize with RXR or interact with coactivators.

4. Structural modeling. Recently, the three-dimensional structure of the LBD of RXXRs (124), RARs (125), and thyroid receptor (TRα1) (126), all RXXR partners, as well as ER (127) and PR (127, 128), have been elucidated. We will focus our analysis of the LBD on the RXXR partners since they are more relevant to the VDR. RARγ and TRα1 receptors were crystallized in the presence of ligand (holodomains), whereas the RXXRs structure was determined in the absence of ligand (apo-domain). Twelve α-helices (H1-H12) arranged as an antiparallel α-helical sandwich comprise the bulk of the LBD of each of the receptors (129). It is likely that the VDR will be arranged in a structurally similar manner, and a model of the VDR helical structure based on the canonical structure of nuclear receptors is shown in Figs. 6 and 7. These three-dimensional structures support the idea that H9 and H10 are essential for the formation of RAR, VDR, TR, or peroxisome proliferator-activating receptor (PPAR) heterodimers with a common RXR subunit. While functional studies support the requirement of these helical sequences in heterodimer formation, H9 and H10 may be insufficient for VDR and PPAR dimerization. This conclusion is experimentally supported by a complete evaluation of the dimerization properties of the carboxy-terminal E region of the VDR (130).

5. Transactivation. An additional function inherent to the E region of the VDR is an activation function termed AF-2. The core of this domain function is located at the extreme carboxy terminus of the protein and is associated with a small subregion defined crystallographically as H12 (117, 118, 124–126, 131). An important observation regarding H12 is the clear repositioning of this α-helix back upon the hydrophobic core and upstream α-helices in the E domain in response to ligand binding (Fig. 7). This repositioning likely “locks” 1,25-(OH)2D3 into its binding pocket and leads to the formation of a complex high-affinity protein surface capable of interacting with specific coactivators such as SRC-1 and others. This requisite interaction between H12 and more upstream α-helices likely accounts for the observed loss of VDR transcriptional capacity after mutation of residues located in either region of the receptor molecule (132, 133). The three-dimensional structure of the VDR will, however, have to be solved directly to confirm these predictions.

C. The regulation of gene expression by the VDR

The proof of the regulation of gene expression by VDR was initially developed by studies of HVDRR where natural mutations in the VDR gene prevented 1,25-(OH)2D3 induction of target genes such as 24-hydroxylase (16, 18, 134). An understanding of the molecular mechanisms through which 1,25-(OH)2D3 and its receptor modulate gene expression is outlined in Fig. 3 and emerged initially through examination of the promoters for genes known to be regulated by this hormone. A major focus of these studies was to demonstrate that 1,25-(OH)2D3 could regulate promoter activity, that specific DNA sequences within the promoter were required, and that a functional VDR was an essential component of the trans-
activation machinery. These principles of direct regulation of gene expression by 1,25-(OH)₂D₃ were established using human (135–137) and rat (138–140) osteocalcin gene promoters as models and were strengthened through subsequent investigation of the osteopontin gene (141), the calbindin genes (142, 143), and the 25-hydroxyvitamin D₃ 24-hydroxylase genes (70, 144, 145). HVDRR mutant VDRs were shown to be incapable of activating such promoter constructs, both supporting the critical role of functional VDR in transactivation as well as defining the defect causing HVDRR (146–148).

1. Direct regulation of transcription by 1,25-(OH)₂D₃. Initial investigations by McDonnell et al. (149) demonstrated that 1,25-(OH)₂D₃ stimulated transcription from the human osteocalcin gene promoter in a dose-dependent fashion consistent with its actions on the expression of the endogenous gene. The sensitivity of this promoter to 1,25-(OH)₂D₃ enabled subsequent definition of the DNA sequence element within the promoter responsible for mediating hormone action (see below). Equally important, the ability of 1,25-(OH)₂D₃ to stimulate this transcriptional activation was de-
pertinent upon the presence of intact VDR. Thus, while the osteocalcin promoter was unresponsive to 1,25-(OH)_2D_3 when introduced into a VDR-negative cell line or cotransfection with an HVDRR mutant VDR, cointransfection of the wild-type VDR via a recombinant expression vector restored hormonal responsiveness. The establishment of this VDR- and hormone-dependent assay in cells enabled a determination of the functional domains of the VDR. As will be seen in subsequent sections of this review, this assay has become essential in establishing the inactivating nature of numerous mutations found in the VDR chromosomal gene in patients with HVDRR.

2. VDREs. Deletion analysis of the human osteocalcin promoter led to the identification of a cis-acting element that was located approximately 500 bp upstream of the transcriptional start site and that mediated 1,25-(OH)_2D_3 induction (135). This study and one by Ozono et al. (137) resulted in definition of the first VDRE, a directly repeated hexanucleotide sequence separated by 3 bp. Parallel studies using the rat osteocalcin gene promoter led to similar conclusions regarding the organizational motif of the VDRE (138–140). Definitive VDREs have since been localized in the mouse osteopontin gene (141), the rat and human 24-hydroxylase genes (70, 144, 145), and the human p21 gene (150). As seen in Fig. 8, each of these elements is comprised of two directly repeated hexanucleotide half-sites and, like the human osteocalcin VDRE, the half-sites are separated by a 3-bp spacer. Studies on the mouse calbindin D_28K (142) and the rat calbindin D_28K (143) and the rat calbindin D_28K (142) genes have also revealed apparent VDREs, although these sequences mediate rather weak 1,25-(OH)_2D_3 induction in their natural promoter environments and do not exhibit structural similarity to the more well characterized VDREs. One possibility is that the VDR binds to these sites, perhaps in combination with a nonconventional protein partner(s) (see below). The exact mechanism whereby the VDR interacts with its VDRE, which is described in the next section, is particularly relevant since a large majority of the mutations found within the VDR gene that lead to HVDRR are found within the VDR DBD and their mechanism is to interfere with DNA binding.

3. Interaction of VDR with VDRE DNA. The definition of VDREs within vitamin D-sensitive gene promoters facilitated subsequent examination of the interaction between the VDR and its DNA-binding sites. An extensive battery of mutations introduced into the VDR and tested for functionality have revealed that two domains within the VDR are required for high-affinity DNA binding in vivo, the DNA-binding or C domain and the ligand-binding or E domain. Interestingly, the molecular basis for abrogation of DNA binding in each of the two regions is fundamentally different. In the DBD, missense mutations found in HVDRR cases or mutations generated by site-directed mutagenesis that lead to a disruption of one or both of the zinc-coordinated finger structures reduce or prevent direct receptor-VDR interaction and/or sensitize the receptor to subsequent proteolytic degradation (151–155). In contrast, loss of DNA binding via LBD mutations within the carboxy-terminal E domain result from the disruption of functions unrelated to DNA binding per se but essential to the formation of a high-affinity receptor DNA complex capable of transactivation (130, 132, 149, 156). Mutations that specifically lead to loss of hormone binding represent one such class. Assuming that the defect is restricted to ligand interaction, this type of mutation results in a protein capable of DNA binding but unable to process the signal that initiates the DNA binding events and subsequent transactivation. Several mutations of this class have been identified in HVDRR and will be discussed below. A second series of mutations disrupts the capacity of VDR to form dimers with partner proteins that include, but are not restricted to, the receptor RXR (see below for discussion). Examples of this type of mutation have been observed in HVDRR (157), although it arises more commonly in thyroid hormone resistance (158). Finally, mutations that lead to truncations within the carboxy-terminal domain also abrogate DNA binding, but do so as a result of the obvious loss of both dimerization and hormone-binding capabilities.

4. Subunit structure of the active VDR heterodimer. The observation that VDREs are comprised of two half-sites led to the prediction that the VDR might bind to these sites as a functional dimer. Indeed, the interaction of each of the classic steroid receptors with their respective hormone response elements (HREs) are known to occur via homodimerization. It was a surprise, therefore, to observe that the VDR did not associate with DNA as a homodimer, but rather as a heterodimer (Fig. 8). This finding, made by Liao et al. (159) and Sone et al. (152, 160), demonstrated that DNA binding of recombinant VDR produced in yeast or in vitro could only be

![Fig. 8. Model of the RXR/VDR heterodimer bound to a consensus VDRE. RXR (R) and VDR (V) are illustrated bound to a directly repeated VDRE located upstream of the start site of transcription. The dark circle represents the 1,25-(OH)_2D_3 ligand, whereas P represents a serine phosphorylation site at residue 208 in the human protein. The nucleotide sequences of several known VDREs and the genes in which they reside are documented below the figure. Each hexanucleotide sequence is separated by a 3-bp spacer.](image)
achieved after reconstitution with mammalian cellular extracts. The factor supplied by the extract, whose presence was confirmed by several additional groups (161, 162), was termed nuclear accessory factor or NAF. NAF was found to be widely distributed in cells and tissues and hypothesized to be an unknown nuclear receptor. Similar heterodimeric activities were identified for the thyroid hormone receptor (TR) and RAR. In 1991 and 1992, four groups of investigators demonstrated that this activity derived from three related members of the nuclear receptor gene family termed retinoid X receptor (RXRα, RXRβ, and RXRγ) (163–166). It was concluded that NAF was a single manifestation of a mixture of one or more of the RXRs expressed in any given cell type. True dimerization between the VDR and RXR has been confirmed through definition of the VDR dimerization domain (130). Utilizing an extensive series of internal deletions of the VDR, two regions located within the E domain were shown to be essential for interaction with RXR. These regions coincide with two subregions within the E domain that are moderately conserved within the entire nuclear receptor gene family. Perlmann et al. (167) suggested recently that a small region of 40 aa lying within the second E/F homology domain (corresponding to H9 and H10 of the crystal structure of the RXR LBD) is sufficient within RXR to form dimers with RAR and TR. This same region is apparently not sufficient, however, to permit formation of RXR-VDR heterodimers, suggesting that the domains responsible for interaction between RXR and other signaling partners may be different.

Interestingly, in view of the asymmetric nature of the VDRE (two directly repeated half-sites) and the heterodimeric nature of the VDR modulatory unit (RXR and VDR), distinct polarity must also exist with respect to the half-site-receptor interaction. Indeed, Jin et al. (130) and Freedman and co-workers (153) demonstrated that RXR binds to the upstream or 5′-half-element and that VDR binds to the downstream half-element on consensus type VDREs (Fig. 8). Whether this polarity is maintained on all VDREs remains to be proven. However, this organization is consistent with that noted for both RXR-TR and RXR-RAR heterodimers bound to their respective HREs (168, 169). Irrespective of the mechanism, the existence of a general permissive partner protein that functions as a central regulator for several endocrine systems suggests the potential for considerable cross-talk between the systems.

5. The transactivation domain of the VDR. As indicated earlier, two regions are believed to mediate the transactivation capacity of the VDR, a carboxy-terminal AF-2 region comprised of H12, which includes residues 416–424, and the E1 region located in the midregion of the receptor comprising H3 and H4, which includes residues from 232 to 272 (30) (Figs. 4 and 6). Additional helices downstream of H4 may be involved as well. Based upon the crystal structure of apo-RXR LBD and holo-RAR LBD (124–126), it is likely that interaction of 1,25-(OH)2D3 with the VDR leads to the folding of H12 back against the hydrophobic core of the E domain and helices therein. This repositioning completes the high-affinity binding site for 1,25-(OH)2D3 and creates a complex surface capable of additional protein-protein interactions (Fig. 7). Indeed, mutations in each of these two regions can selectively abrogate ligand binding, transactivation, or both.

Does the AF-2 domain of RXR contribute to transactivation by the VDR? It is clear that RXR has the potential to function as a signaling partner when complexed to orphan receptors, such as LXR and NGF-IB (167, 170). Additional studies suggest that the AF-2 function of the silent partner is required for activation by the signaling partner (171, 172). This supports the idea that the AF-2s of the silent and the signaling receptors both participate in a common protein surface essential for activation of transcription. Perhaps activation of the silent partner occurs via the process of dimerization with the signaling partner and/or after DNA binding (173). Evidence is rapidly accumulating for such regulatory events.

6. Transcriptional comodulators and cofactors. The transactivation domain of most nuclear receptors interacts directly with additional classes of proteins termed comodulators. These proteins include the positive modulators or coactivators SRC-1 (116), GRIP-1/TIF2 (174, 175), and ACTR (176) and the negative regulators or corepressors NCoR (177) and SMRT (119, 120). Indeed, the AF-2 of the VDR is found to be essential for interaction with SRC-1 (117, 118) and likely is essential for other comodulator interactions as well (Fig. 9). After recruitment of a comodulator into the promoter region by a nuclear receptor, these proteins both induce chromatin structural changes enzymatically or recruit additional proteins capable of similar actions or both. Coactivators appear to exhibit histone acetyltransferase activity (176) and recruit additional histone acetyltransferases such as P/CAF (178) and CBP/p300 (179), each of which functions to enzymatically modify histones such that nucleosomes are destabilized and transcription is facilitated. The ability of CBP to interact simultaneously with a number of unrelated transcription factors also suggests an additional function for these large proteins—that of integrating multiple inputs on a gene promoter so as to produce a coherent output (180, 181). In contrast, corepressors apparently served to recruit deacetylases such as HDAC1 (182–184). Histone deacetylase activity serves to stabilize chromatin and repress transcription (111). In this paradigm, nuclear receptors appear to function, at least in part, to recruit the enzymatic machinery necessary for carrying out the changes in chromatin structure essential for the effective regulation of transcription. Nuclear receptors are, however, capable of additional important contacts. Two groups have shown that a domain within the VDR hinge region makes important contacts with TFIIB (121, 122), a member of the initiation complex per se. This interaction, one of several made by nuclear receptors, appears to enhance transcription through direct stabilization of the transcriptional machinery.

As will be detailed in subsequent sections of this review, mutations that interfere with various steps in the 1,25-(OH)2D-VDR hormone action pathway lead to the syndrome of HVDRR.

IV. Cellular Basis of HVDRR

A. Studies in cultured skin fibroblasts

The syndrome of HVDRR was first recognized as an entity in 1978/1979 (5–7, 185), and since that time a number of cases
of vitamin D resistance have been described, which are detailed in Table 3. The HVDRR cases are referred to throughout this review as F1, F2, etc., where the F denotes the family number (8–19, 25, 134, 186–209). The clinical findings in children affected with HVDRR, along with their failure to respond to the administration of physiological and even supraphysiological doses of vitamin D, suggest that the disease is caused by end-organ resistance to vitamin D. The identification and characterization of the VDR as the mediator of 1,25-(OH)2D3 action and its presence in the major target tissues of vitamin D action led investigators to suspect that the cause of HVDRR was due to a genetic defect in the VDR (6–9). Studies of VDR from HVDRR cases began after the demonstration that VDRs were present in skin (27, 43, 210) and could be studied in cultures derived from human skin biopsies (211).

In 1982, Feldman et al. (16) examined the vitamin D system in cultured skin fibroblasts from two siblings with HVDRR (family F11). This study demonstrated that high-salt extracts of cultured fibroblasts from HVDRR patients had undetectable levels of [3H]1,25-(OH)2D3 binding. The fibroblasts from the affected individuals were also resistant to high concentrations of 1,25-(OH)2D3, since 24-hydroxylase activity, a well-characterized biomarker for 1,25-(OH)2D3 responsiveness, could not be induced after hormone treatment. This approach provided a model for studying HVDRR subjects using cultured dermal fibroblasts to examine both the ligand-binding properties of the VDR as well as cellular responsiveness to 1,25-(OH)2D3 measured by the induction of 24-hydroxylase. Subsequently, a number of other HVDRR cases were examined using cultured skin fibroblasts (11, 188–191) or, in one case, cells derived from bone (192). In some patients the fibroblasts lacked specific [3H]1,25-(OH)2D3 binding (11, 188–192) similar to the cases reported by Feldman et al. (16). On the other hand, some fibroblasts exhibited normal [3H]1,25-(OH)2D3 binding. Nevertheless, the HVDRR cells showed no response to 1,25-(OH)2D3 treatment (11, 18, 190, 192, 197, 203). From these early studies it was apparent that at least two classes of VDR defects exist. Patients who had normal [3H]1,25-(OH)2D3 binding were categorized as “receptor-positive” or “ligand-binding positive” and, in most cases, their defect was later shown to be in the DBD of the VDR. Those cases that showed decreased or absent [3H]1,25-(OH)2D3 binding were denoted “receptor negative” or “ligand binding negative” and the defect was later shown to be in the LBD of the VDR or due to absent VDR.

Studies by Griffin and Zerwekh (190) and Liberman et al. (191, 192) also used 24-hydroxylase activity to demonstrate 1,25-(OH)2D3 resistance. On the other hand, Clemens et al. (189) showed that fibroblasts from HVDRR patients were not growth-arrested after hormone treatment in contrast to fibroblasts from healthy individuals that were growth-arrested. These early observations showed that cells from HVDRR patients were resistant to 1,25-(OH)2D3 and that a variety of abnormalities in the VDR existed.

1. Ligand-binding negative phenotype. As the number of reports on HVDRR increased, the heterogeneous nature of the defects in the VDR became more apparent. Hochberg et al. (17, 25) reported clinical findings in four patients from two unrelated families of Arab origin (F11, F18) who exhibited HVDRR and alopecia. Fibroblasts from three of these patients and several of their parents, as well as an additional unrelated family from Germany (F17), were studied by Chen.
## Table 3. Families and subjects with HVDRR

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient name/description</th>
<th>Ethnicity/origin</th>
<th>Onset age</th>
<th>Affected</th>
<th>Consanguinity</th>
<th>Alopecia</th>
<th>Ligand binding</th>
<th>Molecular basis</th>
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<td>Haitian</td>
<td>?</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>+ Arg73Gln</td>
<td>18, 146</td>
</tr>
<tr>
<td>F20</td>
<td>Kindred 7, patient 7, 7, P7</td>
<td></td>
<td></td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>194, 197, 202, 243</td>
</tr>
<tr>
<td>F21</td>
<td>Patient 1, 1a</td>
<td>Kuwait</td>
<td>1 yr</td>
<td>1</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>14, 259, 260</td>
<td></td>
</tr>
<tr>
<td>F22</td>
<td>Patient 1, 1b</td>
<td>Hispanic</td>
<td>1 yr</td>
<td>1</td>
<td>No</td>
<td>Yes</td>
<td>±</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>F23</td>
<td>Patient 1, 1a</td>
<td>Saudi</td>
<td>1 yr</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>+ Gly46Asp</td>
<td>199, 247</td>
<td></td>
</tr>
<tr>
<td>F24</td>
<td>Patient 2, 2a</td>
<td>Arab</td>
<td>4 months</td>
<td>1</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>195, 201</td>
<td></td>
</tr>
<tr>
<td>F25</td>
<td>Patient 1, 1b</td>
<td>Japanese</td>
<td>2 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>+ Arg80Gln</td>
<td>19, 233</td>
<td></td>
</tr>
<tr>
<td>F26</td>
<td>Patient 2, 1b</td>
<td>Japanese</td>
<td>2 months</td>
<td>1</td>
<td>Yes</td>
<td>No</td>
<td>+ Arg50Gln</td>
<td>19, 233</td>
<td></td>
</tr>
<tr>
<td>F27</td>
<td>Patient 3, 1b</td>
<td>Japanese</td>
<td>2 months</td>
<td>1</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>F28</td>
<td>Patient 4, 4</td>
<td>Persian-Jewish</td>
<td>9 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td>195, 201</td>
<td></td>
</tr>
<tr>
<td>F29</td>
<td>Patient 1, 1a</td>
<td>Saudi</td>
<td>13 months</td>
<td>2</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td>Tyr295stop</td>
<td>13, 202, 245</td>
</tr>
<tr>
<td>F30</td>
<td>Patient 1, 1b</td>
<td>Saudi</td>
<td>13 months</td>
<td>2</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td>Tyr295stop</td>
<td>13, 202, 245</td>
</tr>
<tr>
<td>F31</td>
<td>Patient 2, 2a</td>
<td>Arab</td>
<td>2 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>+ Gly33Asp</td>
<td>146, 203</td>
<td></td>
</tr>
<tr>
<td>F32</td>
<td>Patient 3, 3</td>
<td>Turkish</td>
<td>5 weeks</td>
<td>2</td>
<td>Yes</td>
<td>Yes</td>
<td>+ Gly33Asp</td>
<td>146, 203</td>
<td></td>
</tr>
<tr>
<td>F33</td>
<td>Patient 4, 4</td>
<td>Japanese</td>
<td>16 months</td>
<td>2</td>
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<td>Yes</td>
<td>+ Arg50Gln</td>
<td>204, 233</td>
<td></td>
</tr>
<tr>
<td>F34</td>
<td>Patient 5, 5</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
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<td>+ Arg50Gln</td>
<td>204, 233</td>
<td></td>
</tr>
<tr>
<td>F35</td>
<td>Patient 6, 6</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F36</td>
<td>Patient 7, 7</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>+ Tyr295stop</td>
<td>22, 147</td>
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<tr>
<td>F37</td>
<td>Patient 8, 8</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td></td>
<td></td>
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<tr>
<td>F38</td>
<td>Patient 9, 9</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F39</td>
<td>Patient 10, 10</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F40</td>
<td>Patient 11, 11</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F41</td>
<td>Patient 12, 12</td>
<td>Arab</td>
<td>16 months</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
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</tr>
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</table>
et al. (134). In these studies, HVDRR fibroblasts exhibited negligible [3H]1,25-(OH)2D3-binding, and hormonal treatment failed to induce 24-hydroxylase enzyme activity. These cases were designated as representing the ligand-binding negative phenotype. Interestingly, of the five parents in the study, only the father in family F18 exhibited a phenotype theoretically expected of a heterozygote. His fibroblasts contained half of the normal amount of [3H]1,25-(OH)2D3-binding and also showed a half-maximal response to 1,25-(OH)2D3. The fibroblasts of the other four parents showed a normal complement of VDR and a normal response to 1,25-(OH)2D3. The fibroblasts of the other four parents showed a normal complement of VDR and a normal response to 1,25-(OH)2D3, treatment, which has been the usual pattern found in fibroblasts of heterozygotes.

In 1982, after the development of a monoclonal antibody to the VDR (87, 212, 213), the presence of VDR in some patients with the ligand binding-negative phenotype (11, 15) was assessed by Pike et al. (214). Using a radioligand immunoassay (215), an immunoreactive protein was detected in cell extracts from fibroblasts of ligand binding-negative HVDRR patients (214). The authors speculated that the defect in the VDR was due to a structural abnormality in the LBD preventing [3H]1,25-(OH)2D3 from binding to the receptor and not from defective synthesis of the VDR protein (214).

2. Ligand binding-positive phenotype. A ligand binding-positive case was described by Hirst et al. (18) who studied the VDR in fibroblasts from a Haitian family (F19) with HVDRR. Cultured fibroblasts from two sisters with HVDRR were unresponsive to 1,25-(OH)2D3 treatment despite normal [3H]1,25-(OH)2D3-binding and VDR abundance. In addition, sucrose gradient sedimentation studies revealed a VDR of normal size. However, the protein exhibited a decreased ability to form aggregates in low-salt conditions as compared with normal receptor. Using DNA-cellulose chromatography, the authors demonstrated that the VDR from the HVDRR fibroblasts exhibited a significant decrease in affinity for heterologous DNA. The normal receptor eluted from the DNA-cellulose at 170–173 mm KCl, while the mutant receptor eluted at 105–109 mm KCl. A second HVDRR family (F31), studied by Malloy et al. (203), examined a similar defect in the DNA binding properties of the receptor. The VDR from the affected individuals displayed normal [3H]1,25-(OH)2D3 binding and was normal in size as shown by Western blotting. However, DNA-cellulose chromatography clearly revealed that the VDR had a low affinity for DNA eluting at 100 mm KCl in contrast to the wild-type VDR, which eluted at 200 mm KCl. The patients from F19 and F31 families were therefore categorized as having the ligand binding-positive phenotype and, in addition, had a VDR that exhibited a low affinity for DNA. When the VDR from the parents’ cells were subjected to DNA-cellulose chromatography, two forms of the receptor were found. One receptor form eluted at 200 mm KCl, indicating that it had a high affinity for DNA similar to the wild-type receptor, while the other form eluted at 100 mm KCl, demonstrating a low affinity for DNA similar to the HVDRR patient. This was the first clear evidence showing the heterozygous state of the HVDRR parents. It was suspected that the defects in these cases would likely be due to point mutations in the BD (18, 203), which was later proved correct (146).

Liberman et al. (197) also described four cases (F1, F3, F5, F20) of ligand binding-positive resistance to 1,25-(OH)2D3. Two of the cases (F5, F20) exhibited VDRs with a low affinity for DNA similar to the F19 and F31 families. Gamblin et al. (194) examined 1,25-(OH)2D3 induction of 24-hydroxylase activity in F5 and F20 fibroblasts and demonstrated complete hormone resistance. In the other cases, F1 and F3, Liberman et al. (197) demonstrated that the VDRs had a reduced ability to localize to the nucleus despite showing a normal affinity for DNA. Gamblin et al. (194) further showed that the F1 and F3 fibroblasts exhibited 24-hydroxylase activity when exposed to high concentrations of 1,25-(OH)2D3 in vitro, also exhibited a calcemic response to high doses of calciferols in vivo.

Castells et al. (196) also described a ligand binding-positive
B. Studies in other cells

In addition to cultured skin fibroblasts and bone cells, studies of the VDR from patients with HVDRR have been carried out in a number of other cell types including peripheral mononuclear cells (195), phytohemagglutinin (PHA)-stimulated lymphocytes (200, 206), myeloid progenitor cells (201), Epstein-Barr virus (EBV)-immortalized B lymphoblasts (22, 146, 147, 203), and HTLV-1 virus-immortalized T lymphoblasts (205). It is interesting to note that EBV-immortalized B lymphoblasts, from normal subjects that express wild-type VDR, do not exhibit induction of 24-hydroxylase activity or growth inhibition in response to 1,25-(OH)2D3 (22). On the other hand, PHA-stimulated lymphocytes and HTLV-1-immortalized T lymphoblasts from normal subjects are capable of responding to 1,25-(OH)2D3 (205, 216). In PHA-stimulated lymphocytes, the absence of an inhibitory effect of 1,25-(OH)2D3 on DNA synthesis or lack of induction of 24-hydroxylase activity have been used as markers to rapidly diagnose HVDRR (200, 206). In addition, Takeda et al. (206) showed that PHA-stimulated lymphocytes from parents of children with HVDRR expressed intermediate levels of 24-hydroxylase when treated with 1,25-(OH)2D3.
promoter suggest that the transcription factor SP-1 may play an important role in its expression (97). Indeed, deletion of these elements results in a dramatic reduction in the activity of this promoter after introduction into cultured cell lines. Interestingly, the mouse VDR gene shows a similar, although not identical, organizational profile at the level of the promoter, suggesting a similar mode of regulation (219). Additional studies are in progress to evaluate and identify further important molecular determinants of VDR gene expression. Thus far, no cases of HVDRR have been shown to be caused by mutations in the 5′-prime regulatory region of the VDR gene.

C. Polymorphisms of the VDR gene

Mutations in the coding regions of the VDR gene lead to significant functional consequences, as will be discussed extensively in the next section on HVDRR. On the other hand, two sets of normal variants or polymorphisms have been noted in the VDR gene, and their effects on VDR function are worthy of discussion. The first class represents a set of polymorphisms at the 3′-end of the VDR in intron J (between exons 8 and 9) and exon 9 (Fig. 11). Although multiple polymorphisms have been identified in this region (220), the commonly studied ones are defined by the restriction enzymes BsmI, ApaI, and TaqI. These polymorphisms have been associated with variations in bone mineral density (BMD) in a number of human studies and, although controversial, are hypothetically predictive of osteoporosis risk (220). A meta-analysis of a number of studies concluded that there was a small difference in BMD that was associated with these polymorphisms (221). A poly A microsatellite of variable length found in the 3′-untranslated region in exon 9 is linked to the three sites (220), although the linkage is of variable tightness, depending upon ethnicity (222). Interestingly, the polymorphisms have also been associated with other diseases such as osteoarthritis, hyperparathyroidism, and prostate cancer (223–229). Attempts to define differences in VDR function between the polymorphic variants have not been successful, so that a rationale for disease association is not obvious (230–232).

The second polymorphism class was observed initially by Saijo et al. (233). It occurs in the translation start codon (ATG) located in exon 2 and is referred to as the start codon polymorphism (SCP) or FokI site because it is defined by this restriction enzyme (234). In this polymorphism, thymine is replaced by cytosine, which results in the initiation of translation at an ATG site located 3 aa downstream of the first ATG. The SCP variant alleles generate two VDR gene products differing in length by 3 aa (232, 235). The distribution of the SCP, defined by the restriction enzyme FokI, has been assessed in several population groups (234–237). Interestingly, the most abundant form appears to be the amino-terminal foreshortened protein (designated F). There are indications that this protein may exhibit increased functional activity compared with the longer protein (f) (235), although other work indicates that there is no substantial difference between the activities of the variant forms (232). A potential association between the SCP alleles and BMD has been identified, and the polymorphism apparently correlates with osteoporosis risk (234–236). However, since not all populations exhibit this association (237), additional studies will be required to substantiate whether the SCP is significantly related to BMD. The SCP is inherited independently of the 5′-polymorphisms, and use of the two sets of markers in combination may prove to be a better predictor of osteoporosis risk than either polymorphism used alone.

The system used to number the amino acids in the VDR and thus to identify the site of a mutation in HVDRR cases has been somewhat confusing due to variations in the VDR length caused by the SCP (233). Depending upon the nucleotide sequence at this polymorphic site, the VDR can be comprised of 424 (F variant) or 427 (f variant) aa. Most of the earlier reports of HVDRR cases based their numbering on 424 aa.

![FIG. 11. Polymorphisms in the human VDR chromosomal gene. The structural organization of the human VDR gene is shown. The three 5′-noncoding exons and eight coding exons are depicted as shaded boxes. The location of the start codon polymorphism (SCP) is shown above exon 2 and the FokI polymorphism it generates is shown below the exon. The lowercase f is used to indicate the absence of the FokI restriction site and a 427-aa protein. The uppercase F is used to indicate the presence of the FokI site and a 424-aa protein. The location of the BAT polymorphisms is shown above intron J and exon 9, and the BsmI, ApaI, and TaqI restriction sites are shown below the figure. The presence (uppercase) or absence (lowercase) of these restriction sites does not change the amino acid sequence of the VDR. In addition, there is a variable length poly A microsatellite in exon 9, which is indicated on the figure.](image-url)
aa since this is the most common genotype, and the HVDRR patients and normal subjects usually exhibited the SCP that resulted in the loss of the first 3 aa at the N terminus of the VDR (3). However, since researchers have universally adopted the 427-aa numbering system of Baker et al. (90), the numbering used in this review to describe mutations previously numbered using 424 aa has been adjusted by 3 to correspond to that of the published sequence (90).

VI. HVDRR Mutations Causing the Ligand Binding-Positive Phenotype

A. Initial description of DNA-binding domain (DBD) mutations

Using the information obtained from the biochemical and cellular studies of the HVDRR patients, investigations into the molecular cause of HVDRR began shortly after the human VDR cDNA sequence was reported by Baker et al. (90). From the amino acid sequence data, a highly conserved zinc finger structure was identified in the VDR. Since the zinc finger motif was thought to be the DBD, investigators examined this region of the VDR gene for mutations in the ligand binding-positive HVDRR cases that exhibited low-affinity DNA binding. The development of the PCR by Saiki et al. (238) during this same time period provided investigators a convenient method to amplify genes from small amounts of DNA using a thermostable DNA polymerase that greatly accelerated the sequence analyses.

In 1988, Hughes et al. (146) used PCR to amplify exons of the VDR gene in DNA samples from the F19 and F31 families that exhibited the ligand binding-positive, but DNA binding-defective phenotype (18, 203). In family F19 (18), a missense mutation (CAA to CGA) was found in exon 3, which encodes the second zinc module of the DBD. The mutation substitutes a polar uncharged glutamine for a positively charged arginine at amino acid 73 (Arg73Gln) (Fig. 12). In family F31 (203), a missense mutation (GGC to GAC) was identified in exon 2, which encodes the first zinc module of the DBD. This mutation results in the replacement of a glycine with a highly charged aspartic acid at amino acid 33 (Gly33Asp) (Fig. 12). Both a normal and mutant allele were found in the parents' DNA from both families, which confirmed the genetic transmission and recessive nature of the disease. Using site-directed mutagenesis each mutation was recreated in the wild-type VDR cDNA, and the properties of the mutant VDR were analyzed after expression in COS-1 cells. Both F19 and F31 mutant VDRs exhibited normal [3H]1,25-(OH)2D3 binding, and each exhibited low-affinity binding to calf thymus DNA (146, 148). These experiments confirmed that the mutations gave rise to the ligand binding-positive, DNA binding-defective phenotype seen in the patients' fibroblasts (18, 203). In addition, Sone et al. (148) demonstrated that the mutant VDRs were transcriptionally inactive in cotransfection experiments in CV-1 cells. Using an osteocalcin-CAT reporter plasmid, the authors showed that CAT activity could be induced by the wild-type VDR but not by the two mutant VDRs, proving that these mutations were the cause of vitamin D resistance (148). The VDR mutations described by Hughes et al. (146) were the first natural disease-causing mutations identified in the entire steroid-thyroid-retinoid receptor gene superfamily. Mutations have since been found in many of the classic receptors including TR (158), AR (239), ER (240), GR (241), and mineralocorticoid receptor (MR) (242).

FIG. 12. Model of the DBD of the VDR and location of mutations causing HVDRR. The two zinc finger modules and the amino acid composition of the DBD are shown. Conserved amino acids are depicted as shaded circles. The location of the mutations is indicated by large arrows. Missense mutations are illustrated as large circles, premature stop mutations as ovals, and the one frameshift (Fs) mutation as a star. The location of the intron separating exon 2 and exon 3, which encode the separate zinc finger modules, is indicated by an arrow. Numbers specify amino acid number.
B. Characterization of additional DBD mutations

Since the initial report (146), a number of additional mutations have been identified in the VDR DBD. The location of these mutations within the DBD is illustrated schematically in Fig. 12 and tabulated in Table 4. Sone et al. (243) examined the VDR from two unrelated patients (F5 and F20) previously shown to exhibit a ligand binding-positive and low-affinity DNA binding phenotype by Liberman et al. (191, 197). In both patients, a G-to-A missense mutation (CGG to CAG) was identified in exon 3. This mutation replaces arginine with a glutamine at amino acid 80 located in the second zinc finger module (Arg80Gln). The recreated Arg80Gln mutant receptor bound [3H]1,25-(OH)2D3 normally and exhibited low-affinity binding to calf thymus DNA. In addition, the Arg80Gln mutant receptor was unable to activate gene transcription from a reporter plasmid, demonstrating that this molecular defect is the cause of HVDRR in these cases (243). The same Arg80Gln mutation was also identified in two siblings with HVDRR (F49) by Malloy et al. (21). The F49 family and the families (F5, F20) described by Sone et al. (243) both had origins in North Africa; however, no genetic relationship between these families could be established.

Table 4. Analysis of mutations in HVDRR cases with and without alopecia

<table>
<thead>
<tr>
<th>Family</th>
<th>Mutation</th>
<th>Base change</th>
<th>Exon</th>
<th>Domain</th>
<th>Ligand binding</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>F53</td>
<td>Arg30stop</td>
<td>CGA-TGA</td>
<td>2</td>
<td>DBD</td>
<td>–</td>
<td>Zhu et al. (248)</td>
</tr>
<tr>
<td>F54</td>
<td>Arg30stop</td>
<td>CGA-TGA</td>
<td>2</td>
<td>DBD</td>
<td>?</td>
<td>Mechina et al. (249)</td>
</tr>
<tr>
<td>F31</td>
<td>Gly33Asp</td>
<td>GCC-GAC</td>
<td>2</td>
<td>DBD</td>
<td>+</td>
<td>Hughes et al. (146)</td>
</tr>
<tr>
<td>F45</td>
<td>His35Gln</td>
<td>CAC-CAG</td>
<td>2</td>
<td>DBD</td>
<td>+</td>
<td>Yagi et al. (20)</td>
</tr>
<tr>
<td>F44</td>
<td>Lys45Glu</td>
<td>AAA-GAA</td>
<td>2</td>
<td>DBD</td>
<td>+</td>
<td>Rut et al. (244)</td>
</tr>
<tr>
<td>F23</td>
<td>Gly46Asp</td>
<td>GGC-GAC</td>
<td>2</td>
<td>DBD</td>
<td>+</td>
<td>Lin et al. (247)</td>
</tr>
<tr>
<td>F47</td>
<td>Phe47Ile</td>
<td>TTC-ATC</td>
<td>2</td>
<td>DBD</td>
<td>+</td>
<td>Rut et al. (244)</td>
</tr>
<tr>
<td>F26</td>
<td>Arg50Gln</td>
<td>CGA-CAA</td>
<td>3</td>
<td>DBD</td>
<td>+</td>
<td>Saijo et al. (233)</td>
</tr>
<tr>
<td>F33</td>
<td>Arg50Gln</td>
<td>CGA-CAA</td>
<td>3</td>
<td>DBD</td>
<td>+</td>
<td>Saijo et al. (233)</td>
</tr>
<tr>
<td>F19</td>
<td>Arg73Gln</td>
<td>CGA-CAA</td>
<td>3</td>
<td>DBD</td>
<td>+</td>
<td>Hughes et al. (146)</td>
</tr>
<tr>
<td>F46</td>
<td>Arg73stop</td>
<td>CGA-TGA</td>
<td>3</td>
<td>DBD</td>
<td>–</td>
<td>Wiese et al. (245)</td>
</tr>
<tr>
<td>F55</td>
<td>Arg73stop</td>
<td>CGA-TGA</td>
<td>3</td>
<td>DBD</td>
<td>–</td>
<td>Cockerill et al. (246)</td>
</tr>
<tr>
<td>F5</td>
<td>Arg80Gln</td>
<td>CGG-CAG</td>
<td>3</td>
<td>DBD</td>
<td>+</td>
<td>Sone et al. (243)</td>
</tr>
<tr>
<td>F20</td>
<td>Arg80Gln</td>
<td>CGG-CAG</td>
<td>3</td>
<td>DBD</td>
<td>+</td>
<td>Sone et al. (243)</td>
</tr>
<tr>
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<td>3</td>
<td>DBD</td>
<td>+</td>
<td>Malloy et al. (21)</td>
</tr>
<tr>
<td>F50</td>
<td>Glu92fs</td>
<td>Intron E</td>
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<td>–</td>
<td>–</td>
<td>Hawa et al. (264)</td>
</tr>
<tr>
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<td>Gln152stop</td>
<td>CAG-TAG</td>
<td>4</td>
<td>Hinge</td>
<td>–</td>
<td>Kristjansson et al. (260)</td>
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<tr>
<td>F43</td>
<td>Cys190Trp</td>
<td>TGT-TGG</td>
<td>5</td>
<td>LBD</td>
<td>–</td>
<td>Thompson et al. (263)</td>
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<tr>
<td>F56</td>
<td>Leu233fs</td>
<td>GTC-GTG</td>
<td>6</td>
<td>LBD</td>
<td>–</td>
<td>Cockerill et al. (246)</td>
</tr>
<tr>
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<td>Gln259Pro</td>
<td>CAG-CCG</td>
<td>7</td>
<td>LBD</td>
<td>+</td>
<td>Cockerill et al. (246)</td>
</tr>
<tr>
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<td>Tyr259stop</td>
<td>TAC-TAA</td>
<td>7</td>
<td>LBD</td>
<td>–</td>
<td>Malloy et al. (258)</td>
</tr>
<tr>
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<td>Tyr259stop</td>
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<td>7</td>
<td>LBD</td>
<td>–</td>
<td>Ritchie et al. (147)</td>
</tr>
<tr>
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<td>Tyr295stop</td>
<td>TAC-TAA</td>
<td>7</td>
<td>LBD</td>
<td>–</td>
<td>Wiese et al. (245)</td>
</tr>
<tr>
<td>F30</td>
<td>Tyr295stop</td>
<td>TAC-TAA</td>
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<td>LBD</td>
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<tr>
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<td>Malloy et al. (22)</td>
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<tr>
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<tr>
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<td>Arg391Cys</td>
<td>CCG-TGG</td>
<td>9</td>
<td>LBD</td>
<td>+</td>
<td>Whitfield et al. (157)</td>
</tr>
<tr>
<td>F42</td>
<td>exon 7–9 deletion</td>
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<td>Thompson et al. (263)</td>
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<tr>
<td>F48</td>
<td>None</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Hewison et al. (265)</td>
</tr>
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Saijo et al. (233) described a DBD mutation in three HVDRR patients from two unrelated families (F26 and F33) of Japanese origin. Earlier investigations showed that fibroblasts from the patients had normal [3H]1,25-(OH)2D3 binding, but the VDR exhibited abnormal nuclear binding (200, 204, 207). Sequence analysis identified a G-to-A transition in exon 3 that converted arginine to glutamine at amino acid 50 at the start of the second zinc finger module (Arg50Gln). The parents were identified as carriers of both the normal and mutant alleles using single-strand conformational polymorphism (233). Recreated mutant VDR cDNA expression data were not provided to confirm the functional consequences of this defect on the VDR.

A His35Gln mutation in the first zinc module of the VDR DBD was identified by Yagi et al. (20). This mutation changed a positively charged amino acid to a neutral one. The VDR from the patient’s cells (F45) exhibited normal 1,25-(OH)2D3 binding but, consistent with the location of the mutation in the DBD, the receptors exhibited low-affinity binding to DNA. The patient’s fibroblasts were transiently transfected with a VDRE-CAT reporter plasmid to test for 1,25-(OH)2D3 responsiveness. The transformed cells were unable to induce gene transcription. However, when the patient’s fibroblasts
were cotransformed with the wild-type VDR cDNA and the reporter plasmid, the cells acquired the ability to respond to hormone.

Two mutations in the VDR DBD were reported by Rut et al. (244). One patient (F47) described in a previous report by Lin and Uttley (209) had an A-to-G mutation in exon 2. The missense mutation resulted in lysine at amino acid 45 being replaced by glutamic acid (Lys45Glu). In the same report, Rut et al. (244) examined the VDR gene in a patient (F44) described in a previous report by Simonin et al. (208). They identified a unique T-to-C base change in exon 2 that resulted in phenylalanine at amino acid 47 being replaced by isoleucine (Phe47Ile). The recreated mutant VDRs exhibited normal $[^3]$H$1,25$-(OH)$_2$D$_3$ binding but were transcriptionally inactive (Phe47Ile). The recreated mutant VDRs exhibited normal $[^3]$H$1,25$-(OH)$_2$D$_3$ binding but were transcriptionally inactive (Phe47Ile). The recreated mutant VDRs exhibited normal $[^3]$H$1,25$-(OH)$_2$D$_3$ binding but were transcriptionally inactive (Phe47Ile).

A patient with HVDRR from a Moroccan family (F46) was examined for mutations in the VDR gene by Wiese et al. (245). At the cellular level, this patient exhibited a ligand binding-negative phenotype, suggesting that the mutation would lie in the LBD. The authors discovered an opal mutation (CGA to TGA) in which a C-to-T substitution introduced a premature stop codon at amino acid 73. The Arg73 stop mutation truncates the receptor in the middle of the second zinc finger module, resulting in the production of a 72-aa polypeptide. No evidence for the presence of a truncated VDR in the patient’s cells was demonstrated since both the LBD and monoclonal antibody-binding sites were deleted in the mutant protein. The Arg73 stop mutation occurs in the same codon that causes the Arg73Gln mutation in the D family (F19) (146). In the Arg73 stop mutation, CGA is mutated to TGA, while in the Arg73Gln mutation the CGA is mutated to CAA. The Arg73 stop mutation has also been identified in a young boy (F56) from Greece, who had HVDRR with alopecia (246).

Lin et al. (247) examined the VDR gene for mutations in a patient (F23) with HVDRR previously described by Sakati et al. (199). DNA sequencing uncovered a unique G-to-A base change in exon 2. This mutation resulted in a glycine at amino acid 46 being changed to an aspartic acid (Gly46Asp). The recreated Gly46Asp mutant VDR exhibited the characteristics of a DBD mutation in that the mutant receptor bound $[^3]$H$1,25$-(OH)$_2$D$_3$ normally but displayed a reduced affinity for DNA. The mutant receptor was also shown to be transcriptionally inactive in reporter gene assays. The authors demonstrated that the patient was homozygous for the mutation and the patient’s father was a carrier of the mutant allele using PCR and a restriction fragment length polymorphism (RFLP) generated by the mutation. In contrast to the other DBD mutations described above, the mutation at Gly46 occurs in an amino acid that is not well conserved in the steroid-thyroid-retinoid receptor superfamily. However, Gly46 is conserved among receptors that form heterodimers with RXR, proteins such as TR and RAR.

A young boy (F53) of French-Canadian origin with HVDRR and alopecia has been reported by Zhu et al. (248). The patient’s fibroblasts lacked specific $[^3]$H$1,25$-(OH)$_2$D$_3$ binding and failed to exhibit 24-hydroxylase mRNA induction after treatment with up to 100 nM $1,25$-(OH)$_2$D$_3$. Northern blotting showed that the cells expressed a normal sized VDR mRNA, but Western blotting failed to detect any protein. A C-to-T base substitution was located in exon 2, which changed the codon for arginine (CAG) at amino acid 30 to an opal stop codon (TAG) (Arg30 stop). The 29-aa polypeptide represents the shortest truncated protein produced by a premature stop mutation in the VDR. The mutation eliminated 398 aa including the LBD, the monoclonal antibody epitope, the second zinc finger module, and a portion of the first zinc finger module.

The same Arg30 stop mutation was also identified in two children with HVDRR from a family (F54) living in Brazil (249). One child died at 4 yr of age due to cardiorespiratory insufficiency. Interestingly, the parents, who were first cousins, were phenotypically normal but had slightly elevated levels of serum $1,25$-(OH)$_2$D. The mean value for the father was 73 pg/ml and for the mother, 93 pg/ml (normal range 20–80 pg/ml). The elevated $1,25$-(OH)$_2$D values raise the possibility of mild vitamin D resistance in the heterozygotic parents, a finding that has not been documented previously in other parents of HVDRR children.

C. Structural analysis of DBD mutations

As noted above, the crystal structure of the VDR has not been reported at this time. However, crystallographic studies of the GR (108), RXR, and TR (111) DBD structures have been elucidated and, based on these studies, one can extrapolate the alterations created by the mutations to the VDR DBD (250, 251). Crystallographic analyses of the GR demonstrate that amino acids 457–469 (corresponding to residues 38–50 in the VDR) form an $\alpha$-helix that joins the two zinc finger modules. This $\alpha$-helix packs perpendicularly with a second $\alpha$-helix at the base of the second zinc finger. Together, the hydrophobic residues of these two $\alpha$-helices comprise the hydrophobic core of the DBD. Lys45Glu and Gly46Asp mutations are located in the P box (aa residues 41–46), a region of the receptor likely important in contacting the DNA bases and determining the specificity of the receptor for specific VDREs (Figs. 5 and 12). Rut et al. (244) proposed that the Lys45Glu mutation would disturb the hydrogen bonding between Lys45 and a guanine nucleoside in the VDRE half-site. The conversion of Gly46 to aspartic acid, a bulky, charged amino acid, probably leads to unfavorable electrostatic interactions with the negatively charged phosphate backbone of the DNA helix, which may prevent the receptor from contacting specific nucleotide bases in the VDRE. Alternatively, the Gly46Asp mutation may eliminate the ability of the VDR to specifically recognize VDREs (247). Similarly, the Gly33Asp mutation is expected to have a repelling effect on the negatively charged phosphate backbone due to the negatively charged aspartic acid (244). On the other hand, the substitution of glycine for histidine in the His35Glu mutation most likely eliminates a hydrogen bond donated from the positively charged histidine to the phosphate of a guanine nucleoside in the VDRE (244). The Phe47Ile mutation is a relatively conserved substitution; however, the loss of the phenylalanine ring structure may disrupt the integrity of the hydrophobic core of the DBD and obstruct the formation of the proposed $\alpha$-helical structure at the base of the first zinc finger, such that the VDR could not bind normally to its VDRE (244).
It is interesting to note that four of the DBD mutations, Lys45Glu, Gly46Asp, Phe47Ile, and Arg50Gln, occur in a LysXxxPhePhe[Lys/Arg]Arg sequence motif that has been identified as a binding site for calreticulin (252–254). Calreticulin binds to the VDR (253), and cotransfection of calreticulin expression plasmids with a VDRE/RARE-luciferase reporter construct causes a decrease in the reporter gene activation by VDR in a dose-dependent manner (253). Since calreticulin may modulate VDR transactivation, disruption of the calreticulin-binding site may lead to a decrease in VDR function. The effects these mutations might exert to alter possible calreticulin actions on the VDR are unknown.

**VII. HVDRR Mutations Causing the Ligand Binding-Negative Phenotype**

A. Initial description of ligand-binding domain (LBD) mutations

The first molecular analysis of the ligand binding-negative phenotype was reported by Ritchie et al. (147). These investigators used PCR to amplify exons of the VDR gene in three related HVDRR patients from families F18, F34, and F36 (16, 17, 25, 134). A single nucleotide base change was identified in the codon for tyrosine (TAC) at amino acid 295, which changed the sequence to an ochre termination codon (TAA) (147). The same mutation was found in each patient. The Tyr295 stop mutation truncates 132 aa of the carboxy terminus of the VDR, which results in the deletion of a major portion of the LBD, thereby creating the ligand binding-negative phenotype. The reconstructed mutant VDR exhibited no specific 
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{[3H]}1,25-(OH)_{2}D_{3} \text{ binding and failed to activate gene transcription. The Tyr295 stop mutation was the first mutation identified in the VDR LBD. The location of this mutation and other LBD mutations is shown in Fig. 13 and summarized in Table 4.}

Four additional families (F35, F37, F38, and F39) related to the families described above make up a large kindred in which consanguineous marriages occurred. The entire kindred was fully analyzed by Malloy et al. (22) (see Fig. 2). A total of eight children from this kindred exhibited HVDRR with alopecia and the same hormone binding-negative HVDRR phenotype. All of the affected children were homozygous for the ochre stop mutation at Tyr295, and their parents were heterozygous as determined by analysis of a Rsal RFLP created by the mutation (22). Interestingly, the 30,000 mol wt truncated protein that is predicted to be produced by this mutation could not be detected by Western blot analysis. In addition, Northern blot analysis of RNA obtained from skin fibroblasts or EBV-transformed lymphoblasts from the patients failed to detect any mutant VDR mRNA in all but one case (F35). The absence of mRNA transcripts has been reported in other genetic diseases where a premature stop mutation has been found and, in this case, likely accounts for the absence of the mutant-truncated VDR protein (255–257).

B. Characterization of additional LBD mutations

Family F11, described in earlier papers, had two affected children with HVDRR who exhibited the ligand binding-negative phenotype (16, 17, 25, 134). This family of Christian Arabs lives in the same town as the extended kindred (F18,F34–39) described above who are Muslim Arabs. Although there is no known genetic relationship between family F11 and the large kindred, the Tyr295 stop mutation is the cause of HVDRR in this family as well (258). The Tyr295 stop mutation was also identified by Wiese et al. (245) in two related patients (F29, F30) from Saudi Arabia who were previously studied by Bliziotes et al. (13). These patients are apparently unrelated to the other families with the same mutation.

An analysis of the VDR from the parents of children with the Tyr295 stop mutation led to an interesting observation. As mentioned previously, fibroblasts from the heterozygous parents, with the exception of the father in F18, were shown to have a normal abundance of VDR. In contrast, when the VDR was examined in EBV-transformed lymphoblasts from peripheral blood of the same individuals, the receptor abundance was half of normal controls, which is consistent with the heterozygous state of these individuals (22). The reason for the difference in the level of VDR expression in the two cell types remains unresolved. Interestingly, the VDR abundance in the lymphoblasts of the father in F18 was approximately one-fourth of the level in normal controls, which suggests that an additional problem related to VDR expression may have arisen in that individual.

The first mutation found in the VDR LBD that resulted in an amino acid substitution was described by both Rut et al. (259) and Kristjansson et al. (260). The patient from Kuwait (F21) had HVDRR without alopecia. Preliminary studies by Fraher et al. (14) on the patient’s fibroblasts showed absent 
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{[3H]}1,25-(OH)_{2}D_{3} \text{ binding. However, a later study by Rut et al. (259) showed that the fibroblasts contained normal amounts of } [3H]1,25-(OH)_{2}D_{3} \text{ binding but the affinity of the receptor for } 1,25-(OH)_{2}D_{3} \text{ was significantly reduced [dissociation constant (K}_d\text{) = } 10 \times 10^{-10} \text{ M] compared with normal controls (K}_d\text{ = } 0.7 \times 10^{-10} \text{ M). 1,25-(OH)_{2}D_{3} \text{ resistance was demonstrated by the failure of the patient’s fibroblasts to induce 24-hydroxylase activity when treated with the hormone (14, 259). Molecular analysis of the VDR gene identified a unique G-to-T missense mutation in exon 7 (259, 260). This mutation resulted in replacement of a positively charged arginine residue by a neutral charged leucine at amino acid 274. The recreated Arg274Leu mutant VDR was relatively resistant to vitamin D. However, the mutant VDR was able to activate gene transcription from a VDRE reporter plasmid but required 1,25-(OH)_{2}D_{3} \text{ concentrations approximately 1,000-fold higher than the wild-type receptor (260).}

A second missense mutation in the VDR LBD was described by Malloy et al. (261). The patient in this case exhibited three rare genetic disorders: HVDRR, congenital generalized lipoatrophic diabetes (Berardinelli-Seip syndrome), and persistent Müllerian duct syndrome (262). The patient, a Turkish boy (F51), had rickets and high 1,25-(OH)_{2}D_{3} \text{ levels but did not have alopecia. He was treated with extremely high doses of calcitriol (Rocaltril, 12.5 } \mu \text{g/day) which eventually normalized his serum calcium and ultimately improved his rickets. However, the child died of apparently unrelated problems. The patient’s fibroblasts had normal VDR abundance, but the affinity of the receptor for 1,25-
(OH)\textsubscript{2}D\textsubscript{3} was shown to be decreased by about 2-fold when assayed at 0°C. Induction of 24-hydroxylase mRNA in the patient's cultured fibroblasts required approximately a 5-fold increase in 1,25-(OH)\textsubscript{2}D\textsubscript{3} compared with control cells. Sequence analysis of the VDR gene uncovered a C-to-G missense mutation in exon 8. This mutation leads to replacement of histidine by glutamine at amino acid 305 (His305Gln). Interestingly, [\textsuperscript{3}H]1,25-(OH)\textsubscript{2}D\textsubscript{3}-binding studies of the reconstructed mutant protein demonstrated an 8-fold lower affinity for 1,25-(OH)\textsubscript{2}D\textsubscript{3} compared with the wild-type VDR when the assays were performed at 24°C. In gene transactivation assays, the His305Gln mutant VDR was approximately 5-fold less responsive to 1,25-(OH)\textsubscript{2}D\textsubscript{3} compared with the wild-type VDR. RFLP analysis with AlwNI showed that a sibling with HVDRR was homozygous for the same mutation and that the parents were heterozygous. It is unclear how the HVDRR point mutation is related, if at all, to the two other genetic abnormalities present in this child. The boy’s sister, who also had HVDRR and the same mutation in the VDR, did not exhibit the other genetic defects.

Two novel missense mutations in the LBD have been characterized by Whitfield et al. (157). One patient, a girl (F4), had the classic symptoms of HVDRR but without alopecia. The patient’s fibroblasts were originally examined by Griffin and Zerwekh (190), who showed that the cells had normal 1,25-(OH)\textsubscript{2}D\textsubscript{3} binding but had defective induction of 24-hydroxylase activity. Nucleotide sequencing of the VDR uncovered a T-to-G substitution in exon 8, which changed the codon for isoleucine to serine at amino acid 314 (Ile314Ser). In transactivation experiments, high concentrations of 1,25-(OH)\textsubscript{2}D\textsubscript{3} were required to achieve normal activity. This patient showed a nearly complete cure when treated with pharmacological doses of 25-hydroxyvitamin D3.

The second patient in the study was a young girl (F52) who had HVDRR with alopecia. Sequencing showed that the patient had a C-to-T base change in exon 9, which converted an arginine to cysteine at amino acid 391 (Arg391Cys). In transactivation studies, the mutant VDR required high concentrations of 1,25-(OH)\textsubscript{2}D\textsubscript{3}, as well as increased levels of RXR to achieve normal gene transactivation. The “rescue” achieved by RXR supplementation demonstrates the importance of both 1,25-(OH)\textsubscript{2}D\textsubscript{3} binding and heterodimerization with RXR in VDR-mediated gene activation (157).

Two siblings, a brother and sister from India (F57) that had HVDRR with alopecia, were studied by Cockerill et al. (246). The children’s parents were first cousins. Using cultured fibroblasts, [\textsuperscript{3}H]1,25-(OH)\textsubscript{2}D\textsubscript{3} binding was found to be normal, but 1,25-(OH)\textsubscript{2}D\textsubscript{3} induction of 24-hydroxylase activity was absent. DNA sequence analysis revealed a single base alteration (A to C) that changed glutamine to proline at amino acid 259 (Gln259Pro). Transactivation experiments demonstrated that the recreated Gln259Pro mutant VDR was functionally inactive. In addition, to examine VDR protein-protein interactions and DNA binding, electrophoretic mobility shift assays were performed. Whereas the wild-type VDR formed two complexes (complex A and complex B) in the electrophoretic mobility shift assay, the Gln259Pro mutant VDR showed a reduction in the formation of complex B and an enhancement in complex A. The authors speculated that the Gln259Pro mutation in the VDR affected protein-protein interactions possibly by increasing the affinity of the receptor for an unidentified protein.

An additional mutation has been described in the VDR LBD by Thompson et al. (263). The HVDRR patient (F43) was shown to have a mutation in exon 5. In this case, a unique single base change (TGT to TGG) was found, which changed cysteine to tryptophan at amino acid 190 (Cys190Trp). Further details about this case were not included in this preliminary report.

C. Structural analysis of LBD mutations

From crystallographic studies of the holo- and/or apo-LBDs of RXR, RAR, and TR and from sequence alignments of the nuclear receptors, a generalized canonical structure of the LBD has been developed (129) (see Fig. 13). In the model,
the LBD is composed of helices H1 and H3–H10 with a variable length region between H1 and H3 (loops 1–3). The majority of the conserved residues in the LBD are located in a 34-aa cluster from the C terminus of H3 to the middle of H5, including the E1 region. These conserved residues form the hydrophobic core by holding together H3, H4, H5, H8, and H9 and loops 3–4 and 8–9. Residues in H1, H3, H5, β-turn, loop 6–7, H11, loop 11–12, and H12 form a hydrophobic ligand-binding pocket that accommodates the hormone. Once a ligand enters the pocket, a lid formed by H11 and H12 closes over the pocket.

In HVDRR, six single amino acid substitutions have been identified in the VDR LBD that lead to 1,25-(OH)2D resistance (Figs. 13 and 14). One mutation, Cys190Trp, occurs in loop 1–3, but the consequences of this alteration on the LBD are not readily discernible from the LBD model. A second mutation, Gln259Pro, occurs in H4. In RARγ, this amino acid (Gln259 in RARγ) has been shown to stabilize the hydrophobic core by binding to the main-chain NH groups in aakylation (260). The Ile314Ser mutation occurs in H7 hormone affinity, probably by increasing the flexibility of the transition (261) occurs in loop 6–7, which causes a decrease in ligand binding and 1,25-(OH)2D3 responses (202). This premature stop mutation occurs at Gln 152 and deletes 306 aa of the VDR. As expected, the Gln152 stop mutant VDR was unresponsive to 1,25-(OH)2D3 in gene activation assays. The Gln152 stop mutation was also identified by Wiese et al. (245) in a HVDRR patient (F32) previously reported by Barsony et al. (202).

B. Splice site mutations

Hawa et al. (264) examined the molecular cause of HVDRR with alopecia in a young Greek girl (F50). Using RT-PCR and DNA sequencing, they showed that the patient’s RNA sequence diverged from the wild-type sequence at nucleotide 147. The sequence from exon 4 was deleted and the sequence that followed was from exon 5. Sequence analysis of the VDR chromosomal gene found no mutations in the exons; however, a G-to-C base change was found in the 5′-end of intron E (Fig. 10). This single nucleotide change converts the wild-type sequence from GTAAAGT to GTAACT and eliminates the 5′-donor splice site [consensus sequence: GT(A/G)AGT]. The loss of the 5′-donor splice site caused exon 4 to be skipped in the processing of the VDR transcript. The loss of exon 4 introduced a reading frameshift that resulted in a premature stop codon in exon 5. The mutant protein contains 92 aa of the wild-type sequence and an additional 6 aa due to the frameshift (Glu92fs) (Fig. 12). The shortened VDR had no [3H]1,25-(OH)2D3 binding and failed to induce 24-hydroxylase activity.

A splice site mutation was also identified in a German patient (F56) with HVDRR and alopecia (246). Studies of the patient’s fibroblasts showed absent [3H]1,25-(OH)2D3 binding and failure to induce 24-hydroxylase activity with 1,25-(OH)2D3 treatment. In this case, a cryptic 5′-donor splice site was generated in exon 6. The mutation in this case, a C-to-G transition, changed the sequence from GTCAAGT to GTCACT and eliminates the 5′-donor splice site [consensus sequence: GT(A/G)AGT]. This single base change did not alter the amino acid coding sequence in exon 6 but introduced a splice site that could be recognized by the spliceosome complex during RNA processing. As a result, the mutation caused a 56-bp deletion in exon 6 that led to a frameshift 15 bases into exon 7. The mutant protein contains 233 aa of the wild-type sequence and an additional 4 aa due to the frameshift (Leu233fs) (Fig. 13). The mutation caused the truncation of 194 aa of the VDR leading to a loss of 1,25-(OH)2D3 binding and hormone responsiveness.

VIII. Additional Mutations In The VDR Gene

A. Hinge region mutations

A premature stop codon caused by an amber mutation (CAG to TAG) in exon 4 was found by Kristjansson et al. (260) in a Turkish patient (F32) with HVDRR. Previous studies using fibroblasts from this patient had demonstrated an absence of ligand binding and 1,25-(OH)2D3 responses (202). This premature stop mutation occurs at Gln 152 and deletes 306 aa of the VDR. As expected, the Gln152 stop mutant VDR was unresponsive to 1,25-(OH)2D3 in gene activation assays. The Gln152 stop mutation was also identified by Wiese et al. (245) in a HVDRR patient (F32) previously reported by Barsony et al. (202).

Fig. 14. Schematic illustration of the LBD of the VDR and location of amino acid substitutions causing HVDRR. The α-helices (H1-H12) of the VDR LBD are depicted as shaded rectangles and the single β-turn is drawn as an open rectangle connected to an open triangle. The location of the missense mutations are indicated by arrows. The loops connecting the helices are drawn as solid lines. N, Amino terminus; C, carboxy terminus.
C. Major structural mutations

There has been one case (F42) reported in which a major structural defect in the VDR gene was found to cause HVDRR (263). The defect, a deletion in the VDR gene, was identified by PCR and Southern blotting. The deletion eliminated exons 7, 8, and 9. This is the only case thus far reported in which a partial gene deletion has been shown to be the cause of HVDRR.

D. Vitamin D resistance without a mutation in the VDR

Since the initial description of HVDRR as a genetic disorder, mutations in the VDR gene were suspected to be the cause of 1,25-(OH)\textsubscript{2}D\textsubscript{3} resistance exhibited by HVDRR patients. In addition to mutations in the coding region of the VDR gene, mutations may also be located in the promoter and noncoding regions of the gene that could prevent VDR mRNA transcription or hinder the translation of the VDR protein. However, although the VDR is the principal determinant in the 1,25-(OH)\textsubscript{2}D\textsubscript{3} action pathway, it is possible that target organ resistance to 1,25-(OH)\textsubscript{2}D\textsubscript{3} may also result from mutations in other proteins that are essential to the transactivation process. Some of the likely candidates include transcription factors such as RXR, as well as coactivators or corepressors of VDR activity. Defects in these proteins may disturb the contact between the interacting protein and the VDR and therefore prevent the VDR from binding to VDREs and/or hinder VDR transactivation. Since RXR and other interacting proteins are essential for the activity of many receptors in the retinoid-thyroid subgroup, mutations in these proteins would be expected to exhibit a more complex phenotype than simply vitamin D resistance and HVDRR.

Hewison et al. (265) described a case of HVDRR in which a mutation could not be found in the VDR. The patient (F48), a young girl of English descent, exhibited all of the hallmarks of HVDRR including alopecia. The patient’s fibroblasts expressed a normal sized VDR mRNA and exhibited normal [\textsuperscript{3}H]1,25-(OH)\textsubscript{2}D\textsubscript{3} binding. However, no induction of 24-hydroxylase activity was observed when the fibroblasts were treated with up to 1 \textmu M 1,25-(OH)\textsubscript{2}D\textsubscript{3}. Although the cells were clearly resistant to 1,25-(OH)\textsubscript{2}D\textsubscript{3}, no mutations were found within the coding region of the VDR gene. The patient’s VDR cDNA was recovered using RT-PCR and then expressed in CV-1 cells. In these cells cotransfected with a VDRE reporter plasmid, the patient’s VDR exhibited a normal transactivation response to 1,25-(OH)\textsubscript{2}D\textsubscript{3}. These results clearly demonstrated that the patient’s VDR was normal. The authors suggested that the tissue resistance was not due to a defect in the VDR and that the hormone resistance causing HVDRR was most likely the result of a mutation in an essential protein that participates in the 1,25-(OH)\textsubscript{2}D\textsubscript{3} action pathway. However, the source of the defect causing vitamin D resistance in this case remains unknown.

In Cauca, Colombia, more than 200 patients have been diagnosed with a disease that somewhat resembles HVDRR without alopecia (266). The patients exhibit lower limb deformities due to rickets but are otherwise in good physical condition. Rickets limited to the lower extremities, as in these cases, has not been reported in other HVDRR families. The affected individuals have serum calcium levels that are within the normal range but their serum 1,25-(OH)\textsubscript{2}D levels are unusually high, suggesting target organ resistance. Analysis of 24-hydroxylase activity from fibroblasts of two of the most severely affected patients showed a low induction of enzyme activity by 1,25-(OH)\textsubscript{2}D\textsubscript{3} when compared with normal controls. After RT-PCR, the VDR cDNA was obtained from these two patients and sequenced. No mutations were found. Since the cause of vitamin D resistance in this instance was not due to mutations in the VDR and a functional response to 1,25-(OH)\textsubscript{2}D\textsubscript{3} was demonstrated, it has not been clearly ascertained whether this entity is a variant of HVDRR. However, these cases support the concept that target organ resistance may be due to mechanisms other than mutations in the VDR. The high prevalence of the disease in the population and the localized distribution of the rickets raises the possibility of an environmental cause.

IX. HVDRR Mouse Model

Patients with HVDRR mutations that cause complete resistance to 1,25-(OH)\textsubscript{2}D\textsubscript{3} are surviving models of a VDR gene knockout experiment of nature. However, for practical and ethical purposes, VDR studies have been mostly limited to the use of cultured skin fibroblasts from patients. Fibroblasts are an excellent model system with which to analyze the VDR mutations and their effects on hormone responsiveness. However, the usefulness of this system is limited to VDR functions present only in this cell type. Fibroblasts do not allow investigation of the effects of VDR knockout in other target tissues, such as the intestine, kidney, or bone, or in specific cell types such as osteoblasts. Recently, VDR gene knockout mouse models have been created using targeted gene disruption strategies. Yoshizawa et al. (56) deleted the first zinc finger module by ablating exon 2, while Li et al. (57) deleted the second zinc finger module by ablating exon 3. In each VDR(−/−) null mouse, the animals displayed the classic features of HVDRR. The mice exhibited hypocalcemia and hypophosphatemia and developed rickets and secondary hyperparathyroidism after weaning. Serum levels of 1,25-(OH)\textsubscript{2}D\textsubscript{3} were elevated and 24,25(OH)\textsubscript{2}D\textsubscript{3} levels were low. Alopecia, which developed progressively after birth, was also manifested in these animals. On the other hand, the VDR heterozygous mice with the (+/−) genotype were normal. The VDR knockout mouse model provides a valuable tool with which to examine the effects of the VDR null mutation in various target tissues not readily available for study in humans. Experimental findings using this approach are eagerly awaited.

X. Treatment of HVDRR

A. Vitamin D

As discussed above, HVDRR is caused by a generalized resistance to 1,25-(OH)\textsubscript{2}D\textsubscript{3}. To overcome the resistance to vitamin D, a number of treatment therapies using calcium and active vitamin D metabolites have been attempted. The response has varied widely in different cases. For the most part, it appears that patients with HVDRR without alopecia
are generally more responsive to treatment with vitamin D preparations than those patients with alopecia (26). In a few of the early reports, patients without alopecia responded clinically and radiologically to the administration of pharmacological doses of vitamin D ranging from 5,000–40,000 IU/day (5, 6, 185). Patients without alopecia also responded to 20–200 μg/day of 25(OH)D₃ and 17–20 μg/day of 1,25-(OH)₂D₃ (6). Only three HVDRR patients without alopecia have had their VDR defect characterized at the molecular level. One patient, F51, exhibited a His305Gln mutation in the VDR LBD that decreased the affinity of the receptor for 1,25-(OH)₂D₃ (259–261). This patient (as well as his fibroblasts) responded to high doses of 1,25-(OH)₂D₃ (12.5 μg/day) (261, 262). The high doses of the hormone apparently overcome the low affinity-binding defect and achieved adequate VDR occupancy to mediate normal 1,25-(OH)₂D₃ responses. A second patient, F4, had an Ile314Ser mutation in the VDR LBD and exhibited normal 1,25-(OH)₂D₃ binding but sharply reduced VDR transactivation. The patient originally seen at 2 yr of age presenting with rickets and elevated serum 1,25-(OH)₂D₃ was treated with 1 mg/day of vitamin D₂ until age 18 (185). At age 20, after an uneventful pregnancy, she developed hypocalcemia that was treated successfully with 50 μg/day of 25(OH)D₃. In contrast to these patients who responded to therapy, the third patient, F21, with the Arg274Leu mutant VDR was examined in CV-1 cells for transactivation activity, a positive response was observed at high doses of hormone (260).

In general, HVDRR patients with alopecia appear to be more resistant to treatment with vitamin D metabolites; however, a small number of these patients have been treated successfully using vitamin D. Two patients showed signs of improvement when given vitamin D or 1α(OH)D₃ (10, 187), and one patient responded to 25(OH)D as well as 1α(OH)D₃ and unfortunately later died of pneumonia as a result of the disease (14). Fibroblasts from this patient were also unresponsive to hormone treatment. Interestingly, when the recreated Arg274Leu mutant VDR was examined in CV-1 cells for transactivation activity, a positive response was observed at high doses of hormone (260).

B. Calcium

Since hypocalcemia is a major manifestation of HVDRR, restoration of serum calcium by the administration of calcium salts has been used as a therapy for treating HVDRR patients. In one study by Sakati et al. (199), high-dose oral calcium therapy was given to a patient (F23) who had failed to respond to calciferols. The patient received 3–4 g of elemental calcium orally per day and showed clinical improvement during 4 months of therapy. The patient was later shown to have the Gly46Asp mutation (247). In another study, Balsan et al. (268) successfully used long term intravenous calcium infusions in a child with HVDRR who failed prior treatment with large doses of vitamin D derivatives and/or oral calcium (11). This therapy apparently bypasses the calcium absorption defect in the intestine caused by the mutant VDR. High doses of calcium were infused intravenously during the nocturnal hours over a 9-month period. Clinical improvement accompanied by relief of bone pain was observed within the first 2 weeks of the start of intravenous therapy. Within 7 months, the child gained both weight and height. Eventually, the serum calcium normalized, the secondary hyperparathyroidism was reversed, and the rickets was ultimately cured as assessed by x-ray and bone biopsy. The syndrome recurred, however, when the intravenous infusions were discontinued. Several other investigators have similarly demonstrated beneficial effects of intravenous calcium infusion in HVDRR children (13, 269, 270). In a study by Weisman et al. (269), two patients treated with calcium infusion showed a decrease in serum alkaline phosphatase activity and an increase in their serum calcium and phosphate over a 1-yr period (269). x-Ray analysis showed resolution of the rickets with the appearance of normal mineralization of bone. After radiological healing of the rickets has been achieved with intravenous calcium infusions, high-dose oral calcium therapy has been shown to be effective in maintaining normal serum calcium concentrations in some cases (270). For those HVDRR children that do not respond to high-dose calcitriol, it seems reasonable to initiate this two-step protocol at an early age.

The effectiveness of intravenous calcium to heal rickets suggests that the lack of calcium absorption caused by the mutant VDRs in the intestine is the major defect causing the bone disease. The intravenous calcium infusion bypasses this intestinal defect and provides mineral to the bone-forming site. Apparently, normal bone formation is achieved if the mineral supply is adequate, despite the lack of vitamin D action on the bone cells. Since some intestinal calcium absorption is vitamin D-independent, oral calcium in high dosage has been used as an effective treatment. Once healing of rickets has been achieved by intravenous calcium, the requirement for mineral is decreased, and maintenance calcium treatment by the oral route may adequately protect against rickets.
C. Prenatal diagnosis

A prenatal diagnosis of HVDRR is now possible in pregnant women from high-risk families. Cultured cells from chorionic villus samples or amniotic fluid have been used to determine whether the fetus has HVDRR using [3H]1,25-(OH)2D3 binding, induction of 24-hydroxylase activity, and RFLP analyses of known mutations (271, 272). A summary of the RFLPs generated by mutations in the VDR gene is shown in Table 5.

D. Spontaneous healing of rickets

It is interesting to note that there have been several HVDRR patients who have shown spontaneous improvement in their disease (17, 18, 134). When spontaneous healing of rickets occurs, it usually happens between 7–15 yr of age and is not necessarily associated with the time of puberty. Sometimes the spontaneous recovery occurs after the patient has undergone a relatively ineffective long-term treatment with vitamin D metabolites and mineral replacement. The healing process arises spontaneously and does not appear to be related to the treatment. In some patients, the spontaneous improvement occurred after the treatment was discontinued (18). The patients appear to remain eucalcemic without therapy and show no evidence of osteomalacia or rickets. Cultured skin fibroblasts obtained from an HVDRR patient whose rickets had spontaneously healed continued to exhibit resistance to 1,25-(OH)2D3 (18). Spontaneous improvement has been noted in patients exhibiting the hormone-binding negative phenotype (17, 134) where a Tyr295 stop mutation was identified (22, 147) as well as in the hormone-binding positive phenotype (18) where an Arg73Gln mutation was identified (146). Interestingly, in those patients who showed improvement of their hypocalcemia and rickets, the alopecia persisted (17, 18, 134). It is not uncommon for children to "outgrow" genetic diseases and, perhaps after skeletal growth has been completed, the body is able to compensate for the defective VDR gene by other means.

XI. Analysis, Summary, and Conclusions

HVDRR is a rare recessive genetic disorder caused by mutations in the VDR that result in end organ resistance to 1,25-(OH)2D3 action. The major defect caused by the mutant VDR is a decrease of intestinal calcium and phosphate absorption, which leads to decreased bone mineralization and rickets. Since 1978, more than 50 families exhibiting signs and/or symptoms of HVDRR have been studied (Table 3). Usually, the diagnosis of HVDRR has been based on high circulating levels of 1,25-(OH)2D3 and resistance of cultured skin fibroblasts to 1,25-(OH)2D3 treatment. Many cases have been analyzed for [3H]1,25-(OH)2D3 binding to the VDR and/or transactivation of reporter genes by the VDR, which revealed that the disease was caused by heterogeneous mutations in the VDR gene. A number of cases of HVDRR have not yet been examined for mutations in the VDR gene. Since some of these cases presented late in life, it is possible they may have been due to nonhereditary or acquired resistance to 1,25-(OH)2D3.

Analysis of the syndrome of HVDRR provides many interesting insights into vitamin D physiology and the role of the VDR in mediating 1,25-(OH)2D3 action. Our analysis of the findings leads us to make the following observations.

At the time of this writing, eight missense mutations have been found in the VDR DBD that prevent the receptor from activating gene transcription even though 1,25-(OH)2D3 binding is normal. Six missense mutations have been identified in the LBD that cause reduced or complete hormone insensitivity either by decreasing hormone affinity and/or interfering with heterodimerization with RXR. Four mutations have been found that introduce premature termination codons, which truncate the VDR and lead to complete hormone resistance. Two unique splice site mutations have been demonstrated that also introduce premature termination codons. A partial deletion encompassing exons 7–9 of the VDR gene has also been described.

Despite the many pleiotropic processes regulated by 1,25-(OH)2D3, children with HVDRR exhibit only symptoms that relate to their calcium deficiency and/or alopecia. We conclude that in vivo, the pleiotropic actions of vitamin D can be compensated for by other mechanisms but the calcemic effects cannot.

The improvement in rickets after chronic intravenous cal-

### Table 5. RFLPs generated by mutations causing HVDRR

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence</th>
<th>RFLPa</th>
<th>Normal</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>Arg30Stop</td>
<td>CGA</td>
<td>TGA</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Gly32Asp</td>
<td>GCC</td>
<td>GAC</td>
<td>Bsrl</td>
</tr>
<tr>
<td></td>
<td>His35Gln</td>
<td>CAC</td>
<td>CAG</td>
<td>MspI</td>
</tr>
<tr>
<td></td>
<td>Gly46Asp</td>
<td>GGC</td>
<td>GAC</td>
<td>MseI</td>
</tr>
<tr>
<td></td>
<td>Lys45Glu</td>
<td>AAA</td>
<td>GAA</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Phe47Ile</td>
<td>TTC</td>
<td>ATC</td>
<td>–</td>
</tr>
<tr>
<td>Exon 3</td>
<td>Arg50Gln</td>
<td>CGA</td>
<td>CAA</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Arg73Gln</td>
<td>CGA</td>
<td>CAA</td>
<td>BsaHI</td>
</tr>
<tr>
<td></td>
<td>Arg73stop</td>
<td>CGA</td>
<td>TGA</td>
<td>BglI</td>
</tr>
<tr>
<td></td>
<td>Arg80Gln</td>
<td>CGG</td>
<td>CAG</td>
<td>MspI</td>
</tr>
<tr>
<td></td>
<td>Arg152stop</td>
<td>CAG</td>
<td>TAG</td>
<td>Bsrl</td>
</tr>
<tr>
<td></td>
<td>Cys190Trp</td>
<td>TGT</td>
<td>TGG</td>
<td>–</td>
</tr>
<tr>
<td>Exon 5</td>
<td>Leu233fs</td>
<td>GTC</td>
<td>GTG</td>
<td>Thh111I</td>
</tr>
<tr>
<td></td>
<td>Arg274Leu</td>
<td>GCA</td>
<td>CCA</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Tyr295stop</td>
<td>TAC</td>
<td>TAA</td>
<td>Rsal</td>
</tr>
<tr>
<td>Exon 8</td>
<td>His305Gln</td>
<td>CAC</td>
<td>CAG</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Ile314Ser</td>
<td>ATC</td>
<td>AGC</td>
<td>–</td>
</tr>
<tr>
<td>Exon 9</td>
<td>Arg391Cys</td>
<td>CGC</td>
<td>TGC</td>
<td>FspI</td>
</tr>
</tbody>
</table>

a Restriction enzyme site in normal or mutant sequence is indicated;
b –, Absence of restriction enzyme site.
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Calcium infusion or oral calcium raises interesting questions about the role of vitamin D in bone homeostasis. Correction of hypocalcemia and secondary hyperparathyroidism leads to healing of the rickets, as assessed by x-ray and bone biopsy. Thus, although there are many well defined actions of vitamin D on osteoblasts, the response to normalization of serum calcium suggests that 1,25-(OH)_2D_3 action on osteoblasts is not essential to form normal bone. The implication is that 1,25-(OH)_2D_3 action on bone is mainly due to its effects on intestinal mineral absorption to provide calcium and phosphate for bone formation. The same conclusion was reached by Underwood and DeLuca (273), who showed that the development of rickets is prevented in totally vitamin D-deficient rats by calcium and phosphate infusions in the absence of vitamin D. The ramifications for therapy are that normalization of serum calcium by intravenous and/or oral calcium supplements will bypass the defective intestinal VDR and heal the rickets.

Although 1,25-(OH)_2D_3 is an inhibitor of PTH production, in the HVDRR children, normalizing serum calcium by intravenous infusion is sufficient to suppress their PTH overproduction and does not require 1,25-(OH)_2D_3 action. In addition, intravenous calcium therapy without phosphate is adequate to correct all of the metabolic abnormalities in children with HVDRR. This suggests that the hypophosphatemia in these patients is mainly the result of secondary hyperparathyroidism and not inadequate intestinal phosphate absorption.

A number of interesting issues concerning alopecia and HVDRR are worth noting. Since VDRs have been found in hair follicles (27, 28), 1,25-(OH)_2D_3 action through the VDR appears to be essential for the differentiation of this structure during embryogenesis. Also, Marx et al. (26) have shown that there is some correlation between the severity of rickets and the presence of alopecia. HVDRR patients with alopecia tend to be nonresponsive to calciferols while those without alopecia tend to be responsive to high doses of vitamin D. The alopecia or some degree of hair loss appears to be associated primarily with DBD mutations or premature stop mutations, which usually result in complete hormone unresponsiveness and total resistance. A few patients with LBD mutations did not develop alopecia. Interestingly, the patient from family F48 had alopecia despite not having a mutation in the VDR. Alopecia remains unchanged in patients that undergo successful therapy or that show spontaneous improvement. In families with a history of HVDRR, the absence of body hair in newborns provides initial evidence for the disease. Interestingly, alopecia usually has not been found in other conditions related to defective vitamin D action, including 1α-hydroxylase deficiency (VDDR I) and other forms of vitamin D deficiency. In HVDRR, the hair follicles appear normal on microscopic examination, but without hair shafts present. We suspect that 1,25-(OH)_2D_3 action is necessary for the establishment of the hair synthetic machinery at a critical stage in development.

A prenatal diagnosis of HVDRR is now possible in pregnant women from high-risk families. Cultured cells from choriocarcinoma samples or amniotic fluid have been used to ascertain whether the fetus has HVDRR using [3H]1,25-(OH)_2D_3 binding, induction of 24-hydroxylase activity, and RFLP analyses (271, 272).

The mechanism for the spontaneous improvement in some HVDRR children as they get older is an interesting dilemma. One hypothesis that explains the normalization of the 1,25-(OH)_2D_3 endocrine system, in the face of inactive VDRs, is that some other transcription factor can substitute for the defective vitamin D system. Possibly RAR, RXR, or TR can substitute for a nonfunctional VDR and activate the appropriate target genes to reverse the hypocalcemia and restore the bones to normal. This hypothetical explanation remains untested. However, Whiffen et al. (157) have shown in vitro that addition of RXR can rescue mutant VDR with defects in the dimerization domain and restore hormone responsiveness.

HVDRR is a relatively rare disease compared with androgen (239)- and thyroid (158)-resistant syndromes, which occur more frequently in the population. On the other hand, only a few cases of glucocorticoid (241), mineralocorticoid (242), and estrogen (240) resistance have been reported, while progesterone resistance has not been described. It is therefore interesting to speculate on the reasons for these differences in prevalence of diseases caused by mutations in the steroid hormone receptors. We believe that HVDRR is relatively rare because it is a true recessive disease. Heterozygotes are asymptomatic and for these rare mutations to affect both alleles in an individual, consanguinity is usually required. On the other hand, the number of cases of androgen resistance (androgen insensitivity syndrome or AIS) is greater, in part, because the AR gene is on the X-chromosome and a single mutation would result in the disease in the hemizygous male population. Females with two copies of the AR gene appear phenotypically normal even when one allele is mutated. Males acquire the mutant AR gene from their asymptomatic, heterozygotic mothers and consanguinity is not required. Thyroid hormone resistance (generalized resistance to thyroid hormone or GRTH), on the other hand, is often caused by dominant negative mutations in which one defective allele inactivates the normal allele (158). Like androgen resistance, only a single mutant TR allele is necessary to cause thyroid resistance. In contrast, dominant negative mutations have not been described in HVDRR where heterozygotes exhibit a normal phenotype. Since TR and VDR are thought to act through a common heterodimerization partner, RXR, one might speculate that the difference between GRTH and HVDRR due to a dominant negative mechanism might be due to the ability of TRs to form homodimers while VDRs do not. Few reports of glucocorticoid and mineralocorticoid resistance have been described (241, 242). Total resistance is likely to be lethal while mild cases might be more prevalent in the population than one might expect but are not diagnosed because they are not as easily recognized as androgen insensitivity syndrome, GRTH, and HVDRR (241). Mutations in ER and PR are rarely recognized although a single case of defective ER has been described in a male patient (240). The rarity of clinical cases was originally thought to be due to lethality of the mutation but, since knockout mice survive, this may also be due to lack of ascertainment or interference with successful pregnancy.

In conclusion, the biochemical and genetic analysis of the...
VDR in the HVDRR syndrome has yielded important insights into the structure and function of the receptor in mediating 1,25-(OH)2D3 action. Similarly, studies of children affected with HVDRR continue to provide further insight into the biological role of 1,25-(OH)2D3 in vivo. A concerted investigative approach of HVDRR at the clinical, cellular, and molecular level has proven exceedingly valuable in understanding the mechanism of action of 1,25-(OH)2D3 and improving the diagnostic and clinical management of this rare genetic disease.

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