Vitamin D–Resistant Diseases

Uri A Liberman

ABSTRACT: Hereditary vitamin D receptor defects (HVDRDs) is a more appropriate and precise title for an inborn error of metabolism commonly known as pseudo-vitamin D deficiency or vitamin D dependency, type II. It is a rare autosomal recessive disorder, ~70 kindreds were described, but its main importance is elucidating the physiology of vitamin D and calcium homeostasis in humans. Patients usually develop the clinical and biochemical aberrations, within the first year of life (i.e., muscle weakness, bone pain, deformities, and fractures). The term “hereditary vitamin D receptor defects” (HVDRD) may be the most appropriate and precise to describe this condition. As with many inborn errors of metabolism, HVDRD is a rare disorder; ~70 kindreds have been described since the first publication almost 30 yr ago. The significance of this disease relates to its role in elucidating the physiology of vitamin D and calcium homeostasis in humans. It is an autosomal recessive disease; it is familial, and there are kindreds with more than one sibling being affected; there is frequent consanguinity; and the origin of many affected kindreds is concentrated in a region centered around the Mediterranean. An unusual feature of this disease is total alopecia, documented in about one half of the patients and usually in more severely affected patients. Patients are normal at birth and develop the clinical symptoms and biochemical signs within the first year of life. A few milder cases with late childhood onset were described as well. The clinical, radiological, histological, and most of the biochemical features are similar, if not identical, to vitamin D deficiency (i.e., bone pain, bone deformity expressed in the most rapidly growing bones [this will be a function of age], fractures, and growth retardation). If not treated, this disorder may lead to inanition, severe skeletal deformity, recurrent respiratory infections, and even death. The basic abnormality is defective calcium absorption from the small intestine leading to hypocalcemia, secondary hyperparathyroidism, and hypophosphatemia, and defective mineralization of newly formed bone matrix. The disease is not cured by vitamin D replacement therapy, although some patients respond to very high doses of vitamin D or its metabolites. Cells derived from patients, mainly cultured skin fibroblasts, were used to assess steps in calcitriol action from cellular uptake to bioresponse and to elucidate the molecular aberrations in the vitamin D receptor (VDR). Point mutations in the VDR gene were identified in every patient examined, and the same defect was observed in the obligatory heterozygotes. The functional characterization of the patient’s VDR reflected the localization of the mutation (18 different ones described to date), thus providing vital information about the structure–function relationship in the human VDR and the essentiality of the VDR as the mediator of vitamin D action.


Key words: Rickets-hereditary, vitamin D receptor, inborn errors in the vitamin D receptor, vitamin D resistance, 1,25(OH)2D (calcitriol)

VITAMIN D–RESISTANT DISEASES is an ambiguous term for several reasons. (1) It does not define what is the most distal vitamin D metabolite to which there is resistance. Thus, it will include perturbations in the synthesis of the active hormone, 1,25-dihydroxyvitamin D [1,25(OH)2D; calcitriol]. These disturbances could be primary (i.e., hereditary pseudovitamin D deficiency rickets) or secondarily acquired (i.e., chronic renal failure). (2) The above title does not describe the bio-effect that characterizes the resistant state. However, the accepted term “hereditary vitamin D–resistant rickets” (HVDRR) is confusing as well, because not all causes of rickets and/or osteomalacia resistant to vitamin D or calcitriol involve primary disturbances in calcitriol metabolism or action. For example, rickets resistant to calciferol also describes primary disturbances in phosphate homeostasis as hereditary hypophosphatemic rickets with hypercalciuria (HHRH) or primary bone defects such as hypophosphatasia. The most helpful bio-effect to include in a description is the most direct action known to be abnormal; thus, calcium malabsorption is more useful than rickets to characterize defects in vitamin D action. The term “hereditary hypocalcemic rickets resistant to calcitriol” defines more precisely this abnormality. However, based on our current knowledge of the etiology and pathophysiology of this disease, the term “hereditary vitamin D receptor defects” (HVDRD) may be the most appropriate and precise to describe this condition.

Department of Physiology and Pharmacology and the Felsenstein Medical Research Center, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.
roidism, and hypophosphatemia. The combination of low calcium and phosphorous in the extracellular fluid will cause defective mineralization of newly formed bone matrix, which in children will cause abnormalities in diaphyses, metaphyses, and epiphyses; the latter in particular will cause bone deformities. These bone changes have overt appearances on radiographs. The characteristic features that differentiate this inborn error from vitamin D deficiency are (1) very high serum concentrations of 1,25(OH)2D before or on treatment with vitamin D or its metabolites and (2) the absence of any clinical, radiological, or biochemical response to standard vitamin D replacement therapy. Some patients have shown complete remission of the disease on high doses of vitamin D, 25-hydroxyvitamin D, or the 1α-hydroxylated metabolites plus calcium supplementation. Some patients did not respond to maximal vitamin D therapy. In a few of these patients, a therapeutic response was achieved with large amounts of calcium, either orally almost to the point of intolerance or by continuous intravenous infusions.

Because of the widespread expression of the vitamin D receptor (VDR) in many human tissues, cells from affected patients were used to characterize the intracellular molecular defects. This was done mainly in cells originating from accessible tissues such as fibroblasts grown from skin biopsies and peripheral lymphocytes. In a few cases, keratinocytes and osteoblast-like cells grown from bone biopsies were used. These cells were used to assess most of the steps in calcitriol action from cellular uptake to bioreponse and to elucidate the molecular aberrations in the hormone receptor protein and the nuclear DNA that encodes for it. Hormone–receptor interaction, including binding affinity and capacity, was measured in intact cells, nuclei, or high salt-soluble extracts (so-called cytosol). The molecular defects were studied by isolation, amplification, and sequencing genomic VDR DNA, as well as by cloning and sequencing VDR cDNA. The mutant DNA was recreated in vitro and was transfected into cells that do not express endogenous VDR. Post-transcriptional action of 1,25(OH)2D was tested in cells originating from patients or in cells co-transfected with VDR (either mutant or wildtype) fused to a promoter containing a vitamin D response element (VDRE). The most extensively tested response was calcitriol induction of 25(OH)D₃-24-hydroxylase activity in cultured skin fibroblasts or transfected cells. Several other bioassays or different cells have also been used, including inhibition of cell proliferation, mainly in stimulated T cells, or stimulation of osteocalcin synthesis. Mutations in the VDR gene have been identified in almost every patient studied with this disease. Point mutations caused nonsense changes that introduce a stop codon or led to a frame-shift resulting in a premature stop codon that produced a truncated VDR that lacks hormone binding, DNA binding, or both. Missense VDR mutations were documented in cells from all other patients or kindreds examined. The functional characterization of the patient’s VDR reflected the localization of the point mutation. Post-transcriptional response was severely deficient, from suppressed response under high concentrations of 1,25(OH)₂D₃ or 9-cis retinoic acid receptor (RXR) to absolutely no response. Mutations localized to the DNA-binding domain, N-terminal or zinc-fingers’ regions, showed no binding to DNA and no measurable in vitro response. Mutations localized to the C-terminal region included those that alter hormone binding, expressed as decreased affinity or capacity, up to no detectable binding, and those that interfere with heterodimerization of VDR and RXR or of binding to co-factors. These latter mutations sometimes showed limited binding under specific conditions but with a deficient post-transcriptional response. In vitro responses paralleled the in vivo response to therapy and thus can be used as a predictor of patient responses to high doses of vitamin D or its metabolites. In every kindred in which more than one patient was examined, the same defect was identified in the affected sibling. Obligatory heterozygotes were found to have one normal and one mutant allele with the same molecular defect as the affected patient, but without any clinical or biochemical abnormalities.

Recently, a patient with compound heterozygosity involving two different molecular defects in the ligand-binding domain was described. This boy had total alopecia and severe rickets and could be completely cured by very high doses of 25(OH)D, 250 μg/d initially, followed by 100 and 75 μg/d as a maintenance dose for years, plus calcium supplementation. His VDR had a low hormone-binding capacity with markedly deficient stimulation of 25(OH)D₃-24-hydroxylase. The recreated mutations, each one tested separately in vitro, showed deficient hetrodimerization and different transactivation of two gene promoters. This patient, similar to another one described >20 yr ago, showed that, during remission (normocalcemia, normophosphatemia, normal iPTH), 1,25(OH)₂D₃ production is driven by the substrate [i.e., 25(OH)D concentrations].

As stressed before, studies of patients with hereditary VDR defects are important in delineating basic vitamin D physiology and calcium homeostasis in humans. Some of those are the importance and exclusivity of the VDR as a mediator of vitamin D action on calcium homeostasis; the structure–function relationship of the VDR; and the essential nature of the position of certain amino acids for binding to DNA, to hormone, to RXR, and to co-factors, attesting to the importance of these components of the system. The observation that a defect may have different transcriptional effects on promoters may have implications for designing therapeutic agents acting as selective VDR modulators. The essential role of 1,25(OH)₂D₃ as a negative feedback control on its own production is based on these observations. Finally, the fact that complete cure of rickets in patients resistant to calcitriol could be obtained by supplying enough calcium, either orally or intravenously, suggests that calcitriol is not essential (or may be redundant) in bone mineralization and homeostasis, in contrast to its pivotal and irreplaceable role in calcium homeostasis.

REFERENCES


Address reprint requests to:
Uri A Liberman, MD, PhD
Department of Physiology and Pharmacology
Tel-Aviv University
25 Tagore Street
Tel-Aviv 69203, Israel
E-mail: uliberm@post.tau.ac.il

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