

# Atrichia Caused by Mutations in the Vitamin D Receptor Gene is a Phenocopy of Generalized Atrichia Caused by Mutations in the Hairless Gene

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**Generalized atrichia with papules is a rare disorder characterized by loss of hair shortly after birth and development of cutaneous cysts. Mutations in the hairless gene (HR) cause this phenotype in both mouse and human. Here we present a case of atrichia with papules in a patient with a normal HAIRLESS gene but with mutations in both alleles of the VITAMIN D RECEPTOR. The patient exhibited vitamin D resistant rickets, which was confirmed by an absent response of her fibroblasts to 1,25-dihydroxyvitamin D<sub>3</sub> *in vitro*. Similar to individuals with HAIRLESS mutations, her skin showed an absence of normal hair follicles and the presence of follicular remnants and cysts. The cyst epithelium contained**

**keratin-15- and keratin-17-positive cells suggesting derivation from the hair follicle bulge and the presence of epithelial stem cells. Although hair loss has been reported in association with hereditary vitamin D resistant rickets, we now characterize this alopecia as clinically and pathologically indistinguishable from generalized atrichia with papules, which was previously thought to be caused only by mutations in HAIRLESS. These findings suggest that VDR and HR, which are both zinc finger proteins, may be in the same genetic pathway that controls postnatal cycling of the hair follicle. Key words: alopecia/cysts/hair follicle/rickets/transcription factors. *J Invest Dermatol* 117:612-617, 2001**

**T**he syndrome of atrichia with papular lesions (OMIM 209500), first described in 1954 by Damste and Prakken, is characterized by permanent hair loss beginning within the first few months after birth, and subsequent development of keratin-filled cysts in the skin (Damste and Prakken, 1954). Mutations in the HAIRLESS (HR) gene have been identified as causative in several families with this condition, which is inherited in an autosomal recessive manner and does not affect any extracutaneous sites (Ahmad *et al*, 1998a, b, 1999; Cichon *et al*, 1998; Zlotogorski *et al*, 1998; Kruse *et al*, 1999; Sprecher *et al*, 1999b; Aita *et al*, 2000). The hairless gene encodes a predicted 127 kD protein that contains a zinc finger domain, suggesting it functions as a transcription factor (Cachon-Gonzalez *et al*, 1994; Djabali *et al*, 2000). *Hr* null mice develop hair loss and cysts remarkably similar to patients with atrichia with papules (Montagna *et al*, 1952; Mann, 1971; Panteleyev *et al*, 1999).

Alopecia is a frequent feature of hereditary vitamin D resistant rickets (HVDRR, OMIM 277440), a rare autosomal recessive disorder described in multiple kindreds (Beer *et al*, 1981; Hochberg *et al*, 1985; Marx *et al*, 1986). Similar to families with atrichia with

papules, hair is generally present at birth but then is lost within 12 mo. Hair loss may precede the development of bony rachitic changes. Remarkably, bony changes often resolve and metabolic defects can normalize with age, but the alopecia is permanent (Hirst *et al*, 1985). Mutations in the VITAMIN D RECEPTOR (VDR), which functions as a transcription factor, are responsible for HVDRR associated with atrichia (Rut *et al*, 1994; Hawa *et al*, 1996; Whitfield *et al*, 1996). *Vdr* null mice develop metabolic and bony changes as well as alopecia very similar to humans with the disorder (Li *et al*, 1997; Yoshizawa *et al*, 1997). The alopecia in HVDRR has not been well characterized, and its similarity to the alopecia caused by hairless mutations has not been appreciated.

Here, we studied a patient with HVDRR and atrichia who has distinct mutations in both VDR alleles, with a normal HR gene. For the first time, we demonstrate that the cutaneous phenotype associated with HVDRR is remarkably similar to the generalized atrichia with papules caused by mutations in HR. Similar to patients with HR mutations, follicular remnants in this patient's skin appear to possess hair follicle stem cells, some of which generate cutaneous cysts. Overall, our findings suggest that VDR and HR may be part of the same molecular pathway necessary for normal hair follicle cycling.

## MATERIALS AND METHODS

**Immunohistochemistry** Skin was fixed in formalin and embedded in paraffin. Paraffin sections were immunostained with the mouse monoclonal antibody DAKO clone C8/144B, which recognizes

Manuscript received December 13, 2000; revised April 24, 2001; accepted for publication April 27, 2001.

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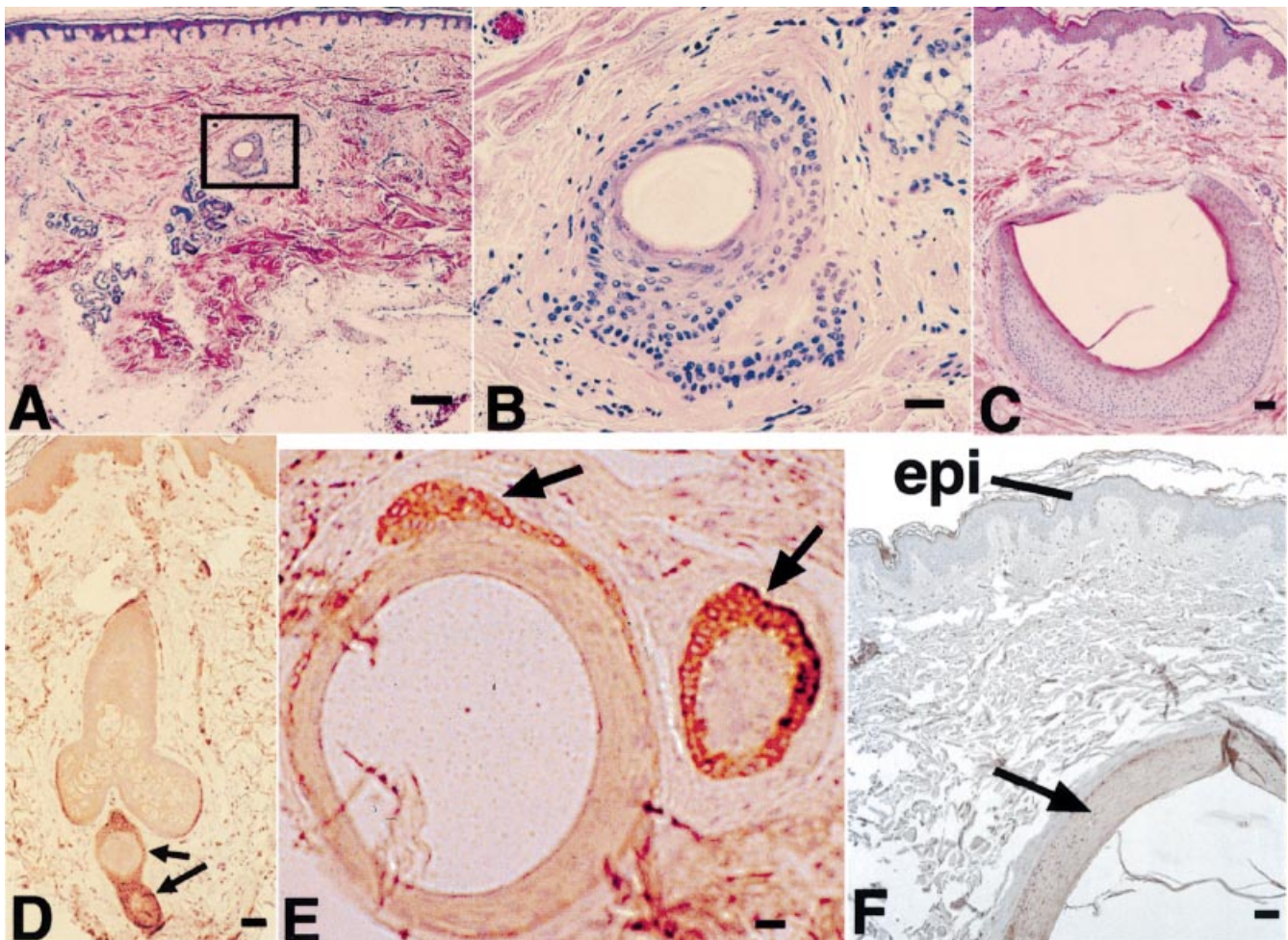
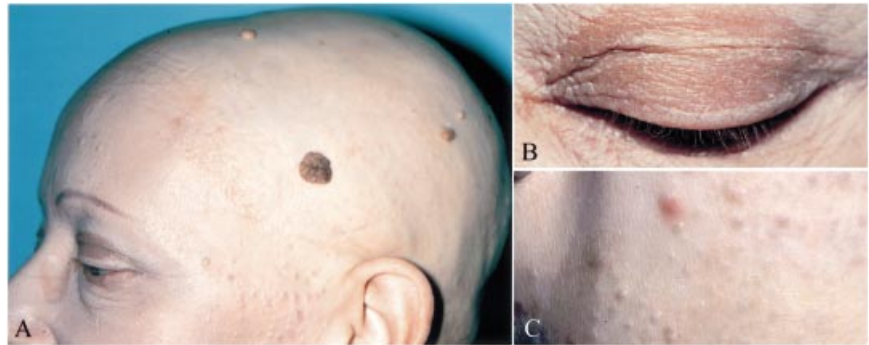
Abbreviations: HR, HAIRLESS; HVDRR, hereditary vitamin D resistant rickets; VDR, VITAMIN D RECEPTOR.

cytokeratin 15 (Lyle *et al.*, 1998), or a rabbit polyclonal antibody raised against cytokeratin 17 that was kindly provided by Dr. Pierre Coulombe, Johns Hopkins University School of Medicine, Baltimore, Maryland (McGowan and Coulombe, 2000). Tissue sections were steamed in citrate buffer (10 mM sodium citrate, pH 6.77) for 15 min prior to incubation. After blocking of endogenous peroxidase with hydrogen peroxidase 6% and washing with blocking agent (1 × phosphate-buffered saline, 0.1% Triton X100, 0.1% bovine serum albumin, and 5% goat serum) for 20 min, the tissue sections were incubated with the C8/144B antibody, diluted 1:40, or anti-keratin 17 (anti-K17) antibody, diluted 1:1000, overnight at 4°C. The avidin-biotin complex technique

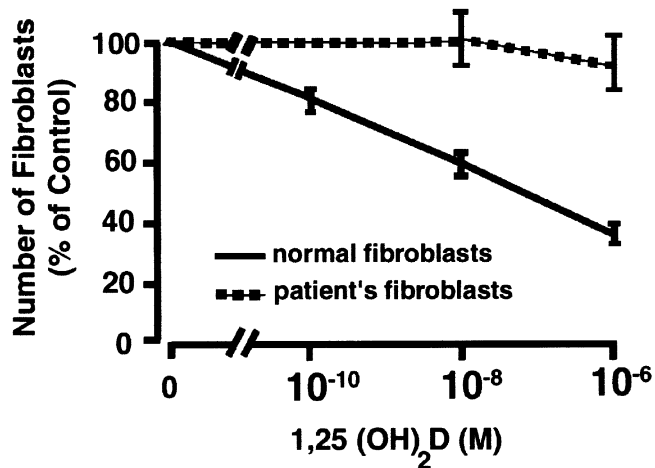
was used for immunohistochemistry. After washing, the secondary antibody (biotinylated antimouse or antirabbit IgG), diluted 1:300, was added and incubated at room temperature. Streptavidin HRP (Gibco BRL), diluted 1:500, was applied to the slides and incubated for 30 min at room temperature. After washing, the sections were developed with diaminobenzidine (Sigma) as the chromogen, and counterstained with hematoxylin.

**Fibroblast proliferation assay** Fibroblasts were isolated from a 4 mm punch biopsy specimen from the patients' scalp, and from normal human foreskin (control). After trypsinization overnight, fibroblasts were plated

**Figure 1. Appearance of patient with atrichia caused by VDR mutations.** (A) Atrichia of scalp; (B) sparing of eyelashes (also frequent in atrichia caused by HR mutations); (C) cysts on neck skin.



**Figure 2. Scalp biopsy from patient with atrichia caused by VDR mutations.** Note the absence of normal follicles (A). (B) Higher magnification of solitary follicular remnant boxed in (A). Note bulges of the outer root sheath. (C) Typical cyst in dermis. (D, E) Immunostaining for K15 (brown/red stain, arrows) confirms the presence of bulge cells in follicular remnant and cyst basal layers. (F) Immunostaining for K17. Cyst epithelium is positive. Scale bars: (A) 150  $\mu$ m; (B, E) 20  $\mu$ m; (C, D, F) 50  $\mu$ m.



**Figure 3.** Fibroblasts from the patient with HVDRR and atrichia are resistant to the effects of 1,25(OH)<sub>2</sub>vitaminD<sub>3</sub>. Increasing concentrations of 1,25(OH)<sub>2</sub>vitaminD<sub>3</sub> suppress growth of normal fibroblasts but not growth of the patient's fibroblasts *in vitro*.

in a 35 mm dish in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum. The second passage cultures were fed with fresh medium and treated with 1,25(OH)<sub>2</sub>D<sub>3</sub> 10<sup>-6</sup>, 10<sup>-8</sup>, and 10<sup>-10</sup> M on the first and fourth day of the culture. Proliferation was assessed by counting cell numbers on the seventh day after dosing. The antiproliferative activity of 1,25(OH)<sub>2</sub>D<sub>3</sub> was expressed as a percentage of the control in the absence of 1,25(OH)<sub>2</sub>D<sub>3</sub>. Experiments were performed in triplicate. Error bars in **Fig 3** represent standard deviation.

**Primer design** For amplification of segments of genomic DNA corresponding to the *VDR* gene, the primers were designed on the basis of flanking intronic sequences. Each primer pair was optimized using the program Primer Designer (Educational Software, version 3.0). The oligomer primers were 20 nucleotides in length, contained no greater than three identical consecutive bases, minimal secondary structure, between 45% and 65% G/C content, and were free of the potential for primer-dimer formation. Amplimers ranged from 196 to 589 bp in size, and were placed in introns of the *VDR* gene to span the exons. Primers were synthesized using an automatic oligonucleotide synthesizer (Applied Biosystems).

**Mutation detection** DNA was prepared from peripheral blood leukocytes according to standard techniques (Sambrook *et al*, 1989). Exons and splice junctions were polymerase chain reaction (PCR) amplified from genomic DNA and sequenced directly in an ABI Prism 310 Automated Sequencer, using an ABI prism dRhodamine Terminator cycle sequencing Ready Reaction Sequencing Kit (PE Applied Biosystems), following purification in Centriflex Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, MD). Exon 4 of the *VDR* gene was amplified with the following set of primers: 5'-AGGAGGAAGTTTC-CTGGAG-3' (sense primer) and 5'-GGCTCCACTAGTGCTTCTCC-3' (antisense primer). Exon 8 of the *VDR* gene was amplified with the following primers: 5'-CTGGCCATTGCTTCTCACAG-3' (sense primer) and 5'-TGCTACGTCTCCCTTCAGGT-3' (antisense primer). Identification of the mutation was performed by visual comparison of the patient's sequence with that of an unrelated, unaffected control individual.

## RESULTS

**Clinical description of patient** The patient is a 55-y-old Caucasian woman with a history of vitamin D resistant rickets, cutaneous cysts, and atrichia. Hair loss first developed within the first 5 wk of life, and by 1 y of age scalp and body hair were nearly completely absent (**Fig 1a**). Only eyelashes are present (**Fig 1b**). Asymptomatic cutaneous cysts first developed at the age of 5 and progressively increased in number (**Fig 1c**). These 0.5–1.0 cm bluish and flesh-colored nodules are concentrated along the jaw line, neck, chest, and upper arms. She is of short stature (150 cm)

and has bowing of weight-bearing bones. Her teeth and nails are normal. Her parents are not consanguineous. A brother, her only sibling, is unaffected.

Calcium, phosphorus, and parathyroid hormone levels were within normal limits at the time of our examination. As a child, these levels were markedly abnormal and resistant to treatment with high doses of vitamin D.

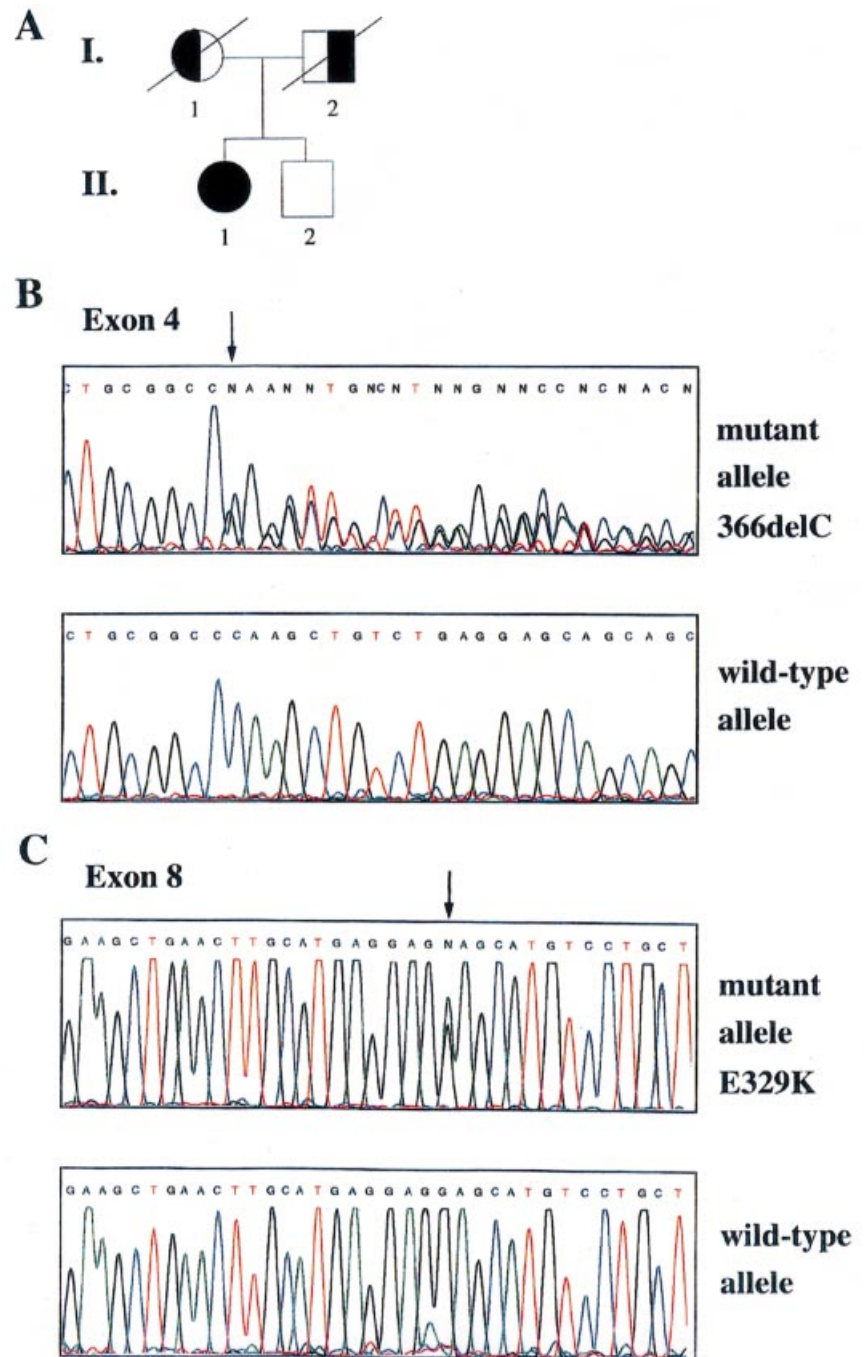
Current management includes incision and cauterization of cysts. Treatment with topical tretinoin and systemic isotretinoin did not prevent the development of cysts.

**Pathologic examination of skin** A scalp biopsy revealed complete absence of normal hair follicles (**Fig 2A**), but occasional small, empty follicular structures were evident in cross-section in the mid dermis, and these were associated with a rudimentary sebaceous lobule (**Fig 2B**) and arrector pili muscle. Scattered diminutive follicles containing orthokeratin without hair shafts were present in the superficial dermis. Some follicles opened directly to the epidermal surface and contained laminated orthokeratin. These lesions were reminiscent of "utricle" seen in mice with mutations in *hr* (Mann, 1971; Panteleyev *et al*, 1998b). A biopsy from neck skin revealed multiple mid dermal cysts lined with an epithelium that resembled outer root sheath (**Fig 2C**). Some cysts contained a thin granular layer along the luminal surface. Granulomatous inflammation surrounded portions of a cyst that contained focal areas of trichilemmal keratinization.

**Analysis of hair follicle stem cell reserve** Panteleyev *et al* (1998b) analyzed the changes in *hr/hr* mouse skin using markers for hair follicle epithelium. They determined that the superficial cutaneous cysts in the hairless mouse appeared to arise from the hair follicle bulge, which is thought to possess hair follicle stem cells. We previously reported that K15 is a marker for the hair follicle bulge area in human hair follicles (Lyle *et al*, 1998). In order to determine whether hair follicle bulge cells are present in the cysts from this patient with atrichia and rickets, we immunostained the skin using an antibody that detects K15. K15-positive cells were present at the bottom of the follicular remnants, which appears to end at the level of the bulge (**Fig 2D**). Interestingly, many cysts possessed K15-positive bulge cells (**Fig 2E**), suggesting that the process leading to cyst formation in HVDRR is similar to that caused by mutations in *HR*. To further evaluate the upper follicular origin of these cysts, we also immunostained the scalp tissue with an antibody that detects K17, which is normally found in the outer root sheath epithelium, not in the bulb (McGowan and Coulombe, 2000). The scalp cysts also expressed K17 (**Fig 2F**).

**Analysis of sensitivity to vitamin D<sub>3</sub>** 1,25-dihydroxyvitamin D<sub>3</sub>, the active form of vitamin D<sub>3</sub>, decreases cellular proliferation in a dose-dependent manner in normal cultured human fibroblasts (MacLaughlin *et al*, 1985; Smith *et al*, 1988). Fibroblasts from patients with HVDRR are resistant to this effect and show no change in proliferation in response to 1,25-dihydroxyvitamin D<sub>3</sub>. In order to evaluate whether fibroblasts from this patient exhibited resistance to the effects of 1,25-dihydroxyvitamin D<sub>3</sub>, we treated fibroblasts isolated from the patient's scalp with 1,25-dihydroxyvitamin D<sub>3</sub> *in vitro*. In contrast to the normal fibroblasts, fibroblasts from this patient showed no response to the addition of 1,25-dihydroxyvitamin D<sub>3</sub> (**Fig 3**). These findings of end-organ resistance to 1,25-dihydroxyvitamin D<sub>3</sub> are most consistent with mutations in the *VDR* gene.

**Analysis of *HR* and *VDR* genes** Because the atrichia with papular phenotype of this patient was identical to that of patients carrying mutations in the *HR* gene, we screened for mutations in this gene in this patient with HVDRR with atrichia. By direct DNA sequencing of this patient's *HR* gene, we found no mutations. Knowing that HVDRR has been associated with mutations in the *VDR* gene, we screened for a mutation in the human *VDR* gene. Exons and splice junctions were PCR amplified from genomic DNA and sequenced directly. Sequence analysis of exon 4 of the *VDR* gene from the affected individual revealed a



**Figure 4. Genetic analysis of the patient.** (A) The pedigree is suggestive of autosomal recessive inheritance. (B) Comparison of mutant and wild-type DNA sequence of exon 4 from *VDR*. Arrow points to the deletion of a single nucleotide at position 366 (366delC), which results in a frame shift and downstream premature termination codon at nucleotide 420. (C) Comparison of mutant and wild-type DNA sequence of exon 8 of *VDR*. Arrow points to a missense mutation at nucleotide 985 leading to conversion of glutamic acid to lysine (E329K).

heterozygous single base pair deletion at nucleotide position 366, designated 366delC. This deletion led to a frameshift and downstream premature termination codon at nucleotide position 420 within exon 4 (Fig 4).

The second mutation was identified in exon 8 of the *VDR* gene. Direct sequence analysis of exon 8 revealed a heterozygous G-to-A transition in the proband. The G-to-A transition occurred at the first base of the glutamic acid (E) residue at position 985 (GAG), leading to a missense mutation converting the glutamic acid residue to a lysine (K) residue (AAG), and was designated E329K. The mutation destroyed the cleavage site for the restriction endonuclease *Mnl*I (GGAG), which was used to confirm the presence of the mutation in genomic DNA, in addition to direct sequencing. To verify that the missense mutation was not a normal polymorphic variant within the *VDR* gene, we screened for the mutation by a combination of direct sequencing and restriction digestion in a

control population consisting of 50 unrelated, unaffected individuals. No evidence for the mutant allele was found in these individuals, suggesting that E329K is a pathogenic mutation. The proband therefore is a compound heterozygote for two different *VDR* mutations (366delC/E329K). Screening of DNA from her unaffected brother revealed that he does not carry mutations in either of the *VDR* genes. The pedigree is consistent with autosomal recessive inheritance. No other family members are available for screening. Given that we were limited to analyzing DNA from the proband and her brother, it is possible that one of the mutations arose *de novo*.

#### DISCUSSION

The presence of hair at birth in both HVDRR and atrichia with papules suggests that hair follicle development is normal in these

conditions. Indeed, both *vdR* and *hr* mutant mice develop normal hair follicles and hair. Subsequent hair loss, beginning 3–4 wk after birth, suggests a defect in hair follicle cycling—either in catagen, telogen, and/or anagen onset. In hairless mice, Panteleyev *et al* (1999) confirmed that a defect in catagen was responsible for the phenotype. Normally during catagen, the epithelial cells in the lower portion of the hair follicle undergo apoptosis, and this results in cessation of hair growth and a precisely orchestrated involution of the lower follicle leading to telogen (Cotsarelis, 1997; Paus and Cotsarelis, 1999). After telogen, the follicle reenters anagen and the hair reforms. In the hairless phenotype, hair regrowth is disrupted because the lower follicle disintegrates during catagen (Panteleyev *et al*, 1998b, 1999), and the normal juxtaposition of epithelial stem cells in the bulge and mesenchymal cells in the hair follicle dermal papilla is not reestablished during telogen. This prevents mesenchymal–epithelial interactions thought to be necessary for reentry into anagen (Cotsarelis *et al*, 1990). The preservation of this patient's eyelashes may reflect the eyelashes' normal brief catagen stage in which the follicle only shortens slightly. In this type of follicle, VDR and HR defects may not sufficiently disrupt hair follicle architecture during catagen to result in aberrant hair follicle cycling. Vibrissae follicles also have short catagen stages (Oshima *et al*, 2001), and these follicles are also often preserved in *hr* mutant mice (Mann and Straille, 1961).

In *vdR* mutant mice also, hair loss begins around 3–4 wk of age (Li *et al*, 1997; Yoshizawa *et al*, 1997). Interestingly, the development of many of the metabolic abnormalities in *vdR*  $-/-$  mice can be prevented on a diet that normalizes mineral ion homeostasis; however, the alopecia still develops (Li *et al*, 1998). This finding supports the concept that the hair defect is a direct result of the *vdR* mutation rather than a secondary effect. Further evidence that *vdR* mutations cause aberrant hair follicle cycling was recently reported by Sakai and Demay (2000) who demonstrated that depilation of 18-d-old *vdR*  $-/-$  mice resulted in profoundly impaired hair regrowth. Thus, both humans and mice with mutations in either the hairless or the vitamin D receptor genes appear to have similar phenotypes of normal hair follicle development with aberrant hair follicle cycling.

Another similarity between *hr* and *vdR* mutant phenotypes is the development of cutaneous cysts. Both *hr* and *vdR* mutant mice develop cutaneous cysts, and humans with HR mutations also develop cysts that account for the papules that are part of the syndrome of atrichia with papules (Ahmad *et al*, 1998a; Sprecher *et al*, 1999a). Cysts are rarely mentioned in clinical descriptions of patients with HVDRR (Beer *et al*, 1981; Hochberg *et al*, 1985; Marx *et al*, 1986), although pathologic descriptions of skin from HVDRR patients are rare and include a report of "normal follicles without hair shafts" (Hochberg *et al*, 1985). Our patient clearly developed numerous cutaneous cysts as well as atrichia in a manner that closely mimics the phenotype of patients and mice carrying mutations in *hr*.

Cutaneous cysts in *hr* mutant mice are thought to arise from follicular remnants that survive after the follicle breaks apart during catagen (Panteleyev *et al*, 1998a, 1999). Based on keratin expression and morphologic evidence, many of these cysts appear to arise from the hair follicle bulge (Panteleyev *et al*, 1998b). The cyst epithelium expresses K17, which is normally found in the hair follicle outer root sheath (Panteleyev *et al*, 1998b; McGowan and Coulombe, 2000). We previously showed that K15 is a marker for bulge cells (Lyle *et al*, 1998) and that it is expressed by many tumors that are thought to originate from the bulge (Jih *et al*, 1999). The K15 and K17 positivity of the cystic epithelium and follicular remnants in this patient with a VDR mutation further supports the concept that HR and VDR mutations lead to indistinguishable cutaneous phenotypes. Furthermore, our findings suggest that epithelial stem cells, which may replenish the epidermis with keratinocytes, are present in HVDRR.

The realization that mutations in the HR and VDR genes cause indistinguishable cutaneous phenotypes of generalized atrichia suggests that these two proteins are components of the same

molecular pathway that is necessary for normal involution of the follicle during catagen. VDR is a member of the steroid binding receptor family and acts as a transcription factor. VDR possesses two zinc finger domains in the amino terminus and a ligand (1,25-dihydroxyvitamin D<sub>3</sub>) binding domain at the carboxy-terminus. The upstream zinc finger domain binds to DNA whereas the downstream domain is important for partnering with other proteins, including RXR. To date, at least eight missense mutations in the zinc finger domains have been described, and all of these patients exhibit HVDRR and atrichia (for review see Malloy *et al*, 1999). Similarly, premature termination mutations affecting these sites also cause HVDRR and atrichia. Interestingly, although most mutations in the ligand binding domain cause HVDRR and alopecia, three mutations have been described that resulted in HVDRR without alopecia. This suggests that the binding of 1,25-dihydroxyvitamin D<sub>3</sub> to VDR may not be necessary for normal catagen, but that the functions of the zinc finger domains, namely DNA binding and heterodimerization with other proteins, are necessary for regulation of the hair cycle. This is further supported by the fact that other types of vitamin D deficiency (caused by 1- $\alpha$ -hydroxylase for example), in which the VDR is normal but ligand binding is absent, do not cause alopecia.

Our patient's upstream mutation is located in the "hinge region" of the gene, which lies between the DNA binding domain and the ligand binding domain. The function of the hinge region is not known, but it may play a role in the binding of VDR to other steroid receptors. VDR, like other members of the steroid binding receptor family (e.g. estrogen receptor, thyroid hormone receptor), is known to partner with RXR. RXR- $\alpha$  ablation in mouse epidermis and hair follicle outer root sheath has recently been shown to cause a very similar phenotype to the *hr* and *vdR* mutant mice (Li *et al*, 2001). These mice also develop alopecia and cutaneous cysts, and have poor hair regrowth after depilation. In addition, *hr*, *vdR*, and *RXR- $\alpha$*  are all expressed in hair follicle keratinocytes (Reichrath *et al*, 1994; Panteleyev *et al*, 2000; Li *et al*, 2001). Thus, we propose that VDR, HR, and RXR- $\alpha$  are all in the same or convergent genetic pathways necessary for activation of genes controlling catagen. One possibility is that these proteins form a complex that binds to transcriptional elements involved in the control of catagen. This concept is supported by the fact that HR also has a zinc finger domain and is known to bind to thyroid receptor and modulate transcription of genes containing thyroid-responsive elements (Thompson and Bottcher, 1997). HR does not bind RXR or other steroid receptors, however, including the corticosteroid and mineralocorticoid receptors. Therefore, another possibility is that these proteins independently bind to separate sites on the same promoter that controls transcription of genes controlling catagen. Clearly, future studies are required to determine the exact relationship between HR and VDR, as well as the components of these genes that control hair follicle cycling.

## REFERENCES

- Ahmad W, Faiyaz ul Haque M, Brancolini V, *et al*: Alopecia universalis associated with a mutation in the human hairless gene. *Science* 279:720–724, 1998a
- Ahmad W, Irvine AD, Lam H, *et al*: A missense mutation in the zinc-finger domain of the human hairless gene underlies congenital atrichia in a family of Irish travellers. *Am J Hum Genet* 63:984–991, 1998b
- Ahmad W, Nomura K, McGrath JA, Hashimoto I, Christiano AM: A homozygous nonsense mutation in the zinc-finger domain of the human hairless gene underlies congenital atrichia. *J Invest Dermatol* 113:281–283, 1999
- Aita VM, Ahmad W, Panteleyev AA, *et al*: A novel missense mutation (C622G) in the zinc-finger domain of the human hairless gene associated with congenital atrichia with papular lesions. *Exp Dermatol* 9:157–162, 2000
- Beer S, Tieder M, Kohelet D, *et al*: Vitamin D resistant rickets with alopecia: a form of end organ resistance to 1,25 dihydroxy vitamin D. *Clin Endocrinol (Oxf)* 14:395–402, 1981
- Cachon-Gonzalez MB, Fenner S, Coffin JM, Moran C, Best S, Stoye JP: Structure and expression of the hairless gene of mice. *Proc Natl Acad Sci U S A* 91:7717–7721, 1994
- Cichon S, Anker M, Vogt IR, *et al*: Cloning, genomic organization, alternative transcripts and mutational analysis of the gene responsible for autosomal recessive universal congenital alopecia [published erratum appears in *Hum Mol Genet* 1998, November; 7 (12):1987–1988]. *Hum Mol Genet* 7:1671–1679, 1998

- Cotsarelis G: The hair follicle: dying for attention. *Am J Pathol* 151:1505–1509, 1997
- Cotsarelis G, Sun TT, Lavker RM: Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* 61:1329–1337, 1990
- Damste TJ, Prakken JR: Atrichia with papular lesions; a variant of congenital ectodermal dysplasia. *Dermatologica* 108:114–121, 1954
- Djabali K, Aita VM, Christiano AM: Hairless is translocated to the nucleus via a novel bipartite nuclear localization signal and is associated with the nuclear matrix. *J Cell Sci* 114:367–376, 2000
- Hawa NS, Cockerill FJ, Vadher S, et al: Identification of a novel mutation in hereditary vitamin D resistant rickets causing exon skipping. *Clin Endocrinol (Oxf)* 45:85–92, 1996
- Hirst MA, Hochman HI, Feldman D: Vitamin D resistance and alopecia: a kindred with normal 1,25-dihydroxyvitamin D binding, but decreased receptor affinity for deoxyribonucleic acid. *J Clin Endocrinol Metabolism* 60:490–495, 1985
- Hochberg Z, Gilhar A, Haim S, Friedman-Birnbaum R, Levy J, Benderly A: Calcitriol-resistant rickets with alopecia. *Arch Dermatol* 121:646–647, 1985
- Jih D, Lyle S, Elenitsas R, Elder D, Cotsarelis G: Cytokeratin 15 expression in trichoepitheliomas and a subset of basal cell carcinomas suggests they originate from hair follicle stem cells. *J Cutan Pathol* 26:113–118, 1999
- Kruse R, Cichon S, Anker M, et al: Novel Hairless mutations in two kindreds with autosomal recessive papular atrichia. *J Invest Dermatol* 113:954–959, 1999
- Li M, Chiba H, Warot X, Messaddeq N, Gerard C, Chambon P, Metzger D: RXR- $\alpha$  ablation in skin keratinocytes results in alopecia and epidermal alterations. *Development* 128:675–688, 2001
- Li YC, Pirro AE, Amling M, Delling G, Baron R, Bronson R, Demay MB: Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia. *Proc Natl Acad Sci U S A* 94:9831–9835, 1997
- Li YC, Amling M, Pirro AE, et al: Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor-ablated mice. *Endocrinology* 139:4391–4396, 1998
- Lyle S, Christofidou-Solomidou M, Liu Y, Elder D, Albelda S, Cotsarelis G: The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. *J Cell Sci* 111:3179–3188, 1998
- MacLaughlin JA, Gange W, Taylor D, Smith E, Holick MF: Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D<sub>3</sub>. *Proc Natl Acad Sci U S A* 82:5409–5412, 1985
- Malloy PJ, Pike JW, Feldman D: The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. *Endocr Rev* 20:156–188, 1999
- Mann SJ: Hair loss and cyst formation in hairless and rhino mutant mice. *Anat Record* 170:485–500, 1971
- Mann SJ, Straille WE: New observations on hair loss in the hairless mouse. *Anat Record* 140:97–102, 1961
- Marx SJ, Bliziotis MM, Nanes N: Analysis of the relation between alopecia and resistance to 1,25-dihydroxyvitamin D. *Clin Endocrinol (Oxf)* 25:373–381, 1986
- McGowan KM, Coulombe PA: Keratin 17 expression in the hard epithelial context of the hair and nail, and its relevance for the pachonychia congenita phenotype. *J Invest Dermatol* 114:1101–1107, 2000
- Montagna W, Chase HB, Melaragno HP: Skin of hairless mice. I. Formation of cysts and the distribution of lipids. *J Invest Dermatol* 19:83–94, 1952
- Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y: Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* 104:233–245, 2001
- Panteleyev AA, Paus R, Ahmad W, Sundberg JP, Christiano AM: Molecular and functional aspects of the hairless (hr) gene in laboratory rodents and humans. *Exp Dermatol* 7:249–267, 1998a
- Panteleyev AA, van der Veen C, Rosenbach T, Muller-Rover S, Sokolov VE, Paus R: Towards defining the pathogenesis of the hairless phenotype. *J Invest Dermatol* 110:902–907, 1998b
- Panteleyev AA, Botchkareva NV, Sundberg JP, Christiano AM, Paus R: The role of the hairless (hr) gene in the regulation of hair follicle catagen transformation. *Am J Pathol* 155:159–171, 1999
- Panteleyev AA, Christiano AM: Patterns of hairless (hr) gene expression in mouse hair follicle morphogenesis and cycling. *Am J Pathol* 157:1071–1079, 2000
- Paus R, Cotsarelis G: The biology of hair follicles. *N Engl J Med* 341:491–497, 1999
- Reichrath J, Schilli M, Kerber A, Bahmer FA, Czarnetzki BM, Paus R: Hair follicle expression of 1,25-dihydroxyvitamin D<sub>3</sub> receptors during the murine hair cycle. *Br J Dermatol* 131:477–482, 1994
- Rut AR, Hewison M, Kristjansson K, Luisi B, Hughes MR, O'Riordan JL: Two mutations causing vitamin D resistant rickets: modelling on the basis of steroid hormone receptor DNA-binding domain crystal structures. *Clin Endocrinol (Oxf)* 41:581–590, 1994
- Sakai Y, Demay MB: Evaluation of keratinocyte proliferation and differentiation in vitamin D receptor knockout mice. *Endocrinology* 141:2043–2049, 2000
- Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989
- Smith EL, Pincus SH, Donovan L, Holick MF: A novel approach for the evaluation and treatment of psoriasis. Oral or topical use of 1,25-dihydroxyvitamin D<sub>3</sub> can be a safe and effective therapy for psoriasis. *J Am Acad Dermatol* 19:516–528, 1988
- Sprecher E, Bergman R, Szargel R, Friedman-Birnbaum R, Cohen N: Identification of a genetic defect in the hairless gene in atrichia with papular lesions: evidence for phenotypic heterogeneity among inherited atrichias. *Am J Hum Genet* 64:1323–1329, 1999a
- Sprecher E, Lestringant GG, Szargel R, et al: Atrichia with papular lesions resulting from a nonsense mutation within the human hairless gene. *J Invest Dermatol* 113:687–690, 1999b
- Thompson CC, Bottcher MC: The product of a thyroid hormone-responsive gene interacts with thyroid hormone receptors. *Proc Natl Acad Sci U S A* 94:8527–8532, 1997
- Whitfield GK, Selznick SH, Haussler CA, et al: Vitamin D receptors from patients with resistance to 1,25-dihydroxyvitamin D<sub>3</sub>: point mutations confer reduced transactivation in response to ligand and impaired interaction with the retinoid X receptor heterodimeric partner. *Mol Endocrinol* 10:1617–1631, 1996
- Yoshizawa T, Handa Y, Uematsu Y, et al: Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nat Genet* 16:391–396, 1997
- Zlotogorski A, Ahmad W, Christiano AM: Congenital atrichia in five Arab Palestinian families resulting from a deletion mutation in the human hairless gene. *Hum Genet* 103:400–404, 1998