The Alopecias Associated With Vitamin D–Dependent Rickets Type IIA and With Hairless Gene Mutations

A Comparative Clinical, Histologic, and Immunohistochemical Study

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Objective: To establish the unique and common clinical and microscopic characteristics of the alopecias associated with vitamin D–dependent rickets (VDDR) type IIA and with hairless gene mutations.

Design: A comparative clinical, histologic, and immunohistochemical study of the alopecias in 6 patients with VDDR IIA and 4 patients with atrichia with papular lesions (APL) and/or alopecia universalis congenita (AUC) (hereinafter “APL/AUC”).

Main Outcome Measures: Clinical data were gathered from medical records, personal interviews, and physical examinations. Histologic and immunohistochemical studies were performed on 6 scalp punch biopsy specimens from each of the 2 studied groups.

Results: The alopecias in VDDR IIA and APL/AUC showed similar clinical, histologic, and immunohistochemical features. The clinical presentation of the VDDR alopecia resembled either the APL phenotype (ie, with papules and milia) or the AUC phenotype (without papules and milia). The main histologic findings included void infundibula; absence of the lower two thirds of the hair follicles, often replaced by vertically oriented irregular epithelial structures or epithelial cysts; irregular epithelial structures, often with small cysts in the middle and lower dermis; and small, medium, and large keratinizing cysts at all levels of the dermis. The larger epithelial cysts in the upper dermis stained positively for cytokeratin (CK) 10, which suggests an infundibular derivation, whereas the remaining irregular epithelial structures and cysts in the middle and lower dermis stained positively most frequently for CK17, CK19, and CD34, which suggests an outer root sheath derivation.

Conclusions: The alopecias associated with VDDR IIA and with hairless gene mutations show striking clinical and microscopic similarities. Disintegration of the lower two thirds of the hair follicles seems to be the underlying defect, and a common pathogenetic pathway might be involved.

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Recent studies have revealed underlying mutations in the human hairless gene in numerous families affected with 2 allelic disorders: atrichia with papular lesions (APL) (Mendelian Inheritance in Man [MIM] 209500) and alopecia universalis congenita (AUC) (MIM 203655).1-13 Apart from the absence of hair, the skin is normal in AUC, whereas patients with APL develop papular or milialike growths over most of their skin during the first years of life.14-16 How impaired function of the hairless protein leads to atrichia is still not fully understood.17-19

The histopathologic features of alopecia in rhino and hairless mice that carry mutations at the hairless locus17 have been well documented.20 The histopathologic characteristics in humans is deduced primarily from case descriptions, some of which were published prior to the discovery of the underlying hairless gene mutations.1,2,4,5,9,11-14,16-25

Alopecia is also a frequent feature of hereditary vitamin D–dependent rickets (VDDR) type IIA (MIM 277440), a rare autosomal recessive disorder described in several kindreds.26-28 Similar to families with APL, hair is generally present at birth but is then lost within 12 months. Miller et al29 have recently described a patient with VDDR IIA and reported striking clinical and histologic similarities to APL. Zlotogorski et al30 also noted clinical similarities to APL in several patients with VDDR IIA, and histologic similarities to APL in 1 patient with VDDR IIA. The present study was performed to determine the unique and common features of the alopecias associated with VDDR IIA and with hairless gene muta-
We observed 6 patients with VDDR IIA, 5 female and 1 male ranging in age from 5 to 18 years (Table 1). One female and 3 male ranging in age from 5 to 15 years, and 4 patients with APL and/or AUC (hereinafter “APL/AUC”), 1 female and 3 male ranging in age from 4 to 18 years (Table 1 and Table 2). The diagnosis of the APL/AUC cases was confirmed by molecular genetic studies showing mutations in the hairless gene. The diagnosis of the VDDR IIA cases was confirmed by molecular genetic studies showing a point mutation in the vitamin D receptor (VDR) gene.

The medical records of each patient were reviewed, and each patient was interviewed and physically examined. The patient's parents were also interviewed. After obtaining informed written consent from each participant, a 4-mm punch biopsy specimen was obtained from their scalps, except for 1 patient with VDDR (patient 2; Table 1), for whom we used a previous biopsy specimen obtained for diagnostic purposes at age 8 months. In 2 patients with APL/AUC from whom new scalp biopsy specimens were obtained (patients 1 and 2; Table 2), we also studied previous specimens obtained for diagnostic purposes 3 years and 1 year earlier, respectively.

The routinely processed specimens were completely serially sectioned in a vertical fashion. The first sections were stained with hematoxylin-eosin. The next 4 sections were stained with anti-keratin monoclonal antibodies to cytokeratin (CK) 10, CK17, CK19, and CD34, all available from DAKO A/S, Copenhagen, Denmark, and by using the avidin-biotin immunoperoxidase technique (Histostain-Plus kit; Zymed Laboratories Inc, San Francisco, Calif). All of the remaining serial sections were also stained with hematoxylin-eosin.

Positive and negative control immunostainings were performed on 2 normal scalp specimens available from our files. In the normal control scalp specimens, CK10 was expressed in the normal control scalp specimens, CK17 was expressed in (+) Suprabasal layers of infundibula and upper parts of irregular epithelial structures, CK19 was expressed in (+) Suprabasal layers of irregular epithelial structures (epithelial cysts not present), CD34 was expressed in (+) Basal layers of irregular epithelial structures

Abbreviations: Plus sign, positive findings; minus sign, negative findings.

*Results pertaining to hair follicle-related structures only.

### Table 1. Histologic and Immunohistochemical Histologic Findings in 6 Cases of Vitamin D–Dependent Rickets Type II Alopecia

<table>
<thead>
<tr>
<th>Patient/Sex/Age, y</th>
<th>Mutation</th>
<th>Age at Biopsy</th>
<th>Histologic Findings*</th>
<th>Immunohistochemical Findings*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/15 Y292X</td>
<td>15 y</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle missing. Several irregular epithelial structures and 2 small epithelial cysts in lower dermis.</td>
<td>(+) Suprabasal layers of infundibula and upper parts of irregular epithelial structures</td>
<td>CK10 (+) Suprabasal layers of infundibula and upper parts of irregular epithelial structures</td>
</tr>
<tr>
<td>2/M/8 Y292X</td>
<td>8 mo</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle replaced by irregular epithelial structures with small epithelial cysts.</td>
<td>(+) Suprabasal layers of infundibula and 1 small epithelial cyst in the upper dermis</td>
<td>CK10 (+) Suprabasal layers of irregular epithelial structures and small epithelial cysts</td>
</tr>
<tr>
<td>3/F/4 Y292X</td>
<td>4 y</td>
<td>Large infundibula devoid of hair shafts. Lower two thirds of hair follicle missing. A large epithelial cyst in upper dermis with granular layer in upper half and pale-staining epithelium devoid of granular layer in lower half.</td>
<td>(+) Innermost layers of upper half of large epithelial cyst</td>
<td>CK10 (+) Suprabasal layers of large epithelial cyst. More in the lower half</td>
</tr>
<tr>
<td>4/F/10 Y292X</td>
<td>10 y</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle missing. Two large epidermal cysts in mid- and upper dermis. Several irregular epithelial structures in mid dermis.</td>
<td>(+) Suprabasal layers of infundibula and large epithelial cysts</td>
<td>CK10 (+) Suprabasal layers of irregular epithelial structures and large epithelial cyst</td>
</tr>
<tr>
<td>5/F/5 Y292X</td>
<td>5 y</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle replaced by irregular epithelial structures and small epithelial cysts.</td>
<td>(+) Suprabasal layers of infundibula</td>
<td>CK10 (+) Suprabasal layers of irregular epithelial structures and small epithelial cysts</td>
</tr>
<tr>
<td>6/F/5 Y292X</td>
<td>5 y</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle replaced by irregular epithelial structures and small epithelial cysts.</td>
<td>(+) Suprabasal layers of infundibula</td>
<td>CK10 (+) Suprabasal layers of irregular epithelial structures and small epithelial cysts</td>
</tr>
</tbody>
</table>

Abbreviations: Plus sign, positive findings; minus sign, negative findings.

*Results pertaining to hair follicle-related structures only.
in the suprabasal layers of the epidermis and in the infundibular portions of the normal hair follicles. Cytokerin 17 was present in the suprabasal cells of the outer root sheath (ORS), usually below the infundibulum. Cytokerin 19 was present in a few basal cell layer segments and some suprabasal cells of the ORS below the infundibulum. We found that CD34 was expressed more conspicuously in the basal cell layer but also in the suprabasal cell layers of the ORS below the infundibulum.

### Table 2. Histologic and Immunohistochemical Findings in 4 Cases of Atrichia With Papular Lesions and/or Alopecia Universalis Congenita

<table>
<thead>
<tr>
<th>Patient/ Sex/Age, y</th>
<th>Mutation</th>
<th>Age at Biopsy, y</th>
<th>Histologic Findings*</th>
<th>Immunohistochemical Findings*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/18</td>
<td>D1012N13</td>
<td>15 (Biopsy 1)</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle missing. In 1 hair follicle, lower two thirds replaced by irregular epithelial structure with small epithelial cysts; 3 midsized cysts with pale-staining epithelium in mid-dermis and upper dermis; 2 of the cysts ruptured and surrounded by a granulomatous reaction.</td>
<td>(+) Suprabasal cells of infundibula. Mid-dermal epithelial cysts not stained. Irregular epithelial structures and ruptured cysts not present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(–) Epithelial cysts present</td>
</tr>
<tr>
<td>18 (Biopsy 2)</td>
<td></td>
<td></td>
<td></td>
<td>(+) Suprabasal layers of infundibula</td>
</tr>
<tr>
<td>2/M/10</td>
<td>3434delC</td>
<td>9 (Biopsy 1)</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle missing. In 3 follicles, lower two thirds replaced by irregular epithelial structures; in 2 more follicles by small pale-staining epithelial cysts; 2 more medium and large epithelial cysts with pale-staining epithelium in mid-dermis.</td>
<td>(+) Suprabasal layers of infundibula. Epithelial cysts not present</td>
</tr>
<tr>
<td>10 (Biopsy 2)</td>
<td></td>
<td></td>
<td></td>
<td>(+) Suprabasal layers of infundibula and innermost layers of epithelial cyst except for epithelial tail</td>
</tr>
<tr>
<td>3/M/4</td>
<td>189-199del and 0478X13</td>
<td>4</td>
<td>Infundibula devoid of hair shafts. Lower two thirds of hair follicle missing. Irregular epithelial structures in mid-dermis, and small epithelial cysts with pale-staining cytoblast.</td>
<td>(+) Suprabasal layers of infundibula</td>
</tr>
<tr>
<td>4/M/14</td>
<td>3434delC</td>
<td>14</td>
<td>Infundibula devoid of hair shafts; 3 irregular pale-staining epithelial structures and small epithelial cysts replacing lower two thirds of hair follicles.</td>
<td>(+) Suprabasal layers of infundibula</td>
</tr>
</tbody>
</table>

Abbreviations: Plus sign, positive findings; minus sign, negative findings.

*Results pertaining to hair follicle–related structures only.

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RESULTS

Our VDDR results are summarized in Table 1 and our APL/AUC findings appear in Table 2.

CLINICAL AND MOLECULAR GENETIC FEATURES

A representative clinical photograph of a patient with APL is seen in Figure 1. A patient with VDDR IIA is shown in Figure 2.

According to the patients’ parents, most of our patients with VDDR IIA were born with normal hair distribution. All patients lost their hair at age 1 to 3 months. At the time of physical examination (ranging from age 8 months to 15 years), all 6 patients with VDDR had universal alopecia except for remaining eyebrows and eyelashes in 4 patients. Three patients (patients 1, 3, and 4; Table 1) had small milia that were either generalized or restricted to skin areas such as the upper body or the scalp and face. A genetic workup demonstrated the same nonsense mutation (Y292X) in the VDR gene in all 6 patients, who belong to 3 related families.31

All 4 patients with APL/AUC were born with normal hair distribution but lost their hair between ages 40 days and 4 months. At the time of physical examination (ranging from age 4 to 18 years), all patients had total absence of hair except for occasional eyelashes, and partial eyebrow hair loss in 1 patient. Generalized milia were present in 2 patients (patients 2 and 4; Table 2), aged 10 and 14 years, respectively. These patients were siblings previously shown to carry a single-base deletion (3434delC) in the hairless gene.7 The clinical presentation in these 2 patients was compatible with APL. Another patient (patient 1, Table 2), aged 18 years, did not demonstrate any milia or papular rash except for acne lesions on the face and chest since puberty. His clinical presentation was compatible with AUC with an underlying homozgyous nonsense mutation (D1012N) in the hairless gene.19 The fourth patient (patient 3, Table 2), aged 4 years, displayed minute papules on both cheeks. These “borderline” findings precluded his definitive assignment to an APL or AUC phenotype. This patient was shown to carry 2 heterozygous mutations (Q478X and 189-199del) in the hairless gene.13

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

The histologic findings in the VDDR and APL/AUC cases were essentially similar (Tables 1 and 2). In all cases, the normal hair follicles were missing. The only remaining normal portions were the infundibular parts, which had normal-looking sebaceous glands but were devoid of hair shafts (Figure 3 and Figure 4). The lower two thirds of the hair follicles below the level of the sebaceous glands were missing, often replaced by vertically oriented irregular epithelial structures with small cystic dilatations (Figure 3) or by small to medium-sized epithelial cysts (Figure 5 and Figure 6). Irregular epithelial structures and small epithelial cysts were also found farther down the dermis (Figures 3 and 4). Medium-sized cysts and occasionally large cysts were the predominant finding in the middle and upper dermis of some of the other cases (Figures 5, 6, 7, and 8). The middle dermal cysts had a laminated corneal layer in their innermost parts without an underlying granular cell layer (Figure 5). In 1 of the cases, a large epithelial cyst had a granular cell layer in its upper half and paler-appearing epithelial cells devoid of a granular cell layer in its lower half (Figures 7 and 8). The immunohistochemical stainings were performed with 4 monoclonal antibodies, each used on a single section of each specimen. Infundibula were usually present in these sections, but some of the other structures present in the hematoxylin-eosin analysis were occasionally absent in the single serial immunohistochemistry sections. This has been noted in the footnotes in Tables 1 and 2.

The immunohistochemical staining patterns in patients with VDDR and APL/AUC were also essentially similar (Figures 9, 10, and 11). Cytokeratin 10 was expressed in the suprabasal layers of the residual infundibula of all cases (Figure 9). All of the large epithelial cysts in the upper dermis stained positively for CK10. In 1 of the large cysts, only the upper half of the cyst, which had a granular cell layer, stained positively for CK10 (Figure 7). This entire cyst stained positively for CK17, and the basal layer of the lower half, which did not have a granular cell layer, stained positively for CD34 and CK19 (Figure 8). In another case, the entire large cyst in the upper dermis stained positively for CK10 except for a lower “epithelial tail,” which stained positively for CK19. Most of the small and midsize epithelial cysts stained negatively for CK10. Also, CK10 was not detected in the irregular epithelial structures except in 1 case in which it was expressed only in their uppermost vertical portions.

Cytokeratin 17 was always expressed in the suprabasal cells of the vertically oriented and deep-seated irregular epithelial structures and the small epithelial cysts (Figure 11). Positive staining for CK17 was also detected in midsize and large epithelial cysts. The residual infundibula were not stained except occasionally in their lowermost parts (Figure 11).

The staining for CK19 was usually positive in the basal cell layer and occasionally in some suprabasal cells of the vertically oriented and deep-seated irregular epithelial structures (Figure 10). In about half of the cases in which irregular epithelial structures were present, CD34 was expressed in the basal cells of these structures. Staining was also detected in the basal cell layer of a medium-sized cyst and in the lower part of a large epithelial cyst in the middle dermis (Figure 8).

COMMENT

Most histologic studies of patients with APL regard the presence of keratinous cysts in the dermis as the dominant feature.1,8,11,12,14,15,22 These dermal cysts may reach the size of milia and occasionally demonstrate granular-layer cells in their innermost layers.1,12 Misciali et al25 described additional tubular epithelial structures devoid of hair shafts extending from the epidermis to the deep dermis and characterized by the presence of clear cells resembling the lower portion of the ORS. Ill-defined epithelial aggregates in the lower dermis have been reported

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in a patient with APL. Serial sectioning of the entire specimen from this patient was included in the present study (patient 3; Table 2) and revealed additional small epithelial cysts with pale-staining cytoplasm. Complete serial sectioning of all of our VDDR IIA and APL/AUC cases in which keratinous cysts were the dominant feature often demonstrated small irregular epithelial structures in the middle dermis as well.

In a recent VDDR IIA case study, researchers found an absence of normal hair follicles along with the presence of...
follicular remnants and multiple middle dermal cysts lined with ORS-like epithelium in the dermis. 29 A few abnormal follicles represented by the remaining parts of their upper portions and a large keratinizing cyst were observed in another VDDR IIA case. 30 These findings were similar to those of APL. 29,30 In the present study, we found a striking histologic similarity between the alopecias in VDDR IIA and APL/AUC cases. The main histologic findings included empty infundibula; absence of the lower two thirds of the hair follicles, often replaced by vertically oriented irregular epithelial structures or epithelial cysts; irregular epithelial structures often with small cysts in the middle and lower dermis; and small, medium-sized, and large keratinizing epithelial cysts at all levels of the dermis.

Histologic resemblance was also noted between hairless mice and VDR knockout mice, especially in relation to the formation of dermal cysts. 32 Recent experiments have demonstrated that the VDR is most likely involved directly in hair-growth signaling and that forceful induction of catagen by hair plucking results in failure of new anagen hair formation in VDR-deficient mice. 33

It has been suggested, on the basis of experimental findings in mice with hairless gene mutations, that the entire ORS except the infundibulum and bulge disintegrates toward the end of the first hair cycle. 17,34 The remaining putative bulge cells proliferate to produce long, downward-oriented epithelial outgrowths and dermal cysts. Additional epithelial cysts are formed by the epi-
The results of the present study seem to extend this proposed scenario in hairless mice to humans with alopecias associated with VDDR IIA and hairless gene mutations. The upper dermal large keratinous cysts in our study often contained a granular cell layer and stained positively for CK10, which is expressed by the normal infundibulum. Therefore, these cysts may have derived from the remaining infundibula, which are equivalent to the utricles in the mouse. The missing lower two thirds of the normal hair follicle was often replaced by vertically oriented irregular epithelial structures with occasional small cystic dilatations or by small to medium-sized epithelial cysts usually without a granular cell layer. These structures stained positively for CK17, which is expressed in the normal ORS, and mostly negatively for CK10. The vertically oriented irregular epithelial structures (or downgrowths) also stained positively for CK19, predominantly in their basal cell layer. CD34 was expressed in the basal cell layer of a few middle dermal cysts and in some of the vertically oriented irregular epithelial structures. Both CK19 and CD34 are normally expressed in follicular germinative cells (or stem cells) and in more differentiated daughter cells. CD34 is also expressed in the suprabasal cells of the normal ORS, and its presence in these cells denotes trichilemmal keratinization. The restriction of CD34 expression to the basal cell layer in the vertically oriented epithelial structures and middle dermal epithelial cysts may indicate a lack of trichilemmal keratinization in these structures. These staining patterns are compatible with an ORS derivation, and since CK19 and CD34 are not expressed only by bulge cells, the suggested bulge-cell derivation of these vertically oriented epithelial structures cannot be confirmed in the present study. Similar staining patterns in the lower dermal cysts and the deep irregular epithelial structures suggest an ORS derivation as well.

The present study and previous reports indicate that although patients with AUC and some with VDDR IIA lack papules or milialike lesions clinically, epithelial cysts...
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REFERENCES


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ARCHIVES Web Quiz Winner

Congratulations to the winner of our December quiz, Firouzeh Niakosari, MD, McMaster University, Hamilton, Ontario. The correct answer to our December challenge was annular atrophic lichen planus. For a complete discussion of this case, see the “Off-Center Fold” section in the January ARCHIVES (Popkin DL, Greene RE, Fung JF. Widespread annular eruption in a black man. Arch Dermatol. 2005;141:93-98).

Be sure to visit the Archives of Dermatology Web site (http://www.archdermatol.com) to try your hand at the Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month’s print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of The Art of JAMA II.