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A case of erythrokeratoderma variabilis without mutations in connexin 31

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Summary Erythrokeratoderma (EK) variabilis is a heterogeneous group of diseases characterized by migratory erythematous patches and hyperkeratotic plaques. Mutations in connexin 31 have recently been found to underlie several cases of EK variabilis. We describe a Japanese girl with extensive lesions that appeared to be a form of EK variabilis, clinically resembling genodermatose en cocardes (Degos). Our patient had characteristic migratory rosette or target-like erythematous keratotic plaques with peripheral scaling in addition to relatively fixed keratotic plaques. Sequencing of the connexin 31 gene did not detect mutations. Skin biopsy showed parakeratotic hyperkeratosis with hypergranulosis. Immunohistochemically, suprabasal keratins, involucrin and profilaggrin were unequivocally expressed, while loricrin expression was greatly diminished and deiminated K1 was undetectable. Our results confirm aetiological heterogeneity in EK. The histological features suggest disruption of keratinocyte terminal differentiation at a very late stage.

Key words: connexin, erythrokeratoderma variabilis, immunohistochemistry, keratin, loricrin

Erythrokeratoderma (EK) represents a heterogeneous group of disorders characterized by asymptomatic, widespread, well-demarcated erythematous and hyperkeratotic lesions with initial onset in infancy or early childhood (McKusick 133200).[1–3] Two major subtypes have been recognized: EK variabilis (Mendes da Costa)[4] and EK progressiva symmetrica (Gottron). Both are inherited as an autosomal dominant trait. Skin lesions of classic EK variabilis consist of two types, migratory erythematous areas and fixed hyperkeratotic plaques, while lesions of EK progressiva symmetrica are relatively fixed erythematous keratotic plaques. In addition to these 'classic' and relatively well-documented types of EK, several rare variants have been reported. Among them is EK en cocardes or genodermatose en cocardes (Degos), another dominantly inherited disorder.[5] This has usually been regarded as a rare variant of EK variabilis, although changeable erythema without hyperkeratosis is not seen.[2,6] The skin lesions are characterized by migratory rosette or target-like plaques with peripheral scales.

Until now, two types of gene abnormalities have been detected in EK: an insertion mutation in the loricrin gene detected in a family with a form of EK progressiva symmetrica,[7] and mutations in connexin 31 found in several pedigrees with EK variabilis.[8,9] We report a further patient with EK who had both transient and fixed erythematous keratotic plaques. We could not detect a connexin 31 gene mutation or mutant loricrin expression, confirming further genetic heterogeneity in EK. Histological examination suggested disruption of keratinocyte differentiation at a very late stage.

Case report

An 8-month-old Japanese girl presented with generalized scaly erythematous lesions. No skin lesions were noted at birth, but the eruption had been present since she was 3 months old. Lesions were initially detected on her heels, and gradually extended to other body sites.

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She was otherwise healthy and the skin condition seemed to be asymptomatic. A detailed family history revealed no similar cutaneous condition in the relatives. There was no history of consanguinity.

Examination revealed multiple, sharply demarcated, slightly raised, scaly, erythematous papules and plaques on her scalp, face, trunk, buttocks and four extremities (Fig. 1a–c). The plaques on the scalp, hands, knees and

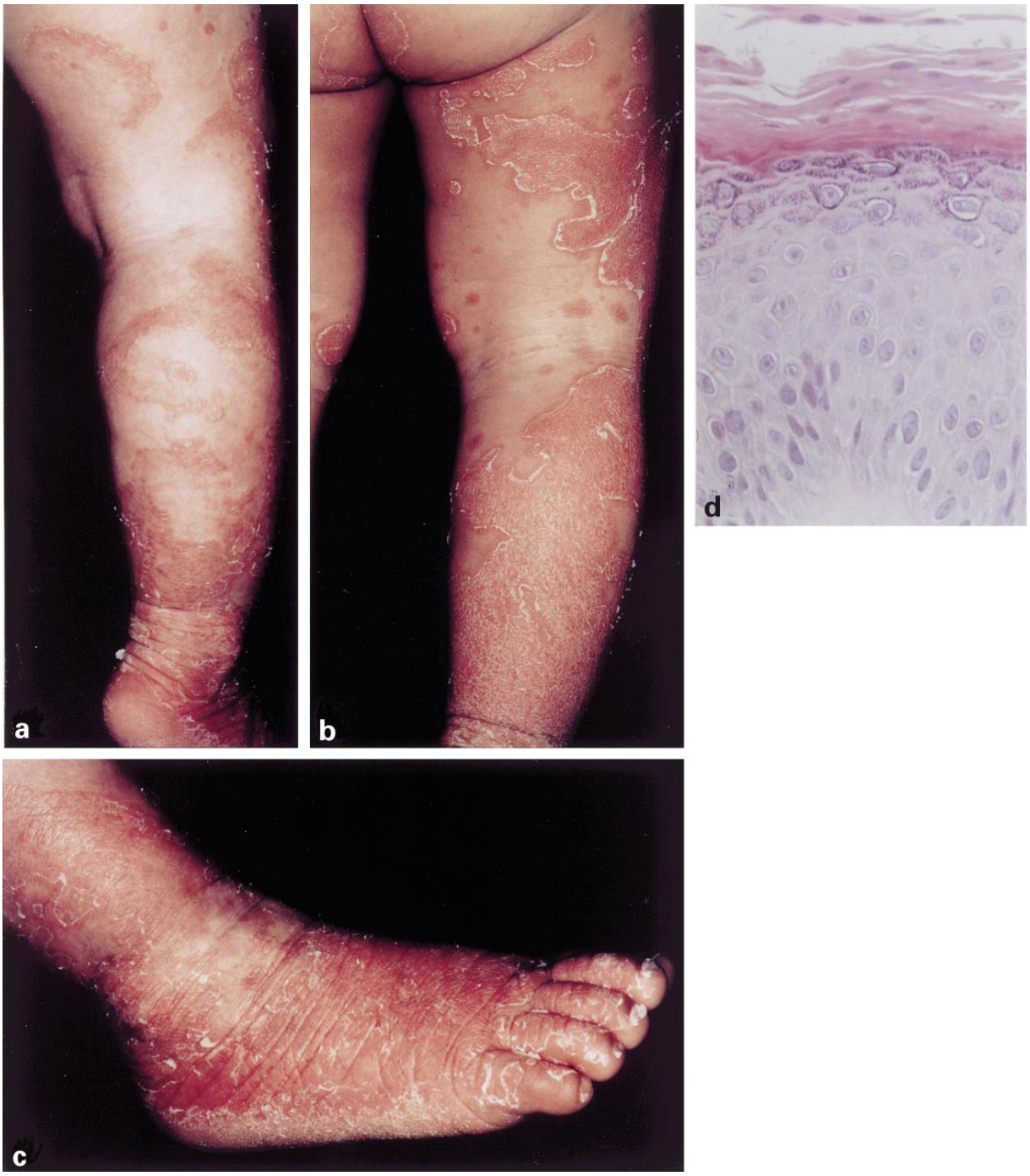

Figure 1. (a) Figurate, targetoid, arciform and geographically bordered erythematous keratotic plaques with large and small lamellar scales are seen on the right leg. (b) The same area is shown 3 weeks later. Note that shapes of the skin lesions have greatly changed over the course of time. (c) A diffuse erythematous keratotic plaque is evident over the right foot. (d) Skin biopsy of one of the plaques on the right thigh revealed parakeratotic hyperkeratosis, moderate hypergranulosis and acanthosis (haematoxylin and eosin; original magnification × 100).

feet were relatively fixed with geographical borders. On the other sites, especially the trunk and thighs, migratory annular or arciform plaques with peripheral whitish lamellar scales were observed. These fleeting lesions started as small red spots, spreading outwards gradually, and lasted for several weeks. Her palm and sole skin was persistently rough, scaly and erythematous. Numerous new annular lesions erupted on her trunk and extremities when she experienced febrile illness such as exanthema subitum or common cold. The nails of all fingers and toes showed spoon nail deformity. No follicular papules were detected. Mucous membranes, teeth and hair were normal, and no other physical abnormalities were noted.

No fungal elements were detected in KOH preparations of the scales. Full blood cell count, liver function tests, serum triglycerides and cholesterol values, glucose, creatinine, serum urea nitrogen, calcium and phosphate concentration and urinalysis were within normal limits. Lactate dehydrogenase isoenzyme pattern was normal. Antinuclear antibodies were not detected. Serum levels of vitamin A, IgE, oestradiol, progesterone and thyroid hormones were all normal. Chest X-ray, electrocardiogram, electroencephalogram and skull magnetic resonance imaging gave normal results. No neurological or ophthalmological abnormalities was detected.

To date, at 2 years of age she is still suffering from both stable and transient skin lesions, but has developed no hair abnormalities such as bamboo hairs. Mental and physical development are also normal. Topical therapy with ointment containing 10–20% urea and 0·0002% tacalcitol cream provided little benefit. Oral retinoids have not been used to date in view of her young age, apparent lack of symptoms and the chronic course of the condition.

Biopsy of a plaque on her right thigh showed hyperkeratosis with parakeratosis, mild hypergranulosis and mild acanthosis (Fig. 1d). A minimal lymphocytic infiltrate was noted around the superficial blood vessels. Electron microscopy showed no abnormal keratin aggregation (not shown). Immunohistochemical studies were performed as described previously.[7,10] As the primary antibodies, antibodies against involucrin (BT601, Biomedical Technologies, Stoughton, MA, U.S.A.), filaggrin (BT576, Biomedical Technologies), loricrin (AF62, Berkeley Antibody Company, Richmond, CA, U.S.A.), epidermal suprabasal keratins (34βB4, Enzo Diagnostics, New York, NY, U.S.A.), deiminated K1 peptide[11,12] and mutant loricrin[13] were used.

In the patient epidermis, involucrin and filaggrin-positive cell layers were thicker than normal, proportional to the thickened spinous and granular cell layers. Loricrin expression, however, was greatly diminished (Fig. 2), while it was normally expressed in the acrosyringial cells. Expression of 34βB4 was positive in the differentiated keratinocytes, as in normal skin (Fig. 3). Deiminated K1, which was conspicuous in the stratum corneum of normal skin, was not detected in patient skin. Mutant loricrin, which was uniquely expressed in a family with EK progressiva symmetrica,[13] was not detected in our patient (not shown).

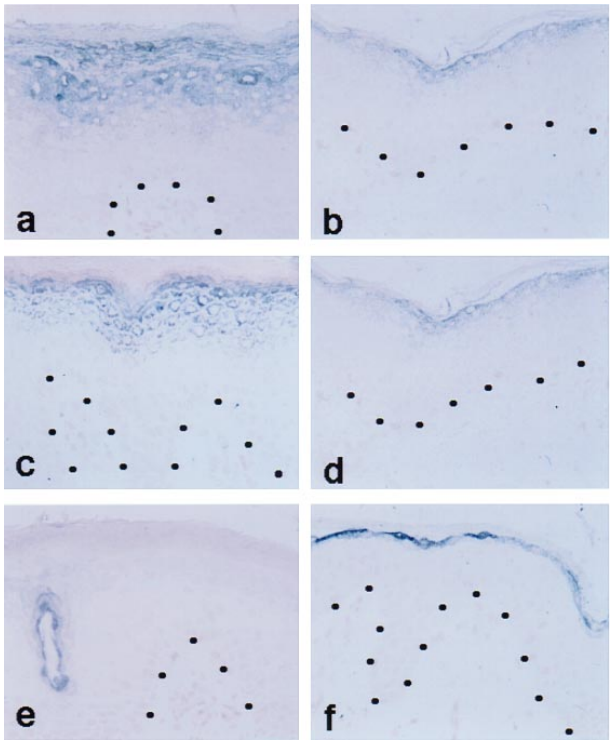

Figure 2. Immunohistochemical staining for involucrin (a,b) (pro)-filaggrin (c,d) and loricrin (e,f) in the patient with erythrokeratoderma (a,c,e) and a normal human subject (b,d,f). Involucrin and filaggrin are expressed in the patient skin as well as in normal skin, but loricrin expression is greatly diminished in the patient epidermis except in the cells around the intraepidermal sweat duct (left in e). Epidermal–dermal junctions are marked with black dots (original magnification × 100).

DNA was obtained from the blood of the patient and her parents. Genomic DNA encompassing the coding sequences of connexin 31 and connexin 26 was amplified by polymerase chain reaction, as described previously.[8,9,14] No sequence variants were detected in either of the genes (not shown).

Discussion

We consider our patient to have a form of EK. The erythematous plaques with peripheral scaling were reminiscent of Netherton syndrome (ichthyosis linearis

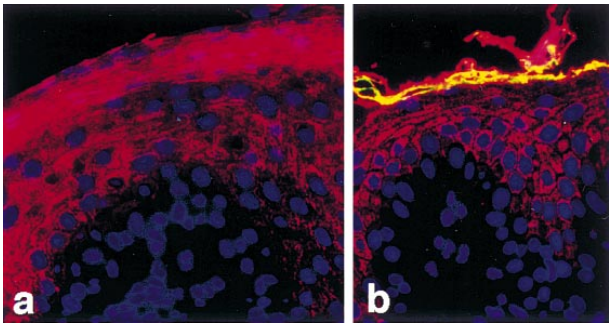

Figure 3. Immunofluorescence staining of 34βB4 (R-phycoerythrin, red) and deiminated K1 peptide (fluorescein isothiocyanate, green) in the patient with erythrokeratoderma (a) and a normal human subject (b). Double-positive cells are seen in yellow. 34βB4 is positive in the suprabasal cell layers both in the patient and in normal skin. Deiminated K1 peptide-positive cell layers, which are double-positive to 34βB4, are seen in the stratum corneum of normal skin, but not in the patient skin. Nuclei are stained with 4,6-diamino-2-phenylindole (blue) (original magnification × 160).

circumflexa), but no hair abnormalities, double-edged scales, eosinophilia or increased IgE levels were detected. Pityriasis rubra pilaris was excluded because of the lack of follicular papules. Hereditary lactate dehydrogenase M-subunit deficiency[15] was excluded based on the normal results of isoenzyme pattern. The annular epidermolytic ichthyosis variant of bullous congenital ichthyosiform erythroderma[16] was also excluded based on the absence of tonofilament clumping on electron microscopy. Keratolytic winter erythema (Oudtshoorn skin disease)[17] was excluded because there was no characteristic seasonal variation, peeling of the palms and soles or necrotic Malpighian layer. Continual peeling skin syndrome[18] was excluded because the stratum corneum surrounding the peripheral scales was rather adherent and could not be peeled manually.

Because of the presence of various subtypes,[1,3,19,20] the classification of EK has been a matter of debate. The classical EK variabilis described by Mendes da Costa is characterized by two types of skin lesions: figurate hyperkeratotic plaques and transient erythematous areas.[4,21] The latter are independent from the former, changing shapes and distribution capriciously within minutes, hours or days. The fleeting erythematous lesions observed in our patient were slightly raised scaly patches with annular or arciform configuration and peripheral lamellar scales, distinct from those in classical EK variabilis. These are very similar to those described in EK en cocardes (Degos),[5] although the involvement of palms and soles seems to be less conspicuous in the previous case reports. Because of marked variability of expression even within a family with EK variabilis,[22] EK en cocardes is usually regarded simply as a variant of EK variabilis.[1,2,6,20] In fact, annular erythematous lesions with scaling have been observed in patients with typical features of EK variabilis.[6,20] Distinction between EK variabilis and EK progressiva symmetrica may also be ambiguous, as was highlighted by the case of two siblings, one with EK variabilis and the other with EK progressiva symmetrica,[23] and by a large family with EK variabilis, including patients with various expressions: some members had keratoderma alone, others had erythema alone, and still others had both types of skin lesions.[24] In view of the variability and the overlap of clinical manifestations of EK, nosology solely based on clinical appearance may not be sufficient. More appropriate classification of this disease spectrum will be established only when the underlying molecular defects are thoroughly identified.

Previous immunohistochemical[25,26] and electron microscopic studies on EK[23,27–29] are difficult to assess, because molecular analyses of the disease were not performed. Heterozygous mis-sense mutations in the connexin 31 gene have recently been detected in five families with EK variabilis.[8,9] An insertion mutation in the loricrin gene has been found in one family with EK progressiva symmetrica with palmoplantar keratoderma.[7] We analysed the connexin 31 gene as well as the connexin 26 gene, which is responsible for palmoplantar keratoderma with deafness,[14,30,31] but no mutations were detected. Mutant loricrin, which is uniquely expressed in loricrin keratoderma,[13] was not detected either. The results of the present histological and immunohistochemical analyses suggest a disruption of keratinization at a very late stage. Keratinocyte differentiation is accompanied by sequential changes of cell morphology, expression and modification of various keratinization-associated proteins.[10,11,32,33] Current evidence indicates that expression of the suprabasal keratins involucrin and profilaggrin precedes expression of loricrin, deimination of K1 and disintegration of nuclei. Because our patient's epidermis displayed unequivocally the set of earlier differentiation markers, but not of late markers, we speculate that the molecular defect of the disease could be some unknown factor(s) that affect or regulate terminal differentiation of keratinocytes.

Mutational analysis of Cx30.3 in this family revealed the unaffected father was a compound heterozygote for E204A/C169W, the mother was wildtype and the affected child was heterozygous for the E204A allele inherited from the father. At present, these appear to be non-disease associated polymorphisms in the Cx30.3

gene suggesting that a mutation in another gene other than Cx31 and Cx30.3 can also underlie EKV.

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