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CLINICAL REPORT

Erythrokeratoderma Variabilis without Connexin 31 or Connexin 30.3 Gene Mutation: Immunohistological, Ultrastructural and Genetic Studies

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Erythrokeratoderma variabilis, characterized by migrating erythema and fixed keratotic plaques, is a rare congenital disorder which has recently been connected with connexin (Cx)30.3 or Cx31 gene mutations. We present a 9-month-old Japanese girl who exhibited the typical clinical features of the disease, but carried no Cx30.3 or Cx31 gene mutations. Histopathologically, regular acanthosis with hyperkeratosis and hypergranulosis was observed in her lesional skin. Upregulation of involucrin and loricrin expression, and a weak expression of Cx26 was immunohistochemically observed in the upper spinous and granular layers. Electron microscopy revealed no abnormality in the keratin filaments, cornified cell envelope or gap junctions. Direct sequencing revealed no pathogenetic mutations in the Cx26, Cx30.3, Cx31 or Cx31.1 genes in this patient. The results indicate that erythrokeratoderma variabilis is pathologically heterogeneous, and that abnormalities in keratinization other than Cx30.3 and 31 gene mutations may underlie some forms of this disease. ***Key words: connexin 26; erythrokeratoderma; gap junction; loricrin.***

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Erythrokeratoderma (EK) is a disorder characterized by erythema and hyperkeratotic circumscribed lesions. It can be classified into several subtypes depending on characteristic clinical features. The group of EK disorders includes EK variabilis, EK symmetrica progressiva, loricrin keratoderma, EK en cocardes, Netherton syndrome, KID syndrome, keratolytic winter erythema, and other rare variants (1–3). The most well-understood major subtypes are EK variabilis and EK symmetrica progressiva. EK variabilis is characterized by erythema migrating for several hours or days, and relatively persistent keratotic plaques. EK symmetrica progressiva is characterized by fixed symmetrical scaly plaques with a predisposition to certain body sites, including the shoulder, cheeks and buttocks. There are many transitional and/or overlapping forms, and therefore the distinctions between each case are not always clear. Recent reports of familial EK linkage analyses have disclosed several loci linked to certain EK subtypes. Connexin (Cx)30.3 and Cx31 gene mutations were reported in familial cases of EK variabilis (4–6). In other EK subtypes, loricrin gene mutations in loricrin keratoderma (7), serine protease inhibitor gene mutation in Netherton syndrome (8), Cx26 gene mutation in KID syndrome (9), and an unknown gene linked to 8p22-p23 in keratolytic winter erythema (10) have been reported. EK is now considered to be pathogenetically heterogeneous and a new classification based on molecular studies has been proposed (2).

Here, we present a Japanese patient with EK variabilis who underwent immunohistological, ultrastructural and genetic investigations. The entire coding regions of Cx26, Cx30.3, Cx31 and Cx31.1 genes were investigated, and no mutations were found.

CASE REPORT

Clinical course

A 9-month-old Japanese girl visited our clinic for treatment of migrating eruptions over her entire body. She presented with an erythematous lesion on her face at 4 months which gradually covered almost her entire body over a period of 2 months. Her general condition was good. There was no familial history of any skin disorder nor consanguinity in the family. On examination, slightly raised, sharply marginated erythematous keratotic plaques were scattered, some confluent, over almost her entire body (Fig. 1). There were no pustular signs. A biopsy specimen from the left lower leg revealed regular acanthosis and hyperkeratosis with hypergranulosis (Fig. 2). Parakeratosis was partially observed. There was slight inflammatory cell infiltration in both the epidermis and the dermis. The diagnosis of EK variabilis was made from the clinical and histopathological findings. Tacarcitol (vitamin D3 analogue) ointment was chosen as a treatment.

Over the next 4 years her skin lesions improved slightly, although occasional deteriorations were observed, mainly in the autumn and winter. Physical examination at the age of 4 revealed annular, scaly erythematous

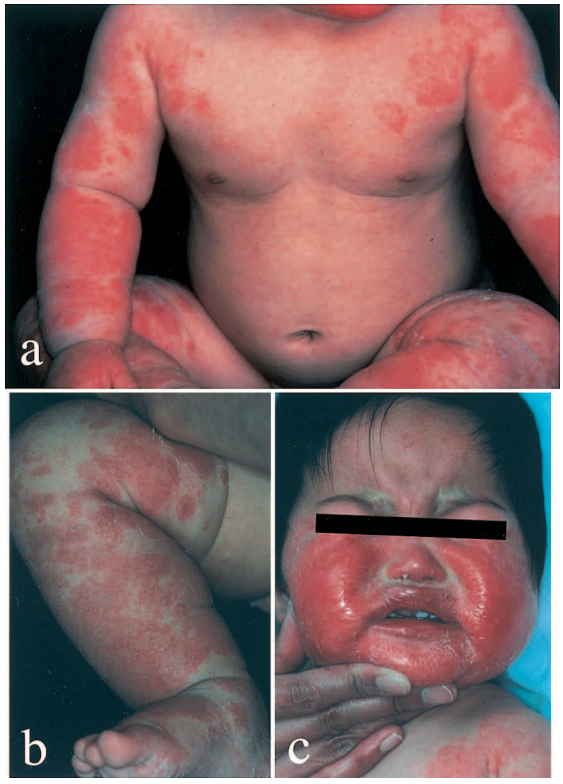

Fig. 1. Clinical features at 9 months of age. Slightly raised, figurative erythema with peripheral scales were scattered and partly fused over almost the entire body, including the arms, trunk (a), legs (b) and face (c).

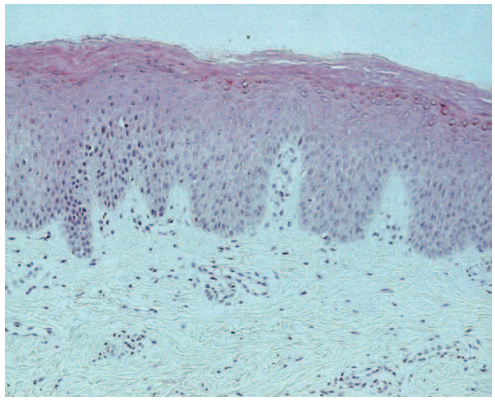

Fig. 2. Histopathology of a skin biopsy taken from a migrating erythema on the leg. Regular acanthosis and hyperkeratosis with hypergranulosis was observed. Partial parakeratosis was also seen. An inflammatory cell infiltrate was largely absent (haematoxylin-eosin, ×200 magnification).

plaques with central healing on the upper trunk, extremities and buttocks (Fig. 3a, b). These eruptions appeared and faded away during intervals of about 2 weeks. Psoriasiform plaques were also seen on the right shoulder and knees (Fig. 3c). Palms and soles were spared from involvement. She complained of occasional slight itching.

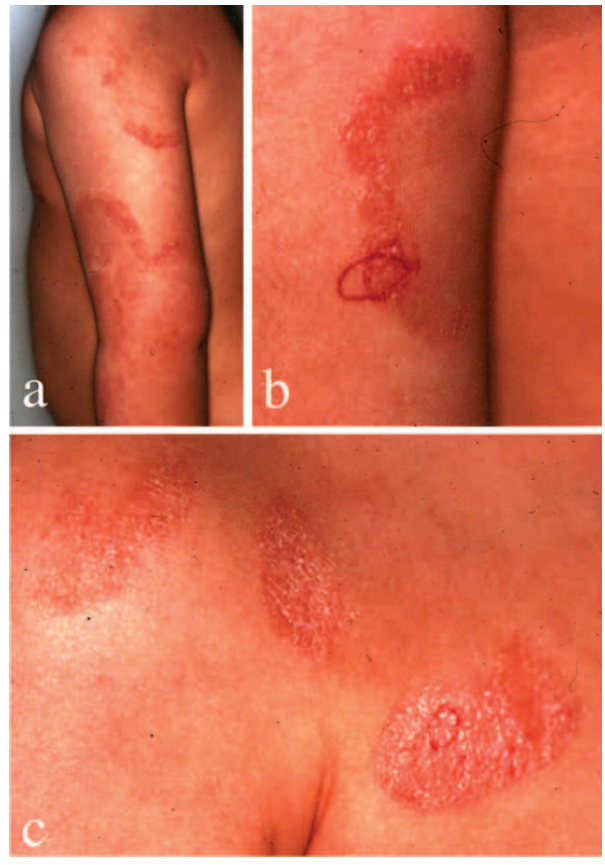

Fig. 3. Clinical features at 4 years of age. Eruptions were mainly confined to the arms, knees and buttocks. Annular, scaly erythematous plaques with central healing were observed on the arms (a, b), along with psoriasiform plaques on the right shoulder (c). The red ink marks in figures (b) and (c) represent the biopsy sites for ultrastructural and immunofluorescence studies.

Electron microscopy

Skin biopsy specimens were taken from the right arm and shoulder at the age of 4 as indicated in Fig. 3. Ultrastructurally, there were no abnormalities of desmosomes, tonofilaments or the cornified cell envelope. Gap junction structures also appeared normal and in typical numbers (Fig. 4).

Immunohistochemistry

Involucrin and loricrin were strongly expressed by immunofluorescence microscopy (Fig. 5), especially in the upper spinous and granular layers. Keratin 1 and keratin 10 (data not shown) expression was normal. Anti-citrulline peptide antibody staining was performed to investigate the activity of peptidylarginine deiminases (11, 12) and showed a normal staining pattern in the cornified cell layer. Cx expression in the patient's epidermis was studied using available antibodies directed to certain Cxs, including Cx26 and Cx43. Weak expression of Cx26 was observed in the upper spinous and granular layers, whereas Cx26 is not expressed in normal

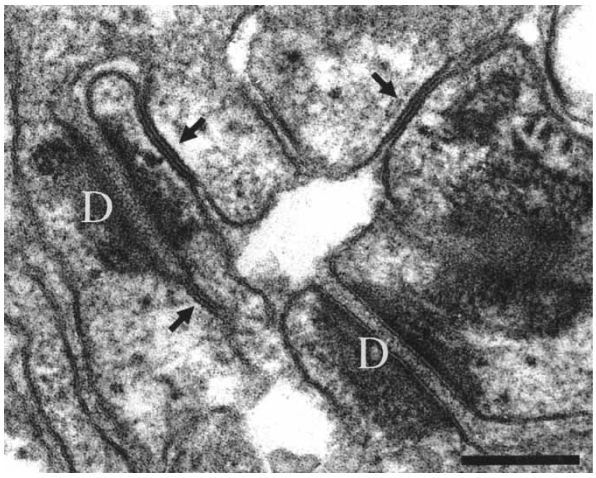

Fig. 4. High power electron microscopic view of a skin biopsy taken from the migrating erythema. Normal numbers of gap junction structures were observed in the periphery of the upper spinous cells. Arrows = gap junctions; D = desmosomes; bar = 200 nm.

human epidermis. Cx43 was expressed in the spinous and granular layers, similar to control skin.

Mutation analysis of Cx genes

Genomic DNA was extracted from peripheral blood of the patient. Using genomic DNA as a template, the entire coding sequences of GJB2 (Cx26 gene), GJB3 (Cx31 gene), GJB4 (Cx30.3 gene) and GJB5 (Cx31.1gene) were amplified by polymerase chain reaction (PCR), as described previously (4–6, 13). DNA sequencing of all the PCR products was carried out with a Genetic Analysis 310A automatic sequencer (Perkin Elmer-ABI, Foster City, CA). No nucleotide changes, including mutations and polymorphisms, were detected in any of the genes.

DISCUSSION

We concluded that our case showed the features of EK variabilis as a result of the presence of a migrating scaly erythema and fixed psoriasiform plaques. Psoriasis was excluded histologically by the absence of infiltrating neutrophils and by the presence of hypergranulosis. In addition, loricrin was normally expressed in the granular layers, in contrast to the absence of loricrin expression in psoriasis (14). EK progressiva symmetrica was excluded by the presence of migrating erythema, which is not usually observed in EK progressiva. Loricrin keratoderma is excluded by the absence of palmoplantar keratoderma with pseudoainhum, and because of normal loricrin expression. Netherton syndrome was also excluded by the absence of double-edged scales, which are characteristic of ichthyosis linearis circumflexa, and normal hair appearance. KID syndrome was excluded because the eyes and the auditory function were not involved. Keratolytic winter erythema was excluded by the clinical absence of

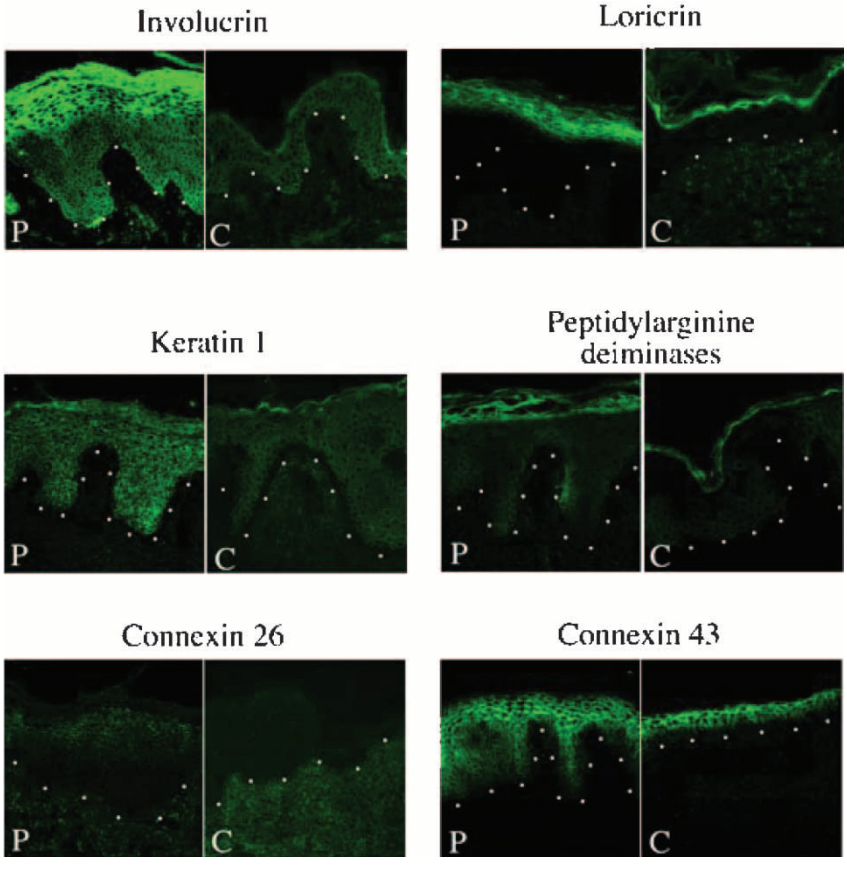

Fig. 5. Immunofluorescence studies of keratinization markers and connexins in a lesional skin biopsy taken as indicated in Fig. 3, compared to normal skin. Involucrin and loricrin expression were upregulated in the upper stratum spinosum and the stratum granulosum. Keratin 1 was normally expressed in all layers except the stratum basale. Peptidylarginine deiminase expression was detected by staining using an anti-citrulline peptide, which is a deiminated protein produced by the enzyme, and it is normally observed in the cornified cells. Cx26 expression was faintly observed within the upper stratum spinosum and the stratum granulosum, in contrast to negative expression in the normal epidermis. Cx43 expression was seen in the spinous and granular layers in a normal pattern similar to control epidermis. P = patient's lesional skin; C = control skin (normal); dotted line = basement membrane zone.

palmar-plantar peeling and the histological absence of necrobiosis of the Malpighian layer.

Another differential diagnosis is EK en cocardes, which exhibits a characteristic annular polycentric scaly erythema that comes and goes over an interval of 3–4 weeks. In our case, annular scaly erythema was observed from 4-year-old. However, this typical target-like erythema is not exclusive in EK en cocardes, but has also been reported in an EK variabilis patient (15). Additionally, in a large EK variabilis pedigree, several affected patients exhibited polycyclic, scaly erythema (3). Because of this clinical overlap between EK variabilis and EK en cocardes, EK en cocardes is often regarded as a clinical variant of EK variabilis (1, 15). Conversely, Findlay & Morrison (16) noted the clinical resemblance between keratolytic winter erythema and EK en cocardes; it is thought that these two disorders may even be identical (2, 16). However, EK variabilis and keratolytic winter erythema are genetically different molecular entities, because EK variabilis is related to connexin gene mutation, whereas keratolytic winter erythema is linked to 8p22-p23, where no connexin genes reside. The diagnostic criterion of EK en cocardes is still controversial and a full elucidation of the genetic defect(s) in EK en cocardes is needed to clarify this problem.

Electron microscopic findings revealed no obvious abnormality in the keratin filament, cornified cell envelope or gap junctions. In a previous report of an EK variabilis case with Cx31 mutation (5), no obvious morphological changes in gap junction structures were reported.

Immunohistochemical results were consistent with the histological findings of acanthosis and hypergranulosis. Increased expression of involucrin and loricrin was thought to reflect the hypergranulosis. Upregulated Cx26 expression was observed in the stratum granulosum and the upper part of stratum spinosum in this patient. Cx expression in normal human skin has been studied in detail (17). Cx43 is expressed in the stratum spinosum and granulosum, and to a lesser extent in the stratum basale. Cx26 is usually not expressed in normal human epidermis (17), as shown in the control skin in Fig. 5. However, upregulated Cx26 expression in the upper spinous and the granular layers has been reported in hyperproliferative conditions, i.e. psoriasis (18), verruca vulgaris (19) and EK variabilis (20). The positive Cx26 staining in the patient's epidermis may therefore be due to hyperproliferation. Normal peptidylarginine deiminase activity detected by an anti-citrulline peptide antibody suggested normal degradation of keratohyaline granules in the patient's epidermis.

Recent studies have revealed several causative mutations in Cx30.3 and Cx31 genes in more typical forms of EK variabilis (4–6). However, in our case, no causative mutations could be found in these genes. Additionally, we investigated other Cx genes in the same Cx β family, Cx26 and Cx31.1, but again, no mutations were found. After mutations in Cx30.3 and Cx31 genes were reported as the causes of EK variabilis, only one EK variabilis case without any Cx30.3 or Cx31 gene mutation has been reported (21). In that case, decreased loricrin expression and peptidylarginine deiminase activity were shown in the upper spinous and granular layers.

In our case, no Cx30.3 or Cx31 gene mutations were found throughout their entire coding sequences. In addition, normal loricrin expression and peptidylarginine deiminase activity were observed in the epidermis. These results suggest that EK variabilis without Cx30.3 or Cx31 gene mutations is more pathogenetically heterogeneous, and that there are still unknown pathogenetic mechanisms underlying this disease other than Cx30.3 or Cx31 gene mutations, defects associated with abnormal loricrin distribution and altered peptidylarginine deiminase expression.

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