Acta Derm Venereol 2010; 90: 274–278

CLINICAL REPORT

Familial Erythrokeratodermia Variabilis with Pustular Lesions: A New Variant?

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We report here a Chinese family with erythrokeratodermia variabilis which had 30 affected members. The patients had characteristic clinical features of stationary and migratory lesions. Some of the patients had adult onset of the disease. Five out of 30 patients noted episodes of pustule-like lesions during their disease course. Histological examination of the proband showed granular cell vacuolation and upper-epidermal neutrophil aggregates. Mitochondria vacuolation was noted in keratinocytes by electron microscopic examination. No GJB3 and GJB4 pathogenic mutation was detected. These unusual presentations suggested a new phenotypic and genetic correlation in this Chinese pedigree of erythrokeratodermia variabilis. *Key words: erythrokeratodermia variabilis; pustule; Chinese.*

(Accepted October 29, 2009.)

Acta Derm Venereol 2010; 90: 274–278.

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Erythrokeratodermia variabilis (EKV) is a rare genodermatosis characterized by variable migratory erythematous and relatively stable keratotic lesions (1). EKV is heterogeneous both clinically and genetically. In some cases, phenotypic and genetic correlation was established in familial or sporadic cases (2). We report here a Chinese pedigree with 30 affected individuals. Clinical features in most of the patients were summarized. Detailed clinical, histological and electron-microscopic (EM) features were described in the proband and in one of her cousins. No pathogenic mutations were identified.

MATERIALS AND METHODS

Peripheral blood samples were obtained from family members of IV1–4 and V1, 2, 7 (Fig. 1), and DNA was extracted. We checked GJB3 and GJB4 mutations by polymerase chain reaction (PCR) and sequencing. Three pairs of primers were designed to amplify all the exons of GJB-3 and GJB-4 according to the Genbank sequence:

GJB3 exon 1F 5'CAACCCGCCCTCATTCCT3',
1R 5'TCCCAGTTTCCCTCCATCCT3'
GJB3 exon 2F 5'GCTGTGCCTCAGTTTCCTA3',
2R 5'ACCTCTGTGCTGCTGTTGT3'
GJB4 F 5'AATCCCTACCCTACCAAGTC3', R 5'TGAGGTCCACCTAATCCC3'

The PCR reaction conditions were: initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C denaturation for 30 s, annealing at 62°C, 56°C and 56°C, respectively, for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. After PCR amplification, the samples were electrophoresed on 2% agarose gel, yielding size products of 888 bp, 1409 bp and 1332 bp, respectively. The PCR products were eluted and purified using QIAquick Gel Extraction Kit (Qiagen, Hilden Germany) according to the manufacturer's instructions. Sequencing was performed using an ABI 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Due to the close clinical resemblance to ichthyosis bullosa of Siemens, we additionally checked Keratin 2 (KRT2) mutations. Three pairs of primers were designed according to the Genbank sequence, to the effect that all the exons of the KRT 2 were covered for amplification and sequencing:

KRT 1F 5'TTGGCAGTCGGAGTCTTG3', 1R 5'CAGGCTGTTATGGGAAATG3'
KRT 2F 5'GCAAGTCTTAGCACAGTTGTT3',
2R 5'GTTCTCAGTTGTCAGGAGGG3'
KRT 3F 5'TGCGGGTGGAAGTGGTAAA3',
3R 5'GGTCACGCTGGAACCAAAA3'

The PCR reaction conditions were: initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C denaturation for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. After PCR amplification, the samples were electrophoresed on 2% agarose gel, yielding product sizes of 599 bp, 491 bp and 616 bp, respectively. The PCR products were eluted and purified using QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. Bi-directional sequencing was performed using an ABI 3730 DNA Sequencer (Applied Biosystems).

This study was approved by the ethics committee of China Medical University. Informed consents were obtained from patients who underwent a skin biopsy or DNA analysis.

CLINICAL REPORTS AND RESULTS

Case 1 (proband)

A 20-year-old woman (V2 in Fig. 1) presented to the dermatology clinic with severe itchy erythematous patches on her body. Her condition started a few months after birth and was most prominent at the age of 5 years, when most of her body surface was slightly red and scaly for a period of time (as recalled by her parents). The rashes usually changed in configuration over a matter of hours to days, and tended to be aggravated during the summer. She had also noticed small pustules arising at the periphery of the red patches a couple of

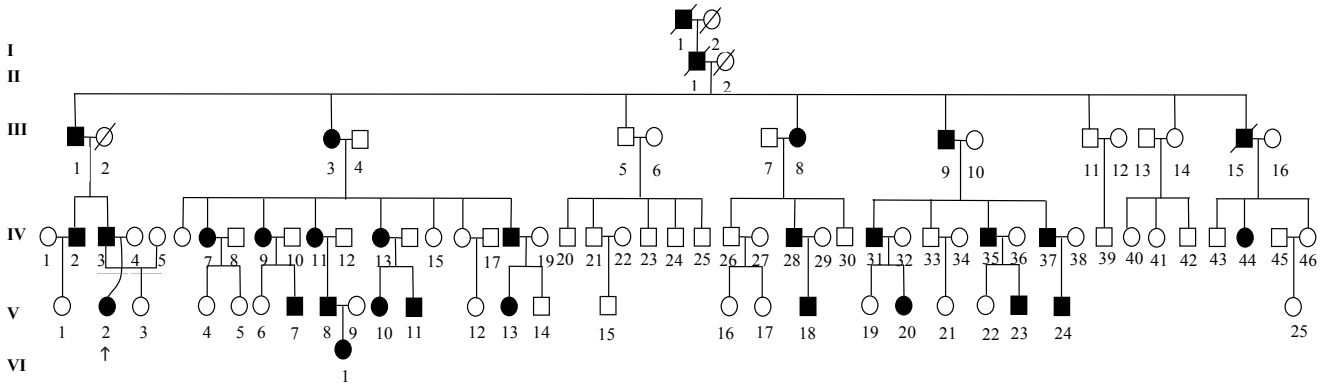

Fig. 1. Pedigree of the proband (V2) and case 2 (V7), indicated by arrows. Filled symbols indicate affected individuals.

times over the years. The pustules usually disappeared after a couple of days. Her past history was unremarkable, except for near-sightedness and astigmatism. She was the first-born child of non-consanguineous parents, and had been born at full-term, following a normal pregnancy and delivery. She had received little treatment, except for a short period of topical steroids a year earlier.

On examination, well-defined, irregular or round scaling patches were noted on her trunk, extremities, buttocks and waists (Fig. 2a). Some of the patches had urticaria-like margins with pigmented central areas covered with scales. Several vesicles/pustule-like lesions approximately 2–5 mm in diameter were noted at the periphery of the patches on her legs (Fig. 2b). Homogeneously keratotic plaques with fine whitish scales were present on the extensor sides of her extremities and her lumbo-sacral area. Her right sole had a hyperkeratotic red patch and a thickened toenail plate (microscopic examination revealed the presence of hyphae in the scrapings, while that from lesions on the legs and back was negative). She also had slight keratosis pilaris on the extensor sides of her extremities. Laboratory tests, including complete blood count, liver and kidney function tests and blood lipids, were normal. A biopsy was taken from the periphery of a red patch (close to a pustule) on her leg. Histological examination revealed basket-woven orthohyperkeratosis, vacuolated granular cells with sparse keratohyalin granules, slight acanthosis, and sparse dyskeratotic keratinocytes in the upper epidermis. There were focal infiltrating neutrophils at the granular layer, mild superficial perivascular infiltrate of mononuclear cells (Fig. 3a). A periodic acid-Schiff stain showed no sign of microbes. EM showed widened in-

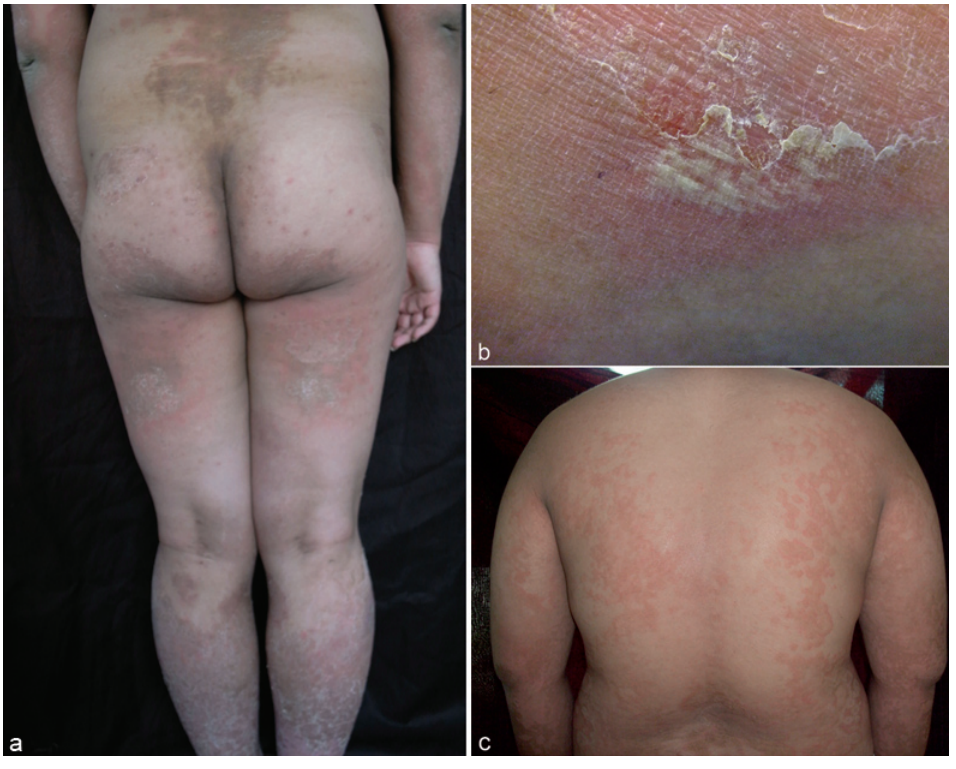

Fig. 2. (a) Red patches and plaques with white scales on the proband's back, buttocks and limbs. Patches on her thigh changed in configuration in days. (b) Pustule-like lesions on the periphery of the scaly red patch on her lower legs (proband). (c) Round plaque with urticaria-like margins in V7.

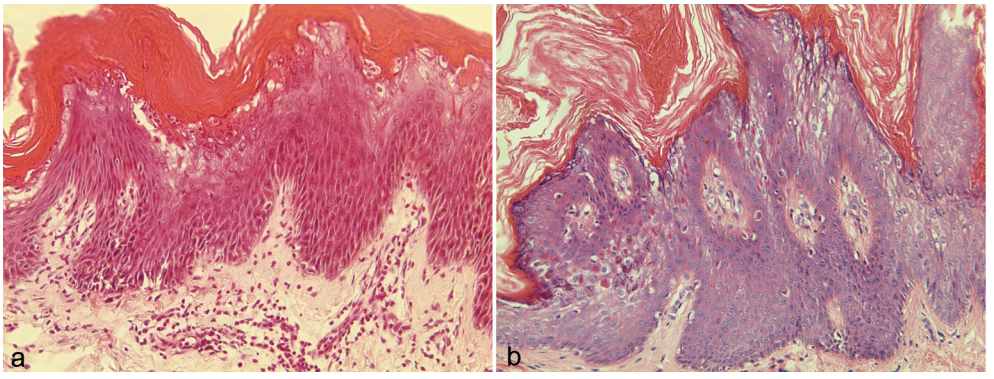

Fig. 3. The proband's skin lesional histology. (a) There was orthohyperkeratosis, slight acanthosis of Malpighian cells and vacuolated granular keratinocytes. There were few dyskeratotic keratinocytes and some neutrophil aggregates in the upper epidermis. (b) More prominent dyskeratotic keratinocytes in the epidermis in V7. (Haematoxylin and eosin (H&E) × 200).

tercellular spaces; prominent mitochondria vacuolation involving nearly the whole epidermal layers (Fig. 4).

Case 2 (V7)

An 18-year-old boy (V7 in Fig. 1) noticed obvious itchy red patches with a "dirty surface that was not easy to scrub off". He also noticed some red patches that changed in size or disappeared after a couple of days. The lesions had first appeared at approximately 16 years of age. The lesions tended to be severe in the summer season or when eating spicy foods. Physical examination revealed well-demarcated red patches on his extremities. Some of the patches were rough and slightly pigmented, with central adherent scales. Circular urticaria-like plaques were noted on his trunk (Fig. 2c). The sides of his neck and axilla region had dark velvet patches that were compatible with acanthosis nigricans. He was overweight, but his general health was otherwise good. Routine laboratory tests were normal, except for an increased level of alamin aminotransferase (109 U/l, normal range: 4–43 U/l). A biopsy was taken from a migratory lesion on his back. Histological examination revealed basket-woven or compact orthohyperkeratosis, vacuolated granular cells with sparse keratohyalin granules, slight acanthosis, prominent papillomatosis and perivascular infiltrates (Fig. 3b). No obvious neutrophilic infiltration was observed in the epidermis. The EM examination also showed widened intercellular spaces and apparent mitochondria vacuolation, similar to that in the proband.

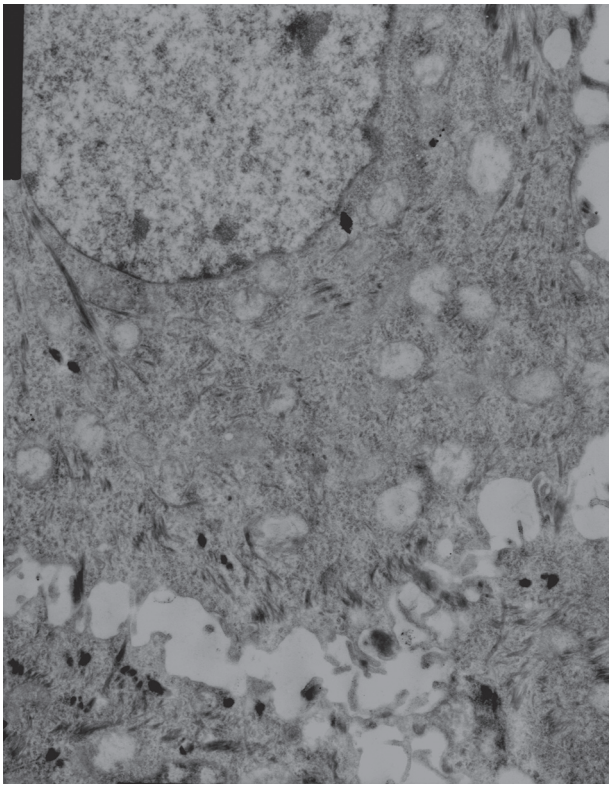

Fig. 4. Electromicrographic manifestation showing mitochondria vacuolation in keratinocytes, involving almost all the layers of epidermal keratinocytes. There were also widened intercellular spaces (× 4000).

The family pedigree is of Han nationality and has 92 members in six consecutive generations (Fig. 1). There are 30 affected family members, including 18 males and 12 females. We were able personally to examine 56, including 19 affected members, mostly from family III_{1-8}. Clinical investigation was carried out by two dermatologists during the months of June to August 2008 (Drs Zhang and Huo). The main features of the examined patients are shown in Table I. In addition, there were some minor findings. Three of the examined patients had dry skin and slight ichthyosis vulgaris. Four of the patients had signs of acanthosis nigricans (all of the four patients were overweight). Five of the patients had hyperhidrosis of the palms and soles. Seven of the patients had near-sightedness. None of the patients had hearing problems.

No pathogenic mutations in GJB3, GJB4 or KRT2 were found in the patients.

DISCUSSION

This family pedigree corresponds to autosomal dominant mode of inheritance as previously described for EKV by Laudau et al. (1). The diagnosis of EKV depends mainly on clinical-pathological manifestations, although the histological changes are not specific.

Table I. *Main and special features in the erythrokeratodermia variabilis pedigree*

Family tree number	Sex	Age at diagnosis, years	Age at onset, years	Symptoms	Aggravating factors	Affected sites	Pustules
III1	M	80	6	Itching	Sun, heat	Extremities, trunk, buttocks, palm and soles	Yes
III3	F	77	3	Burning	Humid, heat	Extremities, palm and soles	Yes
III8	F	73	20	Severe itching	Heat	Extremities	No
III9	M	68	6	Itching	Heat, spicy food	Extremities, palm and soles	Yes
IV2	M	42	4	Itching	None	Extremities, abdomen	No
IV3	M	41	10	Itching	Sun, heat	Extremities, palms and soles	No
IV9	F	48	46	Itching	Sun, heat, spicy food, menopause	Lower legs	No
IV13	F	44	1	Itching	Heat	Extremities, trunk, buttocks, palms and soles	No
IV18	M	39	7	Itching	Humid, heat	Extremities, trunk, palms and soles	Yes
IV28	M	45	14	Itching	Heat	Extremities	No
IV31	M	44	3	Itching	Heat	Extremities, palms and soles	No
IV35	M	39	27	Itching/burning	Spicy food	Lower legs	No
IV37	M	34	8	Itching	Sun, spicy food	Extremities, palms and soles	No
V2[a]	F	17	0.5	Severe itching	Sun, heat, humid	Extremities, trunk, buttocks, palms and soles	Yes
V7	M	18	16	Itching	Sun, heat, humid, spicy food	Extremities, trunk, buttocks, palms and soles	No
V10	F	20	11	Itching	Sun, heat	Extremities	No
V11	M	6	birth	Itching	None	Extremities, trunk, buttocks, palm and soles	No
V13	F	14	5	Severe itching	Spicy food	Extremities, trunk, buttocks, palm and soles	No
V18	M	22	20	Itching	Heat	Extremities	No

[a]Proband.

Clinical differential diagnosis of EKV from other erythrokeratoderma was well described by Landau et al. (1) Diseases with similar clinical manifestations include progressive symmetric erythrokeratoderma, Netherton syndrome, keratitis-ichthyosis-deafness (KID) syndrome, erythrokeratolysis hiemalis (Oudtshoorn disease), etc.

In this Chinese pedigree, the patients had both stationary and migratory patchy lesions, which are typical of EKV. EKV usually starts at an early age, although rare cases of adult onset have been reported (3). Four out of 19 EKV from this pedigree reported onset of the disease in adulthood.

We cannot rule out the possibilities of poor memory or negligence, or the difficulty in recognition of the mild rashes on a type III or IV skin in Chinese people. Itching is the most common symptom in the affected members. Similar to other previous reports, summertime or heat exacerbates the symptoms in most of the patients. Some of the patients noticed that eating spicy food made their lesions worse or feel more itchy. Spicy Chinese food usually contains chilli, which causes sweating or a sensation of the body heating up. The unique finding was that five of the 19 investigated patients (including the proband) noticed episodes of short-term pustule-like lesions. Histologically, the affected skin in the proband had some vacuolated keratinocytes in the supra-epidermis, some dyskeratotic keratinocytes and mild infiltration of neutrophils in the granular layers. EM examination of the specimen from the proband showed widened intercellular spaces and pronounced mitochondria vacuolization. All these histological changes might give rise to the clinical form of blister/pustules.

The general histological profile of EKV is manifested by hyperkeratosis, slight acanthosis and papillomatosis, and some perivascular infiltration of lymphocytes and histiocytes. Vacuolation of granular cells could be observed in some cases. In addition to the general features of EKV, both patients had some patchy vacuolation in the upper epidermis and sparse dyskeratotic cells. The presence of dyskeratotic keratinocytes was also observed in a previous report (4). A unique finding in the proband was the presence of focal infiltrating neutrophils in the granular layer. The ultra-structural changes of EKV vary from one report to the other (5). Two studies showed decreased number of keratinosomes in granular layers (4, 6), whereas another study noticed a normal number of keratinosomes (7). In a Japanese patient with EKV, an increased number of gap junctions was observed (8). In the present two cases, we observed pronounced mitochondria vacuolization and widened intercellular spaces. Interestingly, perinuclear vacuolization and mitochondria vacuolization is observed in erythrokeratoderma progressive symmetrica (PSEK) (9, 10). These overlapping features between EKV and PSEK further question the categorization of PSEK (11).

There are two probable subtypes of EKV that have been summarized (5), one is the erythrokeratoderma with erythema gyratum repens-like lesions (1), the other is erythrokeratoderma *en cocardes*, which has target-like lesions (12). In this Chinese EKV pedigree, we recorded unusual pustule-like lesions in five of the 19 examined members, with compatible histological and EM findings.

EKV is aetiologically heterogeneous. Most EKV patients have pathogenic mutations in one of two neighbouring connexin genes, *GJB3* and *GJB4*, encoding the

gap junction proteins Cx31 and Cx30.3, respectively (13). In some cases, phenotypic and genetic correlations have been established, e.g. two mutations in Cx30.3 (T85P, F137L) were associated with the occurrence of rapidly changing erythematous patches with prominent, circinate, or gyrate borders in affected children. Highly variable intrafamilial phenotypes were also noted, suggesting a strong influence of modifying genetic and epigenetic factors (2). Other studies also demonstrated that not all EKV cases harbour mutations in Cx31 and Cx30.3 (14, 15). Indeed, we could not find any hotspot mutations in members of this Chinese family. Because of some vacuolated keratinocytes and clinical features reminiscent of epidermolytic ichthyosis, we also examined KRT2 with negative results.

Further investigation is required to elucidate the unique phenotypic-(epi-)genetic relationship in this family.

ACKNOWLEDGEMENTS

This work was supported by programme for Changjiang Scholars and Innovative Teams in Universities, Ministry of Education, China (IRT0760). The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; or in the preparation, review, or approval of the manuscript.

Dr Gao had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs L. Zhang and W. Huo contributed equally to this work, and should be regarded as co-first authors.

The authors declare no conflict of interest.

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