Novel Mutation in *GJB4* Gene (Connexin 30.3) in a Family with Erythrokeratodermia Variabilis

Emilie Sbidian[1], Norredine Bousseloua[1], Laurence Jonard[2], Stéphanie Leclerc-Mercier[3], Christine Bodemer[1] and Smail Hadj-Rabia[1]*

*[1]AP-HP Centre de Référence des Maladies Génétiques à Expression Cutanée (MAGEC) et Service de Dermatologie, Hôpital Necker-Enfants Malades, Université Paris V Descartes, 149 rue de Sèvres, FR-75015 Paris, [2]AP-HP, Centre de Référence des Surdités Génétiques, Hôpital Armand-Trousseau, [3]AP-HP Service d'Anatomie Pathologique, Hôpital Necker-Enfants Malades, Université Paris V Descartes, Paris, France. *E-mail: smail.hadj@inserm.fr*

Accepted June 6, 2012.

Erythrokeratodermia variabilis (EKV, MIM: 133200) is a rare autosomal dominant disorder characterized by the association of: (*i*) localized or generalized hyperkeratosis, and (*ii*) transient erythematous areas (1). Hyperkeratotic areas show well-demarcated, geographically outlined, and fixed plaques, in a strikingly symmetrical distribution. The hallmark of EKV is the continual occurrence of transient, sharply outlined, figured red patches of variable intensity that fade within a few hours or days. A very closely related phenotype, characterized by fixed and growing erythematous hyperkeratosis symmetrical plaques, known as progressive symmetrical erythrokeratoderma of Gottron (PSEK, MIM: 133200), was initially seen as a distinct entity (2). The *GJB3* gene, encoding connexin 31, is the major disease-causing gene of EKV (3). Mutations in a second gene, *GJB4*, encoding connexin 30.3, have been reported in both EKV and PSEK (4, 5), illustrating the genetic heterogeneity of these disorders. Thus, van Steensel (6) suggested that PSEK and EKV may be manifestations of the same genetic defect, and proposed the designation "erythrokeratodermia variabilis and progressiva" (EKV-P, MIM: 133200) to indicate the protean nature of the disorder.

We describe here a large Algerian family, in which 9 affected patients presented with EKV-P phenotypes associated with a novel mutation (c.256T>A) in the *GJB4* gene.

CASE REPORTS

The proband, a 48-year-old man, was referred to our department for a 42-year history of skin manifestations. He was the seventh child of non-consanguineous parents originating from Algeria (Patient II-1, Fig. 1a). The lesions started when he was 6 years of age. Symmetrical brown-red, fixed, partly hyperkeratotic macules started on the earlobes, flexural areas of the neck, antecubital, and popliteal fossae, involving the face, the trunk and the extremities (Fig. 1 b, c). Simultaneously, diffuse erythematous patches of body skin, changing in size and shape and regressing over the course of hours or days, were reported. The erythema progressively decreased in intensity, whereas diffusion of hyperkeratotic areas increased. The palms, soles, hair and nails were normal. Audiometric explorations were normal. A clinical diagnosis of EKV was made in view of the characteristic skin lesions, i.e. both fixed symmetrical hyperkeratosis and transient erythematous areas. Skin biopsies showed a massive compact orthokeratotic hyperkeratosis with focal acanthosis and papillomatosis. The granular layer was normal. Keratotic plugging was seen in all the orifices of adnexal structures. The dermis and hypodermis were normal. This characteristic aspect is closer to the histological

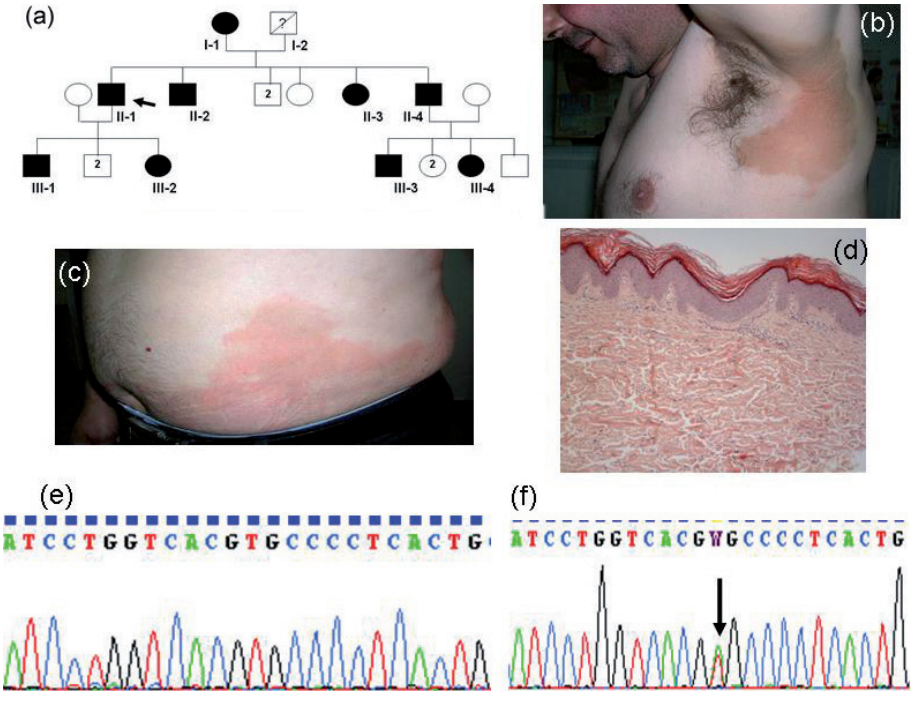

Fig. 1. Clinical, histological and molecular characteristics of the EKV proband (a) Pedigree of the family confirming autosomal dominant inheritance (*black arrow*: proband, black squares; affected males, *black circles*: affected female). The proband presented with both symmetrical brown-red fixed partly hyperkeratotic macules (b) and diffuse erythematous patches of body skin (c). Histopathology (d) shows massive compact orthokeratotic hyperkeratosis with focal acanthosis and papillomatosis. Keratotic plugging was seen in all the orifices of adnexal structures. The granular layer, dermis and hypodermis were normal. Normal sequence (e) compared to the abnormal sequence (f) of the proband. The *black arrow* shows the c.256T>C mutation.

description of lamellar ichthyosis (Fig. 1d). Acitretin (35 mg daily) was started with good effect.

Seven relatives of the proband had highly similar phenotypes (Fig. 1a and S1; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1436).

After informed consent, DNA was extracted from peripheral blood leukocytes of the patient and his 7 affected relatives and 4 unaffected relatives, *GJB3* and, *GJB4* genes were sequenced. A novel heterozygous transition c.256T>A was found in the *GJB4* gene in 8 affected patients (reference sequence NM_153212.1) (Fig. 1 e, f). This mutation causes substitution of a cysteine by a serine at codon 86 (p.Cys86Ser/C86S) in connexin-30.3 protein. The mutation segregates with the disease and was absent in the 4 unaffected relatives. It is noteworthy that the cysteine at position 86 of connexin 30.3 is extremely conserved among species (from human to zebrafish) and the Grantham score is high (112). Moreover, the mutation is not reported in the Single Nucleotide Polymorphisms (SNP) and polymorphisms databases (Leiden Open Variation Database (http://www/lovd.n/2.0/), dbSNP (http://www.ncbi.nlm.nih.gov/SNP/)), nor in 10700 exomes (Exome variant Server (http://evs.gs.washington.edu/EVS/)).

DISCUSSION

To the best of our knowledge, we describe here for the first time the EKV-P phenotype related to a novel mutation (c.256T>A) in the *GJB4* gene. The absolute conservation of the cysteine at position 86 of connexion 30.3 among species, and the Grantham score, strongly suggested the pathogenicity of this *GJB4* mutation. Moreover, the c.256T>A mutation has been reported previously in the *GJB3* gene in 4 EKV families, whereas no such mutation was detected in unaffected family members or in a European control cohort, thus excluding the possibility that these variations represent common polymorphisms (3). Cysteine 86 is a residue that putatively forms sulphur bridges and is located at the transition of E1 to TM2. Loss of a sulphur bridge can be expected to disrupt local conformation and the interaction with *GJB4*. The mechanism of EKV-P pathogenesis is unknown. However, Tattersall et al. (7) suggested that endoplasmic reticulum (ER) stress is the main mechanism of cell death. These results indicate that, *in vivo*, ER stress may lead to abnormal keratinocyte differentiation and hyperproliferation in EKV patient skin.

The intra-familial phenotype of the family described here is consistent with the phenotype described in the literature. EKV may be genetically heterogeneous (8); however, there is no genotype-phenotype correlation, with the exception of the erythema gyratum repens phenotype. The most common Mendes Da Costa EKV type, initially related to *GJB3* gene mutations, and Cram-Mevorah EKV type, usually related to *GJB4* gene mutations, share common features (9). However, minor clinical differences exist: circinate or gyrate borders patches are reported in Cram-Mevorah type. Macari et al. (4) reported 8 affected and 3 healthy subjects in an Israeli family, of Kurdish origin, with EKV. The patients carried a heterozygous missense mutation (F137L) in the GJB4 gene and presented with mild-to-severe phenotypes consisting of well-demarcated erythematous hyperkeratotic plaques with irregular borders and migratory erythematous lesions described as erythema gyratum repens. The palms and soles were spared. Richard et al. (3, 10, 11) reported 5 unrelated families and a sporadic case in which 6 distinct point mutations (G12D, T85P, F189Y, F137L, R22H and F137L) in the *GJB4* gene were identified. As in our series, highly intra- and inter-familial variable phenotypes were described, suggesting the strong influence of modifying genetic and epigenetic factors. All individuals carrying G12D and R22H mutations presented with typical features of EKV, i.e. extensive, symmetrical, well-demarcated hyperkeratotic plaques on the extremities and trunk, and short-lasting erythematous plaques. The palms and soles remained intact. Patients harbouring T85P mutation had fixed hyperkeratotic plaques with palmoplantar keratoderma. T85P and F137L mutations were associated with the occurrence of erythematous patches with prominent raised, circinate borders and rapidly changing configuration, leading to erythema gyratum repens appearance, as reported by Macari et al. (4). In contrast, the major feature in patients with F189Y mutations was severe hyperkeratosis with accentuated skin markings, ridging in large skin folds and hypertrichosis lanuginose. Palms and soles were not involved. Recently, Scott et al. (12) reported a 13-year-old boy with hyperkeratotic plaques and transient erythema on the face and limbs carrying a novel missense mutation (T130M) in the *GBJ4* gene. Interestingly, connexins 30.3 and 31 physically interact to form heterodimeric connexon (9). This interaction provides a novel explanation for the similarity of P-EKV due to either connexin-31 or -30.3 mutation.

The authors declare no conflicts of interest.

REFERENCES

1. Mendes da Costa S. Erythro- et keratodermia variabilis in a mother and a daughter. Acta Derm Venereol 1925; 6: 255–261.
2. Gray LC, David LS, Guill MA. Progressive symmetric erythrokeratodermia. J Am Acad Dermatol 1996; 34: 858–859.
3. Richard G, Smith LE, Bailey RA, Itin P, Hohl D, Epstein EH, et al. Mutations in the human connexin gene GJB3 cause erythrokeratodermia variabilis. Nat Genet 1998; 20: 366–369.
4. Macari F, Landau M, Cousin P, Mevorah B, Brenner S, Panizzon R, et al. Mutation in the gene for connexin 30.3 in a family with Erythrokeratodermia variabilis. Am J Hum Genet 2000; 67: 1296–1301.
5. van Steensel MAM, Oranje AP, van der Schroeff JG, Wagner A, van Geel M. The missense mutation G12D in connexin30.3 can cause both erythrokeratodermia variabilis of Mendes da Costa and progressice symmetric erythrokeratodermia of Gottron. Am J Med Genet A 2009; 149A: 657–661.
6. van Steensel M. Does progressive symmetric erythrokeratoderma exist? Br J Dermatol 2004; 150: 1043–1045.

7. Tattersall D, Scott CA, Gray C, Zicha D, Kelsell DP. EKV mutant connexin 31 associated cell death is mediated by ER stress. Hum Mol Genet 2009; 18: 4734–4745.
8. Richard G, Brown N, Smith LE, Terrinoni A, Melino G, Mackie RM, et al. The spectrum of mutations in erythrokeratodermias – novel and de novo mutations in GJB3. Hum Genet 2000; 106: 321–329.
9. Plantard L, Hubert M, Macari F, Meda P, Hohl D. Molecular interaction of connexin30.3 and connexin 31 suggests a dominant-negative mechanism associated with erythrokeratodermia variabilis. Hum Mol Genet 2003; 12: 3287–3294.
10. Richard G, Lin JP, Smith L, Whyte YM, Itin P, Wollina U, et al. Linkage studies in erythrokeratodermias: fine mapping, genetic heterogeneity and analysis of candidate genes. J Invest Dermatol 1997; 109: 666–671.
11. Richard G, Brown N, Rouan F, van der Schroeff JG, Bijlsma E, Eichenfield LF, et al. Genetic heterogeneity in erythrokeratodermia variabilis: novel mutations in the connexin gene GJB4 (Cx30.3) and genotype-phenotype correlations. J Invest Dermatol 2003; 120: 601–609.
12. Scott CA O'Toole E, Mohungoo MJ, Messenger A, Kelsell DP. Novel and recurrent connexin 30.3 and connexin 31 mutations associated with erythrokeratoderma variabilis. Clin Exp Dermatol 2011; 36: 88–90.