

The Molecular Pathology of Progressive Symmetric Erythrokeratoderma: A Frameshift Mutation in the Loricrin Gene and Perturbations in the Cornified Cell Envelope

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Summary

The erythrokeratodermas (EKs) are a group of disorders characterized by erythematous plaques associated with variable features that include palmoplantar keratoderma. One type of EK is known as “progressive symmetric erythrokeratoderma” (PSEK). We studied members of a family of Japanese origin in which the index case with PSEK had had well-demarcated nonmigratory erythematous plaques on her extremities since birth. Sequence determination of the loricrin gene revealed an insertion of a C following nucleotide 709. The mutation results in a frameshift that changes the terminal 91 amino acids in the wild-type polypeptide into missense amino acids and adds 65 additional residues. This further implicates loricrin defects in the pathogenesis of disorders with palmoplantar keratoderma and pseudoainhum.

Introduction

A clinically and genetically heterogeneous group of disorders known as the “erythrokeratodermas” (EKs) is characterized by widespread erythematous plaques, either stationary or migratory, associated with features that include palmoplantar keratoderma (Nir and Tanzer 1978; Gray et al. 1996; Stevens et al. 1996; Christiano 1997). The genetic defects underlying the EKs are still largely unknown (Stevens et al. 1996; Christiano 1997). One type of EK, known as “progressive symmetric erythrokeratoderma” (PSEK), which is characterized by erythematous and hyperkeratotic plaques inherited in an autosomal dominant fashion, has been noted by some authors to display incomplete penetrance and variable

expressivity (Hopsu-Havu and Peltonen 1970; Nir and Tanzer 1978; Maldonado-Ruiz et al. 1982; Nazzaro and Blanchet-Bardon 1986; Lamprecht et al. 1988; Kudsi and Naeyaert 1990; MacFarlane et al. 1991; Miura et al. 1991; Kiesewetter et al. 1993; Niemi and Kanerva 1993; Gray et al. 1996). Only ~30 cases have appeared in the literature since the initial description, by Darier in 1911, entitled “Erythrokeratodermie Verruqueuse en Nappes, Symetrique et Progressive” (Darier 1911). Non-migratory erythematous plaques develop shortly after birth and are distributed symmetrically over the body, particularly on the extremities (hands and feet), the buttocks, and sometimes the face, together with palmoplantar keratoderma.

EK variabilis (EKV), a second major clinical subtype of EK, was first described by Mendes da Costa in 1925, as relatively fixed patches of hyperkeratosis with erythematous areas “characterized by capriciously formed outlines, like the boundary lines of seacoasts on maps” (Mendes da Costa 1925). EKV is also inherited in an autosomal dominant pattern, with variable expressivity, and the erythematous areas move from hour to hour. The condition is usually evident at birth or within the 1st year of life; lesions predominantly affect the face, buttocks, and extremities; and palmoplantar keratoderma is generally present. The major factor distinguishing between PSEK and EKV is the sharply outlined geographical regions of migratory erythema in the latter (Mendes da Costa 1925; Brown and Kierland 1966; Hopsu-Havu and Tuohimata 1971).

Are the EKs one disorder? Macfarlane and colleagues posed this question in 1991, when they reported clinical and ultrastructural studies of two sisters, in which the younger sister appeared to have EKV whereas the older sister's presentation was more consistent with PSEK (MacFarlane et al. 1991). They speculated that these cases represented variable expression of a single defect and underscored the difficulty in distinguishing the heterogeneous EK phenotypes on the basis of clinical presentation alone. Recently, a genetic locus for EKV was refined to a locus on chromosome 1p32-34 (van der

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Schroeff et al. 1984, 1988; Richard et al. 1996), in a region of candidate connexin genes involved in epidermal differentiation. In contrast to the linkage of EKV to chromosome 1p, no locus for PSEK had previously been defined by genetic linkage studies.

In this study, we report identification of a frameshift mutation in the loricrin gene on chromosome 1q21 in a family with PSEK. Loricrin is the major structural component of the cornified cell envelope, formed beneath the plasma membrane of stratified squamous epithelial cells during terminal differentiation (Hohl et al. 1991; Yoneda et al. 1992). These data establish that this clinical variant of PSEK is genetically distinct from the forms of EKV linked to chromosome 1p, and they further implicate components of the cornified cell envelope in the pathogenesis of disorders typified by palmoplantar keratoderma and pseudoainhum (Maestrini et al. 1996).

Subjects, Material, and Methods

Clinical Description

The proband (III-1; fig. 1) was a 27-year-old female of Japanese origin who was the only child of nonconsanguineous parents. Her father (II-3), two paternal uncles (II-6 and II-7), and her paternal grandfather (I-3) were similarly affected with EK. She presented with erythematous skin lesions on the trunk, face, and extremities and with palmoplantar keratotic lesions (fig. 2A and C). Some clinical and histological features of this case have been published previously in the Japanese literature (Ishida et al. 1986, 1987). Her lesions developed soon after birth and did not change much thereafter. She had no migratory erythematous lesions, nor did she have any blistering. Her hearing was normal, and audiometry showed a normal pattern.

There were well-demarcated red-colored figurate plaques with white pityriasisform scales on the extensor and flexor surfaces of the extremities, abdomen, and buttocks (fig. 2C). Her face showed diffuse erythema. Her palms and soles were hyperkeratotic and had a waxy appearance (fig. 2A). Her palms had a honeycomb pattern as well. Her hair, teeth, and nails appeared normal, and she had a very small right third toe. Pseudoainhum, constricting bands encircling the interphalangeal joints of the digits of the hands and feet, was noted on the distal interphalangeal joints of both 5th fingers, and mild constrictions were noted on the joints of other fingers (fig. 2A).

II-3, the 57-year-old father of the proband, had similar symptoms (fig. 2B and D). He had well-demarcated widespread erythematous plaques on his extremities. His palms and soles were hyperkeratotic, and the 5th fingers of both hands showed mild constrictions on the distal interphalangeal joints. His hair, teeth, nails, and hearing were all normal.

Clinical Samples

DNA was isolated from peripheral blood leukocytes of the proband and both parents, according to standard techniques (Sambrook et al. 1979). Skin biopsies were obtained from involved areas of the thighs (cases 1 and 2) and the knee (case 1), under local anesthesia and after informed consent had been obtained. Normal human skin samples from the thigh, which were obtained during plastic surgery, were used for comparison.

Mutation Identification and Characterization

The sequence of exon 2 of the loricrin gene was PCR amplified from genomic DNA by use of the ExpandTM Long Template PCR System kit with High Fidelity Taq (Boehringer Mannheim). The loricrin gene is encoded by two exons, with the entire coding sequence contained within the second exon. The primers, designed from the published sequence (GenBank M94077) (Maestrini et al. 1996), were 5'-GCTGAGGCTCTGGCACCTGAAAG-3' (forward) and 5'-GCCGGAGAGCTCAATGGC-TTCT-3' (reverse) and produce a PCR product of 1,235 bp. PCR conditions were 1 min at 95°C, 45 s at 65°C, and 2 min at 72°C, for 35 cycles. PCR products were purified on Centriflex gel-filtration cartridges (Advanced Genetic Technologies) and were directly sequenced by use of the ABI PRISMTM Dye Terminator Cycle Sequencing Kit with AmpliTaq[®] DNA Polymerase FS (Perkin Elmer-ABI). The sequences were run on an ABI 310 Prism Sequencer (Applied Biosystems).

Allele-specific oligonucleotides (ASOs) were prepared for specific hybridization to both the wild-type and mutant sequences: wild-type, ASO 5'-TGCGCGCCC-CAGCCGAG-3'; mutant, ASO 5'-TGCGCGCCCCCA-GCCGA-3'. After end labeling of each oligomer, filters were hybridized in 5 × SSPE, 0.5% SDS at 37°C for 30 min and were washed at the annealing temperature of the ASOs (62°C) (in 2 × SSPE, 0.1% SDS) and subsequently with increasing stringency. Signals were obtained after exposure to x-ray film and autoradiography.

Protein and Secondary-Structure Predictions

Computer-assisted analysis of the mutant polypeptide was performed by use of protein-manipulation software from Genetics Computing Group (1994).

Light and Electron Microscopy

Light-microscopic examination was performed either on semithin sections of Epon-embedded samples processed for electron microscopy and stained with toluidine blue or on material that was fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. For transmission-electron microscopy, the skin samples were processed as described elsewhere (Ishida-Yamamoto and Iizuka 1995). Ultrathin

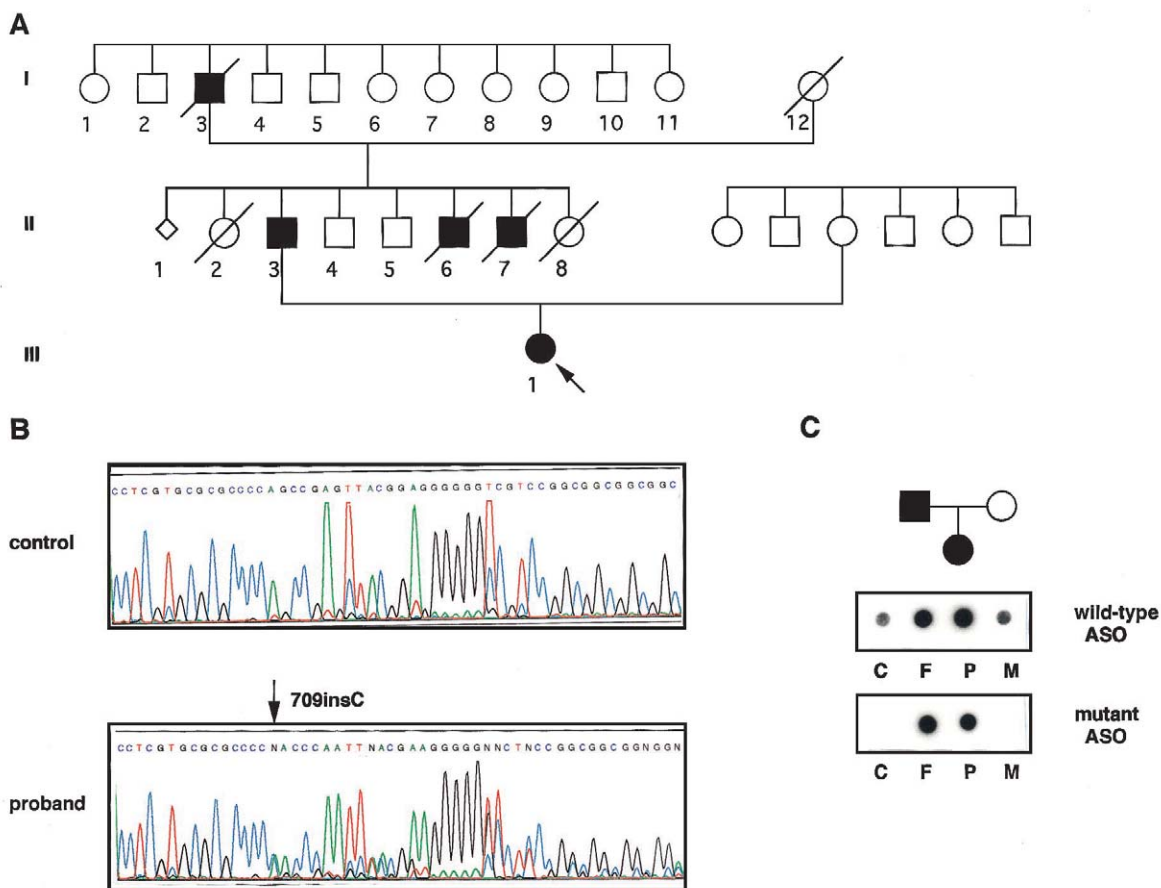


Figure 1 Pedigree and mutation analysis in proband and nuclear family. *A*, Three known generations of Japanese family affected with EK. The proband is represented by the blackened circle (individual III-1) (arrow). Her father (II-3), two paternal uncles (II-6 and II-7), and paternal grandfather (I-3) were similarly affected and are represented by blackened squares. A diagonal line through a symbol indicates that the individual is deceased. *B*, Direct automated sequence analysis of loricrin gene, indicating single-base-pair insertion of a C nucleotide after position 709, indicated, by the arrow in the proband's sequence (lower panel), as 709insC, compared with the control sequence (upper panel). *C*, Mutation confirmed in proband (lanes P) and affected father (lanes F), by ASO hybridization. The wild-type ASO hybridizes with PCR-amplified genomic DNA from the proband, father, mother (lanes M), and an unrelated, unaffected control individual (C) (upper panel), whereas the mutant ASO hybridizes only to the PCR-amplified genomic DNA of the proband and her father (lanes P and lanes F, respectively) (lower panel).

sections were cut and were stained with uranyl acetate and lead citrate.

Immunoelectron Microscopy

Postembedding immunoelectron microscopy was performed as described elsewhere (Ishida-Yamamoto et al. 1996). In brief, the skin samples were frozen without chemical fixation, were subjected to freeze substitution in methanol, and were embedded in Lowicryl K11M (Chemische Werke Lowi). The immunostaining procedures were as described elsewhere (Ishida-Yamamoto et al. 1996). For loricrin staining, a rabbit polyclonal antibody (AF2340) raised against a mouse loricrin N-terminal peptide (Ishida-Yamamoto et al. 1996) was used, and the secondary antibody was 10 or 15 nm of gold-conjugated goat anti-rabbit antibody (Amersham

International). The involucrin antibody (SY5) is a mouse monoclonal antibody (Hudson et al. 1992), and 15 nm of gold-conjugated goat anti-mouse antibody was the secondary antibody (Amersham), whereas 5 nm of gold-conjugated goat anti-rabbit antibody (Amersham) was used for the loricrin antibody AF2340.

Results

Direct automated sequencing of the loricrin gene was performed because the clinical and histological findings (see below) suggested that this gene was a potential candidate. There was a single-base-pair insertion of a C following nucleotide 709 of the loricrin DNA sequence (numbering is according to GenBank M61120) (Hohl et al. 1991) (fig. 1B). The mutation, designated "709insC,"



Figure 2 Characteristic clinical and histological features of PSEK. *A*, Hyperkeratotic and honeycomb-like waxy appearance of palm (case 1). Marked pseudoainhum is noted on the joint of the 5th finger (*arrowheads*), and mild constrictions on the joints of other fingers are also seen. *B*, Diffuse erythema, marked furrows, and small lamellar scales on feet (case 2). Nails appear normal. *C* and *D*, Well-demarcated red-colored figurative plaques with white pityriasiform lamellar scales on buttocks (case 1) and thigh (posterior aspect; case 2). *E* and *F*, Hematoxylin and eosin-stain section from knee lesion (case 1). Note marked hyperkeratosis with parakeratosis, hypergranulosis, and moderate acanthosis. Retained nuclei in the cornified cells are indicated by arrowheads in *F*. Bar = 0.1 mm in *E* and 10 μ m in *F*.

resulted in a frameshift and a delayed termination codon in the loricrin mRNA. The mutation was confirmed in the father and the proband by use of ASO hybridization (fig. 1C). The frameshift caused the replacement of the C-terminal 91 amino acids and extended the chain by an additional 65 residues. The entire wild-type loricrin polypeptide is 315 amino acids in length, so this mutation effectively replaced the carboxy-terminal one-third of loricrin with missense amino acids and removed ap-

proximately one-third of the glutamine and lysine residues involved in isodipeptide cross-link formation.

All biopsy skin samples from involved areas of the thighs and the knee showed similar histological features. Light microscopy showed marked hyperkeratosis with parakeratosis, a thickened granular cell layer, mild acanthosis, and mild lymphocytic infiltration around the superficial blood vessels (fig. 2E and F). The most striking abnormality was the presence of intranuclear granules

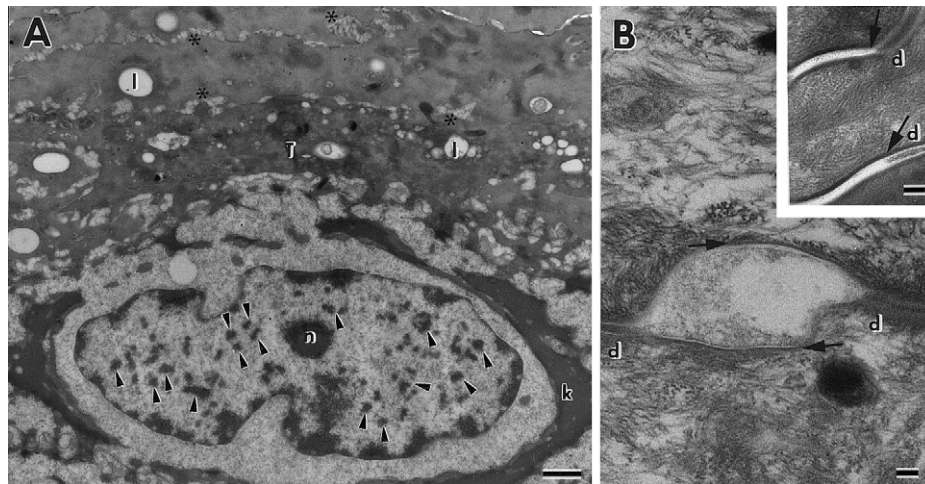


Figure 3 Electron microscopy showing abnormal epidermal differentiation in patient skin. *A*, Superficial granular cell containing many characteristic intranuclear granules (*arrowheads*). Note the presence of a transitional cell layer (T) and increased intercellular substance derived from lamellar granules (*). k = keratohyalin granules; l = lipid droplets; and n = nucleolus. Bar = 1 μ m. *B*, Cornified-cell envelopes (*arrows*) in patient skin, which is thinner than in normal skin (*inset*). d = desmosomes. Bars = 0.1 μ m. Biopsy samples are from the thigh of case 2 (*A* and *B*) and from a healthy individual (*inset* in *B*).

in the upper granular cells (fig. 3A). Although the granular-cell layers were thickened, keratohyalin granules appeared normal (fig. 3A). The transitional cell layer, which is only occasionally observed in normal skin, was a prominent feature between the granular and cornified cells (fig. 3A). Lamellar granules were increased in number in the spinous and granular cells, and the intercellular substance derived from these granules was abundant in both (fig. 3A). Lipid droplets and remnants of cellular organelles were present in the cornified cells (fig. 3A). Thin, electron-dense cell envelopes were seen in the superficial granular cells. However, the increase in thickness of cell envelopes in the horny layer that occurs in normal skin was not observed in the patient's skin (fig. 3B).

To identify the composition of the granules, immunoelectron microscopy was used to show that intranuclear granules in the granular cells were loricrin immunoreactive (fig. 4A). The retained nuclei in the parakeratotic cornified cells also contained loricrin-positive granules (fig. 5A and C). However, cornified cell envelopes were sparsely labeled with the loricrin antibody (fig. 5B and C), which is in complete contrast to the labeling pattern in normal skin, where loricrin antibodies intensely decorate cornified cell envelopes (fig. 5D). The most superficial granular cells or transitional cells of the patient's skin often showed more loricrin labels on the cell envelopes than in the matured cornified cells (fig. 5B). This pattern has not been observed in normal skin and is in keeping with aberrant cornified cell envelope assembly. Involucrin labels on the cornified cell envelopes in the patient's skin were greater than those in normal skin (fig. 5B–D).

Discussion

In this study, we report the molecular basis of PSEK in this family as a delayed termination mutation in the loricrin gene. Furthermore, we have analyzed the impact of this mutation on cornified cell envelope formation at the ultrastructural level, as well as its effect on cross-linking and rigidification of the protein (see below). Although clinically it appeared that this patient had unequivocal diagnostic features of PSEK, we initiated molecular analysis of the loricrin gene on the basis of more subtle clinical and histological similarities with a form of palmoplantar keratoderma known as “Vohwinkel syndrome” (Vohwinkel 1929; Camisa and Rossana 1984; Camisa et al. 1988), in which we elsewhere had identified a different loricrin mutation (Maestrini et al. 1996). These similarities included palmoplantar hyperkeratosis with a honeycomb appearance, pseudoainhum, and ichthyotic lesions on other body sites, although the PSEK cases had significantly more widespread and striking erythematous hyperkeratotic plaques.

The nature of the EK mutation in the loricrin gene is also reminiscent of the VS mutation (Maestrini et al. 1996), in terms of both its location within the gene and its functional consequences. The EK mutation is a 1-bp insertion of a C (709insC) after a stretch of four consecutive C nucleotides, residing only 21 bp upstream of the VS mutation, which is a 1-bp insertion of a G (730insG) after a stretch of six consecutive G nucleotides (Maestrini et al. 1996) (fig. 6). The EK mutation results in a frameshift and delayed termination of transcription, with replacement of the 91 carboxy-terminal amino

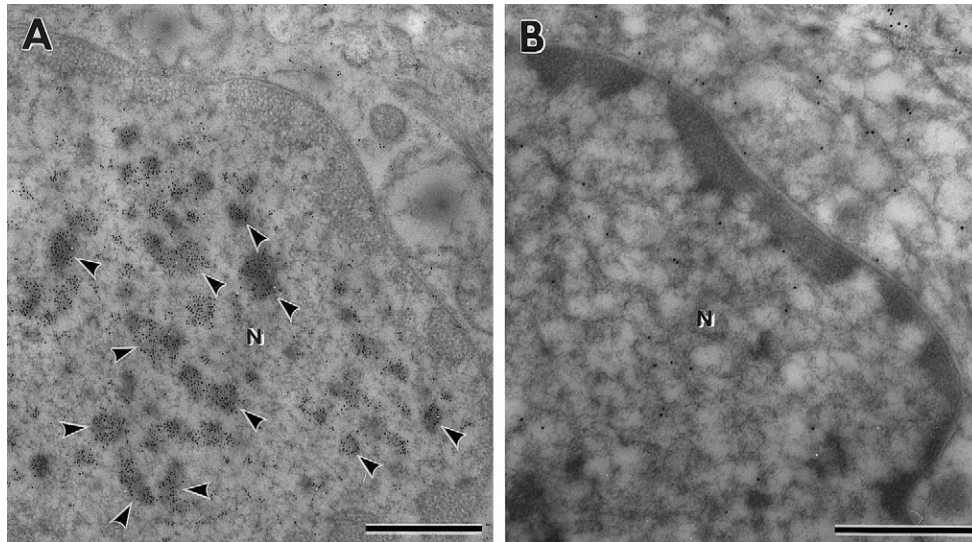


Figure 4 Immunoelectron microscopy showing abnormal loricrin staining in patient skin: loricrin labeling in granular cells. Immunoreaction is heavily accumulated on the intranuclear aggregates (*arrowheads*) in the patient skin. Normal skin shows diffuse cytoplasmic and nucleoplasmic labels not associated with granules. Biopsy samples are from the thigh of case 2 (A) and from a healthy individual (B). N = nuclei. Bars = 1 μ m.

acids by 91 missense amino acids (fig. 6). Furthermore, the mutant transcript overrides the endogenous termination and recognizes a novel termination codon 65 nucleotides downstream, thereby extending the loricrin polypeptide an additional 22 amino acids (fig. 6). This novel C-terminus is remarkably similar to the VS mutation, which creates the identical 84 missense amino acids, in addition to the 22-amino-acid extension (Maestrini et al. 1996).

We predict that the mechanism of action of this mutation on the disruption of the envelope acts in two ways: (1) by impairing and disrupting its ability to cross-link to itself and to other cornified cell envelope components and (2) by diminishing its flexibility, perhaps rendering it more rigidly structured. First, 17 of 23 unique *intramolecular* crosslinks formed by transglutaminases I and III *in vitro* (Candi et al. 1995) are broken by the mutation. Second, the relative number of *intermolecular* cross-links between loricrin and other cornified cell envelope proteins that are disrupted by the mutation (Steinert and Marekov 1994, 1997) are known. It is clear that, although the mutation disrupts most of the loricrin-loricrin intermolecular cross-links, the majority of the cross-links between loricrin and other proteins are left intact, with the exception of the loricrin-involucrin association, in which half the cross-links are broken.

Secondary structure-prediction methods give further insights into the structural changes at the C-terminus that are incurred as a result of the mutation. Very little of the native loricrin protein is either alpha-helix or beta sheet in its structure, and it is predicted that this protein has no alpha or beta secondary structure at all (Chou

and Fasman 1978; Rost and Sander 1993; Rost 1995; Rost et al. 1995, 1996). This result is consistent with the observation, on the basis of circular dichroism, that native loricrin has no secondary structure (Candi et al. 1995). Therefore, we expect that the primary determinant of loricrin structure is cross-linking (Steinert and Marekov 1994). The protein-analysis methods also predict that there will be no significant secondary structure generated in the mutated C-terminal region. Thus, when the native cross-linking is disrupted by the mutation, the mutated region becomes a random coil, causing both increased rigidification and loss of flexibility of the protein, as well as the potential for dominant-negative interactions with other loricrin molecules and/or other envelope components.

We speculate that some normal loricrin molecules are participating in cornified cell envelope formation, since we do not observe erythematous lesions over the entire body surface and since areas of uninvolved skin were clearly evident in these two cases with PSEK. It may be possible that there is some redundancy in the structure of the envelope or that other cornified-cell-envelope precursors may be compensating for the abnormalities in loricrin. This hypothesis is supported by the observation of only a minimal erythematous phenotype lasting only 4–5 d in a mouse model in which the loricrin gene had been inactivated by targeted ablation (de Viragh et al. 1997). In the skin of the loricrin-deficient mice, evidence for compensation by SPR-2 and involucrin was evident in neonates and in 4-d-old mice, respectively, but failed to correct a defect in barrier function (de Viragh et al. 1997). Although we have not performed any quantita-

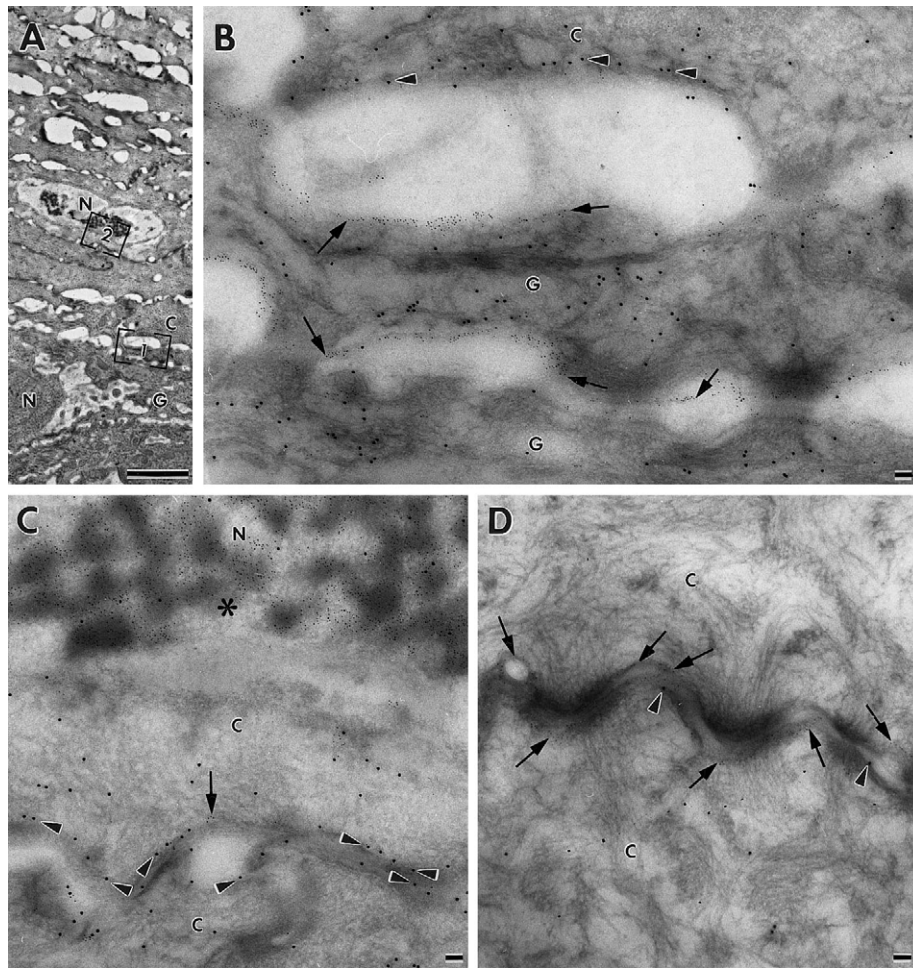


Figure 5 Loricrin (labeled with 5 nm gold) and involucrin (labeled with 15 nm gold) double staining demonstrating aberrant cornified-cell-envelope formation. The areas marked by boxed numerals "1" and "2" in A are shown at a higher magnification in B and C, respectively. Note both the temporal cell peripheral accumulation of loricrin labels (arrows in B) in the most superficial granular cells and disappearance of the labels on the cell envelopes of cornified cells in patient skin. Compare that with rich loricrin labeling in normal cornified-cell envelopes (D). Granular aggregates (*) within the parakeratotic cornified-cell nucleus are densely labeled with the loricrin antibody (C). Involucrin labels (arrowheads) on the cornified-cell envelopes are rich in patient skin (B and C) but not in normal skin (D). G = granular cells; and C = cornified cells. Biopsy samples are from the thigh of case 2 (A–C) and a healthy individual (D). N = nuclei. Bar = 5 μ m in A and 0.1 μ m in B–D.

tive analysis of involucrin expression in the PSEK family, a similar compensatory mechanism may contribute to the apparent increase of involucrin deposition by immunoelectron microscopy, together with an unmasking of involucrin epitopes in the absence of loricrin (fig. 5B and C). Both the explanation of the dramatic phenotypic expression in a palmoplantar distribution despite ubiquitous expression of the loricrin gene and the pathogenesis of the pseudoainhum are matters that remain to be explored.

The clinical features of disorders labeled as EKs vary widely among pedigrees, raising the possibility that the clinical descriptors assigned to these phenotypes serve to group them together in a rather loosely defined fashion (Hopsu-Havu and Peltonen 1970; Nir and Tanzer 1978; Maldonado-Ruiz et al. 1982; Nazzaro and Blanchet-

Bardon 1986; Lamprecht et al. 1988; Kudsi and Naeyaert 1990; MacFarlane et al. 1991; Miura et al. 1991; Kiesewetter et al. 1993; Niemi and Kanerva 1993; Gray et al. 1996). Furthermore, there are several reports in the literature that describe ultrastructural findings in EK; yet, as far as we are aware, none of them has shown findings similar to those in the cases presented in the present study (Nazzaro and Blanchet-Bardon 1986; Lamprecht et al. 1988; Kudsi and Naeyaert 1990; MacFarlane et al. 1991; Miura et al. 1991; Kiesewetter et al. 1993; Niemi and Kanerva 1993; Gray et al. 1996). Therefore, it is clear that the EKs are a heterogeneous group of diseases and that either different mutations in loricrin or mutations in molecules other than loricrin will be responsible for variant phenotypes. Nonetheless, the clinical findings of palmoplantar keratoderma with

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EK  TCGTGC GCGCCCCCAGCCGAGTTACGGAGGGGGTCGTCCGGCGCGCGCGGCAGCGCGGAAG...
      S  C  A  P  Q  P  S  Y  G  G  G  S  S  G  G  G  G  S  G  G
                P  V  E  L  R  R  G  V  V  R  R  R  R  Q  R  R  K

VS  TCGTGC GCGCCCCCAGCCGAGTTACGGAGGGGGGTCGTCCGGCGCGCGCGGCAGCGCGGAAG...
      S  C  A  P  Q  P  S  Y  G  G  G  S  S  G  G  G  G  S  G  G
                V  V  R  R  R  R  Q  R  R  K

wt   S  G  C  F  S  S  G  G  G  G  G  S  S  G  C  G  G  G  S  S
mt   R  L  L  L  Q  R  R  G  R  R  E  L  R  L  R  R  L  L  R

wt   G  I  G  S  G  C  I  I  S  G  G  G  S  V  C  G  G  G  S  S
mt   D  W  Q  R  L  H  H  Q  W  R  G  L  R  L  R  R  W  F  L  W

wt   G  G  G  G  G  G  S  S  V  G  G  S  G  S  G  K  G  V  P  I
mt   R  R  R  R  R  L  L  R  G  W  L  R  E  W  Q  G  R  P  D  L

wt   C  H  Q  T  Q  Q  K  Q  A  P  T  W  P  S  K  *
mt   P  P  D  P  A  E  A  G  A  Y  L  A  V  Q  I  D  P  P  G  Y

mt   G  G  E  G  V  G  G  V  F  Q  G  H  R  W  A  *

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Figure 6 Analysis of amino acid sequences of EK mutation. The two upper lines represent the loricrin mutations in the EK family (present study), 709insC (denoted by boldface on the top line), and in the VS family (Maestrini et al. 1996), 730insG (denoted by boldface on the second line). Single-letter amino acid translation of the sequence is given directly beneath the nucleotide sequence, and the mutant carboxy-terminal loricrin sequence resulting from the frameshift and delayed termination codon is underlined. wt = wild-type sequence; mt = mutant sequence (*underlined*); and * = termination codon.

pseudoainhum, as well as histological and immunohistochemical characteristics including the presence of intranuclear loricrin granules and abnormal cornified-cell envelopes, serve as useful markers to identify a genodermatosis that may be caused by a loricrin mutation.

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References

- Brown J, Kierland RR (1966) Erythrokeratoderma variabilis: report of three cases and review of the literature. *Arch Dermatol* 93:194-201
- Camisa C, Hessel A, Rossana C, Parks A (1988) Autosomal dominant keratoderma, ichthyosiform dermatosis and elevated serum beta-glucuronidase. *Dermatologica* 177:341-347
- Camisa C, Rossana C (1984) Variant of keratoderma hereditaria mutilans (Vohwinkel's syndrome). *Arch Dermatol* 120:1323-1328
- Candi E, Melino G, Mei G, Tarcsa E, Chung S-I, Marekov LN, Steinert PM (1995) Biochemical, structural, and transglutaminase substrate properties of human loricrin, the major epidermal cornified cell envelope protein. *J Biol Chem* 270:26382-26390
- Chou PY, Fasman GD (1978) Empirical predictions of protein conformation. *Annu Rev Biochem* 47:251-276
- Christiano AM (1997) Frontiers in keratodermas: pushing the envelope. *Trends Genet* 13:227-233
- Darier J (1911) Erythrokeratodermie verruqueuse en nappes, symetrique et progressive. *Bull Soc Fr Dermatol Syphilol* 22:252-264
- de Viragh PA, Scharer L, Bundman D, Roop DR (1997) Loricrin deficient mice: upregulation of other cell envelope precursors rescues the neonatal defect but fails to restore epidermal barrier function. *J Invest Dermatol* 108:555
- Genetic Computing Group (1994) Program manual for the Wisconsin package 8.1. Madison, WI
- Gray LC, Davis LS, Guill MA (1996) Progressive symmetric erythrokeratoderma. *J Am Acad Dermatol* 34:858-859
- Hohl D, Mehrel T, Lichti U, Turner ML, Roop DR, Steinert PM (1991) Characterization of human loricrin: structure and function of a new class of epidermal cell envelope proteins. *J Biol Chem* 266:6626-6636
- Hopsu-Havu VK, Peltonen L (1970) Erythrokeratoderma congenitalis progressive symmetrica (Gottron): a case report. *Dermatologica* 141:321-328
- Hopsu-Havu VK, Tuohimata P (1971) Erythrokeratoderma congenitalis progressiva symmetrica (Gottron): an analysis of kinetics of epidermal cell proliferation. *Dermatologica* 142:137-144
- Hudson DL, Weiland KL, Dooley TP, Simon M, Watt FM (1992) Characterization of eight monoclonal antibodies to involucrin. *Hybridoma* 11:367-379

- Ishida A, Hirokawa M, Ara M, Watanabe M, Matsumoto M, Iizuka H, Kumakiri M (1987) Progressive erythrokeratoderma: ultrastructural and biochemical study. *Jpn J Dermatol* 97:785–792
- Ishida A, Watanabe M, Iizuka H, Mizumoto T (1986) Two cases of progressive erythrokeratoderma with hyperkeratosis of the palms and soles. *Jpn J Clin Dermatol* 40:695–699
- Ishida-Yamamoto A, Eady RAJ, Watt FM, Roop DR, Hohl D, Iizuka H (1996) Immunoelectron microscopic analysis of cornified cell envelope formation in normal and psoriatic epidermis. *J Histochem Cytochem* 44:167–175
- Ishida-Yamamoto A, Iizuka H (1995) Differences in involucrin immunolabeling within cornified cell envelopes in normal and psoriatic epidermis. *J Invest Dermatol* 104:391–395
- Kiesewetter F, Simon MJ, Fartasch M, Gevatter M (1993) Progressive partially symmetric erythrokeratoderma with deafness: histological and ultrastructural evidence for a subtype distinct from Schnyder's syndrome. *Dermatology* 186:222–225
- Kudsi S, Naeyaert JM (1990) Progressive symmetric erythrokeratoderma of Darier Gottron. *Dermatologica* 180:196–197
- Lamprecht A, Goecke T, Anton-Lamprecht I, Kuster W (1988) Progressive erythrokeratoderma and cochlear hearing impairment: a case report and review of the literature. *Int J Pediatr Otorhinolaryngol* 15:279–289
- MacFarlane AW, Chapman SJ, Verbov JL (1991) Is erythrokeratoderma one disorder? a clinical and ultrastructural study of two siblings. *Br J Dermatol* 124:487–491
- Maestrini E, Monaco A, McGrath JA, Ishida-Yamamoto A, Camisa C, Hovnanian A, Lathrop M, et al (1996) A molecular defect in loricrin, the major component of the cornified cell envelope, underlies Vohwinkel's syndrome. *Nat Genet* 13:70–77
- Maldonado-Ruiz R, Tamayo L, del Castillo V, Lozoya I (1982) Erythrokeratoderma progressiva symmetrica: report of 10 cases. *Dermatologica* 164:133–141
- Mendes da Costa S (1925) Erythro-er keratoderma variabilis in a mother and daughter. *Acta Derm Venerol* 6:300–304
- Miura Y, Yoshiike T, Sou H, Yaguchi H, Ogawa H (1991) A case report of progressive symmetric erythrokeratoderma and a review of progressive erythrokeratoderma in Japan. *Jpn J Dermatol* 101:635–643
- Nazzaro V, Blanchet-Bardon C (1986) Progressive symmetric erythrokeratoderma: histological and ultrastructural study of patient before and after treatment with etretinate. *Arch Dermatol* 122:434–440
- Niemi K-M, Kanerva L (1993) Histological and ultrastructural study of a family with erythrokeratoderma progresiva symmetrica. *J Cutan Pathol* 20:242–249
- Nir M, Tanzer F (1978) Progressive symmetric erythrokeratoderma. *Dermatologica* 156:268–273
- Richard G, Whyte YM, Smith L, Itin P, Hohl D, Wollina U, Epstein E, et al (1996) Linkage studies in erythrokeratodermas: fine mapping, genetic heterogeneity and analysis of candidate genes. *J Invest Dermatol* 107:481
- Rost B (1995) Prediction-based threading. In: Rawlings C, Clark D, Altman R, Hunter L, Lengauer T, Wodak S (eds) *The Third International Conference on Intelligent Systems in Molecular Biology*. AAAI Press, Menlo Park, pp 314–321
- Rost B, Casadio R, Fariselli P, Sander C (1995) Prediction of helical transmembrane segments at 95% accuracy. *Protein Sci* 4:521–533
- Rost B, Fariselli P, Sander C (1996) Topology prediction for helical transmembrane proteins at 86% accuracy. *Protein Sci* 7:1704–1718
- Rost B, Sander C (1993) Prediction of protein secondary structure at better than 70% accuracy. *J Mol Biol* 232:584–599
- Sambrook J, Fritsch EF, Maniatis T (1979) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Steinert PM, Marekov LN (1994) The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isopeptide cross-link components of the human epidermal cornified cell envelope. *J Biol Chem* 270:17702–17711
- (1997) Direct evidence that involucrin is a major early isopeptide crosslinked component of the keratinocyte cornified cell envelope. *J Biol Chem* 272:2021–2030
- Stevens HP, Kelsell DP, Bryant SP, Bishop DT, Spurr NK, Weissenbach J, Marger D, et al (1996) Linkage of an American pedigree with palmoplantar keratoderma and malignancy (palmoplantar ectodermal dysplasia type III) to 17q24: literature survey and proposed updated classification of the keratodermas. *Arch Dermatol* 132:640–651
- van der Schroeff JG, Nijenhuis LE, Khan PM, Bernini LF, Schreuder GMT, van Loghem E, Volkers WS, et al (1984) Genetic linkage between erythrokeratoderma variabilis and Rh locus. *Hum Genet* 68:165–168
- van der Schroeff JG, van Leeuwen-Cornelisse I, van Haeringen A, Went LN (1988) Further evidence for localization of the gene of erythrokeratoderma variabilis. *Hum Genet* 80:97–98
- Vohwinkel KH (1929) Keratoderma hereditaria mutilans. *Arch Dermatol Syphilol* 158:354–364
- Yoneda K, Hohl D, McBride OW, Wang M, Cehrs KU, Idler WW, Steinert PM (1992) The human loricrin gene. *J Biol Chem* 267:18060–18066