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# Loricrin keratoderma: a novel disease entity characterized by nuclear accumulation of mutant loricrin

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Summary

Loricrin is the major protein of the cornified cell envelope, a structure that replaces the plasma membrane during keratinocyte terminal differentiation. Recently, unique heterozygous, insertion mutations in the loricrin gene have been found to underlie certain congenital skin abnormalities, the phenotypes of which vary considerably. Clinically, these patients can be diagnosed as suffering from an ichthyotic variant of Vohwinkel's syndrome, progressive symmetric erythrokeratoderma, or congenital ichthyosiform erythroderma born as a collodion baby. Common clinical features include hyperkeratosis of the palms and soles with digital constriction. Histologic characteristics are parakeratotic hyperkeratosis with hypergranulosis and nuclear accumulation of mutant loricrin. The unique mutations in the glycine rich domain of the mutant loricrin form arginine rich nuclear localization sequences that disrupts differentiation of keratinocytes. This group of unique genodermatoses caused by distinct loricrin mutations is collectively termed loricrin keratoderma.

Keywords: palmoplantar keratoderma; progressive symmetric erythrokeratoderma, nuclear localization sequences; cornified cell envelopes; ichthyosis

## 1. Cornified cell envelopes and loricrin

Epidermal keratinocytes form cornified cell envelopes that are 15- to 20-nm thick, highly insoluble structures that replace the cell membrane during terminal differentiation [1]. Cornified cell envelopes comprise several cross-linked molecules, including loricrin. Loricrin is a glycine-, serine- and cysteine-rich, highly insoluble basic protein expressed in the superficial granular cells of the epidermis [2] (Fig 1). Human loricrin is a 26 kDa protein and consists of 315 amino acids. The small size of loricrin means that it is localized in both the cytoplasm and nucleoplasm in superficial granular cells. In the cornified cells, loricrin is cross-linked to the cornified cell envelope.

## 2. Genetic Disorders of Loricrin

Unique heterozygous mutations in the loricrin gene have been found to underlie distinct skin abnormalities (Fig 1) (Table I). All of the mutations reported to date are single base pair insertions that lead to the synthesis of aberrant proteins in which the C-terminal amino acid sequences are replaced by missense amino acids.

The first loricrin mutation was identified in patients who had been diagnosed as suffering from Vohwinkel's syndrome (VS) [3]. However, the clinical features are different from original VS, reported by Vohwinkel in 1929 [4] which is now known to be caused by mutations in the connexin 26 gene [5] (OMIM 124500). Patients with loricrin mutations and original VS patients share diffuse palmoplantar hyperkeratosis with a honeycomb appearance and digital constriction bands (pseudoainhum) (Fig 2), but differ in several clinical aspects. Original VS showed a peculiar, linear or starfish-shaped hyperkeratosis over the joints of the extremities and was associated with

a high-tone acoustic impairment. By contrast, patients with loricrin mutations do not have starfish keratoses or impaired hearing, but show different skin lesions in non-palmoplantar areas. It can be described as generalized non-erythrodermic ichthyosis [6-8] or designated as an ichthyotic variant of VS (OMIM 604117). In other cases, generalized, well-demarcated erythematous hyperkeratotic plaques are noted and clinically diagnosed as a form of erythrokeratoderma [9] (OMIM 602036) (Fig 3). Still other cases are born as collodion babies that later go on to develope into ichthyosiform erythroderma, and are considered a form of non-bullous congenital ichthyosiform erythroderma [10]. To avoid confusion with true VS caused by connexin mutations, we have proposed a term "loricrin keratoderma" (LK) [2]. There are yet, no apparent genotype-phenotype correlations are noted (Table I). There are variation in severity among patients even in the same LK family. The reason for this is not clear at this moment. It might be related to differences in intensity of loricrin expression or polymorphism in the loricrin gene.

Despite some clinical variation between patients with loricrin mutations, almost all microscopic, ultrastructural and immunohistological features are shared and prove to be of significant diagnostic value. The epidermal cornified layer is thick and retains round nuclei [2] (Fig 4). Granular cell layer is also thick and the nuclei contain characteristic electron dense granular depositions (Fig 5). These deposits and nuclear debris in the stratum corneum are composed of mutant loricrin (Fig 6, 7).

## 3. Loricrin mutations create nuclear localization signals

In all forms of LK, the defects are caused by single allele mutations and are caused by

nucleotide insertions. LK disease is transmitted in a dominant fashion and LK keratinocytes express both wild and mutant types of loricrin protein generated from both alleles. These frame-shift mutations produce mutant proteins 22 amino acids longer than the wild type protein due to a delayed termination codon (Fig 1). Using an antibody recognizing 18 amino acids that exist only in the mutant loricrin, we have demonstrated its site- and differentiation-specific expression in LK epidermis (Fig 6). Interestingly, mutant loricrin is concentrated within the nuclei (Fig 6, 7), while wild protein is equally distributed in the cytoplasm and nucleoplasm (Fig 8b). This phenomenon was first noted when LK epidermal sections were stained with two different anti-loricrin antibodies, one is against N-terminus and the other is against C-terminus. The former antibody stained the nucleus more intensely than the cytoplasm (Fig 8a). The latter antibody stained the cytoplasm and nucleoplasm equally (Fig 8b). Given the nature of loricrin gene mutations, it is now clear that the N-terminal loricrin antibody recognizes both wild- and mutant loricrin, while the C-terminal antibody only detects wild type molecules (Fig 1).

What it the mechanism for the preferential nuclear accumulation of mutant loricrin? The answer is in the sequence of loricrin gene (Fig 9). Translocation of proteins into the nucleus is specified by short stretches of amino acids known as nuclear localization sequences (NLSs) [11]. There is no clear consensus sequence, but most NLSs are highly enriched in basic amino acids, and may be continuous sequences, as in the SV40 large T antigen, or bipartite, as in nucleoplasmin sequence (Fig 10). Loricrin is a glycine rich protein and the gene contains many GGX codons for glycine. Insertion of one nucleotide within this sequence causes a frame-shift and creates several AGG and

CGG codons that happen to code arginine. Mutant loricrin is therefore rich in arginine and contains a potential consensus bipartite NLSs [6]. We have found that the retained nuclei in the stratum corneum of LK skin are TUNEL, a marker of apoptosis, positive [12]. It has been suggested that keratinization is a form of apoptosis. Mutant loricrin accumulated in the nuclei might interfere with the nuclear apoptotic process in the terminal differentiation of keratinocytes.

Suga et al. has recently generated loricrin mutant transgenic mice by the insertion of a C at nucleotide position 1190 in the mouse loricrin coding sequence [13]. The skin manifestations of the transgenic mice were very similar to the clinical presentation of the LK patients. They also demonstrated that the C-terminus of mouse mutant loricrin contains an NLS. They also crossed the transgenic mice with loricrin knockout mice and identified a severe phenotype. This suggests that the disruptive function of mutant loricrin does not require any interaction with the wild type loricrin.

4. Other loricrin mutations show no or only a minor, transient phenotype

There have been several attempts to create animals with different abnormalities in the loricrin gene. Transgenic mice over-expressing human loricrin or mutant loricrin with a large deletion in the central portion failed to show any phenotypic abnormalities [14]. Koch et al generated loricrin-deficient mice that showed a delay in the formation of the epidermal barrier [15]. At birth, the mice weighted less and developed erythroderma. This phenotype was however transient and lasted for only 4-5 days. The presence of a compensatory mechanism to prevent the development of a more severe skin phenotype has been suggested. Loricrin-null mutations have not been reported in a human disease,

but patients born with a self-healing skin disorder similar to lamellar ichthyosis might be candidates for harboring such mutations.

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Figure legends

Fig 1. Schematic drawing of wild and mutant loricrin and the peptide used to raise an antibody against mutant loricrin.

Fig 2. Hyperkeratotic palm skin and a constriction band (pseudoainhum) of the small finger in a LK patient [9].

Fig 3. Erythematous keratotic plaques on the thigh of a LK patient with an erythrokeratoderma phenotype [9].

Fig 4. Photomicrograph showing marked hyperkeratosis with round condensed nuclei and hypergranulosis. Hyperkeratotic plaques on the thigh of a LK patient [9]. Hematoxylin and eosin staining.

Fig 5. Electron microscopy showing the granular cells in a LK patient [6]. Deposition of electron dense granular material (*) is seen within the nucleus (Nu). K, keratohyalin granule.

Fig 6. Immunofluorescent staining of mutant loricrin in a LK patient [9]. Green, mutant loricrin. Red, DNA.

Fig 7. Immunoelectron microscopy using anti-mutant loricrin antibody. Nuclear deposition in the epidermal spinous cell of a LK patient [9] is positively labeled. 10 nm immunogold labeling. Nu, nucleus.

Fig 8 Loricrin immunostaining of a LK patient [9]. a, antibody against N-terminus of loricrin. Strong nuclear staining is noted. b, antibody against C-terminus of loricrin. Both cytoplasm and nucleus are stained diffusely using the immunogold silver staining method.

Fig 9. Codons for glycine and arginine. Nucleotide sequences of the wild type loricrin

gene and 730 insG mutant. Wild type loricrin gene sequences contain many GGX codons that code for glycine. Insertion of single nucleotide (red) causes a downstream frameshift and GGX codons change the reading frame to CGG and AGG that code for arginine.

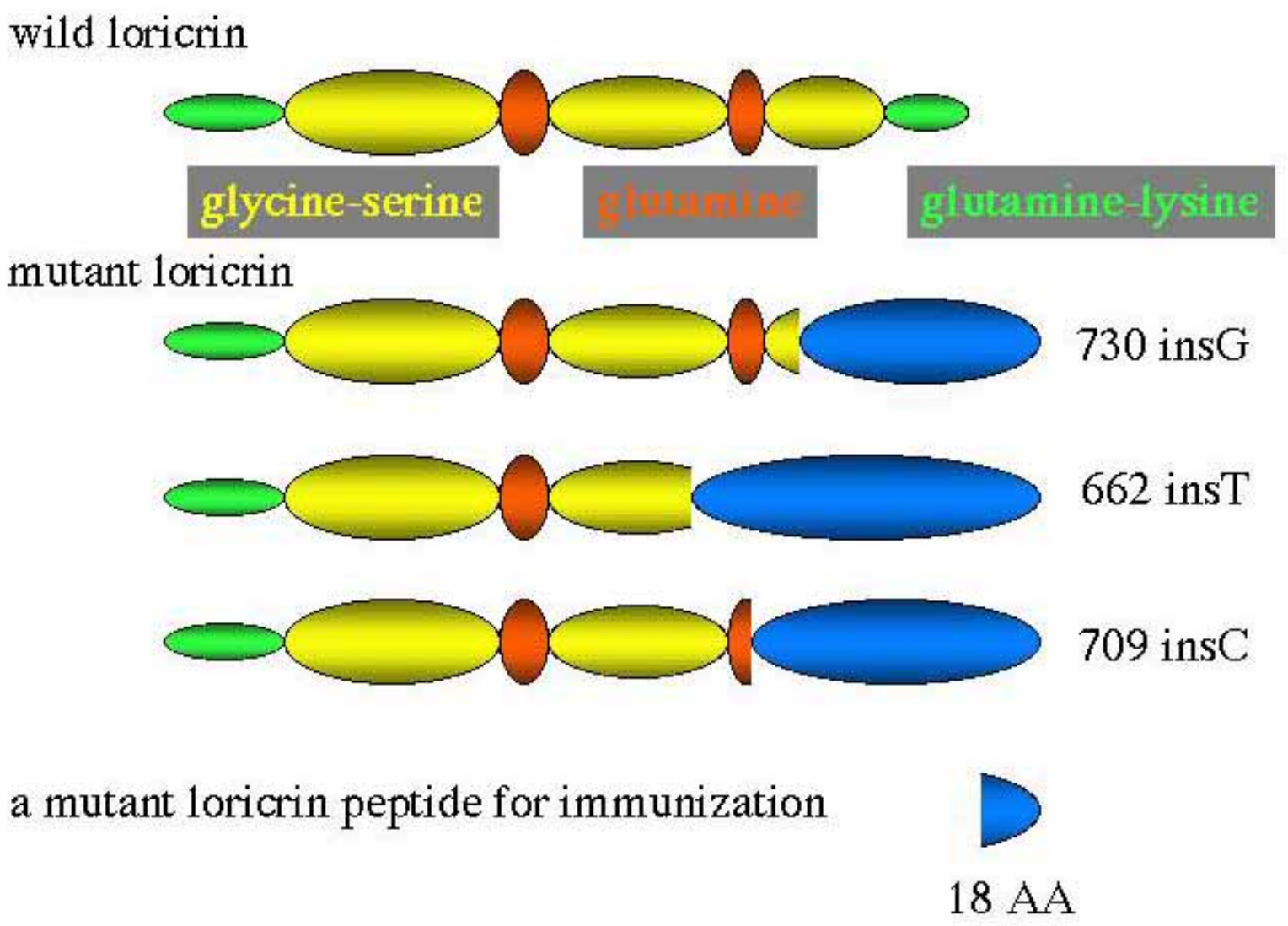
Fig 10. Loricrin amino acid sequence and nuclear localization signals. Classical nuclear targeting sequences contain basic amino acids, lysine (K) and arginine (R). Mutant loricrin is rich in arginine.

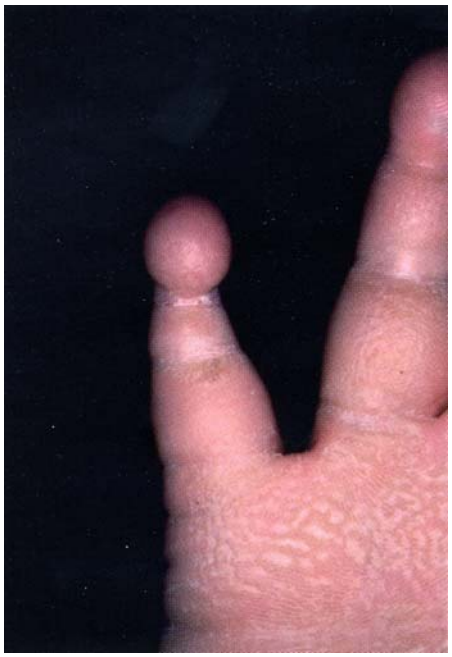
Table I

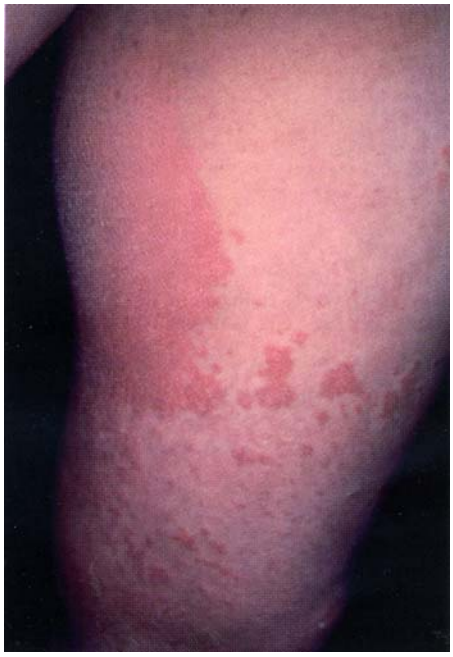
Clinical featrues of loricrin keratoderma

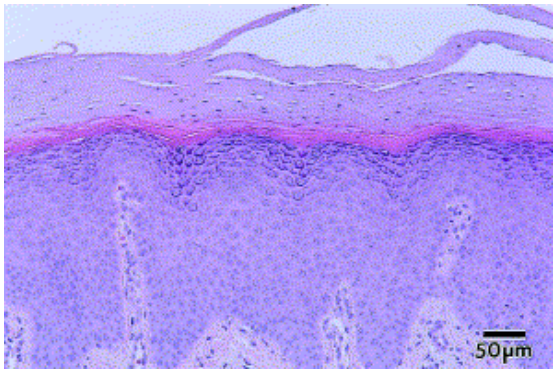
| case number | ethnic backgrounds | gene mutation | honeycomb palmoplantar keratoderma | pseudoainhum and/or autoamputation | other skin abnormalities | reference |
|---|---|---|---|---|---|---|
| 1 | UK | 730 insG | + | severe | diffuse generalized ichthyosis | [3] |
| 2 | Scotland | 730 insG | + | severe ~ mild | covered in whitich material at birth, hyperkeratosis of the knees and elbows, mild to moderate ichthyosis | [6] |
| 3 | Japan | 709 insC | + | severe ~ mild | generalized well demarcated erythematous hyperkeratotic plaques | [9] |
| 4 | Nothern Ireland* | 662 insT | + | mild | hyperkeratosis over the knuckles, generalized non-erythemato ichthyosis | [7] |
| 5 | Japan | 730 insG | + | severe | ichthyosis | [8] |
| 6 | Japan | 730 insG | + | severe | collodion baby, generalized ichthyosiform erythroderma | [10] |
| 7 | UK | 730 insG | + | severe | | [16] |

*personal communication from Dr. Armstrong


wild loricrin
glycine-serine
glutamine
glutamine-lysine
mutant loricrin
730 insG
662 insT
709 insC
a mutant loricrin peptide for immunization
18 AA



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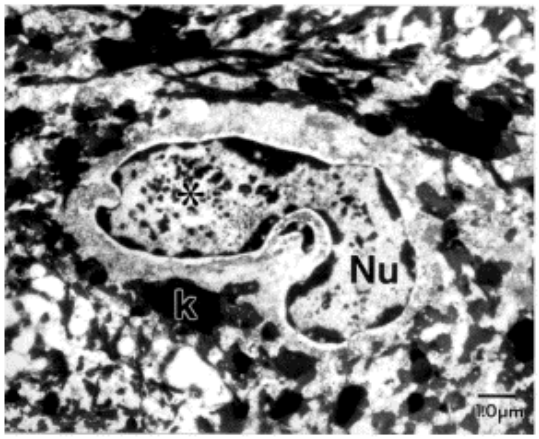
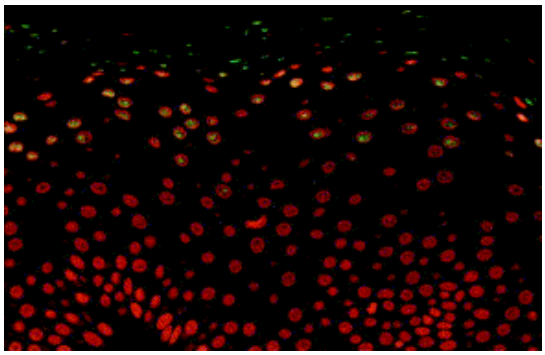
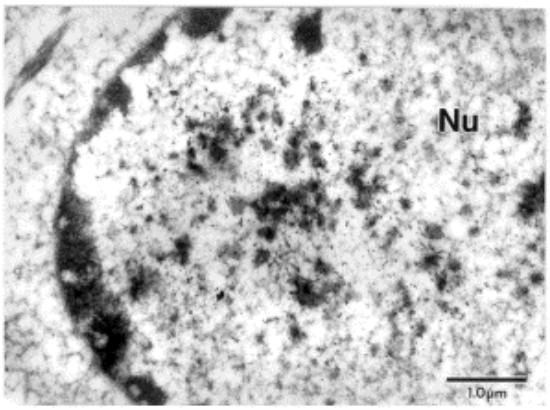
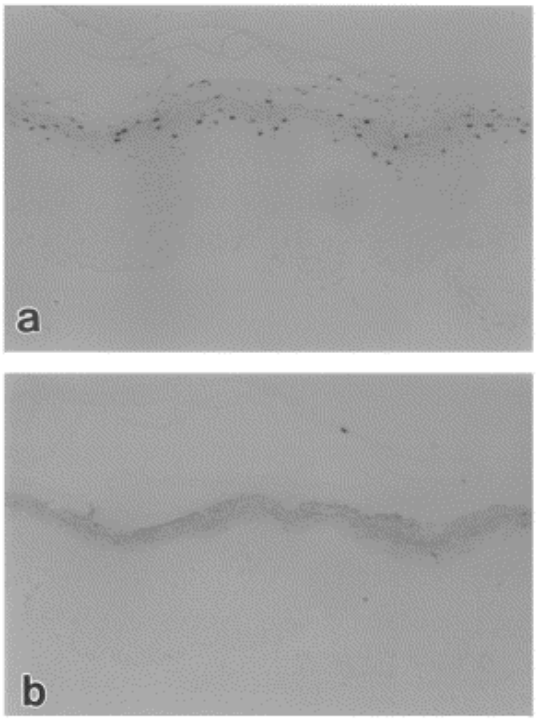

Nu
k
1.0μm




Nu
1.0μm




Gly: GGT, GGC, GGA, GGG
Arg: AGA, AGG, CGT, CGC, CGA, CGG

**wild loricrin**

--- GGA GGG GGG TCG TCC GGC GGC GGC GGC AGC GGC
GGA AGC GGC TCG TTC TCC AGC GGC GGG GGC GGC GGG
AGC TCC GGC TGC GGC GGC GGC TCC TCC GGG ATT GGC
AGC GGC TGC ATC ATC AGT GGC GGG GGC TCC GTC TGC
GGA GGT GGT TCC TCT GGA GGC GGC GGC GGC GGC TCC
TCC GTG GGT GGC TCC GGG AGT GGC AAG GGC GTC CCG
ATC TGC CAC CAG ACC CAG CAG AAG CAG GCG CCT ACC
TGG CCG TCC AAA TAG

**mutant loricrin (730 insG)**

--- GGA GGG GGG GTC GTC CGG CGG CGG CGG CAG CGG
CGG AAG CGG CTC GTT CTC CAG CGG CGG GGG CGG CGG
GAG CTC CGG CTG CGG CGG CGG CTC CTC CGG GAT TGG
CAG CGG CTG CAT CAT CAG TGG CGG GGG CTC CGT CTG
CGG AGG TGG TTC CTC TGG AGG CGG CGG CGG CGG CTC
CTC CGT GGG TGG CTC CGG GAG TGG CAA GGG CGT CCC
GAT CTG CCA CCA GAC CCA GCA GAA GCA GGC GCC TAC
CTG GCC GTC CAA ATA GAT CCC CCA ---

Fig.9

Nuclear Targeting Sequence

SV40 Large T
PKKKRKV

Nucleoplasmin (Bipartite motif)
KRPAATKKAGQAKKKKL

Loricrin Keratoderma (709 insC)

-----PVELRRGVVRRRRQRRKRLLLQRRGRRELR
LRRRLLRDWQRLHHQWRGLRLRRWFLWRRRR
RLLRGWLREWQGRPDLPPDPAEAGAYLAVQIDP
PGYGGEGVGGVFQGHRWA*

Fig.10