

## **UC Davis**

### **UC Davis Previously Published Works**

#### **Title**

Cross-linked envelopes in nail plate in lamellar ichthyosis

#### **Permalink**

<https://escholarship.org/uc/item/0v41z4zq>

#### **Journal**

British Journal of Dermatology, 149(5)

#### **ISSN**

0007-0963

#### **Authors**

Rice, R H  
Crumrine, D  
Hohl, D  
[et al.](#)

#### **Publication Date**

2003-11-01

Peer reviewed

## CONCISE COMMUNICATION

# Cross-linked envelopes in nail plate in lamellar ichthyosis

R.H.RICE, D.CRUMRINE,\* D.HOHL,† C.S.MUNRO‡ AND P.M.ELIAS\*

*Department of Environmental Toxicology, University of California, Davis, CA 95616-8588, U.S.A.*

*\*Dermatology Service, Veterans Affairs Medical Center, Department of Dermatology, University of California, San Francisco, CA 94121, U.S.A.*

*†Department of Dermatology, CHUV, CH-1011 Lausanne, Switzerland*

*‡Southern General Hospital, Glasgow G51 4TF, U.K.*

Accepted for publication 4 March 2003

### Summary

**Background** Corneocytes of the nail plate, like those of the stratum corneum, generate cornified envelopes (CEs) of cross-linked protein that can be visualized readily after removal of non-cross-linked protein by detergent extraction. Defective CE formation occurs in epidermal scale and hair in transglutaminase 1 (TGM1)-negative lamellar ichthyosis (LI) and has been proposed as a diagnostic aid for this syndrome.

**Objectives** (i) To ascertain whether TGM1 is important for CE formation in nail; (ii) to characterize CE abnormalities occurring in LI that may be distinguished from other types of inherited ichthyosis when nail samples are subjected to detergent extraction; and (iii) to evaluate the utility of nails as a diagnostic aid for LI.

**Methods** Nail samples were provided by nine patients previously classified as having TGM1-negative LI, four with other types of ichthyotic conditions and six normal controls. Samples were extracted extensively in sodium dodecyl sulphate under reducing conditions and examined by light and electron microscopy.

**Results** After extraction, defective CE cross-linking was visualized in epidermal corneocytes from seven of nine patients exhibiting TGM1-negative LI, whereas nail samples from patients with the other syndromes were normal. The defects in CE structure resembled those recently reported for LI scale, although in some cases residual CE and CE-associated structures were present.

**Conclusions** Despite the paucity of clinical nail symptoms in LI, TGM1 activity is important for generation of normal CE in nail plate, consistent with its importance in protein cross-linking in interfollicular epidermis and hair. Lack of this activity leads to a strikingly aberrant appearance of CE in LI nail after detergent extraction that is evident ultrastructurally in a large majority of cases. Nail envelopes therefore could provide a useful diagnostic tool in distinguishing LI from other ichthyoses with overlapping clinical features.

**Key words:** corneocytes, transglutaminase 1, transmission electron microscopy

Disorders of the nail can arise from hereditary defects or as manifestations of acquired disease, including pharmaceutical use or systemic exposure to toxic agents.<sup>1,2</sup> The molecular basis for alterations in appearance and integrity, however, is often obscure. Further understanding of deleterious effects on the nail should come from comparison of normal structure with perturbations in genetic diseases. For example, the high

content of keratin tonofilaments imparts structural stability and mechanical resistance to nails, and keratin mutations are now known not only to lead to stratum corneum and hair abnormalities,<sup>3,4</sup> but also to aberrations in nails.<sup>5,6</sup>

The sequence of terminal differentiation in nail resembles keratinocyte maturation in interfollicular epidermis. However, the geometry of cornified cells in each differs substantially, and a granular layer, a prominent feature of epidermis, is lacking in the germinative nail matrix. Although cornified envelopes

Correspondence: Dr R.H.Rice.  
E-mail: rhrice@ucdavis.edu

(CEs) are present in nail corneocytes, the relative contribution of each of the several transglutaminases to their formation is not known.<sup>7</sup> CE formation provides a critical scaffold for the extracellular lamellar bilayers, which subserve the epidermal permeability barrier, as shown most dramatically in transglutaminase 1 (TGM1)-deficient lamellar ichthyosis (LI) syndromes.<sup>8–11</sup> In these syndromes, defective CE structures comprise the basic defect leading to high transepidermal water loss and consequently to the desiccated and scaly appearance of the epidermis. Despite the presence of CE scaffolds, even normal nail minimally impedes transcutaneous water loss due to the absence of a lamellar body secretory system.

Present work explores whether: (i) perturbations of CE formation occur in nail that reflect comparable stratum corneum alterations in ichthyosis syndromes; (ii) lack of TGM1 in LI is reflected in structural nail alterations that reflect those in the epidermis; and (iii) these observed alterations have potential diagnostic utility. For this purpose, nail samples were extracted with detergent to enhance visualization of envelope structures and to avoid artefacts of poor infiltration by transmission electron microscopic embedding medium of such compact epidermal appendages.<sup>7,12</sup>

## Materials and methods

Fingernail clippings were generously provided by nine patients with LI previously classified as TGM1 negative, two TGM1-positive patients<sup>13</sup> with congenital ichthyosiform erythroderma (CIE), one with autosomal dominant ichthyosis (ADI) and one<sup>14</sup> with palmoplantar loricrin keratoderma (KTD, Camisa's syndrome, ichthyosis variant with loricrin mutation). Nail samples from six normal individuals gave results similar to previous observations by light and transmission electron microscopy (TEM).<sup>7</sup> Clippings were cut with scissors to dimensions of  $\approx 1 \text{ mm}^3$  and boiled in a water bath for 6 h in 2% sodium dodecyl sulphate/50 mmol L<sup>-1</sup> sodium phosphate (pH 7.5)/20 mmol L<sup>-1</sup> dithiothreitol. At approximately hourly intervals, the samples were swirled, and the evaporated water was replenished. During treatment, the nail samples became opalescent and flexible. The samples were then rinsed several times in water, fixed in buffered 2% glutaraldehyde, postfixed in 2% osmium tetroxide, and stained *en bloc* with tannic acid and uranyl acetate, dehydrated and infiltrated with Spurr resin as described previously.<sup>15</sup> Thin sections were then examined with a Zeiss 10 A transmission electron microscope operated

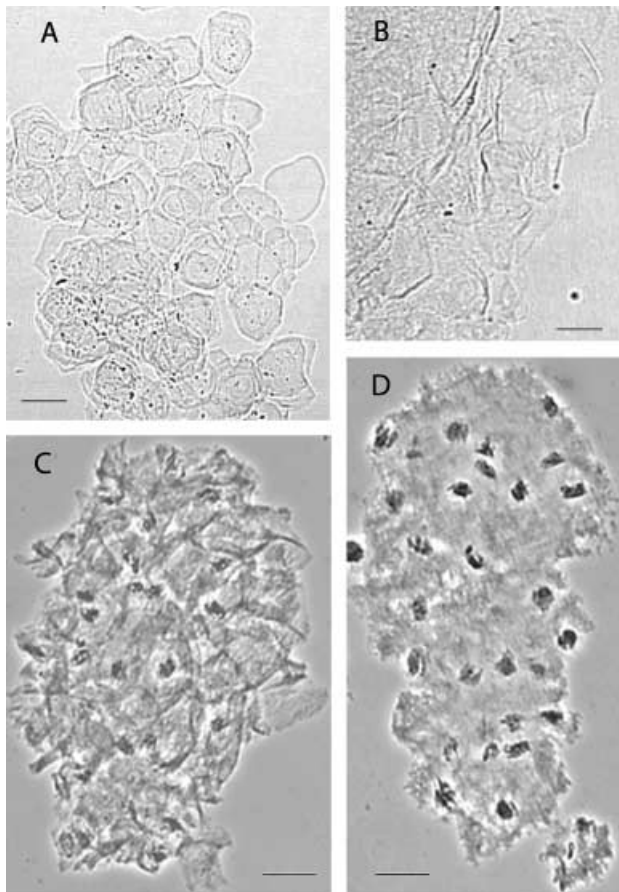
at 60 kV, and cross-sections of cell boundaries were sought for photography. Small pieces from each nail sample after the detergent extraction above were also examined by phase contrast light microscopy; the soft and flexible boiled samples were physically disaggregated with a spatula on the slide before adding the coverslips. Normal and LI epidermal scale (stratum corneum) samples were boiled for 5 min in the detergent solution; the cells became disaggregated and were examined without prior fixation by phase contrast microscopy.

## Results

Samples of normal and LI stratum corneum after boiling were compared by phase contrast light microscopy. The normal stratum corneum contained prominent cross-linked envelopes in which the boundaries of individual cells were clearly visible (Fig. 1A). In contrast, as previously observed,<sup>10</sup> the LI sample contained mostly amorphous material with little structure, although collections of angular fragments of envelope-like projections in disjointed arrangements were visible at the sample peripheries (Fig. 1B).

For comparison, small pieces of the various treated nail samples were examined by phase contrast microscopy. Even after detergent treatment, most still retained a readily discernible cellular architecture, with many of the cornified cells enclosing a prominent nuclear remnant. In the normal samples, CE structures were easily seen at cell borders (Fig. 1C), whereas in the majority of LI nail samples, the cell borders were not discernible (Fig. 1D). A minority of the LI nail samples could not be distinguished reliably from normal nail processed in parallel, but nail samples from two patients with LI were also exceptional in that neither boundaries between cells nor prominent nuclear structures were evident.

TEM provided a more discriminating comparison of the CE in detergent-extracted nail samples from normal individuals vs. the patients with LI. Envelopes from normal samples typically appeared as convoluted doublets, with each corneocyte bounded by an electron-dense outer border (Fig. 2A), similar to those seen in stratum corneum.<sup>10</sup> Some amorphous material, probably reflecting incomplete extraction, remained attached subjacent to the CE. By contrast, nail samples from seven of the nine patients with LI were readily distinguishable from normal in envelope configuration. Of these, two displayed apparently partial or fragmentary envelopes, usually singlet

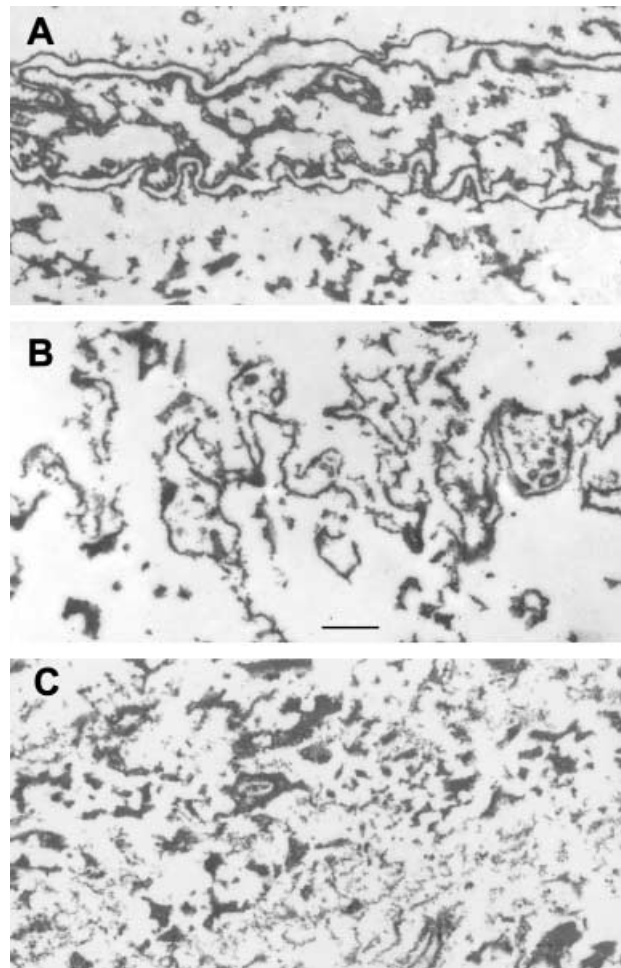


**Figure 1.** Light microscopy of (A,B) detergent-extracted epidermis and (C,D) nail. Samples from (A,C) normal individuals and (B,D) individuals with transglutaminase 1-negative lamellar ichthyosis are compared. Samples were photographed at  $\times 100$  magnification. Scale bars = 25  $\mu\text{m}$ .

structures (Fig. 2B). The remaining five, composed almost entirely of amorphous material, lacked envelopes completely (Fig. 2C) and contained only occasional suggestions of organized structures. Of these five, two lacked even the nuclear remnants that were seen in all the other samples (Fig. 3). Detergent extracted in parallel, nail samples from single patients with ADI and KTD and two patients with CIE were indistinguishable from normal by light or electron microscopy.

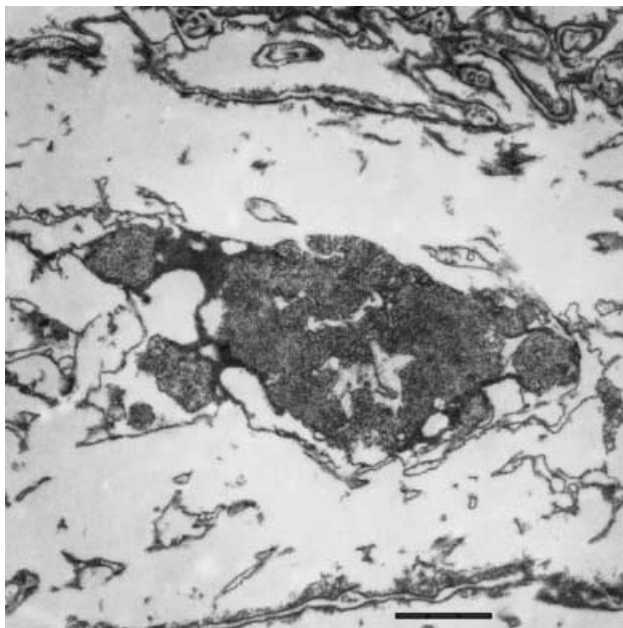
## Discussion

The present results demonstrate firstly that TGM1 is important for the formation of the CE in nail, as it is in stratum corneum. Other transglutaminase activities (or possibly residual TGM1 activity) appear capable of generating an attenuated CE in stratum corneum<sup>10</sup>



**Figure 2.** Variation of envelope structures in detergent-extracted nail samples from patients with lamellar ichthyosis: (A) indistinguishable from normal (two samples); (B) fragmentary (two samples); (C) totally lacking (five samples). Panels A and C have the same magnification as B (scale bar = 0.5  $\mu\text{m}$ ).

and now in nail as well. In two cases of LI having essentially normal nail CE structures, possibly the loss of cross-linking activity was more profound in the epidermis than in the nail. Deficiency of TGM1 clearly resulted in significant abnormalities in nail structure in seven of nine LI samples, but considerable insoluble material, possibly due to residual cross-linking activity, remained even after the extensive detergent extraction. The lack of nuclear remnants in two samples also lacking even partial envelope structures could reflect a more profound lack of TGM1 activity. Alternatively, the residual nuclear structures visible in most of the LI samples could be indicative of another, largely independent cross-linking activity that is present also in hair, where it is responsible for conspicuous nuclear remnants in the medulla.<sup>12</sup> As hypothesized as well for the epidermis, a



**Figure 3.** Nuclear remnant (scale bar = 1  $\mu$ m).

deficit in some other critical envelope component could also give rise to a syndrome characterized by defective CE structures.

CEs of the nail plate, where the corneocytes typically are highly convoluted and have numerous interlocking processes, probably provide considerable intercellular cohesiveness. While none of the samples examined disintegrated under the stress of extraction, nails from patients with LI appeared to disaggregate partially during rinsing with water before fixing and embedding. The nail changes were clinically non-specific, showing hyperplastic features including not only roughness and opacity, but also coarseness and thickening of the nail plate. These observations suggest that the keratin network and residual cross-linking in nails of patients with LI confer sufficient stability to resist ordinary stresses. However, the CEs do comprise a type of adhesive factor, and CE defects could account for the increased nail brittleness in LI similar to that observed in the general population.<sup>16</sup>

Progress in understanding the genetic basis for clinical aberrations in the ichthyoses is continuing.<sup>17</sup> Meanwhile, recent diagnostic improvements have simplified the provisional diagnosis of TGM1-negative LI.<sup>18</sup> Fragile or absent CE in scale has been proposed as a rapid and simple diagnostic test for this syndrome.<sup>19</sup> However, scale samples from some patients show residual envelope-like material, albeit fragmented and

attenuated, probably reflecting the ability of other transglutaminases in LI epidermis to assemble a partial CE and complete corneocyte lipid envelope.<sup>10</sup> As nail does not express lamellar bodies from which the corneocyte lipid envelope originates, LI nail lacked this feature, apparently making the CE more susceptible to disaggregation. Moreover, not only the residual CE, but also the remnant nuclei visible in the nails from other ichthyoses and in normal nail provide a further striking contrast to the featureless amorphous matrix that remained after comparable extraction of two of nine LI nail samples. The present detergent extraction method permits ready observation of structural variation in epidermal appendages that would be difficult to detect otherwise. Thus, nail clipping samples, which are non-invasive, can be utilized for the diagnosis of envelope defects in LI.

### Acknowledgments

We thank Dr Mary L. Williams for providing congenital ichthyosiform erythroderma nail samples, Viviana J. Wong and Felix R. Li for embedding some samples, and Michael R. Dunlap for transmission electron microscopy of some samples. This work was supported by USPHS grants AR19098, AR27130, AR39448, ES04699 and ES05707.

### References

- 1 Daniel CR, Scher RK. Nail changes caused by systemic drugs or ingestants. *Dermatol Clin* 1985; **3**: 491–500.
- 2 Samman PD, Fenton DA. *The Nails in Disease*. Oxford: Butterworth-Heinemann, 1995.
- 3 Fuchs E. Of mice and men: genetic disorders of the cytoskeleton. *Mol Biol Cell* 1997; **8**: 189–203.
- 4 Winter H, Rogers MA, Langbein L *et al*. Mutations in the hair cortex keratin hHb6 cause the inherited hair disease monilethrix. *Nat Genet* 1997; **16**: 372–4.
- 5 Bowden PE, Haley JL, Kinsky A *et al*. Mutation of a type II keratin gene (K6a) in pachyonychia congenita. *Nat Genet* 1995; **10**: 363–5.
- 6 McLean WHI, Rugg EL, Lunny DP *et al*. Keratin 16 and keratin 17 mutations cause pachyonychia congenita. *Nat Genet* 1995; **9**: 273–8.
- 7 Rice RH, Mehrpouyan M, Qin Q, Phillips MA. Transglutaminases in keratinocytes. In: *The Keratinocyte Handbook* (Leigh I, Lane B, Watt FM, eds). Cambridge: Cambridge University Press, 1994: 259–74.
- 8 Huber M, Rettler I, Bernasconi K *et al*. Mutations of keratinocyte transglutaminase in lamellar ichthyosis. *Science* 1995; **267**: 525–8.
- 9 Russell LJ, DiGiovanna JJ, Rogers GR *et al*. Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. *Nat Genet* 1995; **9**: 279–83.

- 10 Elias PM, Schmuth M, Uchida Y *et al.* Basis for the permeability barrier abnormality in lamellar ichthyosis. *Exp Dermatol* 2002; **11**: 248–56.
- 11 Kuramoto N, Takizawa T, Takizawa T *et al.* Development of ichthyosiform skin compensates for defective permeability barrier function in mice lacking transglutaminase 1. *J Clin Invest* 2002; **109**: 243–50.
- 12 Rice RH, Wong VJ, Pinkerton KE. Ultrastructural visualization of cross-linked protein features in epidermal appendages. *J Cell Sci* 1994; **107**: 1985–92.
- 13 Choate KA, Williams ML, Khavari PA. Abnormal transglutaminase 1 expression pattern in a subset of patients with erythrodermic autosomal recessive ichthyosis. *J Invest Dermatol* 1998; **110**: 8–12.
- 14 Korge BP, Ishida-Yamamoto A, Punter C *et al.* Loricrin mutation in Vohwinkel's keratoderma is unique to the variant with ichthyosis. *J Invest Dermatol* 1997; **109**: 604–10.
- 15 Rice RH, Wong VJ, Price VH *et al.* Cuticle cell defects in lamellar ichthyosis hair and anomalous hair shaft syndromes visualized after detergent extraction. *Anat Rec* 1996; **246**: 433–40.
- 16 Kechijian P. Brittle fingernails. *Dermatol Clin* 1985; **3**: 421–9.
- 17 Lefèvre C, Jobard F, Caux F *et al.* Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin–Dorfman syndrome. *Am J Hum Genet* 2001; **69**: 1002–12.
- 18 Hohl D, Aeschlimann D, Huber M. *In vitro* and rapid *in situ* transglutaminase assays for congenital ichthyoses—a comparative study. *J Invest Dermatol* 1998; **110**: 268–71.
- 19 Jeon S, Djian P, Green H. Inability of keratinocytes lacking their specific transglutaminase to form cross-linked envelopes: absence of envelopes as a simple diagnostic test for lamellar ichthyosis. *Proc Natl Acad Sci USA* 1998; **95**: 687–90.