Lamellar Ichthyosis Is Genetically Heterogeneous—Cases with Normal Keratinocyte Transglutaminase

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We recently identified mutations of the keratinocyte transglutaminase gene as a cause of lamellar ichthyosis. In this study we analyzed two sporadic cases of lamellar ichthyosis. Transglutaminase activity measured in membrane extracts from cultured differentiating keratinocytes was within the range observed in normal individuals. Western blot and Northern blot analysis revealed normal size and quantities of keratinocyte transglutaminase protein and mRNA. Sequencing of the 15 exons and their flanking regions demonstrated no deviation from the published sequence except for two silent polymorphisms. These results exclude mutations of keratinocyte transglutaminase as a cause for lamellar ichthyosis in these patients, indicating that lamellar ichthyosis is a genetically heterogeneous disorder. ***Key words: cornified cell envelope/ichthyosis congenita/non-bullous ichthyosiform erythroderma. J Invest Dermatol 105:653–654, 1995***

Recently, we and others showed by immunohistochemistry that some patients suffering from lamellar ichthyosis (LI, ichthyosis congenita, non-bullous congential ichthyosiform erythroderma) completely lack keratinocyte transglutaminase (TGK) expression [1,2]. Furthermore, membrane-bound TGK activity was strongly reduced in cultured keratinocytes from five LI patients due to aberrant TGK mRNA and/or protein synthesis. Direct sequencing of polymerase chain reaction–amplified exons of the TGK gene in these patients revealed mutations that co-segregated with the disease in their families and produced either prematurely terminated proteins or changed highly conserved amino acid residues [3]. Additionally, a genetic linkage analysis in 13 LI families mapped the disease locus to a 9.3-cM region on chromosome 14q11 [4]; in two of these families mutations in the TGK gene were identified [5]. These data provided strong evidence that mutations in TGK can cause LI. Hence, the reduced ability of TGK to crosslink precursor proteins and to form a functional cornified envelope (CE) appears to cause disturbed epidermal homeostasis resulting in generalized hyperkeratosis characteristic of the disease.

In this report, we present two patients with sporadic LI and normal transglutaminase activity, TGK mRNA, and protein synthesis, and no mutation in their TGK genes. This indicates that LI is genetically heterogeneous.

MATERIALS AND METHODS

Both LI-4.5 and LI-5.3 were sporadic cases with a phenotype of LI **(Fig 1)**. Cosanguinity was suspected in family LI-4 of Yugoslavian origin but not evident in the Swiss family LI-5. LI-5.3 was born as a collodion baby, LI-4.5 with non-bullous erythroderma. At 2 weeks of age, both presented as generalized erythematous LI with minor (LI-5.3) and without (LI-4.5) palmoplantar involvement. Histologically, both patients showed acanthosis, orthohyperkeratosis, and a normal granular layer. Cultured keratinocytes from shave biopsies were established and harvested for isolation of DNA, RNA, and protein as described [3]. Immunoblotting, transglutaminase assays, Northern blot analysis, polymerase chain reaction, and DNA sequencing was carried out as described [3,6,7].

RESULTS AND DISCUSSION

The transglutaminase activities in membrane extracts from differentiating keratinocytes of both patients were indistinguishable from those of unaffected individuals **(Table I)**. TG activities in the cytosolic fraction were slightly diminished compared with normal individuals **(Table I)**. Furthermore, normal amounts and sizes of TGK mRNA and protein were found in cultured cells from LI-4.5 and LI-5.3 **(Fig 2)**. DNA sequence analysis of the 15 exons and flanking regions of the TGK gene revealed no changes affecting the deduced amino acid sequence of TGK in both cases. LI-4.5 showed a heterozygous G to C change at position +3365, one nucleotide upstream of the AG of the splice acceptor site of intron 5 (data not shown). Patient LI-5.3 had a heterozygous G to A transversion at position +2698 in exon 4 (data not shown). Both codons, GAG and GAA, encode glutamic acid. These polymorphisms were not identified in 20 alleles sequenced as controls.

Our investigation revealing normal enzyme activity, mRNA and protein synthesis and normal sequences exclude TGK as a candidate gene in the two patients investigated. Thus, LI is genetically heterogeneous. Our results are supported by a recent report of another LI patient with normal TGK immunohistochemistry [2] and, more importantly, a genetic linkage analysis of 15 German LI families. Linkage to the TGM locus on chromosome 14q11 has been excluded conclusively in five of these families (A. Reis, H.C. Hennies, and W. Küster, personal communication). Despite its excessive rarity, autosomal dominant transmission of LI is difficult to exclude formally in our two cases with sporadic LI. However, the clinal phenotype and the histologic picture observed in both patients are unusual for the autosomal dominant variant of LI [8]. Experiments are underway to define the molecular defect(s) in LI cases with normal TGK.

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Abbreviations: CE, cornified cell envelope; LI, lamellar ichthyosis; TGK, keratinocyte transglutaminase.

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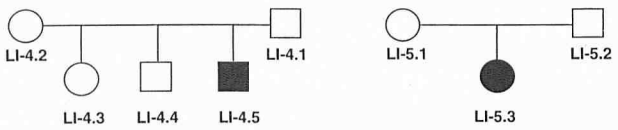

Figure 1. Pedigrees of families LI-4 and LI-5. *Black symbol*, affected person; *open symbol*, unaffected person.

Table I. TG Activity in Keratinocytes from LI-4 and LI-5

Sample	Activity[a] Cytosolic ($\times 10^{-4}$)	Membrane ($\times 10^{-4}$)	Cytosolic:Membrane
LI-4.5	12.9 ± 3.2	192 ± 43	0.07
LI-5.3	11.1 ± 6.5	204 ± 35	0.05
N1[b]	29.3 ± 2.4	247 ± 57	0.11
N2[b]	19.2 ± 3.2	221 ± 97	0.09

[a] Activity is presented as dpm/min per mg protein (3). Results are given as mean ± SD from two different cell passages measured in duplicate.
[b] N1 and N2, healthy controls.

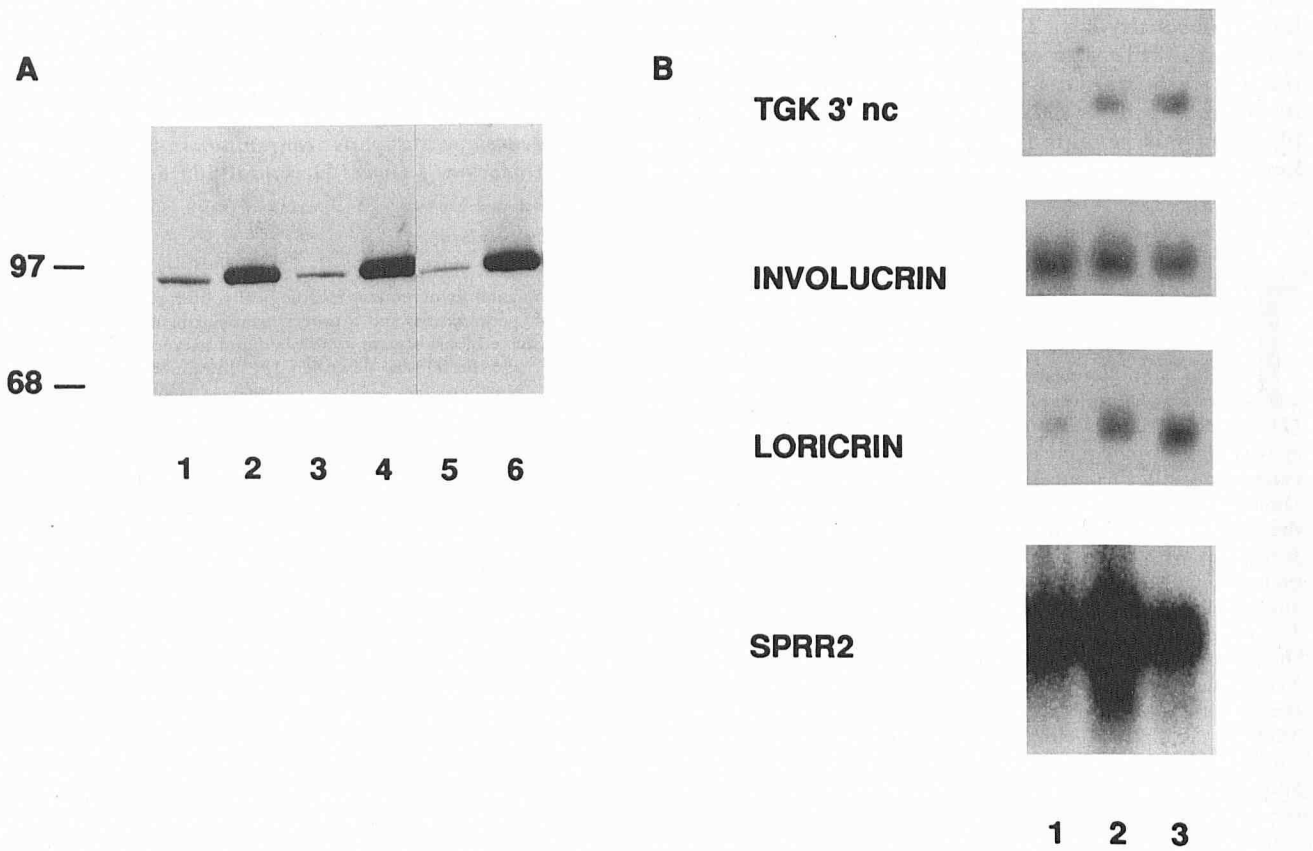

Figure 2. Normal protein and mRNA synthesis in lamellar ichthyosis patients. *A*) Immunoblot analysis using B.C1 antibodies of cytosol (*lanes 1, 3, and 5*) and membrane (*lanes 2, 4, and 6*) extracts from cultured keratinocytes of unaffected individual (*lanes 1 and 2*), patient LI-5.3 (*lanes 3 and 4*), and patient LI-4.5 (*lanes 5 and 6*) reveal normal TGK accumulation. Molecular weight markers are indicated on the left (in kilodaltons). *B*) Northern blot analysis of total RNA from differentiating cultured keratinocytes of unaffected individual (*lane 1*), patient LI-4.5 (*lane 2*), and patient LI-5.3 (*lane 3*) demonstrated mRNAs comparable in size and quantities.

Note Added in Proof: A recent report on linkage analysis of autosomal recessive lamellar ichthyosis (Parmentier L, *et al*: *Hum Mol Genet* 4:1391–1395, 1995) provides further evidence for its genetic heterogeneity.

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