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ORIGINAL PAPER

Strong founder effect for a transglutaminase 1 gene mutation in lamellar ichthyosis and congenital ichthyosiform erythroderma from Norway

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Autosomal recessive congenital ichtyosis (ARCI) is a clinically heterogeneous disorder of keratinisation. It was recently shown that mutations in the *transglutaminase 1* (*TGM1*) gene may be associated with the clinical subtypes lamellar ichthyosis (LI) and non-bullous congenital ichthyosiform erythroderma (CIE). Thirty-six Norwegian families with LI and seven with non-bullous CIE were studied with microsatellite markers linked to the *TGMI* gene. One common halpotype for two markers was found on 74% of disease associated chromosomes. Three individuals homozygous for the common haplotype, two affected by LI and one affected by CIE, were analysed for mutations in the *TGM1* gene. All three patients were found homozygous for a single A to G transition located in the canonical splice acceptor site of intron 5. Probands from the remaining 40 families with LI and CIE were screened for this mutation and the A to G transition was found on 61 out of 72 alleles associated with LI and on 9 out of 15 alleles associated with CIE. These findings suggest a single founder mutation for the majority of patients with LI and CIE in Norway. The 2526A□ G mutation results in the insertion of a guanosine at position 877 (876insG) in the mature cDNA and the frame shift creates a premature termination at codon 293. The mutation was previously observed in one family with a resulting cDNA that included the entire intron 5. These results suggest that the mutation can result in variant transcripts in different individuals.

Keywords: lamellar ichthyosis; non-bullous congenital ichthyosiform erythroderma; *transglutaminase 1 (TGM1)* gene; founder effect

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Introduction

Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of inherited disorders [1] that affect the keratinisation process in the skin. Lamellar ichthyosis (LI) and nonbullous congenital ichthyosiform erythroderma (CIE) are two clinical ARCI subtypes characterised by scaling of the skin. The patients can be born as collodion babies, a membrane which covers the entire body and which cracks after a few days. Everted lips (eclabium) and everted eyelids (ectropion) can be seen in a proportion of the cases.[2,3] The subtype CIE presents with erythroderma and fine white scaling of the skin, whilst LI presents without or with minimal erythroderma and with dark, platelike scales covering the body.[1,3–5] Autosomal recessive ichthyosis (MIM no.242300) can also be classified into four subgroups (type I–IV) based on electron microscopic (EM) features of epidermis.[5–7] According to this classification CIE corresponds to EM type I and LI corresponds to EM type II, respectively.

Linkage analysis has localised the gene for LI to chromosome 14q11[8] and close to the epidermal *transglutaminase 1* (*TGM1*) gene locus.[9–11] Sequence analysis of LI patients revealed mutations in the *TGM1* gene associated with the disease.[12–17] Transglutaminase 1 is an enzyme which is active during terminal differentiation of keratinocytes and is important for the formation of the cornified envelope by a cross-linking reaction of precursor proteins such as loricrin and involucrin. However, a normal transglutaminase activity and a normal *TGM1* gene sequence were found in a proportion of patients with LI indicating genetic heterogeneity.[14,18] This was previously suggested from the ultrastructure analysis of skin biopsies[6,7] and recently confirmed by the identification of a second locus for LI on chromosome 2q.[19] There is evidence of yet a third locus for LI in some families linked to neither *TGM1* nor chromosome 2q.

We present here the combined results from clinical, ultrastructural, and molecular analyses of 43 Norwegian families affected by LI or CIE. The study included clinical examinations, electron microscopy of skin biopsies, analyses with microsatellite markers linked to the *TGM1* gene, and mutation analysis of the *TGM1* gene.

Materials and Methods

Patients and Families

Forty-three Norwegian families comprising 55 members affected by congenital (1st day) onset of LI or CIE were studied. Patients were identified from the past 27 years and blood samples were collected. The patients were examined clinically by one of the authors (TG-D) with the exception of two cases that were examined by local dermatologists (Table 1). A family history of collodion skin supported the diagnosis but this was not taken as a mandatory criterion. Nine patients were clinically evaluated after systemic retinoid treatment had been started. Skin biopsy samples from various body sites (gluteal region, back, upper arm) were prepared for transmission EM.[7] The ultrastructural evaluation (Figure 1, Table 1) was performed by two of the authors (IA-L and IH). In eight families EM evaluation was not performed and the diagnosis was based on the clinical evaluation alone.[4] The geographical origin of the parents of affected individuals was determined. Genealogical investigations identified a common ancestry of four families from West Norway and also of two families in an isolated valley of East Norway. One parent was an immigrant from Germany. The study was approved by the Ethical Committee of Uppsala University, Sweden.

DNA Samples and Haplotype Analysis

Genomic DNA was extracted from peripheral blood cells with phenol-chloroform[20] or by the salt extraction method.[21] Highly polymorphic DNA markers assigned to chromosome 14q11, and linked to the TGM1 locus, were used for allelic association and for haplotype analysis. The polymorphic dinucleotide repeat markers D14S64, D14S264, and D14S275[22] were analysed by radioactivity as described for other microsatellite markers.[23] The intragenic TGM1 intron 14 dinucleotide repeat[10] was typed and visualised by flourescent labelling using an ABI automatic sequencer according to Dupuy and Olaisen.[24] The allele frequencies for the markers used were determined in the Norwegian population. The number of control chromosomes were 304, 96, 101 and 104 for the markers TGM1 intron 14, D14S64, D14S264, and D14S275, respectively. The test for pairwise allelic association was determined by the chi-square method.

cDNA Synthesis and Sequencing

The entire protein coding region of the *TGM1* gene was sequenced from cDNA (exon 2–9) and from genomic DNA (exon 10–15).

Skin fibroblast cultures were established from three patients, one affected by CIE (IR 26) and two affected by LI (IR 1 and IR 13, Table 1). Total RNA was extracted either immediately after harvest or after the cells had been stored at –70°C in guanidinium thiocyanate.[25] For reverse transcription (RT), approximately 5 μg of total RNA was used in the presence of 5 pmol primer (5'-GAA CTC CCT GGA TGA CAA TG) specific for the TGM1 mRNA. After a denaturation step at 70°C for 10 min, the RT reaction was performed in a total volume of 20 μl with 2.5 units of AMV reverse transcriptase (Pharmacia Biotech, Uppsala, Sweden), 0.03 units of RNAse inhibitor (RNAguard, Pharmacia Biotech, Uppsala, Sweden), 10 mM Tris (pH 8.3), 5 mM $MgCl_2$, 1 mM of each deoxynucleotide triphosphate (dNTP) and incubated at 42°C for 1 h followed by 70°C for 10 min. The RT reaction was followed by PCR performed with three different sets of primer pairs. The three resulting amplicons corresponded to nt (–44)– 568, nt 454–1136 and nt 985–1646, respectively, of the TGM1 cDNA (GenBank accession number M90287). The PCR (94°, 1 min; 63°, 1 min; 72°, 2.5 min) was performed in a total volume of 40 μl using 5 μl of the RT reaction, 200 mM of

each dNTP, 1.5 mM $MgCl_2$, 2 units of Taq polymerase and 0.1 mM of each primer and run for 25 cycles. Nested PCR was performed with three internal sets of primers in a total volume of 100 µl with 1 µl of the corresponding RT–PCR reaction diluted 100–500 times. Primers used for the sequencing reactions were the same as used for nested PCR. Two amplicons containing exons 10–14 and exon 15, respectively, were generated by PCR using genomic DNA from the three patients. The fragment containing exons 10–14 was amplified using primers 5'-CTG GGG CCT GTG TGG ACC TTA C and 5'-TGG GGA AGG CCA GAG TGG AAG CA, and exon 15 using primers 5'-AGC TCT TAC TCC CCA CTC CAC and 5'-GGC TCC ATC CGT CTT GGT GTG. A fragment corresponding to exon 5, intron 5 and exon 6 of the

Table 1 Results of clinical and ultrastructural evaluation, haplotyping and mutation analysis in LI and CIE patients from Norway

			Marker alleles				
Proband	*Status*	*EM*	*TGM1*	*D14S64*	*D14S264*	*D14S275*	*Mutations*
IR 1	L1	2	5/5	2/2	5/5	2/2	A2526G/A2526G
IR 2	LI	U	5/5	2/2	5/0	4/2	A2526G/A2526G
IR 3	LI (Ret.)	Nt	5/5	3/1	1/4	3/1	–/–
IR 4	LI	2	5/5	2/2	3/5	5/4	A2526G/A2526G
IR 5	LI	2	5/5	2/2	5/4	2/4	A2526G/A2526G
IR 6	CIE (Ret.)	1	5/5	2/3	5/4	4/2	A2526G/–
IR 7	LI	Nt	5/5	3/3	4/5	2/4	–/–
IR 9	LI	U	5/5	2/2	4/4	1/3	A2526G/A2526G
IR 12	LI	2	5/5	2/3	4/4	1/3	A2526G/A2526G
IR 13	LI	2	5/5	2/2	5/5	2/2	A2526G/A2526G
IR 19	LI	2	5/5	2/2	5/5	3/4	A2526G/A2526G
IR 20	LI	2	5/5	2/2	3/3	4/5	A2526G/A2526G
IR 24	LI	2	5/5	2/2	4/5	5/3	A2526G/A2526G
IR 25	LI	Nt	5/5	2/2	5/5	2/2	A2526G/A2526G
IR 26	CIE	U	5/5	2/2	5/5	2/2	A2526G/A2526G
IR 27	LI	2	7/5	3/2	1/5	5/2	–/A2526G
IR 31	LI	Nt	5/5	2/2	3/5	4/3	A2526G/A2526G
IR 32	LI	2	5/5	2/2	$4/10^{i+}$	4/5	A2526G/A2526G
IR 33	LI	2	5/5	2/2	5/5	2/3	A2526G/A2526G
IR 37	LI (Ret.)	2	5/5	3/3	2/7	5/5	–/–
IR 39	LI	2	5/5	2/2	3/3	3/4	A2526G/A2526G
IR 40	CIE	1	5/5	2/2	3/4	5.4	A2526G/A2526G
IR 42	LI	2	5/5	2/2	5/5	3/3	A2526G/A2526G
IR 43	LI	Nt	5/5	2/2	5/5	3/3	A2526G/A2526G
IR 44	LI	Nt	5/5	3/3	2/2	4/4	–/–
IR 45	LI	Nt	5/5	2/2	5/5	4/2	A2526G/A2526G
IR 46	LI	U	5/5	$-1^{ii}/2$	5/5	5/2	A2526G/A2526G
IR 49	LI	2	5/5	2/4	3/0	2/4	A2526G/A2526G
IR 51;1	CIE (Ret.)	1	5/5	3/3	3/3	1/3	–/–
IR 51;2	CIE (Ret.)	1	1/5	3/3	3/3	1/3	–/–
IR 52	LI (Ret.)	U	5/5	2/2	5/5	2/2	A2526G/A2526G
IR 58	LI (Ret.)	2	5/5	2/2	3/5	4/5	A2526G/A2526G
IR 59	CIE*	2	1/5	3/3	4/4	4/2	–/–
IR 62	LI	2	5#/5	1#/3	4#/3	5#/2	–#/A22526G
IR 68	LI	2	5/5	2/2	5/4	2/4	A2526G/A2526G
IR 70	LI	2	5/5	2/2	5/5	2/4	A2526G/A2526G
IR 74	CIE (Ret.)	1	5.5	2/5	3/4	4/5	A2526G/A2526G
IR 75	LI	2	5/5	2/2	5/3	2/4	A2526G/A2526G
IR 76	LI	2	5/5	$9^{iii+}/3$	3/4	4/2	A2526G/–
IR 77	LI	2	5/5	2/2	3/3	5/5	A2526G/A2526G
IR 92	CIE*	2	5/5	2/2	3/5	2/5	A2526G/A2526G
IR 93	LI† (Ret.)	2	5/5	2/2	3/3	3/4	A2526G/A2526G
IR 94	LI†	Nt	5/5	2/3	5/5	2/5	A2526G/A2526G
IR 95	LI	2	5/5	2/2	Nt/Nt	Nt/Nt	A2526G/A2526G

(Ret.) retinoic acid treatment by the time of diagnosis and biopsy taken for EM.
U: unspecific. Nt: not typed. *CIE phenotypes as infants, but bordering LI phenotypes with age. †: examined by local dermatologists. #: German haplotype. [i]: *de novo* mutation in D14S264, paternal or maternal 4→5 mutation. [ii]: inherited or new mutation. [iii]: paternal *de novo* mutation. +: non-paternity was ruled out. /: parental origin.

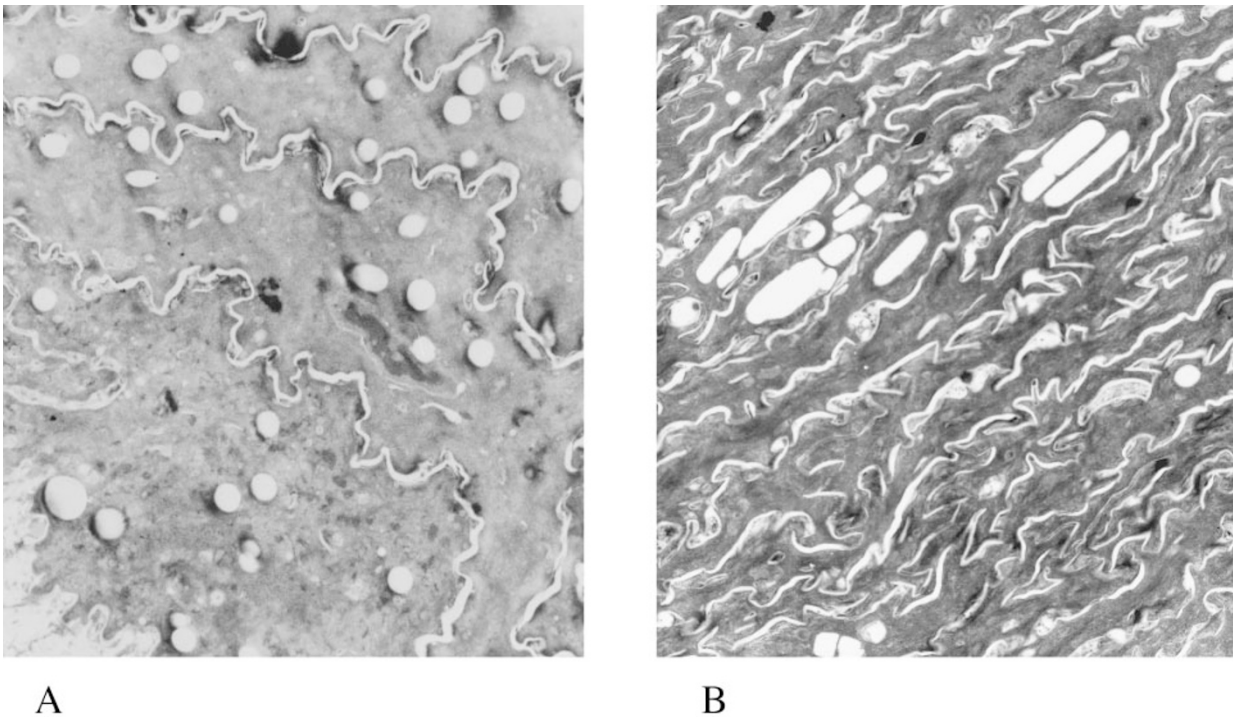

Figure 1 *Ultrastructural features of skin biopsies (magnification approximately × 9 500) from* **A** *a patient with CIE, EM type I and from* **B** *a patient with LI, EM type II. EM type I is characterised by a compact hyperkeratotic horny layer with numerous lipid vacuoles. EM type II is characterised by a massive compact hyperkeratotic horny layer with groups of electron-lucent clefts presenting remnants of cholesterol crystals as a significant diagnostic marker.*

TGM1 gene (nt 2234–2640; GenBank accession number M86360) was amplified in order to confirm the results from the cDNA sequencing. Cycle sequencing was performed on amplicons derived from cDNA and genomic DNA after gel purification on a 1% low melting point agarose gel (Sea plaque, FMC BioProducts, ME, USA). The gel slices were treated with agarase (Boeringer Mannheim GmbH, Mannheim, Germany) following the manufacturers' protocol. Cycle sequencing was performed with the Dye Terminator Cycle Sequencing Core Kit (Perkin Elmer, CA, USA) and analysed on an automated ABI 373 DNA Sequencer.

Restriction Enzyme Analysis

The A to G mutation found in intron 5 creates a new restriction site for *Msp*I on genomic DNA. The presence of the new restriction site was analysed in all 43 families including 55 patients with LI or CIE. A 150 bp DNA fragment spanning the mutation was amplified using primers 5'-GGC TGG GGT ATT GGA GCA GG (forward), and 5'-TGG GTC TCC ACG GCC TCC AT (reverse). The PCR reaction (94°, 30 s; 63°, 30 s; 72°, 30 s) was run for 30 cycles in a total volume of 100 µl. Restriction enzyme digestion of the PCR fragments was performed in a total volume of 30 µl with 30 units of *Msp*I (New England Biolabs, MA, USA) and the buffer supplied. The resulting fragments were separated by electrophoresis on a 12% polyacrylamide gel and visualised by ethidium bromide.

Results

Electron Microscopy (EM)

Ultrastructural diagnosis by EM[7] was performed on dermal specimens from 35 families. The skin biopsy samples were from various body sites including the gluteal region, back and upper arm. Patients from 26 families showed the EM features of type II and patients from four families were classified as EM type I (Figure 1; Table 1). In five families the ultrastructural morphology was unspecific and did not permit EM classification and in eight families biopsies were not taken.

The correlation between the clinical classification and EM classification was consistent except in two cases where the patients initially had CIE phenotypes but bordered on LI phenotypes with age (IR 59 and IR 92, Table 1).

Allelic Association and Haplotype Analysis

Haplotypes over the *TGM1* locus at chromosome 14q11 were constructed with the microsatellite markers D14S64, D14S264, D14S275[22] and the STR marker

Table 2 Marker alleles around the *TGM1* gene in 43 Norwegian families with LI and CIE. Shaded area shows the haplotypes associated with the 2526A → G mutation

Number of chrom.	*Marker alleles*				*Nucleotide*
	TGM1	*D14S64*	*D14S264*	*D14S275*	*2526*
2	5	2	Nt	Nt	G
19	5	2	5	2	G
16	5	2	5	3, 4, 5	G
1	5	–1[i]	5	5	G
1	5	2	10[ii]	5	G
4	5	2	4	4	G
4	5	2	4	1, 2, 3, 5	G
8	5	2	3	4	G
9	5	2	3	2, 3, 5	G
1	5	2	0	2	G
1	5	9[iii]	3	4	G
1	5	3	5	5	G
1	5	3	4	3	G
1	5	4	0	4	G
1	5	5	4	5	G
#1	7	3	1	5	A
#1[iv]	5	1	4	5	A
#2	5	3	4	2	A
2	5	3	4	2	A
1	1	3	4	4	A
6	5	3	2, 1, 7, 3, 5	4, 3, 5, 3, 4	A
1	5	1	4	1	A
2	5	3	3	1, 3	A
1	1	3	4	1	A

#: haplotypes in patients heterozygous for the 2626A → G mutation. Nt: not typed. [i]: inherited or new mutation. [ii]: *de novo* mutation. [iii]: paternal *de novo* mutation. [iv]: German haplotype.

within the *TGM1* gene.[10] The markers span 4.2 cM and their order from the centromere is (TGM1/D14S64/D14S264), D14S275.[8,22] The haplotypes found in the LI and CIE patients are presented in (Table 2). A common haplotype (5–2–5) was identified for the three markers TGM1, D14S64 and D14S264 on 35 out of 87 chromosomes (37%) associated with LI and CIE, versus two out of 90 (2%) on control chromosomes. The chi-square test revealed a significant association between the allele 5 (177 bp) at the TGM1 intron 14 marker locus and LI/CIE ($P < 10^{-6}$), between D14S64 allele 2 (131 bp) and LI/CIE ($P < 10^{-6}$), and between D14S264 allele 5 (243 bp) and LI/CIE ($P < 1.5 \times 10^{-4}$), respectively. In contrast, no associations were found between the corresponding pairs of loci among Norwegian control chromosomes. The combined results from allele association tests and haplotypes of LI/CIE chromosomes suggest a founder effect for a TGM1 mutation. The results also support the locations of the three anonymous markers D14S64, D14S264 and D14S275 on the same side of the *TGM1* gene in the order 14qcen–TGM1–D14S64–D14S264–D14S275–tel.

Sequence Analysis of the TGM1 *Gene and the TGM1 cDNA*

Direct sequencing was performed on cDNA and genomic DNA from three non-related individuals, one affected by CIE (IR26) and two affected by LI (IR 1 and IR 13, Table 1). The patients were homozygous for the haplotype 5–2–5 (TGM1, D14S64, D14S264) associated with LI/CIE in the population studied. Sequencing of TGM1-specific cDNA revealed homozygosity for an insertion of guanosine at position 877 (876insG). The results were verified on genomic DNA of the three patients and revealed a homozygous 2526A G transition (Figure 2) located in the canonical acceptor splice site of intron 5. The cDNA analysis shows that the mutation results in an alternate splicing with the guanosine at position 2526 of intron 5 retained in the spliced transcript. As a consequence, the reading frame is altered and a premature stop codon (UGA) is created at codon 293. Sequencing of the entire protein coding region of the *TGM1* gene in the three patients revealed no other sequence alteration resulting in amino acid substitutions.

Genotyping

The A to G mutation in intron 5 creates a new recognition site for *Msp*I at the genomic level (Figure 3). A PCR fragment spanning the transition was generated from genomic DNA of probands from all 43 families. The resulting amplicon was digested with *Msp*I and the 2526A G mutation was found to be present in 37 out of the 43 probands. In 33 families the affected individuals were homozygous for the mutation and in the remaining four families the patients were heterozygous (Table 1). No clinical differentation could be made on the basis of homozygosity or heterozygosity for the mutation. The transition accounts for 80% (70 out of 87) of LI and CIE alleles in the Norwegian population studied (Figure 4). The mutation was absent in two CIE and four LI families, none of which carried the common haploptype 5–2–5 (TGM1–D14S64–D14S264). There was no strong correlation between the clinical/EM classification and the molecular findings (Table 1). Five patients with CIE and 32 patients with LI were found to have the specific mutation.

Discussion

In this study we show that a single mutation in the *TGM1* gene is responsible for the majority of patients

with LI and CIE in the Norwegian population. The 2526A→G transition was present on 80% of alleles. Genealogical investigations revealed common ancestors in only a few families and consanguinity over the last four generations was excluded in the majority of the families. However, the occurrence of a single ancestral mutation was supported by allelic association and by haplotype analysis. All 70 chromosomes with the 2526A→G mutation carried the same allele at the dinucleotide repeat in intron 14 of the *TGM1* gene. At the neighbouring locus D14S64, 64 out of 70 mutant chromosomes shared one allele, whereas 37 chromosomes shared one allele at D14S264. The more distal marker D14S275 showed a weaker association with only 20 chromosomes carrying 2526A→G and a common allele. The results suggest ancestral recombinations between the different *STR* loci and the *TGM1* gene. The degree of association for marker alleles with the 2526A→G mutation and the haplotypes constructed favours the relative order 14qcen–TGM1–D14S64–D14S264–D14S275–tel.

The observed incidence of LI and CIE in Norway is 1/91,000 over the last 10 years. The number of patients homozygous or compound heterozygous for the 2526A→G mutation were precisely determined over the last 30 years and the corresponding gene frequency for the mutation in Norway was calculated to 0.0022. This is slightly less than was expected (0.0033) from the observed incidence and the explanation may be due to a non-random distribution of the mutant gene.

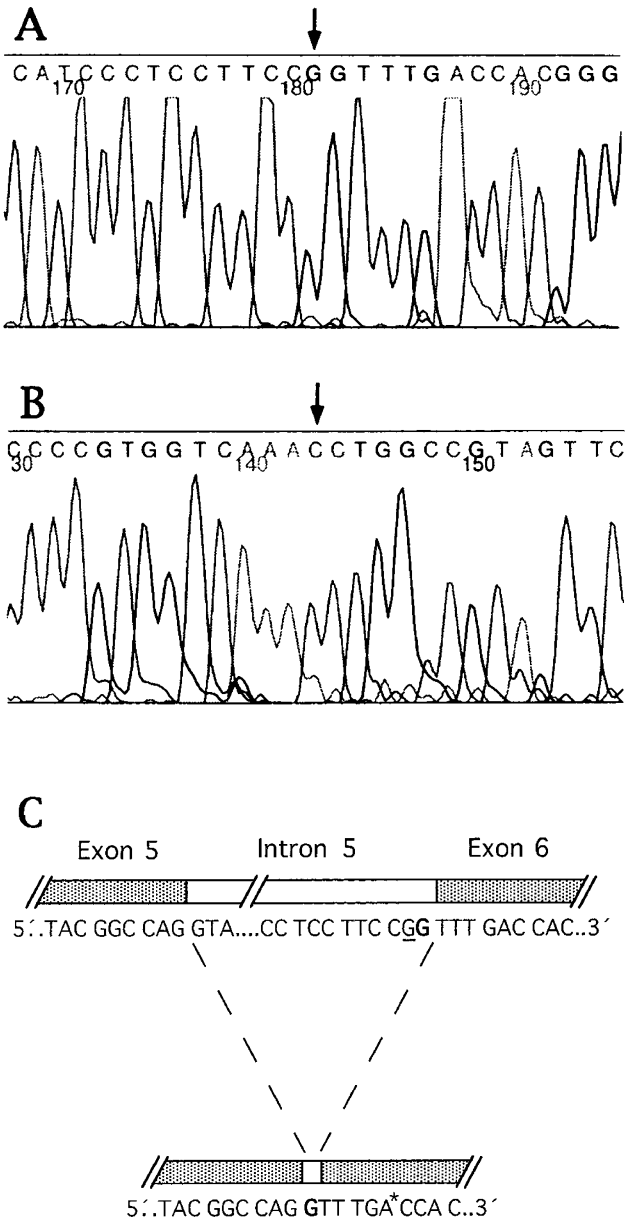

Figure 2 **A** *Print-out from automated sequencing of genomic DNA showing the 2526A→G mutation (arrow) in a homozygous state and* **B** *the cDNA sequence (sequenced by a reverse reaction) with the insertion of a G(C) at position 877 (arrow).* **C** *Schematic presentation of part of the* TGM1 *gene (upper bar) with the resulting cDNA (bottom bar) in patients with the 2526A→G mutation. The transition is underlined (top) and the position of the inserted guanosine in the cDNA is indicated in bold. The resulting frame shift is shown (bottom) and the stop codon is asterisked.*

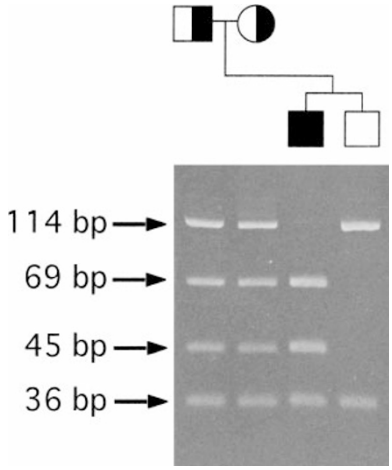

Figure 3 *Detection of the 2526A→G mutation by* Msp*I restriction digestion. An amplicon of 150 bp was generated from genomic DNA in one family with one member affected by CIE. The wild type fragment includes an* Msp*I site as an internal control which results in fragments of 114 bp and 36 bp, respectively. Accordingly, the amplicon with the A to G mutation carries two restriction sites for* Msp*I which results in three fragments of 69 bp, 45 bp and 36 bp, respectively. Lane 1–2, parents (heterozygous); lane 3, affected son (homozygous); and lane 4, a healthy non-carrier son.*

The mutation in the splice acceptor site of intron 5 was shown to result in 876insG in the cDNA. The same A to G transition was previously described on genomic DNA in another family with LI but with a resulting transcript that retained the complete intron 5 sequence.[12] In our patients, intron 5 is indeed spliced but with only one intronic nucleotide retained. Sequencing of cDNA from six Norwegian alleles consistently showed the 876insG mutation. The reason for the different mRNAs from the same genomic alteration is unclear. We did not find the longer and non-spliced transcript in our patients but we can not exclude that it was present in low amounts. It may also have been selected against in the PCR reactions. The strong founder effect of LI and CIE in Norway in combination with a previous report of an LI patient with the same 2426A→G transition suggest that other patients of European descent may carry the same gene defect. This has been shown for phenylketonuria, another recessive condition in which the mutant alleles in Norway are derived from other European countries.[26]

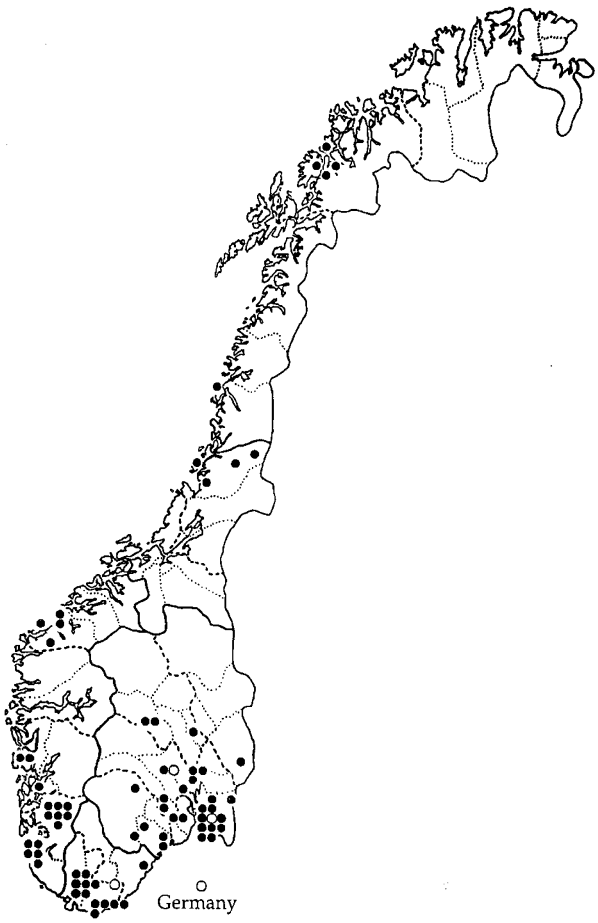

Figure 4 *Map of Norway with parents of CIE and LI patients plotted by their place of birth (parents' residences). Filled circles: 2526A→G carriers. Open circles: carriers of allelic TGM1 mutations according to the affected offspring which are heterozygous for the 2526A→G mutation. Density of the Norwegian population has previously been described by Eiken* et al *1996.*[26]

The A to G transition was absent to two families with CIE and in four families with LI. The haplotypes in these families were different from the majority of haplotypes associated with the mutation but they may carry yet unknown mutations in the *TGM1* gene. In one multiplex family affected by CIE, a *TGM1* gene mutation is not likely since a recombination event between the phenotype and the TGM1 intron 14 marker was seen (IR 51, Table 1). This also supports previous findings of genetic heterogeneity for the disorders.

Classification of the heterogenous group of autosomal recessive congenital ichthyosis has been difficult. The genetic analysis performed in this study revealed that a single mutation was responsible for the disease in the majority of cases with both LI and CIE. This illustrates the importance of combining molecular analyses with clinical and ultrastructural criteria for the primary diagnosis and for the classification of congenital ichthyosis. The results presented here show that a mutation in the *TGM1* gene can result in both LI and CIE. Although in some families the mutation remains unknown, the strong founder effect and an easy detection assay for the molecular defect provide an improvement for the diagnosis and for the detection of carriers in such families.

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