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Genotype/Phenotype Correlation in Autosomal Recessive Lamellar Ichthyosis

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Summary

Autosomal recessive lamellar ichthyosis is a severe congenital disorder of keratinization, characterized by variable erythema of the whole body surface and by different scaling patterns. Recently, mutations have been identified in patients with lamellar ichthyosis in the TGM1 gene coding for keratinocyte transglutaminase, and a second locus has been mapped to chromosome 2. We have now analyzed the genotype/phenotype correlation in a total of 14 families with lamellar ichthyosis. Linkage analyses using microsatellites in the region of the TGM1 gene confirmed genetic heterogeneity. In patients not linked to the TGM1 gene, the second region identified on chromosome 2 and a further candidate region on chromosome 20 were excluded, confirming as well the existence of at least three loci for lamellar ichthyosis. Sequence analyses of the TGM1 gene in families compatible with linkage to this locus revealed seven different missense mutations, five of these unpublished so far, and one splice mutation. No genotype/phenotype correlation for mutations in the TGM1 gene was found in this group of patients, which included two unrelated patients homozygous for the same mutation. Similarly, no clear difference in the clinical picture was seen between patients with TGM1 mutations and those unlinked to the TGM1 locus. Comparison of genetic and clinical classifications for patients with lamellar ichthyosis shows no consistency and thus indicates that clinical criteria currently in use cannot discriminate between the molecularly different forms of the disease.

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Introduction

Lamellar ichthyosis (LI) is a heterogeneous group of severe hereditary disorders of keratinization. Marked differences in the clinical picture and course of LI are observed (Traupe 1989). In most cases, LI is inherited autosomal recessively; a rare form, however, of autosomal dominantly inherited LI has been described as well (Traupe et al. 1984). In the autosomal recessive type of LI (McKusick 1994; MIM 242100), newborns often present a pergament-like collodion membrane. After losing this encasement, patients show a characteristic generalized scaling on the entire body, including scalp and flexural surfaces, and a variable erythema. Various classifications of LI forms have been proposed, according to clinical, histological, and ultrastructural parameters (reviewed in Traupe 1989). Depending on the finding of erythema, two major types of recessive LI were clinically discriminated (Hazell and Marks 1985; Williams and Elias 1986). The erythematous form of LI, also referred to as nonbullous congenital ichthyosiform erythroderma, is characterized by a strong redness of the entire body surface. Patients often also show an ectropion. In most cases, this form of LI is associated with a fine, whitish scaling of the skin and marked palmoplantar hyperkeratosis and hyperlinearity (Traupe 1989). In contrast, a nonerythematous form was described that often exhibits large dark scales and a rough, barklike pattern of the skin (Williams and Elias 1986). A divergent classification of patients with LI is obtained on the basis of histological and ultrastructural investigations. Five types of LI can be distinguished by assessment of ultrastructural depositions in the stratum corneum and of the patterns of membranes and lamellar structures (Anton-Lamprecht 1992).

The horny cells of the stratum corneum are characterized by a stable cell envelope formed of several components that are covalently bound to each other. These components include a number of proteins, such as involucrin, loricrin, and the small proline-rich proteins, along with lipids on the outer surface of the cell envelope. Keratinocyte transglutaminase (TGK), which catalyzes the formation of covalent ϵ-(γ-glutamyl)lysine

bonds, is involved in the cross-linking of structural proteins in the upper granular layer. TGK is encoded by the transglutaminase 1 gene (TGM1) on chromosome 14q11 (Kim et al. 1992; Phillips et al. 1992; Yamanishi et al. 1992). Moreover, epidermal transglutaminase, which is encoded by the transglutaminase 3 gene (TGM3), is present in suprabasal keratinocytes. TGM3 has been localized to chromosome 20q11-q12 (Gentile et al. 1994; Wang et al. 1994).

In patients with autosomal recessive LI, a lack of TGK expression has been shown (Hohl et al. 1993; Huber et al. 1995*a*; Lavrijsen and Maruyama 1995), and linkage of autosomal recessive LI to TGM1 has been described (Russell et al. 1994). Subsequently, mutations have been identified in the TGM1 gene in patients with LI (Huber et al. 1995*a*; Parmentier et al. 1995; Russell et al. 1995). In a number of families with autosomal recessive LI, however, mutations in the TGM1 gene could be ruled out either by showing normal TGK activity (Huber et al. 1995*b*; Lavrijsen and Maruyama 1995) or by excluding linkage to TGM1 (Parmentier et al. 1995; Bale et al. 1996). Furthermore, a second locus for LI (ICR2B) has recently been mapped, in three consanguineous families from Morocco, to a 6.6-cM interval between the markers at D2S325 and D2S137 on chromosome 2q33-q35 (Parmentier et al. 1996).

Here we present the investigation of a total of 14 families with clinically different forms of autosomal recessive LI. Our findings confirm that LI is genetically heterogeneous but do not show consistency of the molecular results with clinical classifications. Mutations in the TGM1 gene were identified in a number of LI patients, but no genotype/phenotype correlation could be derived.

Material and Methods

Patients with LI

A total of 14 families with autosomal recessive LI were analyzed clinically and molecularly. Thirteen of these originate from Germany, and one is of Moroccan extraction. In two families, only one patient each and the patient's parents were available. All others are two- or three-generation families with one or two affected individuals. The family from Morocco is consanguineous, an unresolved relationship is remembered in one of the German families, and the parents of all other patients are unrelated.

Microsatellite Analysis

DNA was prepared according to standard methods, from blood samples drawn from consenting individuals. Linkage analysis on chromosome 14 was performed with the microsatellite located within the TGM1 gene (Compton et al. 1992) and with two microsatellites at anonymous loci, D14S64 and D14S264 (Dib et al. 1996), closely linked to TGM1. On chromosome 20, microsatellite markers at D20S101, D20S107, and D20S119 were analyzed. Genotyped markers on chromosome 2 are at D2S116, D2S325, D2S157, and D2S143 (Dib et al. 1996). Analyses were performed by use of an automated DNA sequencer (ALF, Pharmacia, or 373, Perkin Elmer). Microsatellites were amplified in 15-μl PCR mixtures containing 30 ng DNA, 0.33 μM of each primer, one of which was end-labeled with fluorescent dye, 0.1 mM of each dNTP, and 0.5 U *Taq* DNA polymerase (Perkin Elmer) in 50 mM KCl, 10 mM Tris/HCl (pH 8.0), and 1.5 mM $MgCl_2$. Reactions consisted of 27 cycles of 15 s at 94°C, 15 s at annealing temperature, and 30 s at 72°C. PCR products were separated on 6.6% polyacrylamide sequencing gels and were analyzed by use of the Fragment Manager (Pharmacia) or the Genotyper software (Perkin Elmer).

Linkage Analysis

Linkage analyses were performed with the computer programs in the LINKAGE package, version 5.1 (Lathrop et al. 1984). Independent segregation of the phenotype in question and a locus analyzed is considered significant for LOD scores $Z < -2$. Autosomal recessive inheritance with complete penetrance was assumed in all LI families investigated.

Mutation Analysis

The translated exons 2–15 of the TGM1 gene were amplified by PCR with intronic primers designed according to published TGM1 sequences (Kim et al. 1992; Phillips et al. 1992). DNA was amplified by PCR as described above. For SSCP analyses, ~10% of the volume of the PCR sample was denatured and separated on 8% polyacrylamide gels according to the procedure described by Orita et al. (1989). Electrophoresis was performed at 40 W and 4°C or 15°C for 1.5–6 h. PCR products were sequenced directly either by use of a cycle-sequencing protocol (Amersham) or by solid-phase sequencing with magnetic beads (Dynal) and T7 DNA polymerase (Pharmacia). Sequencing products were analyzed on an automated DNA sequencer (Pharmacia).

A detailed description of the transglutaminase activity assay will be given elsewhere (M. Huber and D. Hohl, personal communication). In brief, frozen skin sections were incubated in 100 mM Tris/Cl pH 8, 1% BSA for 30 min, to block nonspecific binding, and then in 100 mM Tris/Cl pH 8, 5 mM $CaCl_2$, and 12 μM monodansylcadaverine for 1 h, to detect transglutaminase activity. For a negative control in normal human skin, EDTA was added to a final concentration of 20 mM. After the transglutaminase reaction was stopped in PBS/10 mM

EDTA, sections were incubated with rabbit antidansyl antibody (1:100) (Aeschlimann et al. 1993) and monoclonal anti-TGK antibody B.C1 (1:1) (Thacher and Rice 1985) in 12 % BSA/PBS for 3 h. Sections were then incubated with biotinylated horse antimouse antibody (1:100) in 12% BSA/PBS and normal horse serum (1:100) for 30 min and afterwards with fluorescein isothiocyanate–labeled swine antirabbit antibody (1:40) and Texas Red streptavidin (1:400) in 12% BSA/PBS for 30 min.

Results

Clinical Investigations

Fourteen families with autosomal recessive LI, from Germany and Morocco, were investigated clinically, molecularly, and immunohistochemically (fig. 1 and table 1). The clinical picture of the patients analyzed was highly variable. Most were born as collodion babies, and many showed joint contractures and palmoplantar hyperkeratosis. Nine unrelated patients suffered from erythema, accompanied—in the more severely affected cases—by ectropion, whereas five patients from different families presented a nonerythrodermic form of LI. The type of scaling was highly variable, ranging from fine scales of fair color to dark brownish, polygonal, barklike scales (table 1). The LI phenotype breeds true, in all families where ascertainable. Thirteen LI patients from nine different families of German origin, who were revealed to carry inactivating mutations in the TGM1 gene, are described in more detail below.

Patient NE.—The parents of patient NE are not related and have a healthy younger son. The affected girl was born at term, as a collodion baby with ectropion. There was a remarkable improvement of the skin condition after rupturing and solving of the collodion membranes some weeks after birth. At the age of 1 year, only a minimal, superficial, white scaling of the thighs remained visible. There were no clinical signs of generalized erythema, scaling, ectropion, or palmoplantar keratoderma. Similar disorders are not known within the family.

Patient SW.—The female patient has one older brother, one older sister, and one daughter, all of whom are healthy. Ichthyosis was present in the patient at birth, with large brown scaling without erythema. During childhood, the color of scales was light brown to dark brown, but later, scales were of black-brown, especially at flexural folds. Clinical examination of the patient at the age of 33 years presented no visible erythema of the skin, light scales on the entire surface of the body, no ectropion, and normal skin of palms and soles. There were no ichthyotic disorders in the family history and no consanguinity of the parents.

Patients PB and EB.—The patients are brothers, 10 and 17 years of age. Family history was unremarkable, the patients have two unaffected siblings, and the parents were not consanguineous. Both brothers were born as collodion babies. Their clinical presentation was identical, with absent or only slight erythema; platelike, large, dark brown scales of the whole skin, including scalp, face, and the flexural folds; slight ectropion; moderate hyperkeratosis of palms and soles; and hypohidrosis.

Patients FS and JS.—The children are identical female twins, aged 6 years. Consanguinity of the parents is not discerned, but both grandparents originate from small neighboring villages. There is one healthy younger brother. Both children were born in the 31st wk of gestation, as collodion babies with ectropion, eclabium, and contractures of fingers and toes. Clinical investigation presented identical findings in both children, with marked erythema, fine white scaling of the entire skin, including the scalp, a darker, grey-brownish scaling only on the back of the feet, and slight palmoplantar keratosis. Both children have a marked hypohidrosis.

Patient JL.—Both parents and three of the grandparents were born in the same town, but consanguinity of the parents is not known. There are no siblings. At birth, patient JL had no collodion membranes and no marked erythema. The clinical findings in the 35-year-old man showed a generalized erythema of moderate intensity and a fine scaling of the skin, lighter on the arms and the trunk and darker on the lower legs. There is hypohidrosis and marked keratosis on palms and soles. Similar disorders are not remembered in the family.

Patient KM.—The parents are not related, and the patient, a boy, is their only child. He was born in the 34th wk of gestation, with generalized erythema; probably with collodion membrane; partially with platelike keratoses similar to those seen in harlequin ichthyosis; ectropion; and eclabium. Clinical examination at the age of 3 years revealed a marked erythroderma; moderate ectropion; light grey-brown scales of the entire skin with marked, platelike scaling of the scalp; moderate palmoplantar keratoderma; and hypohidrosis. Keratinization disorders are unknown in the family.

Patient VB.—The 5-year-old girl has a healthy older brother. She was born as a collodion baby. Clinical finding showed a moderate erythema; brown scales, especially on the lower limbs; platelike light brown hyperkeratosis of the scalp; marked palmoplantar keratoderma; and generalized hypohidrosis. Similar disorders are not remembered in the family. Consanguinity of the parents is not known, but both grandparents come from a small village of about 3,000 inhabitants.

Patients AW and PW.—The patients are siblings and have one healthy older brother. Their parents are healthy and not related. Both children were born as collodion

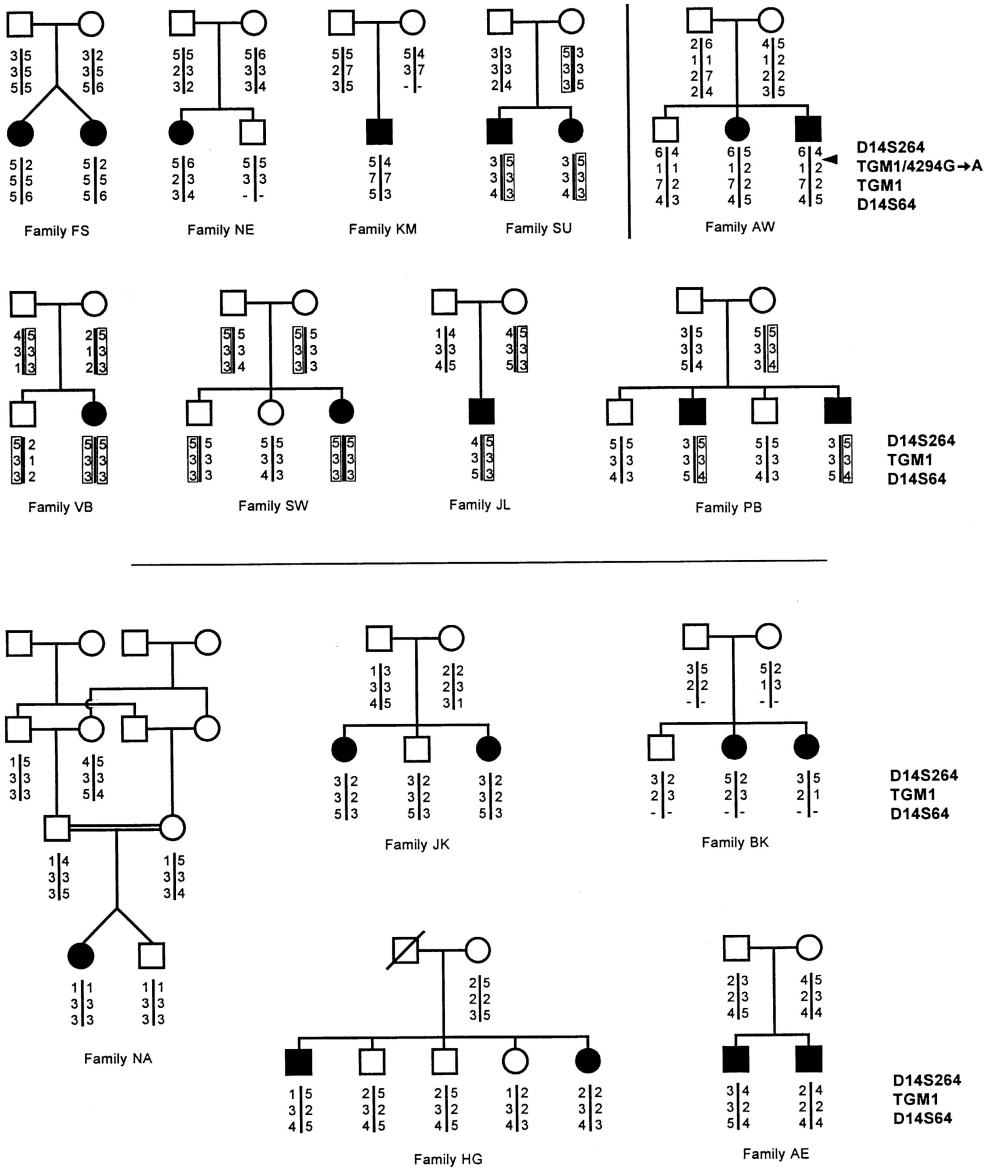

Figure 1 Pedigrees of 14 families with autosomal recessive LI and haplotypes aligned from genotypes at TGM1, D14S264, and D14S64. The phases in the parents are unknown in most families and deduced in the children. *Upper panel:* Families analyzed for mutations in the TGM1 gene. Five families—FS, NE, SU, VB, and PB—are compatible with linkage to TGM1. Family AW is only partly informative for the markers analyzed but the base substitution 4294G→A in the TGM1 gene, which results in mutation G382R, reveals a recombination between D14S264 and TGM1 (*arrowhead*). Simplex families KM and JL are not suitable for linkage analyses. The splice mutation A3447G in the TGM1 gene has been identified seven times in families SU, VB, SW, JL, and PB. The mutation has been found on the same haplotype, 222–174–130, at the loci D14S264, TGM1, and D14S64 in six cases. This haplotype, coded as 5–3–3, is boxed in the figure. The corresponding haplotype in family PB, 222–174–128, is coded as 5–3–4. *Lower panel:* Families with LI not linked to TGM1. Linkage has been excluded in five families by the identification of obligatory recombination events.

babies. Clinically, they presented an almost identical finding, with moderate erythema of the entire skin; slight ectropion; palmoplantar keratoderma; and a platelike brown scaling of the complete skin that is lighter on the scalp, arms, and trunk but darker on the lower legs and in the great body folds. Furthermore, there was a marked hypohidrosis.

Patients SU and JU.—Both are siblings and the only children of healthy parents. A distant relationship of the parents was reported, but consanguinity could not be explicitly determined. The patients noted that they had ectropions, as children, and hypohidrosis until the age of 16 years. Clinical findings at the ages of 30 and 31 years showed identical skin lesions, with large scales of

Table 1

Summary of Clinical Features, Linkage, and Mutation Data for 14 Families with LI

Family	Origin	Consanguinity	Clinical Features		Linkage[a]			Mutations in TGK	TGK Activity
			Erythema	Scaling	TGM1	ICR2B	TGM2/3		
NE*	German	−	−	Whitish, fine	+	NT	NT	R323Q	NA
SW*	German	−	−	Fair color	NI	NT	NT	A3447G/A3447G	NA
PB*	German	−	−	Dark, platelike	+	NT	NT	R315C/A3447G	−
FS*	German	−	+	Whitish, fine	+	NT	NT	V518M	NA
JL*	German	−	+	Fine	NA	NA	NA	G144R/A3447G	−
KM*	German	−	+	Light brown, medium-size	NA	NA	NA	V518M/V518M	−
VB*	German	−	+	Platelike	+	NT	NT	A3447G/A3447G	NA
AW*	German	−	+	Platelike	NI	NT	NT	R225H/G382R	NA
SU*	German	?	+	Barklike	+	NT	NT	R142H/A3447G	NA
NA	Moroccan	+	−	Dark	−	−	−	NT	NA
HG	German	−	−	Platelike	−	−	−	NT	NA
AE	German	−	+	Fair color, fine	−	−	−	NT	NA
BK	German	−	+	Fair color, fine	−	−	−	NT	NA
JK	German	−	+	Light brown, medium-size	−	NI	−	NT	NA

Note.—A detailed clinical description of the patients with mutations in TGK, indicated by an asterisk (*), is given in the text. Linkage analyses were not possible in two simplex families, but mutation analyses in the TGM1 gene were performed in patients from these families as well. NI = not informative; NA = not available; and NT = not tested. A plus sign (+) indicates presence; a minus sign (−) indicates absence; and the question mark (?) indicates that a possible consanguinity could not be clarified.

[a] Assessment for linkage to candidate regions with microsatellites on chromosomes 14 (TGM1), 2 (ICR2B), and 20 (TGM2 and TGM3); see text for details.

the scalp; fine, light scaling of the face; and large brown scales of trunk and limbs, with a barklike pattern of the skin. A patchlike erythema was seen, along with hyperkeratosis of palms and soles and clubbing of the nails.

Linkage Studies

In 12 families with autosomal recessive LI—one of these consanguineous, with four to six family members from two or three generations—linkage analyses were performed by use of microsatellites in the chromosomal region of TGM1 on chromosome 14q (Kim et al. 1992; Phillips et al. 1992; Yamanishi et al. 1992). The remaining two families were simplex families and not suitable for linkage analyses. To directly assess the segregation at the locus TGM1, we analyzed the highly informative microsatellite in intron 14 of the TGM1 gene (Compton et al. 1992). In addition, we inviestigated microsatellites at the anonymous loci D14S64 and D14S264. A cDNA identified by the Whitehead Institute, WI-9158, is identical to the TGM1 coding sequence. D14S64 has been localized on the distal side and D14S264 on the proximal side of WI-9158 by analysis of YAC contigs (Hudson et al. 1995, with supplementary data from the Whitehead Institute/MIT Center for Genome Research, Human Genome Mapping Project).

Five families were compatible with linkage of LI to the locus TGM1 according to genotype and haplotype data for TGM1, D14S64, and D14S264 (fig. 1 and table 1). However, these results give only a clue to linkage, since single families taken into account are too small to give firm evidence. For the loci D14S264 and D14S64, neither recombination to TGM1 (Russell et al. 1994) nor to one another (Dib et al. 1996) has been reported so far. In family AW, haplotypes could not be aligned at these three loci because the mother is uninformative for the microsatellite at TGM1. A mutation identified in exon 7 of the TGM1 gene in the mother and her affected children, however, revealed a recombination between D14S264 and TGM1 in this family (fig. 1). No further recombination event was found in this interval, in the families described here. Family SW was not informative for the three markers. Five families show obligatory recombination events between the LI phenotype and the three microsatellites close to the TGM1 locus (fig. 1).

The families shown not to be linked to TGM1 were additionally analyzed for linkage to two other candidate regions for LI. It has been demonstrated by FISH that the locus TGM3 is located in close proximity to the tissue transglutaminase gene locus (TGM2) on the long arm of chromosome 20 (Wang et al. 1994). The cDNA WI-7847 of the Whitehead Institute, which is identical to the TGM2 coding sequence, has been mapped to the 1.2-cM interval between D20S107 and D20S99 by radiation hybrid mapping (Schuler et al. 1996). The microsatellite markers at D20S101, D20S107, and

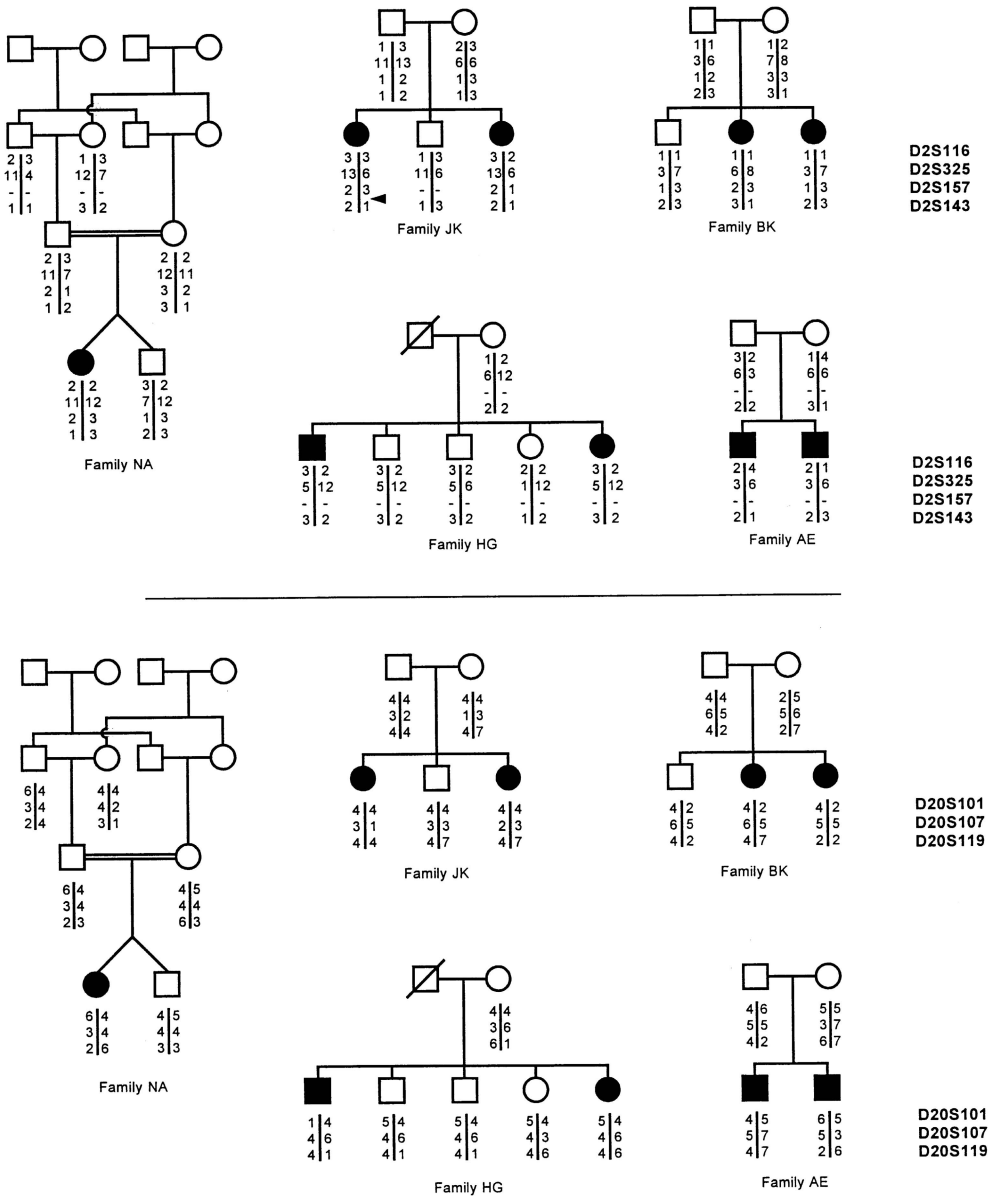

Figure 2 Pedigrees of 5 families with autosomal recessive LI not linked to TGM1 and haplotypes in candidate regions for LI on chromosomes 2 and 20. The phases in the parents are unknown, in most families, and are deduced in the children. *Upper panel:* Haplotypes aligned from genotypes at D2S116, D2S325, D2S157, and D2S143 in the region of the second locus for LI, ICR2B. This locus has recently been mapped between the markers at D2S325 and D2S137 (Parmentier et al. 1996). Linkage to ICR2B could be excluded in families BK, HG, and AE. In family NA, linkage can most likely be excluded by the lack of homozygosity by descent. Family JK is not informative because of a recombination between D2S157 and D2S143 (*arrowhead*). *Lower panel:* Haplotypes aligned from genotypes at D20S101, D20S107, and D20S119 in the region of the TGM2 and TGM3 genes. Linkage to this region could be excluded in four families. In family NA, again, linkage can most likely be excluded by the lack of homozygosity by descent.

D20S119 cover a 13-cM region containing the TGM2 and TGM3 gene loci. Although the number of probands in each of the five LI families analyzed is too small for obtaining effective LOD scores, the transglutaminase gene loci on chromosome 20 could be excluded from linkage. Obligatory recombination events were identified for at least two of the chromosome 20 markers analyzed, and exclusion of linkage is confirmed by haplotype analyses in four of the families (fig. 2). Linkage can most likely be excluded by lack of homozygosity in the consanguineous Moroccan family NA, in which both grandfathers are brothers and both grandmothers are sisters. Furthermore, microsatellites at D2S116, D2S325, D2S157, and D2S143 were genotyped. These loci are

located on chromosome 2q33-q35 in the region to which the second locus for LI, ICR2B, has recently been mapped (Parmentier et al. 1996). Linkage to this locus could also be excluded in at least three of the families not linked to TGM1 (fig. 2 and table 1). In family NA, again, only lack of homozygosity could be demonstrated, and in family JK, which shows a recombination between D2S157 and D2S143, linkage to the second LI locus cannot be excluded (fig. 2).

Mutation Studies

Mutation analyses in the TGM1 gene were performed in nine families with autosomal recessive LI by SSCP analyses and direct sequencing of TGM1 exons. Seven different missense mutations and one splice mutation were found in TGK (fig. 3 and table 1). Besides mutations R142H (Russell et al. 1995) and R323Q (Huber et al. 1995*a*), five novel missense mutations not described so far were detected: G144R (nucleotide exchange 1494G→A in TGM1, according to Kim et al. 1992), R225H (2730G→A), R315C (3515C→T), G382R (4294G→A), and V518M (7915G→A). These mutations were not present on 100 chromosomes of unaffected control individuals of German origin. The splice mutation affecting the 3′ splice site of intron 5, A3447G (according to Kim et al. 1992), has been described by Huber et al. (1995*a*) as A3366G. This mutation was identified on seven chromosomes. Two LI patients, SW and VB, are homozygous for this splice mutation, whereas patients JL, PB/EB, and SU/JU are compound heterozygous for A3447G and one missense mutation. Remarkably, six of seven chromosomes carrying mutation A3447G have the same haplotype, 222–174–130, aligned from genotypes at D14S264, TGM1, and D14S64 (fig. 1). In the seventh case, family PB, the corresponding haplotype is 222–174–128 (i.e., it deviates at D14S64 by only one [CA] repeat). The respective mutations were also found in the parents of affected individuals, and, in all cases, both parents of homozygous patients were shown to be heterozygous for the mutation. This finding indicates that all cases are truly familial and recessively inherited. To confirm that the TGM1 mutations detected in affected individuals really impair the function of the enzyme, we investigated skin sections immunohistochemically from three LI patients (PB, JL, and KM) from whom skin biopsies were available (table 1). All of these patients showed markedly decreased activity of TGK.

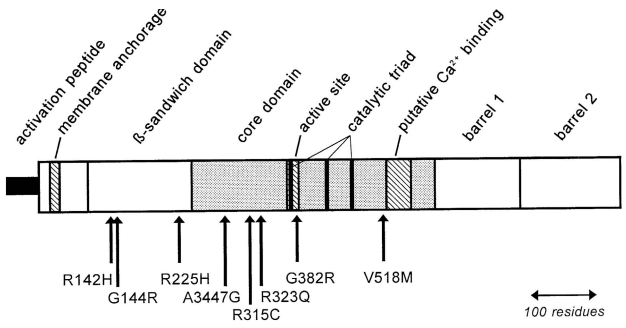

Figure 3 Schematic depiction of the putative secondary structure of the TGK polypeptide, based on the secondary structure of blood coagulation factor XIII A (Takahashi et al. 1986; Yee et al. 1994) whose primary structure is highly similar to TGK. The positions of mutations identified in patients with autosomal recessive LI described here are marked by arrows. In the case of the splice mutation A3447G, the position of the sequence insertion is indicated, which is caused by failure in excision of intron 5 and results in a premature stop codon.

Discussion

Linkage Studies

To address the question whether a genotype/phenotype correlation can be found in patients with autosomal recessive LI, we have analyzed 14 families with LI, from Germany and Morocco. Twelve of these were suitable for linkage studies, and five families could be excluded from linkage to TGM1 (table 1). Two families were uninformative, but the remaining five families were compatible with TGM1 linkage. The families not linked to the TGM1 gene were additionally assessed for linkage to two further candidate regions. Linkage of LI to the loci TGM2 and TGM3 on chromosome 20, however, could be excluded. A second locus for autosomal recessive LI, ICR2B, has been localized to chromosome 2q (Parmentier et al. 1996). This locus could be clearly ruled out as well in three of the families. Consanguineous family NA, from Morocco, can most likely be excluded by lack of homozygosity for the markers at ICR2B, which has originally been identified in Moroccan families.

It has been noted elsewhere that TGK expression is in fact detectable in a number of LI patients (Huber et al. 1995*b;* Lavrijsen and Maruyama 1995), and a total of 15 families not linked to the TGM1 region have been described so far (Parmentier et al. 1995; Bale et al. 1996). The findings presented here substantiate the genetic heterogeneity of LI and demonstrate that mutations resulting in a LI phenotype must exist in at least three different genes. There is no obvious candidate for a new LI locus, and we have therefore embarked on a genomewide search for further loci.

Mutation Studies

SSCP and sequencing analyses of the TGM1 gene were performed in LI patients, and a TGK assay was carried out in three cases in which a skin biopsy was available. Eight different mutations were found, including seven missense mutations and one splice mutation (fig. 3 and

table 1). Among these, mutations R142H and R323Q have been described elsewhere (Huber et al. 1995*a*; Russell et al. 1995). Mutation G144R resides in a region of TGK that obviously exhibits a mutational hot spot. A total of four different mutations have been identified in the residues R142, R143, and G144 (Huber et al. 1995*a*; Russell et al. 1995). The lack of TGK expression found in patient JL strengthens the likelihood that G144R is an inactivating mutation. It resides in a segment supposed to be crucial for the correct structure and function of the enzyme (Kim et al. 1994). Moreover, the residues at positions 142–145 are extremely conserved between various transglutaminases and among many species. The mutation R225H changes a moderately conserved residue, which is also part of the presumed β-sandwich domain of TGK. It might also act by altering the folding of the protein. Missense mutation R315C again affects a highly conserved residue. Furthermore, a mutation has been found in the arginine residue at position 252 of another transglutaminase, blood coagulation factor XIII A, causing inherited factor XIII deficiency (Mikkola et al. 1996). The primary structure of factor XIII A (Takahashi et al. 1986) is highly similar to the structure of TGK, and the position 252 in factor XIII A corresponds to residue 315 in TGK.

Mutation G382R significantly alters a conserved residue. Glycine or alanine residues are found at the corresponding positions in other transglutaminases, but no charged side chains, such as those in the arginine residue, are introduced here. Moreover, G382R affects a residue close to C377 that is part of the supposed catalytic triad of TGK (Yee et al. 1994). It belongs to the highly conserved region around the active site of the enzyme (Takahashi et al. 1986). Remarkably, mutation V518M resides much more distal in the TGM1 gene than any other mutation described so far. It affects a residue even distal to the active site and the potential catalytic triad (fig. 3), and this residue is only moderately conserved in transglutaminases. The mutation, however, has been identified on a total of three chromosomes, patients FS being heterozygous and KM being homozygous for this mutation. Moreover, the lack of TGK activity in patient KM confirms that this is also a truly inactivating mutation.

Splice mutation A3447G results in premature stop codon, as described by Huber et al. (1995*a*). It has been observed on the same haplotype in six cases, which points at a common origin of the chromosomes with mutation A3447G and suggests that this is a rather old mutation. In the brothers PB and EB and in their mother, A3447G was identified on a haplotype identical at D14S264 and TGM1. The allele at D14S64 is different by only 2 bp (i.e., one [CA] repeat). This chromosome could therefore present either the same ancient haplotype, with a mutation in the microsatellite at D14S64, or an older recombination between TGM1 and D14S64. Patients SU and JU are compound heterozygotes for TGM1 mutations, although their parents are related. No details about this distant relationship are known, however, and the mutations were found on different haplotypes, one of these the common haplotype carrying the recurrent mutation A3447G. In two cases, the patient NE and the sisters FS and JS, only one mutation in the TGM1 gene was identified. The apparent absence of the second TGM1 mutation is presumably due to the insufficient sensitivity of the SSCP analysis. In both of these cases, the mutation found has also been described in other LI patients with absent TGK activity. All the mutations identified in the TGM1 gene can be assumed to result in impairment of TGK activity and are therefore the cause for the phenotype of LI.

Genotype/Phenotype Correlation

Autosomal recessive LI is characterized by remarkable clinical heterogeneity. An erythematous and a nonerythematous type of LI were distinguished clinically (Hazell and Marks 1985; Williams and Elias 1986). Moreover, patients might present different patterns and colors of the scales, they might or might not have an involvement of palmoplantar hyperkeratosis, and most but not all were born as collodion babies. We have therefore compared two genetically differentiated groups of LI patients: one group comprising five unrelated patients excluded from linkage to the TGM1 gene (referred to as non–TGM1 LI patients), and one group of nine patients with LI unambiguously caused by TGM1 gene mutations (TGM1 LI).

The group of non–TGM1 LI patients included erythematous as well as nonerythematous cases, and they presented the whole range between fine scales of fair, whitish color and large, dark brown scales (table 1). These differences in the clinical picture could still be explained by different genes causing the disorder. The entire spectrum of LI variants, however, was also seen in TGM1 LI patients (table 1). Patients with erythema from both groups often showed fine and light scaling (six of nine); patients with the nonerythematous form of LI were mostly affected by large, dark brown scales (three of five).

In seeking a genotype/phenotype correlation for specific mutations in the TGM1 gene, we were able to compare two patients who carry the same TGM1 genotype. Both the patients VB and SW are homozygous for the splice mutation A3447G, but they differed in their clinical picture. VB suffers from a moderate erythema. She shows brownish scales, particularly affecting the lower limbs, and marked palmoplantar hyperkeratosis. On the contrary, SW presented no visible erythema, scaling of fair color on the entire surface of the skin, but no in-

volvement of palms and soles. The size and color of scales in patient SW, however, seem to have changed over the course of time, even in the absence of treatment with retinoids. These findings, together with the clinical variability seen within the entire group of TGM1 LI patients, contradict a genotype/phenotype correlation for mutations in the TGM1 gene. Particularly, this is also true for the phenotypical differentiation between the erythematous and the nonerythematous forms of LI.

In summary, no characteristic differences could be detected in the clinical presentation between the TGM1 LI and non–TGM1 LI patients. Each group displayed the whole spectrum of symptoms for LI, such as erythematous and nonerythematous forms, various scaling patterns, and existence or lack of palmoplantar keratoderma. Hence, there are currently no conclusive criteria for the clinical differentiation of LI types corresponding to their genetic classification. There are many gene products involved in the terminal differentiation of the epidermis, and mutations in quite a few of these can be envisaged as a cause for the symptoms seen in LI. Only the identification of further genes for LI will allow a refined genetic classification of autosomal recessive LI. Correspondingly, as those findings give further insight into differentiation processes in the skin, they will provide the basis for further understanding of the etiology of LI.

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