Self-Healing Collodion Baby: a Dynamic Phenotype Explained by a Particular Transglutaminase-1 Mutation

Michael Raghunath,* Hans-Christian Hennies,† Bijan Ahvazi,‡ Melanie Vogel,* Andre Reis,†§ Peter M. Steinert,‡ and Heiko Traupe*

*Department of Dermatology, University Hospital, Münster, Germany; †Gene Mapping Center, Max Delbrück Center for Molecular Medicine, Berlin, Germany; ‡Laboratory of Skin Biology, NIAMS, NIH, Bethesda, Maryland, U.S.A.; and §Institute of Human Genetics, University of Erlangen, Germany

Spontaneous healing with no or only very mild ichthyosis distinguishes the "self-healing collodion baby" from other congenital ichthyoses. In two self-healing collodion baby siblings with markedly diminished epidermal transglutaminase 1 activity we found the compound heterozygous transglutaminase 1 mutations G278R and D490G. Molecular modeling and biochemical assays of mutant proteins under elevated hydrostatic pressure suggest significantly reduced activity in G278R and a chelation of water molecules in D490G that locks the mutated enzyme in an inactive *trans* conformation *in utero*. After birth these water molecules are removed and the enzyme is predicted to isomerize back to a partially active *cis* form, explaining the dramatic improvement of this skin condition. ***Key words: collodion baby/genetics/lamellar desquamation/newborn/skin/transglutaminase 1. J Invest Dermatol 120: 224–228, 2003***

The term "collodion baby" describes an uncommon and transient condition of the newborn. At birth, affected children are encased in a glistening membrane that cracks in a characteristic manner within 48 h and desquamates in large lamellae after a few days leaving an almost normal appearing skin that shows some scaling on the trunk. This phenotype can be initially quite severe and often includes severe ectropion and everted lips (reviewed in Traupe, 1989; Sybert, 1997). Collodion babies require intensive care, suffering from severe temperature dysregulation and increased insensible water loss (Buyse *et al*, 1993). The mortality in the first weeks of life is about 10% (Traupe, 1989). Initially, collodion babies look very much alike at birth, but later take different clinical courses. Most patients evolve into the different types of congenital ichthyosis such as transglutaminase-1 (TGase1) deficient lamellar ichthyosis (OMIM: 242300), trichothiodystrophy, Sjögren–Larsson syndrome, or Gaucher disease (Lui *et al*, 1988). About 10% of all collodion babies heal spontaneously within the first few weeks (reviewed in Traupe, 1989; Sybert, 1997). A minority develops dry skin often present on the trunk only reminiscent of mild forms of ichthyosis vulgaris (Langer *et al*, 1991). This condition is called the self-healing collodium baby (SHCB). In the OMIM catalog SHCB is listed jointly as lamellar exfoliation of newborn or desquamation of newborn together with ichthyosis congenita and lamellar ichthyosis (#242300). In 30%–40% of cases with autosomal recessive congenital ichthyosis, mutations in the *TGM1* gene have been found (Huber *et al*, 1995; 1997; Lavrijsen and Maruyama, 1995; Parmentier *et al*, 1995; Russell *et al*, 1995; Laiho *et al*, 1997; 1999; Hennies *et al*, 1998a; Raghunath *et al*, 1998; Tok *et al*, 1999; Akiyama *et al*, 2001; Cserhalmi-Friedman *et al*, 2001). The genetic basis of SHCB is not known. In this paper, we describe a family of SHCB in which two siblings have the same two heterozygous mutations in *TGM1*. One of these mutations is new and occurs at a novel location that could allow restoration of some TGase1 activity on drying of the skin several days after birth.

Manuscript received March 15, 2002; revised September 26, 2002; accepted for publication October 3, 2002

Reprint requests to: Heiko Traupe, M.D., Department of Dermatology, University of Münster, Von-Esmarch-Str. 58, D-48149 Münster, Germany; Email: traupeh@uni-muenster.de

MATERIALS AND METHODS

Patients The family originates from Kosovo (former Yugoslavia) and has an Albanian ethnic background. The nonconsanguineous parents are free of skin diseases. After 10 miscarriages (each between the twenty-fourth and thirtieth gestational week) the couple had three daughters. The youngest daughter, the index case V.S., was seen by one of us at the age of 2 d in the Department of Paediatrics and a biopsy was taken. At that time point the girl was totally wrapped in a glistening membrane that had already begun to crack. The girl was seen at the age of 9 d in our department (**Fig 1**) with extensive desquamation that was completed within 2 wk. At 4 wk, the extremities and the face appeared completely normal. The older sister N.S. (case 2), also born as a collodion baby, showed mild fine white scaling confined to the trunk at the age of 4 y when she was seen for the first time (**Fig 1**). The clinical diagnosis of SHCB was made in both. The oldest girl, aged 17 y and healthy, was not available for clinical examination.

Immunohistochemical localization of TGase1 protein Cryostat sections were fixed in acetone at −20°C for 10 min and air dried. Sections were incubated with the monoclonal antibody BT-621 against human keratinocyte TGase (Biomedical Technologies, Stoughton, MA) 1:40 for 16 h at 4°C. Bound anti-body was detected in a two-step procedure using biotinylated goat antimouse 1:400 for 90 min (Jackson ImmunoResearch Laboratories, West Grove, PA) and streptavidin DTAF (dto) 1:100 for 30 min.

Histochemical TGase assay The assay (Raghunath *et al*, 1998) allows differentiation between full, residual, and lost epidermal activity in skin cryosections. Four millimeter punch biopsies were taken with informed parental consent from N.S. at the age of 4 y and from V.S. at the ages of 2 d and 4 mo. Thirty normal adult skin samples and skin from three newborns (2 d, 7 d, and 9 d) served as a control group. Briefly, air-dried 5 μm skin cryosections were incubated with the TGase substrate biotinylated cadaverine (Molecular Probes, Leiden, The Netherlands) in Tris buffer (pH 7.4 or 8.4) in the presence of calcium ions. Incorporated

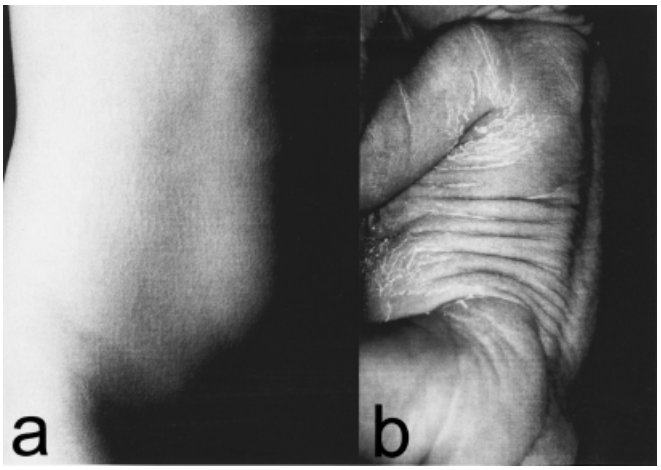

Figure 1. Clinical aspects of the two affected siblings with SHCB. (*a*) N.S. at the age of 4 y: mild scaling on the trunk. (*b*) V.S. at the age of 9 d: extensive desquamation and erythroderma.

Table I. Comparison of specific activities of normal and mutated TGase 1 forms expressed in baculovirus cells at ambient and elevated pressure

	Ambient pressure	Ambient pressure + 115 mm Hg
Cytosolic		
Wildtype	15.5 ± 5.6	12.2 ± 2.5
G278R	0.3 ± 0.1	0.2 ± 0.2
D490G	12.5 ± 2.4	4.7 ± 0.9
Membrane-bound		
Wildtype	5.5 ± 2.0	3.7 ± 1.1
G278R	none	none
D490G	4.7 ± 2.8	0.8 ± 0.5

Specific activities are given as pmol of [14C]putrescine incorporated into succinylated casein/h/pmol of Tgase. Note the striking drop in specific activity at elevated pressure in the D490G mutation.

substrate was visualized using streptavidin-DTAF. Images were taken using a video imaging system and the average exposure time of 1000 ms obtained for the staining intensity of normal adult skin was applied to all other experiments with SHCB patients and neonates.

DNA analyses *TGM1* mutation screening was performed as reported earlier (Hennies *et al*, 1998b) using DNA from ethylenediamine tetraacetic acid blood of both parents and the two affected daughters. Exons 2–15 of the *TGM1* gene were amplified by polymerase chain reaction (PCR) using intronic primers (Philips *et al*, 1992). PCR products showing single-stranded conformation polymorphism (SSCP) changes were directly sequenced on an ABI 377 automatic sequencer.

Structural modeling The three-dimensional model of human TGase1 was generated on the basis of the known atomic structure of human factor XIIIa (fXIIIa) as present in the protein data bank (Bernstein *et al*, 1977) under accession number 1F13 (Ichinose *et al*, 1998). By using the model building program O (Jones *et al*, 1991) the sequence of TGase1 was inscribed onto the structural frame provided by the residues of fXIIIa. The resulting model for TGase1 was then optimized using molecular dynamics.

TGase1 expression and assay Wildtype TGase1 as well as the G278R, D490G mutants were expressed in and purified from baculovirus (Candi *et al*, 1996). Briefly, a full-length wildtype human TGase1 cDNA cloned into the baculovirus vector pVL1392 (PharMingen, San Diego, CA) was used as a template for the two mutant forms (**see Table I**) that correspond to mutations identified in the SHCB patients. The TransformerTM Site-directed Mutagenesis Kit (Clontech) was used for introduction of mutations. The TGase1 cDNAs in the pVL1392 vector were under the transcriptional control of the strong baculovirus polyhedrin promoter. The three TGase1 recombinant virus clones (wildtype and two mutant forms) were obtained by cotransfection of each vector with the modified *Autographa californica* nuclear polyhedrosis virus DNA (BaculoGold DNA, PharMingen). BaculoGold DNA carries a lethal deletion and does not code for viable virus particles by itself. Cotransfection of this DNA with a complementing plasmid construct rescues the lethal deletion of this virus DNA and reconstitutes, by homologous recombination, viable virus particles inside the transfected insect cells. Insect cells (Sf9, PharMingen) were grown in Grace's insect medium supplemented with 10% fetal bovine serum (Life Technologies). Cells were processed as described recently to obtain separate cytosolic and membrane-bound fractions (Candi *et al*, 1998), aliquots of which were used for the TGase assay (Candi *et al*, 1998). In some cases, the reaction was performed in a closed vessel subjected to increased pressure to simulate the higher hydrostatic pressure *in utero* in comparison to the ambient environment after birth (**Fig 4**).

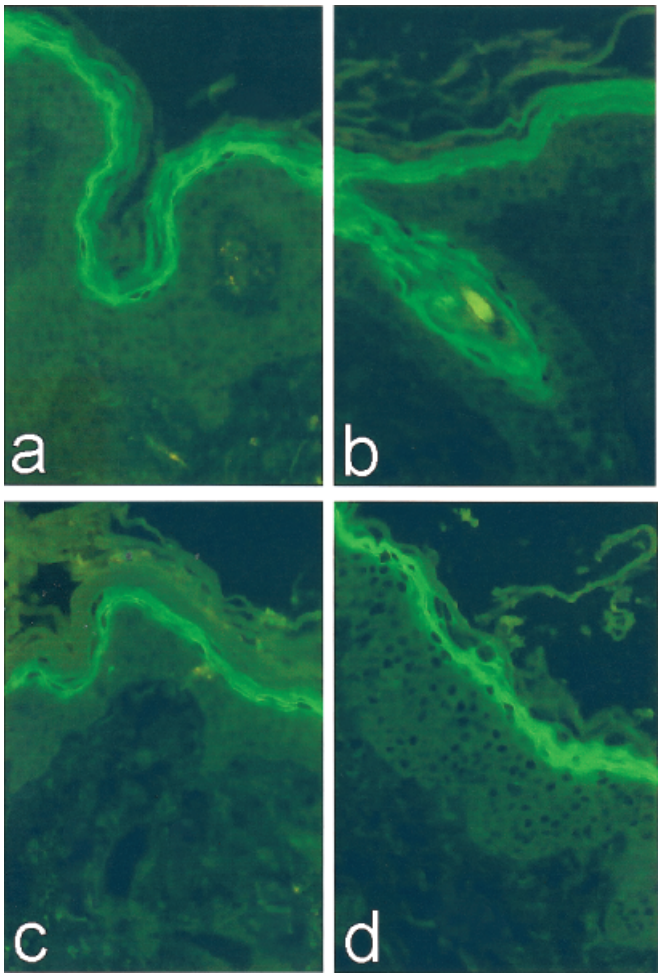

Figure 2. Immunohistochemical detection of TGase1 protein in normal skin and SHCB. (*a*) Normal skin: pericellular linear and continuous localization in the uppermost nucleated two to three keratinocyte layers. No detection in the cornified layer. (*b*) V.S. aged 2 d: pericellular localization in the transitional zone of the granular layer including a hair follicle. Additional strong signal in most of the thickened cornified layer. (*c*) The same child at 4 mo. Distribution pattern comparable to normal skin involving two to three cell layers of the stratum granulosum. No significant staining in the cornified layer. (*d*) Four-year-old sister shows distribution comparable to normal skin with slightly pronounced scaling and apparent absence of protein in the cornified layer.

RESULTS

Immunohistochemical detection of TGase1 protein The visualization of TGase1 as protein revealed a pericellular distribution in keratinocytes of the uppermost three nucleated cell layers of the epidermis in normal adults (**Fig 2*a***) and involvement of up to four cell layers in normal newborns (not

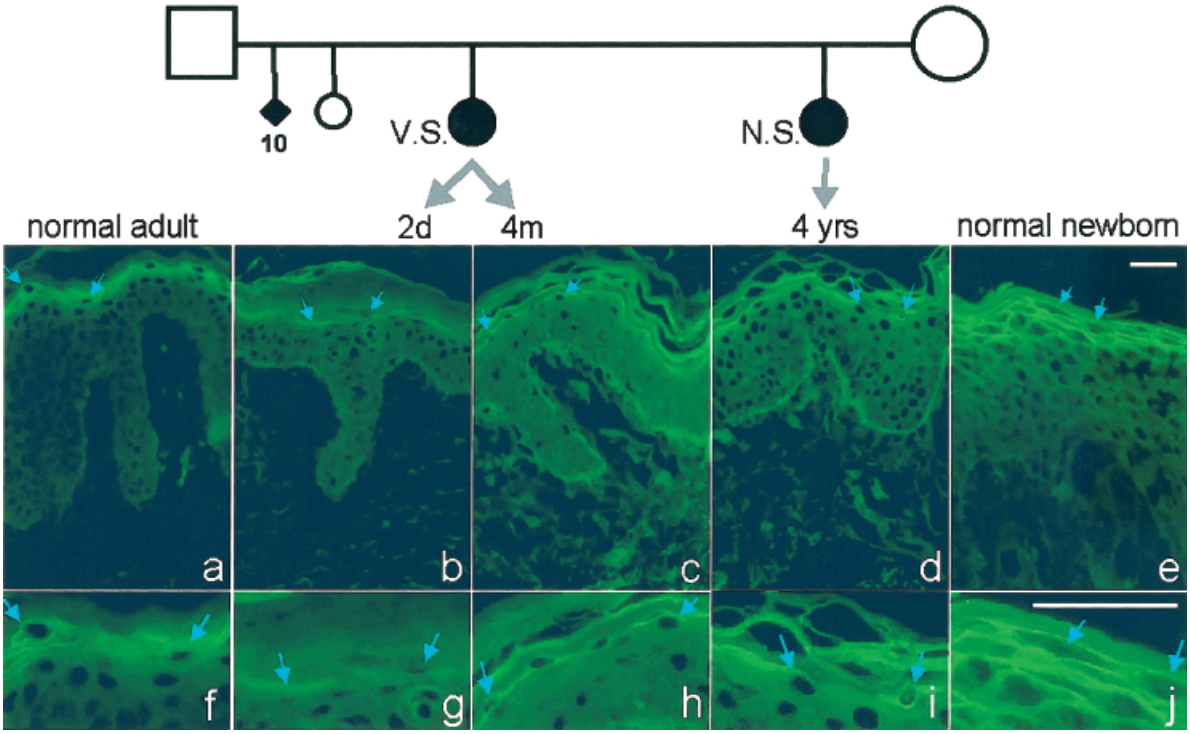

Figure 3. Pedigree of SHCB family and visualization of TGase activity in SHCB in comparison with normal adult and newborn skin. TGase-mediated incorporation of biotinylated cadaverine in cryostat sections was visualized using streptavidin DTAF. The images obtained are arranged according to the family pedigree. Enlarged details of the upper epidermis are given below the respective image. (*a*), (*f*) Normal adult skin: well-defined pericellular linear activity in a continuous fashion along the granular layer. The activity is spread over two cell layers. (*e*), (*j*) Normal 2-d-old newborn: comparably enhanced activity with a strictly pericellular pattern involving up to four cell layers. (*b*), (*g*) SHCB baby at 2 d: faint and patchy activity at the border between the granular and horny layer. The thickened horny layer shows patches of activity. (*c*), (*h*) The skin of the same child at 4 mo shows enhanced activity along a single cell layer bordering the stratum corneum. Separated by a TGase-negative zone a second layer of activity is found in the lower stratum corneum. Comparable findings in the 4-y-old sister (*d*, *i*). *Arrows*: border between nucleated keratinocytes and horny layer. *Bars*: 50 μm.

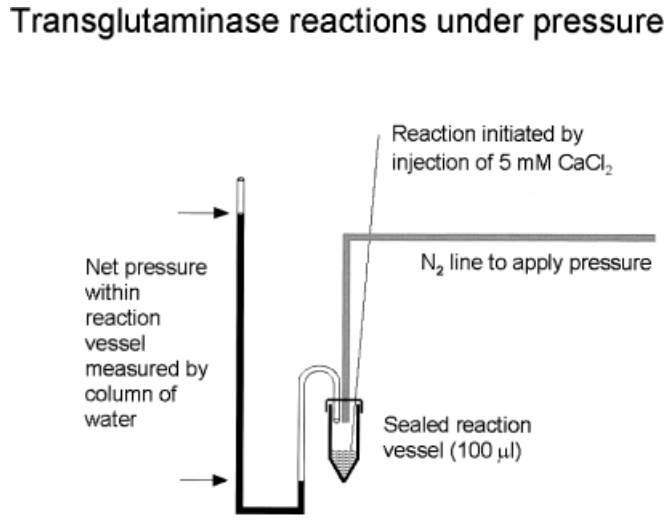

Figure 4. Experimental setting to assess recombinant TGase1 activity *in vitro* under pressure. Both the cytosolic form and the enzyme form bound to insect membranes were used in standard TGase assays. The reaction was performed in a closed vessel. Increased pressure was achieved by application of pressurized nitrogen and adjusted to a water column of 160 cm (about 115 mmHg). The reactants were equilibrated at the increased pressure for 10 min, and the reaction was initiated by injection of $CaCl_2$, 5 mM.

shown) with no immunoreactivity in most of the horny layer. The SHCB patients V.S. and N.S. showed a comparable pattern at the age of 4 mo and 4 y, respectively (**Fig 2*c, d***). At 2 d of age, however, the skin of V.S. showed immunoreactivity restricted to the uppermost nucleated cell layer and most of the horny layer (**Fig 2*b***) with a staining intensity comparable to the other skins.

Histochemical TGase activity Skins of normal adults showed pericellular linear TGase activity confined to the uppermost two or three nucleated cell layers; neonates showed involvement of one or two cell layers more (**Fig 3*a, f, e, j***). In contrast to the immunohistochemical findings, all SHCB patients showed alterations in activity and distribution pattern of the enzyme in comparison to normal skins. In particular, V.S. at the age of 2 d showed only very faint activity in patchy distribution along the border between the granular and horny layer (**Fig 3*b, g***). Patchy activity was also observed in the horny layer (**Fig 3*b, g***). Four months later, the skin of the same patient showed still patchy but far stronger activity along the same localization but an additional thin zone of activity within the horny layer (**Fig 3*c, h***). The 4-y-old SHCB patient N.S. showed the same pattern as the 4-mo-old sister (**Fig 3*d, i***).

DNA analyses Two point mutations were identified in exon 5 and 10 by SSCP analysis and subsequent sequencing. A substitution of G by A at position nt 3400 and A to G at position nt 7367 in the *TGM1* gene leads to the mutations G278R and D490G, respectively. Both children were compound heterozygous for these mutations. The father was heterozygous for G278R, and the mother was heterozygous for D490G (data not shown).

Modeling of the G278R mutation The G278 residue has been conserved in all TGase enzymes. It resides in the catalytic core domain near the interface with the β-barrel 1 and 2 domains (Yee *et al*, 1994) (**Fig 5**). The Cβ and Cγ hydrophobic part of the much larger mutant arginine side chain packs against the aromatic ring of Y277 (Y314 in fXIIIa), potentially forms an

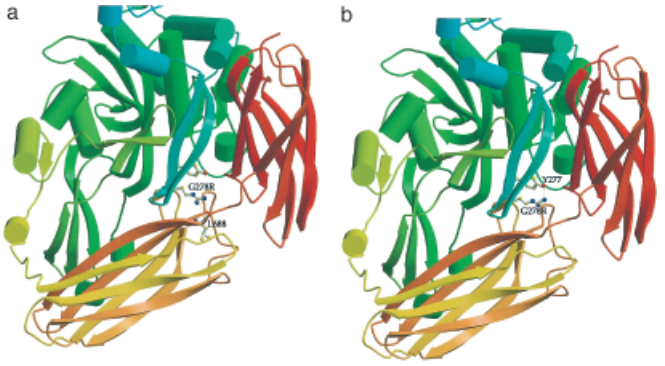

Figure 5. Modeling of the G278R mutation. Part of the predicted three-dimensional structure of TGase1 (based on the fXIIIa modeling) in the vicinity of the mutation. G278 lies near the interface of the catalytic core domain and the β-barrel 1 and 2 domains. Insertion of the bulkier arginine residue will probably cause deformation and allow the formation of an inappropiate H-bond with Y277 (*a*). Also, the arginine will form H-bonds with L288 at the interface of the barrels, and thereby interfere with the required movement during enzyme activation (Yee *et al*, 1994) (*b*). These changes should result in loss of enzyme activity, as revealed by direct assays of the mutant (**Table I**).

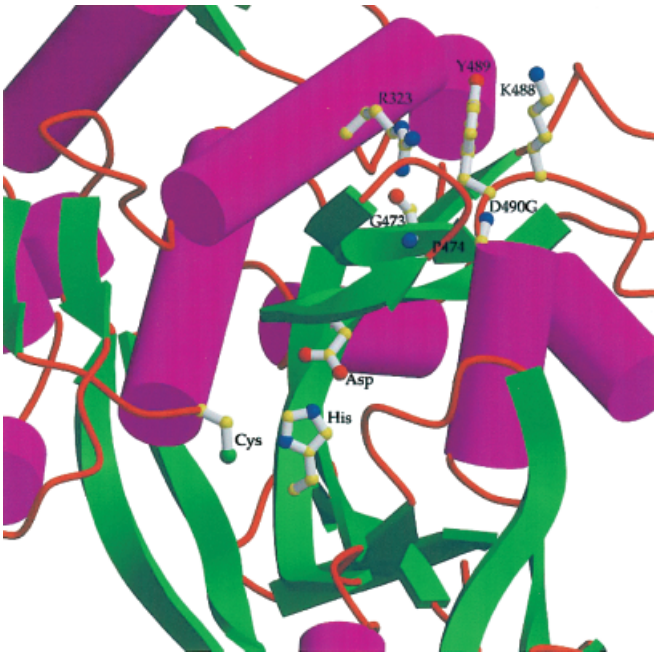

Figure 6. Modeling of the D490G mutation. This mutation is located just above the active site/catalytic triad of the core domain. The loss of the aspartic residue will interfere with the orientation of the two *cis* G473/P474 and K488/Y489 peptide bonds required for correct folding of this domain and they will fail to correctly interact with R323. Water molecules can reversibly interact with the K488/Y489 residues to flip the *cis* bonds (active enzyme form) over to the *trans* (inactive enzyme form) configuration. We predict that with the glycine mutant form, additional water molecules introduced under increased hydrostatic pressure could unusually stabilize the *trans* configuration, resulting in lost activity. In "dry" normal skin this process reverses, allowing retention of some TGase1 activity (**Table I**).

H-bond with the hydroxyl group of Y277, and thereby is likely to cause considerable local misfolding (**Fig 5*a***). In addition, the mutant arginine residue may form two improper interactions. The first involves a potential H-bond with the backbone carbonyl oxygen of the conserved L688 (L627 in fXIIIa). Second, an arginine at position 278 will probably interfere with the residues R687 and R689 that flank L688. Together, these are predicted to derange the linkage between the two β-barrel domains (**Fig 5*b***) and should explain why the G278R mutation leads to an inactive enzyme (**Table I**).

Modeling of the D490G mutation The conserved D490 residue (D427 in fXIIIa) forms a set of complex interactions that are essential for the proper folding of the catalytic core domain of TGase1 (Yee *et al*, 1994). It is immediately preceded by the nonproline *cis* peptide bond pair formed by K488/Y489. This *cis* bond has likewise been conserved in all TGase enzymes. Thus, the orientation of D490 is governed by this bond. In addition, all TGase enzymes have a second *cis* proline peptide bond pair formed by residues G473/P474. Together, these two pairs in the *cis* configuration are essential for the correct three-dimensional organization of the active site region of the catalytic core domain (Weiss *et al*, 1998) (**Fig 6**). Chelation of several water molecules through the carbonyl backbone oxygen of K488 with the backbone nitrogen of Ser476 force the G473/P474 pair to isomerize to the *trans* configuration, which has been predicted to inactivate the enzyme (Ichinose *et al*, 1998). Removal of the waters will allow isomerization back to the active *cis* form. Thus, TGase1, like fXIIIa, is predicted to occur as an equilibrium between the two forms. The D490 residue forms two sets of H-bonds with (i) the guanidium nitrogen of R323 and (ii) the nitrogen backbone of G473. Further, the carbonyl oxygen of G473 forms an H-bond with R323. Mutation of R323 leads to lamellar ichthyosis (Tok *et al*, 1999) and the equivalent residue (R260) in fXIIIa also leads to disease (Kungsadalampai *et al*, 1999). Thus, D490 is involved in a three-dimensional network of H-bonds including the two essential *cis* peptide bond pairs. Therefore, the D490G mutations would be expected to introduce local flexibility and loss of key H-bonds, and result in some loss of enzyme activity (**Table I**). Furthermore, we predict that water molecules will occupy the cavity created by the smaller side chain of glycine. Modeling potential electron density maps predicts that water molecules could chelate with K488 and R323 to form an unusually stabilized inactive *trans* configuration. Thus, under high hydrostatic water pressure, the D490G mutant enzyme could be inactive, but under lower pressure conditions removal of the water molecules will reestablish an equilibrium with the *cis* form and allow some enzyme activity.

Specific activities of TGase1 mutants Following expression and purification, specific activities were measured (**Table I**). When the TGase assays were performed under standard solution conditions, the G278R mutant form was inactive, but the D490G mutant retained up to 80% of wildtype activity. Under conditions of increased pressure equivalent to about 115 mmHg, the wildtype TGase1 activities were generally reduced to 80% (cytosolic) and 70% (bound to insect membranes). This may in part be due to nonproductive deamidation of the glutamine substrate by water under conditions of increased pressure. The D490G mutant was much more sensitive, however, in that the activity was reduced to 40% (cytosolic) and 20% (membrane bound), respectively. In direct comparison to wildtype TGase1 activity at increased pressure, the D490G mutant showed residual activities of only 39% (membrane bound) and 22% (cytosolic) (**Table I**).

DISCUSSION

The benign and self-limited clinical course of SHCB distinguishes itself from (TGase1-deficient) lamellar ichthyosis. Nevertheless, both conditions are listed under a joint entry number in the OMIM catalog (#242300) indicating a relation between both conditions. Accordingly, Langer *et al* (1991) suggested that SHCB could represent a minimal expression of lamellar ichthyosis. Our data, as exemplified with one family, show that this concept is valid and how this dynamic phenotype can be explained on the molecular level. From the two compound heterozygous *TGM1* mutations in our SHCB family G278R has been reported

previously in combination with R286Q in a patient with lamellar ichthyosis (Cserhalmi-Friedman *et al*, 2001). Three-dimensional modeling predicted that the G278R mutant enzyme has no activity. This was confirmed by a direct assay of the specific activity of the recombinantly expressed mutant enzyme.

Analysis of the novel D490G mutation is more complex. In part, this residue interacts with R323, which has been reported mutated in cases of classical lamellar ichthyosis (Tok *et al*, 1999) as well as for the equivalent residue in fXIIIa (Kungsadalampai *et al*, 1999). More significantly, our analyses reveal that mutation of residue D490 could influence the normal water molecule mediated *cis–trans* isomerization of two *cis* peptide bond pairs in the active site region of TGases. In particular, we postulate that, in the D490G mutant form, water molecules particularly stabilize the inactive *trans* form. In support of this idea, we showed that under normal ambient pressure conditions this mutant retains about 80% of normal activity, but under increased pressure conditions wherein more water molecules would be expected to occupy the space created by the smaller glycine residue the D490G mutant is almost inactive (**Table I**).

With respect to the SHCB phenotype we speculate the following. *In utero*, we would expect the average hydrostatic water pressure to be of the order of 100 mmHg, under which conditions the D490G mutant enzyme might be maximally hydrated and therefore largely constrained in the inactive *trans* configuration. Therefore, we would expect that, together with the G278R mutation, the compound heterozygous siblings would have very low total TGase1 activity, resulting in a collodion membrane phenotype at birth consistent with diagnosis of lamellar ichthyosis. After birth, the skin becomes less hydrated, so that the D490G mutant enzyme may revert to the partially active *cis* configuration. This regained TGase1 activity, amounting to upwards of 40% of total normal, may be sufficient to sustain a minimal threshold to allow an improved epidermal phenotype. These ideas are fully in accord with the observed relative histochemical improvement of TGase1 activity in the younger patient V.S. during her first 4 mo of life, and her older sister N.S. who had diminished TGase1 activity but with mild pityriasiform scaling on the trunk. Immunohistochemistry confirmed that the reduced and patchy distribution of TGase1 activity in the granular layer in both SHCB patients was generally not based on the physical lack of enzyme. With regard to normal stratum corneum we can only speculate that the almost complete absence of TGase1 activity and immunoreactivity is a result of local inactivation or proteolytic degradation of the enzyme. These processes might be disturbed in SHCB. Alternatively, the double contoured activity zones in SHCB might reflect shedding of mutated TGase1 populations in different hydration and thus activity states depending on periodic changes of skin conditions (local therapy, environmental factors).

In summary, we propose that the reported cases of SHCB are caused by a novel mutation in the *TGM1* gene that affects an important *cis–trans* isomerization property of TGase enzymes. The resulting tardy modification of the cornified envelope will lead to clinical manifestation only in the prenatal or immediate perinatal period, or when the skin is otherwise challenged. Only after birth when the skin is exposed for the first time in life to air is sufficient TGase1 activity acquired to build a more normal cornified layer.

We thank the family for kind cooperation. The work of H.T. and M.R. is supported by grants from the Deutsche Forschungsgemeinschaft (Tr 228/6-1) and by the German self support group Selbsthilfe Ichthyose e.v.

REFERENCES

Akiyama M, Takizawa Y, Suzuki Y, Ishiko A, Matsuo I, Shimizu H: Compound heterozygous TGM1 mutations including a novel missense mutation L204Q in a mild form of lamellar ichthyosis. *J Invest Dermatol* 116:992–995, 2001

Bernstein FC, Koetzle TF, Williams GJ, *et al:* The protein data bank: a computer based archival file for macromolecular structures. *J Mol Biol* 112:535–542, 1977

Buyse L, Graves C, Marks R, Wijeyesekera K, Alfaham M, Finlay AY: Collodion baby dehydration: the danger of transepidermal water loss. *Br J Dermatol* 129:86–88, 1993

Candi E, Melino G, Mei G, Tarcsa E, Chung SI, Marekov LN, Steinert PM: Biochemical structural and tranglutaminase substrate properties of human loricrin, the major epidermal cornified cell envelope protein. *J Biol Chem* 270:26382–26390, 1996

Candi E, Melino G, Lahm A, Ceci R, Rossi A, Kim I-G, Ciani B, Steinert PM: Transglutaminase 1 mutations in lamellar ichthyosis. *J Biol Chem* 273:13693–13702, 1998

Cserhalmi-Friedman PB, Milstone LM, Christiano AM: Diagnosis of autosomal recessive lamellar ichthyosis with mutations in the TGM1 gene. *Br J Dermatol* 144:726–730, 2001

Hennies HC, Küster W, Wiebe V, Krebsova A, Reis A: Genotype/phenotype correlation in autosomal recessive lamellar ichthyosis. *Am J Hum Genet* 62:1052–1061, 1998a

Hennies HC, Raghunath M, Wiebe V, Vogel M, Velten F, Traupe H, Reis A: Genetic and immunohistochemical detection of mutations inactivating the keratinocyte transglutaminase in patients with lamellar ichthyosis. *Hum Genet* 102:314–318, 1998b

Huber M, Rettler I, Bernasconi K, *et al:* Mutations of the keratinocyte transglutaminase in lamellar ichthyosis. *Science* 267:523–528, 1995

Huber M, Yee VC, Burri N, Vikerfors E, Lavrijsen AP, Paller AS, Hohl D: Consequences of seven novel mutations on the expression and structure of keratinocyte transglutaminase. *J Biol Chem* 277:21018–21026, 1997

Ichinose A, Tsukamoto H, Izumi TI, *et al:* Arg260-Cys mutation in severe factor XIII deficiency conformational change of the A subunit is predicted by molecular modeling and mechanics. *Br J Haematol* 101:264–272, 1998

Jones TA, Zou Y, Cowan SW, Kjeldgaard M: Improved methods for building protein models in electron density maps and the location of error in these models. *Acta Cryst A* 47:110–119, 1991

Kungsadalampai S, Chelvanayagam G, Baker R, Tiedmann K, Kuperan P, Boeard PG: Identification and characterization of two missense mutations causing factor XIIIA deficiency. *Br J Haematol* 104:37–43, 1999

Laiho E, Ignatius J, Mikkola H, *et al:* Transglutaminase-1 mutations in autosomal recessive congenital ichthyosis: private and recurrent mutations in an isolated population. *Am J Hum Genet* 66:529–538, 1997

Laiho E, Niemi KM, Ignatius J, Kere J, Palotie A, Sarialho-Kere U: Clinical and morphological correlations for transglutaminase-1 gene mutations in autosomal recessive congenital ichthyosis. *Eur J Hum Genet* 7:625–632, 1999

Langer K, Konrad K, Weninger M, Wolff K: Collodion baby with transition to mild lamellar ichthyosis. Clinical course, histopathology and ultrastructural findings. *Hautarzt* 42:34–38, 1991

Lavrijsen AP, Maruyama T: Absent transglutaminase TGK expression in two of three patients with lamellar ichthyosis. *Arch Dermatol* 131:363–364, 1995

Lui K, Commens C, Chong R, Jaworski R: Collodion babies with Gaucher disease. *Arch Dis Child* 63:854–856, 1988

Online Mendelian Inheritance in Man OMIM: (TM). McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2000 *World Wide Web URL. http://www.ncbi.nlm.nih.gov/omim/*

Parmentier L, Blanchet-Bardon C, Nguyen S, Prudhomme JF, Dubertret L, Weissenbach J: Autosomal recessive lamellar ichthyosis: id. *Hum Mol Genet* 44:1391–1395, 1995

Philips MA, Stewart BE, Rice RH: Genomic structure of keratinocyte transglutaminase: recruitment of new exon for modified function. *J Biol Chem* 267:2288–2286, 1992

Raghunath M, Hennies HC, Velter F, Wiebe V, Steinert PM, Reis A, Traupe H: A novel *in situ* method for the detection of deficient transglutaminase activity in the skin. *Arch Dermatol Res* 290:621–627, 1998

Russell LJ, DiGiovanna JJ, Rogers GR, Steinert PM, Hashem N, Compton JG, Bale SJ: Mutation in the gene for transglutaminase-1 in autosomal recessive lamellar ichthyosis. *Nat Genet* 9:279–283, 1995

Sybert VP: Disorders of the epidermis. In: Sybert VP, ed. *Genetic Skin Disorders.* New York, Oxford: Oxford University Press, 1997: pp 5–128

Tok J, Garzon MC, Cserhalmi-Friedman RP, Lam HM, Spitz JL, Christiano AM: Identification of mutations in the transglutaminase 1 gene in lamellar ichthyosis. *Exp Dermatol* 8:128–133, 1999

Traupe H: Isolated congenital ichthyoses. In: Traupe H, ed. *The Ichthyoses. A Guide to Clinical Diagnosis, Genetic Counseling and Therapy.* Berlin: Springer, 1989: pp 103–154

Weiss MS, Metzner HJ, Hilgenfeld R: Two non-proline *cis* peptide bonds may be important for factor XIII function. *FEBS Lett* 423:291–296, 1998

Yee VC, Trong I, Bishop PD, Pderson LC, Stenkamp RE, Teller DC: Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. *Proc Natl Acad Sci USA* 91:7296–7300, 1994