Ichthyosis Bullosa of Siemens Is Caused by Mutations in the Keratin 2e Gene

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Ichthyosis bullosa of Siemens is a blistering disorder with autosomal dominant inheritance. The disease resembles bullous congenital ichthyosiform erythroderma but is less severe. Keratins K1 and K10 have been implicated in bullous congenital ichthyosiform erythroderma. Linkage analysis pointed to the involvement of a keratin type II gene (12q11–13) in ichthyosis bullosa of Siemens. Mutations in the highly conserved regions of K1, a member of the type II gene cluster, were excluded. The gene coding for keratin 2e is also located in the type II gene cluster and the expression of the gene coincides with the occurrence of epidermolytic hyperkeratosis. Sequence analysis revealed the presence of mutations in the K2e gene in patients with ichthyosis bullosa of Siemens. Three different mutations were detected, one in the 1A domain and two in the 2B domain of the rod. Furthermore, histologic and ultrastructural examination of skin biopsies indicated that ichthyosis exfoliativa is identical to ichthyosis bullosa of Siemens. This was confirmed by the results of the molecular analysis. In the family diagnosed as ichthyosis exfoliativa, a mutation was detected that was identical to the mutation found in one of the families with ichthyosis bullosa of Siemens.  

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Patients with bullous congenital ichthyosiform erythroderma (BCIE), often referred to as epidermolytic hyperkeratosis, suffer from fragility of the differentiating keratinocytes in the suprabasal layers of the skin. BCIE is caused by mutations in the genes coding for keratin 1 or keratin 10 that are expressed in the suprabasal cells [1–4].  

Ichthyosis bullosa of Siemens (IBS) has been described as an entity distinct from BCIE [5–7]. In the former disease hyperkeratosis and blistering are milder than in BCIE. Furthermore, IBS can be differentiated from BCIE by the absence of congenital erythroderma. Light- and electron microscopic examination show the features of epidermolytic hyperkeratosis, confined to the stratum granulosum and the upper part of the stratum spinosum, whereas in BCIE these findings are present in the whole suprabasal compartment. Although BCIE and IBS have been described as two diseases, it has also been hypothesized that they are allelic disorders [7,8].  

Autosomal dominant ichthyosis exfoliativa (IE) is a recently described type of bullous ichthyosis [9]. Only one family has been published so far (family 2 in the present study). The clinical symptoms of IE are very similar to IBS. However, according to the original paper, signs of epidermolytic hyperkeratosis were absent.  

Linkage analysis in two families with IBS and the family with IE already suggested that both disorders are due to a defect in a member of the type II keratin gene cluster (K1–K8) on chromosome 12q11–13. K1 is a good candidate because mutations in the gene can cause BCIE. However, sequence analysis gave no indications for mutations in the conserved regions: the H1 region of the head domain of the type II keratins, the helix initiation peptide of the 1A rod domain, the helix termination peptide of the 2B rod domain, the L12 linker between the 1B and 2A rod domains, and an area close to the stutter in the 2B helix. The mutations causing BCIE are exclusively detected in these regions of K1 and K10 [1–3,10–12].  

The keratin 2e gene is expressed in the upper spinous layers, from the third or fourth cell layer onwards [13]. Only these layers show epidermolytic hyperkeratosis in IBS patients [5–7]. Sequence analysis of the K2e gene in IBS patients of three different families and in patients of the family with IE revealed three different mutations either in the helix initiation peptide or in the helix termination peptide. As was suggested by the clinical picture, IE and IBS are identical disorders.

MATERIALS AND METHODS

Patients Four families were included in the analysis. Family 1 has been previously reported [7] as suffering from IBS. The affected individuals had brownish wrinkled hyperkeratosis and superficial blistering since early childhood. Blistering was more pronounced during hot and humid weather and could be provoked by mild trauma. Erythroderma had never been present in any of the affected individuals. Skin lesions were localized especially on the extensor surfaces of arms and legs and around the umbilicus, knees, and ankles. In the hyperkeratotic regions superficially denuded areas were observable. Occasionally, fresh blisters ranging in size from 0.5 to 2 cm appeared. In contrast to patients of the families 2–4, two affected family members of family 1 had brownish wrinkled hyperkeratosis since birth.

members also suffered from chronic relapsing pusular eruptions, surrounded by an erythematous flare. Light microscopic examination revealed epidermolytic hyperkeratosis limited to the upper part of the epidermis. On ultrastructural examination the cells of the granular and upper spinous layer showed marked edema, whereas keratohyaline granules were coarse. Keratinocytes in the upper spinous layer displayed aggregates of tonofilaments forming V shapes or shells around the nuclei.

Family 2 has been reported previously as ichthyosis exfoliativa [9]. We re-examined the family, including histologic and ultrastructural analyses. The patients showed dark grey hyperkeratotic lesions with denuded areas. Superficial blistering occurred spontaneously, especially during the summer, but occurred also after trivial trauma. There was no history of erythroderma in any of the patients. Light- and electromicroscopic examination of a hyperkeratotic area showed the features of epidermolytic hyperkeratosis limited to the granular and upper spinous layers. The features consist of keratohyaline clumps, perinuclear vacuolization, and aggregates of tonofilaments forming perinuclear shells (Fig. 1a-b). Histopathologic examination of a developing blister showed marked extra- and intracellular edema limited to the keratinocytes of the upper spinous layer. Periodic acid Schiff—positive deposits were seen within the subcorneal split. The number of tonofilaments and keratohyaline granules were reduced. The few tonofilament bundles were localized at the periphery. Tonofilament clumping could be observed focally in the upper spinous layer (Fig. 1c).

From family 3 four affected individuals were examined. They showed skin lesions similar to the patients of families 1 and 2. The light and electron-microscopic findings in all patients were the same as in families 1 and 2. The diagnosis of the disease was IBS.

From family 4 a 21-year-old man had blistering since the age of 3 years. Erythroderma was never present. He suffered from the same skin lesions as the patients from families 1, 2, and 3. Histologic examination showed the features of epidermolytic hyperkeratosis limited to the upper part of the spinous layer. The other family members could not be examined.

Genomic DNA The genomic DNA, used for amplification of the fragments of the K2e gene, was isolated from peripheral blood according to the method described in [14].

Amplification of DNA Fragments A fragment of 1.8 kb including the region coding for the H1 and 1A domain of K2e was amplified with primers 5'-CTTGTGACTTTTCTCCCTGGA-3' (sense) and 5'-TTCTGCAGTTGCCCATCGAG-3' (antisense). The polymerase chain reaction (PCR) was performed in a volume of 50 µl containing 0.5—1 µg of genomic DNA, 250 ng of each of the primers, 200 µM of dATP, dGTP, dCTP, and dTTP, 10 mM Tris/HC1, pH 8.5, 50 mM KCl, 1 mM MgCl2, 0.01% gelatine, 10% dimethyl sulfoxide, and 0.5 U Taq Polymerase (Perkin Elmer). A PCR program with 35 cycles of 30 seconds at 94°C, 1 min at 60°C, and 2 min at 72°C was followed. The fragment including the region coding for the C-terminal part of the 2B domain was amplified with the primers 5'-GCAGTGTAAGAATGTGCAAGATG-3' (sense) and 5'-CTGTGACTTTCCTCCCTGGA-3' (antisense). The reaction conditions were as described above with the exception of an annealing temperature of 55°C instead of 60°C. Primer sequences were deduced from the cDNA sequence reported in [13].

Cycle Sequencing Purification of the PCR fragments and cycle sequencing were performed as described in [15]. Sequencing of the 1A fragment was performed with the primer 5'-GGTGGCCCTGGCCCTGGA-3' and of the 2B fragment with the sense primer used for the amplification reaction.

Allele-Specific PCR The allele-specific PCR to detect the transversion of A to C in the 1A domain (family 1) was performed with the sense primer used for amplification of the 1A domain (see above) and the antisense primer 5'-TTGTGACTTTGAGGTTATGGTCG-3'. The last nucleotide at the 3' end of the primer corresponds to the mutation. The conditions for the amplification were as described above with the annealing temperature of 62°C instead of 60°C and 2 mM MgCl2.

Screening for the Mutation Present in Families 2 and 3 Screening for the G to A mutation in families 2 and 3 was performed through amplification of a 1.6 kb fragment with the primer 5'-CATTGGAATGACCTGAGGAGG-3' (sense) and the primer 5'-TTCCTCAGTTGGCCCAACCTG-3' (antisense, intron VII). The latter primer deviates in one nucleotide (underlined) from the sequence of the intron. This destroys an Nalm restriction site, which simplifies the analysis. For amplification, the intron primer was end-labeled with 32P. The PCR was performed in a reaction mixture of 25 µl with the components in the same concentration as described above. Amplification involved 30 cycles of 30 seconds at 94°C, 1 min at 55°C, and 1 min at 72°C. For the digestion with Nalm 10 X restriction buffer (supplied by the manufacturer) was added to the end concentration of 1 X and 100 mM spermidine was added to give a concentration of 1 mM. After the addition of 1 U

Figure 1. Skin biopsy of a hyperkeratotic lesion in a patient from family 2. a) The epidermis is acanthotic and orthohyperkeratotic. In the stratum granulosum and the upper part of the stratum spinosum perinuclear halos and keratohyaline clumps are observed. Scale bar, 50 µm. b) Ultrastructurally keratinocytes of the upper spinous layer displayed pronounced aggregates of tonofilaments forming shells around the nuclei. Scale bar, 1 µm. c) Skin biopsy of a developing blister in a patient from family 2. The granular and upper spinous layer show a marked extra- and intracellular edema. Serous deposits were seen within the subcorneal split reflecting early blistering. Clumping of tonofilaments and coarse keratohyaline granules are less obvious. Scale bar, 50 µm.
Figure 2. Segregation of the mutation in the K2e gene in family 1. The mutation is detected by allele-specific amplification. In each of the affected persons the mutated allele could be amplified but not in any of the unaffected family members. Persons in the pedigree marked with a bar are included in this analysis.

MnlI (New England Biolabs) the reaction mixture was incubated at 37°C for 4 h. The fragments were separated on an 8% nondenaturing polyacrylamide gel.

Screening for the Mutation Detected in Family 4 The transition of T to C detected in the patient of family 4 creates a HpaII restriction site. The testing for the presence of the HpaII site was performed after amplification of the surrounding region with the primers 5'-GTTGAGACCTTGAGGG3' (sense) and 5'-TTCTCCTTTCCAGTGGCCC-3' (antisense, intron VII). For the amplification the protocol was identical to that described in "amplification of DNA fragments." The digestion with 2 U HpaII was performed as described for the digestion with MnlI. Fragments were analyzed on a 2.5% agarose gel.

Prediction of Protein Structure The Chou-Fasman algorithm [16] (Caos Camn Center, Nijmegen) was used for the prediction of protein structure.

Linkage Analysis Two-point linkage analysis was performed with the Mlink program of the Linkage program package (version 5.03 [17]). The gene frequency of IBS was assumed to be 0.000005. The mutations were treated as polymorphisms with a frequency of 0.001.

RESULTS

To screen for the presence of mutations in the keratin 2e gene, we started with the direct sequencing of the regions coding for the helix boundary peptides and the H1 domain from two patients of families 1, 2, and 3, from the single patient of family 4, and from two unaffected persons.

Family 1 A heterozygous transversion of adenosine (A995) to cytosine (C) was detected in patients of family 1. This transversion leads to the substitution of a proline for glutamate at position 187 (numbering according to [13]) of the predicted protein sequence. Through allele-specific PCR we studied the inheritance of this mutation in the family to substantiate its disease-causing nature. The mutant allele could be detected in all affected family members and not in the unaffected persons (Fig 2). Linkage analysis between the mutation and the disease gave a maximum lod score of 3.42 at a recombination fraction of zero. The absence of this mutation in control people indicated that the mutation is not a common polymorphism.

Families 2 and 3 In both families 2 and 3 the sequence coding for the helix termination region of the 2B rod domain contained an identical mutation. The GAG codon for glutamic acid493 is changed to an AAG codon leading to the incorporation of a lysine in the predicted K2e protein. The mutation destroyed an MnlI restriction site making it possible to follow the inheritance of the mutation in the families. In family 2 the mutation completely co-segregated with IE (Fig 3a). In family 3 the father was the first affected member and thus a new mutation must have occurred. His three children all were affected and had the mutation (Fig 3b). His parents and five brothers and sisters all were unaffected and lacked the mutation. Together with the absence of the nucleotide change in 50 control people this indicates that the detected mutation causes the disorder. A lod score of 5.46 and 0.54 at no recombination was calculated in two-point linkage analysis between the mutation and IBS in families 2 and 3, respectively.

The mutated G1510 is part of a CpG dinucleotide. These are known to have a 42-fold higher mutation rate than expected from random mutation [18]. The same mutation also was detected in a familial IBS patient by McLean et al.[8]

Family 4 A different mutation was detected in the sequence coding for the helix termination peptide in a familial IBS patient: the transition of T1502 to C leads to the substitution of proline for leucine490. Although the segregation of the mutation in the family could not be studied because of the lack of blood samples of the relatives, it is likely that this mutation causes the disease, because of the nature of the amino acid substitution (see below) and because the mutation could not be detected in 50 unaffected persons. An HpaII site is created by the transition.

Nature of the Mutations In family 1 a nonpolar residue, proline, has been substituted for an uncharged but polar residue, glutamine, of the helix initiation peptide. This means the change of a relatively hydrophilic into a hydrophobic amino acid, which can be expected to induce structural changes. Furthermore, proline is known to be interruptive in protein structure. The Chou-Fasman algorithm [16] for prediction of protein structure predicted the mutation to cause a substantial decrease in the length of the α-helix in the corresponding region of the protein.

In both families 2 and 3 the mutation results in the substitution of a basic lysine for the acidic glutamic acid493 in the helix termination peptide of the 2B rod domain. The change in charge of the conserved residue can be expected to have a strong effect on the secondary structure of the protein. This is also indicated by the decrease of the length of the helix structure, which is predicted by the Chou Fasman algorithm.

Although the substitution of proline for leucine490 in family 4 does not result in a change of charge or polarity, the incorporation of proline can be expected to have a disruptive influence (see above). Also this substitution leads to a marked shortening of the α-helix in the predicted protein structure.

DISCUSSION

Ichthyosis exfoliativa was published as a disorder distinct from IBS on histopathologic grounds [9]. The identity of the clinical symptoms of both diseases and the linkage to the type II keratin gene cluster induced us to include IE in this study. The results of the histologic and ultrastructural re-examination of skin biopsies as well
as the mutation analysis demonstrated the identity of IBS and IE. In the
remaining part of the discussion we will only use IBS to indicate the
present disorder.

Linkage studies already suggested that IBS is caused by a defect in
a type II keratin gene located in the gene cluster on chromosome
12q11-13.3 The detection of the mutations in IBS patients finally
proved the hypothesis that a defect in keratin K2e causes IBS. The
mutations show a dominant phenotype as do all but one mutation in
keratin genes identified so far [19]. In one of the families (family 1)
we found a mutation in the helix initiation peptide of the 1A domain.
In three other families (families 2–4) two different amino acid
substitutions were detected in the helix termination peptide of the
2B domain. The mutated glutamine in family 1 and the
leucine490 substituted in family 4 both are conserved in all type II
keratins. The mutated glutamic acid in family 2 and 3 is not only
conserved in the type II keratins but in the type I as well. These
mutations further underline the importance of the integrity of the
helix boundary peptides for the appropriate KIF (keratin intermediate
filament) formation (cf. [10–12,20–23]).

The helix boundary peptides are two out of six regions where a close
interaction occurs between neighboring molecules in the KIF and thus
specify the correct alignment of these molecules [11,24,25]. According to the prediction of the structure of the mutated
K2e molecules, the mutations cause a change in the corresponding
part of the α-helical rod domain. All of the three mutations
result in the incorporation of a residue with a lower helix
propensity parameter than the normal residues [16]. The changes in
structure can be expected to affect both the heterodimerization
through impairment of the coiled coil formation and the higher-
order filament assembly, reflected in the disturbed cytoskeleton in
keratinocytes of the upper spinous and granular layer of the
epidermis (cf. [20,22,23,26]). The clarification of the genetic defect causing IBS finally demonstrates that IBS is distinct from BCIE. The divergent expression patterns of K2e and K1 or K10 can explain the clinical and histologic differences between both diseases. Defects in filament formation
are in the skin of IBS patients only visible in the granular layer and
the upper spinous layer [5–7,9]. This coincides with the
expression of the K2e gene in normal skin [13]. In BCIE, filament
clumping is present in the whole suprabasal compartment that expresses K1 and K10.

A substitution of lysine for the glutamic acid corresponding to the
Glu493 in K2e has been introduced in K14 and subsequently
expressed by transient transfection in SCC-13 cells. The phenotype is
milder than with transfection of a mutated K14, causing the severe
EBs-Dowling-Meara in patients [20]. This suggests that the nature
of the mutations can explain the milder epidermolytic hyperkerato-
sis of IBS compared to that of BCIE.

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