A Novel Helix Termination Mutation in Keratin 10 in Annular Epidermolytic Ichthyosis, a Variant of Bullous Congenital Ichthyosiform Erythroderma

Yasushi Suga,*† Karynne O. Duncan,‡ Peter W. Heald,‡ and Dennis R. Roop*†

Departments of *Cell Biology and †Dermatology, Baylor College of Medicine, Houston, Texas; ‡Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut

Annular epidermolytic ichthyosis is a distinct phenotypic variant of bullous congenital ichthyosiform erythroderma that has recently been described in two separate kindreds. Individuals with this variant present with bullous ichthyosis in early childhood and hyperkeratotic lichenified plaques in the flexural areas and extensor surfaces at later ages. Characteristically, they also develop intermittent bouts of annular and polycyclic, erythematous, scaly plaques on the trunk and proximal extremities. We now describe a third kindred with annular epidermolytic ichthyosis. Molecular analysis of this family revealed a novel mutation resulting in an isoleucine to threonine substitution at residue 107 (codon 446) within the highly conserved helix termination motif at the end of the rod domain of keratin 10. ***Key words: disease/genetics/intermediate filaments/keratin. J Invest Dermatol 111:1220–1223, 1998.***

Bullous congenital ichthyosiform erythroderma (BCIE) is an autosomal dominant disorder manifest by widespread blistering and an ichthyotic erythroderma at birth that persists into adulthood. Histologically there is diffuse epidermolytic degeneration in the lower spinous layer of the epidermis. This disorder is caused by mutations in either keratin 1 or 10 (Kl or Kl0) (Cheng *et al*, 1992; Chipev *et al*, 1992; Rothnagel *et al*, 1992). Ichthyosis bullosa of Siemens (IBS) is an autosomal dominant disorder characterized by a more benign course in which affected individuals are often born with diffuse erythema and blistering that becomes more localized and actually improves with age. In addition to the hyperkeratotic, lichenified flexural plaques, these individuals also exhibit a localized Mauserung or "molting effect." Histologically, the hyperkeratotic plaques reveal a more superficial epidermolytic hyperkeratosis in the upper spinous and granular layers of the epidermis. IBS is caused by mutations in the keratin 2e gene (K2e) (Kremer *et al*, 1994; McLean *et al*, 1994; Rothnagel *et al*, 1994). Sahn *et al* (1992) reported a new clinical phenotype that resembles clinical and histologic features of both BCIE and IBS. They called this phenotype annular epidermolytic ichthyosis (AEI). Recently, we described a second kindred with clinical and histologic features similar to AEI (Joh *et al*, 1997). Molecular analysis revealed a novel mutation in the K10 gene (a dinucleotide mutation within the 2B segment of the rod domain) in affected family members. Therefore, we proposed that AEI should be considered a variant of BCIE (Joh *et al*, 1997). We now report a third kindred with similar clinical and histologic findings as the other two families described as having AEI. Molecular analysis of DNA from affected and unaffected family members in our kindred revealed yet another novel mutation within the helix termination motif of K10.

Manuscript received July 15, 1998; revised September 8, 1998; accepted for publication September 10, 1998.

Reprint requests to: Dr. Dennis R. Roop, Baylor College of Medicine, Department of Cell Biology, One Baylor Plaza, Houston, TX 77030.

Abbreviations: AEI, annular epidermolytic ichthyosis; BCIE, bullous congenital ichthyosiform erythroderma; IBS, ichthyosis bullosa of Siemens.

MATERIALS AND METHODS

Patients The proband is an 11 y old male (individual IV.2 in **Fig 1**), born to nonconsanguinous parents, who was referred to us for evaluation and treatment of severe atopic dermatitis. He had no skin abnormalities at birth. At the age of 7 mo he was noted to have very dry skin, especially in the flexural areas and over the extensor surfaces. He never had frank blistering or erythroderma. Over time, the plaques in the flexural areas and over the extensor surfaces expanded centrifugally and developed marked central hyperkeratotic lichenification. He also had intermittent episodes of erythematous, scaly, annular and serpiginous plaques that would develop over the proximal extremities and trunk (**Fig 2*a***). Hair, nails, mucous membranes, and palms and soles were normal. Thus, the clinical features are compatible with a diagnosis of AEI.

His mother (III.3) and younger sister (IV.1) both had mild hyperkeratotic plaques over the knees and elbows, but no other cutaneous findings. His father (III.4) was unaffected. His maternal uncle (III.1) and maternal grandfather (II.4) had lesions of similar clinical appearance and severity as our proband, but both were deceased (**Fig 1**).

A biopsy was performed from the edge of a flexural, hyperkeratotic, lichenified plaque and blood was obtained for DNA analysis.

Sequencing Genomic DNA was isolated from whole blood of the proband and the family members as described previously (Rothnagel *et al*, 1992). The 2B region of K10 was amplified from genomic DNA using specific oligonucleotides (5′-AAGTGCTGAAACTGAAATGGTGCC-3′ and 5′-TCTACCCTCTCTCCTCCCTTCCTC-3′) corresponding to bp 4373–4396 and to 4837–4860 of the published sequence (Rieger and Franke, 1988). Polymerase chain reaction (PCR) conditions were 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C, for 30 cycles. PCR products were purified using a PCR Purification Kit (Qiagen, Santa Clarita, CA) and were directly sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Kit with AmpliTaq DNA Polymerase FS (Perkin Elmer-ABI, Foster City, CA). The sequences were run on an ABI Prism 377 DNA Sequencer (Perkin Elmer-ABI). The sequencing primer was 5′-ATAAGCGTCACCATACTC-3′, corresponding to bp 4463–4480 of the published sequence (Rieger and Franke, 1988).

PCR-restriction fragment length polymorphism (RFLP) analysis The mutation destroys an Apo I site (5′-PuAATTPy-3′) in the 2B region of K10

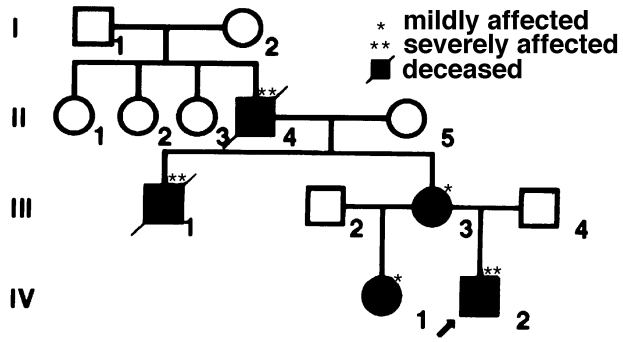

Figure 1. Pedigree of the AEI family. □, Men; ○, women. Solid and open symbols denote affected and unaffected individuals, respectively. The *arrow* indicates the proband (individual IV-2).

in the mutant allele. Therefore, segregation of the mutation was confirmed by RFLP analysis with Apo I. The 2B region of K10 was amplified by PCR with specific oligonucleotides and digested with Apo I (New England Biolabs, Beverly, MA) at 50°C overnight.

RESULTS

Histopathology is consistent with superficial epidermolytic hyperkeratosis Histopathologic examination of a skin biopsy specimen from a flexural, hyperkeratotic, lichenified plaque revealed orthokeratosis, acanthosis, papillomatosis, and a thickened granular layer (**Fig 2*b***). Also prominent were coarse keratohyaline granules, blurring of the cell boundaries, and vacuolar degeneration of the keratinocytes in the superficial spinous and granular layers, reminiscent of those findings in IBS.

Sequence analysis reveals a novel I107T mutation within the highly conserved helix termination motif of K10 Because the histologic analysis of the proband was most consistent with a diagnosis of IBS, we initially screened the K2e gene for mutations. The failure to find obvious mutations in the K2e gene prompted us to examine both the K1 and the K10 genes. Direct automated sequence analysis revealed a T to C transition at position 107 (codon 446) within the 2B segment of the rod domain of K10, which converted an isoleucine residue (ATT) to a threonine (ACT) [I107T], indicated by the *arrow* in **Fig 3(*a*)**. This mutation was found in all affected family members examined and was absent in unaffected members (**Fig 4**).

RFLP analysis reveals that the I107T mutation segregates with the AEI phenotype The I107T mutation abolishes an Apo I site (5′-PuAATTPy-3′) in the mutant allele. To further confirm that this mutation was not a polymorphism and was indeed associated with the disease phenotype, 50 normal controls were screened by RFLP analysis, as well as affected and unaffected family members. A 490 bp fragment containing the 2B region of K10 was amplified by PCR and digested with Apo I. The normal allele was digested into 330 and 160 bp fragments, but the 490 bp fragment from the mutant allele was resistant to digestion. All affected individuals in this family were heterozygous for the mutant and the wild-type allele, and produced three (490, 330, and 160 bp) fragments in this assay. Unaffected individuals were homozygous for the wild-type allele, and produced only two (330 and 160 bp) fragments. This mutation was not detected in the 50 normal DNA analyzed (data not shown). Thus, the Apo I RFLP cosegregates with expression of the disease phenotype, in agreement with the autosomal dominant inheritance pattern of this disease.

DISCUSSION

In this study, we describe a novel mutation in the BCIE variant, AEI, which is the first reported substitution of this highly conserved isoleucine residue (I107T) within the helix termination motif of a type I keratin gene. Although we have not presented functional data documenting that this mutation is pathogenic, several lines of evidence suggest that it is. First, this mutation was only detected in family members with clinical symptoms, and not in unaffected family members. Second, this mutation was not observed in any of the 100 normal K10 alleles that were screened in this study and there are no published reports of silent polymorphisms at this site. Third, this isoleucine residue is absolutely conserved in all type I and type II keratins sequenced to date, as well as in several other intermediate filament proteins (Hatzfeld and Weber, 1991; Irvine *et al*, 1997).

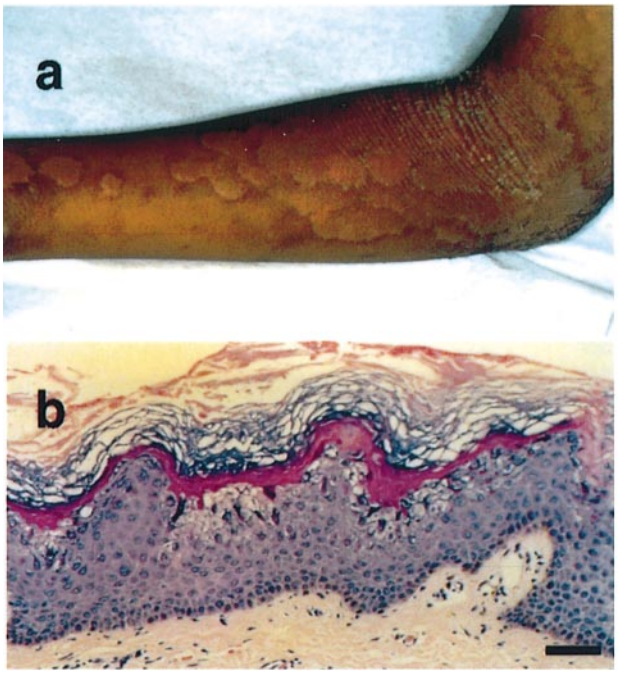

Figure 2. Clinical presentation and histopathology of the proband. (*a*) Typical flexural presentation, showing the hyperkeratotic lichenified plaques with verruciform columnar scales in linear array over the antecubital fossae, with surrounding, concentric, scaly, annular, and polycyclic plaques. (*b*) Histopathology of hyperkeratotic lichenified flexural plaque. Hyperkeratosis, acanthosis, and thickened granular layer. Keratinocytes in upper spinous and granular layers of epidermis demonstrate cytoplasmic vacuolization and prominent keratohyaline granules. *Scale bar.* 50 μm.

Cross-linking experiments predict important regions of overlap between the helix initiation and termination motifs of neighboring keratin molecules (Steinert *et al*, 1993); however, to date, the overwhelming majority of the K10 mutations identified in BCIE occur within the helix initiation motif (Rothnagel and Roop, 1995; Corden and McLean, 1996). In fact, due to the lack of reported mutations in the helix termination motif of type I keratins, we previously speculated that substitutions within this region might be well tolerated and fail to produce an obvious phenotype (Rothnagel *et al*, 1994). This study clearly documents that mutations in the helix termination motif of K10 can be pathogenic; however, based on a survey of reported cases of BCIE, it is also apparent that substitutions at this particular residue of K10 are not as disruptive as those occurring in the helix initiation motif of K10, or those occurring in either the helix initiation or the termination motifs of K1 (Rothnagel and Roop, 1995; Corden and McLean, 1996).

Prior to this study, only three mutations had been identified in the 2B segment of K10, and these occurred within residues located just upstream of the helix termination motif. Chipev *et al* (1994) initially described a family in which the index patient was diagnosed with ichthyosis hystrix (localized) BCIE at the age of 5 mo and molecular analysis detected a L103Q mutation. Her son presented with blistering at birth and her granddaughter had blistering and generalized hyperkeratosis, especially over the flexural surfaces. Unfortunately, the phenotypic severity was not reported for this case and the histologic findings were not presented. Syder *et al* (1994) reported an atypically mild BCIE family that had a K100E mutation just proximal to the end of

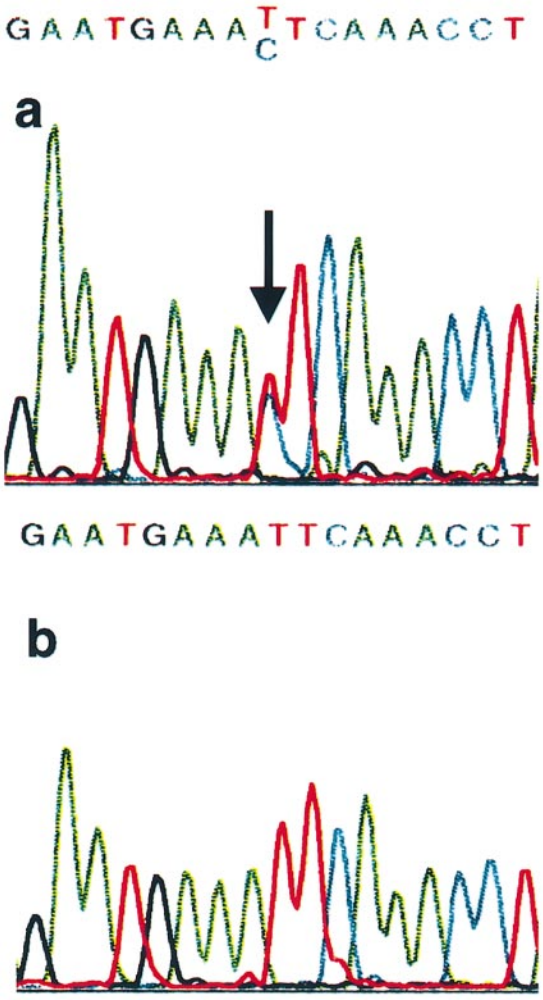

Figure 3. Sequence analysis of the 2B segment of HK10 in the proband and unaffected family members. Direct genomic sequencing of the proband reveals an ATT to ACT mutation at residue 107 (*a*), compared with the control sequence obtained from an unaffected family member (*b*). Numbering of the amino acids is with respect to the 2B segment of the rod domain.

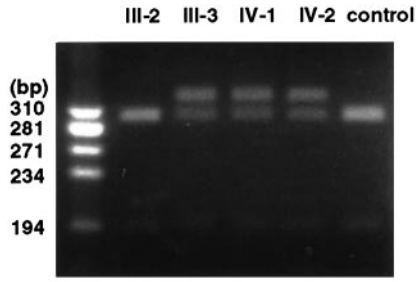

Figure 4. RFLP analysis demonstrating segregation of the mutation with the AEI phenotype. Apo I digestion of the normal allele produces 330 and 160 bp fragments. All affected patients in the family are heterozygous for the 490, 330, and 160 bp fragments. The unaffected persons are homozygous for the 330 and 160 bp fragments. Pedigree numbers correspond to those indicated in **Fig 1**.

the 2B rod domain of K10. Although the clinical description did not report the development of annular and polycyclic erythematous, hyperkeratotic plaques, the histologic findings were somewhat similar to AEI. The third mutation in the 2B segment of K10 was discovered during our initial analysis of the molecular basis of AEI, and consisted of a R83E substitution (Joh *et al*, 1997). This mutation clearly resides outside of the putative overlap region of helix initiation and termination motifs. Therefore, it was not surprising that the clinical course of the disease in this family was much milder than typically described for BCIE patients.

This study describes the fourth mutation, I107T, in the 2B segment of K10, which represents the first mutation in the helix termination motif of a type I keratin. Although the I107T mutation lies within the highly conserved helix termination motif, it does not appear to reside within the putative overlap region with the helix initiation motif. This isoleucine, however, is located in the "a" position of the heptad repeat and the substitution of a threonine would be expected to seriously impair the formation of a regular two-strand coiled-coil in this crucial part of the molecule. This may cause the molecule to terminate earlier than normal or the length may be the same as normal but the conformation may be less regular over the last 2–3 heptads. This could impede the next stage in the molecular hierarchy where the head-to-tail overlap occurs (Steinert and Parry, 1993; Steinert *et al*, 1993). Having said this, it is surprising that the histopathologic findings in this case are so similar to IBS, i.e., cytolysis is restricted to the upper spinous and granular layers of the epidermis. This suggests that the dominant negative interfering effects of this K10 mutant are minimal in the lower spinous cells. Perhaps this particular K10 mutation does not substantially alter the keratin filament network until K5 and K14 subunits, which are known to persist into the spinous layer, are gradually depleted. Alternatively, this K10 mutant may have little effect on K1/K10 dimers, but primarily cause instability when it dimerizes with K2e, which is synthesized in the upper spinous and granular layers. Of course, this assumes that K2e has no other type I dimerization partner except K10.

Clearly, there are still many unanswered questions concerning how individual keratin mutations cause disease. For this reason, it is important to continue to document the phenotypes caused by different keratin mutations. This information is not only useful for correlating genotypes with phenotypes in genetic counseling, but is also essential for inclusion in future molecular modeling and structure/function studies.

We appreciate the interest and participation of the family members in this study. We also thank Drs. Joseph Rothnagel and David Parry for comments on the manuscript. This work was supported by NIH grant (HD25479) to D.R.R. Y.S. was partially supported by The Naito Foundation grant (94-453).

REFERENCES

Cheng J, Syder AJ, Yu QC, Letai A, Paller AS, Fuchs E: The genetic basis of epidermolytic hyperkeratosis: a disorder of differentiation specific epidermal keratin genes. *Cell* 70:811–819, 1992

Chipev CC, Korge BP, Markova N, Bale SJ, DiGiovanna JJ, Compton JG, Steinert PM: A leucine-proline mutation in the H1 subdomain of keratin 1 causes epidermolytic hyperkeratosis. *Cell* 70:821–828, 1992

Chipev CC, Yang JM, DiGiovanna JJ, Steinert PM, Marekov L, Compton JG, Bale SJ: Preferential sites in keratin 10 that are mutated in epidermolytic hyperkeratosis. *Am J Hum Gen* 54:179–190, 1994

Corden LD, McLean WHI: Human keratin disease: Hereditary fragility of specific epithelial tissues. *Exp Dermatol* 5:297–307, 1996

Hatzfeld M, Weber K: Modulation of keratin intermediate filament assembly by single amino acid exchanges in the consensus sequence at the C-terminal of the rod domain. *J Cell Sci* 99:351–362, 1991

Irvine AD, McKenna KE, Bingham A, Nevin NC, Hughes Ä: A novel mutation in the helix termination peptide of keratin 5 causing epidermolysis bullosa simplex Dowling-Meara. *J Invest Dermatol* 109:815–816, 1997

Joh GY, Traupe H, Metze D, *et al*: A novel dinucleotide mutation in keratin 10 in the annular epidermolytic ichthyosis variant of bullous congenital ichthyosiform erythroderma. *J Invest Dermatol* 108:357–361, 1997

Kremer H, Zeeuwen P, McLean WH, *et al*: Ichthyosis Bullosa of Siemens is caused by mutations in the Keratin 2e gene. *J Invest Dermatol* 103:286–289, 1994

McLean WH, Morley SM, Lane EB, *et al*: Ichthyosis bullosa of Siemens – a disease involving keratin 2e. *J Invest Dermatol* 103:277–281, 1994

Rieger M, Franke WW: Identification of an orthologous mammalian cytokeratin gene. *J Mol Biol* 204:841–856, 1988

Rothnagel JA, Roop DR: Analysis, diagnosis, and molecular genetics of keratin disorders. Curr Opinion Dermatol. 211–218, 1995

Rothnagel JA, Dominey AM, Dempsey LD, *et al*: Mutations in the rod domains of keratin 1 and 10 in epidermolytic hyperkeratosis. *Science* 257:1128–1130, 1992

Rothnagel JA, Traupe H, Wojcik S, *et al*: Mutations in the rod domain of keratin 2e in patients with ichthyosis bullosa of Siemens. *Nature Genet* 7:485–490, 1994

Sahn EE, Weimer CE, Garen PD: Annular epidermolytic ichthyosis: a unique phenotype. *J Am Acad Dermatol* 27:348–355, 1992

Steinert PM, Parry DAD: The conserved H1 domain of the type II keratin I chain plays an essential role in the alignment of nearest neighbor molecules in mouse and human keratin1/keratin 10 intermediate filaments at the two-to-four-molecule level of structure. *J Biol Chem* 268:2878–2887, 1993

Steinert PM, Marekov LN, Fraser RDB, Parry DAD: Keratin intermediate filament structure: closslinking studies yield quantitative information on molecular dimensions and mechanism of assembly. *J Mol Biol* 230:436–452, 1993

Syder AJ, Yu QC, Paller AS, Giudice G, Pearson R, Fuchs E: Genetic mutations in the K1 and K10 genes of patients with epidermolytic hyperkeratosis. *J Clin Invest* 93:1533–1542, 1994