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Research Article

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Genetic Mutations in the K1 and K10 Genes of Patients with Epidermolytic Hyperkeratosis

Correlation between Location and Disease Severity

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Abstract

Epidermolytic hyperkeratosis (EH) is a skin disease caused by mutations in the genes encoding K1 and K10, the differentiation-specific keratins of epidermis. To explore the heterogeneity of mutations and to assess whether a correlation exists between disease severity and the extent to which a mutation interferes with keratin network formation, we determined the genetic bases of four severe incidences of EH and one unusually mild case. Two severe cases have the same mutation, K10-R156:C, at a conserved arginine that we previously showed was mutated to a histidine in two unrelated EH families. An additional severe case has a mutation six residues away, still within the amino end of the α-helical rod domain of K10. The other severe case has a mutation in the conserved carboxy end of the K1 rod. In contrast, affected members of the atypically mild family have a mutation just proximal to the conserved carboxy end of the K10 rod. By genetic engineering and gene transfection, we demonstrate that each mutation is functionally responsible for the keratin filament aberrations that are typical of keratinocytes cultured from these patients. Moreover, we show that the mild EH mutation less severely affects filament network formation. Taken together, our studies strengthen the link between filament perturbations, cell fragility, and degeneration. (*J. Clin. Invest.* 1994. 93:1533–1542.) Key words: keratins • epidermolytic hyperkeratosis • skin disease • mutations

Introduction

The major structural proteins of epidermis are keratins, which form an extensive cytoskeletal architecture of 10-nm intermediate filaments (IFs).[1] Based on sequence, these proteins fall into two groups, type I and type II (1), which form heteropolymers. K5 (type II) and K14 (type I) are the pair expressed in the mitotically active, basal layer (2). As keratinocytes commit to differentiate, they switch off expression of K5 and K14 and induce expression of a new pair, K1 (type II) and K10 (type I) (3).

Keratins have a central rod domain that contains four largely α-helical segments, helix 1A, 1B, 2A, and 2B (4). The rod is flanked by nonhelical head (amino) and tail (carboxy) segments that are variable in size and sequence. The helices contain heptad repeats of hydrophobic residues, producing a hydrophobic seal on the helical surface, enabling the coiled-coil heterodimerization of in register, parallelly aligned molecules of K5 and K14, or K1 and K10 (5–7). In vitro, dimers associate in antiparallel fashion to form two types of tetramers: a form where the amino ends of two dimers overlap in a near half-staggered alignment and an unstaggered form (8–11). IFs are ropelike in structure, with putative strings of tetramers forming protofilaments, which intertwine to form protofibrils, which in turn intertwine to yield a single 10-nm filament (12–14).

In vitro, IF assembly occurs in the absence of auxiliary factors. Mutagenesis studies have identified the sequences important for IF formation (15–26). The most critical residues are in the conserved ends of the rod, where even subtle point mutations can affect IF assembly (20, 24, 25). In contrast, proline mutations more central in the rod are often not as severe as conservative substitutions at the rod ends (25).

Transgenic animals expressing mutant human K14 or K10 genes exhibit abnormalities similar to the autosomal dominant human skin diseases, epidermolysis bullosa simplex (EBS; K14 mutations) or epidermolytic hyperkeratosis (EH; K10 mutations) (27–29). Both diseases are typified by skin blistering arising from mechanical stress-induced cytolysis in either the basal (EBS) or suprabasal (EH) epidermal layers. Without a proper keratin network, the keratinocyte seems to be fragile and prone to degeneration (27).

Characterization of the basal-specific keratin genes of EBS patients led to the first direct genetic evidence that this disease arises from point mutations in the K5 or K14 genes (30–32). Patients with severe, i.e., Dowling-Meara, EBS frequently have point mutations within the conserved ends of the rod (30, 32, 33). Two moderate cases with Koebner EBS have point mutations more centrally within the rod (31, 34). Affected members of families with the mildest form, Weber-Cockayne EBS, often have point mutations either in the portion of the K5 head domain adjacent to the rod (35) or within the nonhelical linker segment connecting helices 1B and 2A (36, 37). In vitro mutagenesis studies show a correlation between the location of the EBS mutation and disease severity (38), in agreement with earlier random mutagenesis experiments (25).

EH is an autosomal dominant skin disorder of marked clinical heterogeneity with respect to severity of blistering, keratoderma, and erythroderma, and with respect to the extent of

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1. *Abbreviations used in this paper:* EBS, epidermolysis bullosa simplex; EH, epidermolytic hyperkeratosis; IF, intermediate filament.

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body involvement. Families with varying severity have genetic defects which map to the keratin gene cluster on chromosome 12q (39, 40), and patients have point mutations in their K1 or K10 genes (41–43). Thus far, four different mutations have been described. Two are in helix 1A of K10, and one of these, K10 R156:H, affects the same conserved arginine which when mutated in K14 gives rise to EBS (41, 42). Of the other two, one is in the conserved end of helix 2B of K1 (42), and the other is in the K1 head domain (43). Here, we report the identification of K1 or K10 point mutations in five additional incidences of EH of varying degrees of severity. We also present functional studies to assess the extent to which each of these mutations perturbs keratin network formation in vivo.

Methods

Blood samples and biopsies. Blood samples (15–20 cm^3) from all EH patients and family members were used for isolation of genomic DNA. Blood DNAs from 95 unaffected individuals were used as controls. Punch biopsies (4 mm) were obtained from clinically normal skin of EH individuals. One biopsy was used to culture keratinocytes, while the other biopsy was halved and prepared for routine ultrastructural studies or immunoelectron microscopy (44). Postembedding immunogold labelings were performed using rabbit anti-human K1 antisera (45) followed by protein A–conjugated 15-nm colloidal gold (Amersham Corp., Arlington Heights, IL) (44). Prepared grids were counterstained briefly with lead citrate and examined in an electron microscope (JEOL-CX; Jeol Ltd., Tokyo, Japan).

K10/K1 sequence analysis. PCR were performed on genomic DNAs to amplify the following fragments encompassing K1 and K10 exons: (*a*) 840 bp encoding the amino-terminal end of the K10 rod; primers were K10-5′ (exon 1–sense), 5′-CTGTTCGATACAGCTCAAGCAAGCAC-3′ and K10-I1 (intron 1–anti), 5′-CTCCCATCCTCTTAAGACAAGTAGC-3′; (*b*) 1,208 bp encoding the carboxy-terminal end of the K10 rod; primers were K10-7 (exon 3–sense), 5′-TAGGGTGCTGGATGAGCTGACCCT-3′ and K10-I61 (intron 6–anti), 5′-ACCCTCTCTCCTCCCTTCCTCTCA-3′; and (*c*) 1,209

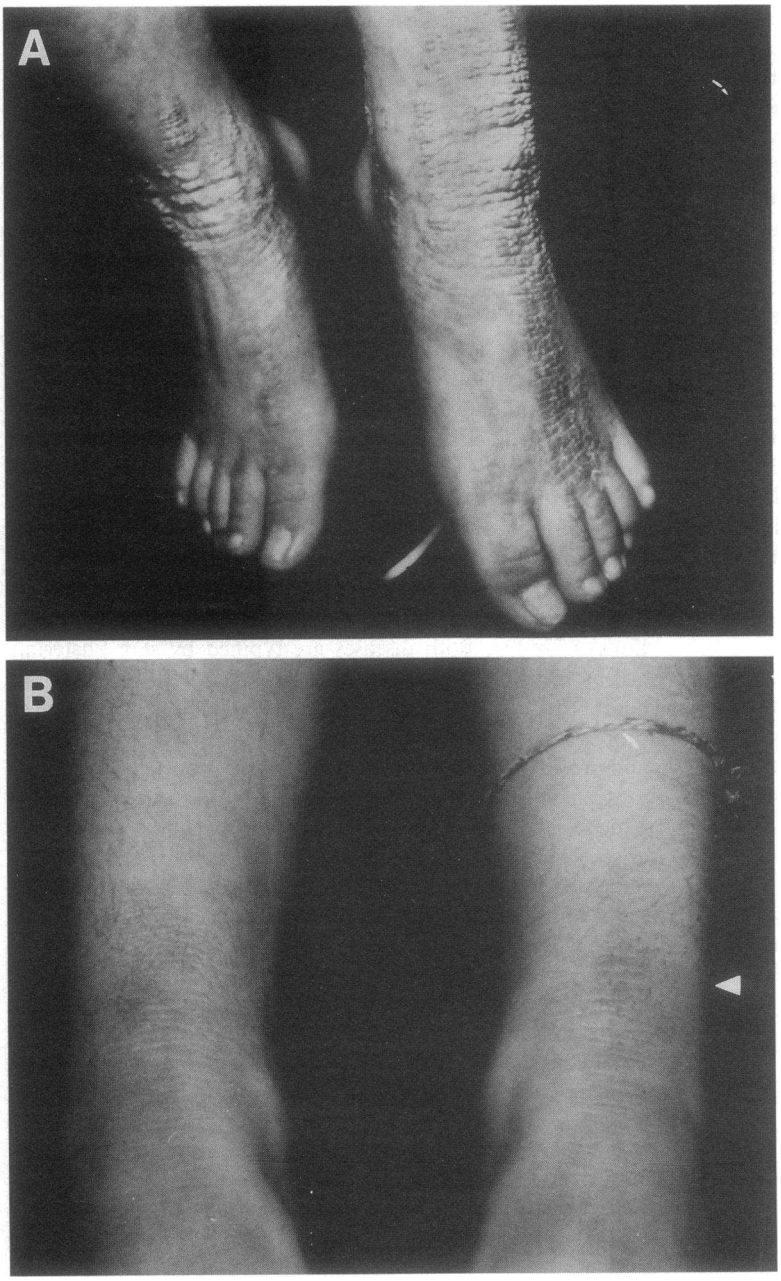

Figure 1. Clinical features of EH cases. (*A*) Ankles of severe EH case. Note gross verrucous thickening of skin, also seen in skin of knees, elbows, Achilles tendons, neck, axillary, inguinal, periumbilical areas, wrists, and dorsal surface of hands and feet. Features were similar for EH1, EH2, EH3, and EH13, all classified as severe. (*B*) Ankles of affected member of EH7 family. Note extremely mild thickening of skin (*arrowhead*). More moderate thickening was seen in skin overlying knees, elbows, and Achilles tendons.

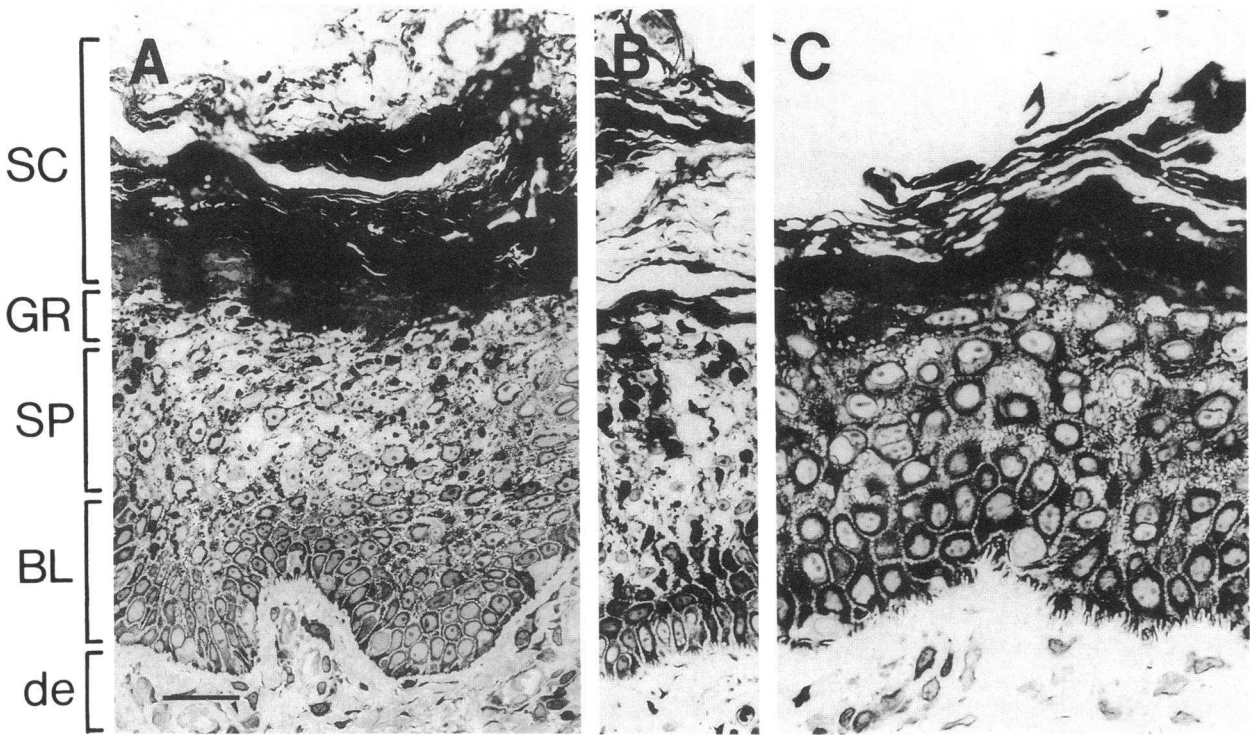

Figure 2. Heterogeneity in morphology of EH skin. Toluidine blue–stained semithin (0.75-μm) sections of skins from EH13 (*A*), EH2 (*B*), and EH7.9 (*C*). Note normal dermis and basal layer in all samples. Note extensive cell degeneration and cytolysis in suprabasal layers of EH13 and EH2. Cell degeneration is evident in EH7.9 skin but is markedly milder. Histologies in *A* and *B* are representative of severe cases. Histology in *C* was similar for all three affected members of the EH7 family. Bar = 45 μm. BL, basal layer; SP, spinous layers; GR, granular layers; SC, stratum corneum; and de, dermis.

bp encoding helix 2B and the carboxy end of K1; primers were K1-I61 (intron 6–sense), 5′-GGTGGGTGTGAGATGACTTAAGGCC-3′ and K1-10 (exon 9–anti), 5′-CCACTGATGCTGGTGTGGCT-TGTGC-3′. PCR primers were designed from the published K1 (46) and K10 (47) sequences. PCR conditions were 1 cycle of 5 min at 97°C, 35 cycles of 1 min at 94°C, 30 s at 60°C, and 1 min at 72°C. Products were passed through microconcentrators for purification (Centricon-100; Amicon, Beverly, MA). Sequencing was performed using a thermal cycle sequencing kit (CircumVent; New England Biolabs Inc., Beverly, MA) on ~ 2 μl of purified PCR product, and reactions were separated by electrophoresis through 6% polyacrylamide gels.

After confirming a mutation in EH7.7 genomic DNA, the entire rod domains of K1/K10 were amplified from cultured keratinocyte cDNA, subcloned, and sequenced (48). For other cases, the K1 and K10 rod domains or portions thereof were also sequenced.

Functional studies. Site-directed mutagenesis (49) was used to engineer mutations in wild-type human K5 or K14 cDNAs cloned into Bluescript KS+ (Stratagene, La Jolla, CA). Fragments encompassing the mutation were then subcloned into the mammalian expression vector, pJK14P or pJK5P. SCC-13 human epidermal keratinocytes (K14+, K5+) or PtK2 potoroo kidney epithelial cells (K18+, K8+) were transiently transfected and subjected to double immunofluorescence as described (15).

Results

Diagnosis of EH patients. 10 autosomal dominant incidences of EH were chosen for study, of which two, EH4 and EH6, had previously been shown to contain the 156R:H mutation in K10 (41). All but one case displayed clinical features at birth, with severe verrucous thickening of the skin and a predisposition to suprabasal blistering, predominantly in flexural areas (Fig. 1 *A*). Family EH7 was unusual in that onset of clinical signs did not appear until ~ 2 yr of age. Affected members then began to display moderate verrucous thickening of the flexural skin (Fig. 1 *B, arrowhead*) and even milder thickening of the axillary, inguinal, periumbilical areas, wrists, and palmoplantar regions. While linear bands of mild hyperkeratosis were evident at popliteal and antecubital areas, clinical blistering never occurred. These features became more severe during childhood, but then showed significant improvement by adulthood, leaving only the persistence of moderate keratoderma on the elbows. The diagnosis was extremely mild EH.

Histological abnormalities were in agreement with the clinical diagnoses. In all cases, the basal epidermal layer appeared normal, whereas suprabasal layers showed a gradient of anomalies. In severe cases, vacuolization and cytolysis began in the lower spinous layers and increased during differentiation (Fig. 2, *A* and *B*). In contrast, only a few isolated spinous cells of EH7 skin showed signs of degeneration (Fig. 2 *C*). All cases showed a thickened granular layer and stratum corneum. Ultrastructural analyses confirmed the diagnoses of EH (see also references 50 and 51) (Fig. 3). Most notably, basal cells were normal (Fig. 3 *A*), while spinous cells possessed vacuoles and areas of clear cytoplasm, indicative of degeneration (Fig. 3, *A* and *B, asterisks*). In severe cases, cells had aberrant keratin tonofibrillar networks, displaying amorphous and nonfilamentous clumps of keratin that labeled with antibodies against K1 (Fig. 3 *B*). In contrast, the cytoskeletons of EH7 suprabasal cells exhibited only mild aggregates of filaments, sometimes organized in perinuclear shells (Fig. 3 *C*).

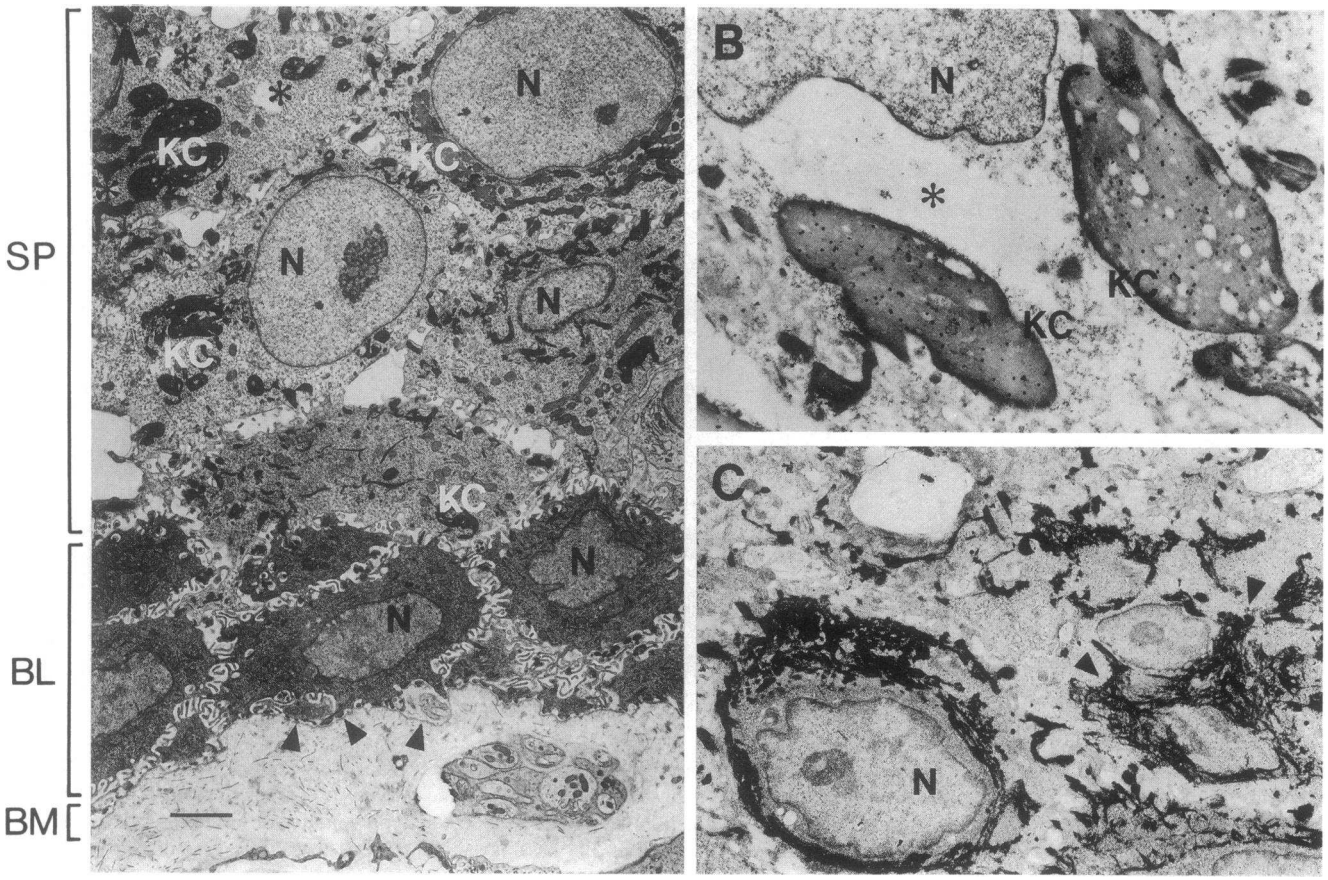

Figure 3. Ultrastructural differences in severity of EH cases. Skin biopsies were fixed and processed for electron microscopy. *A* shows EH13 skin. Note amorphous clumps of keratin (*KC*) and cell degeneration (*asterisks*) in suprabasal but not basal layers. Arrows denote basement membrane (*BM*). *B* shows EH13 suprabasal cell, immunogold-labeled with anti-K1 antibody. *C* shows suprabasal cells of EH7.9 skin. Note perinuclear lateral aggregations of tonofibrils. No amorphous clumps were seen in this sample, nor in EH7.7 or EH7.8. Bar in *A* = 2.3 μm *B* = 0.8 μm and *C* = 1.8 μm. N, nucleus; BL, basal layer; and SP, spinous layer.

Point substitutions in the K10 and K1 rod domains of severe EH incidences. To identify putative K1 and K10 gene mutations, we used PCR to amplify exons corresponding to the rod segments of EH K10 and K1 polypeptides. Two severe incidences, EH3 and EH13, had a C to T transition in the coding strand corresponding to nucleotide 466 of the K10 gene (see reference 47 for the human K10 genomic sequence). This transition was in exon 1, in the first base position of codon 156, and

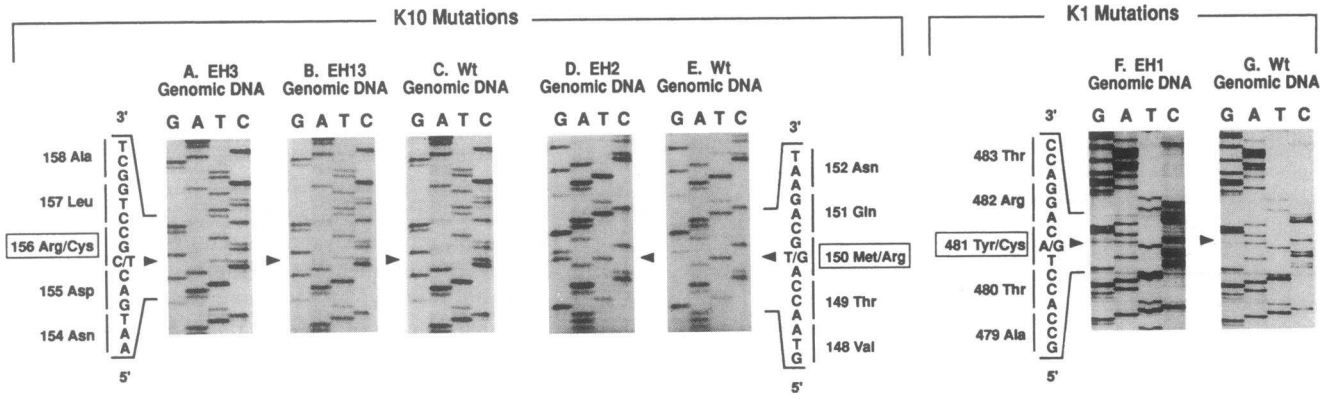

Figure 4. Point mutations in one of the K10 or K1 alleles in EH patients. PCR-amplified fragments from two independent rounds were subjected to DNA sequencing either directly or after subcloning. Shown are coding strand sequences encompassing regions where point mutations were found. Sequences are from: *A–E,* total K10 PCR mixtures of EH3 (*A*), EH13 (*B*), wild-type (*wt*) control for *A* and *B* (*C*), EH2 (*D*), wild-type control for *D* (*E*); *F–G,* total K1 PCR mixtures of EH1 (*F*) and wild-type control for *F* (*G*). Arrowheads denote sites of autosomal dominant mutations of EH patient DNAs, or the equivalent sites of control DNAs.

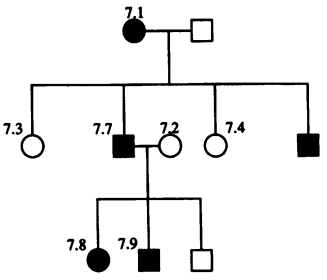

Figure 5. EH7 family tree. Affected members (*black boxes,* male, or *black circles,* female) from three generations have mild EH. Unaffected members are denoted by open symbols.

is predicted to convert R156 (CGC) to C156 (TGC) (Fig. 4, *A* and *B*). We verified that these transitions were not due to polymerase errors during PCR by repeating the amplification and sequence analyses. As expected for a dominant mutation, both C and T appeared in the sequencing ladder. In contrast, the PCR-amplified sequence from control genomic DNA showed only a C at this residue (Fig. 4 *C*). Mutations at this residue were interesting in light of the fact that EH4 and EH6 had R156:H mutations (41), as did another incidence of EH (42). Moreover, the equivalent R:C and R:H mutations have been found in the K14 gene of EBS patients (30, 33, 52).

Interestingly, another patient (EH2) with severe blistering had a K10 mutation, M150:R, a mere six residues away from the R156:C mutation (Fig. 4 *D,* compare with control in *E*). In contrast, yet another severe case (EH1) had a mutation in K1, Y481:C, rather than K10 (Fig. 4 *F,* compare with control in *G*). This mutation was only eight residues upstream from that of a previously reported case of EH (42).

A K10 mutation associated with mild EH. The pedigree for EH7 is shown in Fig. 5. When K10 cDNAs from EH7.7 mRNAs were cloned and sequenced, some had an A to G transition at nucleotide 1315, creating a 439K:E mutation in K10 (Fig. 6). Other clones were wild-type (not shown), suggesting that one K10 allele was normal and one abnormal, in agreement with the autosomal dominant inheritance pattern of the disease.

The position of the EH7 mutation in K10 was 10 residues upstream from the equivalent Y481:C mutation in K1. This location was unusual in that no K10 mutations in this vicinity had thus far been reported for EH. Since this case was also an extremely mild EH case, we checked genomic DNAs of additional family members for this mutation. All affected members had both A and G residues at nucleotide 1315. While the logarithm of the odds score on such a small family was not statistically significant (i.e., < 3), over three generations a perfect correlation existed between the heterozygous appearance of this mutation and the presence of the disease phenotype (Fig. 6).

The four new EH mutations are not polymorphisms. To verify that these new EH mutations are not merely polymorphic variations in the population, we took advantage of the fact that these mutations either obliterate or create restriction endonuclease sites. By selecting appropriate primers for each mutation, we generated a genomic PCR DNA encompassing these sites. Digestion of the PCR DNAs with the relevant restriction endonuclease allowed distinction between the wild-type sequence and the putative EH mutation. Fig. 7 provides representative examples of the data generated. In all cases, only affected EH individuals gave the distinctive heterozygous banding pattern typical of one mutant and one wild-type allele. In a total of 190 wild-type alleles, the loss or gain of the relevant restriction site did not occur. Collectively, these data suggested that these mutations are restricted to individuals affected with EH.

Further analyses revealed no additional K1 or K10 mutations relative to wild-type controls. However, some polymorphisms were detected, including the previously reported G/S polymorphism at codon 126 in the glycine-serine–rich head domain of K10 (41) and a polymorphism in the 463R codon (CGT; CGC) of K1. Other polymorphisms in the highly variable head and tail domains of epidermal keratins have been described (43, 53, 54). In addition, all of our sequences deviate at three specific locations from the GenBank sequences (see also 43, 46, 47): (*a*) nucleotide 317 of K10 is a G instead of an A, leading to S106 (AGT) rather than N106 (AAT); (*b*) nucleotide 1224 of K10 is a G instead of a C, leading to Q408 (CAG) rather than H408 (CAC); and (*c*) nucleotide 1072 of K1 is a T instead of an A, leading to Y358 (TAC) rather than N358 (AAC).

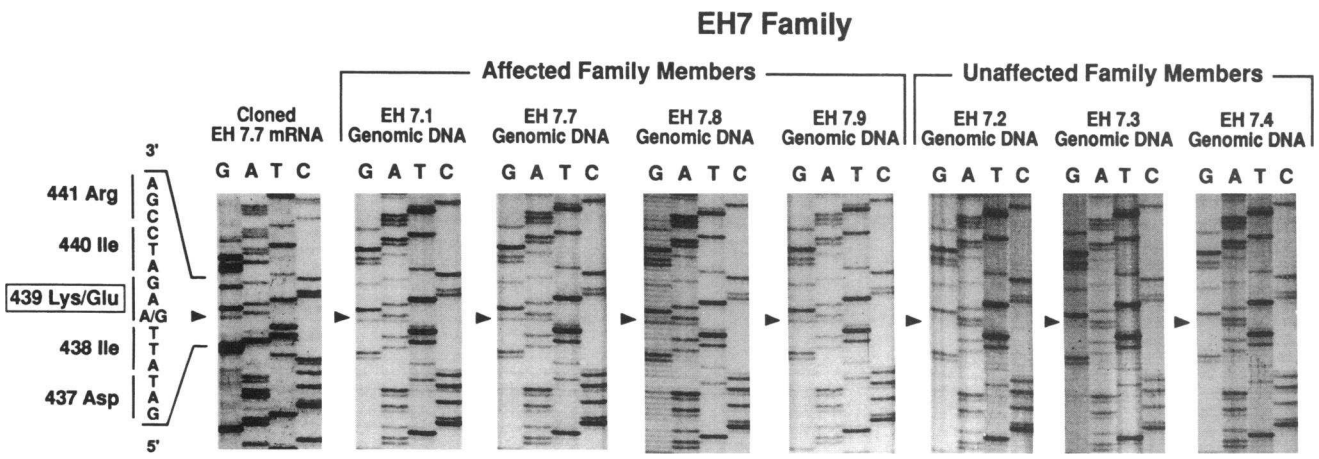

Figure 6. Sequencing the K10 and K1 alleles from EH7 family members. The entire K10 and K1 rod segments, or portions thereof, were PCR-amplified from blood genomic DNAs of EH7 members. Amplified fragments from two independent rounds were subjected to DNA sequencing either directly or after subcloning. Shown are K10 coding strand sequences encompassing nucleotide 1315, which revealed an A to G transition in one of two alleles (*arrowhead*) of affected members. Sequences are from: far left, K10 PCR mixture of cloned allele from 7.7; rest, K10 PCR mixture of total genomic DNAs of affected and unaffected members of EH7 family.

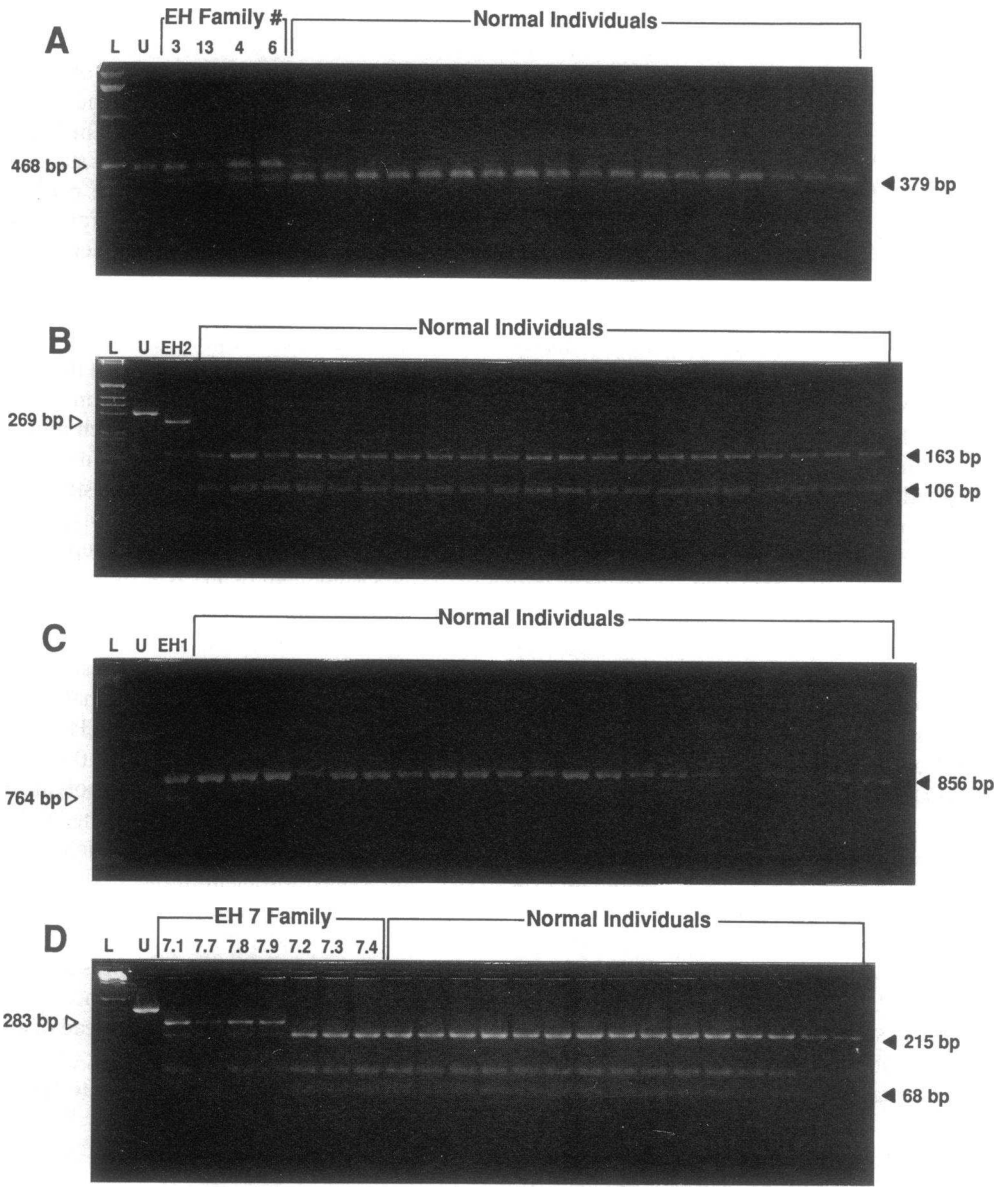

Figure 7. Heterozygous obliteration or creation of restriction endonuclease sites by EH mutations. Genomic DNAs from blood of 95 normal individuals were used as controls. For each mutation, DNAs from EH patients and controls were amplified to produce a fragment encompassing the mutation. Fragments were then digested with a restriction endonuclease whose site was either created or obliterated as a consequence of the mutation. Fragments were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. *L*, DNA ladder; *U*, undigested PCR fragment from control DNA; rest, digested PCR fragments from EH patients or representative controls as indicated. Note that all controls always behaved as wild-type. Open arrowheads indicate bands unique to mutant alleles. Closed arrowheads indicate bands characteristic of wild-type allele. (*a*) As a consequence of the K10 R156:C mutation present in EH3 and EH13, a 468-bp fragment was generated, and an AciI site (between 379 and 89 bp of this fragment) was obliterated. Shown also are EH4 and EH6, previously shown to have a K10-R156:H mutation (41). This also obliterates the site. (*b*) The K10-M150:R mutation present in EH2. As a consequence of the mutation, a 269-bp fragment was generated, and an NlaIII site (between 163 and 106 bp of this fragment) was obliterated. (*c*) The K1-Y481:C mutation present in EH1. As a consequence of the mutation, a PstI site was created, reducing an 856-bp fragment to 764 bp. (*d*) The K10-K439:E mutation present in affected members of the EH7-9 family. As a consequence of the mutation, a 283-bp fragment was generated, and an MseI site (between 215 and 68 bp of this fragment) was obliterated.

Severe EH mutants cause tonofilament clumping, and the mild EH mutant causes more subtle perturbations in keratinocytes. To test whether our new mutations could cause the perturbed epidermal keratin networks seen in our EH patients, we tested the ability of each mutation to interfere with filament network formation in transiently transfected cultured epidermal keratinocytes (15, 30, 41). In culture, keratinocytes are basallike, and hence the endogenous keratins are K5 and K14, not K1 and K10. Consequently, to appropriately test the effects of a mutant on its wild-type background in vivo, we engineered each of the mutations in the corresponding basal keratin cDNA. This assay was likely to be valid, since residues K1-Y481, K10-M150, K10-R156, and K10-K439 are identical in their K5 or K14 counterparts, and since the sequences surrounding these residues are highly conserved between the type II keratins (K1 and K5) on the one hand and the type I keratins (K10 and K14) on the other.

To track expression of the transgene, an epitope tag was used that does not interfere with the functionality of the keratins (15, 38). The epitope tagged wild-type keratins integrated into the SCC-13 network without perturbation (Fig. 8, *A* and *B*). This was true for cells transfected with either the type I (shown) or the type II (not shown) keratin gene. In contrast, the basal keratin equivalents of K10-R156:C/H and M150:R displayed a marked ability to disrupt SCC-13 keratin networks (Fig. 8, *C–E*). The Y481:C mutant also perturbed these networks, but since transient expression of the type II keratin is significantly less than the type I keratin (38), perturbations were only dramatic in PtK2 epithelial cells, which have a more fragile network (Fig. 8 *F*) (15).

The equivalent of the K439:E mutation was significantly milder in its effects. Thus, even though this mutant was expressed comparably with other type I mutants, K439:E-containing SCC-13 keratin networks were largely unaffected (not

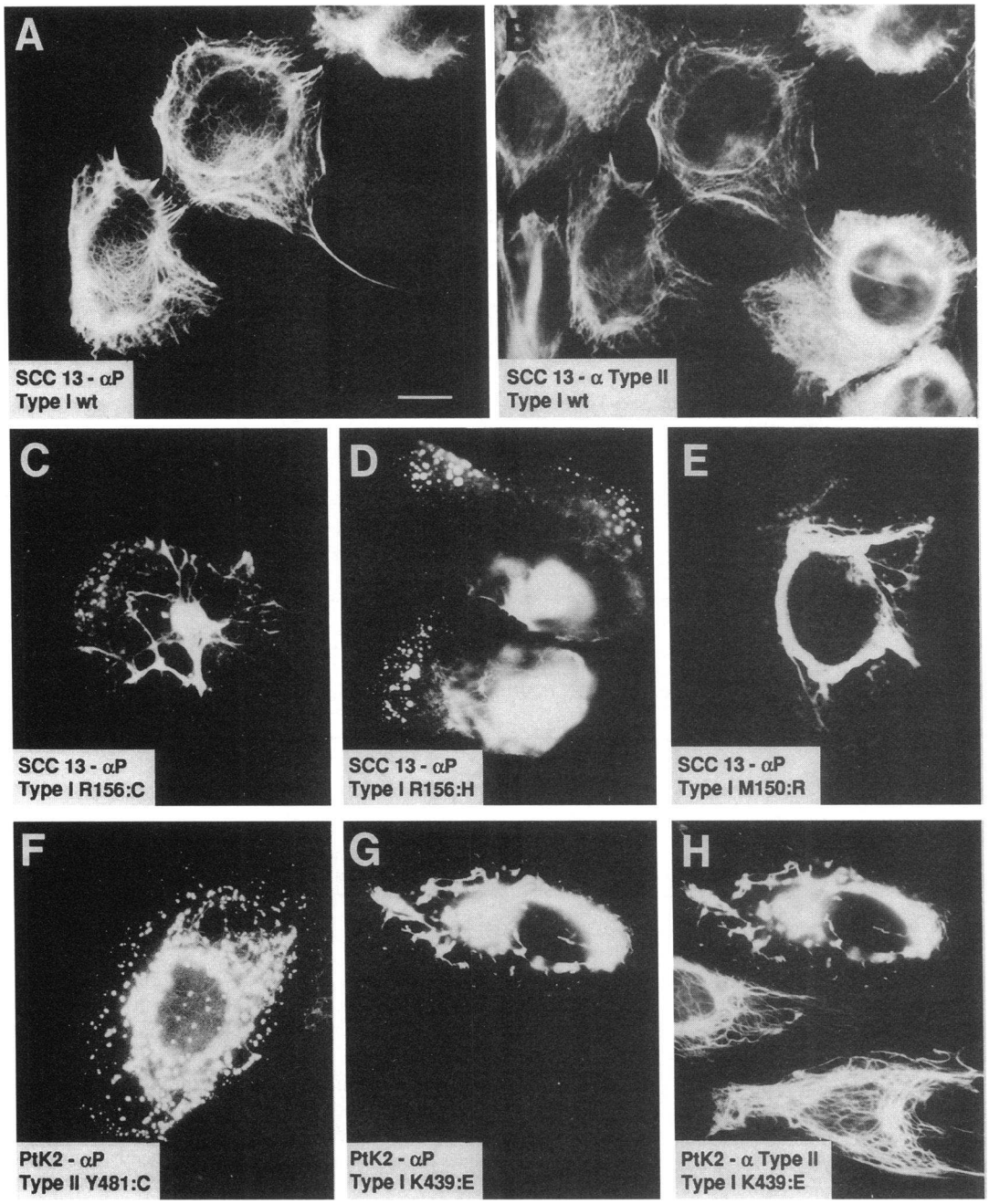

Figure 8. EH point mutations genetically engineered into cloned keratin cDNAs perturb keratin network formation in transfected epithelial cells. The four EH mutations were engineered in cloned human type I and type II epidermal keratin cDNAs in which sequences encoding the five carboxy-terminal keratin residues had been replaced by sequences encoding the antigenic portion of neuropeptide substance P (see Results and references 15 and 38). The SV40 major early promoter and enhancer were used to drive transient expression in epidermal keratinocytes (SCC-13) and kidney epithelial cells (PtK2). Cells were fixed with methanol and stained with antibodies to either the transgene product (*α-P*) or endogenous keratins (*α-Type II*). Note that double immunofluorescence is shown for one example each of transfected SCC-13 and PtK2 cells; in general, colocalization of transgene product and endogenous keratin network was observed. Shown are cells transfected with the basal epidermal counterparts of: *A* and *B*, wild-type K10 (i.e., K14); *C*, K10-R156:C (i.e., K14-R125:C); *D*, K10-R156:H (i.e., K14-R125:H); *E*, K10-M150:R (i.e., K14-M119:R); *F*, K1-Y481:C (i.e., K5-Y469:C); *G* and *H*, K10-K439:E (i.e., K14-405K:E). Bar represents 12 μm for *A–E*, 18 μm for *F*, and 14 μm for *G* and *H*.

shown). Despite the milder effects of the K439:E mutation, this single amino acid substitution was nevertheless sufficient to cause aberrant keratin networks in transiently transfected PtK2 cells (Fig. 8 *G*). As seen by double immunofluorescence, the mutant behaved in a dominant negative fashion, acting to perturb the keratin network in the presence of endogenous wild-type keratin (Fig. 8, *G* and *H*).

Discussion

Fig. 9 illustrates the locations of our new EH mutations relative to the predicted secondary structures of the keratins. Residues 150 and 156 of K10 are located in the amino end of helix 1A; residue 481 of K1 is located in the carboxy end of helix 2B; and residue 439 of K10 is somewhat more central in helix 2B. The

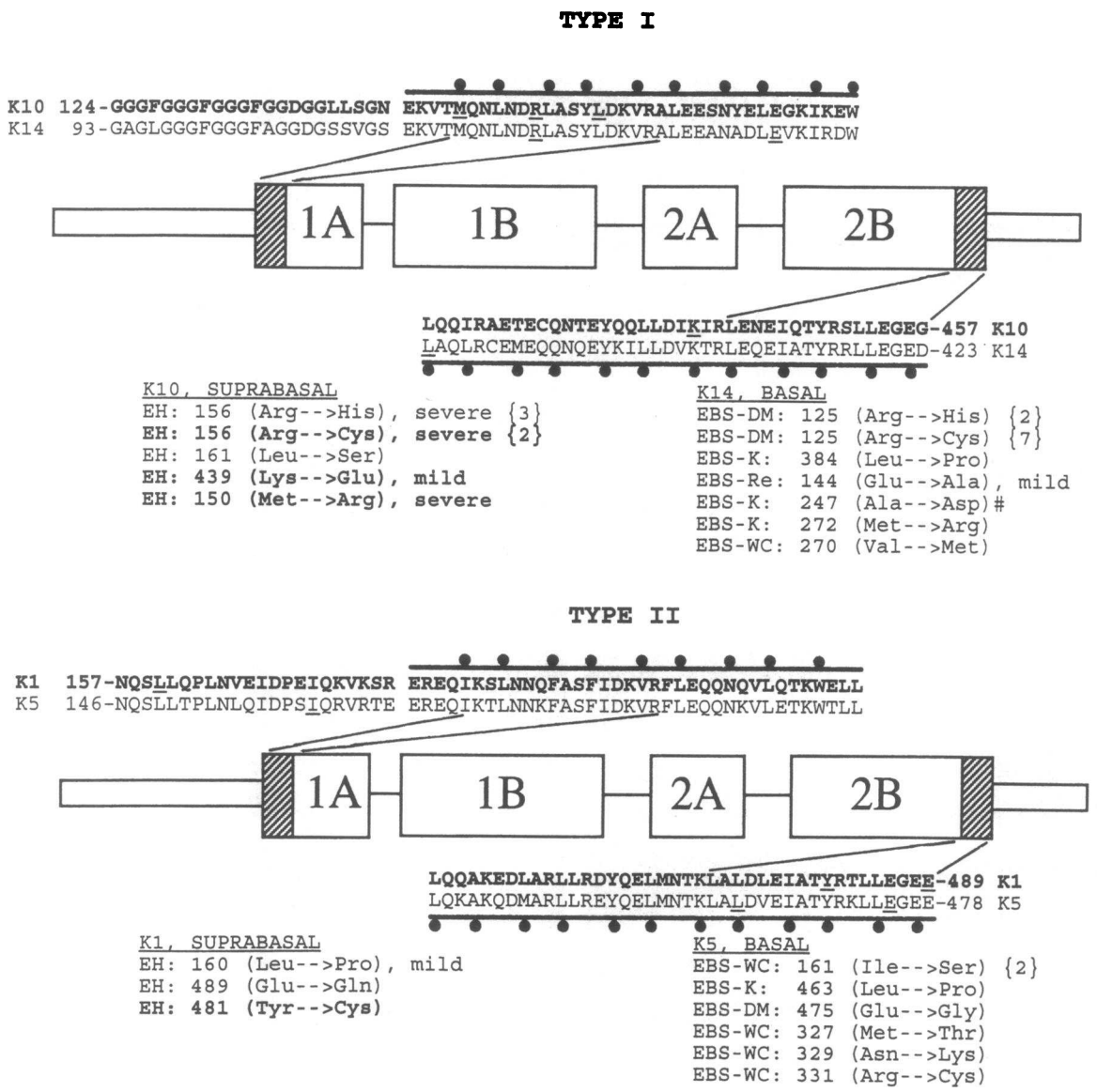

Figure 9. The location of EH and EBS mutations relative to the structure of the keratin polypeptide. Stick figure depicts secondary structures predicted for human epidermal keratins (63–65). Large boxes encompass the central 310–amino acid residue rod domain, predicted to be largely α-helical and containing heptad repeats of hydrophobic residues. The rod is subdivided into smaller helices (1A, 1B, 2A, and 2B) as a consequence of three short nonhelical linker segments (L1, L1-2, and L2, as denoted by thin lines in order from left to right). Hatched bars denote highly conserved end domains of the rod. Sequences corresponding to the ends of the α-helical rod are given, with overhead dots denoting hydrophobic residues comprising the heptad repeats. Smaller open bars denote nonhelical head and tail domains. Shown are locations of published EH (K10 and K1) and EBS (K14 and K5) mutations. Mutated residues that fall into or near the highly conserved rod ends are underlined. #, E. M. Hutton and E. Fuchs, unpublished observations.

arginine at position 156 of K10 is conserved throughout evolution and is surrounded by conserved residues. Given that four of the seven EH mutations we have thus far identified are at this residue, the frequency with which mutations occur at this conserved site appears to be extraordinarily high in EH, as it is in EBS. Two factors seem to contribute to this phenomenon: (*a*) the arginine residue is structurally important for filament assembly, and (*b*) the residue is a hot spot for mutagenesis by CpG methylation and deamination (52, 55).

Residue M150 of K10 is somewhat less conserved than R156, although it is always either leucine, methionine, or isoleucine in 73 of 73 published IF sequences, and it is methionine in most of the lower eukaryotic IF proteins (56 and references therein). It is also predicted to be the first hydrophobic residue in the string of heptad repeats that make up the coiled-coil rod domain. Similarly, the tyrosine residue at position 481 of the K1 polypeptide is also predicted to be a hydrophobic participant in the coiled-coil and it resides in the last heptad repeat of the rod that maintains both *a* and *d* positions as hydrophobic. This tyrosine is remarkably conserved throughout evolution, being present in 83 of 83 published IF sequences, and it is surrounded by highly conserved residues.

Residue K439 of K10 is in the heptad just upstream from that of Y481 in K1. While the residue falls outside the highly conserved end of helix 2B, the residue itself is conserved (81 of 83 sequences). It is also unusual in that it is one of only a few charged residues appearing at positions *a* or *d* of the heptads. It is likely relevant that the effects of K10-K439:E on filament

network formation were noticeably less severe than those of the other EH mutations. The finding correlates well with the mild degree of filament perturbations detected in our ultrastructural analysis of the skin from patient EH7.7. It also parallels the clinical features of EH in this family, which are significantly milder than the other cases we studied. Collectively, these findings suggest that, as in EBS (38), the severity of the EH phenotype depends upon the specific mutation in the K1 or K10 sequence and the degree to which the mutation affects 10-nm filament structure.

Why should the K10-K439:E mutation have a lesser effect on IF structure than the other EH mutations we examined? As judged by sequence comparisons, the major difference between K439:E and the other mutations is that (*a*) it does not lie within as highly a conserved stretch of residues as those that surround the other EH mutations, and (*b*) it does not reside within the first 20 or last 20 residues of the α-helical rod domain of the keratin polypeptide. Recently, it was proposed that the proximal ends of the 1A helices in one keratin heterodimer may overlap slightly with the distal ends of the 2B helices of a second dimer linked longitudinally (11). K439 resides outside this putative overlap and, consequently, it might have a milder effect on filament elongation than those which are a part of the overlap (57). Given our previous studies demonstrating that mutations in the putative overlap region generate shortened keratin filaments when combined with their wild-type partners in vitro (30, 38), this hypothesis is intriguing.

Thus far, all 19 of the severest cases of both EBS and EH, including 14 cases of R156-K10 or R125-K14 mutations, have point mutations in the highly conserved rod ends of one of their basal (EBS) or suprabasal (EH) keratins. Additional studies on EH mutations have recently been reported (58–60), and the number of EH mutations in the highly conserved rod ends has now risen even higher. Of the 10 EH cases we have thus far examined, only 4 cases did not show mutations within the highly conserved ends of the K1/K10 rod domain. One of these is the mild EH7 case. However, at least one other was a severe case in which we found no mutation in the highly conserved ends of the K1/K10 rod domain. Thus, although further studies will be necessary to define the genetic basis of these three cases, it seems likely that even though a strong trend exists between rod end mutations and severe EH or EBS, this correlation will not be absolute.

As additional EH mutations are mapped, it should be possible to develop recombinant DNA or immunologic screening methods to rapidly localize keratin gene or protein defects, respectively. Such screening methods could improve genetic counseling and facilitate prenatal diagnosis. In addition, this technology could also be applied to chorionic villus samples from first trimester fetuses, providing even earlier diagnosis and a lower risk of fetal death than electron microscopy methods which are currently used on intrauterinely obtained skin specimens at 18–20 wk gestational age (for review see reference 61). The possibility of cures for autosomal dominant structural gene defects is limited. However, since keratinocytes can be cultured from a skin biopsy of an EBS or EH patient, it may be possible to develop methods to either (*a*) genetically remove the defective gene in cultured cells, and then graft these repaired cells back onto the patient, or (*b*) graft on foreign keratinocytes which have been genetically manipulated to bypass the patient's immune system. Procedures for culturing keratinocytes and grafting them onto the same patient are used routinely in burn operations (62), and thus a portion of the necessary technology is already in place. Since there should be a tremendous selective advantage for healthy keratinocytes over cytolysis-prone keratinocytes, it is possible that EH might be a disease that is amenable to gene therapy.

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