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A Mutation in the V1 End Domain of Keratin 1 in Non-Epidermolytic Palmar-Plantar Keratoderma

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Mutations in keratin 9 have been found in families with an epidermolytic form of palmar-plantar keratoderma (PPK). In another form of PPK (Unna-Thost type), epidermolysis is not observed histologically. We studied a pedigree with this non-epidermolytic form of PPK. By gene linkage analysis, the type I keratin locus could be excluded but complete linkage with the type II keratin region was found. Sequence analysis identified a single base change in the amino-terminal V1 variable subdomain of keratin 1, which caused a lysine to isoleucine substitution. This non-conservative mutation completely cosegregated with the disease and was not observed in 50 unrelated unaffected individuals. An examination of keratin amino-terminal sequences revealed a previously unreported 22-residue window in the V1 subdomain that is conserved among most type II keratins. The altered lysine is an invariant residue in this conserved sequence. Previously described keratin mutations affect the central regions important for filament assembly and stability, and cause diseases characterized by cellular degeneration or disruption. This is the first disease mutation in a keratin chain variable end region. The observation that it is not associated with epidermolysis supports the concept that the amino-terminal domain of keratins may be involved in supramolecular interactions of keratin filaments rather than stability. Therefore, hyperkeratosis associated with this mutation may be due to perturbations in the interactions of the keratin end domain with other cellular components. ***J Invest Dermatol 103:764–769, 1994***

Palmar-plantar keratoderma (PPK) (tylosis) defines a group of heterogeneous disorders characterized by severe thickening of the epidermis of palms and soles. One type of PPK, also called Unna-Thost Disease [1,2], is inherited as an autosomal dominant disorder (McKusick 148400). The disease is characterized by diffuse hyperkeratosis of the palms and soles, usually becoming evident by 12 months of age. This non-epidermolytic type of PPK (NEPPK) can be similar in clinical presentation to localized epidermolytic hyperkeratosis of the palms and soles as described by Vorner [3] (McKusick 144200). On light microscopy, affected skin from patients with NEPPK show orthokeratotic hyperkeratosis (thickened stratum corneum) and acanthosis (epidermal hyperplasia). In contrast, the epidermolytic type of PPK (EPPK) exhibits hyperkeratosis, but is characterized by a distinctive vacuolar degeneration of the granular layer of the epidermis.

Keratins constitute the major differentiation product of epidermal cells and are either acidic (type I) or neutral/basic (type II). They are expressed in specific type I/type II pairs characteristic of the stage of differentiation of the keratinocyte. Three inherited disorders of the skin have been shown to be caused by mutation in genes encoding epidermal keratins. Epidermolysis bullosa simplex is caused by mutation in either of the keratins 5 or 14 that are expressed in basal epidermal cells (for review, see Fuchs [4]). Generalized epidermolytic hyperkeratosis can be due to mutation in either keratin 1 or 10 [5–10] expressed in suprabasal epidermal cells. Most recently mutations in keratin 9, expressed in the suprabasal cells of palm and sole epidermis, have been reported in EPPK [11,12].

Using linkage and mutation analysis in a large family in which the disease was segregating, we tested the hypothesis that NEPPK is another disorder of epidermal keratin. We identified a unique mutation in the amino-terminal V1 end domain of keratin 1 as the likely cause of the disease. This is the first mutation in an end domain of a keratin. The absence of cell fragility associated with this mutation distinguishes it from those mutations causing generalized epidermolytic hyperkeratosis, EPPK, and epidermolysis bullosa simplex. This finding demonstrates that keratin end domains have a significant role in the function of keratin intermediate filaments in the epidermis.

MATERIALS AND METHODS

Patients and Clinical Material This family was ascertained as part of a continuing effort to identify the etiology of a variety of ichthyoses. Blood and skin samples were obtained with informed consent under a protocol approved by the Institute IRB. Patient diagnosis was established by examination by a dermatologist, review of medical records, or reliable family information. Skin biopsies were obtained from four adult patients and examined with light and electronmicroscopy.

Linkage Analysis NEPPK was modeled as an autosomal dominant, fully penetrant disorder. Lod scores were fully robust over a wide range of disease allele frequencies. KRT10 and microsatellite marker allele frequencies were established from unrelated individuals in the NEPPK family and many other reference families in our laboratory. Lod scores for the KRT1-V1 mutation were calculated using an allele frequency of 0.01 for the mutant allele based

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Abbreviations: NEPPK, non-epidermolytic palmar-plantar keratoderma; PPK, palmar-plantar keratoderma.

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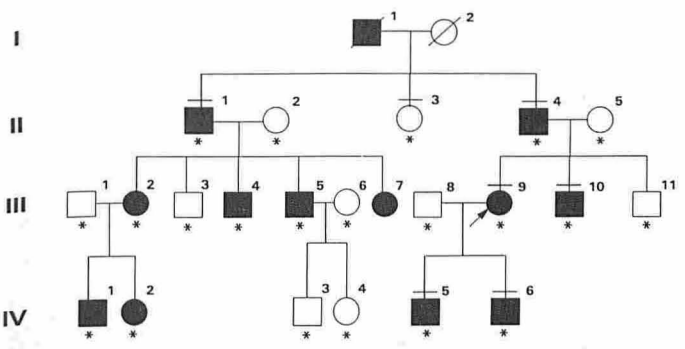

Figure 1. Pedigree of NEPPK family. *Solid symbols* are people with NEPPK; *open symbols,* unaffected subjects. *Arrow* indicates proband; *horizontal bar* above symbol indicates individual was examined by JJD; *asterisk* below symbol indicates individuals on whom DNA was analyzed; *vertical slash* through symbol indicates deceased individual.

on observing no control individuals with the mutation in 100 alleles tested. Computations were performed using the LINKAGE package of programs [13].

DNA Marker Analysis For the Southern blot analysis, 100 μg of genomic DNA was digested with the restriction enzyme MspI and the prepared blots probed with D12S14 and D12S17 obtained from American Type Culture Collection (peFD 33.2 and pYNH 15). The microsatellite markers were amplified by PCR in 40 μl reaction volumes containing 600 ng genomic DNA, 200 nM of each primer, 200 μM of each dGTP, dATP, and dTTP, 25 μM dCTP, 0.2 μl of [^{32}P]-α-dCTP(3000 Ci/mmol), 1 U AmpliTaq DNA polymerase, 1 × buffer containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ overlaid by mineral oil. After an initial denaturing step of 5 min at 95°C, amplification was performed for 32 cycles at 93°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min. A final incubation for 10 min at 72°C was made to ensure elongation of the products. Four microliters of a 1:1 polymerase chain reaction (PCR) product and formamide dye was run on a 6% denaturing polyacrylamide gel. The gels were then dried and autoradiograms exposed overnight at −70°C.

PCR-Based Allele Specific Assays PCR-based allele specific assays (PASA) to detect single-base variants [14] were developed to detect the K1 and K2e variants. The two downstream primers used to distinguish the Lys (**AAA**) to Ile (**ATA**) mutation in KRT1 were 5′-ACACTTATGGAGATGCTTT-3′ (969-951) and 5′-ACACTTATGGAGATGCTT**A**-3′ respectively, with 5′-GTCTCTATGCTTGGGGTAGAGGAGTGTTTAGCTCC-3′ (655-689) used as the common upstream primer. Numbers refer to nucleotide positions in Genbank sequence M98776. Amplification was performed for 35 cycles (denaturation 1 min at 95°C, 30 seconds annealing at 55°C, and 1 min extension at 72°C) with 0.1 mM dNTP, 1.5 mM $MgCl_2$, 125 ng DNA per 25 μl mix and 0.1 μM primers. Primers to distinguish the keratin 2e polymorphism (Gly [**GGC**] versus Asp [**GAC**]) were 5′-ACAACAAATGAATGTTG**G**-3′ (690-707) and 5′-ACAACAAATGAATGTTG**A**-3′, respectively, with 5′-CTTCTTCTTATAATCCTCCACAAG-3′ (852-829) as the common downstream primer. Nucleotide positions are from Genbank sequence S43646. PCR conditions were the same as above, except for 50°C annealing temperature.

Keratin Gene Amplification and Sequencing The keratin 1 gene exons were analyzed as described previously [8]. To amplify complete keratin 2e exons, intron sequences were required. Partial intron sequences were obtained by sequence analysis of PCR-amplified DNA that had been prepared using primers based on the published keratin 2e sequence [15]. The primer sequences for preparation of DNA fragments containing keratin 2e exons and those for sequencing analysis from these templates are available from us on request.

Homology and Secondary Structure Analysis Alignment of keratin sequences was performed using the Macintosh GeneWorks 2.0 (Intelligenetics) utility. Secondary structure predictions utilized algorithms available in the MacVector 4.0 program (Kodak/IBI).

RESULTS

Clinical Studies We investigated a three-generation family in which there were 13 affected individuals **(Fig 1)**. After obtaining informed consent, a complete dermatologic examination was performed on six affected patients, and blood for DNA obtained from 11 affected family members, five unaffected, and five spouses.

The inheritance pattern in the family was consistent with autosomal dominant inheritance, although the observed segregation ratio of 0.75 was probably due to ascertainment bias. In adults the disease manifested as moderate to severe thickening of the skin on palms and soles **(Fig 2*A*)**. The area of hyperkeratosis usually extended along the Achilles tendon of the foot and occasionally along the extensor tendon of the great toe. However, involvement stopped abruptly at the wrist flexure and at the border of the dorsal aspect of the hands and feet. The border of the palmar and plantar hyperkeratosis was separated from the normal-appearing skin by an erythematous halo. There were discrete hyperkeratotic pads over several of the knuckles of the hands. Some adult patients experienced mild limitation of extension of the digits. Patients had a slight beaking (concave) deformity of the nails. Three of the four adult patients who were examined had dermatophyte infection of the toenails and feet, and two had involvement of the palms. Hyperkeratosis of the umbilicus and nipple areolae were present, as well as very mild thickening and dryness of the knees and elbows.

Two affected children were examined. These patients had presented at birth with mild thickening of the palms and soles. On examination, one toddler with a history of atopic dermatitis had eczematous changes involving several areas including blistering and peeling of the plantar surface. Both children had generalized dryness with fine, powdery scale, and hyperkeratosis of the areolae and umbilicus. There was no family history of esophageal carcinoma or breast cancer, as has been associated with PPK in other families [12,16,17].

A 3-mm punch biopsy of the affected palms from four adults and one biopsy from an affected elbow were obtained and examined. There was hyperkeratosis of the stratum corneum with no evidence of epidermolysis characteristic of epidermolytic hyperkeratosis **(Fig 2*B*)**. On electron microscopic examination, cells of the granular and spinous layers did not show the aggregated tonofilaments or large keratohyalin granules characteristic of epidermolytic hyperkeratosis.

Linkage Studies The chromosomal region of the type I keratins was the initial candidate location for the NEPPK gene in this family. Analysis of the segregation of KRT10 size polymorphism [18], and of microsatellite markers D17S579 and D17S800, showed obligate recombination between each of these loci and the NEPPK locus, excluding approximately 40 cM in this region **(Table I)**. Linkage to the type II keratin region on chromosome 12 was then investigated using size variations in the glycine-rich carboxy-terminal V2 subdomain [19] of KRT1, and restriction fragment length polymorphisms in the D12S14 and D12S17 loci. Each of these proved uninformative in this family. Therefore, five microsatellite markers (D12S85, D12S87, D12S90, D12S96, D12S103) mapped to 12q [20] were investigated for linkage to NEPPK. D12S87 was uninformative, but the remaining loci showed complete cosegregation with NEPPK, with D12S90 and D12S103 each giving maximum lod scores ≥ 2.4, and $Z = 3.0$ for D12S96 **(Table I)**. A haplotype constructed from the D12S96, D12S103, and D12S90 loci gave a multipoint lod score of 3.61 at $\Theta = 0$ with NEPPK. As neither the genetic nor physical relationship of these loci to the keratin cluster was known, we typed the microsatellites in a large reference pedigree in which KRT1 was completely informative [6,21]. Although D12S85, D12S96, and D12S103 were largely uninformative in the reference pedigree, linkage between D12S90 and KRT1 was proved ($Z = 5.63$, $\Theta = 0.03$). This result implicated the type II keratin loci in the etiology of NEPPK.

Mutation Analysis in Keratins K1 and K2e Having shown tight linkage between the type II keratin gene cluster at 12q11-q13 and markers linked to NEPPK, we undertook mutational analysis of the type II keratin genes whose expression is associated with suprabasal palmar/plantar epidermis. A good disease-gene candidate was keratin 2e, which has been shown to be expressed at high levels in

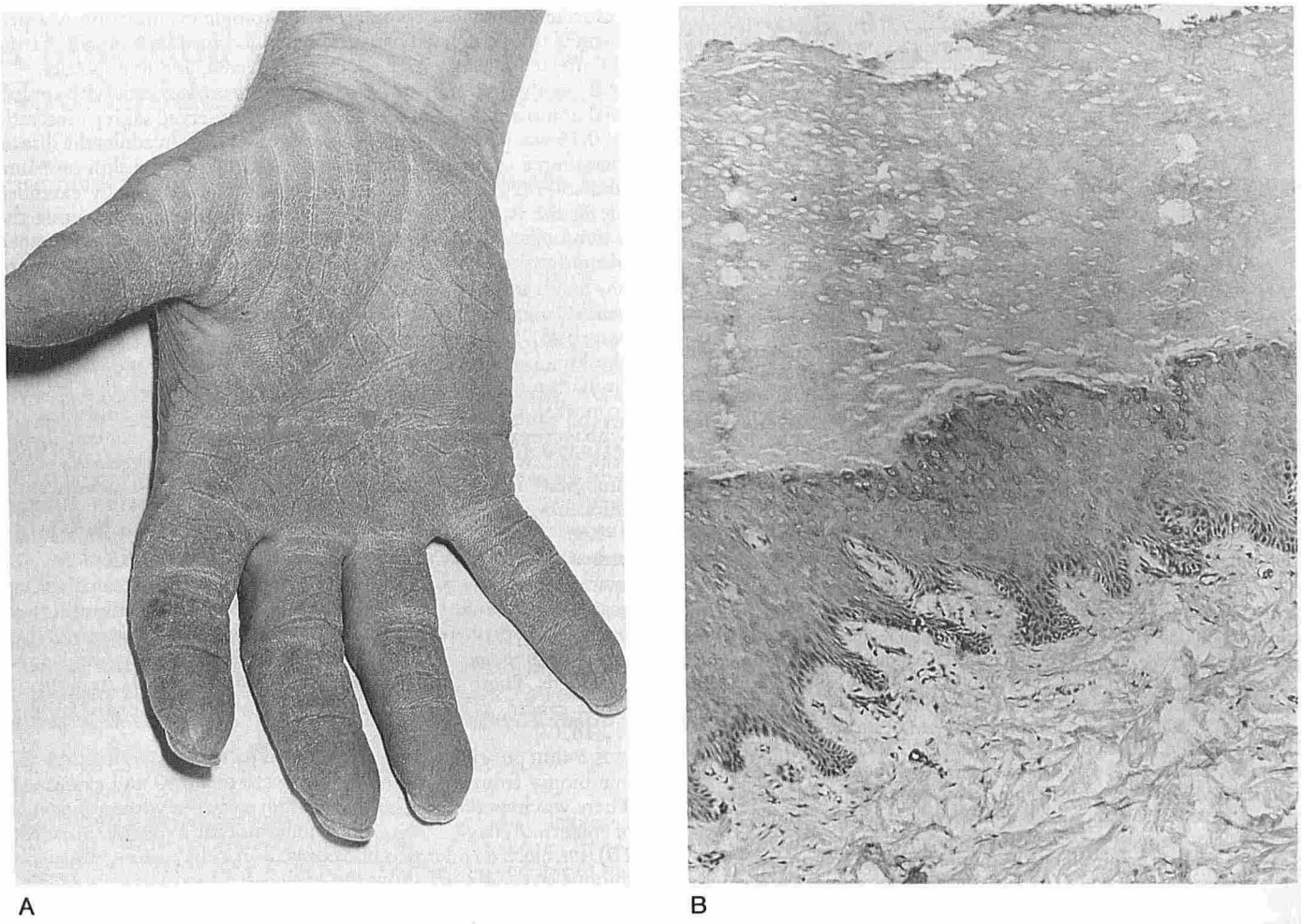

Figure 2. NEPPK. *A*) Patient with NEPPK showing palmar hyperkeratosis. *B*) Hematoxylin and eosin stained section of formalin-fixed skin from palm of patient affected with NEPPK.

palm and sole epidermis, and also at detectable, but much lower, levels in truncal epidermis [15]. Keratin 1, another potential candidate, is a significant type II keratin constituent of palmar/plantar epidermis, as well as a major component of keratin intermediate filaments (KIF) throughout the epidermis. A variety of point mutations in KRT1 had already been shown to be associated with the distinct disorder, generalized epidermolytic hyperkeratosis.

We examined keratin 2e for mutations in NEPPK by amplifying each exon using PCR from the purified genomic DNA of affected and unaffected family members. These amplified products were then sequenced directly. The primers used in our analysis were derived from keratin 2e intron sequences. We observed sequence heterogeneity at a single base position (G versus A), which indicated the presence of either a glycine residue [15] or an aspartic acid in position 6 of the L1 subdomain. However, this polymorphism did not segregate with the disease in the family and was also observed in 16 alleles of 98 control DNA alleles (49 individuals) tested by a PCR-based allele specific assay (PASA) (see *Materials and Methods*).

Table I. Linkage Analysis of NEPPK

	θ						
	0	0.05	0.10	0.20	$\hat{Z}$	$\hat{\theta}$	Exclusion Range
Chromosome 17							
KRT10	$-\infty$	−1.7	−0.9	−0.3			$\theta \leq 0.03$
D17S579	$-\infty$	−4.4	−2.8	−1.3			$\theta \leq 0.14$
D17S800	$-\infty$	−1.4	−0.6	−0.1			$\theta \leq 0.04$
Chromosome 12							
D12S17	0.6	0.6	0.6	0.5	0.6	0	
D12S85	1.9	1.7	1.6	1.2	1.9	0	
D12S96	3.0	2.7	2.4	1.7	3.0	0	
D12S103	2.6	2.4	2.2	1.7	2.6	0	
D12S90	2.4	2.2	2.0	1.5	2.4	0	
Keratin 1 mutation							
KRT1-K73I	4.2	3.9	3.5	2.7	4.2	0	

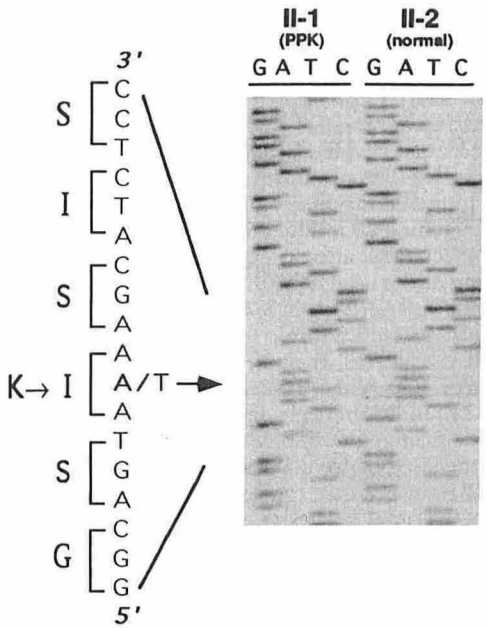

Figure 3. Sequence analysis showing the K73I mutation in the DNA of an individual affected with palmar/plantar keratoderma. The autoradiogram from direct analysis of PCR-amplified DNA shows sequence heterogeneity (both A and T) at a position yielding codons for both lysine and isoleucine at amino acid residue 73 of the keratin 1 gene of affected individuals. Only the normal A is seen in the DNA from an unaffected family member.

The sequence of the KRT1 gene was analyzed. DNA from an affected individual revealed a single base substitution (AAA to ATA) causing a lysine to isoleucine change in codon 73 (designated K73I) located in the variable subdomain, V1, of the amino-terminal end domain of keratin 1 **(Fig 3)**. No sequence variations were found in the H1 subdomain, nor in the entire rod domain, regions where all previous disease-causing mutations in keratin genes have been identified **(Fig 4)**, nor in the V2 or H2 regions of the carboxy-terminal domain. A previously described common polymorphism at position 11 of the E2 domain (arginine, AGG or lysine, AGA) was segregating in the family [8].

To confirm that the K73I variation segregated with NEPPK, a PCR allele-specific assay was developed using a common upstream primer, and either a wild-type specific reverse primer (terminating in a complimentary T) or a mutation-specific reverse primer (terminating in an A). As seen in **Fig 5**, amplification with the wild-type primer generated an amplified fragment from the DNA of both normal and affected individuals, whereas the mutant-specific primer only yielded an amplification product from the DNA of affected individuals. Complete cosegregation of the K73I mutation with the disease in the family was observed yielding a lod score of 4.2 at $\Theta = 0$ **(Table I)**. The PASA analysis was used to demonstrate that this variant was not present in 50 unrelated individuals not having PPK (data not shown).

Mutated Lysine Lies in a Highly Conserved Portion of the V1 Subdomain of Most Type II Keratins To assess the significance of the K73I mutation, the amino-terminal sequences of keratins were evaluated for sequence homologies. Common subdomain features have been described for type I and type II keratins and are indicated in the representation of keratin 1 in **Fig 4** [22,23]. The K73I mutation occurs within the 143-residue E1/V1 region of the protein **(Fig 4)**. Sequence alignment revealed striking conservation over a 22-residue contiguous region within the V1 subdomain of seven human type II keratins **(Fig 6)** and four homologous keratins in other species (not shown). Only keratin 7 and keratin 8 lack this sequence. The mutation in the NEPPK family alters the invariant lysine at position 15 of this sequence. Preceding this residue is the only variable position within the conserved region, which separates a 13-residue conserved segment from an 8-residue conserved segment. We propose this 22-residue conserved sequence be termed the keratin ISIS box. A search of sequence databases for the existence of the keratin ISIS box in other proteins revealed only very partial homologies with either segment.

DISCUSSION

We describe a family with hyperkeratosis of the palms and soles, and limited involvement of the areola, umbilicus, and knuckle pads on the dorsal aspects of the finger joints. Histologic examination revealed the absence of epidermolysis, and defined this family as having NEPPK. Gene linkage and mutation analysis showed that a point mutation in exon 1 of KRT1, resulting in the substitution K73I, is the likely cause of the disease.

Mutations in KRT9 have been described in epidermolytic hyperkeratosis limited to the palms and soles (EPPK) [11,12]. Although the palmar-plantar involvement of the family in this report resembles those with KRT9 mutations, the histologic findings are different. Those families in whom KRT9 mutations have been identified have a characteristic histologic picture termed "epidermolytic hyperkeratosis," whereas four biopsies from the family reported here lack this distinctive histologic feature. Further, our family also has involvement of areola and umbilicus, mild involvement over the knuckles of the hands, and mild thickening and dryness of knees and elbows—features that have not been reported in the KRT9-mutated families. The NEPPK in our family is consistent with that described previously [1,2] and called Unna-Thost disease. Our data suggests that NEPPK is distinct genetically from EPPK (Vorner type) as well. The involvement of locations other than palms and soles in our NEPPK family are inconsistent with the reported expression of keratin 9, which is limited to palmar/plantar epidermis and a few glandular structures [24]. On the other hand, keratin 1 is widely expressed throughout the epidermis.

We and others have reported mutations in the epidermal keratins KRT1 [5,6,8,9] and KRT10 [5,7,10] in generalized epidermolytic hyperkeratosis. The clinical phenotype in this family differs from

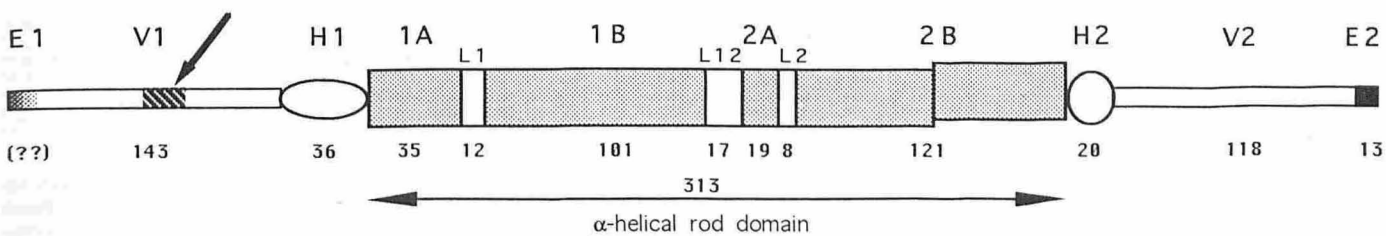

Figure 4. Structural features of keratin 1. All keratins have a central rod domain (about 310 residues) comprised of helical coiled-coil segments (designated 1A, 1B, 2A, 2B) interrupted by non-helical short linker segments (L1, L12, L2). The central region is flanked by the homology regions H1 and H2 (the latter in type II keratins only) and by variable length end domain sequences that can generally be subdivided into end sequences (E1, E2) and variable regions (V1, V2) on the basis of amino acid character. The number of residues in the subdomains of keratin 1 are shown. A precise E1/V1 boundary in keratin 1 cannot be defined. The K73I mutation *(arrow)* and the conserved 22 residue segment *(stripes)* are shown in V1.

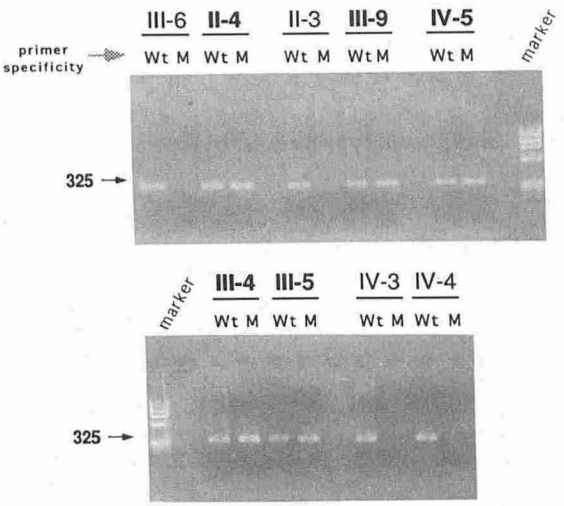

Figure 5. PCR allele specific assay for the presence of the K73I mutation. The normal or mutant alleles were amplified from genomic DNA in separate PCR reactions using either the wildtype-specific reverse primer (left lane of each pair) or the mutant-specific reverse primer (right lane of each pair) and a common forward primer. Unaffected family members yielded amplification of the expected 325 bp DNA fragment for the wildtype-specific primers only, whereas affected individuals (bold) gave bands with both sets of primers. Analyzed individuals are designated according to the pedigree in **Fig 1**.

the others in whom KRT1 mutations have been defined. Adult patients in the family reported by Compton *et al* [21] in whom a keratin 1 mutation was found in the H1 subdomain [6] had primarily palmar and plantar hyperkeratosis. They often experienced blistering, especially with trauma. Children in the family had moderate involvement of the flexural areas of the skin and the corners of the lips, with a ridge pattern of scale. In the report by Yang *et al* [8] keratin 1 mutations were reported in three epidermolytic hyperkeratosis pedigrees. One family had clinical findings nearly identical to the one described in Compton *et al* [21]. Two patients in different kindreds had severe generalized disease with erythroderma, hyperkeratosis, and contractures of the palms and digits. The mother and son described by Rothnagel *et al* [5] with a KRT1 mutation had disseminated hyperkeratotic lesions over joints, hands, and feet and rare neonatal blistering. Histopathologic examination confirmed epidermolytic hyperkeratosis in each of these reported cases, in contrast to the family presented here.

The specific region of the keratin 1 protein affected by mutation may be a major determining factor in the different clinical and histologic consequences of the KRT1 mutation. All of the 31 different mutations in keratins described previously [25] have been in four subdomains of the central α-helical rod domain of the proteins (H1, 1A, L12, 2B), and all have been in disorders characterized by loss of cell integrity (epidermolysis bullosa simplex, generalized epidermolytic hyperkeratosis, EPPK). The mutation described in this paper is the first outside these regions, and the disease in this family is both clinically and histologically distinct.

Several observations make it likely that mutations in the H1, 1A, L12, and 2B subdomains affect KIF stability and integrity. These subdomains are involved in dimerization of type I and II keratin chains to form the basic molecule, and the higher-order interactions between molecules in KIF. A recent structural model for molecule interactions in KIF, based upon filament assembly and cross-linking studies, predicts an overlap between the first 10 residues of 1A of one molecule with the last 10 residues of the 2B subdomain of an adjacent molecule, residues where the majority of disease mutations have been found. A role for the H1 subdomain in filament assembly has also been suggested [26]. In addition, single-amino acid substitutions corresponding to disease mutations in the H1, 1A, and 2B subdomains have been shown to impair the capacity of synthetic peptides to disaggregate preformed filaments [6,8,10,27], and KIF assembled *in vitro* with keratin chains carrying 1A mutations have abnormal structure [28,29].

In contrast, the function of the variable end domains of keratins is poorly understood. Evidence against a role in filament assembly, however, has been reported. In an *in vitro* study of K5/K14 KIF formation [30] deleting the first 71 residues of the V1 region of keratin 5 (including 20 residues of the 22-residue ISIS box) had no detectable effect on filament assembly or structure. In another study [31], deletion of the head domain of keratin 7 (which lacks an ISIS box) did not interfere with K7/K18 filament formation. This argues against a significant role for V1 or the ISIS box in filament structure. It is expected, therefore, that the V1 region must have another function in epidermal cells. Our identification of a highly conserved 22-residue sequence in V1 suggests a common function of this segment among most type II keratins **(Fig 6)**. If so, then the absence of the ISIS box from keratins 7 and 8 implies specific differences in KIF interactions in simple epithelia and other cells expressing only these type II keratins.

How might the K73I mutation affect the function of keratin 1 and IFs? The V1 region consists mostly of short glycine loops flanking the conserved segment [32]. Secondary structure predictions of V1 are largely unrevealing because of the relative lack of order inherent in such structures. However, analysis of the region encompassing the ISIS box predicts a non-helical structure: a short β-turn, followed by a β-sheet and then another turn. The introduction of isoleucine for lysine predicts a shortening of the second turn. Whether it is the change in secondary structure or the non-conservative substitution of a hydrophobic isoleucine for charged lysine, normal interactions of KIF within the epidermis may be disturbed. Although the nature of interactions of KIF within the epidermis have not been fully elucidated, one possibility is an effect on KIF-desmosome junctions. An effect on post-translational modification, for example, phosphorylation, could also alter protein function. The presence of a charged lysine in proximity to serine is characteristic of phosphorylation sites. However, the specific motifs recognized by known kinases are not found in the ISIS box of keratin 1, and phosphorylation was in fact not detected in one study [33]. An alternative possibility is that the invariant lysine in the V1 region is involved in crosslinking of keratins onto the cornified envelope via the action of epidermal transglutaminases [34]. The inability to crosslink a mutated keratin at this site may interfere with normal function of the cornified layer, and lead to hyperkeratosis.

Our study of NEPPK has revealed the first keratin mutation where the effect on epidermal differentiation, namely, hyperkeratosis, is probably not due to destabilization of KIF assembly. The specific disruption of keratin 1 function remains to be determined. Further correlation of specific keratin mutations with clinical and histologic phenotypes should continue to contribute to progress in

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                                        I
CONSENSUS                GGFGSRSLYNLGG.KSISISVA
                         1          13       22
HK1   51GGGSFGAG GGFGSRSLVNLGGSKSISISVA RGGGRGSGF89
HK2p  42ACGFRSGA GSFGSRSLYNLGSNKSISISVA AGSSRAGGF80
HK2e  48GGGGGFGG GGFGSRSLVGLGGTKSISISVA GGGGGFGAA86
HK3   42AYGFRSGA GGFGSRSLYNLGGDKSISISVA AGGSRAGGF80
HK4   37GGAGRCSS GGFGSRSLYNLRGNKSISMSVA GSRQGACFG75
HK5   50SLAGACGV GGYGSRSLYNLGGSKRISISTR GGSFRNRFG88
HK6b  45GLGGACGG AGFGSRSLYGLGGSKRISIGGG SCAISGGYG83
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Figure 6. Alignment of a portion of the E1/V1 end domain sequences of seven human type II keratins obtained from GenBank data files. Sequences are aligned to maximize homology and shaded residues denote regions of particular identity or homology. For each keratin, the starting and ending residue positions within the sequence are given. The isoleucine substitution for lysine 73 of keratin 1 found in the NEPPK pedigree is indicated. Consensus residues (>70%) are given for the 22 residue segment of greatest homology, termed the keratin ISIS box (see text).

understanding the relationship between keratin structure, KIF formation, and KIF function in normal and diseased skin.

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