LETTER TO THE EDITOR

Two Novel Mutations in the Keratin 1 Gene in Epidermolytic Hyperkeratosis

To the Editor:

Epidermolytic hyperkeratosis (EHK, MIM113800) is a rare autosomal dominant genodermatosis, although many cases occur sporadically as new mutations. It is characterized by blistering and erythema at birth, and development of hyperkeratosis with increasing age. Ultrastructural analyses reveal clumping of the keratin intermediate filaments (KIF) within suprabasal keratinocytes of the epidermis. Previous studies have demonstrated that EHK is caused by mutations in the genes encoding the keratin 1 (K1) or the K10 proteins (Cheng et al, 1992; Chipev et al, 1992; Rothnagel et al, 1992; McLean and Lane, 1995; Korge and Krieg, 1996). Clinically, EHK has been divided into the palmoplantar type (palm sole, PS) and the nonpalmoplantar type (nonpalm sole), and each type has been subdivided into three subtypes according to the nature and severity of scaling (DiGiovanna and Bale, 1994; DiGiovanna et al, 1998). The mutation/clinical phenotype correlations available suggest that the PS and nonpalm sole types are due primarily to mutations in the KRT1 and KRT10 genes, respectively. The more severe PS-1 and PS-2 cases are caused by mutations that change sequences encoding the beginning of the 1A rod domain segment, or towards the end of the 2B rod domain segment (Yang et al, 1994, 1999; Arin et al, 1999, 2000; Cserhalmi-Friedman et al, 2000). In this study, we describe two separate cases of EHK, classified as PS-2, caused by two different novel mutations: Asn187Lys located in exon 1, which is the 8th residue of the 1A rod domain segment; and Leu475Pro located in exon 7, which is the 10th residue from the end of 2B rod domain segment of K1.

Two unrelated children, EHK-SJ and EHK-AS, have diffuse thick keratoderma on both palms and soles as well as moderate scaly patches on their entire bodies, but the morphology of their hair, nails, and teeth are normal. Light microscopic examination revealed vacuolar degeneration in the spinous and granular layers. As both sets of parents were normal, these represent sporadic cases. Both were classified as having the PS-2 form of EHK. DNA was extracted (Chipev et al, 1994; Yang et al, 1994) from both the affected and unaffected family members, and from 50 unrelated normal individuals used as controls.

Following sequencing of all exons of the KRT1 and KRT10 genes, patient EHK-SJ had a single nucleotide substitution on one allele of codon 187 in exon 1 of the KRT1 gene (AA -> A), so that the sequence gel of Fig 1(a) shows both the wild-type C and a mutant A at nucleotide position 564 (GenBank NM-006121; relative to the translation start point) in the proband. The mutant allele encodes a lysine residue instead of a wild-type asparagine residue in position 8 of the 1A rod domain segment. This nucleotide substitution does not create or destroy a restriction enzyme site, which was used to confirm that the unaffected parents and 50 other unrelated unaffected persons do not possess this new mutation. The same mutation in the KRT2e gene was reported in ichthyosis bullosa of Siemens (Whittock et al, 2001). In patient EHK-AS, we found a single nucleotide substitution on one allele of codon 475 in exon 7 of the KRT1 gene (CTG to CCG) so that the sequence gel of Fig 1(b) shows both a wild-type T and a mutant C at nucleotide position 1457 (GenBank NM-006121; relative to the translation start point) in the proband. The mutant allele encodes a proline residue instead of a wild-type leucine residue in position 113 of 2B rod domain segment. This nucleotide substitution destroys an MspI restriction enzyme site (C/CGG), which was used to confirm that the unaffected parents and 50 other unrelated unaffected persons do not possess this new mutation.

Figure 1. (a) EHK-SJ. DNA sequences showing C to A change (arrow), which introduces an Asn187Lys amino acid substitution in the 1A rod domain segment of K1. (b) EHK-AS. DNA sequences showing T to C change (arrow), which introduces a Leu475Pro amino acid substitution in the 2B rod domain segment of K1.

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Next, we synthesized four 18-residue peptides corresponding to the beginning of the 1A and the end of 2B region of wild-type K1, or sequences with the described amino acid substitutions, respectively. These were used for an assay that uses synthetic peptides to interfere with KIF assembly (Chipev et al, 1992): wild-type peptides disassemble KIF, whereas peptides bearing identified substitutions caused by novel mutations often do not. In both cases, we found that the wild-type 1A or 2B peptides interfered with the formation of KIF in vitro; however, neither mutant peptide interfered with KIF assembly (Fig 2). From this we can conclude that the substitutions resulting from the observed mutations affect normal KIF structure. The three-dimensional structures of the 1A (Strelkov et al, 2002) and 2B (Herrmann et al, 2000) domains of the related IF protein vimentin have been solved. As the amino acid sequences of vimentin and K1 are conserved in these regions, their structures are likely to be similar. An asparagine residue in position 8 of the 1A domain has been conserved in all IF proteins. Its side chain projects laterally outward from the axis of the coiled-coil, where it presumably interacts with residues on an adjacent molecule. Indeed, we have predicted that this residue forms H-bonds with residues in the L2 linker region in the A11 alignment mode of two adjacent molecules (Mehran et al, 2001). By modeling analyses, the substitution of a larger positively charged lysine residue will interfere with such interactions. Thus this Asn187Lys substitution in patient 2B sequences unravel and fold back along the axis of the coiled-coil, where it presumably interferes with KIF assembly. Likewise, a leucine residue in position 113 of the 2B rod domain segment has been conserved in all IF chains. Based on the extent model of this region in vimentin, this leucine residue marks the point at which the coiled-coil 2B sequences unravel and fold back along the axis of the coiled-coil. Insertion of an aberrant cis peptide bond of a proline residue at this point will prevent this refold. Accordingly, the Leu475Pro substitution will cause failed KIF assembly at the two-molecule level of assembly.

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