A Novel H1 Mutation in the Keratin 1 Chain in Epidermolytic Hyperkeratosis

Jun-Mo Yang, Kiebang Nam, * Ki-Beom Park, Won-Serk Kim, Kee-Chan Moon, † Jai K. Koh, †
Peter M. Steinert,‡ and Eil-Soo Lee

Department of Dermatology, Samsung Medical Center, †Basic Research Center, Samsung Biomedical Research Institute, †Department of Dermatology, Asan Medical Center, Seoul, Republic of Korea; and ‡Laboratory of Skin Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Health, Bethesda, Maryland, U.S.A.

We report a novel mutation in a case of epidermolytic hyperkeratosis that results in a proline for arginine substitution in the penultimate residue position of the H1 subdomain of the keratin 1 chain, which is near the beginning of the rod domain. This causes a severe clinical disease classified as PS-2. Therefore, the H1 subdomain is probably equally important for the maintenance of keratin intermediate filament integrity as the rod domain. Since earlier concepts had implied that mutations in the H1 subdomain produce milder disease, this case suggests that attempts to correlate mutations with disease presentation remain problematic. Key words: type II keratin/H1 subdomain/Epidermolysis.
case (Fig 1a). Because she had moderate to severe thick scaly patches with erythema on her entire body, including palms and soles, as well as mild digital contractures, this case was classified PS-2 or perhaps borderline PS-1.

**Identification of an Arginine to Proline Substitution in the H1 Subdomain** We found no sequence variations from normal in all exons of the K10 gene. In the K1 gene, however, we found a single nucleotide substitution in one allele in the affected child (CGA to CCA) so that the sequence gel of Fig 1b shows both a wild type G and a mutant C in position 2 of codon 178. The mutant allele encodes a proline residue instead of a wild type arginine residue in position 35 of the H1 subdomain. This nucleotide substitution destroys an XhoI restriction enzyme site (CCTCGAG), which was then used as a test for the presence of the mutation in polymerase chain reaction–amplified DNA. In this analysis, the normal alleles yield fragments of 368 and 132 bp, but the affected allele yields fragments of 300, 368, and 132 bp (Fig 1c). Because none of the other three family members and 50 other unrelated unaffected persons display the XhoI undigested fragment, the G to C substitution in the affected child represents a new mutation.

**The Proline Substitution Introduces Major Structural Change to the Keratin 1 Sequence** We have devised an in vitro disassembly assay using synthetic peptides corresponding to sequence domains of keratins (Chipew et al, 1992). Due to a competition reaction, wild type peptides disassemble the KIF into small oligomers, but peptides with amino acid substitutions found in keratin diseases often compete less and, as a result, only partly disassemble the KIF. The degree of disassembly is monitored by light scattering (turbidity). In this study, we found that both wild type and mutant full-length as well as short H1 synthetic peptides caused virtually complete disassembly of K1/K10 KIF (Table I). Based on our earlier observations with this assay, i.e., substitutions located near the ends of synthetic peptides often do not disassemble KIF in vitro (Steinert et al, 1993b), however, we made longer peptides that span the junction of the H1 and 1A sequence region. In this case, the peptide bearing the proline substitution was essentially unable to disassemble the KIF (Table I). This means that the proline substitution at this residue position does indeed impart a major structural change to the synthetic peptide and to the intact keratin 1 chain itself. From this, we can conclude that the KIF of the mutant allele of the patient are defective, which leads to the severe pathology of PS-2 EHK.

**Complexity of Assignment of Disease Severity with Location of Amino Acid Substitutions** Previous point mutational analyses revealed that proline substitutions in end-domain sequences resulted in less severe consequences for KIF structure than substitutions in rod-domain sequences (Letal et al, 1992). This concept was supported by the findings that substitutions in the H1 subdomain of the K1 or K5 chains resulted in less severe forms of pathology in EHK or EBS (Compton, 1994; McLean and Lane, 1995). This work, however, shows that H1 substitutions close to the beginning of the rod domain may also cause a severe phenotype. Also in this regard, it has been noted that a very common substitution in the tenth residue position of the 1A rod-domain segment of type I keratin chains (often R10H) can lead to widely differing degrees of severity in several types of keratinopathies, including NPS-1, NPS-2, or NPS-3 in EHK (Compton, 1994; McLean and Lane, 1995). This means that other unknown etiologic

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**Table I. Peptide Disassembly Assay**

<table>
<thead>
<tr>
<th>Peptide Sequence</th>
<th>% Light scattering remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 No peptide</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>2 Wildtype H1</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>3 Wildtype short H1</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>4 Mutant H1</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>5 Mutant short H1</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>6 Wildtype IA</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>7 Wildtype short H1 + wildtype IA</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>8 Mutant short H1 + wildtype IA</td>
<td>89 ± 8</td>
</tr>
</tbody>
</table>

*The data are the average ± SD of three to five separate measurements.
*The P in bold identifies the proline substitution in a region predicted to form an α-helix (but not coiled-coil) at the penultimate residue position of the H1 subdomain.

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**Figure 1. Characteristics of EHK-SMC.** (a) Pedigree. (b) DNA sequences showing G to C change, which introduces an R178P amino acid substitution. (c) XhoI digestion of polymerase chain reaction–amplified DNA of family members. The mutation abolishes an XhoI site in the patient. Only the affected individual shows the undigested 500-bp fragments.
factors must also influence disease severity. Therefore, the association of disease phenotype with mutation/substitution location should be made with caution.

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