A Glutamate to Lysine Mutation at the End of 2B Rod Domain of Keratin 2e Gene in Ichthyosis Bullosa of Siemens

JUN-MO YANG1,2, EUN-SO LEE3, HYO-JUNG KANG2, GWANG-SEONG CHOI3, KOZO YONEDA4, SEUNG-YONG JUNG1, KI-BEOM PARK1, PETER M. STEINERT5 and EIL-SOO LEE1

1Department of Dermatology, College of Medicine, Sungkyunkwan University, Samsung Medical Centre, 2Clinical and Basic Research Centre, Samsung Biomedical Research Institute, 3Department of Dermatology, Ajou University School of Medicine, Seoul and Suwon, Republic of Korea, 4Department of Dermatology, College of Medicine, Kyoto National University, Kyoto, Japan and 5Laboratory of Skin Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Health, Bethesda, MD, USA

Ichthyosis bullosa of Siemens is a rare autosomal dominant skin disorder whose clinical findings are quite similar to those of epidermolytic hyperkeratosis. The differences between those two diseases include absence of erythroderma and different distributions in the skin in ichthyosis bullosa of Siemens. Recent studies have confirmed that ichthyosis bullosa of Siemens is caused by the mutation in the keratin 2e (K2e) gene, which is expressed in the upper spinous and granular layers. We have identified a sporadic case of ichthyosis bullosa of Siemens; based on diagnosis by histopathological findings, the K2e gene of the patient was analysed. Direct sequencing of PCR products revealed a single base change in sequences encoding the highly conserved end of the 2B rod domain segment of the K2e gene. This mutation results in substitution of the codon for glutamic acid by a codon for lysine in position 493 in K2e (E493K). Mutations of the K2e gene involving five different residue positions (Q187P, T485P, L490P, E493D, E493K and E494K) are known to cause ichthyosis bullosa of Siemens. Of these sites, E493, which is conserved in type I and type II keratin genes, is the most frequently altered amino acid in the K2e gene. These data together suggest that this codon constitutes a hot spot for mutations in the K2e gene. Key words: keratin 2e; mutation; ichthyosis bullosa of Siemens.

(Accepted April 30, 1998.)

Jun-Mo Yang, Department of Dermatology, College of Medicine, Sungkyunkwan University, Samsung Medical Centre, 50 Ilwon Dong, Kangnam Ku, Seoul 135-710, Korea.

The expression of individual pairs of keratins is specific to the body site location and the differentiation stage of the keratinocytes. The expression of keratin 2e in ichthyosis bullosa of Siemens is caused by the mutation in the keratin 2e (K2e) gene, which is expressed in the upper spinous and granular layers. We have identified a sporadic case of ichthyosis bullosa of Siemens; based on diagnosis by histopathological findings, the K2e gene of the patient was analysed. Direct sequencing of PCR products revealed a single base change in sequences encoding the highly conserved end of the 2B rod domain segment of the K2e gene. This mutation results in substitution of the codon for glutamic acid by a codon for lysine in position 493 in K2e (E493K). Mutations of the K2e gene involving five different residue positions (Q187P, T485P, L490P, E493D, E493K and E494K) are known to cause ichthyosis bullosa of Siemens. Of these sites, E493, which is conserved in type I and type II keratin genes, is the most frequently altered amino acid in the K2e gene. These data together suggest that this codon constitutes a hot spot for mutations in the K2e gene. Key words: keratin 2e; mutation; ichthyosis bullosa of Siemens.

Ichthyosis bullosa of Siemens (IBS) (MIM number 146800) (1) is a rare autosomal dominant skin disorder that is highly penetrant and clinically evident from birth in affected individuals (2–4). The clinical findings of IBS are similar to those of epidermolytic hyperkeratosis. Patients with IBS are born with widespread blistering, but absence of erythroderma, and exhibit a wide variety of symptoms (2–4). The clinical distinction between epidermolytic hyperkeratosis and IBS was made by Siemens in 1937 (5). He noted that the skin of IBS patients was unusually fragile, that blistering occurred primarily in the granular layer, and that there was a tendency to shed the outer layers of the epidermis, producing localized denuded areas. He coined the term “Mauserung” to describe this distinctive clinical finding.

Keratins are the major gene product of keratinocytes, and form the intermediate filament cytoskeletal network in these cells (6). The major epithelial keratins of “soft” epithelia are the type I (keratins 9–20) and type II (keratins 1–8) chains.
Acta Derm Venereol (Stockh) 78

peptide derived from the K2e protein, KIF were rapidly disassembled (Fig. 2b) compared to the controls (Fig. 2a), whereas a peptide containing the E→K substitution reported herein did not disassemble the KIF (Fig. 2c).

DISCUSSION

A convincing case for the role of KIF in the maintenance of the structural integrity of the epidermis has been made in recent years, with the findings that point mutations in a variety of epidermal keratins cause serious pathology. For example, mutations in the K5 or K14 genes lead to various forms of epidermolysis bullosa simplex of simplex (19). In epidermolysis bullosa simplex, cytolysis occurs in the basal cell layer where K5 and K14 are specifically expressed. Similarly, mutations in K1 or K10 lead to suprabasal cytolysis and result in epidermolytic hyperkeratosis (18, 20). Point mutations in the palm- and sole-specific K9 gene are responsible for epidermolytic palmo-planter keratoderma (21). Indeed, mutations in more than 10 different keratin chains are now known to cause a wide variety of “keratinopathy” diseases (22).

Linkage studies suggested that IBS is caused by a defect in a type II keratin gene located on chromosome 12q11-13. Based in part on its histopathology of blistering in the granular layer of the epidermis, the K2e gene seemed a strong candidate. Subsequent mutation analyses confirmed the involvement of the K2e gene, and, including the present study; mutations in 15 different cases have been described. To date, five different residue positions have been changed (Q187P (12), T485P (14), L490P (12), E493D (10), E493K (10→13) and E494K (13)). Remarkably, ten cases involve the same codon: 493. This codon encodes a glutamic acid residue and is located in position 117 toward the end of the 2B rod domain, or the “helix-termination” segment of the keratin chains. This residue position has been highly conserved in keratin chains. It is thought to be critical for the formation of the heterodimeric coiled-coil molecule, and for the maintenance of the overlap between neighbouring molecules within the KIF structure (22). We have shown that synthetic peptides corresponding to the end of the 2B rod domain can disassemble pre-formed KIF, or prevent their assembly in vitro, whereas peptides containing amino acid substitutions seen in keratin diseases do not interfere with structure or do so only poorly (18, 23). The E→K substitution results in alteration of both the charge and shape of the side chain, and presumably disrupts the interactions required for the higher-order levels of KIF structure. The presence of the abnormal protein and KIF results in a disturbed cytoskeleton in keratinocytes of the upper spinous and granular layers of the epidermis, leading to the pathology of IBS.

Our studies have identified a mutation involving the same critical glutamic acid residue in IBS. This is the first report of this mutation in a Korean (non-Western) patient. Together, these data indicate that this residue position is a hot spot for mutations in the K2e gene. This observation will greatly facilitate analyses of further cases of this disease.

About 25% of epidermolytic hyperkeratosis patients do not exhibit mutations within the rod domains of K1 or K10 (22). Interestingly, Rothnagel et al. (10), studying two families originally diagnosed with epidermolytic hyperkeratosis, could not identify K1 or K10 mutations; instead, they identified mutations in the K2e gene. This result implies that some epidermolytic hyperkeratosis patients may have been incorrectly diagnosed, and an analysis of their K2e genes may now allow...

RESULTS

Clinical description of patient

The clinical and histological descriptions have been published elsewhere (17). In brief, a 3-year-old Korean boy had blistering and dark grey hyperkeratosis since 100 days after birth. This was a new sporadic case, since no one in his family had symptoms. Blisters developed after mechanical trauma and occurred more frequently during summer. Nails and hair were normal. Light and electron microscopic examinations were consistent with IBS.

Upon sequencing of all exons of the K2e gene, we found a single nucleotide substitution in one allele of the affected child (GAG to AAG) in codon 493 (Fig. 1a). The mutant allele encodes a lysine residue instead of wild type glutamic acid residue in position 117 of the 2B rod domain segment. Neither his parents nor his brother showed this change. A PCR amplification of specific allele assay was employed to amplify selectively the wild type allele or the allele carrying the mutation (see MATERIALS AND METHODS). Only the affected individual (III-1) in this family shows the PCR product with mutant primer.

Fig. 1. (a) DNA sequences of exon 7 of the K2e gene of IBS-AJ. This shows G to A in the first nucleotide position of codon 493, which indicates an E493K mutation in one allele. (b) PCR amplification of specific allele assay. The numbering indicates the patient IBS-AJ (III-1), his healthy brother (III-2), the healthy parents (II-1 and II-2), and his only living grandparents (I-1, I-2, I-3). Only the affected individual (III-1) in this family shows the PCR product with mutant primer.

42Stop
a differential diagnosis to be made at the genetic level to distinguish between epidermolytic hyperkeratosis and IBS.

ACKNOWLEDGEMENTS

We thank the family for their participation and co-operation. This work was supported by Samsung Biomedical Research Institute grant C9 6 0 2 1 (Yang).

REFERENCES


