

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

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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.

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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 epidermal keratins of “soft” epithelia are the type I (keratins 9–20) and type II (keratins 1–8) chains.

The expression of individual pairs of keratins is specific to the body site location and the differentiation stage of the keratinocytes. The basal layer keratinocytes of the epidermis express keratins K5 and K14. Their expression decreases with terminal differentiation, while K1 and K10 expression is sustained at a higher level, together with a variable amount of K2e (6). This is a protein of molecular weight 65.8 kDa encoded by a 2.6-kb mRNA species (7). Another protein of similar molecular weight, K2p, has been identified. K2p has a slightly different amino acid composition, is encoded by a different gene, and is expressed specifically in the hard palate and gingiva (8).

Linkage (9) and mutation analyses (10–14) have identified the mutations in the K2e gene which cause IBS and ichthyosis exfoliativa. Ichthyosis exfoliativa is a recently described autosomal dominant type of bullous ichthyosis. The clinical symptoms of ichthyosis exfoliativa are very similar to IBS, but signs of epidermolytic hyperkeratosis are absent (15).

We have studied one unrelated patient with biopsy-proved IBS and report the mutation at the end of the 2B domain in the K2e gene.

MATERIALS AND METHODS

Source of DNA, direct PCR amplification of keratin 2e gene and sequencing procedure

The methods for DNA extraction, PCR amplification, and for direct DNA sequencing are exactly the same as in a previous report (16). The primers used for amplifying exon 7 of the K2e gene were: primer I6(+), 5'-GTG ATC CAG AGG CTG CAG GGG GAG ATC-3', 21B(+), 5'-AGG GTG TCA TCA ACG GTG ACA-3', and 20B(-), 5'-CTG GGA GAG ATG AGA GGA AGG-3'. Primers I6(+)/20B(-) were used for the first PCR amplification reaction, and 21B(+)/20B(-) for the reamplification reaction. The latter primer was also used for sequencing.

Screening assay for K2e mutation

The mutation in the investigated patient did not destroy or create any restriction enzyme sites. A PCR amplification of specific alleles assay was developed. The method for this assay is the same as in a previous report (16). The primers AJwt(-), specific for the wild type base G, and AJmt(-), specific for the mutation A, were each used with 21B(+) as the second primer. The primers used for PCR amplification of specific alleles assay were: AJwt(-), 5'-TGC CCT CAC CTC GAC TCC TC-3', and AJmt(-), 5'-TGC CCT CAC CTC GAC TCC TT-3'.

Electron microscopy

Synthetic peptides of a sequence corresponding to the end of the 2B rod domain segment of the wild type K2e chain, or containing the E→K substitution reported in this paper, were used in a disassembly assay of K1/K10 keratin intermediate filaments (KIF) (14).

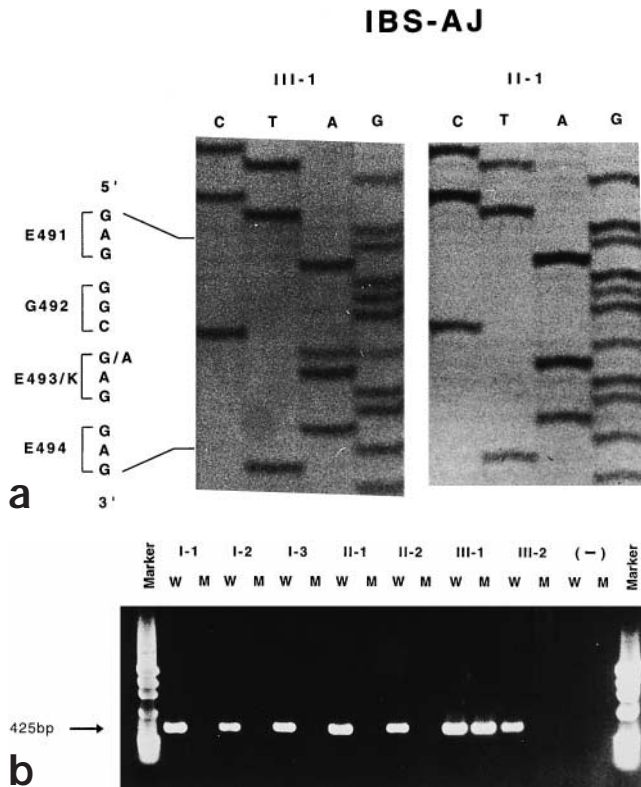


Fig. 1. (a) DNA sequences of exon 7 of the K2e gene of IBS-AJ. This shows G to A in the first nucleotide position of codon 493, which indicates an E493K mutation in one allele. (b) PCR amplification of specific allele assay. The numbering indicates the patient IBS-AJ (III-1), his healthy brother (III-2), 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.

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 alleles assay was employed to amplify selectively the wild type allele or the allele carrying the mutation (see MATERIALS AND METHODS). Only the affected individual had a PCR product with both the mutant and wild type-specific primers, whereas other unaffected family members and 50 normal controls were negative for the primer bearing the mutation (Fig. 1b).

Using an established KIF disassembly assay with a wild type peptide derived from the K2e protein, KIF were rapidly dis-

sembled (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 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 palmoplantar 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

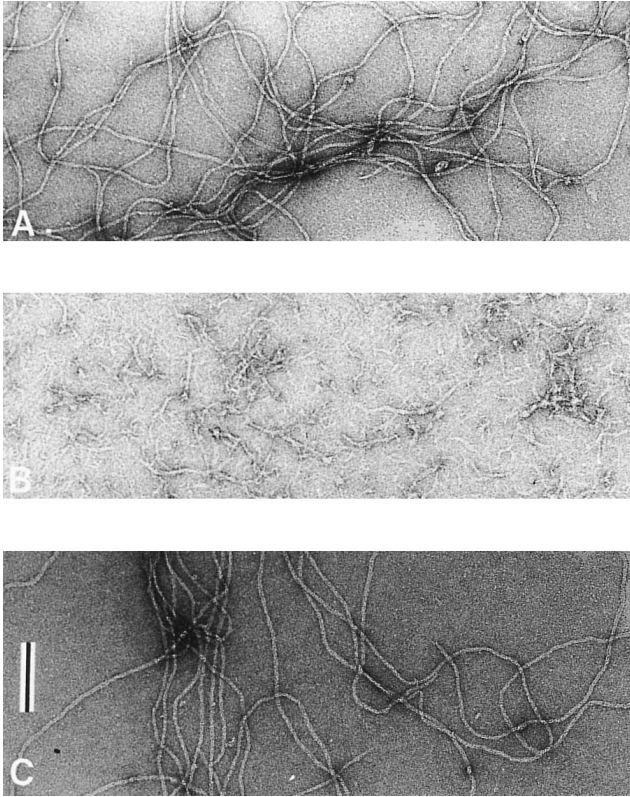


Fig. 2. Electron microscopy of keratin intermediate filament (KIF). (a) Normal KIF composed of K1/K10 chains. (b) As (a), but reacted with wild type K2e peptide for 30 min. (c) Reacted with K2e peptide containing the E→K substitution for 6 h. Negatively stained with uranyl acetate. Bar: 100 nm.

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

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