A Novel Mutation in the L12 Domain of Keratin 5 in the Köbner Variant of Epidermolysis Bullosa Simplex

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We have identified a novel mutation within the linker L12 region of keratin 5 (K5) in a family with the Köbner variant of epidermolysis bullosa simplex. The pattern of inheritance of the disorder in this family is consistent with an autosomal dominant mode of transmission. Affected individuals develop extensive and generalized blistering at birth or early infancy but in later years clinical manifestations are largely confined to palmo-plantar surfaces. Direct sequencing of polymerase chain reaction products revealed a T to C transition within codon 323 of K5 in affected individuals, resulting in a valine to alanine substitution of the seventh residue within the L12 linker domain. This mutation was not observed in unaffected family members or in 100 K5 alleles of unrelated individuals with normal skin. The other critical regions of K5 and K14 were unremarkable in this family except for common polymorphisms that have been previously described. The valine at position 7 of the L12 domain is absolutely conserved in all type II keratins, and in other intermediate filament subunits as well, which suggests that this residue makes an important contribution to filament integrity. Secondary structure analysis revealed that alanine at this position markedly reduces both the hydrophobicity and the β-sheet nature of the L12 domain. This is the first report of a mutation at this position in an intermediate filament subunit and reinforces the importance of this region to filament biology.

Key words: disease/genetics/intermediate filaments.


Several genetic disorders of the integument, characterized by cell cytolysis as a result of physical trauma, have been shown to be associated with keratin mutations (reviewed in Rothnagel, 1996). Electron microscopy of these disorders invariably reveals abnormal keratin filaments with tonofilament clumping and a collapsed keratin filament cytoskeleton, often with a perinuclear distribution (Anton-Lamprecht, 1994). Keratins are intermediate filament proteins that are expressed as coordinate pairs of type I and type II subunits in a tissue- and differentiation-state-specific manner (Steinert, 1993). Keratins have a conserved domain structure, which is common to all intermediate filament proteins, consisting of a central α-helical rod domain flanked by nonhelical N- and C-terminal domains (Steinert and Roop, 1988). The α-helical domain is interrupted by three nonhelical linker peptides denoted as L1, L12, and L2, which separate the rod domain into the 1A, 1B, 2A, and 2B helical segments. Mature keratin filaments are assembled from coiled-coil heterodimers, each consisting of a type I (keratins K9–K21) and a type II (K1–K8) subunit held together through interactions between the α-helical domains (Hatzfeld and Weber, 1990; Steinert, 1990). The dimers in turn polymerize both laterally and longitudinally to form the 10 nm filaments. Mutations in keratin subunits generally do not interfere with dimer formation or polymerization but instead act dominantly to reduce the tensile strength of the mature filament.

Epidermolysis bullosa simplex (EBS) is a rare genetic disease charac-

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Abbreviation: EBS-K, epidermolysis bullosa simplex-Köbner.
Weber–Cockayne variant of EBS. Here we report a family diagnosed with the more severe Köbner form of EBS (EBS-K) and demonstrate the presence of a novel mutation in the L12 region of K5 in affected individuals.

MATERIALS AND METHODS

Polymerase chain reaction and DNA sequencing Genomic DNA was extracted and purified from whole blood using the QIAamp blood kit according to the manufacturer’s instructions (QIAGEN, Hilden, Germany). The L12 region of K5 was amplified from genomic DNA using specific oligonucleotides (5′-CACACCGAGAAACAGATGAC-3′ and 5′-CATTCCTAGTGTCGTCATGGCC-3′ corresponding to bp 101–121 of intron 4 and to 59–79 of intron 5 of the sequences determined by Stephens et al (1997). The sequencing primer (5′-TGACCGACTCCAAATCTC-3′) corresponds to bp 118–135 of the published intron 4 sequence (Stephens et al, 1997). The polymerase chain reaction reactions were preheated (95°C, 5 min), 2.5 units of Taq polymerase added, and cycled 35 times (94°C, 45 s; 56°C, 30 s; 72°C, 45 s) with a final extension at 72°C for 10 min. Polymerase chain reaction products were purified using QIAEX II (QIAGEN) and directly sequenced using the Thermo Sequenase dye terminator cycle sequencing system (Amersham Life Science, Cleveland, OH). Sequencing products were analyzed on an ABI Prism 377 automated sequencer (Applied Biosystems, Foster City, CA).

Mutation analysis by restriction enzyme digestion The L12 region of K5 was amplified using the oligonucleotides and polymerase chain reaction conditions listed above and the purified DNA digested with MspAl I (New England Biolabs, Beverly, MA). The digest products were separated on a 2% agarose TAE gel (Sambrook et al, 1989) in the presence of ethidium bromide and visualized by UV light.

RESULTS

Clinical description of the EBS family We studied a multigeneration family in which a diagnosis of EBS-K was made on the basis of a clinical history of generalized blistering with minimal oral involvement developing at birth or early in infancy and associated with a histopathology of basal layer degeneration. The index case, an 18 y old male, also suffered from microcytosis (cause not identified) and cystic acne. His treatment included oral retinoids (Roaccutane), which was complicated by increasing epidermal fragility requiring dosages to be maintained below 0.5 mg per kg per d. There was significant variation in the severity of symptoms exhibited by affected family members. Although the proband and his mother still suffer from generalized blistering, other family members reported that blistering rarely occurred outside of palmoplantar surfaces. All reported that blistering was more severe and generalized during childhood.

Identification of a valine to alanine substitution in the L12 region of K5 We sequenced the coding regions of both K5 and K14 of affected and unaffected family members. This analysis revealed a number of base changes from the published sequences that were present in both affected and unaffected individuals and represent allelic differences in the population (Wanner et al, 1993); however, we noted a single base substitution (GTG→GCG) in codon 323 of K5 that was only present in affected individuals (Fig 1). The T to C transition results in the expression of an alanine residue instead of valine at position 7 (V7A) of the L12 domain. The base change also creates a restriction enzyme site recognized by MspAl I and this was exploited to rapidly screen for the mutation in the EBS–K family (Fig 2) and in the normal population (data not shown). Notably, this change was not observed in unaffected family members or in the DNA from 50 unrelated and unaffected control individuals.

The valine to alanine substitution produces a significant structural change in L12 The affected valine residue at position 7 of the K5 L12 domain occurs at the beginning of a conserved motif of alternating apolar-nonpolar residues (VXLXMD in type II keratins) that has been recognized in all L12 sequences and suggests a β-type structure for this region (North et al, 1994). The V7 residue is absolutely conserved in all type II keratins and in many other intermediate filament proteins as well (Fig 3). The equivalent residue in the type I keratins is also valine or another apolar residue such as leucine (Conway and Parry, 1988). The strict conservation of valine at this site suggests...
that any change is likely to be deleterious although a priori, the alanine substitution at position seven. The graphs show the propensity for α-helix (red curve) and β-sheet (blue curve) formation for the normal L12 sequence (a) and the mutant L12 sequence (b). Both curves are the average of a residue-specific attribute over a window of four residues. The threshold value for the continuation of a given structure is arbitrarily set at 1.0.

DISCUSSION

The L12 linker peptide demarcates the end of the 1B and the beginning of the 2A α-helical segments and is found in all intermediate filament proteins, but its contribution to filament assembly and/or function has not been unequivocally determined. Current filament assembly models predict that the L12 region is in close proximity to the L2 and H2 regions in the staggered alignment of coiled-coiled dimers, or with the L12 region of a neighboring pair of subunits in the nonstaggered antiparallel alignment (Steinert, 1993). In the antiparallel alignment, the four L12 regions are in a position to contribute to tetramer stability and in turn to filament stability through hydrophobic interactions with one another. Prior to this study, four other mutations in the L12 region of K5 had been reported and interestingly, all occur in the C-terminal half of the peptide (Fig 3) where they are predicted to promote α-helical formation at the expense of the normal β-sheet structure (Rugg et al, 1993; Chan et al, 1994; Matsuki et al, 1995). It has been postulated that these structural changes would result in a more rigid molecule with less resistance to torsional stress (McLean and Lane, 1995). These mutations may also impair the normal packing of dimers in the filament resulting in diminished interactions between residues critical for maintaining tensile strength. In vitro modeling of K5 L12 mutations (M11T and N13K) have shown that these substitutions tend to cause filament unravelling and result in loosely packed, shortened filaments with an apparent increase in diameter (Chan et al, 1994). These authors concluded that the L12 region plays a prominent role in stabilizing lateral associations between heterodimers. Given that the V7A mutation found in this study also has a marked affect on the secondary structure of L12, it is likewise postulated to interfere with lateral interactions, leading to a weakening of the keratin filament network and the subsequent clinical manifestations in these patients.

It should be noted that although some of the patients in this study display symptoms consistent with the Köbner form of EBS, a diagnosis of EBS–Weber–Cockayne may be equally appropriate for other members of this family, particularly older individuals. By comparison, the other K5 L12 mutations have all been identified in patients with the classical EBS–Weber–Cockayne symptoms of mild blistering limited to palmoplantar epidermis (Rugg et al, 1993; Chan et al, 1994; Matsuki et al, 1995). This suggests that the V7A mutation has a more profound effect on filament function than the other K5 L12 mutations, and further underscores the importance of this valine residue to the biology of the L12 domain. Similarly, both K14 L12 mutations (V8M and M10R) were also associated with more severe symptoms (Rugg et al, 1993; Humphries et al, 1993), with the M10R substitution identified in a family diagnosed with EBS-K (Humphries et al, 1993). Taken together these studies provide an indication of the relative contribution of each of these L12 residues to filament stability.

In this study we have identified a novel mutation within the L12 region of K5 that is associated with a clinical phenotype. Although we have no functional data proving that this mutation is pathogenic, both genetic evidence and structural analysis data strongly suggest that it is. This mutation was only observed in individuals with clinical symptoms. Notwithstanding, the predicted changes in secondary structure, the substitution of alanine for valine also produces a significant side chain alteration at this position. The absence of the extended branched side chain of valine could result in the loss of critical interactions with residues of proximal subunits and a subsequent reduction in filament stability (Alber, 1989; Zhu et al, 1993).
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