Novel N160I Mutation of Keratin 9 in a Large Pedigree from Hungary with Epidermolytic Palmoplantar Keratoderma

Márta Csikós, Péter Holló, Krisztina Becker, Emőke Rácz, Attila Horváth and Sarolta Kárpáti
Department of Dermatology, Semmelweis University, Mária u. 41, H-1085 Budapest, Hungary. E-mail: csimar@bor.sote.hu
Accepted March 17, 2003.

Sir,

The keratin 9 gene (KRT9) encodes the type I keratin 9, an intermediate filament protein expressed only in the terminally differentiated epidermis of the palms and soles (1). Mutations in KRT9 are a cause of epidermolytic palmoplantar keratoderma Vörner (EPPK), an autosomal dominant disease characterized by diffuse thickening of the entire palmoplantar epidermis bordered with erythematosus margins (2–4). Most of the known EPPK mutations are located in the evolutionarily highly conserved residue of the critical aminoterminal end in the rod domain of keratin 9 (4–12).

A large Hungarian family with EPPK confirmed by histopathological and ultrastructural studies is presented. The proband, a 22-year-old woman, showed a novel missense mutation 479A→T, N160I in the aminoterminal end of the rod domain of the KRT9, which was confirmed in the other affected members of the family.

MATERIAL AND METHODS
All participating family members gave their written informed consent to the histology and genetic studies. A skin biopsy was obtained from the sole under local anaesthesia. Specimens were processed for light and electron microscopy. DNA was isolated from peripheral blood lymphocytes according to standard techniques.

A 429 bp fragment containing the major part of exon 1 in
the functional KRT9 gene was amplified by PCR using sense primer 5'-TTGGCTACAGCTACGGGAGGAT-3’ and anti-sense primer 5’-TGAGATCATCAATAGTTAATAT-3’ as described previously (5). Amplification conditions were: 95°C for 5 min, 95°C for 45 s, 60°C for 30 s and 72°C for 30 s for 40 cycles in a TouchGene thermal cycler (Techne Cambridge Ltd, UK).

PCR products were sequenced directly using the ABI Prism 310 automated sequencing system (Applied Biosystem). Numbering of the nucleotides was according to GenBank Homo sapiens mRNA for keratin 9 (accession number: NM 000226).

Restriction endonuclease digestion with Tsp509 I was carried out in accordance with the manufacturer’s recommendations (New England BioLabs Inc.). The fragments were separated by 2% ethidium bromide-stained agarose gel.

One hundred alleles from 50 healthy unrelated individuals were screened for the mutation by restriction enzyme analysis to exclude the possibility of a polymorphism.

CASE REPORT

The 22-year-old proband was born after an uncomplicated pregnancy. A confluent thickening of the palmar and plantar skin became obvious shortly after birth and progressed with age. Otherwise her skin, oral mucosa, teeth, hair and nails were normal. The hyperkeratotic area was surrounded by an erythematous border (Fig. 1a). Her mother and several other members of the family had the same disease (see below). Epidermolytic changes in the high spinous and granular layers as well as a prominent thickening of the stratum corneum were detected (Fig. 1b). On ultrastructural analysis, characteristic tonofilament clumping and cytolysis were observed within the stratum spinosum and granulosum (results not shown).

Fig. 1. (a) Diffuse, sharply defined hyperkeratosis on the sole with erythematous border. (b) Epidermolytic changes in the spinous and granular layers with massive thickening of stratum corneum (haematoxylin-eosin staining).

Direct nucleotide sequencing of the PCR fragment containing exon 1 detected a heterozygous A-to-T transversion at nucleotide position 479, leading to an asparagine (AAT)-to-isoleucine (ATT) change at amino acid residue 160 in the keratin 9 protein of our patient (Fig. 2). Since 479A→T abolishes a Tsp509 I restriction endonuclease recognition site, the mutation has been confirmed using this restriction enzyme. The 429 bp PCR product was digested by Tsp509 I in two small fragments (275 bp, 154 bp) in the wild type alleles, and because of the abolished recognition site the 429 bp product remained unchanged in the mutated allele (Fig. 3). Segregation of the mutation was observed in the affected members of the family.

DISCUSSION

The palmoplantar keratodermas have recently been reviewed and reclassified (13). EPPK is distinguishable from non-EPPK by the following criteria: (i) the presence of epidermolytic changes in the epidermis, (ii) the disease being apparent at birth (in control to later onset for non-EPPK), (iii) the erythematous border of the hyperkeratotic area, and (iv) findings of a mutation in the KRT9 gene (2, 4, 13).

Type I keratin 9 is expressed only in the terminally differentiated epidermis of the palms and soles, beside the suprabasal epidermal cell-specific type I keratin 10 and the type II keratin 1, which contribute to the cytoskeleton (1). The highly conserved boundary motifs of the central rod domain appear to be essential for correct filament assembly, and missense mutations in these regions result in filament aggregation and the

Fig. 2. Sequence analysis of the KRT9 gene. Upper sequence shows the mutant, lower sequence the wild type allele (reverse sequence). The heterozygous A-to-T transversion at nucleotide position 479 converts an asparagine (N) to an isoleucine (I) change at amino acid residue 160 in the keratin 9 protein (top).
most severe dominant skin fragility syndromes such as EPPK (14, 15).

Our case fulfilled the clinical, histopathological and ultrastructural criteria of EPPK. A novel dominant inheritance missense mutation (479A→T, N160I) in exon 1 of the KRT9 caused the EPPK phenotype. Analysis of the data collected in the literature shows that most of the mutations disclosed are located in a narrow range between codons 156 and 171 in the 1A domain and only one insertional mutation was found in the 2B domain of keratin 9 (4 – 12). The majority were missense mutations and only one gene defect generated a premature stop codon (12). These mutations are usually specific for individual families, and only three recurring mutations have been identified. Out of the keratin 9 mutations reported previously, the incidence of R162W substitution is estimated at 36% (10/28 total cases), suggesting that this major hot spot mutation is the most frequent in EPPK patients. Mutations in codon 160 of keratin 9 – containing our novel mutation – account for more than 14% of mutations identified in EPPK. Genetic analyses of many pedigrees suggest that new, de novo mutations rarely occur in this disorder (4 – 12).

ACKNOWLEDGEMENTS

We thank Melinda Szöke (H-MED Diagnostic and Research Laboratory, Hungary) for performing direct sequence analysis and Menyhárt Ferencné for technical assistance. The work was supported by a grant from the Hungarian National Scientific Research Program (OTKA T032139).

REFERENCES