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

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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 erythematous 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′-TTGGCTACAGCTACGGCGGAGGAT-3′ and antisense primer 5′- TGAGATCATCAATAGTGTTATAAT-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 nucleotids 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).

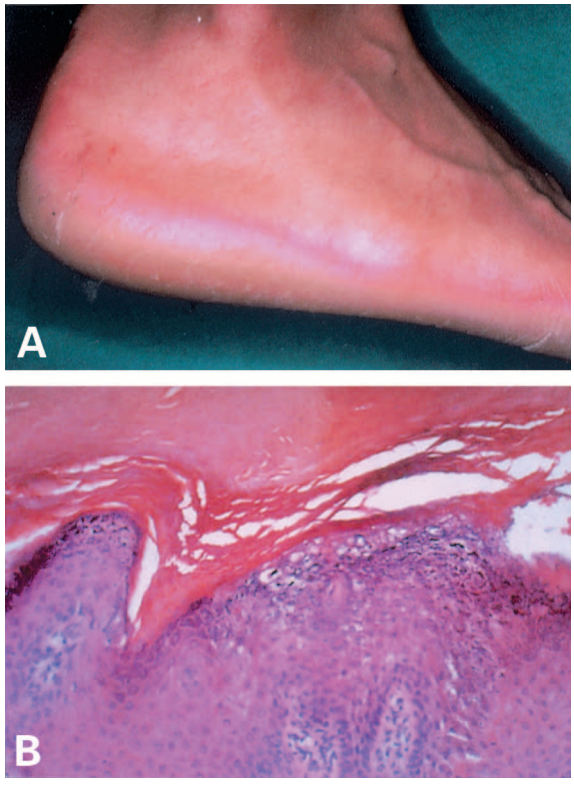

Fig. 1. (a) Diffuse, sharply defined hyperkeratosis on the sole with erythematous border. (b) Epidermolytic changes in the spinous and granular layers with massive thickening of stratum corneum (haematoxylin-eosin staining).

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

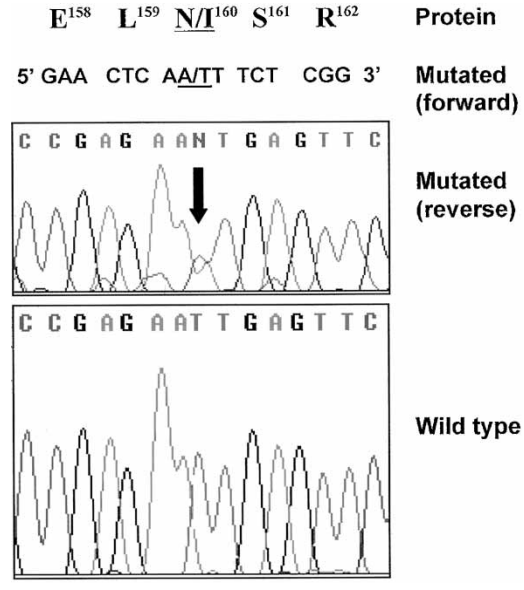

Fig. 2. Sequence analysis of the KRT9 gene. Upper sequence shows the mutant, lower sequence the wild type allele (reverse sequence). The heterozygous A-to-T transversion at nucleotide position 479 converts an asparagine (N) to an isoleucine (I) change at amino acid residue 160 in the keratin 9 protein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

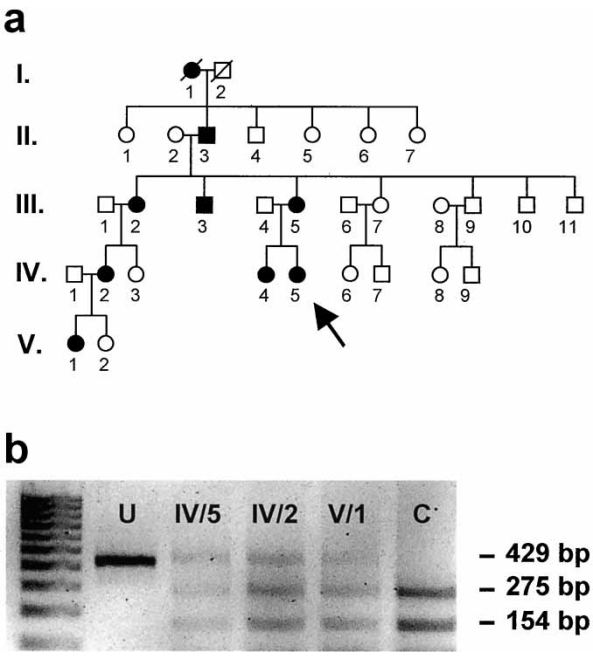

Fig. 3. (a) Pedigree of the family: the affected proband is indicated by the arrow. Segregation of disease was compatible with a fully penetrant autosomal dominant inheritance. (b) Verification of mutation: 479A→T abolishes a Tsp 509 I restriction endonuclease recognition site. The 429 bp PCR product was digested by Tsp509 I into two small fragments (275 bp, 154 bp) in the wild type allele and because of the abolished recognition site, the 429 bp product remained uncut in the mutated allele.

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).

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REFERENCES

1. Fuchs E, Green H. Changes in keratin gene expression during terminal differentiation of the keratinocyte. Cell 1980; 19: 1033–1042.
2. Vörner H. Zur Kenntnis des Keratoma hereditarium palmare et plantare. Arch Derm Syph 1901; 56: 3–31.
3. Reis A, Kuster W, Eckardt R, et al. Mapping of a gene for epidermolytic palmoplantar keratoderma to the region of the acidic keratin gene cluster at 17q12-21. Hum Genet 1992; 90: 113–116.
4. Reis A, Hennies HC, Langbein L, et al. Keratin 9 gene mutations in epidermolytic palmoplantar keratoderma (EPPK). Nat Genet 1994; 6: 174–179.
5. Covello SP, Irvine AD, McKenna KE, et al. Mutations in keratin K9 in kindreds with epidermolytic palmoplantar keratoderma and epidemiology in Northern Ireland. J Invest Dermatol 1998; 111: 1207–1209.
6. Coleman CM, Munro CS, Smith FJ, et al. Epidermolytic palmoplantar keratoderma due to a novel type of keratin mutation, a 3-bp insertion in the keratin 9 helix termination motif. Br J Dermatol 1999; 140: 486–490.
7. Yang JM, Lee S, Kang HJ, et al. Mutations in the 1A rod domain segment of the keratin 9 gene in epidermolytic palmoplantar keratoderma. Acta Derm Venereol 1998; 78: 412–416.
8. Endo H, Hatamochi A, Shinkai H. A novel mutation of a leucine residue in coil 1A of keratin 9 in epidermolytic palmoplantar keratoderma. J Invest Dermatol 1997; 109: 113–115.
9. Rothnagel JA, Wojcik S, Liefer KM, et al. Mutations in the 1A domain of keratin 9 in patients with epidermolytic palmoplantar keratoderma. J Invest Dermatol 1995; 104: 430–433.
10. Navsaria HA, Swensson O, Ratnavel RC, et al. Ultrastructural changes resulting from keratin-9 gene mutations in two families with epidermolytic palmoplantar keratoderma. J Invest Dermatol 1995; 104: 425–429.
11. Bonifas JM, Matsumura K, Chen MA, et al. Mutations of keratin 9 in two families with palmoplantar epidermolytic hyperkeratosis. J Invest Dermatol 1994; 103: 474–477.
12. Szalai S, Szalai Cs, Becker K, et al. Keratin 9 mutations in the coil 1A region in epidermolytic palmoplantar keratoderma. Ped Dermatol 1999; 16: 430–435.
13. Kuster W, Becker A. Indication for the identity of palmoplantar keratoderma type Unna-Thost with type Vorner. Thost's family revisited 110 years later. Acta Derm Venereol 1992; 72: 120–122.
14. Letai A, Coulombe PA, Fuchs E. Do the ends justify the means? Proline mutations at the ends of the keratin coiled-coil rod segment are more disruptive than internal mutations. J Cell Biol 1992; 116: 1181–1195.
15. Corden LD, McLean WH. Human keratin diseases: hereditary fragility of specific epithelial tissues. Exp Dermatol 1996; 5: 297–307.