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Haploinsufficiency of desmoplakin causes a striate subtype of palmoplantar keratoderma

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Desmosomes are highly organized intercellular adhesive junctions that are particularly prominent in epidermis and other tissues experiencing mechanical stress. Desmoplakin, a constitutive component of the desmosomal plaque, is the most abundant protein present in such junctions and plays a critical role in linking the intermediate filament network to the plasma membrane in these tissues. Here we report the first mutation in the gene encoding desmoplakin. The identified mutation, resulting in a null allele and haploinsufficiency, was observed in genomic DNA from a kindred with the dominantly inherited skin disorder, striate palmoplantar keratoderma. Affected individuals had a linear pattern of skin thickening on the fingers and palms and circumscribed areas of skin thickening on the soles. Affected skin demonstrated loosening of intercellular connections, disruption of desmosome–keratin intermediate filament interactions and a proportion of rudimentary desmosomal structures. The disorder mapped to chromosome 6p21 with a maximum lod score of 10.67. The mutation was a heterozygous C→T transition in exon 4 of the desmoplakin gene and predicted a premature termination codon in the N-terminal region of the peptide. This is the first reported mutation of desmoplakin and also the first inherited skin disorder in which haploinsufficiency of a structural component has been implicated. It identifies dosage of desmoplakin as critical in maintaining epidermal integrity.

INTRODUCTION

The hereditary palmoplantar keratodermas (PPKs) are a clinically and genetically heterogeneous group of disorders characterized by thickening of the skin of the palms and soles with prominent hyperkeratosis (1–3). Striate PPK (MIM 148700) is a rare but clinically distinctive, autosomal dominant subtype of focal PPK characterized by a linear pattern of skin thickening on the palms and flexor aspects of the fingers and areate or island-like areas of thickening on the soles (4). It is usually an isolated cutaneous condition although rarely abnormalities of the nails, teeth and hair have been noted (5). Genetic linkage in one kindred with the striate phenotype has been reported to chromosome 18q12, a region which contains a cluster of desmosomal cadherin genes (6–8).

We have studied a large kindred with a striate PPK phenotype and have excluded linkage to the previously reported desmosomal cadherin locus on chromosome 18q21, thus confirming genetic heterogeneity within this subtype. Subsequently, microsatellite typing was performed with markers mapping to other candidate regions, and genetic linkage to chromosome 6p21 was demonstrated. The gene encoding desmoplakin, a constitutive desmosomal plaque protein, maps to this region (9). Here we report the first mutation of desmoplakin which results in a functionally null allele and desmoplakin haploinsufficiency.

RESULTS

Clinical features

We studied a large kindred with a striate PPK phenotype (Fig. 1). Affected individuals developed palmoplantar thickening in the first or early second decade of life. This was most prominent in a linear pattern along the flexor aspects of the fingers and over pressure points on the soles (Fig. 2). There was a tendency to a co-existent milder, more diffuse background palmoplantar thickening in some. Individuals were prone to develop fissuring although there was no history of frank blister formation. Heavy manual work was a notable exacerbating factor. There were no skin changes outside the palmoplantar areas and indeed no abnormalities of the hair, nails or teeth were noted.

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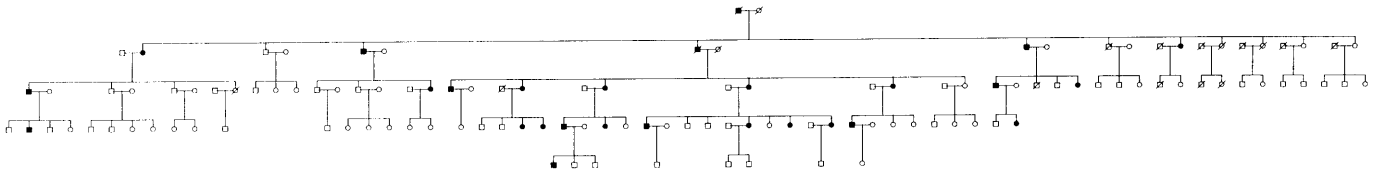

Figure 1. Pedigree of the hereditary palmoplantar keratoderma kindred demonstrating autosomal dominant inheritance of the disorder spanning five generations.

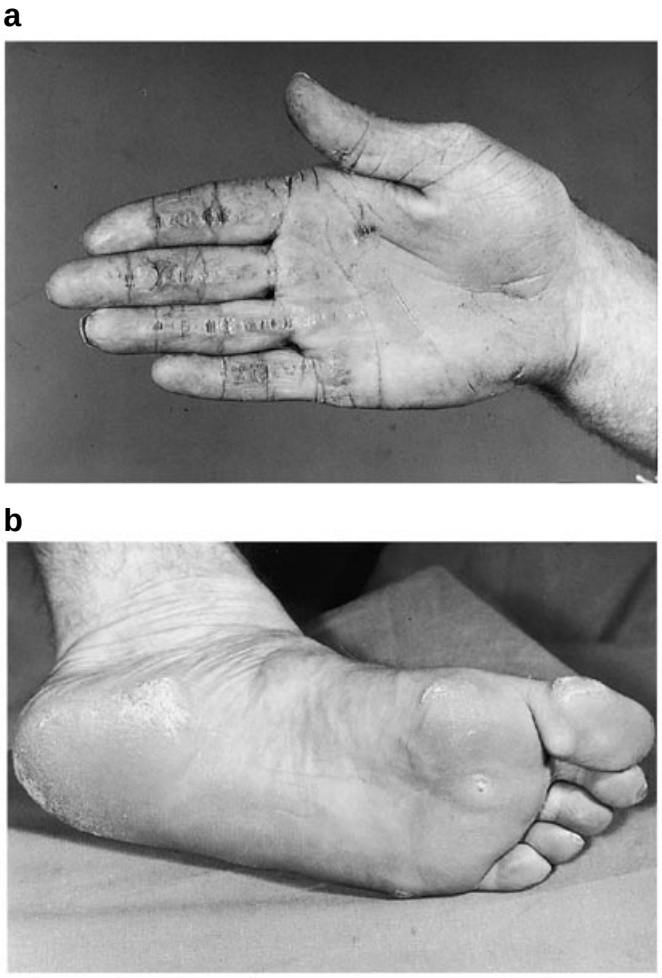

Figure 2. (**a**) Striking linear bands of thickened skin are noted on the central flexor aspects of the fingers extending into the central palmar region. There is associated fissuring and a milder background palmar hyperkeratosis. (**b**) Focal areas of thickened plantar skin are noted over the medial aspects of the heel, forefoot and big toe. In addition, circumscribed, thickened areas are noted over the metatarsal heads centrally, the plantar aspects of the big toe and lateral forefoot. These sites represent those most exposed to frictional stresses and pressure effects.

Microscopy

Light microscopy of affected palmar skin demonstrated both hyperkeratosis and acanthosis with darkly staining granular or filamentous material within the cytoplasm of spinous cells and associated loosening of intercellular connections (Fig. 3). Electron microscopy confirmed abnormal bunching of the keratin filament network. In some areas, the peripheral keratin network was sparse or lacking, with loss of connections with desmosomes. In addition, a proportion of desmosomes were small and rudimentary and the intercellular spaces were widened, although contact between cells was usually maintained through peripheral microvillus-type processes (Fig. 4). The ultrastructure of clinically uninvolved arm skin from the same individual was normal. Examination for desmoplakin immunoreactivity showed a patchy reduction in peripheral cellular staining in the mid-spinous layer in affected skin compared with controls.

Genotyping and linkage analysis

Genetic linkage for the striate phenotype has been reported to chromosome 18q12, a region which contains a cluster of desmosomal cadherin genes (6–8). Accordingly, microsatellite typing was performed with *D18S536* and *D18S36* which map to this genetic interval but linkage was excluded in this kindred (Table 1). Subsequently, further microsatellite typing was performed with markers mapping to other candidate regions including the keratin gene clusters on chromosome 12q and 17q and the desmoplakin locus on chromosome 6p21. Complete co-segregation of the disease phenotype was shown with markers *D6S263* and *D6S470*, with a maximum two-point lod score of 10.67 (Table 1). Recombination events placed the disease locus between *D6S477* and *D6S443* within which lies desmoplakin.

Table 1. Two-point lod scores for chromosome 6 and 12 markers

Marker	Z_{max} value					
	0.0	0.01	0.05	0.1	0.2	0.3
D6S1713	–∞	4.09	5.58	5.68	4.87	3.48
D6S1574	–∞	8.08	8.15	7.63	6.14	4.29
D6S477	–∞	5.97	6.13	5.73	4.56	3.16
D6S263	7.31	7.17	6.59	5.83	4.22	2.55
D6S470	10.67	10.50	9.80	8.89	6.90	4.66
D6S443	–∞	2.53	3.49	3.49	2.73	1.58
D18S536	–∞	–5.16	–2.48	–1.42	–0.54	–0.17
D18S536	–∞	–9.30	–4.08	–2.17	–0.82	–0.55

Mutation analysis

RNA was extracted from cultured keratinocytes of three affected individuals and three normal controls. After reverse transcription, the desmoplakin cDNA was PCR amplified and sequenced. This analysis failed to detect a mutation but identified a polymorphic change at nucleotide 8804 (G→C) which was present in a heterozygous state in one normal control and in an apparently homozygous state in one affected patient. Subsequent sequencing

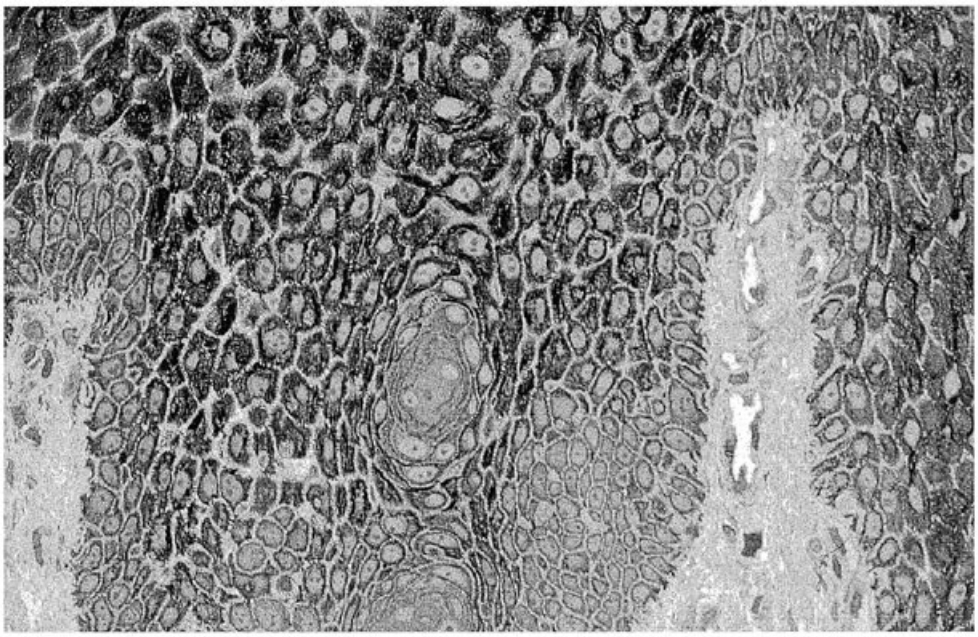

Figure 3. Photomicrograph of a semi-thin section of clinically affected palm skin showing the major abnormalities in the spinous layer of the epidermis. The intercellular space is widened, giving the impression, in places, of cell–cell separation, and the cells contain abnormal cytoplasmic densities. The cells in the basal layer and periluminal part of an acrosyringium in an epidermal ridge at the centre of the micrograph are normal in appearance. Richardson's stain, original magnification ×100.

of the exons of desmoplakin following amplification of genomic DNA demonstrated heterozygous polymorphic changes at nucleotides 333, 2963 and 8804 in each affected patient. In each case, cDNA sequencing showed apparent homozygosity at these nucleotides. A more significant change was detected in exon 4 at nucleotide 1323 with a heterozygous C→T transition in all affected patients sequenced which was not present in five unaffected family members tested (Fig. 5). This change had not been evident on cDNA screening. The mutation was confirmed using PCR amplification of specific alleles in all affected individuals and excluded from unaffected family members and 100 unrelated normal control alleles. This C→T transition converts a glutamine residue to a stop codon (Q331X). The absence of RNA transcribed from the mutant allele in keratinocytes would suggest that nonsense-mediated mRNA decay is significant in this kindred, resulting in a functionally null allele and desmoplakin haploinsufficiency.

DISCUSSION

This is the first human genetic disease to be attributed to a mutation of desmoplakin and, in addition, it represents the first inherited skin disorder in which haploinsufficiency of a structural epidermal protein has been implicated as the disease-causing mechanism. It identifies desmoplakin as a critical contributor to both desmosome–keratin intermediate filament interactions and to intercellular adhesion mechanisms. Desmosomes are highly organized intercellular adhesion complexes present in almost all epithelia (10–12). Important components include the desmosomal cadherins (desmogleins and desmocollins), the constitutive plaque proteins (desmoplakin and plakoglobin) and a number of accessory plaque proteins such as plakophilin 1. Desmoplakin, the most abundant plaque protein, lacks a transmembranous domain and acts as a critical linker protein for connecting keratin intermediate filaments to desmosomes (13). Previous studies have shown that the C-terminal domain of desmoplakin associates directly with keratin intermediate filament networks and that the N-terminal head domains of type II keratins have a special significance in establishing these connections (14–19). The N-terminal domain of desmoplakin, however, governs its association with the desmosomal plaque (13,15,19). This domain has been shown to bind directly to plakoglobin and to play a role in organizing the desmosomal cadherin–plakoglobin complex (19,20). Recent studies are consistent with the model that in desmosomes, desmoplakin and plakoglobin anchor to desmosomal cadherins and to each other, forming an ordered array of non-transmembranous proteins that associate with intermediate filament networks (19,20).

In the kindred we report, the ultrastructural findings are consistent with the proposed function of desmoplakin both as a critical linker protein anchoring intermediate filaments to desmosomes and also in influencing the hierarchy of protein interactions which give rise to effective intercellular adhesion. Furthermore, the abnormal desmosomes, lacking inner plaques and normal connections with keratin filaments (Fig. 4c), are ultrastructurally similar to the junctions described in A431 epithelial cells stably expressing desmoplakin N-terminal polypeptides (13). Desmoplakin is a member of the plakin family of proteins (21). Homozygous null mutations of plectin, another member of the plakin family, give rise to epidermolysis bullosa simplex with muscular dystrophy and neurodegeneration (22,23). Heterozygotes, however, manifest no disease phenotype. Furthermore, null mutations in both alleles of plakophilin 1, a major desmosomal accessory plaque protein, recently were reported in a novel ectodermal dysplasia/skin fragility syndrome (24). Although the affected individual had more widespread cutaneous and ectodermal involvement and more

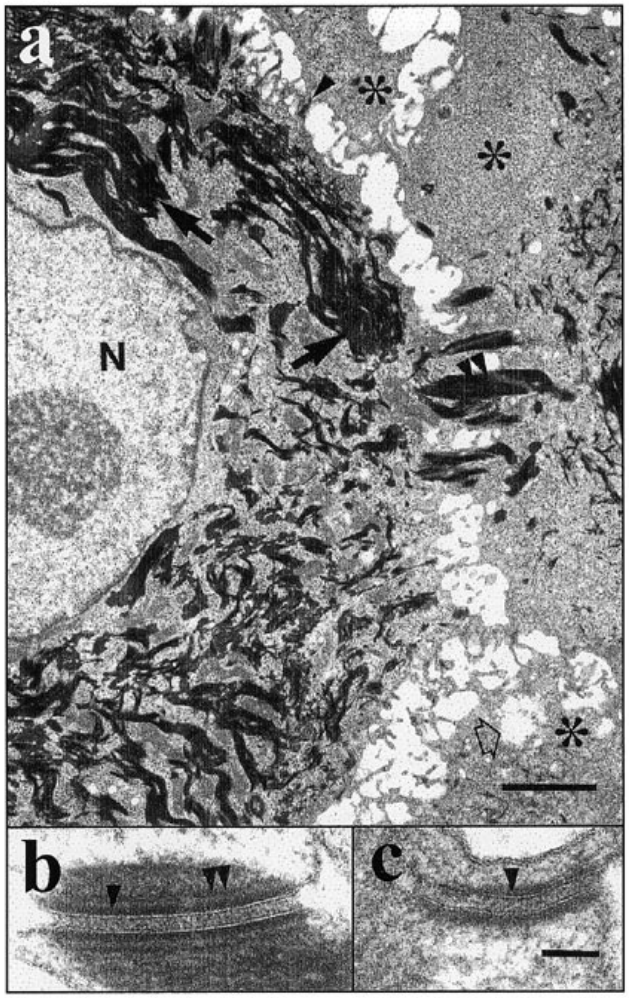

Figure 4. Ultrastructural features of cells in the mid-spinous layer of lesional epidermis, as shown in Figure 3. (**a**) The cell with nucleus (N) contains abnormal aggregates of tonofilaments or keratin intermediate filaments (KIF) (large arrows) in its cytoplasm. The KIF make irregular connections with desmosomes of normal size (double arrowheads) but do not appear to associate with smaller, atypical junctions (single arrowhead). The peripheral cytoplasm of neighbouring cells (asterisks) is largely devoid of normal KIF and desmosomes, and contains a possible focus of lysis (open arrowhead). In areas where desmosomes are absent, contact between cells is made by fine microvillus-like surface projections. (**b**) Normal appearing desmosome with well-developed inner plaque (double arrowheads) and outer plaque (single arrowhead). (**c**) Desmosome-like junction lacking inner cytoplasmic plaque and normal association with tonofilaments. The outer plaque appears normal (arrowhead). Magnification bars: (a) 2 μm, (b and c) 0.1 μm.

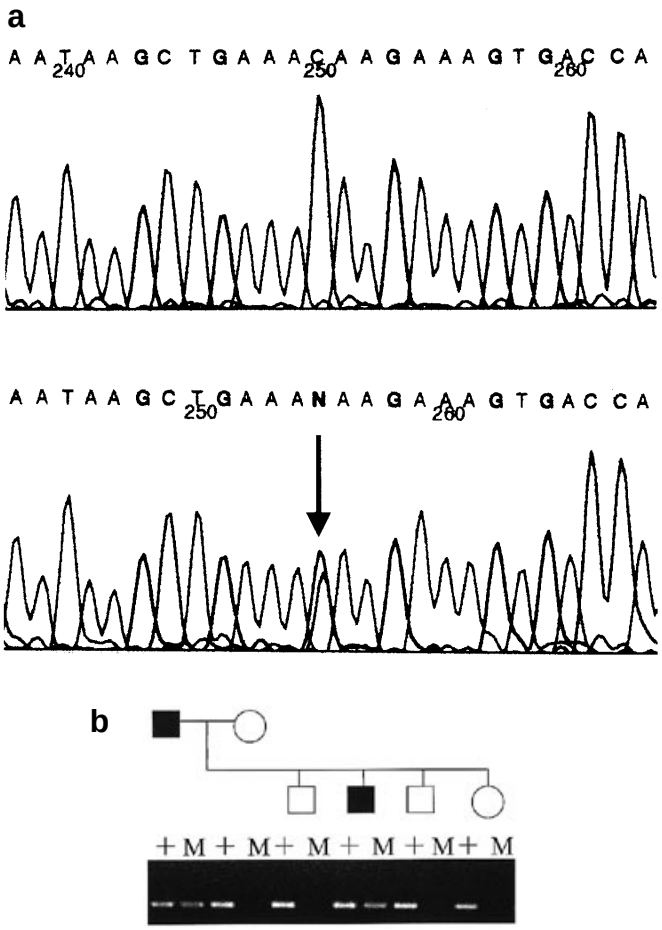

Figure 5. (**a**) Nucleotide sequencing reveals a heterozygous C→T transition in an affected individual (bottom panel) which is not present in a normal control (top panel). This changes a glutamine residue (CAA) to a stop codon (TAA). (**b**) Verification of the mutation by PCR amplification of specific alleles in a section of the pedigree. A 312 bp fragment was amplified using a common sense primer, DP4F, and allele-specific antisense primers, DPNR (wild-type, lanes +) or DPMUTR (mutant-specific, lanes M). The mutant-specific primer amplified product only in the affected father and his affected son but not in the unaffected individuals. All affected family members amplified product with the mutant-specific primer while unaffected family members and 50 normal controls failed to do so.

extensive loss of cell–cell adhesion than the kindred we report, the heterozygous parents of this child were phenotypically normal. The existence, therefore, of clinical manifestations due to haploinsufficiency of desmoplakin in the kindred we describe clearly suggests that desmoplakin interactions are even more critical. Desmoplakin is expressed during early embryogenesis and desmosomes are found not only in epidermis but also in a wide variety of other tissues, including simple epithelia, myocardial and meningeal cells and dendritic reticulum cells (10,11). The restricted clinical phenotype in this kindred, not only in its limitation to skin but also in a striate distribution, is interesting. One normal allele may permit sufficient desmoplakin production to maintain structural integrity at most sites, but at sites exposed to intense and repetitive mechanical stresses, as occurs on the palms and soles, the reduced desmoplakin level may become inadequate and clinically significant.

MATERIALS AND METHODS

Genotyping and linkage analysis

Microsatellite markers were amplified by PCR using γ-^{32}P-labelled forward primers, analysed on 6% polyacrylamide sequencing gels and visualized by autoradiography. Two-point lod scores were calculated using the MLINK program of FASTLINK assuming autosomal dominant inheritance with complete penetrance, zero mutation rate and a disease allele frequency of 0.001.

Mutation detection

Keratinocytes were cultured from skin biopsies taken from uninvolved arm skin. Total RNA was extracted from ~1×10^6 cells using a Purescript RNA isolation kit (Gentra) and reverse transcribed to cDNA. Genomic DNA was extracted from peripheral blood lymphocytes using standard guanidine methods. Primer sets were designed to amplify the desmoplakin-coding region from cDNA, and each exon of desmoplakin from genomic DNA using intronic primers (full data not shown). PCR products were purified using a Wizard kit (Promega) and sequenced using the ABI PRISM Ready Reaction System (Big Dye Terminators) on an ABI377 automated sequencer.

The primers used to amplify and sequence exon 4 were: DP4F, 5′-ctcctgtgtcatcttgagtaacc-3′; and DP4R, 5′-cctgaaaagagtttgc-caaccc-3′. PCR was performed with Qiagen *Taq* polymerase and 0.3 μM primers (97°C for 3 min followed by 40 cycles of 97°C for 1 min, 56°C for 1 min and 72°C for 1 min, and then 72°C for 10 min), amplifying a product of 504 bp.

Verification of mutation

The identified mutation does not alter any known restriction enzyme site. The mutation was confirmed using PCR amplification of specific alleles. A 312 bp fragment was amplified using a common sense primer (DP4F) and either a wild-type (DPNR) or mutant (DPMUTR) antisense primer (97°C for 3 min; followed by 36 cycles at 97°C for 30 s, 55°C for 30 s and 72°C for 30 s and then 72°C for 10 min). PCRs were performed with 0.3 μM primers using Qiagen *Taq* polymerase, and the products were resolved by agarose gel electrophoresis. The primers used were as follows: DP4F, 5′-CTCCTGTGTCATCTTGAGTAACC-3′; DPNR, 5′-GGACAAGTTGGTCACTTTCTTG-3′; and DPMUTR, 5′-GGACAAGTTGGTCACTTTC**C**T**A**-3′. Primer DPMUTR has the mutated base at the 3′ end (bold) and a single base mismatch three bases from its 3′ end (underlined) to discourage amplification of the wild-type allele.

Light and electron microscopy

Skin specimens were processed largely according to previous methods (25). Briefly, the samples were fixed in half-strength Karnovsky fixative (26) (containing 2% formaldehyde and 2.5% glutaraldehyde in 0.04 M cacodylate buffer), followed by further fixation in 1.3% osmium tetroxide, dehydration in a graded ethanol series and embedding in Epon resin. For light microscopy, semi-thin sections (1 μm) were stained with Richardson's solution (27). For electron microscopic examination, ultra-thin sections were stained with uranyl acetate and lead citrate and observed in a JEOL 100CX transmission electron microscope operating at an accelerating voltage of 80 kV.

Immunofluorescence microscopy

Skin biopsies were snap-frozen immediately in liquid nitrogen and then mounted in Tissue Tec. Cryostat sections (10 μm) were collected onto APES-coated slides and air dried, and antibody labelling was then performed as described elsewhere (28). The primary antibodies used were 11-5F and NW 38 [desmoplakins I and II (15,29)]. Incubation with the primary antibody was for 2 h at room temperature. After washing in phosphate-buffered saline (PBS), secondary antibody conjugated to fluorescein isothiocyanate (FITC) and diluted 1:100 was applied and incubated for 1 h at room temperature. The sections were then washed and briefly incubated in 1:1000 propidium iodine. Finally the slides were washed, mounted in glycerol/PBS containing antifade and viewed under a fluorescence microscope.

GenBank accession number

Human desmoplakin I mRNA has been deposited in the GenBank sequence database with accession no. M77830. Nucleotide numbering throughout refers to the mRNA sequence.

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