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Recessive mutation in desmoplakin disrupts desmoplakin–intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma

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Desmosomes are major cell adhesion junctions, particularly prominent in the epidermis and cardiac tissue and are important for the rigidity and strength of the cells. The desmosome consists of several proteins, of which desmoplakin is the most abundant. Here, we describe the first recessive human mutation, 7901delG, in the desmoplakin gene which causes a generalized striate keratoderma particularly affecting the palmoplantar epidermis, woolly hair and a dilated left ventricular cardiomyopathy. A number of the patients with this syndromic disorder suffer heart failure in their teenage years, resulting in early morbidity. All tested affected members of three families from Ecuador were homozygous for this mutation which produces a premature stop codon leading to a truncated desmoplakin protein missing the C domain of the tail region. Histology of the skin revealed large intercellular spaces and clustering of desmosomes at the infrequent sites of keratinocyte adhesion. Immunohistochemistry of skin from the patients showed a perinuclear localization of keratin in suprabasal keratinocytes, suggesting a collapsed intermediate filament network. This study demonstrates the importance of desmoplakin in the attachment of intermediate filaments to the desmosome. In contrast to null *Desmoplakin* mice which die in early development, the truncated protein due to the homozygous 7901delG mutation in humans is not embryonic lethal. This suggests that the tail domain of desmoplakin is not required for establishing tissue architecture during development.

INTRODUCTION

Palmoplantar keratodermas (PPKs) are a diverse group of skin diseases characterized by hyperkeratosis of the palmoplantar epidermis (1). To date, the majority of PPKs have been attributed to mutations in structural proteins such as the keratins, desmosomal proteins and connexins. Keratins are the major intermediate filaments (IFs) of keratinocytes and are mutated in a spectrum of epidermal disorders (2). Desmosomes are cell–cell junctions found predominantly in the heart and skin. They link IF networks to the cell membrane and function in maintaining tissue architecture and integrity. Desmosomes consist of a number of membrane proteins, two of which, desmoglein 1 and desmoplakin, have been shown to underlie autosomal dominant non-syndromic striate PPK (3,4). Connexins are the major proteins of gap junctions which are involved in intercellular communication. To date, mutations in two connexins, Cx26 and Cx31, have been associated with epidermal disease (5,6). Interestingly autosomal dominant mutations in Cx26 underlie Vohwinkel's syndrome with the mutilating keratoderma associated with hearing loss. Many other forms of PPK occur in syndromes associated with cancer, neuropathy, hair abnormalities and heart failure.

As in epidermal disease, structural proteins have previously been implicated in dilated cardiomyopathy, with the involvement of dystrophin documented in X-linked dilated cardiomyopathy (7). The dystrophin protein is thought to bind actin filaments via an N-terminus binding domain. Mutations in α-cardiac actin itself have also been shown to cause dilated cardiomyopathy (8). Other structural proteins associated with dilated cardiomyopathies include laminin, metavinculin, α-dystroglycan, and α- and γ-sarcoglycan (9).

Three families from Ecuador were studied that showed evidence of autosomal recessive inheritance of a syndromic disease affecting the hair, skin and heart. The epidermal

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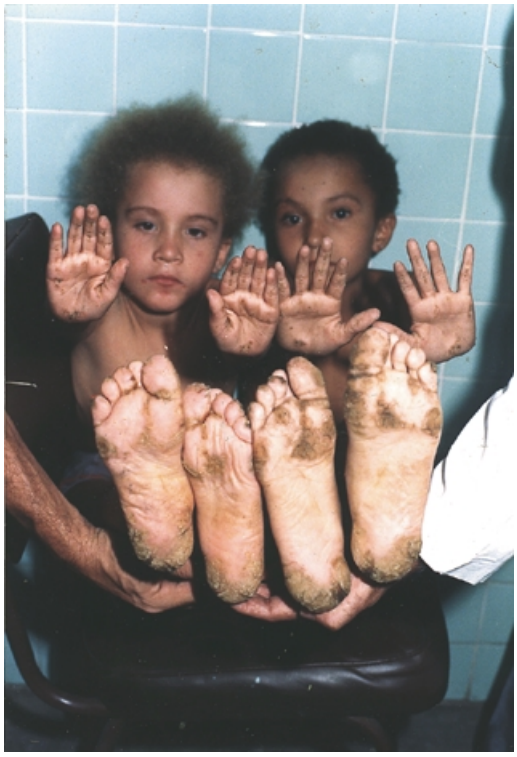

Figure 1. Clinical features showing the striate pattern of palmoplantar hyperkeratosis and woolly hair in two affected family members.

disease was a striate form of PPK and the heart disease was diagnosed as a left ventricle dilated cardiomyopathy. All affected individuals had clinical manifestations of all three disorders. A genome scan was performed on these three families, the affected members of which showed homozygosity at 6p23–p24 where desmoplakin is found. Mutation screening of desmoplakin revealed a single nucleotide deletion in the tail domain leading to a premature stop codon and truncation of the tail domain of the protein.

RESULTS

Clinical features

The skin disease presented as a PPK with some non-volar involvement particularly at sites of pressure or abrasion (10). All epidermal lesions had the characteristic longitudinal hyperkeratosis of a striate PPK (Fig. 1). The hair was curly and woolly in texture. Cardiological investigation including electrocardiographic and echocardiographic examinations of a number of the affected family members has been reported with the diagnosis of a left ventricular dilated cardiomyopathy often resulting in heart failure during adolescence (10). Dilated cardiomyopathy is characterized by cardiac enlargement and disrupted cardiac contraction.

Autozygosity mapping

Since there was evidence of consanguinity within two of the families and all three families were from the same region of Ecuador, we hypothesized that all affected family members were likely to have inherited the same ancestral disease allele on both maternal and paternal chromosomes. In addition, the syndrome is rare as only one other family with similar clinical manifestations has been reported in the literature (11). Towards the identification of the genetic basis of this syndrome, an autozygosity genome scan was performed using 386 microsatellite marker loci to genotype all potentially informative individuals from the three Ecuadorian pedigrees. Homozygosity in affected individuals was demonstrated with one marker, *F13A1*, which maps to the chromosomal region 6p23–p24. None of the unaffected family members were homozygous for the disease-associated *F13A1* allele segregating in each family. Interestingly, affected members from the two larger pedigrees (A and B) were homozygous for the same allele whereas the affected individual from the smaller pedigree was homozygous for a different allele. Homozygosity for all affected individuals was not seen for any other marker in the genome scan. To confirm linkage and to establish whether all three families had inherited the same ancestral disease haplotype, further markers from 6p23–p24 were genotyped across the pedigrees. In addition, microsatellites were identified from genomic sequence of a bacterial artificial chromosome (BAC) clone (GenBank accession no. AL031058) which harboured a potential candidate gene for this disorder, *Desmoplakin* (*DSP*). Microsatellite 1 (Des.mic.1) was found in intron 1 and microsatellite 3 (Des.mic.3) in intron 23 of the *DSP* gene. Further haplotype analysis with these additional microsatellite markers confirmed linkage and did indeed reveal a common disease haplotype segregating in all three families (Fig. 2).

Denaturing high performance liquid chromatography (DHPLC) and sequence analysis

Desmoplakin maps within the disease haplotype segregating in the three Ecuadorian families. Functionally, desmoplakin is a good candidate for the disease gene. Desmoplakin is the most abundant protein of desmosomes and is important in the attachment of the IF network to the plasma membrane and in intercellular adhesion. Though haploinsufficiency mutations in *DSP* do not result in cardiomyopathy or woolly hair, they are implicated in non-syndromic dominant striate PPK in which carriers present with a similar but less extensive pattern of keratoderma (4,12). Mutations in other desmosomal proteins have been reported to show similar epidermal phenotypes to that segregating in the Ecuadorian families. For example, a mutation in *Desmoglein 1* also results in autosomal dominant non-syndromic striate PPK (3) and studies of desmosomal junctions in hair suggest that defects in desmosomal proteins may produce a hairshaft dysplasia (13).

Genomic DNA from one affected, one unrelated and an individual heterozygous for the disease haplotype was used to PCR amplify each of the 24 exons of *DSP* using conditions previously described (12). PCR products were then subjected to heteroduplex formation and analysed using DHPLC. In all individuals genotyped as carriers, a heteroduplex was observed in the PCR product amplified using primer 24e (Fig. 3a). No further heteroduplexes were observed in carriers in the remainder of the gene. Sequence analysis of exon 24 revealed a deletion of a G nucleotide at position 7901 (7901delG with reference to GenBank accession no. M77830) (Fig. 3a) which

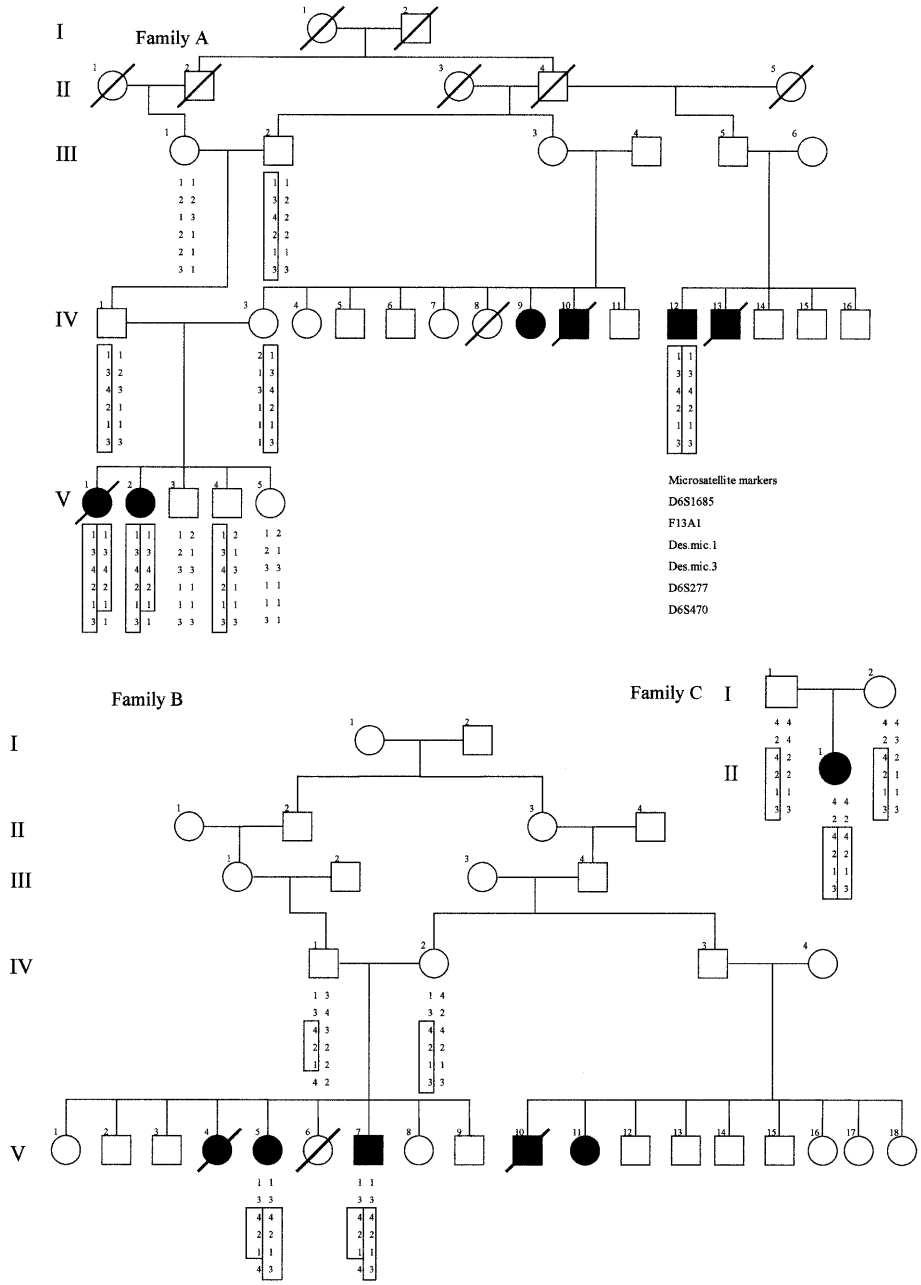

Figure 2. Pedigree structure and haplotype analysis of the three families investigated in this study. Filled symbols represent family members affected with the keratoderma, woolly hair and cardiomyopathy. A diagonal slash across the symbol indicates that the individual is deceased. Haplotypes were constructed with microsatellite markers mapping to 6p23–p24 and showed the co-segregation of a common disease haplotype (boxed) in all three families.

segregated with the disease in all three families with all affected family members homozygous for this sequence variant. This change was not seen in 100 unrelated normal controls.

Effect of the mutation on the desmoplakin protein

The mutation 7901delG, is predicted to cause a premature stop codon to be inserted 18 amino acids downstream of the deletion and would result in the truncation of the C domain in the tail region of the protein (Fig. 3b). This region of the desmoplakin protein has been shown to interact with IFs to anchor them to the desmosome (14,15). Western blot analysis using an anti-desmoplakin antibody confirmed the predicted truncation of the desmoplakin protein in cultured keratinocytes from one of the affected family members (Fig. 3d).

Histopathology investigation of epidermis

Histology of affected patient skin revealed large intercellular spaces between suprabasal keratinocytes supporting the finding of a cell adhesion defect underlying the disease

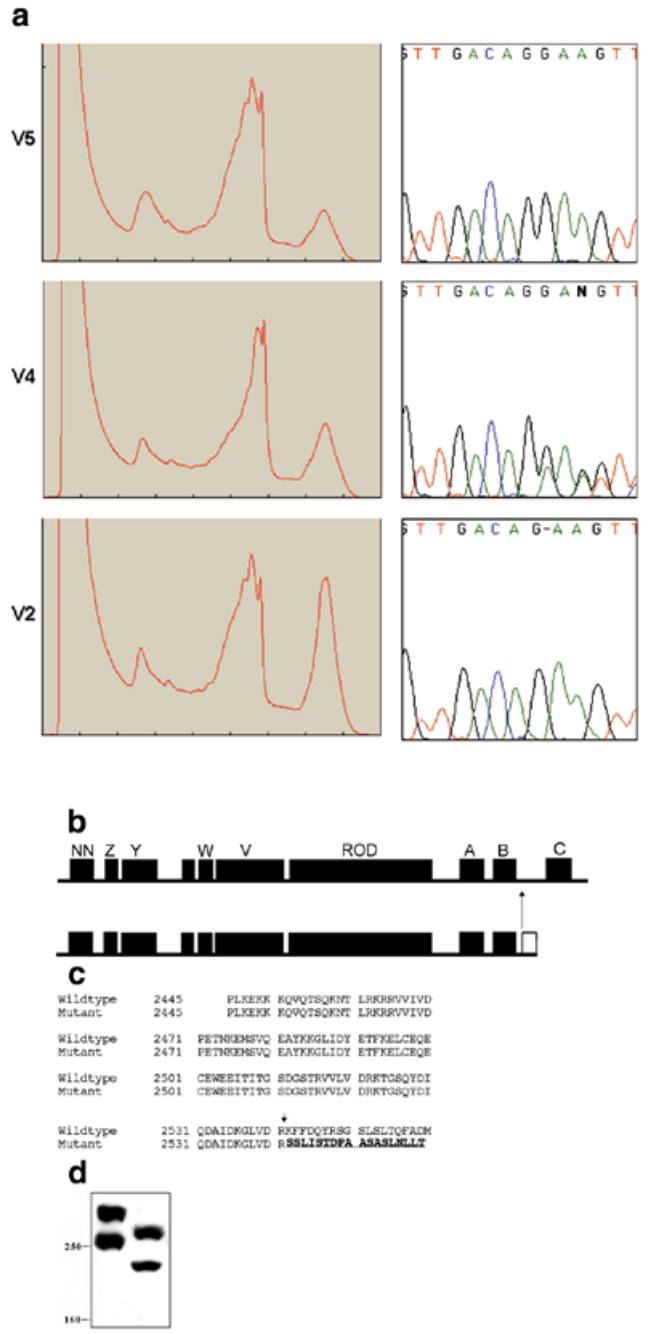

Figure 3. 7901delG mutation in desmoplakin. (**a**) Left panels show heteroduplex analysis. DNA heteroduplex formation of the exon 24e PCR-amplified DNA in a heterozygous gene carrier (family A: V4) compared with that of a homoduplex seen in an affected individual (family A: V2) and also a non-gene carrier (family A: V5). DNA sequence traces of exon 24e for the same three individuals to the right of heteroduplex traces. Individual V2 has a homozygous deletion of a guanosine residue at nucleotide position 7901, individual V4 is heterozygous for 7901delG and individual V5 is homozygous wild-type. (**b**) Schematic representation of the structure of the desmoplakin protein shows that 7901delG introduces a unique sequence of 18 amino acids (shown by unfilled box) and results in the truncation of the protein with loss of the C domain. (**c**) Amino acid sequence of part of exon 24 comparing mutant and wild-type alleles of *DSP* (introduced 18 amino acids downstream shown in bold text). (**d**) Western blot analysis of desmoplakin protein in cultured keratinocytes from an affected individual (right) compared with control keratinocytes (left). Note that *DSP* exists as two isoforms with the 7901delG mutation resulting in the truncation of both isoforms.

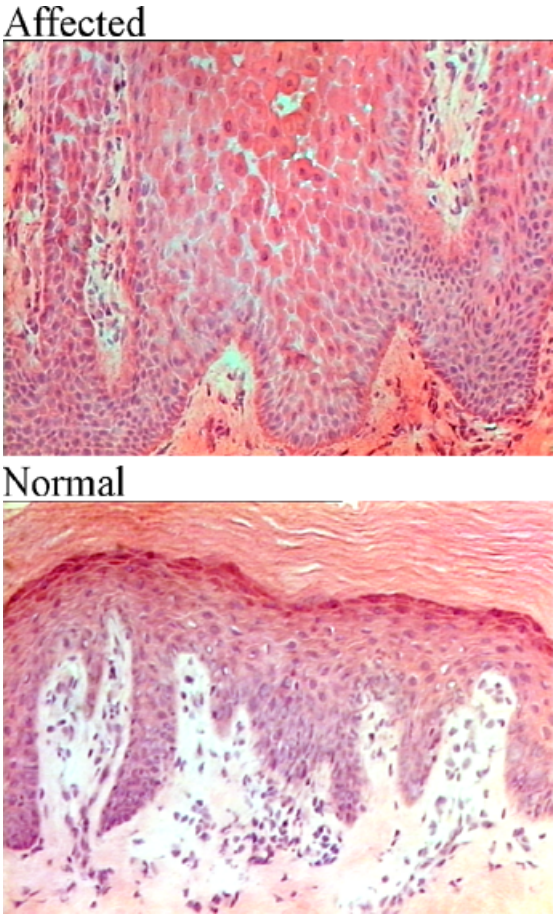

Figure 4. Histology of palmoplantar epidermis (top) from an affected individual showing large intercellular spaces between the suprabasal keratinocytes and (bottom) from a normal individual.

(Fig. 4). Basal cells, however, appeared normal. Electron microscopy of affected palm showed clumping of desmosomes at the infrequent sites of cell–cell adhesion (data not shown).

Immunohistochemistry on affected palm skin

Desmoplakin has an abnormal distribution in affected epidermis compared with that seen in a palm biopsy from an unaffected unrelated individual. In suprabasal keratinocytes, desmoplakin appeared mainly at points of cell–cell contact (Fig. 5a). Another desmosomal protein, plakoglobin, showed a similar redistribution (data not shown). The type II keratin, KRT1, a major IF protein of suprabasal keratinocytes, no longer showed a normal filament network, but had a membranous and perinuclear localization (Fig. 5d). Basal and lower suprabasal keratinocytes which do not show the large intercellular spaces have an apparently normal desmoplakin and keratin distribution.

DISCUSSION

Here we report the first recessive mutation in desmoplakin which causes a syndromic disease comprising of a keratoderma, hair abnormality and cardiomyopathy. Desmoplakin is

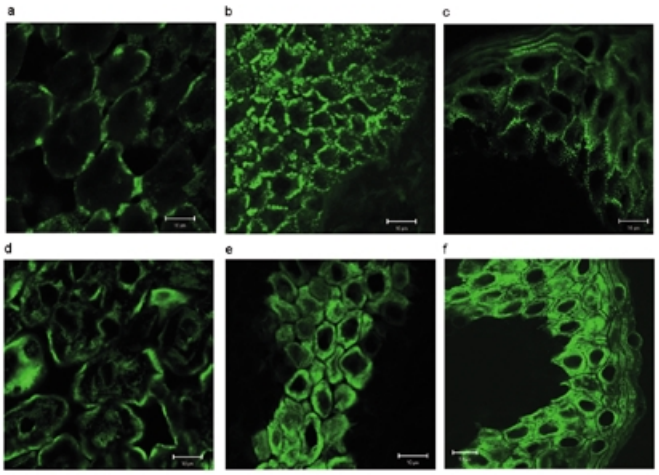

Figure 5. Immunofluorescence confocal microscopy analysis of palmoplantar epidermis. Desmoplakin labelling (11-5F) in (**c**) epidermis from an unaffected individual and (**a**) suprabasal and (**b**) basal and lower suprabasal epidermal layers from an affected individual shows the characteristic membraneous staining. Note that in (a) desmoplakin immunofluorescence is more punctate, being present only at the more infrequent points of cell–cell contact. Immunostaining for keratin 1 (LHK1) in (**f**) epidermis from an unaffected individual and (**e**) the basal and lower suprabasal layers of skin from an affected individual shows the characteristic diffuse cytoplasmic staining corresponding to the keratin IF network. In contrast, immunostaining for keratin 1 in suprabasal layers of skin from (**d**) an affected individual reveals condensed punctate intracytoplasmic staining. Bar, 10 μm.

the most abundant of the desmosomal proteins and is characteristic of all desmosomes which are major cell–cell junctions particularly abundant in the epidermis and in heart muscle cells (16,17). This study supports a key role for desmoplakin in desmosomal cell adhesion and for stabilization of desmosome–IF interactions. The recessive *DSP* mutation described here results in a truncated desmoplakin protein missing the C domain of the tail region. *In vitro* experiments have shown that the tail domain of desmoplakin is responsible for the binding of IFs specifically to the head domain (14,15). In contrast, the head domain of desmoplakin is hypothesized to act as a point of anchorage to the desmosome via interactions with desmocollin 1 and other desmoplakin molecules (16,18). Alternate splicing of the protein produces two isoforms, DPI and DPII, which differ in the length of the rod domain (19) so the mutation 7901delG will affect both isoforms (Fig. 3d).

From gene targeting studies in mice, desmoplakin has been shown to be important in desmosome stabilization for establishing ordered tissue development as homozygous *Dsp* null mice die in early embryogenesis (17). In contrast to mice, this recessive human mutation in desmoplakin is not embryonic lethal, which may reflect differences in mouse and human development or more likely that the truncated desmoplakin molecule has some residual desmosomal function. Clues as to the mechanism of the mutation can be found in the study of the epidermal phenotype. In affected individuals, disease is restricted to areas subjected to stress, such as the suprabasal palmoplantar epidermis and other epidermal sites prone to pressure or abrasion. This suggests that 7901delG/7901delG cells are still able to maintain the IF network without the desmoplakin tail domain unless they are stressed. This is illustrated by the normal histology of the basal cells which are less stressed than the suprabasal cells in affected individuals. In addition, unlike individuals harbouring haploinsufficiency *DSP* mutations associated with non-syndromic striate PPK (4,12), heterozygotes for 7091delG display no evidence of keratoderma. This could lead us to speculate that either desmoplakin–IF binding affinity is reduced but not lost, or that loss of desmosome–IF binding via desmoplakin may be compensated for by other desmosomal proteins. *In vitro* studies have shown that plakoglobin and plakophilin 1 are able to bind to IFs but with a lower affinity than desmoplakin (16).

As truncation of the desmoplakin protein appears to impair binding of keratins to the desmosome in the epidermis under stressed conditions, it is likely that the same mechanism causes disruption of hard keratins in the hair and desmin filaments in the heart muscle. Mutations in IF proteins found in the skin and heart show similar phenotypes, for example, keratin 9 mutations in an epidermolytic form of autosomal dominant diffuse PPK (20) and desmin mutations in autosomal dominant dilated cardiomyopathy (21,22). In normal cardiac tissue, actin filaments are held together by Z bands which help in transmitting tension along the myofibrils. Desmin is localized around these Z bands to provide support during muscle contraction (23). We hypothesize that desmin filament networks could be disrupted as a result of 7901delG, leading to fragile myofibrils and impaired cardiac contraction.

During the course of this study, a mutation in another desmosomal component, plakoglobin, has been shown to cause autosomal recessive diffuse PPK with arrhythmogenic right ventricular cardiomyopathy and woolly hair (Naxos disease) (24). The mutation results in a truncation of the plakoglobin protein and may affect stable association with desmosomal cadherins. It is not clear why desmoplakin and plakoglobin mutations affect just the skin, heart and hair as they are expressed in other tissue types or why these two desmosomal syndromes result in clinically distinct keratodermas and heart disorders. These data underlie the importance of cellular junctions in maintaining tissue integrity and provide further evidence for the role of structural proteins in the aetiology of genetic skin diseases and cardiomyopathies.

MATERIALS AND METHODS

Human material

With informed consent, venous blood samples from patients and their families were collected in 0.25 M EDTA and genomic DNA was extracted using a Nucleon BACC3 kit (Nucleon Biosciences, Coatbridge, UK). Genomic DNA extracted from 100 unrelated individuals was used as a control population. Skin biopsy material was obtained and processed using standard procedures from one affected family member.

Autozygosity mapping and haplotyping

A 10 cM genome scan was carried out using Research Genetics Map Pairs Weber Screening Set version 9 (Research Genetics, Huntsville, AL). One primer for each microsatellite pair was tagged with M13 DNA sequence. PCR was performed in a total reaction volume of 20 μl using standard cycling conditions. Microsatellite profiles were visualized and analysed using a Li-Cor 4200

GeneReadIR automated sequencer (MWG, Milton Keynes, UK). To confirm linkage, additional microsatellite markers spanning the area were identified via the National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). Two microsatellites were also identified from the sequence of a BAC clone mapping to 6p23–p24 (GenBank accession no. AL031058). The primer sequences for Des.mic 1 and 3 are as follows: Des.mic.1: forward, 5′-CCCATCTATGCATAATGCAACC-3′, reverse, 5′-GTCCTCACGGTAGTGCTACAAG-3′; Des.mic.3: forward, 5′-CGCTTTTGATCATGGCCCTAGTG-3′, reverse, 5′-CTCACCTGTTACAGCTAGATG-3′.

Heteroduplex formation, DHPLC and sequence analysis

Each exon of the human desmoplakin gene was amplified by PCR as described previously (12). Heteroduplex formation was carried out by heating the PCR products for 5 min at 94°C, followed by cooling to 40°C at a rate of 0.03°C/s. DHPLC was performed using a Transgenomic WAVE DNA Fragment analysis system (Transgenomic, Crewe, UK). Following DHPLC, each variant PCR product was purified using a PCR purification column (Qiagen, Crawley, UK), directly sequenced by PCR cycle sequencing using Big-Dye terminator chemistry and analysed on an ABI/PE Biosystems 377 automated sequencer (ABI/PE, Warrington, UK).

Immunohistochemistry

We performed immunohistochemistry on 4 mm sections of snap frozen palm skin biopsies. The primary antibodies used were desmoplakin (11-5F, 1:50) (25), plakoglobin (PG11E4, 1:100; Zymed, San Francisco, CA) and keratin 1 (LHK1), followed by FITC-conjugated rabbit anti-mouse (Dako, Cambridge, UK) secondary antibody. The sections were viewed under a confocal microscope.

Western blot

Keratinocytes from patient and normal skin were cultured until confluency. Cells were scraped off into 1× SDS sample buffer then boiled for 3 min and sonicated three times for 10 s each. The proteins were separated using 5% SDS–PAGE then blotted onto Hybond-P PVDF membrane (Amersham Pharmacia Biotech, St Albans, UK). The primary antibody used was 11-5F at a dilution of 1:500 followed by horseradish peroxidase-conjugated rabbit anti-mouse (Dako). This was visualized using ECL-plus kit (Amersham Pharmacia Biotech).

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