Short Report

Novel homozygous mutation in \textit{DSP} causing skin fragility–woolly hair syndrome: report of a large family and review of the desmoplakin-related phenotypes


Desmoplakin is an important cytoskeletal linker for the function of the desmosomes. Linking desmoplakin to certain types of cardiocutaneous syndromes has been a hot topic recently. Skin fragility–woolly hair syndrome is a rare autosomal recessive disorder involving the desmosomes and is caused by mutation in the desmoplakin gene (\textit{DSP}). We report five members from a large family with skin fragility–woolly hair syndrome. The index is a 14-year-old girl with palmoplantar keratoderma, woolly hair, variable alopecia, dystrophic nails, and excessive blistering to trivial mechanical trauma. No cardiac symptoms were reported. Although formal cardiac examination was not feasible, the echocardiographic evaluation of the other two affected younger siblings was normal. Homozygosity mapping and linkage analysis revealed a high LOD score region in the short arm of chromosome 6 that harbors the \textit{DSP}. Full sequencing of the \textit{DSP} showed a novel homozygous c.7097 G>A (p.R2366H) mutation in all affected members, and the parents were heterozygous. This is the report of the third case/family of the skin fragility–woolly hair syndrome in the literature. We also present a clinical and molecular review of various desmoplakin-related phenotypes, with emphasis on onset of cardiomyopathy. The complexity of the desmoplakin and its variable presentations warrant introducing the term ‘desmoplakinopathies’ to describe all the phenotypes related to defects in the desmoplakin.

Conflict of interest

None to declare.
Desmoplakin is a member of the plakin family of cytoskeletal linkers. Along with other proteins, plakins are crucial for the function of the desmosomes (1, 2). Desmosomes have dual roles in mediating adhesion between cells and in linking the intermediate filaments (IFs) of one cell to those of its neighbor, thereby establishing an integrated scaffold across the entire epithelium (3). The desmosomes are found predominantly in the epidermis and heart (1, 3). Desmoplakin links the transmembrane cadherins via plakoglobin to the cytoplasmic IF network (4, 5). It forms a dumbbell-shaped homodimer with a central coiled oligomerization rod domain (amino acids: 1057-1945) flanked by two globular domains, the N-terminal desmosomal-binding domain (amino acids: 1-1056), and the C-terminal IF-binding domain (amino acids: 1946-2871). The C-terminal domain (C-tail) is composed of three plakin repeat subdomains (A, B, C), as well as a glycine-serine-arginine-rich domain thought to regulate desmoplakin binding to IFs (2, 3, 6). In gene targeting studies in mice, desmoplakin was shown to be crucial not only in anchoring IFs to desmosomes, but also in desmosome assembly and/or stabilization (7). There are two isoforms of the desmoplakin protein as a result of alternative splicing, DP I (322 kDa) and DP II (259 kDa) with DP II lacking approximately two-thirds of the central rod domain. Both are encoded by the DSP gene on 6p24.3, which consists of 24 exons and spans 45 kDa of genomic DNA (3, 8–10). Both isoforms of desmoplakin are widely expressed in numerous tissues, and desmoplakin II expression is lower in simple epithelia (8, 11). The complexity of the desmosomal structure and the large number of proteins involved partly explain the genetic and clinical heterogeneity of desmosomal disorders. As the most abundantly expressed component of the desmosome (8), desmoplakin is a great example of clinical heterogeneity related to a single gene defect. Herein, we report using linkage analysis a Saudi family with skin fragility–woolly hair syndrome due to a novel mutation in the DSP gene.

Materials and methods

Human subjects

A total of five patients from two related families with the dermatological disease were included. The parents of the two families consist of two brothers married to two sisters. The parents are healthy cousins from southern Saudi Arabia. The study was approved by the Research Advisory Council at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia (RAC # 2040042). Written informed consent was given by the parents for the project and photography.

DNA isolation, genotyping and DSP sequencing

DNA was extracted from whole blood using the Gentra DNA Extraction Kit (Valencia, CA) following the manufacturer’s instructions. DNA samples were processed on the Affymetrix Gene Chip Human Mapping 250K array (Santa Clara, CA) following the manufacturer’s instructions. Data generated by the 250K Affymetrix SNP chip were used for linkage analysis and homozygosity mapping using EasyLinkage (v5.08) and Genotyping Console (v3.0.2) software, respectively. Three independent runs were performed using EasyLinkage that varied in the marker set while maintaining the same marker distance and minor allele frequency (0.2cM and 0.0001, respectively) as well as the fully penetrant autosomal recessive model. After DNA extraction from blood, the exons and exon/intron boundaries of the DSP were amplified by polymerase chain reaction and sequenced.

Results

Clinical evaluations

The index patient is a 14-year-old Saudi girl who was seen at King Faisal Specialist Hospital and Research Centre for evaluation regarding unknown form of skin disease. The antenatal history was unremarkable. Shortly after birth, the patient was noted to have pustules on the face, hands and hips. At that time, septic workup was negative. She underwent dermatological evaluation (Fig. 1a–d) in 1998 and the impression was that she might have some form of ectodermal dysplasia vs pachyonychia congenita. Notably, the family reported easy blistering of the skin with minimal trauma. In follow-up at the age of 10 years, she was found to have lusterless scanty curly hair, impetigo of the right nasal area and crusted erosion on the back and buttocks, and thickened nails with subungual hyperkeratosis with wedging. Additionally, she had palmoplantar keratoderma (PPK) with fissuring and superficial scars on the legs. The teeth and oral cavity were normal, and there was normal sweating. The growth parameter showed height on the 3rd percentile and the weight below the 3rd percentile, and the head circumference was just below the 3rd percentile.

A 3-mm punch skin biopsy was performed in the initial phase (1998) from a blister. There was an intra-epidermal blister with variably necrotic roof
Fig. 1. Clinical appearance of skin fragility—woolly hair appearance of two patients as well as the skin biopsy are shown. Note the severe involvement of the skin in the proband (at the age of 2 years) with keratoderma and hyperkeratosis of the palms and soles (a, b), nail dystrophy, erosions, and ruptured blister (c, d). Woolly and scant hair as well as mild perioral erosions in patient 2 (at the age of 5 years) are also shown (e, f). Skin biopsy findings (proband) depict in low magnification A where blistering is noted in the higher stratum spinosum. In frame B the edge of the blister highlights the spongiotic nature and level of the separation. Spongiosis is more prominent further away from the main vesicle C where a small vesicle is noted.
and moderate spongiosis in the adjacent epidermis (Fig. 1). Acantholysis was present and dyskeratosis was absent. Microscopic intra-epidermal vesicles were also noted in the areas of spongiosis. Perivascular inflammation was minimal, limited to the papillary dermis and mixed, including rare neutrophils and eosinophils. The histology, although peculiar, did not suggest any definitive condition. Skin immunofluorescence using specific anti-desmoplakin antibodies was not available. Laboratory investigations showed normal complete blood count, normal renal and liver function. In addition, selenium and zinc levels were normal. Thyroid function and quantitative serum amino acid concentrations were also unremarkable. The patient was treated with 20% urea cream on palms, soles and nails. No cardiac symptoms were reported and the cardiac auscultation was normal. However, no electrocardiography (ECG) or echocardiography was performed due to refusal by the patient. There was no family history of sudden death and the family pedigree is given in Fig. 2a. The clinical findings in the other affected patients in the two families are summarized in Table 1.

### Genotyping and DSP sequencing

Multipoint parametric linkage analysis assuming a recessive model of inheritance and complete penetrance revealed linkage on chromosome 6. A maximum LOD score of 4.7 was achieved between regions 6p25.1-p24.1 (Fig. 2b). Desmoplakin gene, a cause of skin fragility–woolly hair syndrome, lies in this region, and thus appeared to be a strong candidate gene. Direct sequencing of the DSP gene revealed novel homozygous mutation namely c.7097 G>A (p.R2366H) (Fig. 2c). This homozygous mutation was found in all the affected members and was absent in 400 chromosomes from 200 unrelated healthy control individuals of the same ethnic origin. The parents were confirmed carriers of the mutation.

### Discussion

As a large linker molecule in various tissues, it is not surprising that there are a number of human genetic disorders associated with desmoplakin mutations, with varying degrees of severity (8). Table 2 summarizes the different clinical presentations of the defect within the DSP. Clearly, the phenotypes involve primarily the skin and/or heart with some overlapping of the clinical features. The autosomal dominant phenotypes are arrhythmogenic right ventricular dysplasia, and keratosis palmoplantaris striata II; while skin fragility–woolly hair syndrome, dilated cardiomyopathy with woolly hair and keratoderma (Carvajal-Huerta syndrome), and lethal acantholytic epidermolysis bullosa are autosomal recessive. The first disease to be attributed to desmoplakin gene defect is striate keratosis palmoplantaris striata II (12), while the first recessive human desmoplakin gene disease to be described is the dilated cardiomyopathy with woolly hair and keratoderma caused by the 7901delG mutation (13).

The clinical presentation in our patients with the involvement of the skin, nails and hair and the family pedigree suggest an autosomal recessive condition like the ectodermal dysplasia with skin fragility, and skin fragility–woolly hair syndrome caused by mutations in the plakophilin 1 gene (PKP1) and the DSP, respectively. Moreover, homozygous mutations in another desmosomal component, plakoglobin, cause PPK, woolly hair and dilated cardiomyopathy (Naxos disease). The two families in this report with five members affected were large enough to perform linkage analysis and obviate the need to do candidate gene approach. The c.7097 G>A (p.R2366H) mutation found in exon 24 affects the C-terminal domain of desmoplakin. Using the PolyPhen algorithm, it is predicted to be possibly damaging (24). All reported mutations in the DSP are illustrated according to the phenotype and localization of the mutation within the desmoplakin protein in Fig. 3. In addition to this report, only two cases/families with skin fragility–woolly hair syndrome were reported. Whittock et al. (18) reported two cases with compound heterozygosity in the DSP causing the skin fragility–woolly hair syndrome. In the first case, both N287K and C809X mutations were in the N-terminal domain. While in the second one, the mutation Q664X involved the N-terminal domain, and the mutation R2366C was in the C-terminal domain (18). It appears that the first indication of the disease is the skin blistering, which usually starts immediately after birth. In this syndrome, heterozygous carriers of C809X, N287K, Q664X, or R2366C mutations displayed no phenotypic abnormalities. Similarly, the heterozygous carriers of the R2366H in our families have no clinical manifestations. Whittock et al. (18) concluded that desmoplakin haploinsufficiency can be tolerated in some cases, but that in combination with a missense mutation on the other allele, the consequences are a severe genodermatosis with specific clinical manifestations (18). The C-terminal tail is essential in the function of desmoplakin as it interacts with the...
Fig. 2. Family pedigree, linkage analysis, and the sequence chromatogram. The pedigree (a) above shows two consanguineous branches of the large family. Squares and circles denote males and females, respectively. Linkage analysis (b) is shown confirming a peak of high LOD score (4.7) flanking the region 6p25.1-p24.1. The sequence chromatogram (c) shows the c.7097 G>A (p.R2366H) missense mutation in the affected members. Normal and carrier sequencing data are shown for comparison.
Novel homozygous mutation in DSP

Table 1. Clinical characteristics of all the affected members in the two families

<table>
<thead>
<tr>
<th>Family 1</th>
<th>Family 2</th>
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<tbody>
<tr>
<td></td>
<td>Patient 1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>14</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
</tr>
<tr>
<td>Skin fragility</td>
<td>++</td>
</tr>
<tr>
<td>Erosions and blisters</td>
<td>++</td>
</tr>
<tr>
<td>Perioral erosion/crusting</td>
<td>+</td>
</tr>
<tr>
<td>Woolly hair</td>
<td>+</td>
</tr>
<tr>
<td>PPK</td>
<td>++</td>
</tr>
<tr>
<td>Nail wedging</td>
<td>+</td>
</tr>
<tr>
<td>Teeth</td>
<td>Normal</td>
</tr>
<tr>
<td>Perspiration</td>
<td>Normal</td>
</tr>
<tr>
<td>Heart</td>
<td>No cardiac symptoms</td>
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PPK, palmoplantar keratosis.
aImprovement by the age of 5 years.
bCould not perform echocardiogram due to refusal by the patient/family.

IF cytoskeleton. There is evidence from previous studies of a genotype–phenotype correlation in terms of cardiac involvement, as truncating mutations as well as homozygous missense mutations involving the C-terminal domain predispose to cardiomyopathy (6, 20). Although the desmoplakin-related phenotype in our family is clinically going along the skin fragility–woolly hair syndrome, the diagnosis is not beyond doubt for two reasons. First, despite numerous attempts to obtain full cardiological evaluation in our family, we could not perform echocardiography except only on two youngsters (patients 2 and 3). Second, patients with the recessive missense mutation in the desmoplakin C-terminus (p.Gly2375Arg), which is located only nine amino acids C-terminally of the...
Table 2. Clinical and molecular characteristics of different syndromes caused by defects in the desmoplakin gene, and brief comments about similar dermatological syndromes caused by other genes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Inheritance (OMIM #)</th>
<th>Clinical features</th>
<th>Mutations/reference</th>
<th>Comments/other genes</th>
</tr>
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<tbody>
<tr>
<td>ARVD8</td>
<td>AD (607450)</td>
<td>Characterized by non-ischemic ventricular arrhythmias of RV origin. The diagnosis relies on EKG and angiographic criteria, and pathologic findings of fibrofatty replacement of ventricular myocardium. Majority of cases involve the right ventricle. Single report in which there is predominantly left ventricular involvement.</td>
<td>RV: V30M (14), Q90R (14), W233X (14), S299R (14), R1255K (15), R1775I (15), R2339Q (16), R2834H (14), 423-1 G&gt;A (15)</td>
<td>ARVD is genetically heterogeneous with several loci that have been mapped. It has an overall prevalence of 1:1000. Penetrance for ARVD8 is ~50%</td>
</tr>
<tr>
<td>Skin fragility–woolly hair syndrome</td>
<td>AR (607655)</td>
<td>Diffuse PPK, woolly hair, variable alopecia, and no cardiac anomalies. Only two cases reported. The family in this report is the third.</td>
<td>CH: N287K/C809X (18), Q664X/R2366C (18) Homozygous: R2366H (this report)</td>
<td>Ectodermal dysplasia and skin fragility syndrome (OMIM 604536) is an AR disorder caused by mutations in the plakophilin 1 gene (PKP1)</td>
</tr>
<tr>
<td>Cardiomyopathy, dilated, with woolly hair and keratoderma (Carvajal-Huerta syndrome)</td>
<td>AR (605676)</td>
<td>Total of five patients/families reported with onset of the dermatological findings usually at birth or in the first year of life. Cardiomyopathy has variable onset and severity, and may cause sudden death.</td>
<td>Homozygous: R1267X (19), G2375R (20), 7901delG (13) Homozygous: 2516del4/3971del4 (21) CH:2516del4/3971del4 (21)</td>
<td>Mutation in the plakoglobin gene causes Naxos disease (OMIM 601214), an AR condition characterized by woolly hair, cardiomyopathy, and a clinically distinct keratoderma.</td>
</tr>
<tr>
<td>Keratosis palmoplantaris striata II (PPKS2)</td>
<td>AD (612908)</td>
<td>Rare form of PPK characterized by linear bands of skin thickening on the palms and flexor aspects of the fingers, and circumscribed areas of thickening on the soles. Cardiomyopathy was reported in a patient with I608ins30bp (Q331X) (12), (939 + 1G&gt;A (2), l608ins30bp (22)</td>
<td>Homozygous: R1934X/6370delTT (4) Homozygous: 2874delTT (23)</td>
<td>PPKS1 (OMIM 148700) and PPKS3 (OMIM 607654) are caused by mutations in the DSG1 and KRT1 genes, respectively.</td>
</tr>
<tr>
<td>Epidermolysis bullosa, lethal acantholytic</td>
<td>AR (600638)</td>
<td>Three newborns with a lethal and rapidly progressive generalized epidermolysis, and universal alopecia. One newborn with cardiomyopathy (2874delT)</td>
<td>CH: R1934X/6370delTT (4) Homozygous: 2874delT (23)</td>
<td>These mutations lead to absence of the complete C-terminus and loss of desmoplakin–IF interaction.</td>
</tr>
<tr>
<td>Cardiomyopathy, dilated, with woolly hair, PPK, extensive skin blistering and enamel abnormalities</td>
<td>AR (No MIM #)</td>
<td>One case was reported. In addition to the phenotype, there was extensive mucocutaneous blisters, nail dystrophy, and enamel dysplasia.</td>
<td>6310delA/A2655D (6)</td>
<td>Widespread left and right ventricular involvement with severe myocardial abnormalities.</td>
</tr>
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</table>

ARVD8, arrhythmogenic right ventricular dysplasia, familial, 8; EKG, electrocardiogram; IF, intermediate filament; PPK, palmoplantar keratoderma.
mutation in our patients, did show a cardiomyopathy/sudden death between the age of 15 and 30 years (20).

Dilated cardiomyopathy with woolly hair and keratoderma (Carvajal-Huerta syndrome) has been reported in five cases/families. The small number of cases reported with DSP mutations precludes us from understanding the phenotypic spectrum of the skin and heart involvement in skin fragility–woolly hair syndrome, and Carvajal-Huerta syndrome. Reviewing all the dilated cardiomyopathy with woolly hair and keratoderma cases with regards to the onset of cardiac signs is crucial in assessing whether some patients with classic skin fragility–woolly hair syndrome may go on to develop cardiomyopathy later in life. In the family reported by Carvajal-Huerta (25), the onset of abnormal ECG ranged between 8 and 34 years, with the majority before 17 years of age. Several individuals died before the age of 17 years (25). Alcalai et al. (20) reported an Arab family with dilated cardiomyopathy with woolly hair and keratoderma affecting a 16-year-old girl. The onset of cardiomyopathy was not clear, but eight members died suddenly between the ages of 15 and 30 years on whom no cardiac evaluation was performed. Uzumcu et al. (19) reported a child with the same syndrome who had severe cardiac phenotype with early onset (before 3 years of age) and rapid progression that resulted in patient death. The patient reported by Tanaka et al. (21) developed the cardiomyopathy within the first year of life and it was progressive in nature. Finally, Mahoney et al. (6) reported a case with similar phenotype in a child who died at the age of 14 years from undiagnosed cardiomyopathy. It is apparent from these reports that the onset of cardiomyopathy was variable and usually in the
first two decades of life. In addition, there was always the risk of sudden death from the cardiac involvement. Cardiomyopathy was also reported in other desmoplakin phenotypes like keratosis palmoplantaris striata II and lethal acantholytic epidermolysis bullosa (22, 23). These observations confirm that the desmoplakin-related phenotypic spectrum is probably to expand (and hopefully better understood) as more cases are reported.

In conclusion, the clinical evaluation of such cases always presents a diagnostic dilemma because of the overlapping phenotypes with other genes like the plakoglobin and plakophilin 1. The luxury of having a large family with five affected members has allowed the use of linkage analysis as a diagnostic tool. Other diagnostic approaches would include the use of skin immunohistochemistry or direct sequencing of the candidate genes.

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References