ELECTRONIC LETTER

Loss of desmoplakin isoform I causes early onset cardiomyopathy and heart failure in a Naxos-like syndrome

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Background: Desmosomes are cellular junctions important for intercellular adhesion and anchoring the intermediate filament (IF) cytoskeleton to the cell membrane. Desmoplakin (DSP) is the most abundant desmosomal protein with 2 isoforms produced by alternative splicing.

Methods: We describe a patient with a recessively inherited arrhythmogenic dilated cardiomyopathy with left and right ventricular involvement, epidermolytic palmoplantar keratoderma, and woolly hair. The patient showed a severe heart phenotype with an early onset and rapid progression to heart failure at 4 years of age.

Results: A homozygous nonsense mutation, R1267X, was found in exon 23 of the desmoplakin gene, which results in an isoform specific truncation of the larger DSPI isoform. The loss of most of the DSPI specific rod domain and C-terminal area was confirmed by Western blotting and immunofluorescence. We further showed that the truncated DSPI transcript is unstable, leading to a loss of DSPI. DSPI is reported to be an obligate constituent of desmosomes and the only isoform present in cardiac tissue. To address this, we reviewed the expression of DSP isoforms in the heart. Our data suggest that DSPI is the major cardiac isoform but we also show that specific compartments of the heart have detectable DSPII expression.

Conclusions: This is the first description of a phenotype caused by a mutation affecting only one DSP isoform. Our findings emphasise the importance of desmoplakin and desmosomes in epidermal and cardiac function and additionally highlight the possibility that the different isoforms of desmoplakin may have distinct functional properties within the desmosome.

Desmosomes are major cell adhesion junctions, particularly abundant in tissues undergoing constant physical stress, such as the epidermis and the heart. The desmosome appears in electron micrographs as a symmetrical structure between two cells, and consists of electron dense plaques on the cytoplasmic surface of the cell membrane with a less dense central portion spanning the extracellular space. The desmosomal junction comprises numerous proteins from at least three different families: the cadherins, the armadillo proteins, and the plakins.¹ Desmoplakin (DSP) is the most abundant of these, and is present in all desmosomal junctions. Important protein-protein interactions with plakoglobin, plakophilins, and filaments composed of cytokeratin, vimentin, or desmin are essential for the stability and integrity of this junctional complex.²⁻³ DSP is a cytoplasmic plaque protein lacking transmembrane domains and exists as two isoforms, DSPI and DSPII, created by alternative splicing of the same transcript.⁴⁻⁵ These isoforms share common

C-terminal and N-terminal globular head and tail domains, but differ in the length of the rod domain that links them.⁶ It is thought that the rod domain in DSPI may be folded or coiled so that the distance between the head and tail domains *in vivo* is the same in both isoforms.² This may be important for dimer formation through coiled coil interactions in the rod domain and for head to head interactions.³⁻⁶ The assembly and organisation of DSPI and DSPII between the outer and inner dense plaque determines the width of the desmosome.²

Both isoforms are expressed in skin but only the larger isoform, DSPI is reported to be expressed in the heart.⁷ It is through the C-terminal tail of desmoplakin binding to the keratin intermediate filament (IF) cytoskeleton that the cell membrane is anchored, via an 18-amino acid sequence conserved in the head domain of type II epidermal keratins.⁸⁻¹⁰ The N-terminal head domain of desmoplakin plays an important role in organising the desmosomal complex by binding other plaque proteins, such as plakoglobin (γ -catenin) and plakophilin, and in recruiting other desmosomal components, such as the desmosomal cadherins via plakoglobin binding, into discrete clusters at the plasma membrane.¹⁰⁻¹¹ It may also play a role in segregating components of desmosomes and adherens junctions during their assembly.¹² The importance of desmoplakin in both IF attachment and desmosome assembly/stability is further supported by mouse knockouts, which are embryonic lethal.¹³

Loss of junctional integrity by mutations in desmosomal proteins causes skin, hair and heart defects in humans. A 2 bp deletion in the plakoglobin gene (*JUP*, 17q21), leading to a C-terminal truncation of plakoglobin, causes autosomal recessive Naxos disease, characterised by arrhythmogenic right ventricular cardiomyopathy (ARVC), mild epidermolytic palmoplantar keratoderma (EPPK) and woolly hair.¹⁴ Recessive mutations in the desmoplakin gene (*DSP*, 6p24) have been associated with Carvajal syndrome, which presents with dilated left ventricular cardiomyopathy, striate palmoplantar keratoderma (SPPK), and woolly hair. The desmoplakin mutation 7901delG, leading to loss of the keratin binding domain, has been found to be the underlying cause of this syndrome.¹⁵ Additional studies have shown that this mutation leads to poor cell adhesion.¹⁶ Subsequently, the desmoplakin mutation Gly2375Arg has been identified in a similar syndrome manifesting with ARVC, skin disorder, and woolly hair.¹⁷ Other mutations causing haploinsufficiency of desmoplakin have been reported to manifest as non-syndromic SPPK, and dominant mutations in desmoplakin and plakophilin-2 have been associated with non-syndromic ARVC.¹⁸⁻²¹

Abbreviations: ARVC, arrhythmogenic right ventricular cardiomyopathy; DP, desmoplakin; EPPK, mild epidermolytic palmoplantar keratoderma; IF, intermediate filament; PPK, palmoplantar keratoderma; SPPK, striate palmoplantar keratoderma

We now present for the first time the loss of the DSPI isoform resulting from a homozygous nonsense mutation in a patient with a novel Naxos-like syndrome. Expression analysis of DSP isoforms in the heart suggests that, though DSPI is the major cardiac isoform, specific compartments of the heart may express different combinations of isoforms with DSPII. Our findings highlight the possibility that the different isoforms of desmoplakin may have distinct properties within the desmosome and that DSPII may be unable to fully compensate for the loss of the DSPI protein.

MATERIALS AND METHODS

Subjects and medical evaluation

All four individuals from the consanguineous Turkish family were clinically evaluated at the Division of Medical Genetics of the Child Health Institute and the Department of Pediatric Cardiology, Istanbul Medical Faculty. The study was approved by the institutional review board of the Child Health Institute, and informed consent was obtained from each participant.

Haplotype analysis and mutation screening

Genomic DNA was isolated from peripheral blood collected in EDTA from patient and family members using the DNA isolation kit for mammalian blood (Roche, Istanbul, Turkey). Microsatellite markers for haplotyping were chosen from the Ensembl Genome Browser (<http://www.ensembl.org>). Markers were amplified and products run on 8% polyacrylamide gels. After silver staining, the genotypes were determined by two researchers. Exon–intron organisation of the *DSP* and *JUP* genes were analysed using the UCSC Blat Search (<http://www.genome.ucsc.edu/blat>) and intronic primer pairs were designed to amplify the coding regions of the genes. For haplotyping and mutation analysis genomic DNA was amplified by PCR using a thermal cycler (DNA Engine PTC-200 thermal cycler; MJ Research, Medtek, Turkey). Reaction mixtures (25 µl) contained 100 ng of genomic DNA, 2 mmol/l MgCl₂, 10 mmol/l Tris-HCl, 50 mmol/l KCl, 200 nmol/l of each primer, 0.2 mmol/l dNTPs, and 0.5 U of *Taq* DNA polymerase (MBI Fermentas, Elips, Turkey). For PCR, the reaction mixtures were initially denatured at 94°C for 4 minutes, and amplified for 30–34 cycles with denaturation at 94°C for 30 seconds, annealing between 64°C and 54°C for 30 seconds, extension at 72°C for 60 seconds, and a final extension step at 72°C for 4 minutes. Sequencing of PCR reactions in both directions was performed by Macrogen Inc., Seoul, Korea. Primer sequences and detailed amplification protocols for *DSP* and *JUP* genes are available upon request.

The presence of the *DSP* R1267X mutation and the *JUP* L697M polymorphism in the family was reconfirmed by additional, independent sequence analyses of all family members. For *DSP* R1267X, we established a PCR enzyme digestion test. A 1348 bp fragment of exon 23 was amplified (primers DSP23F1 5'-AAGATGCACATTGGTCTGGG-3' and DSP23R1 5'-TGCTGTTTCCTCTGAGACACC-3') and digested with *Bts*I (New England Biolabs, Germany), which cuts the mutated allele at the site of the mutation, resulting in fragments of 813 bp and 535 bp in length.

Antibodies for immunofluorescence and Western blotting

Western blotting against desmoplakin was performed using the polyclonal AHP320 antibody raised against the pATH/TrpE fusion protein of human desmoplakin C-terminal domain (nucleotides 4247–5228; amino acids 1415–1743; Serotec, UK). Immunofluorescence was performed using either 11-5F (to localise DSPI and DSPII), or the DSPI specific antibody DP2.17 (labelled DSPI; Research Diagnostics, Inc.). The epitope for the 11-5F monoclonal antibody, which was

raised against bovine nasal epithelial desmoplakins I and II,²² has not been mapped, although it has previously been shown to recognise an epitope within the first 2541 amino acids of the full length desmoplakin protein (2871 amino acids).¹⁵ The DSPI specific antibody DP2.17 (labelled DSPI) reacts specifically with DSPI. Although the exact location of the epitope has not been mapped, it must fall within the DSPI specific region of the rod domain of the full length desmoplakin protein.

Immunofluorescence

Snap frozen skin biopsies of palm skin from patients and normal controls were cut into 5 µm sections and left to defrost and air dry. Sections were blocked for 15 minutes at room temperature using phosphate buffered saline (PBS) containing 3% normal rabbit serum, 0.1% Triton X, and then underwent 3×5 minute washes in PBS. Primary antibodies (desmoplakin protein monoclonal antibody 11-5F used at 1:50 dilution;²³ and desmoplakin I protein monoclonal antibody DP 2.17 used at 1:25 dilution; Research Diagnostics) were diluted in PBS, added to the sections and left to incubate at 37°C for 1 hour. The sections underwent 3×5 minute washes in PBS. A rabbit antimouse FITC secondary antibody was added and left to incubate in the dark at room temperature for 1 hour. Sections then underwent 3×5 minute washes in PBS before being counterstained with propidium iodide (1 µg/ml) for 30 seconds. After being washed as described above, the sections were mounted using Immu-Mount aqueous mounting medium (Thermo Shandon, Pittsburgh, USA) and visualised under UV light using Leica DC200 software (Leica Imaging systems Ltd, Cambridge, UK).

Western blotting

Snap frozen patient tissue biopsies were cut into 5 µm sections and the Cryo-M-Bed embedding compound (Bright Instrument Company Ltd, Huntingdon, UK) was scraped away from the tissue, then 2–4 tissue sections from each patient (with an approximate total surface area of 50 mm²) were scraped into 100 µl 2× Laemmli buffer (100 mmol/l Tris-HCl pH 6.8, 200 mmol/l dithiothreitol, 4% (w/v) sodium dodecyl sulphate (SDS), 0.2% (w/v) bromophenol blue, 20% (v/v) glycerol) then heated to 100°C for 3 minutes and centrifuged at 15 700 *g* for 5 minutes. Polyacrylamide gel electrophoresis was performed according to the standard methods: 5 µl of each sample was subjected to electrophoresis on an 8% SDS gel in 1× running buffer (0.025 mol/l Tris, 0.19 mol/l glycine, 0.1% (w/v) SDS). Separated proteins were then transferred to Hybond-C nitrocellulose membrane (Amersham Pharmacia Biotech) using 1× transfer buffer (0.025 mol/l Tris, 0.19 mol/l glycine). Membranes were blocked with PBS containing 10% (w/v) milk powder and 0.1% (v/v) Tween 20 for 1 hour prior to primary antibody incubation, which was carried out overnight at 4°C in PBS containing 5% (w/v) milk powder and 0.1% (v/v) Tween 20. Secondary antibodies were conjugated with horseradish peroxidase (Dako). Proteins were then visualised using ECL Plus kit (Amersham Pharmacia Biotech). Antibodies used for Western blotting were plakoglobin monoclonal antibody (Transduction Labs, UK) used at 1:1000 dilution with rabbit antimouse secondary antibody, and AHP320 desmoplakin polyclonal antibody (Serotec, UK) used at 1:200 dilution with goat antirabbit secondary antibody.

DSP isoform expression

Primers designed to specifically amplify DSPI cDNA or DSPII were used to perform PCR on a cardiovascular cDNA panel (BD Pharmingen, UK). The primers used are given in table 1.

Table 1 Primers used for PCR on cardiovascular cDNA panel

DSP isoform	Primer name	Primer sequence (5'-3')*	Size of PCR product (bp)	AT (°C)
DSPI (WT + R1267X)	DSP F1	GCAGATGTTTCATGCTCGGTA (3268-3287)	800	65
	DSP R1	GGCCTGCAAGATGCTGTCAT (4049-4068)		
DSPI (WT only)	DSP F1	GCAGATGTTTCATGCTCGGTA	930	65
	DSP R2	CATTGCTCAGAGCGCTGC (4179-4198)		
DSPI and DSPII	DSP F1	GCAGATGTTTCATGCTCGGTA	DSPI 2440; DSPII 644	60
	DSP R3	TGTACCACCTGAGTACACTG (5689-5706)		

*Position of primers in according to M77830 is given in parentheses. AT, annealing temperature; WT, wild type.

PCR was carried out using cDNA from the Clontech human cardiovascular multiple tissue cDNA panel (BD Biosciences) in a total volume of 25 µl containing 0.5 ng cDNA. Cycling conditions were 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, primer annealing for 30 seconds, and 72°C extension for 45 seconds, with a final extension of 72°C for 5 minutes. When primers F1 and R3 were used in combination, an extension time of 45 seconds allowed the amplification of isoform II but did not allow amplification of the 2440 bp isoform I fragment. PCR products were subjected to electrophoresis on 2% agarose with 0.1 ng/ml ethidium bromide gels, and visualised under UV light using Alpha-Imager software.

RESULTS

A patient with a novel Naxos-like syndrome

The 3½ year old patient was born to consanguineous parents and referred to our clinic because of chronic fatigue, cardiac arrhythmias, and a suggestive dilated cardiomyopathy. He was clinically evaluated by 12 lead ECG, 24 hour Holter ECGs, and echocardiography. The patient presented with left and right ventricular dilated cardiomyopathy and thinning of the ventricular walls (fig 1B). A progressive severe reduction of the left ventricular ejection fraction (<25%) was present, and a mitral valve regurgitation diagnosed. The 12-lead ECG patterns were in accordance with a severe cardiomyopathy and showed widening of the QRS complexes, right axis

Figure 1 Clinical findings of the cardiocutaneous syndrome. (A) The patient presented with typical woolly hair and epidermolytic palmoplantar keratoderma of the palms and soles. Written informed consent was given for the publication of these images. (B) Echocardiography showing a significant dilated right ventricular outflow tract and M-mode photograph showing left ventricular dilatation with depressed systolic function. (C) Representative 12 lead ECG, showing right axis deviation, sinus tachycardia, and ventricular ectopic beats.

deviation, and sinus tachycardia with ventricular ectopic beats (fig 1C). The Holter ECGs showed frequent runs of ventricular tachycardias with couplets and bigemini early ventricular beats. An arrhythmogenic dilated cardiomyopathy with involvement of the left and right ventricles was diagnosed, and the patient was put on anticongestive treatment and amiodarone. Additionally, he had woolly hair and palmoplantar keratoderma (PPK) (fig 1A); histological analysis of a skin biopsy indicated an epidermolytic type of PPK. The clinical phenotype of the disease resembled aspects of both Naxos disease and Carvajal syndrome, although the patient presented with an unusual severe cardiac phenotype with early onset (before 3 years of age) and rapid progression. The patient died from heart failure, and permission for a postmortem examination was denied.

The consanguineous parents and the patient's 6 year old brother did not show any cardiac or any hair or skin abnormalities. Therefore, the final diagnosis of an autosomal recessive cardiocutaneous syndrome was given characterised by arrhythmogenic left and right ventricular cardiomyopathy with EPPK, and woolly hair.

Molecular analysis of plakoglobin and desmoplakin

Two genes, those encoding plakoglobin and desmoplakin, represented excellent candidate genes for the Naxos-like syndrome in this patient. Haplotype analysis of polymorphic

markers located in the *DSP* and *JUP* gene regions on chromosome 6p24 and 17q21, respectively, showed homozygosity for one parental allele at both loci in the patient (fig 2A, B). Sequencing of the *DSP* gene identified a homozygous stop mutation R1267X (C3799T) in exon 23 (fig 2C, D). This mutation was not present in 100 ethnically matched control chromosomes. The parents were heterozygous for the mutation, as was the patient's healthy brother. Two different donor sites within exon 23 are used to create DSPI and DSPII isoforms, and the R1267X mutation is located in the DSPI specific part of the isoform (fig 3A), therefore, the 1604 amino acid truncation of the protein caused by the mutation would affect only the DSPI isoform.

Additionally, mutation analysis of the *JUP* gene using direct sequence analysis identified a non-synonymous polymorphism, L697M (T2089A), in exon 13 of the gene (fig 2E). The patient was homozygous for the A allele, his brother was homozygous for the T allele, and both parents were heterozygous. No further alteration in *JUP* was identified. The frequency of the T2089A polymorphism for different genotypes in the Turkish population was determined as: TT 0.57 (63/111), AT 0.36 (40/111), and AA 0.07 (8/111). L697M is located at the very end of the C-terminal part of the plakoglobin protein. This part of the C-terminus is variable and not conserved between species. T2089A is located at position +4 after the acceptor splice site in exon 13. Although

Figure 2 Molecular analyses of the Naxos-like patient. Haplotypes of polymorphic markers in the candidate regions on chromosomes 6p24 (*DSP*) (A) and 17q21 (*JUP*) (B). The allele carrying the *DSP* mutation and *JUP* SNP are coloured in red. (C) PCR/enzyme digestion analysis of the *DSP* C3799T mutation. Mutation carriers (father (F), mother (M), and brother (B)) are detected by the presence of an undigested wild type fragment (1348 bp) and two fragments of the digested mutant allele. The homozygous index patient (I) shows complete digestion. Sequence analyses of (D) the C3799T mutation in *DSP*, and (E) the T2089A polymorphism in *JUP*. Heterozygous and homozygous carriers are shown in comparison.

Figure 3 The R1267X mutation causes loss of DSPI isoform. (A) Usage of different donor splice sites in exon 23 generates the DSPI and DSPII isoforms. The R1267X mutation is located in the DSPI specific part of exon 23. (B) Western blot showing desmoplakin (DSP) and plakoglobin (Pg) expression in the Naxos-like patient (Pat) compared with normal skin (NS) and palmoplantar skin (PP). Only the smaller isoform of desmoplakin, DSPII, is present in our patient whereas both isoforms are expressed in NS and PP skin. (C–F) Immunofluorescence to confirm the absence of DSPI in the patient's skin. Antibody 11-5F stains both isoforms of desmoplakin and shows that at least one of the two isoforms is expressed in normal skin (C) and the skin of the patient (D) at the plasma membrane. The DSPI antibody however, is specific for the unique protein sequence of the rod domain of DSPI and shows that the epitope for this isoform is absent in the patient skin (F), but present in normal skin (E). (G) RT-PCR carried out on normal skin and skin of an R1267X heterozygote to ascertain whether the R1267X transcript is stable or degraded by nonsense mediated mRNA decay. F1 and R1 PCR primers were designed to specifically amplify the DSPI transcript. PCR products represent the total amount of present DSPI transcript (mutant and WT). Levels of DSPI (F1/R1) and GAPDH transcript were measured by densitometry. (H) Western blot of different heart areas showing a prominent signal for DSPI in all four heart chambers: left ventricle (LV), left atrium (LA), right ventricle (RV), right atrium (RA), and skin control (SC). DSPII signals are undetectable or barely detectable.

we were not able to see any visible splicing error in our initial RT-PCR analysis performed on mRNA of the heterozygous mother, we cannot exclude minute effects on splicing. Using a splice prediction programme, we showed that the value of the splice site was decreased for the rare A allele from 0.94 to 0.85 (splice site prediction by Neural Network).²⁴ As the patient presented with an extreme severe cardiac phenotype, it will be an interesting working hypothesis in the future that homozygosity for this rare A allele may have a negative modifier effect on the cardiac phenotype due to changes in the efficiency of the acceptor splice site.

Immunofluorescence performed on patient skin

Immunofluorescence was performed on skin from the patient and compared with that of a normal site matched control. Comparison was also made to a skin biopsy taken from a patient with SPPK, woolly hair, and dilated left ventricular cardiomyopathy with 7901delG mutation in *DSP*.¹⁵ The overall morphology of the skin from our patient resembled

normal interfollicular skin with a normal distribution of desmoplakin, plakoglobin, and keratin 1.

To further analyse the DSP R1267X mutation, a Western blot against plakoglobin, DSPI, and DSPII was performed, using skin biopsy tissue from this patient compared with normal interfollicular and normal palmoplantar skin. Plakoglobin was present at similar levels in normal and patient skin. The DSP blot revealed that, as predicted with the R1267X mutation, the patient was missing the larger DSPI isoform (fig 3B). However, the epitope for the antibody is located in the C-terminal of the protein and will be lost in DSPI as a consequence of truncation. In order to confirm that the only full length DSP isoform present in the patient skin was DSPII, immunofluorescence was carried out using the primary antibody 11-5F (which reacts with both DSPI and DSPII) and an antibody specific to DSPI (fig 3C–F). The epitope for the DSPI antibody is located in the alternatively spliced region of the rod domain, which is not present in DSPII. The immunofluorescence performed using 11-5F

Figure 4 DSP isoform distribution in human heart. Specific amplification of DSPI, DSPII, and control GDH on a cardiovascular cDNA panel from various compartments of the human adult and fetal heart. Additional amplification of skin cDNA was used as an internal control.

showed that at least one of the two isoforms is expressed in the skin of the patient with a normal membranous distribution. In normal interfollicular skin, DSPI is expressed throughout the suprabasal layers and localised to the membrane, but in patient skin, DSPI staining was negative. Therefore, the only full length DSP isoform detected in the skin (and other tissues including the heart) of this patient was DSPII.

In order to determine whether the mutant protein is completely lost, or present in a truncated state, it was necessary to ascertain whether the transcript is stable. As patient material was limited, this was achieved by performing PCR on cDNA from the skin of the mother, who is heterozygous for the mutation R1267X. Primers were designed to specifically amplify DSPI. As the reverse primer is 5' of the mutation, both the wild type and R1267X alleles were amplified and the PCR product obtained was representative of the total amount of DSPI transcript present (fig 3G). The result of this semiquantitative analysis showed that the amount of DSPI transcript in the heterozygous mother was significantly reduced by about 50% compared with the normal control, suggesting an unstable transcript (fig 3G). Therefore, a complete absence of the DSPI transcript in the homozygous patient is likely.

DSPI and DSPII expression in human heart

Previous studies have suggested that although both isoforms DSPI and DSPII are expressed in the skin, only DSPI is present in the heart.⁷ All of the desmoplakin-desmin interactions in the heart must therefore be mediated by the C-terminal part of DSPI; the loss of DSPI in this patient would therefore result in a complete loss of desmoplakin-desmin interactions in the heart, disrupting the desmosomal plaque. Western blotting of different cardiac compartments displayed a prominent signal for DSPI in all four heart chambers, whereas DSPII signals were undetectable or barely detectable (fig 3H). To verify this, we designed a PCR based method to specifically amplify DSPI or DSPII on a cardiovascular cDNA panel. DSPI was shown to be ubiquitously expressed throughout the heart and aorta (fig 4). Surprisingly, a DSPII transcript was found in the left atrium and ventricle, interventricular septum, sinistra auricle, and apex of the heart, but at a much lower expression level than that of DSPI. Expression was almost absent in the right

atrium and right ventricle. It is also noteworthy that the DSPII transcript was detected in human fetal heart tissue.

DISCUSSION

Recent discoveries of human mutations in desmosomal proteins have highlighted the importance of the desmosome and its function in cell-cell connection, especially in mechanically stressed tissues. Disruption of these intercellular junctions has been shown to cause particular heart, skin, and hair phenotypes. Although various key players of the desmosomal junction and their interactions have been identified, the complex structural organisation of desmosomes is still incompletely understood. The description of a patient with a cardiocutaneous syndrome caused by the loss of the DSPI isoform provides novel insights into the function of DSP isoforms, the most abundant desmosomal proteins.

The striking difference between the clinical phenotype of the patient presented here and previously reported cases with cardiocutaneous syndromes, such as Naxos disease and Carvajal syndrome, is the severity and early onset of the cardiac phenotype, which led to heart failure before the age of 4 years. Characteristic features were the left and right ventricular dilatation and the associated contractile and electrical dysfunction. Unfortunately, postmortem examination or biopsy could not be performed and therefore, we cannot comment on putative structural changes in the myocardium of the patient. We were only able to get a small skin biopsy for immunofluorescence and Western blot analysis, but there was not sufficient additional material to perform electron microscopy for ultrastructural changes.

The identified homozygous nonsense mutation R1267X (C3799T) in exon 23 of the DSP gene is the first isoform specific mutation described so far. Both parents and the healthy brother were heterozygous carriers of the mutation and we did not find it in any ethnically matched control samples. Results from Western blotting, immunofluorescence, and the semiquantitative isoform specific PCR on cDNA suggested that the patient had no normal DSPI protein and that the mutated DSPI mRNA was unstable, possibly degraded by nonsense mediated RNA decay. The isoform specific RT-PCR was carried out on skin from a R1267X heterozygote. The experimental design allowed a semiquantitative measurement of the total amount of DSPI transcript to ascertain whether the R1267X transcript is stable. Placing the reverse primer (R1) 3' of the mutation would have allowed us to distinguish if the mutated allele is present on mRNA by sequence analysis of the product. Unfortunately, the material obtained was not sufficient to perform this experiment.

Interestingly, heterozygous loss of function mutations in DSP have been described in families with autosomal dominant SPPK without any cardiac phenotype.^{18,19} The reported N-terminal mutations Q331X and IVS7+1 G→A cause haploinsufficiency of both DSP isoforms. The reduced DSP expression seems to be sufficient for normal cardiac function in this dominant disorder. In contrast, recessively inherited complete loss of function of both DSPI alleles also caused severe heart disease in our patient. Both parents and the healthy brother of our index patient were heterozygous carriers of the DSP R1267X mutation but none showed a dermatological phenotype as described for autosomal dominant SPPK caused by haploinsufficiency of DSP. The reason for this may be that the R1267X mutation does not affect the DSPII isoform in our family and that normal DSPII expression in skin is sufficient to compensate for the haploinsufficiency of DSPI in heterozygous carriers. In contrast, mutations described in autosomal dominant SPPK cause haploinsufficiency of both DSP isoforms, leading to the dermatological phenotype.

It is well known that DSP1 is an obligate constituent of all desmosomes and the only isoform present in cardiac tissue.⁷ This implies that the cardiac desmosomes of the patient contain no desmoplakin. However, we found that DSPII is differentially expressed in adult and fetal heart. A DSPII transcript was found in fetal heart, adult left atrium and ventricle, interventricular septum, sinistra auricle, and apex of the heart. Very low expression levels were observed in the right atrium and ventricle. Although the DSPII transcript was present in the heart, it is not necessarily translated, and care must therefore be taken when applying these data to DSP protein expression.

An essential role for desmoplakin for the assembly of desmosomes and cytoskeletal linkage in early development was shown in desmoplakin knock out mice. In one study, DSP^{-/-} mice died at E6.5 due to the disruption of IF linkage to the desmosomes.¹³ In humans, DSPII expression, as in our patient, may be sufficient for embryonic development and functional integrity of the desmosomal junction. There has been speculation about the functional properties of the different DSP isoforms. As DSPII lacks 618 amino acids of the rod domain, it is thought that DSPII forms less stable dimers in vivo and that it may play a unique role in desmosome assembly and maintenance.⁴ Furthermore, it is unclear if DSP1 and DSPII can form heteromers. Arguments against this possibility were different lengths from head to tail, differences of charged residue patterns, and heptad break-points along the rod.^{4,6} Recently it has been suggested that the rod domain in DSP1 may be folded or coiled so that the distance between the head and tail domains in vivo is the same in both isoforms.² This may be important for dimer formation through coiled coil interactions in the rod domain and head to head interactions.^{2,3} The shorter DSPII could be responsible for the width of the inner plaque, while the lateral associations between the longer rod domains of DSP1 could determine the structure of different proposed plaque models.²

Our data on the expression patterns of DSP1 and DSPII transcripts in human heart could indicate different functional properties of these isoforms and therefore explain their compartment dependent expression levels in the heart. The study of this patient indicates that in humans DSPII expression alone is sufficient for cardiac development, but it cannot fully compensate for the loss of DSP1, leading to a severe dilated cardiomyopathy early in childhood. The lack of compensation could be explained by (a) a reduced overall doses of DSP, (b) differences of DSPII expression levels in different compartments of the heart, or (c) an important functional difference between DSP1 and DSPII. As haplo-insufficiency in SPPK does not lead to a cardiac phenotype, it is unlikely that reduced DSP levels are mainly responsible. Only the absence (or very low levels) of DSPII expression in some compartments of the heart would explain the missing compensation of DSPII, but could not completely explain the clinical phenotype of the patient with the involvement of both ventricles of the heart. Therefore, it is likely that DSP1 and DSPII have different functional properties in the heart, and that for this reason DSPII cannot compensate the loss of DSP1.

It is believed that cardiac remodelling occurs during the first few years of human life and the intercellular junctions in the heart are undergoing structural changes that are not complete until about 6 years of age.²³ The first symptoms in our patient were reported shortly after 3 years of age, indicating that the dysfunction of the myocardium started or rapidly progressed during the time of cardiac remodelling. It would be expected that the cardiomyopathy is more pronounced in the areas of increased mechanical stress, such as the left ventricle. One explanation for the additional right

ventricular involvement in our patient may be the very low expression levels of compensating DSPII in the right ventricle. Further studies are needed to define the functional effects and regulatory mechanisms of DSP1/II isoforms during human fetal development and cardiac remodelling.

In the course of haplotyping candidate gene regions, we identified homozygosity at both the plakoglobin and DSP loci. We did not identify an additional mutation in plakoglobin, but the patient was homozygous for the rare allele of a non-synonymous SNP, T2089A (L697M), in exon 13 of the gene. It is tempting to speculate that this change could influence the binding or assembly properties of the plakoglobin C-terminus. Whether the extreme severity of the cardiac phenotype is an effect of the DSP1 loss alone or modifying factors such as the JUP T2089A variant are contributory remains to be elucidated.

In summary, these findings further emphasise the importance of desmoplakin and desmosomes in epidermal and cardiac biology but they also highlight the possibility that the different isoforms of desmoplakin may have distinct properties within the desmosome and that each isoform may be unable to completely compensate for defects in the other.

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