Desmoplakin Gene Variants and Risk for Arrhythmogenic Cardiomyopathy
Usefulness of a Functional Biochemical Assay

Desmoplakin (DSP), a cytolinker of the plakin family, is an essential intracellular component of the cell-cell adhesion complexes desmosomes. Its main function is the anchoring of intermediate filaments (IFs), cytokeratins (Ks), and desmin in epithelial cells and cardiomyocytes respectively, to desmosomes. Tissue-specific gene deletion in mouse has shown that DSP is indispensable for maintenance of the cytoarchitecture and cell resilience in both skin and heart. Numerous human DSP variants have been linked to inherited diseases that variably result in skin fragility, palmoplantar keratoderma, woolly hair, and arrhythmogenic or dilated cardiomyopathy. Homozygous or compound heterozygous mutations that truncate the DSP tail, which bears several domains that synergistically bind to IFs (Figure [A]), are generally pathogenic. For example, severe C-terminal truncations affecting the PRD (plakin repeat domain) 2/B or preceding regions cause lethal acantholytic epidermolysis bullosa, while shorter truncations often result in Carvajal syndrome, characterized by palmoplantar keratoderma, hair defects, and dilated left ventricular cardiomyopathy. Heterozygous DSP mutations exhibit a complex genotype-phenotype relationship that is sometimes questionable. The factors that ultimately determine the apparent skin- or heart-specific impact of certain DSP variants are also unclear. Furthermore, it is exceptional that a heterozygous mutation specifically weakening the interaction of DSP with IFs is pathogenic in the skin or heart.

Recently, we found that the amino acid substitution p.(Gly2375Arg) in the DSP PRD 2/B drastically reduces the binding of the carboxyl (C) terminus of DSP to Ks, desmin, and vimentin. This substitution was associated with arrhythmogenic right ventricular cardiomyopathy, skin fragility, and woolly hair in a homozygous patient. This result suggests that analysis of the IF-binding properties of variants affecting the DSP tail could explain or even predict clinical phenotypes, especially cardiomyopathy that has usually a later onset than skin diseases. To gain better insights into the molecular basis of DSP-related human diseases, we have here assessed the impact of 7 published amino acid substitutions modifying the DSP C-terminus (Figure [A]) on its IF-binding activity by using a novel fluorescence binding assay. The mutations were selected based on the variable associated skin and cardiac phenotype(s) (Figure [B]). The difficulty to estimate the pathogenicity of these variants is illustrated by the frequent ambiguous conclusions of the ClinVar and arrhythmogenic right ventricular cardiomyopathy databases (Figure [B]). Prediction of the effect of the tested amino acid substitutions on the protein structure/function with different programs (PolyPhen-2, SIFT, and PROVEAN) was also not univocal (data not shown). Our biochemical assays revealed that like p.(Gly2375Arg) the amino acid substitutions p.(Gln2295His) and p.(Arg2366Cys) in the DSP PRD 2/B almost abolished the IF-binding activity of the DSP C-terminus to both Ks and type III IF proteins (Figure [C and D]). Furthermore, the substitution p.(Ala2655Asp), located in the

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A. Schematic structure of the DSP tail and EGFP-DSP C-terminus used in fluorescence overlay assays. PRDs (plakin repeat domains; blue), a linker (yellow), and a C-extremity (E, red). The indicated numbers correspond to the domain borders (amino acids) of human DSP I (GenBank: NP_004406). The position of the analyzed amino acid substitutions is shown.

B. Table showing the cDNA/protein changes, conservation, combination with other mutations, clinical phenotype, and pathogenicity evaluation for each case.  

<table>
<thead>
<tr>
<th>Case No</th>
<th>cDNA/protein change</th>
<th>Conservation*</th>
<th>In combination with</th>
<th>Clinical phenotype</th>
<th>Pathogenicity evaluation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.6885A&gt;T p.(Gln2295His)</td>
<td>PRDs 2 Ident.</td>
<td>TTN p.(Pro10595Thr) TMEM43 c.297+13G&gt;A</td>
<td>Dilated cardiomyopathy</td>
<td>Uncertain, unknown</td>
</tr>
<tr>
<td>2</td>
<td>c.7027G&gt;A p.(Glu2343Lys)</td>
<td>PRDs 2 None</td>
<td>DSP p.(Gln664*)</td>
<td>ARVC</td>
<td>N. a., unknown</td>
</tr>
<tr>
<td>3</td>
<td>c.7096C&gt;T p.(Arg2366Cys)</td>
<td>PRDs 2 Cons.</td>
<td>ARVC criteria not met</td>
<td>Blistering, skin fragility, PPK, nail dystrophy, woolly hair</td>
<td>Pathogenic, N. a.</td>
</tr>
<tr>
<td>4</td>
<td>c.7534C&gt;T p.(Asp2512Tyr)</td>
<td>Linkers None</td>
<td>DSG2 p.(Phe388Cys)</td>
<td>None</td>
<td>Uncertain, unknown</td>
</tr>
<tr>
<td>5</td>
<td>c.7623C&gt;G p.(Arg2541Ser)</td>
<td>Linkers None</td>
<td></td>
<td>ARVC</td>
<td>N. a., pathogenic</td>
</tr>
<tr>
<td>6</td>
<td>c.7916G&gt;A p.(Arg2639Gln)</td>
<td>PRDs 1/3 Cons.</td>
<td></td>
<td>ARVC criteria not met</td>
<td>Conflicting, pathogenic</td>
</tr>
<tr>
<td>7</td>
<td>c.7964A&gt;C p.(Ala2655Asp)</td>
<td>PRDs 1/3 Semi-cons.</td>
<td></td>
<td>Mucocutaneous blisters, PPK, nail dystrophy, enamel dysplasia, woolly hair; fibrosis in both heart ventricles postmortem</td>
<td>N. a., N. a.</td>
</tr>
</tbody>
</table>

C. Fluorescence scans of 10% sodium dodecyl sulfate polyacrylamide gels loaded with extracts (30 μg protein) from transfected or not 293T cells expressing the indicated EGFP-tagged proteins. M, markers. (Continued)

D. Graph showing the relative binding of various amino acids and linker proteins in control and transfected conditions. The graphs indicate statistically significant differences between the conditions. (Continued)
indicates that the combination of palmoplantar keratodermas at the age of 5 years. Nevertheless, in connection with the different IF proteins was systematically normalized to 1.0 and compared with that of EGFP-DSP C-terminus with amino acid substitutions in the same concentration range. MeanaSD, n=23 (each independent experiment was performed in triplicate); 1-way ANOVA-Dunnett test: *: 0.01, **: 0.001, and ***: ≤0.0001, respectively. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; DSG2, desmoglein 2; EGFP, enhanced green fluorescent protein; K, keratin; PPK, palmoplantar keratoderma; TMEM43, transmembrane protein 43; and TTN, titin.

PRD 3/C, also significantly weakened the interaction of the DSP C-terminus with all IF proteins. In contrast, the substitutions p.(Glu2343Lys) and p.(Arg2639Gln) had only a weak and nonsystematic impact on the binding of the DSP C-terminus to the tested IF proteins. Finally, both p.(Asp2512Tyr) and p.(Arg2541Ser) substitutions had no significant effect (Figure [C and D]). Of note, p.(Arg2639Gln) and p.(Asp2512Tyr) substitutions were found in healthy controls (http://exac.broadinstitute.org and https://www.ensembl.org).

In brief, only 3 out of the 7 tested mutations had a clear impact on DSP IF-binding activity. None of them specifically affected the interaction of the DSP C-terminus with either Ks or desmin/vimentin, suggesting that the mode of interaction of DSP with type VII and III IFs is similar. This contention is supported by 2 additional observations. First, truncation of the last 51 C-terminal amino acids strongly diminished the binding of the DSP C-terminus to all tested IF proteins (data not shown). Second, we found that the conserved coil 1 domain of Ks, desmin and vimentin provides the main binding site(s) for the DSP C-terminus (unpublished data). Consequently, it is probable that homozygous or compound heterozygous variants affecting the IF-binding properties of DSP will ultimately lead to a combined skin and heart phenotype, like in the homozygous carrier of DSP p.(Gly2375Arg).4 This prediction should apply to the DSP compound heterozygous cases 3 and 7 (Figure [B]). Both patients express one DSP variant coding for the deleterious amino acid substitution p.(Arg2366Cys) or p.(Ala2655Asp) (Figure [D]) and a second variant encoding a truncated DSP that cannot interact with IFs, p.(Gln664*) or p.(Thr2104Glnfs*12), respectively. Case 7 initially showed only defects of the skin and skin appendages but died from an undiagnosed cardiomyopathy at the age of 14 years. Case 3 had cutaneous, nail and hair defects, but no apparent cardiac abnormalities at the age of 5 years. Nevertheless, in connection with our findings and conclusions, a recent review indicates that the combination of palmoplantar keratoderma and hair defects is a clinical warning marker for later cardiac involvement.5

Our biochemical assay can assess the functional impact of the DSP tail variants that will facilitate their classification when analyzed in combination with clinical findings, family history, and comprehensive genetic analyses.

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure.4

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Disclosures
None.

REFERENCES