Desmosomal Genes

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Desmosomes are mirroring, transmembrane protein chains that connect the intermediate filament networks of neighbouring cells. Each chain continuously (dis)assembles due to the turnover of five desmosomal protein types: desmoplakin, plakoglobin, plakophilins, desmocollins and desmogleins. The expression of two genes is critical to the formation of all desmosomes: namely *DSP,* encoding two differently spliced desmoplakin proteins (DPI and DPII) and *JUP,* encoding plakoglobin (PG). Meanwhile, plakophilins, desmocollins and desmogleins are expressed in a tissue-specific manner and are therefore encoded by multiple genes.

Keywords: cardiocutaneous syndromes ; genotype–phenotype correlation ; functional analysis of genetic variants

1. Introduction

The plakophilin and desmocollin gene family each contain three subtypes (*PKP1, PKP2* and *PKP3,* encoding proteins PP1, PP2 and PP3 and *DSC1, DSC2* and *DSC3,* encoding proteins DC1, DC2 and DC3), while the desmoglein gene family contains four subtypes (*DSG1, DSG2, DSG3* and *DSG4,* encoding proteins DG1, DG2, DG3 and DG4). Keratinocytes of the skin and adnexes express both DP isoforms and PG, in addition to any of the aforementioned PP1-3, DC1-3 and DG1-4 combinations. Each specific composition is in accordance with the differentiation status of keratinocytes in the epidermis [1]. Desmosomal proteins are crucial for epidermal integrity and proper epidermal proliferation and differentiation, and irregularities thereof may cause a skin phenotype [2]. Desmosomal proteins also accommodate hair growth and irregularities thereof. The hair follicle contains keratinocytes in an inner and an outer root sheath. In straight hairs, shafts are straight and homogenous, without clear delimitations, but in coiled hair, these shafts have retro-curvatures [3][4][5]. This curve is achieved via a rotation mechanism of aberrantly proliferating and differentiating cells in the inner root sheath [2][6]. Unlike the skin, the protein composition of desmosomes in cardiac tissue is fixed and consists of DPI, PG, PP2, DC2 and DG2. In the heart, desmosomal anomalies typically disrupt the mechanical continuity of cardiac muscle fibres, which is essential for proper conductance and cardiac muscle contraction. Desmosomes are crucial for the anchorage of cardiomyocytes at the intercalated disc parallel to the direction of strain, where they internally dock the desmin network [7]. Thus far, variants in desmosomal genes *DSP*, *JUP* and *DSC2* have been associated with a cardiocutaneous phenotype.

2. Reported DSP Variants

DSP encodes for two differently spliced DP proteins: DPI (332 kDa) and a smaller DPII isoform (260 kDa) that contains a shorter rod domain [8]. The latter is created by an alternative donor splice site in exon 23. The N-terminal plakin-domain binds with PPs and PG, while domains B and C at the C-terminal side bind to intermediate filaments (**Figure 1;** full report in **Table 1**). In cardiac muscle, *DSP* is predominantly spliced into DPI, while the skin contains both isoforms equally. The ClinVar database reported 3290 variants in *DSP*. Of these, 495 were claimed (likely) pathogenic; 1026 (likely) benign; 161 show conflicting interpretations and 1608 have an unknown significance. Only 48 variants were substantiated by functional evidence, including data from transgenic mouse models (see **Figure 1**). This indicates that over 98% of all *DSP* variants were merely predicted by in silico algorithms. For the majority of variants (36/48), the predictions on protein level were correct, while only partially correct in 2/48 variants and incorrect in 5/48 variants. In 5/48 variants, the functional evidence was too elusive to draw conclusions. Moreover, the in silico prediction algorithms frequently contradicted one another, providing little help in assessing the pathogenicity of *DSP* variants.

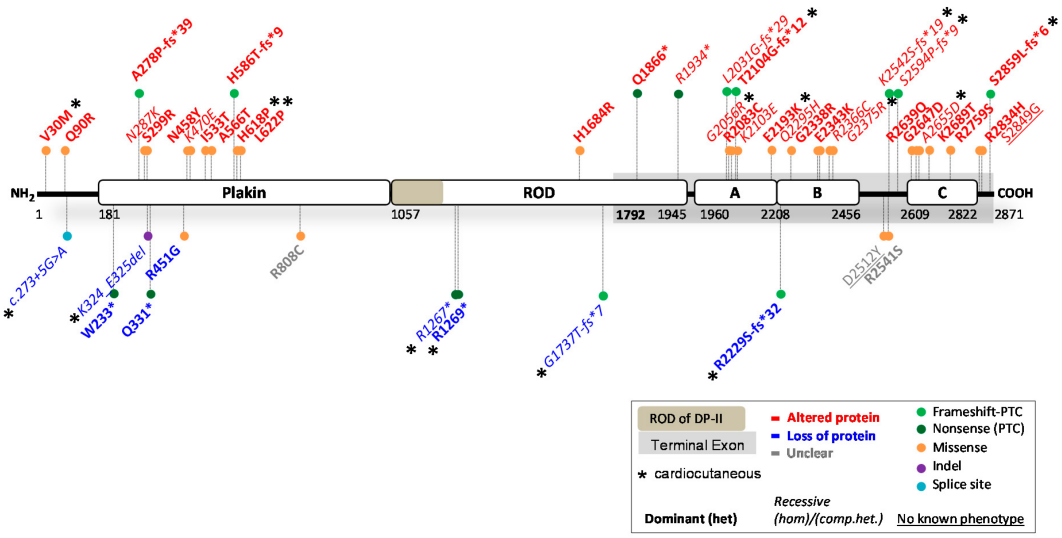

Figure 1. Location of functionally investigated *DSP* variants.

Table 1. Functionally analysed *DSP* variants.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.88G > A	p.(Val30Met)	N-terminus	B	>Protein expressed -PolyPhen-2> Benign (0.000) -SIFT> NOT tolerated -MutPred2> Benign (0.092)	*Altered DP function;* Mutant DP protein expressed, normal size and amount (WB) [9][10][11].	Binding to PG abolished (Co-IP); DP localization in cytoplasm (transfection) [9]; DP normal in myocardial and epidermal tissue. Exhibit weaker binding to iASPP (transfection) [11]. Mouse *DSP*$^{WT/88G > A}$ [9].	Match	(het) PPK; (het)
c.269A > G	p.(Gln90Arg)	N-terminus	B	>Protein expressed -PolyPhen-2> Probably damaging (0.967) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.757)	*Altered DP function;* Mutant DP protein expressed, normal size and amount (WB) [9][11].	Binding to PG abolished (Co-IP); DP localization to cytoplasm (transfection) [9]. Mouse *DSP*$^{WT/269A > G}$ [9]	Match	n.s.
c.273 + 5G > A	Multiple splice products	Intron splice site (N-terminus)	US	-Human Splicing Finder> Broken WT donor site - MaxEntScan> Alteration of WT donor site, probably affecting splicing > Altered splicing, out of frame> PTC > NMD	*Partial loss of DP:* 20% less DP product on WB. No alternatively spliced transcripts discovered in patient-derived cells ([12][13], but did so in in vitro splicing assay (transfection). However, not functional [13].	-In combination with c.6687del> Reduced DP protein on blot and staining in explanted heart and hiPSC-CMs and primary KCs [13][14]. -Dislocation of DP after 2D mechanical stretch; resulted in reduced count and density of desmosomes (EM) in dynamic EHTs leading to lower force and stress [14].	Partial match, normal splicing left.	(comp.het) PPK with *DSP:* c.6687delA

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.699G > A	p.(Trp233*)	Plakin-domain	P	>PTC > NMD, no protein -MutPred-LOF> Borderline pathogenic (0.55385)	*Partial loss of DP*; Mutant RNA not detected in patient cells. Mutant DP is unstable (transfection-WB) [9].	Perinuclear aggregates of DP (transfection IF) [9].	Partial match, but not with transfection IF/WB	n.s.
c.832del	p.(Ala278Profs*39)	Plakin-domain	P	>PTC > NMD, no protein	*Altered DP function;* Truncated DP normally expressed and protein runs at 60 kDa (315 aa) (transfection-WB) -Leads to truncated *DSP* mRNA, also indicating that mRNA translation following the truncation was completely impaired.	c.832del overexpression led to upregulation of PG and downregulation of β-catenin in the nuclei, without affecting their expression in the cytoplasm (transfection) [15].	Mismatch	n.s.
c.861T > G	p.(Asn287Lys)	Plakin-domain	LP	>Protein expressed -PolyPhen-2> Probably damaging (0.997) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.699)	*Altered DP function;* Mutant DP expressed [16].	Aberrant DP and Cx43 localization (transfection-IF) [16].	Match	(hom)PPK; (hom)WH; (hom)EBS
c.897C > G	p.(Ser299Arg)	Plakin-domain	LP	>Protein expressed -PolyPhen-2> Probably damaging (0.999) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.672)	*Altered DP function;* Mutant DP expressed [11].	Exhibit weaker binding to iASPP = desmosome regulator (transfection) [11].	Match	n.s.
c.939 + 1G > A	p.(Gln331*)	Donor site intron 7 (Plakin-domain)	P	>PTC > NMD, no protein	*Partial loss of DP;* Absence of detection of mRNA in multiple patient KCs, reported by two studies, suggests efficient NMD [17][18]. Only 20% DPI and 50% DPII is left on WB [19].	-Major abnormalities in the spinous layer of the epidermis. The intercellular space is widened and KCs contain abnormal cytoplasmic densities [18]. -Small desmosomes and fewer in number; perinuclear keratin distribution [7]. - DC3 seems reduced on WB; volume densities of desmosomal proteins seem different from control [19].	Match	(het) PPK

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.969_974del	p.(Lys324_Glu325del)	Plakin-domain	LP	>Protein expressed -MutPred-Indel> Benign (0.19566)	*Partial loss of DP*; Reduced expression of both native DP isoforms in cytoskeletal and cytoplasmic protein fractions (WB) [10], suggesting instable protein> incomplete degradation.	DP expression was significantly reduced in myocardial tissue and epidermal biopsies (IF) [10].	Mismatch	(hom)PPK; (hom)WH
c.1348C > G	p.(Arg451Gly)	Plakin-domain	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.756)	*Partial loss of DP;* 50% reduced DPI&II protein in EHTs (WB) [20]. -mRNA levels of *DSP* not reduced compared to WT [20]. > instable protein degradation.	50% reduced DP signal and 70% reduced Cx43 in myocardial tissues (IF); Proteolytic degradation by calpain, leading to DP insufficiency [20].	Mismatch	n.r.
c.1372A > T	p.(Asn458Tyr)	Plakin-domain	US	>Protein expressed -PolyPhen-2> Possibly damaging (0.939) -SIFT> Tolerated -MutPred2> Benign (0.323)	*Altered DP function;* Mutant DP expressed [16].	Altered EB1 binding and Cx43 localization (transfection IF) [16].	Match	n.o.
c.1408A > G	p.(Lys470Glu)	Plakin-domain	US	>Protein expressed -PolyPhen-2> Benign (0.082) -SIFT> Tolerated -MutPred2> Benign (0.408)	*Altered DP function;* Conformational alternation, but overall folded structure of DP is remained [21]. Mutant DP expressed (WB) [16].	Mutant is incorporated into the desmosome [10].	Match	n.s./n.o.
c.1598T > C	p.(Ile533Thr)	Plakin-domain	US	>Protein expressed -PolyPhen-2> Probably damaging (0.998) -SIFT> NOT tolerated -MutPred2> Benign (0.442)	*Altered DP function;* Mutant DP expressed (WB) [16].	Altered EB1 binding and Cx43 localization (transfection IF) [16].	Match	n.o.
c.1696G > A	p.(Ala566Thr)	Plakin-domain	US	>Protein expressed -PolyPhen-2> Benign (0.007) -SIFT> Tolerated -MutPred2> Benign (0.153)	*Altered DP function;* Mutant DP expressed (WB) [16].	Mutant is incorporated into the desmosome [10].	Match	n.o.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.1853A > C	p.(His618Pro)	Plakin-domain	LP	>Protein expressed -PolyPhen-2> Possibly damaging (0.602) -SIFT> NOT tolerated -MutPred2> Benign (0.540)	*Altered DP function;* Mutant DP expressed (WB) [22].	Mutant localizes to membrane, affected Cx43 localization (transfection studies/skin biopsies). Desmosome aggregation [22].	Match	(het) PPK; (het) (het) EBS
c.1865T > C	p.(Leu622Pro)	Plakin-domain	LP	>Protein expressed -PolyPhen-2> Probably damaging (0.998) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.828)	*Altered DP function;* Mutant DP expressed (WB) [22].	Mutant localizes to membrane, affected Cx43 localization (transfection studies/skin biopsies). Desmosome aggregation [22].	Match	(het) PPK; (het) (het) EBS
c.1755dup	p.(His586Thrfs*9)	Plakin domain	P	>PTC > NMD, no protein	*Altered DP function;* Truncated DP protein, (65 kDa) (WB), truncation of ROD and C-terminus [23].	n.r.	Mismatch	n.s.
c.2422C > T	p.(Arg808Cys)	Plakin-domain	US	>Protein expressed -PolyPhen-2> Benign (0.047) -SIFT> NOT tolerated -MutPred2> Benign (0.409)	*Unclear;* Conformational alteration (transfection), but overall folded structure of DP is remained [21]. Needs further confirmation in patient cells, whether expressed or not.	n.r.	Unclear	n.s.
c.3799C > T	p.(Arg1267*)	ROD domain (DPI)	P	>PTC > NMD, no protein -MutPred-LOF> Borderline pathogenic (0.5552)	*Partial loss of DPI;* Instable mutant DPI protein (NMD?) [24]. -Highly reduced *DSP* mRNA expression (NMD?) [24]. -Complete loss of DPI in patient skin, DPII has normal expression as expected (WB) [24].	n.r.	Match	(hom)PPK epidermolytic; (hom)WH

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.3805C > T	p.(Arg1269*)	ROD domain (DPI)	P	>PTC > NMD, no protein -MutPred-LOF> Borderline pathogenic (0.55487)	*Partial loss of DPI;* Broken down by NMD. DPI/DPI-II protein ratios lower in variant carriers compared with WT individuals. -DPI/DPII expression ratio reduced by 28% in mutant cells. 15-fold lower mutant than WT [10].	Decreased DP expression in endomyocardial biopsies. DPI deficiency (IF) [10].	Match	(het) PPK; (het)
c.5051A > G	p.(His1684Arg)	ROD domain (DPI)	US	>Protein expressed -PolyPhen-2> Possibly damaging (0.956) -SIFT> NOT tolerated -MutPred2> Benign (0.256)	*Altered DPI function:* No effect on amount or size of DPI protein on WB [25]. DPII should not be affected.	Affects action potential and duration; multiple ion channel dysfunction in hiPSC-CMs [25].	Match	n.r.
c.5208_5209del	p.(Gly1737Thrfs*7)	ROD domain (DPI)	P	>PTC > NMD, no protein	*Partial loss of DPI/Unclear?* Truncated DPI protein predicted to run at similar height as DPII, yet no increase in this band was observed in skin biopsies (WB) [26]. DPII should not be affected, but data are unclear.	n.r.	Unclear	(hom) PPK acantholytic; (hom)WH;
c.5596C > T	p.(Gln1866*)	ROD domain (DPI)	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Altered DPI function;* Truncated DPI protein (160 kDa) observed in skin biopsies [27]. DPII should not be affected.	n.r.	Match	n.s.
c.5800C > T	p.(Arg1934*)	ROD domain (DPI)	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Altered DPI function;* Truncated DPI protein (243 kDa) (WB) [28]. DPII should not be affected. -Aberrant mRNA transcripts. Not NMD.	Stable expressed DP protein, which is recruited into desmosomes, although more punctate staining was observed (IF) [28].	Match	(comp.het) (leth EBS, PPK and with *DSP:* c.6091_6092de
c.6091_6092del	p.(Leu2031Glyfs*29)	PRD (A domain)	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Altered DP function;* Truncated DP-I protein (228 kDa) (WB) [28]. Not clear what happens with DPII. -Aberrant mRNA transcripts. Not NMD.	Stable expressed DP protein, which is recruited in desmosomes, although more punctate staining was observed (IF) [28].	Match	(comp.het) (lethal) EBS, PI and WH, with *D* c.5800C > T [28]

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.6166G > C	p.(Gly2056Arg)	PRD (A domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.872)	*Altered DP function*; Expressed in insoluble fraction of bacterial cells (transfection WB) [29]. Low expression in HeLa cells. Likely expressed mutant.	n.r.	Probable match	(hom) PPK
c.6247C > T	p.(Arg2083Cys)	PRD (A domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> NOT tolerated -MutPred2> Benign (0.443)	*Altered DP function*; Expressed in soluble fraction of bacterial cells (transfection WB), thus correctly folded [29]. Likely expressed mutant, needs confirmation in patient cells.	n.r.	Probable match	n.r.
c.6307A > G	p.(Lys2103Glu)	PRD (A domain)	US	>Protein expressed -PolyPhen-2> Possibly damaging (0.860) -SIFT> Tolerated -MutPred2> Benign (0.417)	*Altered DP function*; Expressed in soluble fraction of bacterial cell transfection, thus correctly folded (WB) [29]. Likely expressed mutant, needs confirmation in patient cells.	n.r.	Probable match	n.r.
c.6310del	p. (Thr2104Glnfs*12)	PRD (A domain)	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Altered DP function;* Several truncated DP proteins shown on WB, but mutant is predicted to be 238 kDa [30]	Fibrosis and fat deposition in the heart with reduction in Cx43, disorganized IDs, but staining of DP, PG and DG2 seemed normal; severe reduction of DPI&II on IF ex vivo skin. β-catenin expression was also reduced on IF in skin [30].	Match	(comp.het) EBS PPK and WH: with *DSP:* c.79 A
c.6577G > A	p.(Glu2193Lys)	PRD (A domain)	US	>Protein expressed -PolyPhen-2> Possibly damaging (0.950) -SIFT> Tolerated -MutPred2> Benign (0.346)	*Altered DP function*; Expressed in insoluble fraction in bacterial cells (transfection WB) [29]. Likely expressed mutant, needs confirmation in patient cells.	n.r.	Probable match	(comp.het) Alo PPK, with *DSP*:c.7567del

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.6687del	p.(Arg2229Serfs*32)	PRD (B domain)	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Partial loss of DP:* NMD of product (WB, NMD inhibitor exp.), 50% reduced protein levels [13][14]. -mRNA 50% reduced [14].	-Reduced DP protein on blot and staining in explanted heart, hiPSC-CMs and primary KCs [13] [14]. -Mislocalisation of DP after 2D mechanical stretch; in combination with c.273 + 5G > A resulted in reduced count and density of desmosomes in hiPSC-derived dynamic EHTs leading to lower force and stress [14] -Faster differentiation observed in primary KCs of patients. Mechanical stretch provoked cell-contact defects [13].	Mismatch NMD active in terminal exon!	(het)PPK; (comp.het)WH: *DSP*: c.273 + 5
c.6885A > T	p.(Gln2295His)	PRD (B domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (0.999) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.833)	*Altered DP function;* Likely truncated DP protein expressed.	Severe binding deficiency with intermediate filaments (transfection IF) [31].	Probable match	n.r.
c.7012G > A	p.(Gly2338Arg)	PRD (B domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.910)	*Altered DP function*; Insoluble fraction in bacterial cell transfection (WB) [29]. Likely expressed mutant, needs confirmation in patient cells.	n.r.	Probable match	n.r.
c.7027G > A	p.(Glu2343Lys)	PRD (B domain)	US	>Protein expressed -PolyPhen-2> Benign (0.077) -SIFT> Tolerated -MutPred2> Benign (0.386)	*Altered DP function;* Likely truncated DP protein expressed. Soluble fraction in bacterial cell transfection, thus correctly folded (WB) [29].	Altered binding with vimentin and keratin8/18 (transfection IF) [31].	Probable match	n.r.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.7096C > T	p.(Arg2366Cys)	PRD (B domain)	LP	>Protein expressed -PolyPhen-2> Probably damaging (0.980) -SIFT> NOT tolerated -MutPred2> Benign (0.622)	*Altered DP function;* Likely truncated DP protein expressed [31]. Soluble fraction of bacterial cell transfection (WB) [29]. High expression in HeLa cells. Needs confirmation in patient cells.	Severe binding deficiency with intermediate filaments (transfection IF) [31]. No binding deficiency with vimentin (IF transfection) [29].	Probable match	(hom)EBS; (hom)PPK; (hom)WH
c.7123G > C	p.(Gly2375Arg)	PRD (B domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.938)	*Altered DP function;* Truncated DP protein expressed [32]. Insoluble fraction of bacterial cell transfection (WB) [29].	Co-alignment with IFs severely affected. Diffuse cytosolic distributed [32]. Targeting to IFs affected (transfection-IF) [29].	Match	(hom)EBS; (hom)PPK; (hom)WH
c.7534G > T	p.(Asp2512Tyr)	Linkers	US	>Protein expressed -PolyPhen-2> Probably damaging (0.998) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.825)	*Unclear;* Likely truncated DP protein expressed. Needs further confirmation in patient cells.	No binding deficiency with IFs (transfection-IF) [31].	Unclear	n.o.
c.7623G > T	p.(Arg2541Ser)	Linkers	US	>Protein expressed -PolyPhen-2> Benign (0.010) -SIFT> NOT tolerated -MutPred2> Benign (0.265)	*Unclear;* Likely truncated DP protein expressed. Needs further confirmation in patient cells.	No binding deficiency with IFs (transfection-IF) [31].	Unclear	n.r.
c.7623del	p.(Lys2542Serfs*19)	Linkers	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Altered DP function;* Severe reduction of both DPI&II (WB), both truncated proteins detected [10].	Normal DP immunoreactivity in epidermal and myocardial tissue (IF)/or almost no signal depending on homozygous or heterozygous patient [10].	Match	(hom)PPK; (hom)WH
c.7780del	p.(Ser2594Profs*9)	Linkers	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Altered DP function;* Truncated DP protein, 18 aa downstream of deletion (WB) [33].	Partial disruption with intermediate filament binding (IF) [33]; KCs have alteration in morphology, elasticity, adhesion capabilities and viscoelastic properties [34][35].	Match	(hom)PPK; (hom)WH

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.7916G > A	p.(Arg2639Gln)	PRD (C domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (0.978) -SIFT > Tolerated -MutPred2> Benign (0.484)	*Altered DP function;* Likely truncated DP proteins expressed [31]. Expressed in soluble fraction in bacterial cells (transfection WB) [29].	Altered binding with desmin and keratin8/18 (transfection IF) [31]. No binding deficiency with vimentin (IF transfection) [29].	Probable match	n.r.
c.7940G > A	p.(Gly2647Asp)	PRD (C domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (0.980) -SIFT> NOT tolerated -MutPred2> Benign (0.619)	*Altered DP function:* Both in insoluble and soluble fraction in bacterial cells (transfection WB) [29]. Likely expressed mutant.	n.r.	Probable match	n.r.
c.7964C > A	p.(Ala2655Asp)	PRD (C domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (0.999) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.754)	*Altered DP function;* Likely truncated DP proteins expressed, as full loss of protein is not expected due to recessive inheritance.	Severe binding deficiency with intermediate filaments (transfection IF) [31].	Probable match	(hom)EBS; (hom)PPK; (hom)WH
c.8066A > C	p.(Lys2689Thr)	PRD (C domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT > Tolerated -MutPred2> Benign (0.625)	*Altered DP function:* Expressed in soluble fraction in bacterial cells (transfection WB), thus correctly folded [29]. High expression in HeLa cells. Likely expressed mutant. Needs confirmation in patient cells.	No binding deficiency with vimentin (transfection IF) [29].	Probable match	n.r.
c.8275C > A	p.(Arg2759Ser)	PRD (C domain)	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT > Tolerated -MutPred2> Benign (0.350)	*Altered DP function:* Expressed in soluble fraction in bacterial cells (transfection, thus correctly folded WB) [29]. Likely expressed mutant. Needs confirmation in patient cells.	n.r.	Probable match	n.r.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.8501G > A	p.(Arg2834His)	C-terminus	US	>Protein expressed -PolyPhen-2> Probably damaging (0.972) -SIFT> NOT tolerated -MutPred2> Benign (0.189)	*Altered DP function;* C-terminally truncated DP protein (WB) [9].	Aberrant IF localization; DP localization at cell membrane; affects other junctional proteins [9]; Arg2834His blocked the GSK3β phosphorylation cascade and reduced DP–GSK3β interactions in KCs and in hearts of Arg2834His DP mice [2]. Mouse *DSP*$^{WT/8501G > A}$ [9][36][37]	Match	n.s.
Engineered variant	p.(Ser2849Gly)	C-terminus	n.a.	>Protein expressed -PolyPhen-2> Probably damaging (0.978) -SIFT> NOT tolerated -MutPred2> Benign (0.344)	*Altered DP function;* Mutant DP protein detected (WB), normal size.	Mutant DP exhibits increased anchorage of keratin/desmin [38] filaments and fosters calcium independency [39].	n.a.	unknown
c.8576_8577del	p.(Ser2859Leufs*6)	C-terminus	LP	>PTC > terminal exon, NOT NMD > protein expressed	*Altered DP function;* Highly reduced mutant DP protein detected in insoluble fraction (WB), none in soluble fraction, but normal size (only 2859 + 6 aa, compared to wildtype 2871 aa)	GSK3β, normally phosphorylates Ser2859Leu, translocated to the soluble fraction of patient extract where its high activity (dephosph). Ser9 was associated with the phosphorylation (Ser33/37-Thr41) and degradation of β-catenin; abolition of β-catenin phosphorylation in the non-soluble fraction was associated with its translocation into CMs nuclei [40].	Match	(hom and het) I

Abbreviations: altered protein function > variant annotated in red; partial loss of protein = variant annotated in blue; unclear > variant annotated in grey; US (uncertain significance); LP (likely pathogenic); P (pathogenic); n.a. (not applicable); n.s. (not specified); aa (amino acids); n.r. (not reported); n.o. (not observed); WT (wildtype); WB (Western blot); IF (immunofluorescence); EM (electron microscopy); NMD (nonsense mediated mRNA decay); fs (frameshift); */PTC (premature termination codon); Co-IP (co-immunoprecipitation); hiPSC (human induced pluripotent stem cells); CMs (cardiomyocytes); EHTs (engineered heart tissues); KCs (keratinocytes); EBS (epidermolysis bullosa simplex); PPK (palmoplantar keratoderma); WH (woolly hair); CM (cardiomyopathy, not further specified); DCM (dilated cardiomyopathy); ACM (arrhythmogenic cardiomyopathy); NCCM (non-compaction cardiomyopathy); LQTS (long QT syndrome); LV (left ventricle); RV (right ventricle); het (heterozygous> phenotype observed in heterozygous carriers); hom (homozygous > phenotype observed in homozygous carriers); comp.het (compound heterozygous > phenotype observed in compound heterozygous carriers).

2.1. *DSP* Variants Causing DP Reduction

Complete loss of DPI&II is probably incompatible with human life, as it causes early embryonic lethality in mice [41]. More importantly, it has not been functionally observed in human patients. However, several variants that cause protein reduction (<100% native DPI/DPII left) in humans and animal models have been reported [42][43][44]. In total, 9/48 variants resulted in variable degrees of DP reduction and inflicted disease in either a dominant (n = 5) or recessive (n = 4) mode of inheritance. This occurred due to two splice-site variants (c.273 + 5G > A and c.939 + 1G > A), four nonsense-inducing variants (c.699G > A, c.3799C > T, c.3805C > T and c.5208_5209del) located before the terminal exon, and one nonsense-inducing variant in the terminal exon (c.6687del) that was unexpectedly targeted by NMD. Moreover, one missense (c.1348C > G) and one in-frame indel variant (c.969_974del) resulted in DP protein reduction, probably due to instable protein degradation. All of the aforementioned variants affected both isoforms, except for variants c.3799C > T, c.3805C > T and c.5208_5209del. The latter are located in the ROD domain of DPI and therefore only affect DPI, but not DPII. Interpreting the phenotype of patients, DP deficiency (≤50% native DPI) seems to be associated with severe cardiomyopathy, while DP deficiency in the skin (≤50% native DPI and DPII) is mostly associated with PPK and WH. Recessive variants that caused loss of DPI but not DPII, or extreme deficient levels of both DPI and DPII (<20%), were associated with skin fragility [26].

2.2. *DSP* Variants Causing an Altered DP Protein

The majority of functionally investigated *DSP* variants (36/48) led to an altered DP protein, due to 23 dominantly and 13 recessively inherited variants. Missense variants were the predominate source for altered DP proteins (n = 27), while the remaining nine variants were due to nonsense or nonsense-inducing variants. Out of the 36 variants, two variants were located near the N-terminus, ten were located in the plakin domain, three in the ROD domain of DPI, six in domain A, five in domain B, two in the linkers, five in domain C and three near the C-terminus. All but one (c.6687del) of the nonsense-inducing variants in the terminal exon of *DSP* skipped NMD. As expected, the phenotype of patients with an altered DP protein indicated that a recessive mode of inheritance was more severe than a dominant mode of inheritance, mostly due to absence of native protein in the former. Cardiomyopathy was observed in 31/36 of the variant carriers, while it went unobserved or unreported in the others. In the skin, 11/36 variants resulted in PPK (recessive n = 9, dominant n = 2) often with WH (recessive n = 7, dominant n = 2). However, PPK and WH were frequently not observed or not reported in the studies primarily focused on the cardiac phenotype. Furthermore, 10/36 variants caused skin fragility, mostly due to a recessive inheritance (n = 7, dominant n = 3). Variants located in the plakin domain frequently affected the binding efficiency to PG [9][10][11], PPs [45] or intermediate filament anchorage [16][46]. Variants located in domains A, B or C almost always affected the binding affinity to intermediate filaments, especially when causing severe property alterations in domains B and C or loss of these through C-terminal truncation. The functional evidence of the remaining 3/48 variants was inconclusive as to whether it resulted in protein reduction or an altered DP protein.

2.3. Potential Therapeutic Avenues

Given the contradicting results from in silico algorithms and several inconsistencies between the prediction and functional evidence, functional assays of the remaining *DSP* variants would be strongly encouraged. The nine variants causing DP reduction indicated that the disease severity tends to be dose-dependent in nature, both in the heart as well as in the skin. Hence, strategies to increase native DP protein levels, especially in the heart, would be of benefit to patients. Injections with *DSP* mRNA in DP-deficient zebrafish have been promising in regaining cardiac function [47]. Besides strategies like RNAi or CRISPR that eliminate protein expression from mutant alleles in patients with altered DP proteins, strategies should simultaneously aim to increase native DP protein levels. For most of the functionally investigated variants, it remains unclear whether they cause a dual organ phenotype, as it is not often assessed or specified.

3. Reported JUP Variants

The *JUP* gene encodes for the 82 kDa PG protein, also known as ɣ-catenin. PG contains an N-terminal head-domain, 12 armadillo domains and a C-terminal tail-domain (**Figure 2**). PG belongs to the catenin protein family and is highly homologous to β-catenin, a potent transcription factor of the canonical Wnt/β-catenin signalling pathway. PG is an important desmosomal protein, comprising the outer dense plaque of the desmosome and connecting the transmembrane DG and DC proteins to DP and PP. β-catenin and PG can be substituted for one another, as β-catenin can be incorporated into desmosomes, while PG can also act as a nuclear transcription factor [48]. The ClinVar database has reported 838 variants in *JUP*. Of these, 30 are claimed (likely) pathogenic; 307 (likely) benign; 70 show conflicting interpretations, and 431 have an unknown significance. Merely eight variants where substantiated by functional evidence, including data from transgenic mouse and zebrafish models (see **Figure 2**). As for *DSP* variants, effects of over 98% of all *JUP* variants were merely predicted by algorithms. The predictions on protein level were correct in 4/8 variants, incorrect in 3/8 variants, while the functional data remained inconclusive in 1/8 variants.

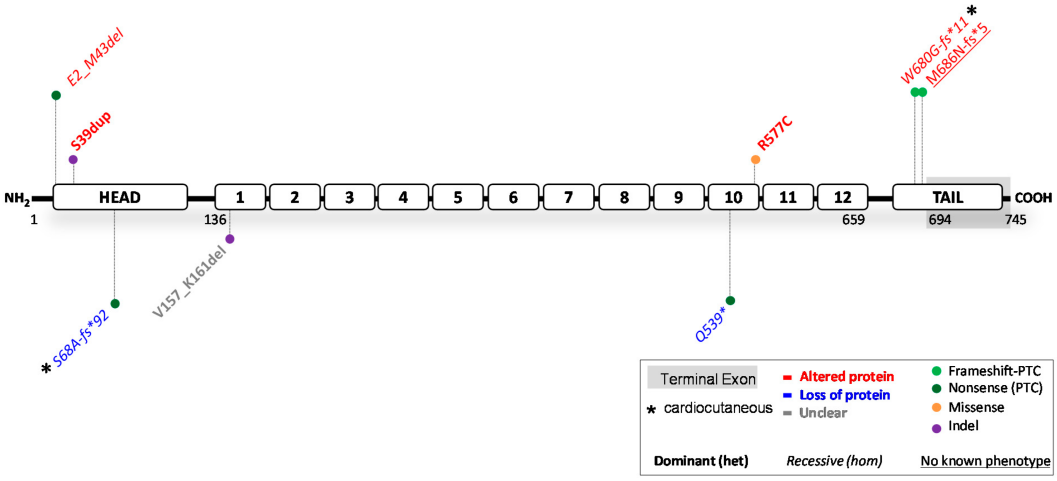

Figure 2. Location of functionally investigated *JUP* variants.

Table 2. Experimental investigation of *JUP* variants.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin	Heart
c.71C > A	p.(Glu2_Met43del) not p.(Ser24*) as predicted	Head domain	LP	>PTC > NMD, no protein -MutPred-LOF> too short sequence for prediction	*Altered PG function;* Truncated N-terminal protein (lacking the first 42 aa, translation re-initiation Met43) with reduced expression in the skin (WB) [49]. -Similar *JUP* mRNA levels as in control [49].	Reduced PG expression (IF; WB) and disrupted distribution of DP and DG1 (IF) [49].	Mismatch	(hom)EBS; (hom)PPK; (hom)WH	n.o.
c.116_118dup	p.(Ser39dup)	Head domain	US	>Protein expressed -MutPred-Indel> Pathogenic (0.76492)	*Altered PG function:* Mutant PG protein size similar as WT (82 kDa; WB) [50][51]. See comment on biological effect [51].	-Patient heart displayed a decrease in signal of DP, PG and Cx43 (IF) [50] - Transfection of HEK293 with mutant construct showed increased size of PG (90 kDa) due to ubiquitin binding (WB); cytoplasmic localization of mutant-PG (IF); higher proliferation and lower apoptosis; fewer and smaller desmosomes in mutant PG cells (EM) [50] -Additional binding properties of mutant PG to TAIP-2 and HRC-BP (Co-IP and yeast-two-hybrid) [50] - Diminished cell stiffness, but not cell adhesion [51].	Match	n.o.	(het) ACM

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin	Heart
c.201del	p.(Ser68Alafs*92)	Head domain	P	>PTC > NMD, no protein	*Loss of PG?:* Highly reduced levels of *JUP* mRNA (normal splicing), no WB performed [52]	-Absence of PG protein staining in skin biopsies (IF); small desmosomes and wide intercellular spaces (EM) [52].	Match	(hom)EBS; (hom)PPK; (hom) alopecia	(hom) ACM
c.469-8_469-1del	p.(Val157_Lys161del)	Armadillo Domain 1	LP	>Protein expressed -MutPred-Indel> Pathogenic (0.78939)	*Unclear;* 15 nucleotides shorter cDNA fragments when compared to controls, no WB performed [53].	Cryptic splice acceptor site activation in exon 4 [53].	Unclear	n.r.	(het) ACM
c.1615C > T	p.(Gln539*)	Armadillo Domain 10	P	>PTC > NMD, no protein - MutPred-LOF> Borderline pathogenic (0.62246)	*Loss of PG;* No truncated, or full-length PG protein detected in patient's skin extracts (WB) [54]. -Apart from the strong reduction of *JUP* (90% reduction), *DSP* and *DSG1* mRNAs were also markedly decreased [54].	-Complete loss of PG protein in the patient's skin (WB and IF, both with N-terminal and C-terminal antibody); No skin barrier formation; significant reduction of DP and DG3 in patient skin (IF). Only few, abnormal desmosomes were formed [54]. - Strong reduction PG in the myocardium [54].	Match	(hom) lethal EBS	n.o. at young age
c.1729C > T	p.(Arg577Cys)	Armadillo Domain 10	LP	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> Tolerated - MutPred2> Pathogenic (0.720)	*Altered PG function:* Mutant PG had similar size as WT and was not reduced on blot according to the study [55].	-On WB, DG2 and Cx43 protein levels were reduced in mutant expression cells, and desmosomal junctions were destabilized (transfection studies) [55].	Match	n.r.	(het) ACM

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin	Heart
c.2038_2039del	p.(Trp680Glyfs*11)	Tail domain	P	>PTC > NMD, no protein	*Altered PG function;* C-terminal truncated PG protein is abundantly expressed (56 aa missing) (WB of biopsied LV and RV of multiple patients) [51][56][57].	-Reduced Cx43 and PG in patient ventricles and absence of phosphorylated Cx43 (IF; WB). A decreased number of gap junctions in patient's myocardium (EM) [57]. - Diminishes cell adhesion, but not the cell compliance [51]. -Mouse *JUP* knockin c.2038_2039del [58]. -The mouse data contradict the human data and suggest that mutant mRNA is broken down by NMD in mice, and not much protein is produced (WB does show truncated protein). The authors reason that although the deletion is located in exon 11, the PTC is located in the terminal exon (exon 12) > homozygous mice die at postnatal day 1, while cardiac development went normal, mice had severe skin fragility. -Fusion of the last 5 exons in mice, produced the truncated protein fully, did not cause lethality; however, mice did not develop cardiac dysfunction at 11 months of age.	Mismatch Mismatch human: mice	(hom)PPK; (hom)WH [59]	(hom) ACM [59]

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin	Heart
c.2057_2058del	p.(Met686Asnfs*5)	Tail domain	n.a.	>PTC > NMD, no protein	*Altered PG function;* Transfected myocytes showed a C-terminal 75 kDa truncated protein (WB) [60].	Cardiac specific Zebrafish *JUP*$^{WT/2057\_2058del}$ [60]. - The zebrafish mutated myocytes showed significant reduction of I_{Na} and I_{K1} current densities. EM showed disruption of cell–cell contact. (GAL4/UAS transactivation system was used to induce cardiac specific expression of the human 2057_2058del variant in zebrafish) [60].	Mismatch, but no patients were traced.	n.r.	(hom) ACM claimed, but no patients were traced

Abbreviations: altered protein function > variant annotated in red; partial loss of protein = variant annotated in blue; unclear > variant annotated in grey; US (uncertain significance); LP (likely pathogenic); P (pathogenic); aa (amino acids); n.r. (none reported); n.o. (none observed); NMD (nonsense mediated mRNA decay); fs (frameshift); * or PTC (premature termination codon); WB (Western blot); IF (immunofluorescence); EM (electron microscopy); co-IP (co-immunoprecipitation); WT (wildtype); LV (left ventricle); RV (right ventricle); EBS (epidermolysis bullosa simplex); PPK (palmoplantar keratoderma); WH (woolly hair); ACM (arrhythmogenic cardiomyopathy); hom (homozygous > phenotype observed in homozygous carriers); het (heterozygous > phenotype observed in heterozygous carriers).

3.1. JUP Variants Causing PG Reduction

Complete loss of PG induces lethality within embryogenesis in mice due to severe heart defects or immediately post-natal due to severe skin fragility [61][62]. Highly suppressed PG protein levels (<10%) also lead to ACM [63], while 40% protein levels do not induce cardiac dysfunction in mice [64]. This suggests that threshold levels for cardiomyopathy in mice span between 10–40% of native protein. Two human variants (c.201del and c.1615C > T, nonsense) were reported to induce complete PG depletion in homozygous patients [52][54]. These carriers developed severe and sometimes lethal skin fragility, in combination with PPK and alopecia in homozygous c.201del carriers. Cardiomyopathy was not observed in any but one patient with old age [52]. Meanwhile, no other patients with PG reduction (≤50% protein) have been reported. The above results suggest that (near) PG depletion is strongly correlated to skin fragility. However, the dose effect of PG reduction on the development of skin features is unknown and warrants further functional studies. In contrast, while PG depletion induces cardiac lethality during embryogenesis in mice, the limited data currently suggest that the human heart may be protected, perhaps due to functional compensation of β-catenin [48][58]. To accurately assess the cardiac penetrance in patients, more variants predicted to cause reduced or depleted PG levels need to be investigated.

3.2. *JUP* Variants Causing an Altered PG Protein

Five functionally investigated variants (5/8) resulted in an altered PG protein. These resulted from three recessively inherited nonsense-inducing variants, resulting in either a N-terminal (Glu2_Met43del) or C-terminal (Trp680Glyfs*11 and Met686Asnfs*5) truncated protein. In addition, two dominantly inherited variants, Ser39dup and Arg577Cys, caused ACM and resulted in proteins comparable to the size of native PG. Unlike the C-terminal truncations, the recessive N-terminal truncation induced skin fragility with PPK and WH, but no cardiac dysfunction. The variant causative for this N-terminal truncation c.71C > A, introduces a PTC at Ser24, but translation re-initiation took place at position Met43, which resulted in deletion of the first 42 amino acids. This suggests that any nonsense-inducing variant located between *JUP*:c.1_126 will likely cause translation re-initiation and a similar phenotype and effect on protein. Opposingly, the two recessive C-terminal truncations correlated with ACM, PPK and WH, but did not induce skin fragility in patients. Moreover, two homozygous PG mouse knockin Trp680Glyfs*11 models were developed, one with and one without fusion of the final five exons. In mice without fusion of exons, this variant resulted in NMD, and only very low levels of C-terminal truncated protein were expressed. These mice died on postnatal day one due to severe skin fragility, induced by depleted PG. Due to their short lifespan, the effect on cardiac function in later stages of development is unclear. In mice with fusion of exons, high levels of C-terminal truncated protein were observed, similarly as in patients. Nonetheless, even at 11 months of age, mice failed to develop cardiac dysfunction [58]. These data suggest that there may be little resemblance between the

cardiac function of humans and mice with regard to *JUP* variants. More variants need to be functionally investigated to draw definitive conclusions.

3.3. Potential Therapeutic Avenues

Currently, too little functional evidence is available to adequately address potential therapeutic strategies that would benefit the cardiac function of patients with disease-causing *JUP* variants. Meanwhile, strategies to increase native protein in patients with skin fragility seem appropriate for all patients with PG depletion and patients with PG proteins that lack the N-terminus. Whether a skin phenotype is only observed in the case of biallelic *JUP* variants, as the eight functionally investigated variants now suggest, needs additional functional evidence. Furthermore, almost half of the functionally investigated variants were falsely predicted, which further pressed the need for more functional studies.

4. Reported DSC2 Variants

The *DSC2* gene encodes for two transmembrane cadherin isoforms: DC2a (99 kDa) and DC2b (93 kDa). The DC2a isoform contains the complete intracellular segment (ICS), whereas this domain is 53 amino acids shorter in DC2b [65], due to alternative splicing of exon 16 (**Figure 3**). Both isoforms are first processed into a precursor protein, followed by a mature protein that can be incorporated into desmosomes. Maturely processed DC2 serves as a transmembrane desmosomal protein, important for extracellular cell–cell attachment. The ClinVar database has reported 1209 variants in *DSC2*. Of these, 102 were claimed (likely) pathogenic; 409 (likely) benign; 85 show conflicting interpretations and 613 have an unknown significance. Notably, merely 15 variants were substantiated by functional evidence, including data from transgenic mouse and zebrafish models (see **Figure 3**). The same trend seen for *DSP* and *JUP* variants, is also observed for *DSC2*, indicating that over 98% of all variants have not been functionally investigated. The predictions on protein level were correct in 11/15 variants, while incorrect in 3/15 variants and unclear in 1/15 variants.

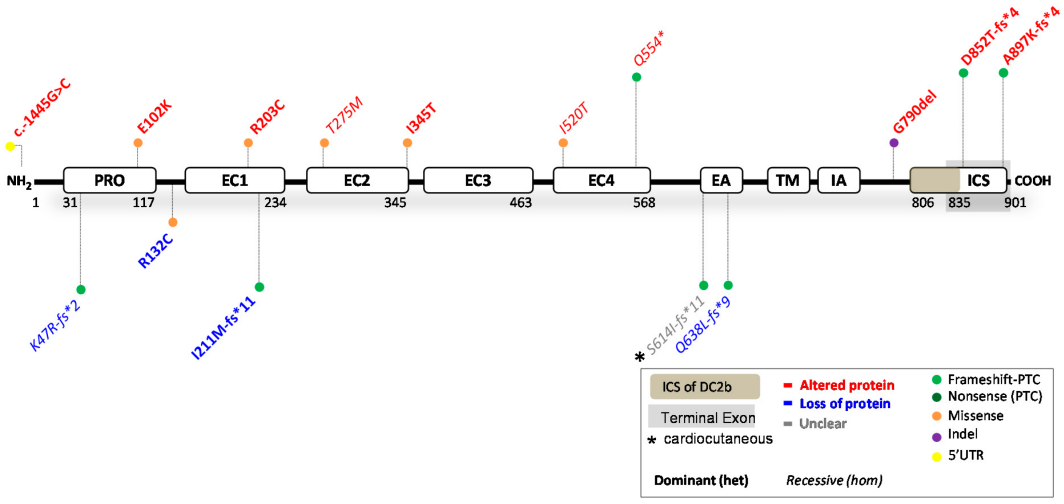

Figure 3. Location of functionally investigated *DSC2* variants.

Table 3. Functionally analysed *DSC2* variants.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.-1445G > C	NC_000018.10:g.31103416C > G	5′UTR	B	Not applicable (5′UTR), cannot be predicted	*Altered DC2 function;* n.s. -Luciferase assay >a decreased transcriptional activity for HEK cells transfected with the DC2 mutant (c.-1445C) construct [66].	Altered transcription factor binding in the presence of the mutant allele.	Mismatch by definition	n.r.
c.140_147del	p.(Lys47Arg fs*2)	PRO peptide-domain	LP	>PTC > NMD, no protein	*Partial loss of DC2:* Patient had reduced levels (>50%) of DC2 in skin biopsy (WB) [67].	n.r. -of note: a relative with only *DSC2*:c.1559T > C (missense) had no phenotype [67].	Match	n.r.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.304G > A	p.(Glu102Lys)	PRO peptide-domain	LB	>Protein expressed -PolyPhen-2> Benign (0.016) -SIFT> Tolerated -MutPred2> Benign (0.158)	*Altered DC2 function;* n.s., but mutant expressed in cells.	IF shows that the mutant protein localizes in a dotted pattern predominantly in the cytoplasm (COS-1 cells, neonatal rat CM transfection) [68].	Match	n.r.
c.394C > T	p.(Arg132Cys)	In between PRO peptide and EC1-domain	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.823)	*Partial loss of DC2;* 50% reduced levels of *DSC2* mRNA in explanted heart and hiPSC-CMs; reduced DC2 protein in explanted heart (WB) [69]	-Reduced levels of all desmosomal genes in explanted heart and hiPSC-CMs, reduction of PG at ID in heart; mutant hiPSC-CMs had shortened action potential durations associated with reduced calcium current density and increased potassium current density [69]. -Increased PPARγ expression and contractile and electric disturbances observed in patient hiPSC-CMs [70] -Zebrafish *DSC2*$^{WT/c.394C > T}$ [69].	Mismatch	n.r.
c.609C > T	p.(Arg203Cys)	EC1 domain	US	>Protein expressed -PolyPhen-2> Probably damaging (1.000) -SIFT> NOT tolerated -MutPred2> Pathogenic (0.899)	*Altered DC2 function;* Complete defect in processing into the mature form [71]. (WB)	-The mutant protein remains in an unprocessed pro-protein form (COS-1 cells transfection) [71]. -In HL-1 cells, the mutant protein fails to localize at the desmosomes of intercalated disc structures [71].	Match	n.r.
c.631-2A > G	p.(Ile211Met fs*11)	EC1 domain	P	>PTC > NMD, no protein	*Partial loss of DC2:* Patient heart tissue shows 60% DC2 reduction (WB) [72] -Reduced mRNA *DSC2* (only 3% compared to WT) [72]	-n.r. -Zebrafish KD of *DSC2* and *DSC2*$^{WT/631-2A > G}$ [72].	Match	n.o.

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.824C > T	p.(Thr275Met)	EC2 domain	US	>Protein expressed -PolyPhen-2> Probably damaging (0.999) -SIFT> NOT tolerated -MutPred2> Benign (0.563)	*Altered DC2 function;* Partial defects in processing into the mature form [71]. (WB)	-Only a proportion of the partly functional DC2 mutant protein is still incorporated into the desmosomes; affects PG at the intercalated disc (COS-1 cells transfection) [71].	Match	n.r.
c.1034T > C	p.(Ile345Thr)	EC2 domain	US	>Protein expressed -PolyPhen-2> Possibly damaging (0.756) -SIFT> NOT tolerated -MutPred2> Benign (0.591)	*Altered DC2 function;* n.s., but mutant expressed in cells.	-In transfected neonatal rat cardiomyocytes and HL-1 cells, the mutant protein localizes in the cytoplasm (IF) [68].	Match	n.r.
c.1559T > C	p.(Ile520Thr)	EC4 domain	LB	>Protein expressed -PolyPhen-2> Probably damaging (0.973) -SIFT> NOT tolerated -MutPred2> Benign (0.601)	*Altered DC2 function;* Protein is expressed, similar size as wildtype (WB) [67]. Unsure if the protein is really altered, or that it maintains all functions [67].	n.r. -of note: a relative with only *DSC2*:c.1559T > C (missense) had no phenotype [67].	Match	n.r.
c.1660C > T	p.(Gln554*)	EC4 domain	P	>PTC > NMD, no protein - MutPredLOF> borderline pathogenic (0.51161)	*Altered DC2 function;* Truncated DC2 protein (75 kDa), wildtype is 150 kDa (transfection WB). Less mature protein, more pre-protein than normal [73].	-Heart biopsies shows DC2 staining in hom-carriers (protein is expressed); mutant protein localizes only partially at cell membrane and predominantly in cytoplasm (transfection IF WB) [73]. -Transfected cells show that the secreted truncated isoforms are not anchored in the plasma membrane [74].	Mismatch	Mild PPK at pressure points, in one hom- and one het-carrier (possibly secondary to farm work)
c.1841del	p.(Ser614Ile fs*11)	EA domain	P	>PTC > NMD, no protein	*Unclear?;* Truncated isoforms expressed (transfection IF, WB), but needs patient cell confirmation.	Transfected cells show that the secreted truncated isoforms are not anchored in the plasma membrane [74].	Unclear	(hom) Mild PPK, WH [75]

HGVS Nomenclature (DNA)	HGVS Nomenclature (Protein)	Protein Domain	ACMG Class	In Silico Predictions	Functional mRNA and Protein Studies	Biological Effect	Prediction: Functional	Skin
c.1913_1916 del	p.(Gln638Leu fs*9)	EA domain	P	>PTC > NMD, no protein	*Partial loss of DC2:* Strong DC2 protein reduction in patient heart tissue (<10% left-WB, also IF) [74]. -*DSC2* mRNA was decreased in patient heart tissue (qPCR)	-Patients' explanted heart shows degradation of sarcomeres and mitochondria; widened intercellular spaces and accumulation of lipid droplets (EM); Transfected cells show that the secreted truncated isoforms are not anchored in the plasma membrane [74].	Match	n.o.
						-Slight LV dysfunction with abnormal calcium release [76]. -Mouse model > [76]		
c.2368_2370 del	p.(Gly790del)	In between IA and ICS domains	US	>Protein expressed -MutPred-Indel> NOT pathogenic (0.4309)	*Altered DC2 function:* No reduction of DC2 protein levels [76].	Hom-mice (G790del) showed enlarged LV and a decreased fractional shortening. Abnormal intracellular calcium release, but no clear ACM phenotype. Het-mice showed no arrhythmias.	Match	n.r.
c.2553del	p.(Asp852Thr fs*4)	ICS domain DC2a only	US	-PTC > terminal exon, not NMD > protein expressed	*Altered DC2a function;* Truncation of the last 47 aa of the DC2a isoform [77].	The mutant protein DC2a lost its ability to bind to PG (HL-1 cells transfection) [77].	Match	n.r.
c.2687_2688 insGA	p.(Ala897Lys fs*4)	ICS domain DC2a only	B	-PTC > terminal exon, not NMD > protein expressed	*Altered DC2a function;* -Premature termination of the protein [78]. -Does not exhibit defects in processing into the mature form [71].	-Cytoplasmic localization of the mutant protein (HL-1 cells transfection) [78]. -This mutant protein is processed into its mature form and can be incorporated into desmosomes; impaired binding to DP and PG (COS-1 cells transfection) [71].	Match	n.r.

Abbreviations: altered protein function > variant annotated in red; partial loss of protein = variant annotated in blue; unclear > variant annotated in grey; US (uncertain significance); B (benign); LB (likely benign); LP (likely pathogenic); P (pathogenic); n.s. (not specified); aa (amino acids); n.r. (none reported); n.o. (none observed); WT (wildtype); WB (Western blot); IF (immunofluorescence); EM (electron microscopy); NMD (nonsense mediated mRNA decay); fs (frameshift); * or PTC (premature termination codon); hiPSC-CMs (human induced pluripotent stem cell derived

cardiomyocytes); KD (knockdown); ID (intercalated disc); PPK (palmoplantar keratoderma); CM (cardiomyopathy); ACM (arrhythmogenic cardiomyopathy); NCCM (non-compaction cardiomyopathy); HCM (hypertrophic cardiomyopathy); LV (left ventricle); hom (homozygous > phenotype observed in homozygous carriers); comp.het (compound heterozygous > phenotype observed in compound heterozygous carriers); het (heterozygous > phenotype observed in heterozygous carriers).

4.1. *DSC2* Variants Causing DC2 Reduction

No patients with complete absence of DC2 protein have been reported. Instead, four variants (4/15), located before the terminal exon, resulted in both DC2a and DC2b protein reduction. The recessively (c.1913_1916delAGAA; ≤10% protein left [74]) and dominantly (c.631-2A > G; 40% protein left) inherited nonsense-inducing variants both caused ACM in patients. Compound heterozygosity of out-of-frame indel variant c.140_147delAACTTGT resulted in NCCM and hypertrophy, which is the only functionally investigated *DSC2* variant associated with cardiomyopathy other than ACM [67]. The missense variant c.394C > T caused 50% protein reduction via instable protein degradation and caused ACM in a dominant mode of inheritance. Altered electrical properties, a key characteristic of ACM, have been observed in patient hiPSC-CMs containing this missense variant [69]. Moreover, dominantly inherited variants c.394C > T and c.631-2A > G were also investigated in a zebrafish model. The ACM phenotype in both models was rescued by injecting human wildtype but not mutant *DSC2* mRNA [69][72]. One of these studies additionally showed that gradual knockdown of *DSC2* resulted in dose-dependent cardiac disease severity [72]. This seems to corroborate with the human data, suggesting that cardiomyopathy occurs in situations with ≤50% of native DC2 protein: and the higher the reduction, the more severe the phenotype. Furthermore, in mice, neither complete nor heart-specific knockout of *DSC2* resulted in any altered viability or cardiac phenotype [79], which emphasizes differences in disease susceptibility among species. None of the DC2 protein-reducing variants caused a skin phenotype, indicating that near loss of the DC2 protein is well tolerated by the skin. Based on the few investigated variants and contradicting results of animal models, incisive conclusions are still difficult to draw.

4.2. *DSC2* Variants Causing an Altered DC2 Protein

Ten variants (10/15) resulted in an altered DC2 protein, which predominantly caused ACM via a dominant (n = 7) or recessive (n = 3) mode of inheritance. Heterozygous variant c.-1445G > C in the 5′UTR affected transcription factor binding mechanisms. Meanwhile, five variants had pronounced effects on the processing of DC2 precursor proteins. For instance, artificial transfection experiments containing missense variants Glu102Lys, Arg203Cys, Thr275Met and Ile345Thr showed punctate cytoplasmic staining, with no or partial ability to be incorporated into desmosomes [71][73][77]. Moreover, nonsense variant Gln554* escaped NMD and resulted in a C-terminal truncated protein, affecting both isoforms [73]. This variant also affected the processing of DC2 precursor proteins, and while a small proportion of maturely processed proteins was incorporated into the desmosome, a larger proportion of precursor proteins remained in the cytoplasm. It is still uncertain whether missense variant Ile520Thr will induce similar alterations that affect the processing of DC2 precursor proteins [67]. Opposingly, nonsense-inducing variants, Asp852Thrfs*4 and Ala897Lysfs*4, only affected isoform DC2a and caused a C-terminal truncated protein. Both were fully processed into a mature protein form, were incorporated into desmosomes, but lost their ability to bind to DP [71] and PG [71][77]. The latter suggests a similar perturbing binding interface for Gln554*. No protein processing information is available on variant Gly790del, other than that it is expressed and translated into a transgenic mouse model. Neither the heterozygous nor homozygous mice showed structural or functional defects in the ventricles or lethal arrhythmias, and only homozygous aged mice showed slight left ventricular dysfunction. This mouse model therefore does not represent the phenotypic severity of the heterozygous Gly790del patients with ACM. In most (7/10) variants, apart from recessive variants Gln554*, Thr275Met and Ile520Thr, ACM was observed in a dominant mode of inheritance. Only recessive inheritance of variant Ser614Ilefs*11 caused PPK and WH, but the functional data were unclear as to whether it causes protein reduction or an altered protein function in patients [74][75]. A skin phenotype was furthermore not observed or went unreported in the other variants.

4.3. Potential Therapeutic Avenues

Whether *DSC2* variants can truly cause a cardiocutaneous phenotype remains somewhat elusive, given that only one investigated variant was associated with PPK and WH, and others that do associate with a skin phenotype were not investigated [75]. It seems that extreme deficiency in DC2 is well-compensated for by other desmocollins in the skin (i.e., DC1 and DC3). Nonetheless, more variants should be investigated to draw decisive conclusions. Meanwhile, with the limited functional evidence in mind, patients with DC2 reduction might benefit from native protein-increasing therapeutic strategies. Taken into account, over-administration may be detrimental to humans, as *DSC2* overexpression caused severe cardiac dysfunction in mice [80].

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