SUPPLEMENTAL MATERIAL

Table S1

Immunological studies

RNA sequencing analysis of normal human PBMCs

Values are given as RPKM

Transcript	$CD3^+$	CD4+	CD8+	T-reg	CD19+	CD14+	CD56+
DSP	0,013	0,010	0,000	0,000	0,086	0,000	0,000

FACS analyses on PBMCs from DSP mutation carrier

Parameter	Unit	Value	Reference range
B Cells			
naive B cells	/nl	0.247	
naive B cells	%	82.7	63.3-88.0
marginal zone-like B cells	/nl	0.015	
marginal zone-like B cells	%	5.2 P ↓	6.1-16.9
IgM only memory B cells	/nl	0.002	
IgM only memory B cells	%	0.7	
switched memory B cells	/nl	0.012	
switched memory B cells	%	4.0 P ↓	4.1-18.7
transitional B cells	/nl	0.010	
transitional B cells	%	3.2	0.6-3.4
CD21low CD38low B cells	/nl	0.005	
CD21low CD38low B cells	%	1.7	0.9-7.6
switched plasmablasts	/nl	0.004	
switched plasmablasts	%	1.3	0.4-3.6
CD19+ B cells	/nl	0.30	0.10-0.40
NK Cells			
NK cells	/nl	0.25	0.10-0.40

NK cells, % of lymphocytes	%	9	5-25
Naive CD45RA+, % of CD4+	%	66	
T Cells			
memory CD45RO+, % of CD4+	%	34	
naive CD45RA+, % of CD8+	%	67	
memory CD45RO+, % of CD8+	%	33	
γ/δ TCR+ T cells, relative	%	22 P ↑	< 10
α/β TCR+ T cells, relative	%	78 P ↓	> 90
γ/δ TCR+ T cells	/nl	0.50	
α/β TCR+ T cells	/nl	1.80	
TCRαβ+ CD4- CD8-, % of TCRαβ+ CD3+	%	1.40	< 2.00
CD19+ B cells, % of lymphocytes	%	10	5-25
CD4/CD8 ratio		0.5 P ↓	1.1-3.0
CD8- CD4- T cells, % of T cells	%	17.30 P ↑	< 15.00
CD8+ CD4+ T cells, % of T cells	%	0.61	< 10.00
CD3+ T cells	/nl	2.30	0.80-3.50
CD3+ T cells, % of lymphocytes	%	81	60-85
CD4+ T cells	/nl	0.65	0.50-1.20
CD4+ T cells, % of lymphocytes	%	23 P ↓	30-60
CD4+ % T cells	%	28.19	
CD8+ T cells	/nl	1.24 P ↑	0.30-0.80
CD8+ T cells, % of lymphocytes	%	43	20-40
CD8+ % T cells	%	53.89	
Lymphocyte proliferation/function			
PHA, patient	cpm	54479	
PHA, healthy control	cpm	31723	
IL-2, patient	cpm	5795	
IL-2, healthy control	cpm	6015	
anti-CD3, patient	cpm	19591	
anti-CD3, healthy control	cpm	5833	
PWM, patient	cpm	4831	
PWM, healthy control	cpm	5482	

SAC, patient	cpm	1096	
SAC, healthy control	cpm	2003	
Tetanus, patient	cpm	810	
Tetanus, healthy control	cpm	94	
Candida, patient	cpm	1763	
Candida, healthy control	cpm	339	
Diphtherie, patient	cpm	30	
Diphtherie, healthy control	cpm	31	
Medium, patient	cpm	31	
Medium, healthy control	cpm	25	
Tetanus SI, patient		26.1	> 3.0
Tetanus SI, healthy control		3.8	> 3.0
Candida SI, patient		56.9	> 3.0
Candida SI, healthy control		13.6	> 3.0
Diphtherie SI, patient		1.0 P-	> 3.0
Diphtherie SI, healthy control		1.2 P-	> 3.0
PHA SI, patient		1027.9	> 50.0
PHA SI, healthy control		991.3	> 50.0
IL-2 SI, patient		109.3	> 30.0
IL-2 SI, healthy control		188.0	> 30.0
Anti-CD3 SI, patient		369.6	> 30.0
Anti-CD3 SI, healthy control		182.3	> 30.0
PWM SI, patient		91.2	> 20.0
PWM SI, healthy control		171.3	> 20.0
SAC SI, patient		20.7	> 10.0
SAC SI, healthy control		62.6	> 10.0
Medium, patient	cpm	53	
Medium, healthy control	cpm	32	

CD8+ lymphocytosis, relative reduction of CD4+ cells, decreased CD4+/CD8+ ratio; increase of $\gamma\delta$ T cells vs. $\alpha\beta$ T cells; reduced switched mature B cells, delayed B cell maturation.
Normal absolute numbers of CD4+ cells, T cells, B cells, NK cells, monocytes, and granulocytes; normal fraction of CD45RA+ CCR7+ naive T cells; lymphocytes display normal proliferation reactions towards 2/3 recall antigens and alltested T and B cell mitogens compared to a healthy control subject, and no evidence for defective signaling.

Serum autoantibody screening of DSP mutation carrier

Parameter	Unit	Value	Reference range
anti-myosin_IgM	titer	1:160	< 1:40
anti-myosin_IgG	titer	negative	< 1:40
anti-troponin I_IgM	titer	1:80	< 1:40
anti-troponin I_IgG	titer	1:40	< 1:40
antinuclear Ab (ANA)/HEp-2-IF	titer	negative	< 1:160
anti-ds-DNA-Ab/Crithidien-IF	titer	negative	< 1:10

Table S2

Cardiac magnetic resonance imaging (CMR) of brothers heterozygous for DSP truncating mutation Arg1458Ter

	Patient 12		Patient 11	
Date	01-04-2017	04-10-2017	18-05-2017	06-09-2017
LV-EF (%)	59	47	65	64
LV-EDV (ml/m2)	103 (62-102)	104 (62-102)	88 (82-113)	120 (82-113)
LV-ESV (ml/m2)	42 (18-39)	57 (18-39)	29 (9-41)	44 (9-41)
LV myocardial mass (g/m2)	83 (42-98)	73 (42-98)	NA	88 (45-81)
T2 (Edema)	normal 1,6/1,5 (<2,0)	normal 1,8/1,9 (<2,0)	borderline 2,0/2,2 (<2,0)	borderline 2,2/1,8 (<2,0)
Early enhancement	2,8/5,2/5,0 (<5,0)	<5,0	2,9/2,0/3,7 (<5,0)	0,8/0,0 (< 5,0)
Late gadolinium enhancement	subepicardial basal & apical	subepicardial postero-septal & lateral	subepicardial anterior, posterior & septal	subepicardial anterior, posterior & septal
LVEDD (mm)	53 normal	57 enlarged	53 normal	58 enlarged
Dyskinesia	none	postero-septal	none	NA
Focal wall abnormalities	none	none	none	none

EDV = enddiastolic volume; EF = ejection fraction; ESV = endsystolic volume; LV = left ventricle; LVEDD = left ventricular enddiastolic diameter

Table S3

Results of endomyocardial biopsies (EMB) of brothers heterozygous for DSP truncating mutation Arg1458Ter

		Patient 12	**Patient 11**	
Date		30-03-2017	22-05-2017	20-09-2017
Location		RV	RV	RV
Fibrosis		None	Moderate interstitial	Moderate interstitial
Myocyte necrosis		None	None	None
Immunhistochemistry CD3 CD68/MHC II		 Elevated None	 Slightly elevated Slightly elevated	 Normal Moderately elevated
Myocardial viral [§] or other infectious [$] genomes		None	None	None

RV = right ventricle

[§] parvovirus B19 (PVB19), enteroviruses (EV), human herpesviruses (HHV6/7), Epstein-Barr virus (EBV), adenoviruses (AdV2/5), human cytomegalovirus (CMV), herpes simplex viruses (HSV1/2), varicella zoster virus (VZV), mumps virus

[$] Toxoplasma gondii, Borrelia burgdorferi

Table S4

Screening of DSP mutation carriers for systemic or intramyocardial infections

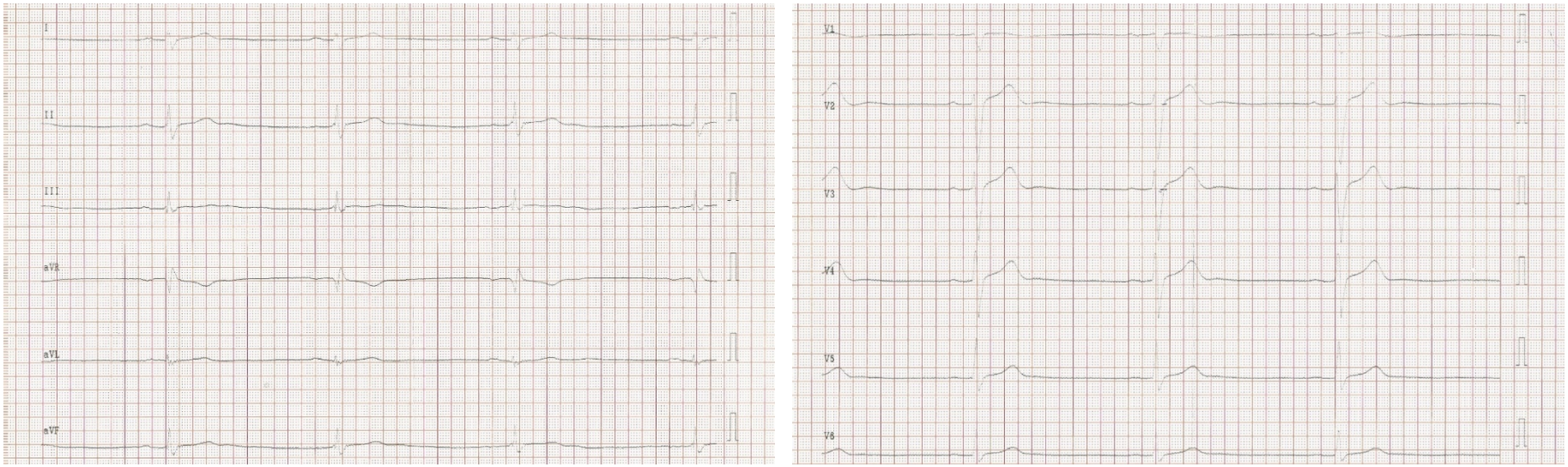
Serum antibodies		
Enterovirus group (EV)	IgG ∅	IgA ∅
Adenovirus group (AdV)	IgG +	IgM ∅
Human herpesvirus 6 (HHV6)	IgG +	IgM ∅
Herpes simplex virus types 1/2 (HSV1/2)	IgG +	IgM ∅
Parvovirus B19 (PVB19)	IgG ∅	IgM ∅
Varicella zoster virus (VZV)	IgG +	IgM ∅
Human Cytomegalovirus (HCMV)	IgG ∅	IgM ∅
Epstein-Barr virus (EBV)	EBNA1, VCA IgG ∅	IgM ∅
Borrelia burgdorferi	IgG ∅	IgM ∅
Chlamydia pneumoniae	IgG ∅	IgM ∅
Mycoplasma pneumoniae	IgG +	IgM ∅
Haemophilus influenzae	IgG + 4.48 IE	

Viral genomes in sputum	
Influenza A virus (H1, H3, H1N1)	Multiplex PCR ∅
Influenza B virus	Multiplex PCR ∅
Respiratory syncytial virus (RSV) types A/B	Multiplex PCR ∅
Coronavirus (HKU1, NL63, OC43)	Multiplex PCR ∅
Parainfluenza virus types 1-4	Multiplex PCR ∅

Nested PCRs on patient PBMCs	
Enteroviridae (RNA)	
Coxsackie group A/B viruses	nPCR ∅
Echoviruses	nPCR ∅
Herpesviridae (dsDNA)	
Herpes simplex virus types 1(2 (HSV1/2)	nPCR ∅
Human herpesvirus type 6/7 (HHV6/7)	nPCR ∅

Epstein-Barr virus (EBV)	nPCR ∅
Cytomegalovirus (HCMV)	nPCR ∅
Varizella zoster virus (VZV)	nPCR ∅
Adenoviridae (dsDNA)	
Adenovirus group (AdV)	nPCR ∅
Parvoviridae (ssDNA)	
Parvovirus B19 (PVB19)	nPCR ∅
Non-viral infectious agents	
Chlamydia pneumonia	nPCR ∅
Toxoplasma gondii	nPCR ∅
Borrelia burgdorferi	nPCR ∅
Eubacteria	nPCR ∅

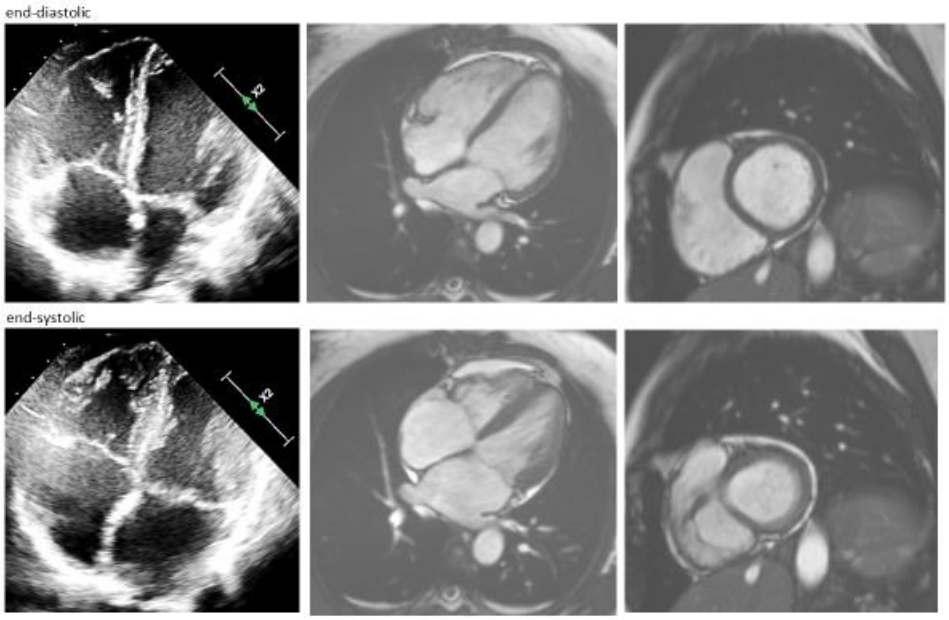
Nested PCR on patient EMBs	
Enteroviridae (RNA)	
Coxsackie group A/B viruses	nPCR ∅
Echoviruses	nPCR ∅
Herpesviridae (dsDNA)	
Herpes simplex virus types 1/2 (HSV1/2)	nPCR ∅
Human herpesvirus type 6 (HHV6)	nPCR ∅
Epstein-Barr virus (EBV)	nPCR ∅
Cytomegalovirus (HCMV)	nPCR ∅
Adenoviridae (dsDNA)	
Adenovirus group (AdV)	nPCR ∅
Parvoviridae (ssDNA)	
Parvovirus B19 (PVB19)	nPCR ∅
Non-viral infectious agents	
Chlamydia pneumonia	nPCR ∅
Toxoplasma gondii	nPCR ∅
Borrelia burgdorferi	nPCR ∅
Eubacteria	nPCR ∅



ECG of patient 11 at admission; 2017-05-15

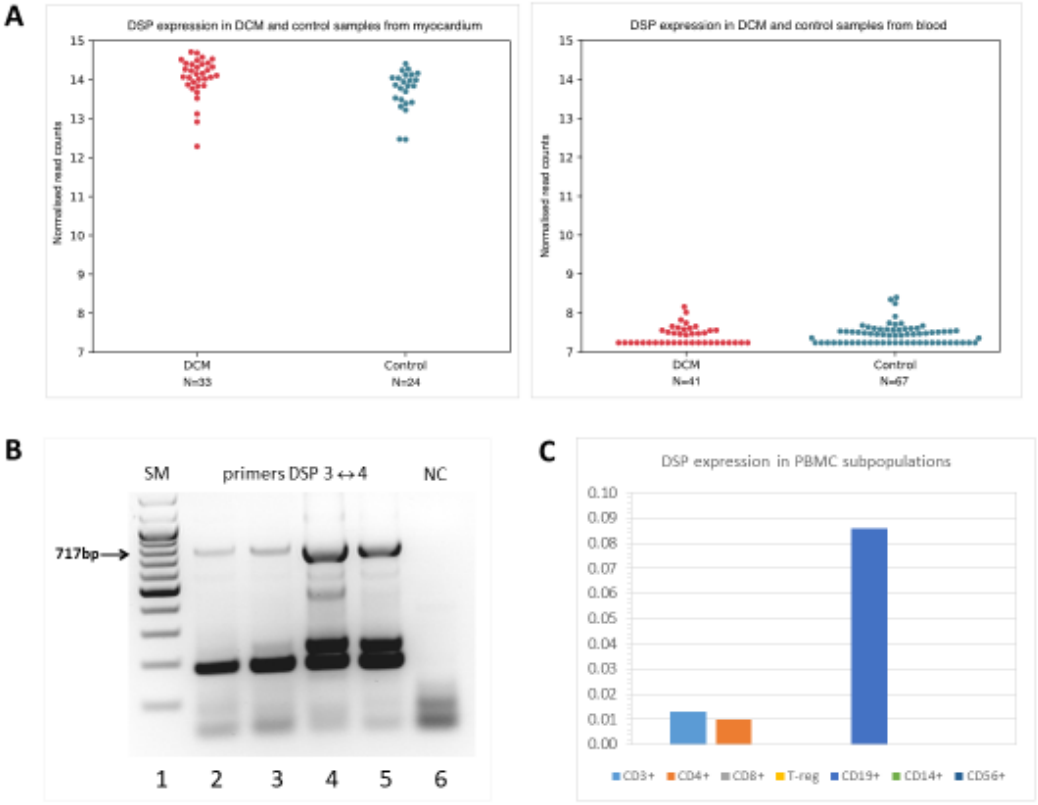
Fig. S1

Figure S2. Echocardiography and cardiac MRI (cMRI) of the mother, patient 2, suffering from recurrent myocarditis.



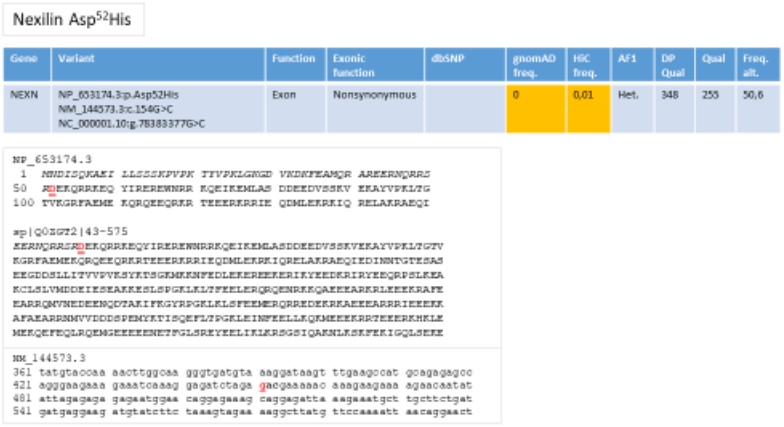
Upper panel from left to right, enddiastolic: echocardiographic 4-chamber view, cMRI 4-chamber view and 2- chamber view. Lower panel from left to right, end-systolic: echocardiographic 4-chamber view, cMRI 4-chamber view and 2- chamber view. Both children and her mother, who has not reported any clinical symptoms or signs resembling those of her sons, or suggesting heart failure or arrhythmias, carry the Arg1458Ter mutation of desmoplakin (DSP).

Figure S3. DSP expression in myocardium and PBMCs of patients and controls.



A The expression of DSP transcripts was tested in left ventricular myocardium and blood from patients with dilated cardiomyopathy (DCM) with RNAseq. DSP is highly expressed in heart tissue. In blood DSP was detected at low levels. No difference could be detected between DCM patients and controls. **B** In PBMCs derived from patient 11 DSP transcripts were detected by PCR. (lane 1: size marker, 100 bp ladder; lanes 2/3: cDNA from PBMCs of patient 11; lane 4: cDNA from PC3 cells; lane 5: cDNA from 293T cells; lane 6: negative control). **C** RNAseq analysis of sorted human PBMC subpopulations suggests that DSP transcripts are restricted to immune cell subtypes $CD3^+$, $CD4^+$ and $CD19^+$.

Figure S4. Heterozygous variant NEXN p.Asp52His.



The nonsynonymous variant NEXN c.154G>C, p.Asp52His was classified as variant of unknown significance (VUS). It is present in both brothers and their mother.