**Supplemental Methods**

*Clinical parameters*

All ECGs were recorded at standard settings (25 mm/s, 10 mm/mV) and digital callipers were used for analysis. SAECG was considered positive when at least one of the three parameters was positive in the absence of QRS duration above 110 ms1. When both CMR and echocardiogram data were available, the results of the former were used to assess for the presence of TFC.1.Left ventricular (LV) dilatation, wall motion abnormalities and systolic dysfunction were defined as per current guidelines2, 3.

*Genetic Testing and Analysis*

Whole exome sequencing (WES) was performed in 138 AC probands, who were previously found negative for desmosomal gene variants. Genomic DNA was extracted from whole blood, paraffin-embedded cardiac tissue and saliva samples using commercially available kits (Qiagen and DNA Genotek). WES was performed utilizing the Agilent SureSelectXT Target Enrichment for Illumina paired-end multiplexed sequencing method and Agilent SureSelectXT Human Exon V5 protocol. Enriched DNA libraries were sequenced on the Illumina NextSeq500 platform as paired-end 75 base reads at a minimum of 30x coverage. Bioinformatic analysis of WES data including copy number variation was carried out with an in-house developed pipeline utilizing standard analysis software including NovoAlign (Novocraft Technologies Sdn Bhd), Picard (<http://picard.sourceforge.net/>) and Genome Analysis Toolkit (GATK) (https://www.broadinstitute.org/gatk/).  HaplotypeCaller 3.1 package in GATK was used for variants calling and generation of a multi-sample joint genotyping. Variant annotation was done with ANNOVAR 4 and Variant Effect Predictor (VEP) tool from Ensembl5.  For *in silico* prediction of pathogenicity of missense variants we used multiple bioinformatics tools, including HumVar-trained PolyPhen-2 model 6, SIFT 7, and MutationTaster 8. Variants were also annotated with minor allele frequencies reported in the Genome Aggregation Database (gnomAD, <http://gnomad.broadinstitute.org/>) 9 and those with a minor allele frequency (MAF) higher or equal to 0.0001 (0.01%) were filtered from further analysis.

*Haplotype Analysis*

﻿In order to investigate whether a common haplotype was shared among families with the p.Leu115Ile variant, genetic data on chromosome 2 astride of the *DES* gene were selected with the NCBI Map Viewer and analyzed for five carriers (A-II-2, A-II-3, A-II-4, A-II-5, B-II-2) from two different families and one gene-negative individual (A-IV-1) on whom we had whole exome data available. In addition, another three variant carriers (B-I-2, C-I-1, C-II-1) and two unaffected family members (A-III-2, C-I-2) were genotyped only for specific SNPs around p.Leu115Ile.

*Histology and Immunohistochemistry*

To determine the effects of *DES* variants on proteins previously implicated in AC pathogenesis in post-mortem cardiac tissue, ventricular myocardial tissue samples from either explanted heart or autopsy material were included in this study. Samples from those cases were fixed in formalin and preserved in paraffin blocks. Cardiac tissue from deceased individuals with no clinical history or pathological evidence of heart disease was used as control. Standard haematoxylin and eosin staining was performed in all patient samples. Immunohistochemical analysis of key proteins previously implicated in the molecular pathogenesis of classic ARVC in myocardial tissue was carried out10. Formalin-fixed, paraffin-embedded tissue sections (5 µm) were deparaffinised, dehydrated, rehydrated and heated in citrate buffer to enhance specific immunostaining. After being cooled to room temperature, the tissue sections were simultaneously permeabilized and blocked by incubation in phosphate-buffered saline (PBS) containing 1% Triton X-100, 3% normal goat serum and 1% bovine serum albumin. The sections were then incubated first with a primary antibody and then with indocarbocyanine-conjugated goat anti-mouse or anti-rabbit rabbit antibodies. Primary antibodies included mouse monoclonal N-cadherin (Sigma-Aldrich, concentration 1:400), rabbit polyclonal Cx43 (Sigma, 1:400), mouse monoclonal plakoglobin (Sigma-Aldrich, 1:1000), mouse monoclonal desmoplakin (Fitzgerald, 1:10), rabbit polyclonal SAP97 (Santa Cruz Biotechnology, 1:50), rabbit polyclonal anti-GSK3β (Cell Signalling Technology, 1:80) and monoclonal anti-DES (AbCam, 1:200). N-cadherin was stained as a housekeeping molecule for quality control purposes. Images were obtained using a Nikon A1R confocal microscope in a 40x magnification.

*Plasmid generation*

The plasmids pET100D-DES, pmRuby-N1-DES, and pmRuby-N1-DES-p.Y122C have been previously described11-13. Specific mutations were inserted into these plasmids using QuikChange Lightning site-directed mutagenesis lit (Agilent Technologies) according to the manufacturer’s instructions. The cDNAs of all generated plasmids were sequenced using Sanger sequencing (Macrogen).

*Cell culture and confocal microscopy*

SW-13 cells (ATCC) were cultured in Dulbecco's modified eagle's medium (DMEM, Thermo Fisher Scientific) supplemented with 10% foetal calf serum and penicillin/streptomycin. Human induced pluripotent stem cells (hiPSC), generated from a healthy donor (NP00040-8, UKKi011-A, European Bank for induced pluripotent Stem Cells, https://ebisc.org/) were kindly provided by Dr. Tomo Saric (University of Cologne). HiPSCs were cultured in Essential 8 medium (Thermo Scientific Fisher) on vitronectin coated cell culture plates. The differentiation into cardiomyocytes was induced by the Wnt signalling agonist CHIR99021 in combination with the GSK3 inhibitor IWP2 (Sigma-Aldrich) as previously described14. SW-13 cells were transfected using Lipofectamin 3000 (Thermo Scientific Fisher) and hiPSC-derived cardiomyocytes were electroporated using the 4D Nucleofector system (CA 137, Lonza) in combination with the P3 Primary Cell 4D-Nucleofector kit (Lonza). 24 h after transfection, after washing with PBS the cells were fixed with 4% paraformaldehyde (10 min) and permeabilized with 0.1% Trition X-100 (15 min). F-actin in transfected SW-13 cells, expressing wild-type or mutant desmin conjugated to the red fluorescent protein mRuby, were stained using phalloidin-Alexa488 (Thermo Scientific Fisher). α-Actinin, as a cardiomyocyte marker, was stained in hiPSC-derived cardiomyocytes using primary antibodies (#7732, Sigma-Aldrich) in combination with secondary anti-mouse-IgG antibodies conjugated to Alexa488 (Thermo Scientific Fisher). Nuclei were stained using 4’,6-diamidin-2-phenylindole (DAPI, 1 µg/mL). Confocal microscopy has been done as previously described13.

*Desmin purification and atomic force microscopy*

Recombinant wild-type and mutant desmin molecules were expressed in *Escherichia coli* (BL21 Star DE3) as previously described11. Ion exchange chromatography using HiTrap DEAE FF columns and immobilized metal affinity chromatography using HisTrap FF crude columns (both GE Healthcare) were applied in combination with the Äkta FPLC system (GE Healthcare) for purification. Recombinant desmin was stored at -80°C. After stepwise dialysis desmin filament formation was induced by adding of sodium chloride (100 mm NaCl, 45 mm Tris-HCl, pH 7.0) and incubation at 37°C for 1 h. Atomic force microscopy was performed as previously described11.

*Statistical analysis*

The statistical analysis was performed using the nonparametric Mann–Whitney test using

GraphPad Prism V8.3 (GraphPad Software). Indicated values represent means ± standard derivation (SD). P-values < 0.05 were considered as significant.

**References**

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