SUPPLEMENTARY MATERIAL

Table S1 Technical information of analysis and interpretation of CES v3

A virtual gene panel for cardiomyopathies (389 genes)	
AARS2, ABCC6, ABCC9, ABHD5, ACAD8, ACAD9, ACADS, ACADVL, ACTA1, ACTC1, ACTN2, ADAR, ADCY5, AGK, AGL, AGPAT2, AHCY, AIP, ALG1, ALMS1, ALPK3, ANK1, ANKRD1, ANKRD11, ANKS6, ANO5, ARSB, ATAD3A, ATPAF2, BAG3, BAZ1B, BBS2, BCS1L, BMP2, BOLA3, BRAF, BRCA1, BRCA2, BRCC3, BRIP1, BSCL2, CALR3, CAP2, CAV1, CAV3, CAVIN1, CDH23, CDKN1C, CHKB, CISD2, CLIP2, CLN3, CLPB, COA5, COA6, COG7, COL7A1, COQ2, COX10, COX14, COX15, COX6B1, COX7B, CPT1A, CPT2, CRYAB, CSRP3, CTNNA3, D2HGDH, DES, DLD, DMD, DMPK, DNAJC19, DOLK, DPM3, DSC2, DSG2, DSP, DTNA, ELAC2, ELN, EMD, ENPP1, EPB42, ERBB3, ERCC2, ERCC3, ERCC4, ERCC6, ERCC8, EYA4, FAH, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FASTKD2, FBXL4, FHL1, FHL2, FIG4, FKRP, FKTN, FLAD1, FLNC, FOXRED1, FTO, FXN, GAA, GABRD, GATA4, GATA5, GATAD1, GBE1, GJA5, GLA, GLB1, GMPPB, GNE, GNPTAB, GNS, GPC3, GPC4, GSN, GTF2H5, GTF2I, GTF2IRD1, GTPBP3, GUSB, GYG1, GYS1, HADH, HADHA, HADHB, HAMP, HAND2, HBB, HCCS, HFE, HGSNAT, HJV, HMGCL, HPS1, HRAS, HSD17B10, IDH2, IDS, IDUA, IFIH1, IGF2, IL12B, INSR, ITGA7, ITPA, JPH2, JUP, KANSL1, KAT6B, KBTBD13, KCNAB2, KCNJ8, KCNQ1, KLF1, KRAS, LAMA2, LAMA3, LAMA4, LAMB3, LAMC2, LAMP2, LDB3, LIAS, LIMK1, LMNA, LTBP4, LZTR1, MAP2K1, MAP2K2, MC2R, MEFV, MEN1, MLYCD, MMACHC, MMP1, MPLKIP, MRAP, MRPL3, MRPL44, MRPS22, MTFMT, MTO1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOCD, MYOM1, MYOT, MYOZ2, MYPN, NAGA, NAGLU, NBAS, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFA2, NDUFA4, NDUFA6, NDUFA9, NDUFAF1, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NEB, NEBL, NEK8, NEU1, NEXN, NF1, NNT, NPPA, NRAS, NUBPL, NUP107, OBSCN, OPA1, PALB2, PCCA, PCCB, PDGFRA, PDHA1, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PGM1, PHKA2, PHKG2, PHYH, PIGT, PKP2, PLN, PMM2, PNPLA2, POLG, POLG2, POMGNT1, POMK, POMT1, POMT2, PPA2, PPARG, PPCS, PPP1CB, PRDM16, PRKAG2, PSEN1, PSEN2, PTPN11, PYGL, PYGM, RAB3GAP2, RAD51, RAD51C, RAF1, RBM20, RERE, RFC2, RIT1, RMND1, RNASEH2A, RNASEH2B, RNASEH2C, RNF113A, RRM2B, RYR1, RYR2, SAMHD1, SARDH, SCN5A, SCO2, SDHA, SDHAF1, SDHB, SDHD, SELENON, SGCA, SGCB, SGCD, SGSH, SHOC2, SKI, SLC19A2, SLC19A3, SLC22A5, SLC25A20, SLC25A3, SLC25A4, SLC2A10, SLC30A10, SLC40A1, SLC4A1, SLX4, SMC1A, SOS1, SOS2, SPEG, SPTA1, SPTB, STAR, SUFU, SURF1, SYNE1, SYNE2, TACO1, TAF1A, TCAP, TERT, TFR2, TGFB1, TGFB3, TK2, TMEM126A, TMEM43, TMEM70, TMPO, TNNC1, TNNI3, TNNI3K, TNNT2, TPM1, TPM2, TPM3, TREX1, TRNT1, TSFM, TTN, TTPA, TTR, TWNK, TXNRD2, UBR1, UQCRFS1, USP9X, VCL, VCP, VHL, VPS13A, WFS1, XK, XRCC2, XRCC4, XYLT1, XYLT2, YARS2	
Methodology (II:6)	
Application:	CES_v3
Sample type:	Blood
Reference genome:	GRCh38/hg38
SOPHiA DDM:	5.10.15--b181-0202a8
Pipeline ID / Revision number / Splitting ID:	ILL1XG1G5_CNV_exome_NextSeq / v5.5.57 / GEN1GN1FSQ2

Quality report (II:6)

Target region coverage

25×	50×	100×	200×	500×	1000×	Coverage 10% quantile	Coverage Heterogeneity
99.38%	97.21%	74.85%	9.98%	0.28%	0.17%	75×	0.84%

Methodology (III:5)

Application:	CES_v3
Sample type:	Blood
Reference genome:	GRCh38/hg38
SOPHiA DDM:	5.10.21--b307-358e31e
Pipeline ID / Revision number / Splitting ID:	ILL1XG1G5_CNV_exome_NextSeq / v5.5.59 / GEN1GN1FSQ2

Quality report (III:5)

Target region coverage

25×	50×	100×	200×	500×	1000×	Coverage 10% quantile	Coverage Heterogeneity
99.37%	98.10%	80.33%	20.67%	0.55%	0.19%	82×	0.96%

The information in the sections listed above for individuals II:6 and III:5 was generated in separate reports by SOPHiA DDM software (available on the request).

Table S2 Primer pairs for Sanger sequencing and qPCR

Sanger sequencing			
exon	**forward primer (5'-3')**	**reverse primer (5'-3')**	**T_a (°C)**
326*	GCAGAGTCCTGGGGTACGTT	TACACGCACCCATCTTAGGC	63
326-330*	TGGAAGGTCACGTCTCTCAA	GACGAGACACTGGGCAATATG	60
quantitative real-time PCR			
exon	**forward primer (5'-3')**	**reverse primer (5'-3')**	**amplicon length (bp)**
326a	GGGGCTCACCAACACCATA	GCCACCTTGCATGGACAATA	129
326d	GTAGCACTTGCACGCAGAAC	GTGGTTGCTCAGGAGAGGAT	86
330	CCATGAAAGGCTGACAGAGTT	GTGCCGCTAATCTGCTTCTC	60
1 (*ERH* gene)	TGGGGAGGGGAAAACGTATG	GCTGCTGTAGCGAAGAGAGT	91
2 (*NEXN* gene)	AGGTGCAAATATATACAGAGCTTCA	GAGACTTACCTCAGCCTTTTGG	90
8 (*SETX* gene)	AGCTTGCGTTGTTGATAATGGT	TGATTCTGGATCGCCTTGGA	87

The amplicons for Sanger sequencing were generated using the specific primer pairs (listed above with *) with additional overhang of known universal primers (UNI-F: TGTAAAACGACGGCCAGT and UNI-R: CAGGAAACAGCTATGACC) at the 5' end of forward and reverse primer, respectively. The UNI-F and UNI-R were then used in the termination reaction for Sanger sequencing.

For quantitative real-time PCR, the primer pairs were designed to amplify DNA regions of a length between 85 and 130 bp and with the annealing temperature (T_a) 60°C. The exon 1 of the *ERH* gene served as an endogenous control and the exon 2 of the *NEXN* gene or the exon 8 of the *SETX* gene served as a control region of diploid state (based on the outputs of CES v3). The overhangs of universal primers were not added to the specific primer sequences for this application.

SUPPLEMENTARY MATERIAL

Detailed clinical information on investigated family members

Individual I:1 (1917-1982) without any detailed clinical information available, died suddenly at the age of 65. No autopsy was done, no molecular genetic analysis done. He had two sisters who died at an advanced age. Their offspring are healthy.

Individual I:2 (1924-2007) was reported as a healthy female. No molecular genetic analysis was done. She had four brothers and two sisters, all of them died at an advanced age. Their offspring are healthy.

Individual II:1 (*1943) is the oldest male of six siblings. He suffers from coronary artery disease without signs of dilated cardiomyopathy (DCM). He underwent coronary artery bypass grafting (CABG) together with aortic valve surgery and repeated percutaneous coronary interventions (PCI). The targeted molecular genetics analyses by real-time PCR and Sanger sequencing excluded the presence of both *TTN* gene deletions. His offspring are reported healthy.

Individual II:2 (*1951) is a female with a mild hypertrophy of the left ventricle and combined aortic valve disease without signs of DCM. The targeted molecular genetics analyses by real-time PCR and Sanger sequencing excluded the presence of both *TTN* gene deletions. One of her sons died of gastric carcinoma (unrelated condition to DCM) at the age of 45, the second son is healthy.

Individual II:3 (1953-1998) received the diagnosis of DCM at the age of 41 years. He suffered from repeated decompensated heart failure states and had documented frequent ventricular ectopy treated with amiodarone at that time. He died of terminal heart failure while being on the waiting list for heart transplant. The molecular genetic analysis was not performed, nevertheless he is an obligatory carrier of the longer intragenic *TTN* deletion disrupting the exon 326. His daughter (III:5) was diagnosed with heart failure at the age of 39 years without previous pregnancies, received the left ventricular assist device (Heart Mate3) for terminal heart failure and underwent heart transplantation one year later. She harbours an out-of-frame deletion NM_001267550.2(TTN):c.80623_85481del in the exon 326 of the *TTN* gene.

Individual II:4 (*1955) is a healthy female. The targeted molecular genetics analyses by real-time PCR and Sanger sequencing excluded the presence of both *TTN* gene deletions. Her daughters are reported as healthy.

Individual II:5 (*1957) was diagnosed with DCM at the age of 66 years. Additionally he has documented persistent atrial fibrillation, now well controlled with amiodarone (CHA_2DS_2-VASc score 2), secondary severe mitral valve regurgitation and signs of pulmonary arterial hypertension aggravate his state. This individual carries an out-of-frame deletion NM_001267550.2(TTN):c.80623_85481del in the exon 326 of the *TTN* gene. His daughter III:9 (*1988) harbours this deletion as well, has no signs of DCM to date. Mild mitral valve regurgitation resulting from morphological Morbus Barlow is documented. The eldest daughter (*1977) and the youngest daughter (*1994) are reported as healthy and do not carry this deletion.

Individual II:6 (*1964) was diagnosed with heart failure at age of 57 years, being on pharmacotherapy he has until now no progress towards terminal heart failure. He harbours the out-of-frame deletion NM_001267550.2(TTN):c.85464_88084del with the breakpoints in exons 326 and 330 of the *TTN* gene.

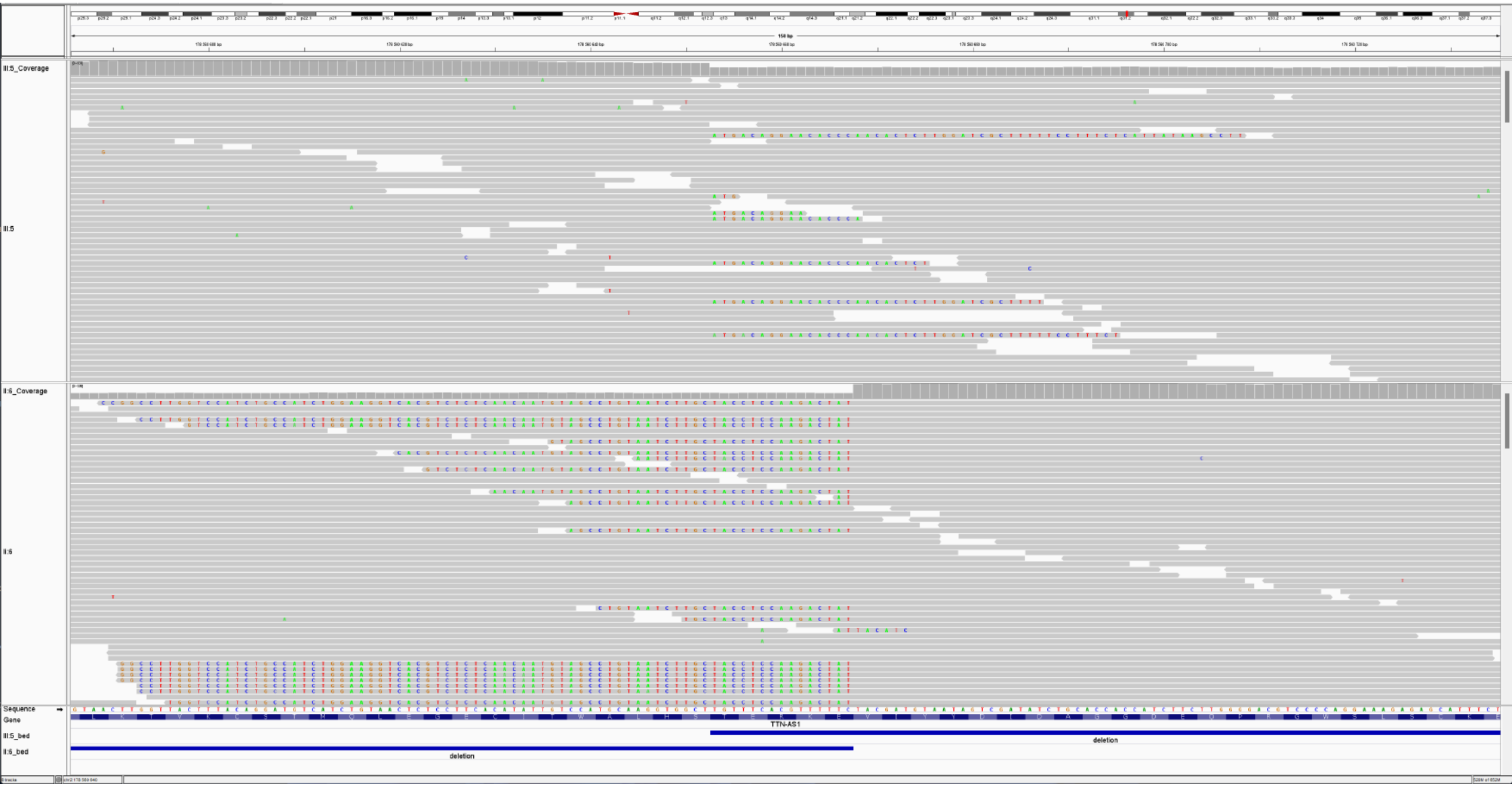
II:5_Coverage
II:5
II:6_Coverage
II:6
Sequence
Gene
TTN-AS1
II:5_bed
deletion
II:6_bed
deletion

Figure S1: **The overlap of two distinct intragenic TTN deletions.** The overlapped 15-bp region of the *TTN* gene deletions is focused in the middle of the figure. The overlap is visualized at the ends of horizontal blue lines (described as III:5_bed and II:6_bed on the left) at the bottom of the figure. The breakpoints are visible as the remarkable decrease of the coverage in the fields above the mapped reads (described as III:5_Coverage, II:6_Coverage on the left).