**SUPPLEMENTAL INFORMATION**

**Targeted panel sequencing in pediatric primary cardiomyopathy supports a critical role of *TNNI3***

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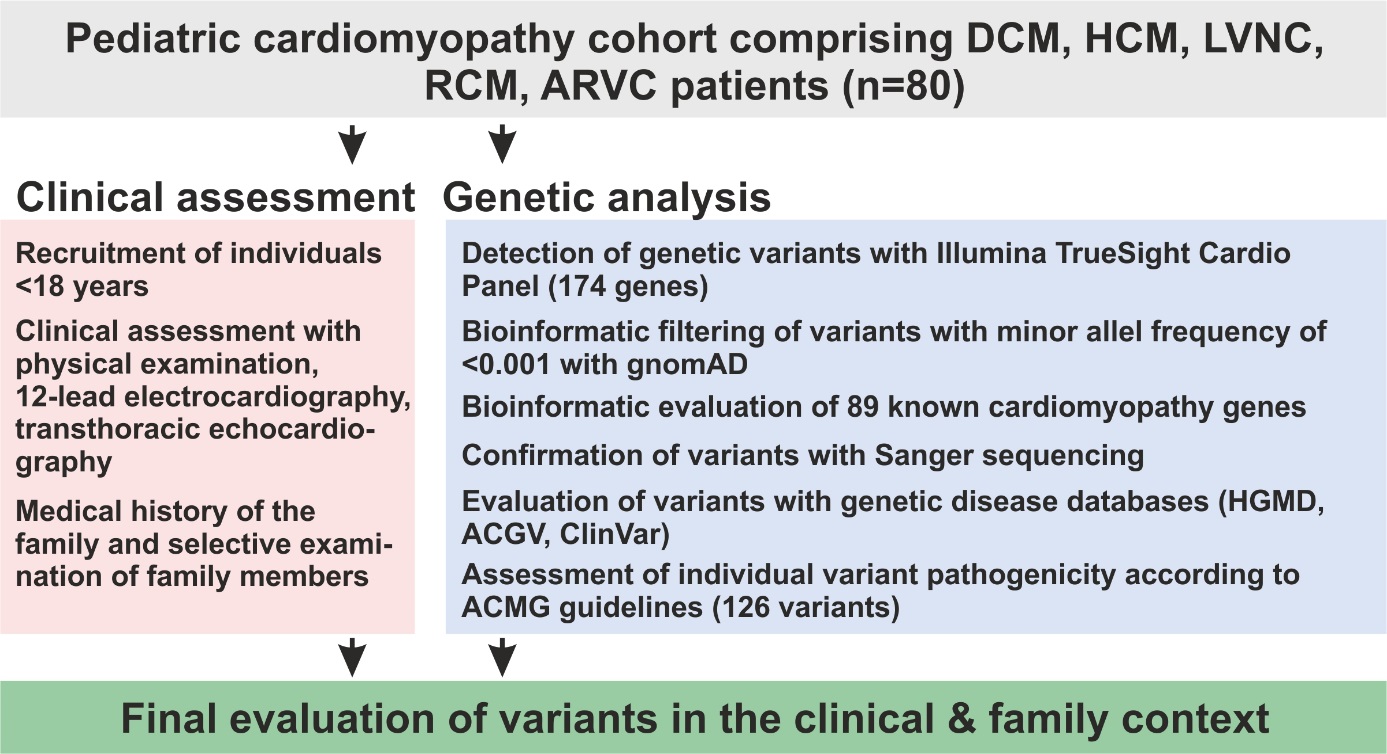
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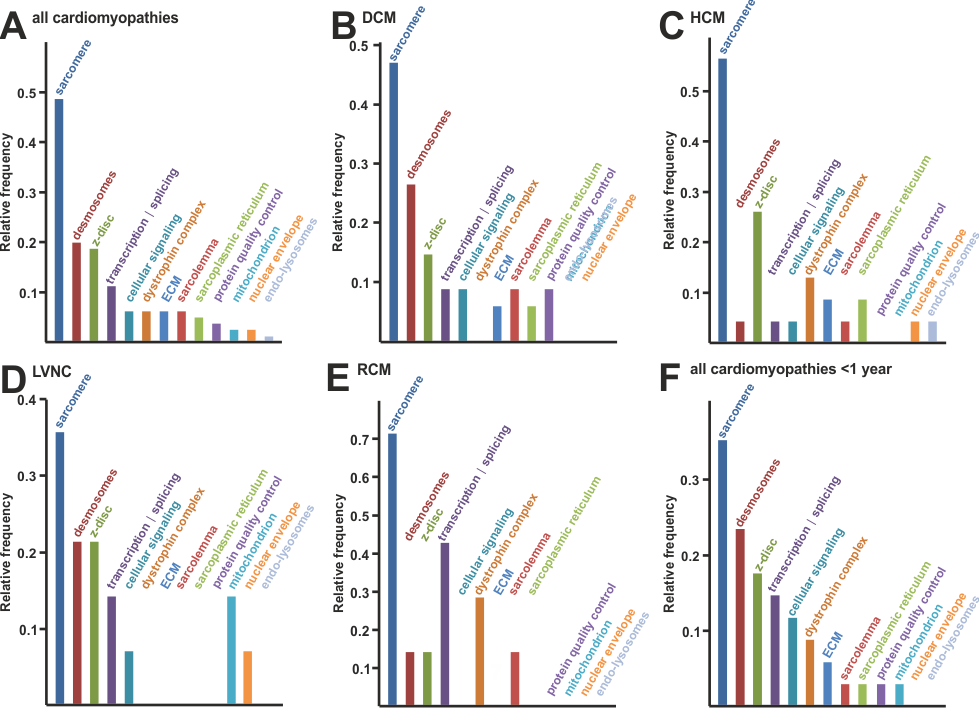
**SUPPLEMENTAL FIGURES S1-S6**

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**Figure S1. Flow chart of study design and genetic evaluation approach.**

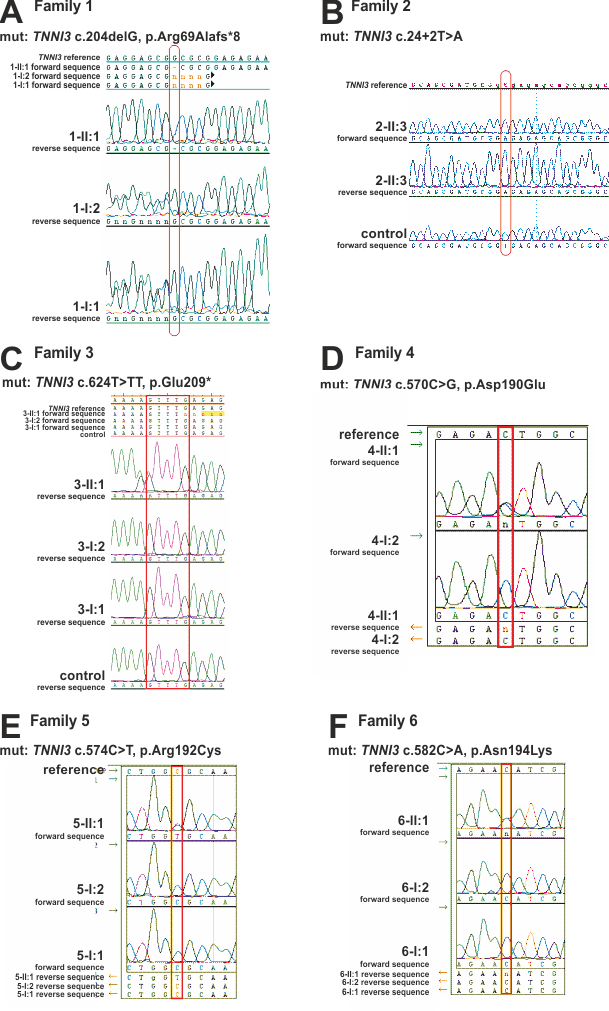
Patients of the cohort were classified for cardiomyopathy (DCM, HCM, LVNC, RCM, and ARVC) after cardiological assessment. DNA samples of index patients were genetically investigated with a NGS CardioPanel approach. Accumulated clinical and genetic information was used for final assessment of detected variants. For interpretation of sequence variants according to ACMG criteria a frequency threshold of <0.0001 was applied.

Abbreviations: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), left ventricular noncompaction cardiomyopathy (LVNC), arrhythmogenic right ventricular cardiomyopathy (ARVC), Genome Aggregation Database (gnomAD), human gene mutation database (HGMD), atlas of cardiac genetic variation (ACGV), National Center for Biotechnology (NCBI) database ClinVar, American College of Medical Genetics (ACMG) guidelines



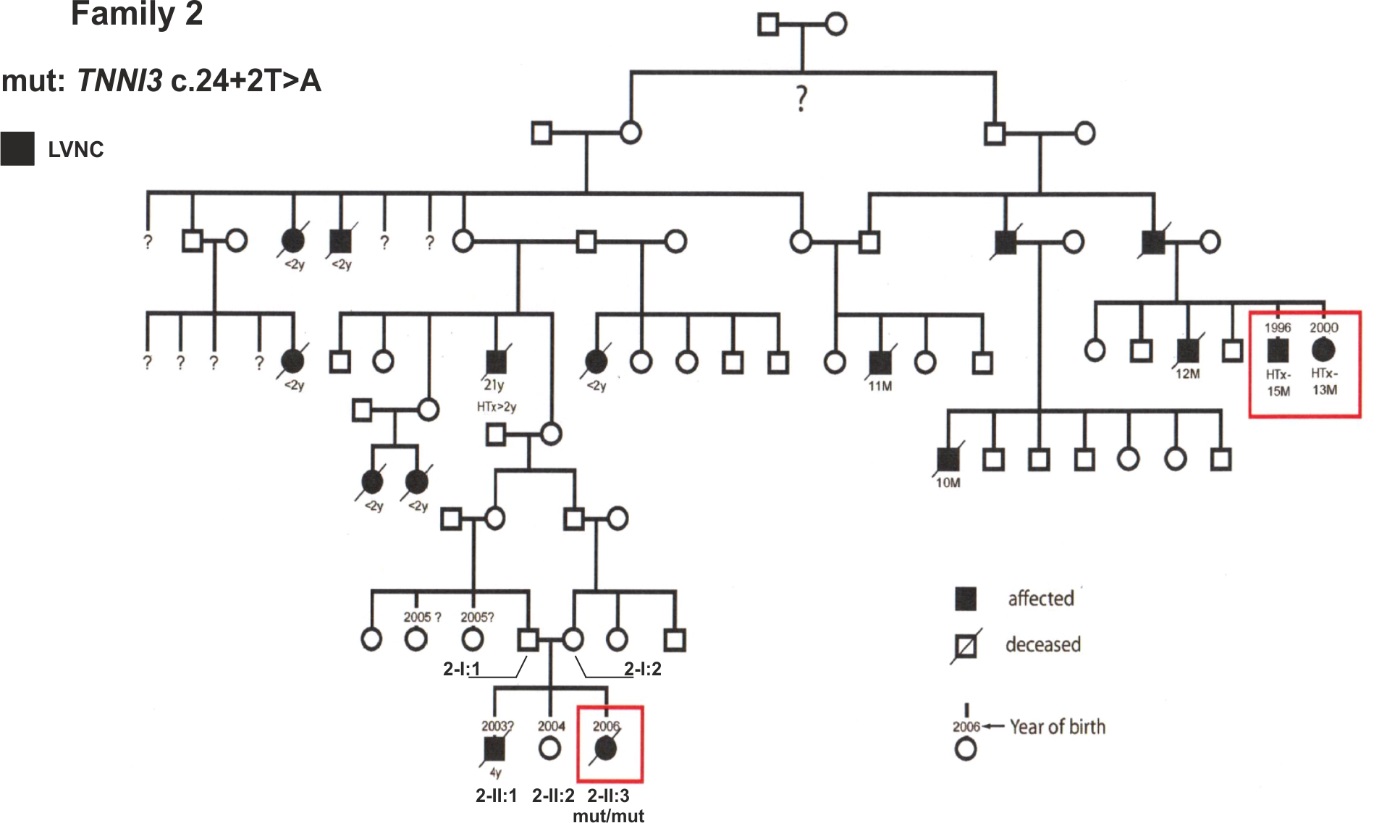
**Figure S2. Relative frequency of patients per functional group.**

**A,** Detected variants were classified according to molecular functional groups (see Table S1). The relative frequency of patients with VOI per functional group is plotted for the entire cohort. **B-E,** The relative frequency of patients with VOI per functional group for CMP subtypes DCM, HCM, LVNC, and RCM. **F,** The relative frequency of patients with VOI per functional group shown for patients with diagnosis of CMP <1 year of age.

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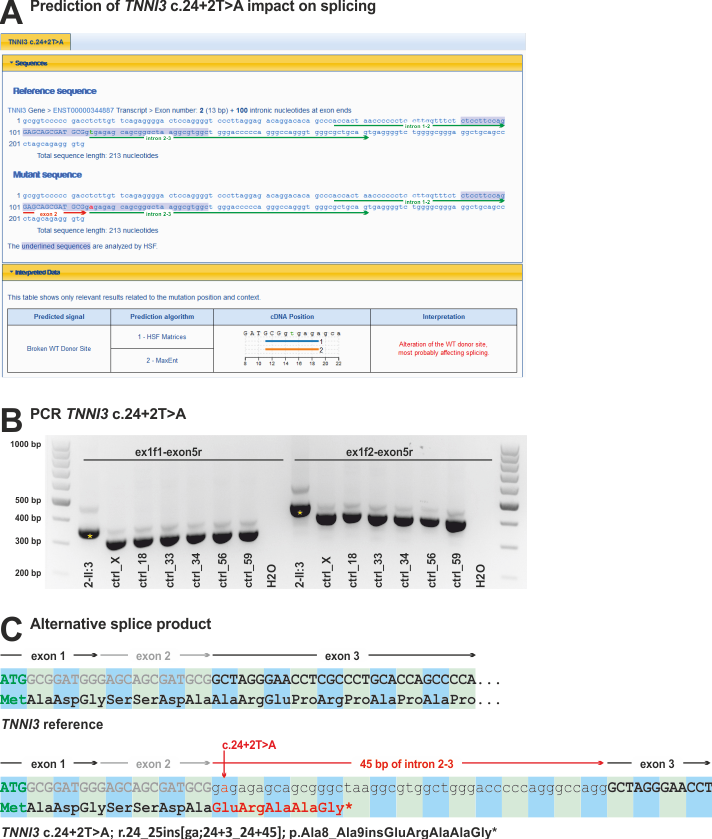
**Figure S3. Molecular genetic analysis of families with hetero- or homozygous *TNNI3* variants.**

**A-F,** Molecular genetic confirmation of *TNNI3* variants in family 1-6 with Sanger sequencing in the index patient and first degree family members, if available. The genetic alteration is highlighted with a red box.

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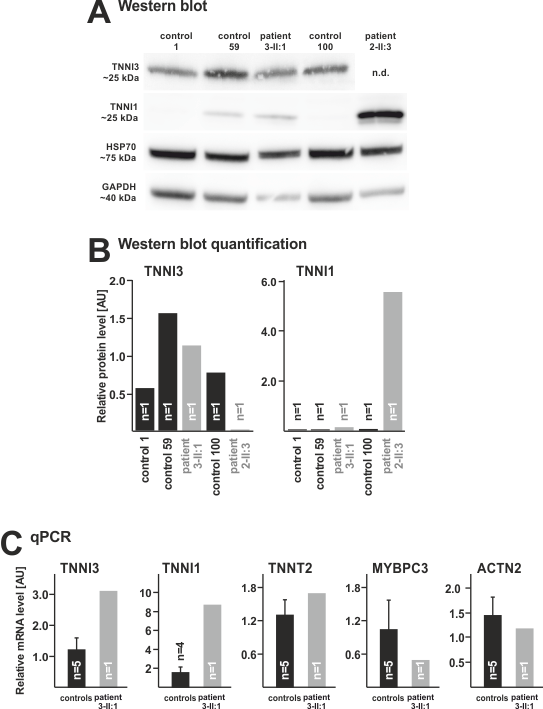
**Figure S4. Extended pedigree of family 2.**

**A,** The complete pedigree of family 2 shows consanguineous marriage of individual 2-I:1 and 2-I:2. Throughout previous generations 14 individuals in total developed early onset CMP leading to childhood death or HTX. None of the other affected or unaffected family members were available for detailed clinical assessment or genetic testing.

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**Figure S5. Molecular analysis of the splice site variant *TNNI3* c.24+2T>A.**

**A,** Analysis of the *TNNI3* variant c.24+2T>A with Human Splicing Finder ([www.umd.be/HSF3](http://www.umd.be/HSF3)) predicts a negative effect of this variant. **B,** The endpoint PCRs ex1f1-exon5r and ex1f2-exon5r were established on cDNA isolated from heart tissue of the patient and controls. Patient 2-II:3 shows for both PCRs an increased fragment size of approx. 50 bp (yellow star) compared to controls. **C,** Sanger sequencing identified an insertion of 45 bp between exon 2 and exon 3. On protein level, this inserts five amino acids GluArgAlaAlaGly\* (TNNI3 p.Ala8\_Ala9insGluArgAlaAlaGly\*) before a stop codon terminates translation.

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**Figure S6. Functional analysis of a heart biopsy from individual 3-II:1 with the heterozygous *TNNI3* variant c.624T>TT, p.Glu209\*.**

**A,** Western blot reveals expression of TNNI3 protein in the 3-II:1 patient heart biopsy and no upregulation of TNNI1 protein. **B,** Quantification of protein levels confirms normal TNNI3 expression in the 3-II:1 patient. TNNI1 protein level did not change in the 3-II:1 heart biopsy but was markedly higher in patient 2-II:3 (homozygous splice site variant *TNNI3* c.24+2T>A). TNNI3 values were adapted from Fig.4C-D. **C,** Quantitative PCR (qPCR) identifies transcriptional upregulation of *TNNI3* and *TNNI1*. The transcript of *MYBPC3* is mildly reduced.

**Supplemental Tables S1-S5**

**Table S1: Primers used in this study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Transcript** | **Primer** | **Primer sequence** | **Length (nucleotides)** |
|  |  |  |  |  |
| **Primer for genomic exon amplification** | | | |  |
| *TNNI3* | NM\_000363.4 | ghTNNI3\_ex2\_f | aagtgggtttgcgagtca | 18 |
|  |  | ghTNNI3\_ex2\_r | ccatcaccaccaagaccc | 18 |
|  |  | ghTNNI3\_ex5\_f | ggagcttgagaatgggtggg | 20 |
|  |  | ghTNNI3\_ex5\_r | gagccaagactccacagacc | 20 |
|  |  | ghTNNI3\_ex8\_f(a) | agatacttaggcatccagggtag | 23 |
|  |  | ghTNNI3\_ex8\_r(a) | acagccaagagtgcttcacat | 21 |
|  |  | ghTNNI3\_ex8\_f(b) | gctactattgacctgagaatcc | 22 |
|  |  | ghTNNI3\_ex8\_r(b) | acagccaagagtgcttcacat | 21 |
|  |  |  |  |  |
| **Primer for cDNA exon amplification for splice site analysis TNNI3 c.24+2T>A** | | | |  |
| *TNNI3* | NM\_000363.4 | chTNNI3\_ex1\_f1 | TCACTGACCCTCCAAACG | 18 |
|  |  | chTNNI3\_ex1\_f2 | GGGAGTCTCAAGCAGCCC | 18 |
|  |  | chTNNI3\_ex5\_r | TGGCAGCGGGTGCTCAGA | 18 |
|  |  |  |  |  |
| **Primer for cDNA amplification with quantitative PCR** | | | |  |
| *TNNI1* | [NM\_003281](http://www.ncbi.nlm.nih.gov/nuccore/NM_003281) | qhTNNI1\_ex5-6\_f | GGATGAGGAGCGATACGACA | 20 |
|  |  | qhTNNI1\_ex5-6\_r | GGCGCTTGAACTTCCCAC | 18 |
| *TNNI3* | NM\_000363.4 | qhTNNI3\_ex3-4\_f | CACCAGCCCCAATCAGACG | 19 |
|  |  | qhTNNI3\_ex3-4\_r | CTGCAATTTTCTCGAGGCGG | 20 |
| *TNNT2* | [NM\_000364](http://www.ncbi.nlm.nih.gov/nuccore/NM_000364) | qhTNNT2\_ex14-15\_f | GAGCTGTGGCAGAGCATCTA | 20 |
|  |  | qhTNNT2\_ex14-15\_r | ATCCTGTTTCGGAGAACATTG | 21 |
| *MYBPC3* | [NM\_000256](http://www.ncbi.nlm.nih.gov/nuccore/NM_000256) | qhMYBPC3\_ex11-12\_f | GCATGAGGCGCGATGAGAAGA | 21 |
|  |  | qhMYBPC3\_ex11-12\_r | CAGCCAGTTCCACGGTCAGC | 20 |
| *ACTN2* | [NM\_001278343](http://www.ncbi.nlm.nih.gov/nuccore/NM_001278343) | qhACTN2\_ex13-14\_f | TTGGAACACCTGGCTGAGA | 19 |
|  |  | qhACTN2\_ex13-14\_r | GCCGACTCGTAATCCTTCTG | 20 |

**Table S2: Functional classification and description of analyzed genes**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Functional classification** | **Gene name** | **Gen** | **Protein** | **Chromo-**  **some** | **Transcript ID** | **Exons** | **Function** |
|  |  |  |  |  |  |  |  |
| **Sarcoplasmic reticulum** | calreticulin 3 | *CALR3* | CALR3 | Chr19 | [ENST00000269881.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000269058;r=19:16479057-16496192;t=ENST00000269881)  [NM\_145046](http://www.ncbi.nlm.nih.gov/nuccore/NM_145046) | 9 | Ca2+ binding protein within SR |
|  | dolichol kinase | *DOLK* | DOLK | Chr9 | [ENST00000372586.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000175283;r=9:128945530-128947619;t=ENST00000372586) [NM\_014908](http://www.ncbi.nlm.nih.gov/nuccore/NM_014908) | 1 | dolichol kinase within SR; involved in GPI-anchor, N- and O-linked protein glycosylation |
|  | phospholamban | *PLN* | PLN | Chr6 | [ENST00000357525.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000198523;r=6:118548298-118560730;t=ENST00000357525) [NM\_002667](http://www.ncbi.nlm.nih.gov/nuccore/NM_002667) | 2 | regulation of cardiac ATP2A2 Ca2+ ATPase, critical regulator Ca2+ homeostasis |
|  | ryanodine receptor 2 | *RYR2* | RYR2 | Chr1 | [ENST00000366574.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000198626;r=1:237042205-237833988;t=ENST00000366574) NM\_001035.2 | 105 | Ca2+ channel releasing Ca2+ from SR into cytoplasm, regulation of cardiomyocyte contractility |
|  |  |  |  |  |  |  |  |
| **Sarcolemma** | ATP binding cassette subfamily C member 9 | *ABCC9* | ABCC9 | Chr12 | [ENST00000261200.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000069431;r=12:21922969-21941402;t=ENST00000261200)  [NM\_020297](http://www.ncbi.nlm.nih.gov/nuccore/NM_020297)  [ENST00000261201.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000069431;r=12:21922969-21941402;t=ENST00000261201) [NM\_005691](http://www.ncbi.nlm.nih.gov/nuccore/NM_005691) | 38 | regulation of KCNJ11 potassium channel |
|  | caveolin 3 | *CAV3* | CAV3 | Chr3 | [ENST00000343849.2](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000182533;r=3:8733800-8746765;t=ENST00000343849) NM\_033337 | 2 | scaffolding protein of caveolae, regulation of G-protein alpha subunits and potassium channels, sarcolemma repair |
|  | hyperpolarization activated cyclic nucleotide gated potassium channel 4 | *HCN4* | HCN4 | Chr15 | [ENST00000261917.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000138622;r=15:73319859-73369264;t=ENST00000261917) NM\_005477.2 | 8 | hyperpolarization-activated ion channel with slow activation and inactivation, exhibiting weak selectivity for potassium over sodium ions, cAMP regulated |
|  | hemochromatosis | *HFE* | HFE | Chr6 | [ENST00000357618.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000010704;r=6:26087405-26094230;t=ENST00000357618)  NM\_000410.3 | 5 | transferrin receptor binding, regulation endocytosis of iron loaded transferrin |
|  | junctophilin-2 | *JPH2* | JPH2 | Chr20 | [ENST00000372980.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000149596;r=20:44176817-44187093;t=ENST00000372980) NM\_020433.4 | 6 | formation of junctional membrane complexes between plasma membrane and SR, regulator of Ca2+ signaling |
|  | sodium channel protein type 5 subunit alpha | *SCN5A* | SCN5A | Chr3 | [ENST00000413689.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000183873;r=3:38548066-38649628;t=ENST00000413689)  NM\_001099404.1 | 28 | mediates voltage-dependent Na+ permeability of excitable membranes |
| **Z-disc** | actinin alpha 2 | *ACTN2* | ACTN2 | Chr1 | [ENST00000366578.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000077522;r=1:236747700-236748140;t=ENST00000366578)  NM\_001103.2 | 21 | F-actin crosslinking protein |
|  | ankyrin repeat domain 1 | *ANKRD1* | ANKRD1 | Chr10 | [ENST00000371697.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000148677;r=10:90912096-90921276;t=ENST00000371697) NM\_014391.2 | 9 | myofibrillar stretch-sensor z-disk, transcription factor that negatively regulates cardiac gene expression |
|  | cysteine and glycine rich protein 3 | *CSRP3* | CSRP3 | Chr11 | [ENST00000533783.1](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000129170;r=11:19182030-19202042;t=ENST00000533783) [NM\_003476](http://www.ncbi.nlm.nih.gov/nuccore/NM_003476) | 7 | scaffolding protein at z-disc, actin binding, regulation of actin dynamics myogenic transcriptional cofactor |
|  | four and a half LIM domains 1 | *FHL1* | FHL1 | ChrX | [ENST00000394155.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000022267;r=X:136204721-136209954;t=ENST00000394155) NM\_001159702.2 | 8 | mechanosensor at z-disc, transcriptional cofactor |
|  | four and a half LIM domains 2 | *FHL2* | FHL2 | Chr2 | [ENST00000344213.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000115641;r=2:105360828-105399061;t=ENST00000344213) NM\_201555.1 | 7 | mechanosensor at z-disc, transcriptional cofactor |
|  | LIM domain binding 3 | *LDB3* | LDB3 | Chr10 | [ENST00000429277.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000122367;r=10:86668692-86734995;t=ENST00000429277) NM\_001171610.1 | 14 | Interaction with actin, stabilization sarcomere during contraction |
|  | myozenin 2 | *MYOZ2* | MYOZ2 | Chr4 | [ENST00000307128.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000172399;r=4:119135784-119187789;t=ENST00000307128) NM\_016599.4 | 6 | z-disc organization by interaction with several proteins, myofibrillogenesis, calcineurin signaling |
|  | myopalladin | *MYPN* | MYPN | Chr10 | [ENST00000358913.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000138347;r=10:68106155-68211699;t=ENST00000358913) [NM\_032578](http://www.ncbi.nlm.nih.gov/nuccore/NM_032578) | 20 | scaffold at z-disc; binds nebulette, nebulin, -actinin |
|  | nexilin F-actin binding protein | *NEXN* | Taz  N | Chr1 | [ENST00000334785.11](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000162614;r=1:77888628-77943224;t=ENST00000334785) NM\_144573.3 | 13 | F-actin binding, maintenance z-disc and sarcomere integrity |
|  | PDZ and LIM domain 3 | *PDLIM3* | PDLIM3 | Chr4 | [ENST00000284770.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000154553;r=4:185500660-185535558;t=ENST00000284770) [NM\_014476](http://www.ncbi.nlm.nih.gov/nuccore/NM_014476) | 7 | crosslinking of F-actin with -actinin-2 |
|  | titin-cap, telethonin | *TCAP* | TCAP | Chr17 | [ENST00000309889.2](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000173991;r=17:39664187-39666555;t=ENST00000309889) [NM\_003673](http://www.ncbi.nlm.nih.gov/nuccore/NM_003673) | 2 | binds titin at z-disc, part of mechanosensing system, important for interaction of z-disc and T-tubules |
|  | vinculin | *VCL* | VCL | Chr10 | [ENST00000211998.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000035403;r=10:74104928-74108975;t=ENST00000211998) NM\_014000.2 | 22 | F-actin binding, mechanosensing, cell-matrix and cell-cell adhesion, linker of z-disc proteins to catenin cadherin dystroglycan |
|  |  |  |  |  |  |  |  |
| **Sarcomere thick filament** | myosin binding protein C, cardiac | *MYBPC3* | MYBPC3 | Chr11 | [ENST00000545968.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000134571;r=11:47331406-47352702;t=ENST00000545968) NM\_000256.3 | 35 | integrates myosin heavy chain and F-actin, modulation of muscle contraction and sarcomere organization |
|  | myosin heavy chain 6 | *MYH6* | MYH6, MHC- | Chr14 | [ENST00000405093.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000197616;r=14:23382019-23407592;t=ENST00000405093) NM\_002471.3 | 39 | major component of cardiac muscle thick filament, muscle contraction, fast myosin isoform, ATPase activity |
|  | myosin heavy chain 7 | *MYH7* | MYH7, MHC- | Chr14 | [ENST00000355349.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000092054;r=14:23412738-23435718;t=ENST00000355349) NM\_000257.2 | 40 | major component of cardiac muscle thick filament, muscle contraction, slow myosin isoform, ATPase activity |
|  | myosin light chain 2 | *MYL2* | MYL2 | Chr12 | [ENST00000228841.12](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000111245;r=12:110910819-110920577;t=ENST00000228841) [NM\_000432](http://www.ncbi.nlm.nih.gov/nuccore/NM_000432).3 | 7 | stabilizes S1 neck region of myosin heavy chain |
|  | myosin light chain 3 | *MYL3* | MYL3 | Chr3 | [ENST00000292327.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000160808;r=3:46857872-46863483;t=ENST00000292327) NM\_000258.2 | 7 | stabilizes S1 neck region of myosin heavy chain |
|  | myosin light chain kinase 2 | *MYLK2* | MYLK2 | Chr20 | [ENST00000375985.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000101306;r=20:31819373-31834689;t=ENST00000375985) NM\_033118.3 | 13 | essential for muscle contraction cycle, phosphorylates specific position of myosin light chain |
|  | titin | *TTN* | titin | Chr2 | [ENST00000589042.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000155657;r=2:178663627-178667307;t=ENST00000589042) NM\_001267550.1 | 363 | central sarcomere scaffolding protein required for assembly, protein interaction platform, regulation of sarcomere resting length and passive stiffness |
| **Sarcomere thin filament** | actin, alpha 1, skeletal muscle | *ACTA1* | ACTA1 | Chr1 | [ENST00000366684.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000143632;r=1:229431249-229434098;t=ENST00000366684) [NM\_001100](http://www.ncbi.nlm.nih.gov/nuccore/NM_001100) | 6 | globular G-actin form F-actin fibers; essential part of the contractile apparatus thin filament |
|  | actin, alpha, cardiac muscle 1 | *ACTC1* | ACTC1 | Chr15 | [ENST00000290378.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000159251;r=15:34788096-34796139;t=ENST00000290378) NM\_005159.4 | 7 | globular G-actin form F-actin fibers; essential part of the contractile apparatus thin filament |
|  | troponin C1, slow skeletal and cardiac type | *TNNC1* | TNNC1 | Chr3 | [ENST00000232975.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000114854;r=3:52451415-52452834;t=ENST00000232975) NM\_003280.2 | 6 | binds the switch region of troponin I in a Ca2+ dependent manner to activate contraction |
|  | troponin I3, cardiac type | *TNNI3* | TNNI3 | Chr19 | [ENST00000344887.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000129991;r=19:55151770-55157732;t=ENST00000344887) NM\_000363.4 | 8 | inhibitory subunit of troponin, regulation thin filament Ca2+ sensitivity |
|  | troponin T2, cardiac type | *TNNT2* | TNNT2 | Chr1 | [ENST00000236918.11](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000118194;r=1:201359008-201377680;t=ENST00000236918) [NM\_001276345](http://www.ncbi.nlm.nih.gov/nuccore/NM_001276345) | 16 | binding of troponin complex to tropomyosin |
|  | tropomyosin 1 | *TPM1* | TPM1 | Chr15 | [ENST00000403994.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000140416;r=15:63042632-63066093;t=ENST00000403994) NM\_001018005.1 | 9 | coiled coil protein that lies along thin filament and blocks myosin binding sites on actin under resting calcium concentrations |
| **Nuclear envelope** | emerin | *EMD* | EMD | ChrX | [ENST00000369842.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000102119;r=X:154379625-154381281;t=ENST00000369842) [NM\_000117](http://www.ncbi.nlm.nih.gov/nuccore/NM_000117) | 6 | formation and stabilization of cortical nuclear actin network, cellular signaling |
|  | lamin A/C | *LMNA* |  | Chr1 | [ENST00000368300.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000160789;r=1:156082600-156083613;t=ENST00000368300) NM\_170707.3 | 12 | nuclear lamina and chromatin organization, critical for nuclear dynamics |
|  | transmembrane protein 43 | *TMEM43* | TMEM43 | Chr3 | [ENST00000306077.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000170876;r=3:14124940-14143679;t=ENST00000306077) NM\_024334.2 | 12 | nuclear envelope structure at inner nuclear membrane, interaction with emerin, mechanotransduction, gene expression |
|  |  |  |  |  |  |  |  |
| **Mitochondria** | cytochrome c oxidase assembly homolog | *COX15* | COX15 | Chr10 | [ENST00000370483.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000014919;r=10:99711844-99732100;t=ENST00000370483) [NM\_001320975](http://www.ncbi.nlm.nih.gov/nuccore/NM_001320975) | 9 | heme A biosynthesis, porphyrin synthesis |
|  | DnaJ heat shock protein family (Hsp40) member C19 | *DNAJC19* | DNAJC19 TIM14 | Chr3 | [ENST00000382564.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000205981;r=3:180984278-180989710;t=ENST00000382564)  [NM\_145261](http://www.ncbi.nlm.nih.gov/nuccore/NM_145261) | 6 | peptide translocation inner membrane to matrix, subunit of HSP40/HSP70 complex, mitochondrial chaperone |
|  | frataxin | *FXN* | FXN | Chr9 | [ENST00000377270.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000165060;r=9:69035259-69074213;t=ENST00000377270)  [NM\_000144](http://www.ncbi.nlm.nih.gov/nuccore/NM_000144) | 5 | heme biosynthesis, protection against iron-catalyzed oxidative stress, iron storage |
|  | hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit | *HADHA* | HADHA | Chr2 | [ENST00000380649.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000084754;r=2:26191335-26195169;t=ENST00000380649) [NM\_000182](http://www.ncbi.nlm.nih.gov/nuccore/NM_000182) | 20 | f[atty acid beta-oxidation](http://www.uniprot.org/uniprot/?query=organism:9606+pathway:436.659) and in [lipid metabolism](http://www.uniprot.org/uniprot/?query=organism:9606+pathway:436). |
|  | cytochrome c oxidase assembly protein | *SCO2* | SCO2 | Chr22 | [ENST00000252785.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000130489;r=22:50523568-50525606;t=ENST00000252785)  [NM\_001169111](http://www.ncbi.nlm.nih.gov/nuccore/NM_001169111) | 2 | copper chaperone, delivering copper to COX2 |
|  | succinate dehydrogenase complex flavoprotein subunit A | *SDHA* | SDHA, SDH2 | Chr5 | [ENST00000264932.10](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000073578;r=5:218241-256700;t=ENST00000264932) [NM\_004168](http://www.ncbi.nlm.nih.gov/nuccore/NM_004168) | 15 | complex II mitochondrial electron transport chain, transfer of electrons from succinate to ubiquinone, involved tricarboxylic acid cycle |
|  | tafazzin | *TAZ* | TAZ | ChrX | [ENST00000601016.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000102125;r=X:154418475-154421722;t=ENST00000601016) [NM\_000116](http://www.ncbi.nlm.nih.gov/nuccore/NM_000116) | 11 | synthesis/remodeling of cardiolipin, [phospholipid](https://en.wikipedia.org/wiki/Phospholipid)-[lysophospholipid](https://en.wikipedia.org/wiki/Lysophospholipid" \o "Lysophospholipid) [transacylase](https://en.wikipedia.org/wiki/Transacylase), important for mitochondrial energy production |
|  |  |  |  |  |  |  |  |
| **Desmosome** | desmocollin 2 | *DSC2* | DSC2 | Chr18 | [ENST00000280904.10](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000134755;r=18:31058840-31102415;t=ENST00000280904)  [NM\_024422](http://www.ncbi.nlm.nih.gov/nuccore/NM_024422) | 16 | part of desmosome junctions, cell-cell adhesion, interaction intermediate filaments |
|  | desmoglein 2 | *DSG2* | DSG2 | Chr18 | [ENST00000261590.12](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000046604;r=18:31498043-31549008;t=ENST00000261590) NM\_001943.3 | 15 | part of desmosome junctions, cell-cell adhesion, interaction intermediate filaments |
|  | desmoplakin | *DSP* | DSP | Chr6 | [ENST00000379802.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000096696;r=6:7541575-7586717;t=ENST00000379802) NM\_004415.2 | 24 | anchoring of intermediate filaments to desmosomes, essential for desmosomal plaque formation |
|  | plakophilin 2 | *PKP2* | PKP2 | Chr12 | [ENST00000070846.10](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000057294;r=12:32790745-32896840;t=ENST00000070846) NM\_004572.3 | 14 | links cadherins to intermediate filaments, mechanical stabilization desmosomes, signaling function |
|  | junction plakoglobin | *JUP* | JUP | Chr17 | [ENST00000393931.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000173801;r=17:41754604-41786698;t=ENST00000393931) NM\_002230.2 | 14 | cytoplasmic component of desmosomes, linker of cadherins to F-actin |
|  |  |  |  |  |  |  |  |
| **Regulation of transcription and splicing** | EYA transcriptional coactivator and phosphatase 4 | *EYA4* | EYA4 | Chr6 | [ENST00000355167.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000112319;r=6:133241351-133532120;t=ENST00000355167) NM\_172105.3 | 20 | tyrosine phosphatase of histone H2AX; impact for DNA repair, stress response and apoptosis |
|  | GATA zinc finger domain containing 1 | *GATAD1* | GATAD1 | Chr7 | [ENST00000287957.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000157259;r=7:92449718-92457161;t=ENST00000287957) [NM\_021167](http://www.ncbi.nlm.nih.gov/nuccore/NM_021167) | 5 | part of chromatin complex recruited to methylated H2K4me |
|  | NK2 homeobox 5 | *NKX2-5* | NKX2-5 | Chr5 | [ENST00000329198.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000183072;r=5:173232109-173235357;t=ENST00000329198) NM\_004387.3 | 2 | transcriptional activation of myocardial lineage together with GATA4, transcriptionally controlled by PBX1 |
|  | PR/SET domain 16 | *PRDM16* | PRDM16, MEL1 | Chr1 | [ENST00000270722.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000142611;r=1:3069211-3438621;t=ENST00000270722) [NM\_022114](http://www.ncbi.nlm.nih.gov/nuccore/NM_022114).3 | 17 | transcriptional regulator of adipocyte development, interaction e.g. PPAR, C/EBP and PGC1, regulator of TGF- signaling, has histone methyltransferase activity (H3K9me1, H3K9me3) |
|  | RNA binding motif protein 20 | *RBM20* | RBM20 | Chr10 | [ENST00000369519.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000203867;r=10:110644397-110839469;t=ENST00000369519) [NM\_001134363](http://www.ncbi.nlm.nih.gov/nuccore/NM_001134363).1 | 14 | mRNA splicing regulator of a specific target genes, important regulator of TTN slicing |
|  | T-box 20 | *TBX20* | TBX20 | Chr7 | [ENST00000408931.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000164532;r=7:35231500-35253631;t=ENST00000408931) NM\_001077653.2 | 8 | transcriptional activator/repressor in cardiac development, interacts with GATA4 and NKX2-5, repression of TBX2 |
|  |  |  |  |  |  |  |  |
| **Dystrophin complex** | desmin | *DES* | DES | Chr2 | [ENST00000373960.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000175084;r=2:219420122-219423843;t=ENST00000373960) [NM\_001927](http://www.ncbi.nlm.nih.gov/nuccore/NM_001927).3 | 9 | intermediate filament; connecting z-discs, sarcomere, sarcolemmal cytoskeleton, nucleus and mitochondria; sarcomeric microtubule anchor |
|  | dystrophin | *DMD* | DMD | ChrX | [ENST00000357033.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000198947;r=X:31932116-33211554;t=ENST00000357033) NM\_004006.2 | 79 | anchors ECM to F-actin cytoskeleton, ligand dystroglycan |
|  | dystrobrevin alpha | *DTNA* | DTNA | Chr18 | [ENST00000444659.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000134769;r=18:34493290-34891844;t=ENST00000444659) [NM\_001390](http://www.ncbi.nlm.nih.gov/nuccore/NM_001390).4 | 22 | interacts with dystrophin |
|  | sarcoglycan beta | *SGCB* | SGCB | Chr4 | [ENST00000381431.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000163069;r=4:52020706-52038482;t=ENST00000381431) [NM\_000232](http://www.ncbi.nlm.nih.gov/nuccore/NM_000232).4 | 6 | linker between F-actin and ECM |
|  | sarcoglycan delta | *SGCD* | SGCD | Chr5 | [ENST00000435422.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000170624;r=5:156326757-156759604;t=ENST00000435422) NM\_000337.5 | 8 | linker between F-actin and ECM |
|  | sarcoglycan gamma | *SGCG* | SGCG | Chr13 | [ENST00000218867.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000102683;r=13:23180952-23325165;t=ENST00000218867)  [NM\_000231](http://www.ncbi.nlm.nih.gov/nuccore/NM_000231) | 8 | linker between F-actin and ECM |
|  |  |  |  |  |  |  |  |
| **Cellular signaling** | B-Raf proto-oncogene, serine/threonine kinase | *BRAF* | BRAF | Chr7 | [ENST00000288602.10](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000157764;r=7:140719327-140783157;t=ENST00000288602) [NM\_004333](http://www.ncbi.nlm.nih.gov/nuccore/NM_004333).4 | 18 | phosphorylates MAP2K1, part of MAPK signaling pathway, transmission of mitogenic signals |
|  | CBL proto-oncogene | *CBL* | CBL | Chr11 | [ENST00000264033.5](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000110395;r=11:119206276-119308149;t=ENST00000264033) [NM\_005188](http://www.ncbi.nlm.nih.gov/nuccore/NM_005188).3 | 16 | E3 ubiquitin-protein ligase, ubiquitination leads to proteasomal degradation, negative regulator of cell surface receptors e.g. FGFR1/2, EGFR, KIT |
|  | HRAS proto-oncogene, GTPase | *HRAS* | HRAS | Chr11 | [ENST00000610977.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000276536;r=CHR_HSCHR11_1_CTG8:532242-537287;t=ENST00000610977) [NM\_001130442](http://www.ncbi.nlm.nih.gov/nuccore/NM_001130442) | 5 | small GTPase, RAS signaling pathway, growth factor signaling |
|  | KRAS proto-oncogene, GTPase | *KRAS* | KRAS | Chr12 | [ENST00000311936.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000133703;r=12:25204789-25250931;t=ENST00000311936) [NM\_004985](http://www.ncbi.nlm.nih.gov/nuccore/NM_004985) | 5 | small GTPase, RAS signaling pathway, growth factor signaling, regulating cell proliferation, critical for tumor development |
|  | mitogen-activated protein kinase kinase 1 | *MAP2K1* | MAP2K1 MEK1 | Chr15 | |  | | --- | |  |   [ENST00000307102.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000169032;r=15:66386817-66492312;t=ENST00000307102) [NM\_002755](http://www.ncbi.nlm.nih.gov/nuccore/NM_002755) | 11 | essential component of MAPK pathway, growth factor signaling, regulating diverse cellular functions (growth, survival, proliferation), upstream activated by RAF1, downstream activation of ERK1/2 |
|  | mitogen-activated protein kinase kinase 2 | *MAP2K2* | MAP2K2 MEK2 | Chr19 | [ENST00000262948.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000126934;r=19:4090321-4124129;t=ENST00000262948) [NM\_030662](http://www.ncbi.nlm.nih.gov/nuccore/NM_030662) | 11 | essential component of MAPK pathway, growth factor signaling, regulating diverse cellular functions (growth, survival, proliferation), upstream activated by RAF1, downstream activation of ERK1/2 |
|  | mindbomb E3 ubiquitin protein ligase 1 | *MIB1* | MIB1 | Chr18 | [ENST00000261537.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000101752;r=18:21741320-21870957;t=ENST00000261537) [NM\_020774](http://www.ncbi.nlm.nih.gov/nuccore/NM_020774) | 21 | ubiquitination of Delta receptors, positively regulates the Delta-mediated Notch signaling |
|  | NRAS proto-oncogene, GTPase | *NRAS* | NRAS | Chr1 | [ENST00000369535.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000213281;r=1:114704469-114716894;t=ENST00000369535) [NM\_00252](http://www.ncbi.nlm.nih.gov/nuccore/NM_002524)4 | 7 | small GTPase, RAS signaling pathway, growth factor signaling, regulating cell proliferation, critical for tumor development |
|  | protein kinase AMP-activated non-catalytic subunit gamma 2 | *PRKAG2* | PRKAG2 | Chr7 | [ENST00000287878.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000106617;r=7:151568694-151876848;t=ENST00000287878) [NM\_016203](http://www.ncbi.nlm.nih.gov/nuccore/NM_016203).3 | 16 | AMP/ATP-binding subunit of AMPK, regulation of cellular energy metabolism by sensing ATP levels |
|  | protein tyrosine phosphatase, non-receptor type 11 | *PTPN11* | PTPN11 | Chr12 | [ENST00000635625.1](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000179295;r=12:112418914-112509913;t=ENST00000635625) [NM\_001330437](http://www.ncbi.nlm.nih.gov/nuccore/NM_001330437) | 15 | regulator unfolded protein response in ER, dephosphorylation EIF2AK3/PERK, tumor development |
|  | Raf-1 proto-oncogene, serine/threonine kinase | *RAF1* | RAF1 | Chr3 | [ENST00000251849.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000132155;r=3:12600199-12608867;t=ENST00000251849) [NM\_002880](http://www.ncbi.nlm.nih.gov/nuccore/NM_002880).3 | 17 | serine/threonine-protein kinase linking membrane-associated RAS GTPases with MAPK signaling, key regulator of cell fate decision |
|  | SHOC2, leucine rich repeat scaffold protein | *SHOC2* | SHOC2 | Chr10 | [ENST00000369452.8](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000108061;r=10:110919603-111011670;t=ENST00000369452) NM\_007373.3 | 9 | regulatory subunit of protein phosphatase complex 1, activation RAF1 kinase, MAPK signaling pathway activation, growth factor signaling |
|  | SOS Ras/Rac guanine nucleotide exchange factor 1 | *SOS1* | SOS1 | Chr2 | [ENST00000402219.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000115904;r=2:38981553-39120463;t=ENST00000402219) [NM\_005633](http://www.ncbi.nlm.nih.gov/nuccore/NM_005633).3 | 23 | guanine nucleotide exchange factor (GEF) for RAS GTPases, promotion of RAS activation, regulates MAPK3 phosphorylation |
|  | transforming growth factor beta 3 | *TGFB3* | TGFB3 | Chr14 | [ENST00000238682.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000119699;r=14:75958202-75981191;t=ENST00000238682) [NM\_003239](http://www.ncbi.nlm.nih.gov/nuccore/NM_003239).2 | 7 | regulates mesenchymal development, activates TGFBR2, regulates cell adhesion ECM formation |
|  |  |  |  |  |  |  |  |
| **Protein quality control** | BCL2 associated athanogene 3 | *BAG3* | BAG3 | Chr10 | [ENST00000369085.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000151929;r=10:119651370-119677819;t=ENST00000369085) [NM\_004281](http://www.ncbi.nlm.nih.gov/nuccore/NM_004281).3 | 4 | activator of autophagy and HSP70 chaperone system, nucleotide exchange factor HSP70 complex, adaptor protein |
|  | crystallin alpha B | *CRYAB* | CRYAB HSPB5 | Chr11 | [ENST00000616970.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000109846;r=11:111908629-111912010;t=ENST00000616970) NM\_001885.1 | 4 | detection of misfolded proteins, targeting to the HSP70 complex, prevention of protein aggregate formation, cardioprotective, stabilization of cyto- and nucleoskeleton, stress inducible |
|  | heat shock protein family B (small) member 8 | *HSPB8* | HSP22 HSPB8 | Chr12 | [ENST00000281938.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000152137;r=12:119178642-119194748;t=ENST00000281938) [NM\_014365](http://www.ncbi.nlm.nih.gov/nuccore/NM_014365).2 | 3 | detection of misfolded proteins, induction of autophagy, prevention of protein aggregate formation, chaperone activity, disassembly of stress granules with BAG3/HSP70 complex |
|  |  |  |  |  |  |  |  |
| **ECM components** | fibrillin 1 | *FBN1* | FBN1 | Chr15 | [ENST00000316623.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000166147;r=15:48408306-48645849;t=ENST00000316623) [NM\_000138](http://www.ncbi.nlm.nih.gov/nuccore/NM_000138).4 | 66 | structural component of 10-12 nm diameter ECM microfibrils, structural and regulatory function in load-bearing connective tissue, regulation TGF- and BMP signaling |
|  | laminin subunit alpha 2 | *LAMA2* | LAMA2 | Chr6 | [ENST00000421865.2](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000196569;r=6:129490736-129492450;t=ENST00000421865) [NM\_000426](http://www.ncbi.nlm.nih.gov/nuccore/NM_000426).3 | 65 | ECM glycoprotein, mediates interaction to dystrophin complex |
|  | laminin subunit alpha 4 | *LAMA4* | LAMA4 | Chr6 | [ENST00000230538.11](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000112769;r=6:112253361-112254588;t=ENST00000230538) [NM\_001105206](http://www.ncbi.nlm.nih.gov/nuccore/NM_001105206).2 | 39 | ECM glycoprotein, constituent of basement membrane |
|  |  |  |  |  |  |  |  |
| **Glycosyl- transferases** | fukutin | *FKTN* | FKTN | Chr9 | [ENST00000223528.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000106692;r=9:105558141-105596730;t=ENST00000223528) NM\_006731 | 10 | biosynthesis of phosphorylated O-mannosyl trisaccharide, required for -dystroglycan synthesis, protein glycosylation |
|  | fukutin related protein | *FKRP* | FKRP | Chr19 | [ENST00000318584.9](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000181027;r=19:46746046-46758485;t=ENST00000318584) [NM\_001039885](http://www.ncbi.nlm.nih.gov/nuccore/NM_001039885) | 4 | biosynthesis of phosphorylated O-mannosyl trisaccharide, required for -dystroglycan synthesis, protein glycosylation |
|  |  |  |  |  |  |  |  |
| **Cytoskeleton** | Alstrom syndrome protein 1 | *ALMS1* | ALMS1 | Chr2 | |  |  | | --- | --- | |  | [ENST00000613296.4](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000116127;r=2:73603299-73610793;t=ENST00000613296) |   [NM\_015120](http://www.ncbi.nlm.nih.gov/nuccore/NM_015120).4 | 23 | centrosome and basal body associated protein, pericentriolar and cilia transport, interaction -actinin, endosomal transport |
|  |  |  |  |  |  |  |  |
| **Endo-lysosomal system** | glucosidase alpha, acid | *GAA* | GAA | Chr17 | [ENST00000302262.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000171298;r=17:80101704-80119879;t=ENST00000302262) [NM\_000152](http://www.ncbi.nlm.nih.gov/nuccore/NM_000152) | 20 | hydrolysis of lysosomal glycogen to glucose |
|  | galactosidase alpha | *GLA* | GLA | ChrX | [ENST00000218516.3](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000102393;r=X:101397803-101407908;t=ENST00000218516) [NM\_000169](http://www.ncbi.nlm.nih.gov/nuccore/NM_000169) | 7 | hydrolysis of -D-galactose residues in -D-galactosides |
|  | lysosomal associated membrane protein 2 | *LAMP2* | LAMP2 CD107b | ChrX | [ENST00000434600.6](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000005893;r=X:120436494-120469306;t=ENST00000434600)  [NM\_001122606](http://www.ncbi.nlm.nih.gov/nuccore/NM_001122606).1 | 9 | binding of target protein and delivery to lysosomes, protein degradation, required fusion autophagosomes and lysosomes, antigen presentation |
|  |  |  |  |  |  |  |  |
| **Others** | transthyretin | *TTR* | TTR | Chr18 | [ENST00000237014.7](http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000118271;r=18:31591726-31599011;t=ENST00000237014) [NM\_000371](http://www.ncbi.nlm.nih.gov/nuccore/NM_000371).3 | 4 | thyroid hormone-binding protein, transport of thyroxine from bloodstream to peripheral organs |

**Table S3: Family history and screening for cardiomyopathy in the 80 families of the pediatric index patients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Patient family ID** | **Diagnosis** | **Family screening**  [yes - 1, no - 0] | **Cardiomyopathy in family history or screening**  [positive - 1, negative - 0] | **Affected family members**  [father - 1, mother - 2, siblings - 3, other - 4, N/A - 5] | **Family screening father**  [affected - 1, unaffected - 2, N/A - 3] | **Family screening mother**  [affected - 1, unaffected - 2, N/A - 3] |
|  |  |  |  |  |  |  |
| CMP-01 | DCM | 1 | 1 | 3 | 2 | 2 |
| CMP-02 | HCM | 1 | 1 | 2 | 2 | 1 |
| CMP-03 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-35 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-04 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-09 | DCM | 1 | 0 | 5 | 3 | 3 |
| CMP-36 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-05 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-06 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-07 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-37 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-08 | HCM | 1 | 1 | 1 | 1 | 2 |
| CMP-38 | HCM | 1 | 1 | 4 | 2 | 2 |
| CMP-39 | HCM | 0 | 0 | 5 | 3 | 3 |
| CMP-40 | RCM | 0 | 0 | 5 | 3 | 3 |
| CMP-41 | RCM | 0 | 0 | 5 | 3 | 3 |
| CMP-42 | HCM | 1 | 1 | 1 | 1 | 2 |
| CMP-43 | HCM | 0 | 0 | 5 | 3 | 3 |
| CMP-44 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-45 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-46 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-47 | LVNC | 0 | 0 | 5 | 3 | 3 |
| CMP-10 | LVNC | 1 | 0 | 5 | 2 | 2 |
| CMP-11 | RCM | 1 | 1 | 2 | 2 | 1 |
| CMP-48 | DCM | 1 | 1 | 3 | 2 | 2 |
| CMP-49 | LVNC | 1 | 1 | 2 | 2 | 1 |
| CMP-12 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-50 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-51 | HCM | 1 | 1 | 2 | 2 | 1 |
| CMP-13 | LVNC | 1 | 1 | 1 | 1 | 2 |
| CMP-52 | HCM | 1 | 0 | 5 | 2 | 2 |
| CMP-53 | HCM | 1 | 1 | 3 | 2 | 2 |
| CMP-54 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-55 | HCM | 1 | 0 | 5 | 2 | 2 |
| CMP-14 | LVNC | 1 | 0 | 5 | 2 | 2 |
| CMP-56 | DCM | 1 | 1 | 1 | 1 | 2 |
| CMP-15 | LVNC | 1 | 1 | 2 | 2 | 1 |
| CMP-57 | HCM | 0 | 0 | 5 | 3 | 3 |
| CMP-58 | RCM | 1 | 0 | 5 | 2 | 2 |
| CMP-59 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-16 | DCM | 1 | 1 | 3 | 2 | 2 |
| CMP-17 | ARVC | 1 | 1 | 1 | 1 | 2 |
| CMP-60 | HCM | 1 | 1 | 1 | 3 | 2 |
| CMP-61 | HCM | 1 | 0 | 5 | 2 | 2 |
| CMP-62 | LVNC | 1 | 0 | 5 | 2 | 2 |
| CMP-18 | DCM | 0 | 0 | 5 | 3 | 3 |
| CMP-19 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-20 | DCM | 1 | 1 | 1 | 1 | 2 |
| CMP-21 | RCM | 1 | 0 | 5 | 2 | 2 |
| CMP-63 | DCM | 1 | 0 | 5 | 3 | 2 |
| CMP-64 | DCM | 1 | 0 | 5 | 3 | 2 |
| CMP-22 | HCM | 1 | 1 | 1 | 3 | 2 |
| CMP-23 | LVNC | 0 | 0 | 5 | 3 | 3 |
| CMP-65 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-24 | LVNC | 1 | 0 | 5 | 2 | 2 |
| CMP-66 | LVNC | 1 | 0 | 5 | 2 | 2 |
| CMP-67 | HCM | 1 | 1 | 1 | 1 | 2 |
| CMP-68 | HCM | 1 | 1 | 1 | 3 | 2 |
| CMP-25 | RCM | 1 | 0 | 5 | 2 | 2 |
| CMP-69 | HCM | 1 | 1 | 2 | 3 | 1 |
| CMP-70 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-71 | HCM | 1 | 0 | 5 | 2 | 2 |
| CMP-26 | DCM | 1 | 1 | 1 | 1 | 2 |
| CMP-72 | LVNC | 1 | 0 | 5 | 3 | 2 |
| CMP-27 | LVNC | 1 | 1 | 2 | 2 | 1 |
| CMP-28 | HCM | 1 | 0 | 5 | 2 | 2 |
| CMP-73 | HCM | 1 | 0 | 5 | 3 | 2 |
| CMP-29 | RCM | 1 | 0 | 5 | 2 | 2 |
| CMP-30 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-74 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-75 | DCM | 1 | 1 | 3 | 3 | 3 |
| CMP-76 | LVNC | 1 | 0 | 5 | 2 | 2 |
| CMP-31 | HCM | 1 | 1 | 1 | 1 | 2 |
| CMP-77 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-78 | HCM | 1 | 0 | 5 | 2 | 2 |
| CMP-79 | HCM | 0 | 0 | 5 | 3 | 3 |
| CMP-32 | DCM | 1 | 0 | 5 | 2 | 2 |
| CMP-33 | ARVC | 1 | 0 | 5 | 2 | 2 |
| CMP-34 | LVNC | 1 | 1 | 2 | 2 | 1 |
| CMP-80 | DCM | 1 | 1 | 3 | 2 | 2 |

N/A - information not available.

**Table S4: Genetic variants found in 80 index patients with pediatric cardiomyopathy**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Transcript** | **cDNA alteration** | **Protein alteration** | **Diagnosis** | **Patient ID complex genotypes** | **gnomAD allele frequency** | **Pathogenicity** | **de novo** | **novel** |
|  |  |  |  |  |  |  |  |  |  |
| ***Pathogenic and likely pathogenic genetic variants*** | | | | | | | | | |
| *ACTC1* | NM\_005159.4 | c.328G>A | p.A110T | DCM, HTX | CMP-77 | 0 | Likely pathogenic  (PM2, PM6, PP2-3) | yes | no |
| *ACTN2* | NM\_001103.2 | c.574C>T | p.R192\* | LVNC, HTX | CMP-10 | 0.000008122 | Pathogenic  (PVS1, PM2, PM6) | yes | yes |
| *DSC2* | NM\_024422.3 | c.1034T>C | p.I345T | ARVC | CMP-33 | 0.000008133 | Likely pathogenic  (PS3, PM2, PP3) | no | no |
| *DSG2* | NM\_001943.3 | c.1016delA | p.V340\* | ARVC | CMP-33 | 0.00000814 | Likely pathogenic  (PVS1, PM2) | no | yes |
| *LAMP2* | NM\_001122606.1 | c.222\_223delTA | p.Y74\* | HCM |  | 0 | Pathogenic  (PVS1, PS4, PM6) | yes | no |
| *MYBPC3* | NM\_000256.3 | c.772G>A  (splice variant) | p.E258K / p.Glu258fsX41 | HCM | CMP-42 | 0.00001851 | Pathogenic  (PS1, PS4) | ? | no |
|  | NM\_000256.3 | c.927-2A>G | - | HCM | CMP-31 | 0.00003235 | Pathogenic  (PS3-4, PM4, PP1) | no | no |
|  | NM\_000256.3 | c.1504C>T | p.R502W | HCM | CMP-38 | 0.00005411 | Pathogenic  (PS1, PS4) | ? | no |
|  | NM\_000256.3 | c.1805C>T | p.T602I | LVNC | CMP-27 | 0.000009848 | Likely pathogenic  (PS1, PM2) | no | no |
|  | NM\_000256.3 | c.2308G>A | p.D770N | HCM | CMP-69 | 0.00001625 | Pathogenic  (PS1, PS4, PM1) | no | no |
|  | NM\_000256.3 | c.2572A>C | p.S858R | DCM, HTX | CMP-09 | 0 | Likely pathogenic  (PS2, PM2, PM5) | ? | yes |
| *MYH7* | NM\_000257.2 | c.677C>T | p.A226V | HCM |  | 0 | Likely pathogenic  (PS4, PM1, PP3) | ? | no |
|  | NM\_000257.2 | c.1063G>A | p.A355T | HCM | CMP-22 | 0 | Pathogenic  (PS1, PS4, PM1) | ? | no |
|  | NM\_000257.2 | c.1283C>A | p.A428D | LVNC | CMP-27 | 0 | Likely pathogenic  (PM1-2, PP1, PP3) | no | yes |
|  | NM\_000257.2 | c.1357C>T | p.R453C | HCM |  | 0 | Pathogenic  (PS1, PS3-4) | ? | no |
|  | NM\_000257.2 | c.1987C>T | p.R663C | HCM | CMP-28 | 0 | Pathogenic  (PS1, PS4, PM2) | no | no |
|  | NM\_000257.2 | c.1988G>A | p.R663H | HCM | CMP-43 | 0.00001443 | Pathogenic  (PS1, PS4, PM1) | ? | no |
|  | NM\_000257.2 | c.2710C>T | p.R904C | DCM | CMP-44 | 0.000004061 | Pathogenic  (PS1, PS4, PM1) | ? | no |
| *PKP2* | NM\_004572.3 | c.1716delG | p.R573Efs\*4 | ARVC |  | 0 | Likely pathogenic (PSV1, PM2) | no | yes |
| *PRKAG2* | NM\_016203.3 | c.1199C>A | p.T400N | HCM |  | 0 | Pathogenic  (PS1, PS3, PM2) | ? | no |
| *TAZ* | NM\_000116.3 | c.355G>A | p.V119M | LVNC | CMP-24 | 0 | Likely pathogenic  (PM1-2, PM5) | no | yes |
| *TNNI3* | NM\_000363.4 | c.24+2T>A (homozygous) | - | LVNC | CMP-01 | 0.00001254 | Pathogenic  (PVS1, PS3, PM2) | ? | yes |
|  | NM\_000363.4 | c.570C>G | p.D190E | HCM |  | 0 | Likely Pathogenic  (PM1-2, PM5) | ? | no |
|  | NM\_000363.4 | c.574C>T | p.R192C | RCM, HTX | CMP-58 | 0 | Pathogenic  (PS1, PS3-4, PM2, PM6) | yes | no |
|  | NM\_000363.4 | c.582C>A | p.N194K | DCM | CMP-80 | 0 | Pathogenic  (PS1, PM1-2, PM6) | yes | no |
|  | NM\_000363.4 | c.624dupT | p.E209\* | RCM, HTX | CMP-29 | 0 | Likely pathogenic  (PM1-2, PM4, PM6) | yes | yes |
| *TNNT2* | NM\_000364 | c.620\_622delAGA | p.K207del | DCM, HTX | CMP-12 | 0 | Likely Pathogenic  (PS1, PM1-2) | ? | no |
|  | NM\_000364 | c.620-622delAGA | p.K207del | DCM, HTX | CMP-74 | 0 | Pathogenic  (PS1, PM1-2, PM6) | yes | no |
|  | NM\_000364.2 | c.812+1G>A | - | HCM |  | 0 | Pathogenic  (PS1, PS3, PM2) | no | no |
| *TPM1* | NM\_001018005.1 | c.257C>T | p.A86V | LVNC | CMP-34 | 0 | Likely pathogenic  (PM1-2, PP1\_moderate, PP3) | no | yes |
| *TTN* | NM\_001267550.1 | c.68329+2\_68329+3insTT | - | RCM, HTX | CMP-25 | 0.00009578 | Likely pathogenic  (PM1-2, PM4) | no | yes |
|  | NM\_001267550.1 | c.85891delG | p.A28631Lfs\*3 | DCM, HTX |  | 0 | Likely pathogenic  (PM1-2, PM4) | no | yes |
|  |  |  |  |  |  |  |  |  |  |
| ***Genetic variants of uncertain significance (VUS)*** | | | | | | | | | |
| *ACTN2* | NM\_001103.2 | c.278G>A | p.R93Q | LVNC | CMP-24 | 0.00002525 | Uncertain significance | no | no |
| *BAG3* | NM\_004281.3 | c.280A>T | p.I94F | DCM |  | 0.0006493 | Uncertain significance | ? | no |
|  | NM\_004281.3 | c.881G>A | p.R294H | DCM, HTX |  | 0.00003663 | Uncertain significance | ? | no |
|  | NM\_004281.3 | c.1634C>G | p.P545R | DCM |  | 0.00009385 | Uncertain significance | no | no |
| *CBL* | NM\_005188.3 | c.805A>G | p.M269V | LVNC | CMP-24 | 0.00001218 | Uncertain significance | no | yes |
| *DMD* | NM\_004006.2 | c.2169-7\_2169-4delGTCT | - | HCM | CMP-43 | 0.00003417 | Uncertain significance | ? | no |
|  | NM\_004006.2 | c.2273A>C | p.D758A | RCM, HTX | CMP-25 | 0.0002632 | Uncertain significance | no | yes |
|  | NM\_004006.2 | c.5723A>T | p.D1908V | HCM, HTX |  | 0.0002101 | Uncertain significance | no | yes |
|  | NM\_004006.2 | c.8996C>T | p.A2999V | RCM, HTX | CMP-29 | 0.0000112 | Uncertain significance | no | yes |
| *DSC2* | NM\_024422.3 | c.304G>A | p.E102K | DCM |  | 0.0007482 | Uncertain significance | no | no |
|  | NM\_024422.3 | c.363G>T | p.K121N | LVNC |  | 0.00001221 | Uncertain significance | no | no |
|  | NM\_024422.3 | c.802A>G | p.T268A | DCM |  | 0.0001444 | Uncertain significance | no | no |
| *DSG2* | NM\_001943.3 | c.1003A>G | p.T335A | DCM |  | 0.0005089 | Uncertain significance | ? | no |
|  | NM\_001943.3 | c.2001+3C>G | - | LVNC |  | 0.000008136 | Uncertain significance | no | yes |
| *DSP* | NM\_004415.2 | c.2774G>A | p.R925Q | RCM, HTX |  | 0.0002309 | Uncertain significance | no | no |
|  | NM\_004415.2 | c.4961T>C | p.L1654P | LVNC, HTX |  | 0.00002849 | Uncertain significance | no | no |
|  | NM\_004415.2 | c.5178C>A | p.N1726K | DCM, HTX |  | 0.0006893 | Uncertain significance | no | no |
|  | NM\_004415.2 | c.7916G>A | p.R2639Q | DCM, HTX | CMP-74 | 0.000853 | Uncertain significance | no | no |
|  | NM\_004415.2 | c.7994C>T | p.T2665M | DCM | CMP-80 | 0.00004338 | Uncertain significance | no | no |
|  | NM\_004415.2 | c.8524C>T | p.R2842C | HCM | CMP-69 | 0.000007384 | Uncertain significance | no | no |
| *DTNA* | NM\_001390.4 | c.1757C>T | p.P586L | HCM |  | 0.0001263 | Uncertain significance | no | no |
| *EYA4* | NM\_172105.3 | c.59A>G | p.D20G | RCM |  | 0.000008132 | Uncertain significance | ? | no |
|  | NM\_172105.3 | c.971-3T>C | - | LVNC, HTX | CMP-10 | 0.00006461 | Uncertain significance | no | yes |
| *FBN1* | NM\_000138.4 | c.902G>T | p.G301V | DCM, HTX |  | 0.0001876 | Uncertain significance | ? | no |
| *FHL1* | NM\_001159702.2 | c.283C>T | p.R95W | HCM |  | 0.0006232 | Uncertain significance | no | no |
| *FHL2* | NM\_201555.1 | c.143G>A | p.G48D | DCM |  | 0 | Uncertain significance | no | yes |
|  | NM\_201555.1 | c.337C>T | p.R113C | DCM |  | 0.000395 | Uncertain significance | ? | no |
| *JPH2* | NM\_020433.4 | c.572C>G | p.P191R | DCM |  | 0.0005276 | Uncertain significance | no | no |
|  | NM\_020433.4 | c.1306C>T | p.R436C | DCM |  | 0.00003236 | Uncertain significance | no | no |
|  | NM\_020433.4 | c.1896G>C | p.E632D | RCM, HTX | CMP-58 | 0.00007797 | Uncertain significance | no | no |
| *JUP* | NM\_002230.2 | c.1714C>T | p.R572W | DCM, HTX | CMP-09 | 0.00001219 | Uncertain significance | ? | no |
| *LAMA4* | NM\_001105206.2 | c.514G>A | p.G172S | HCM | CMP-69 | 0.0002224 | Uncertain significance | ? | no |
|  | NM\_001105206.2 | c.1959T>C | p.D653 | ARVC | CMP-33 | 0.0009604 | Uncertain significance | no | yes |
|  | NM\_001105206.2 | c.2171G>A | p.R724K | DCM, HTX | CMP-12 | 0.0007326 | Uncertain significance | ? | yes |
|  | NM\_001105206.2 | c.4645A>T | p.N1549Y | HCM |  | 0.0001988 | Uncertain significance | ? | yes |
| *LDB3/ZASP* | NM\_001171610.1 | c.66C>A | p.D22E | HCM | CMP-22 | 0.00003236 | Uncertain significance | no | yes |
|  | NM\_001171610.1 | c.664G>A | p.A222T | HCM | CMP-42 | 0.0003646 | Uncertain significance | ? | no |
|  | NM\_001171610.1 | c.778G>A | p.G260S | DCM |  | 0.0000469 | Uncertain significance | ? | yes |
|  | NM\_001171610.1 | c.982G>A | p.A328T | LVNC | CMP-34 | 0.000004062 | Uncertain significance | no | yes |
|  | NM\_007078 | c.1792T>C | p.C598R | HCM | CMP-31 | 0 | Uncertain significance | no | yes |
|  | NM\_001171610.1 | c.1978C>A | p.P660T | HCM | CMP-22 | 0.000007219 | Uncertain significance | no | yes |
| *LMNA* | NM\_170707.3 | c.986G>C | p.R329P | HCM | CMP-22 | 0 | Uncertain significance | ? | yes |
| *MYBPC3* | NM\_000256.3 | c.961G>A | p.V321M | DCM |  | 0.0003261 | Uncertain significance | no | no |
|  | NM\_000256.3 | c.2873C>T | p.T958I | HCM | CMP-42 | 0.000156 | Uncertain significance | ? | no |
| *MYH6* | NM\_002471.3 | c.3010G>T | p.A1004S | HCM | CMP-43 | 0.0009523 | Uncertain significance | ? | no |
|  | NM\_002471.3 | c.5348G>A | p.R1783H | HCM |  | 0.00003248 | Uncertain significance | no | no |
| *MYH7* | NM\_000257.2 | c.475G>A | p.D159N | LVNC, HTX |  | 0 | Uncertain significance | ? | yes |
|  | NM\_000257.2 | c.1425G>T | p.Q475H | LVNC |  | 0 | Uncertain significance | no | no |
|  | NM\_000257.2 | c.2890G>C | p.V964L | DCM |  | 0.0004256 | Uncertain significance | no | no |
|  | NM\_000257.2 | c.3169G>A | p.G1057S | HCM |  | 0.000008121 | Uncertain significance | no | no |
|  | NM\_000257.2 | c.4501G>T | p.E1501\* | DCM |  | 0 | Uncertain significance | no | yes |
|  | NM\_000257.2 | c.5767A>G | p.K1923E | DCM |  | 0 | Uncertain significance | ? | yes |
| *MYL2* | NM\_000432.3 | c.421G>A | p.A141T | DCM, HTX |  | 0 | Uncertain significance | ? | no |
| *MYLK2* | NM\_033118.3 | c.4G>A | p.A2T | DCM, HTX | CMP-77 | 0.0009884 | Uncertain significance | no | no |
|  | NM\_033118.3 | c.425G>T | p.G142V | LVNC, HTX | CMP-10 | 0.00008924 | Uncertain significance | no | yes |
| *MYOZ2* | NM\_016599.4 | c.659T>C | p.M220T | HCM | CMP-22 | 0 | Uncertain significance | no | yes |
| *MYPN* | NM\_001256267.1 | c.259C>G | p.P87A | DCM |  | 0.0000433 | Uncertain significance | no | no |
|  | NM\_001256267.1 | c.802C>T | p.P268S | RCM, HTX | CMP-58 | 0.0004756 | Uncertain significance | no | no |
|  | NM\_001256267.1 | c.970C>T | p.H324Y | HCM | CMP-28 | 0 | Uncertain significance | no | yes |
|  | NM\_001256267.1 | c.2150C>T | p.T717M | DCM, HTX |  | 0.00004467 | Uncertain significance | ? | yes |
|  | NM\_001256267.1 | c.3913A>G | p.M1305V | HCM | CMP-42 | 0.00005412 | Uncertain significance | ? | no |
| *NEXN* | NM\_144573.3 | c.893C>G | p.T298R | HCM | CMP-42 | 0.0001605 | Uncertain significance | ? | no |
|  | NM\_144573.3 | c.1572\_1574delAGA | p.E525del | DCM, HTX |  | 0.0001556 | Uncertain significance | ? | yes |
|  | NM\_144573.3 | c.1572\_1574delAGA | p.E525del | DCM | CMP-01 | 0.0001556 | Uncertain significance | ? | yes |
|  | NM\_144573.3 | c.1619T>C | p.M540T | DCM, HTX |  | 0.000004072 | Uncertain significance | ? | yes |
| *NKX2-5* | NM\_004387.3 | c.355G>T | p.A119S | DCM |  | 0.0009725 | Uncertain significance | no | no |
| *PKP2* | NM\_004572.3 | c.1536T>A | p.N512K | DCM, HTX |  | 0.00001446 | Uncertain significance | no | yes |
|  | NM\_004572.3 | c.2200A>G | p.T734A | DCM, HTX |  | 0.00001083 | Uncertain significance | ? | no |
|  | NM\_004572.3 | c.2365A>G | p.I789V | DCM |  | 0.0003105 | Uncertain significance | ? | yes |
| *PRDM16* | NM\_022114.3 | c.1110C>A | p.D370E | LVNC, HTX |  | 0.000004112 | Uncertain significance | ? | yes |
|  | NM\_022114.3 | c.2296G>A | p.G766S | HCM | CMP-22 | 0.0002788 | Uncertain significance | ? | no |
|  | NM\_022114.3 | c.2372G>A | p.G791D | RCM, HTX |  | 0 | Uncertain significance | no | yes |
|  | NM\_022114.3 | c.2447A>G | p.N816S | RCM, HTX | CMP-25 | 0.0002405 | Uncertain significance | no | no |
| *RAF1* | NM\_002880.3 | c.974A>C | p.Q325P | DCM | CMP-44 | 0.00001804 | Uncertain significance | ? | no |
| *RBM20* | NM\_001134363.1 | c.298C>T | p.L100F | DCM |  | 0 | Uncertain significance | no | yes |
| *RYR2* | NM\_001035.2 | c.1699G>C | p.A567P | DCM, HTX |  | 0.000008299 | Uncertain significance | ? | yes |
|  | NM\_001035.2 | c.4273A>G | p.T1425A | HCM | CMP-38 | 0.00005876 | Uncertain significance | ? | no |
|  | NM\_001035.2 | c.8162T>C | p.I2721T | HCM |  | 0.0005919 | Uncertain significance | no | no |
|  | NM\_001035.2 | c.9655G>A | p.V3219M | DCM |  | 0.00007581 | Uncertain significance | ? | no |
| *SCN5A* | NM\_001099404.1 | c.998+5G>A | - | DCM |  | 0.000213 | Uncertain significance | ? | no |
|  | NM\_001099404.1 | c.1577G>A | p.R526H | HCM |  | 0.00006255 | Uncertain significance | ? | no |
| *SOS1* | NM\_005633.3 | c.3841\_3843dupAAG | p.E1281dup | DCM | CMP-01 | 0.000004065 | Uncertain significance | ? | yes |
| *TAZ* | NM\_000116.3 | c.29C>G | p.P10R | LVNC | CMP-24 | 0 | Uncertain significance | ? | yes |
| *TBX20* | NM\_001077653.2 | c.208G>A | p.G70S | DCM |  | 0 | Uncertain significance | no | yes |
|  | NM\_001077653.2 | c.994C>T | p.P332S | DCM, HTX |  | 0.0000366 | Uncertain significance | no | yes |
| *TGFB3* | NM\_003239.2 | c.293C>T | p.S98L | DCM |  | 0.0009523 | Uncertain significance | no | no |
| *TMEM43* | NM\_024334.2 | c.1177C>T | p.R393W | LVNC |  | 0.000008133 | Uncertain significance | no | yes |
| *TNNC1* | NM\_003280.2 | c.25G>A | p.V9I | DCM, HTX |  | 0 | Uncertain significance | ? | yes |
| *TNNI3* | NM\_000363.4 | c.307C>T | p.R103C | HCM | CMP-22 | 0.000007216 | Uncertain significance | ? | no |
| *TNNT2* | NM\_000364.2 | c.808G>A | p.V270I | DCM |  | 0 | Uncertain significance | no | yes |
| *TPM1* | NM\_001018005.1 | c.340G>C | p.E114Q | DCM, HTX |  | 0 | Uncertain significance | no | yes |
| *TTN* | NM\_001267550.1 | c.25064-4A>G | - | RCM |  | 0.000008577 | Uncertain significance | no | yes |
|  | NM\_001267550.1 | c.39709+1G>T | - | RCM, HTX |  | 0 | Uncertain significance | no | yes |
| *VCL* | NM\_014000.2 | c.590C>T | p.T197I | HCM |  | 0.0002526 | Uncertain significance | no | no |

Codes for pathogenicity prediction were used according to American College of Medical Genetics (ACMG) guidelines.

**Table S5: Selected genetic variants and complex genotypes**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Transcript** | **cDNA Position** | **Protein position** | **Diagnosis** | **Patient ID of complex genotypes** | **gnomAD allele frequency** | **Pathogenicity** | **de novo** | **novel** |
|  |  |  |  |  |  |  |  |  |  |
| ***Truncating TTN variants*** | | | | | | | | | |
| *TTN* | NM\_001267550.1 | c.25064-4A>G\* | - | RCM |  | 0.000008577 | Uncertain significance | no | yes |
|  | NM\_001267550.1 | c.39709+1G>T\* | - | RCM |  | 0 | Uncertain significance | no | yes |
|  | NM\_001267550.1 | c.68329+2\_68329+3insTT\* | - | RCM | CMP-25 | 0.00009578 | Likely pathogenic | no | yes |
|  | NM\_001267550.1 | c.85891delG | p.A28631Lfs\*3 | DCM |  | 0 | Likely pathogenic | no | yes |
|  |  |  |  |  |  |  |  |  |  |
| ***Homozygous variants*** | | | | | | | | | |
| *NEXN* | NM\_144573.3 | c.1572\_1574delAGA | p.E525del | DCM, HTX |  | 0.0001556 | Uncertain significance | ? | yes |
| *TNNI3* | NM\_000363.4 | c.24+2T>A\* | p.Ala8\_Ala9insGluArgAlaAlaGly\* | LVNC | CMP-01 | 0.00001254 | Pathogenic | ? | yes |
|  |  |  |  |  |  |  |  |  |  |
| ***Hemizygous variants*** | | | | | | | | | |
| *DMD* | NM\_004006.2 | c.2169-7\_2169-4delGTCT\* | - | HCM | CMP-43 | 0.00003417 | Uncertain significance | ? | no |
|  | NM\_004006.2 | c.2273A>C | p.D758A | RCM | CMP-25 | 0.0002632 | Uncertain significance | no | yes |
|  | NM\_004006.2 | c.5723A>T | p.D1908V | HCM |  | 0.0002101 | Uncertain significance | no | yes |
|  | NM\_004006.2 | c.8996C>T | p.A2999V | RCM | CMP-29 | 0.0000112 | Uncertain significance | no | yes |
| *FHL1* | NM\_001159702.2 | c.283C>T | p.R95W | HCM |  | 0.0006232 | Uncertain significance | no | no |
| *TAZ* | NM\_000116.3 | c.29C>G | p.P10R | LVNC |  | 0 | Uncertain significance | ? | yes |
|  | NM\_000116.3 | c.355G>A | p.V119M | LVNC | CMP-24 | 0 | Likely pathogenic | no | yes |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| ***Index patients with compound heterozygous variants*** | | | | | | | | | |
| *DSC2* | NM\_024422.3 | c.304G>A | p.E102K | DCM |  | 0.0007482 | Uncertain significance | no | no |
|  | NM\_024422.3 | c.802A>G | p.T268A | DCM |  | 0.0001444 | Uncertain significance | no | no |
|  |  |  |  |  |  |  |  |  |  |
| *MYBPC3* | NM\_000256.3 | c.2572A>C | p.S858R | DCM | CMP-09 | 0 | Likely pathogenic | ? | yes |
|  | NM\_000256.3 | genomic deletion of *MYBPC3* gene | - | DCM | CMP-09 | 0 | Pathogenic | no | yes |
|  |  |  |  |  |  |  |  |  |  |
| ***Index patients with >1 pathogenic and/or likely pathogenic variant*** | | | | | | | | | |
| *MYBPC3* | NM\_000256.3 | c.1805C>T | p.T602I | LVNC | CMP-27 | 0.000009848 | Likely pathogenic | no | no |
| *MYH7* | NM\_000257.2 | c.1283C>A | p.A428D | LVNC | CMP-27 | 0 | Likely pathogenic | no | yes |
|  |  |  |  |  |  |  |  |  |  |
| *DSC2* | NM\_024422.3 | c.1034T>C | p.I345T | ARVC | CMP-33 | 0.000008133 | Likely pathogenic | no | no |
| *DSG2* | NM\_001943.3 | c.1016delA | p.V340\* | ARVC | CMP-33 | 0.00000814 | Likely pathogenic | no | yes |

\* Variant affects splice site

**Table S6: Phenotypes and genotypes of families with *TNNI3* variants**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Echocardiogramm** | | | | | | | |  |  |  |
| **ID** | **Genotype** | **Age (years)** | **IVSD (mm)** | **LVPWd (mm)** | **LVEDd**  **(mm)** | **LVFS**  **(%)** | **LVEF**  **(%)** | **LVEF**  **plan. (%)** | **CMP, Description** | **NC/C ratio >2:1** | **12 lead ECG** | **Arrhyth-mias** | **Medical History** |
|  | | | | | | | | | | | | | | |
| ***Family 1*** | | | | | | | | | | | | | | |
| **1-I:1** | heterozygous,  *TNNI3* c.204delG, p.Arg69Alafs\*8 | 33 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | no symptoms, no medications |
| **1-I:2** | heterozygous,  *TNNI3* c.204delG, p.Arg69Alafs\*8 | 29 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | no symptoms, no medications |
| **1-II:1** | homozygous,  *TNNI3* c.204delG, p.Arg69Alafs\*8 | 1.2 | 7 (+3.5) | 6.9  Z((+5.9) | 38 (+4.4) | 11 | 26 | 25 | **DCM**, severe MR and TR, RVSP 46 mmHg | no | SR, iRBB | no | symptomatic with CHD after vaccination; sus-pected myocarditis; implantation of LVAD at 1.2 y, HTX at 1.8 y |
| ***Family 2*** | | | | | | | | | | | | | | |
| **2-II:3** | homozygous,  *TNNI3* c.24+2T>A,  p.(Ala8+GluArgAlaAlaGly) | 1 | 7 (+4.4) | 6 (+2.9) | 33 (+5.2) | 17 | 37 | 40 | **LVNC**, LV hypertrophy with trabecula-tions and deep recesses in the apex and lateral wall | yes | SR, normal | no | histology from LV biopsy: deep recesses covered by endocardium highly suspicious of LVNC; see pedigree in Figure S2; death at 1.3 y after LVAD implantation and sepsis |
| ***Family 3*** | | | | | | | | | | | | | | |
| **3-I:1** | no *TNNI3* variant | 33 | N/A | N/A | N/A | N/A | N/A | N/A | MRI: mild dilated LA, function and size of LV normal | N/A | SR, normal | no | Arterial hypertension, no symptoms, no medications |
| **3-I:2** | no *TNNI3* variant | 38 | 9 | N/A | 44 | 35 | 64 | 62 | normal | no | SR, normal | no | no symptoms, no medications |
| **3-II:1** | heterozygous,  *TNNI3* c.624T>TT, p.Glu209\* | 4 | 4 (-0.4) | N/A | 25 (-1.4) | 37 | 69 | 77 | **RCM**, biatrial enlargement, PHT, RVSP 50-80 mmHg | no | SR, biatrial abnormality | no | LVAD implantation at 4 y, HTX at 4.5 y, death at 5 y |
| ***Family 4*** | | | | | | | | | | | | | | |
| **4-I:1** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | died from SCD at age 30 with pathology of HCM at autopsy |
| **4-I:2** | no *TNNI3* variant | 39 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | SR, normal | N/A | chronic obstructive pulmonary disease, NYHA II |
| **4-II:1** | heterozygous,  *TNNI3* c.570C>G, p.Asp190Glu | 15 | 37 (+24) | 16 | 29 (-3,6) | 43 | 76 | 77 | **HCM**, PHT | no | pacemaker: atrial stimulation, WPW | WPW, AV-reentry tachycardia | multiple family members with SCD< 40 y, LGE: septal and inferoseptal; ICD implantation at 11 y, NYHA II-III |
| ***Family 5*** | | | | | | | | | | | | | | |
| **5-I:1** | no *TNNI3* variant | 32 | 13 | N/A | 53 | 22 | 43 | 57 | normal | no | discordant T-waves in III, slow R progression | 2 PVCs | no symptoms, no medications |
| **5-I:2** | no *TNNI3* variant | 30,4 | 8 | N/A | 50 | 38 | 68 | N/A | normal | no | SR, normal | normal | no symptoms, no medication |
| **5-II:1** | heterozygous,  *TNNI3* c.574C>T, p.Arg192Cys | 2,7 | 4 (-0.3) | N/A | 24 (-1.8) | 25 | 52 | 44 | **RCM**, severe biatrial enlargement, TI °II, RVSP 50 mmHg | no | SR, LVH, right atrial abnormality | no | Cardiac decompensation, postcapillary PHT, biventricular VAD implantation at 2.7 y, HTX at 3.4 y |
| ***Family 6*** | | | | | | | | | | | | | | |
| **6-II:1** | heterozygous,  *TNNI3* c.582C>A, p.Asn194Lys | 9 | 5,5 (-0.3) | 5,9 (0) | 54,2 (+4.6) | 18 (<28) | 45 (>55) | 30 (>55) | **DCM**, mild MR, LA slightly enlarged | no | SR, normal | no | no symptoms; twin brother died of acute heart failure and DCM at 9 y |

Echocardiogram: z-scores for children in brackets; LVPWd, diastolic left ventricular posterior wall thickness; IVSd, diastolic interventricular septal thickness; LVEDd, left ventricular end diastolic diameter; LVFS left ventricular fractional shortening; LVEF, left ventricular ejection fraction; SR, sinus rhythm; iRBB, incomplete right bundle branch block; PVCs, premature ventricular beats; LA, left atrium; NC/C ratio, noncompacted to compacted ratio; MR, mitral valve regurgitation; TR, tricuspid valve regurgitation; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction; DCM, dilated cardiomyopathy; RCM, restrictive cardiomyopythy; PHT, pulmonary hypertension; LVH, left ventricular hypertrophy; RVSP, right ventricular systolic pressure; MRI, cardiac magnetic resonance imaging; HTX, heart transplantation; LVAD, left ventricular assist device; N/A - not available.

**Table S7: Genetic variants in *MYH7***

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Diagnosis** | **HTX** | **Sex** | **Age at diagnosis** | **cDNA position** | **Protein position** | **ACMG pathogenicity** | **Novel** | **ClinVar** | **Age ≤18 years†** | **Age >18 years‡** | **PubMed ID** |
|  |  |  |  |  |  |  |  |  |  |  |  |
| HCM | no | m | 11,8 | c.1988G>A | p.R663H | Pathogenic | no | yes | yes | yes | 10750581 |
| DCM | no | f | 0,0 | c.2710C>T | p.R904C | Pathogenic | no | yes | yes | yes | 20573160  29212898 |
| LVNC | no | m | 5,9 | c.1425G>T | p.Q475H | Uncertain significance | no | no | no | yes | 21750094 |
| LVNC | yes | m | 0,2 | c.475G>A | p.D159N | Uncertain significance | yes | no | - | - | - |
| DCM | no | f | 0,3 | c.2890G>C | p.V964L | Uncertain significance | no | yes | no | yes | 23349452  19412328 |
| HCM | no | m | 7,4 | c.1357C>T | p.R453C | Pathogenic | no | yes | yes | yes | 1552912  17495353 |
| DCM | no | m | 6,2 | c.5767A>G | p.K1923E | Uncertain significance | yes | no | - | - | - |
| HCM | no | m | 15,2 | c.1063G>A | p.A355T | Pathogenic | no | yes | yes | yes | 12707239  20031618 |
| HCM | no | m | 16,2 | c.3169G>A | p.G1057S | Uncertain significance | no | yes | no | yes | 15358028 |
| LVNC | no | f | 11,7 | c.1283C>A | p.A428D | Likely pathogenic | yes | no | - | - | - |
| HCM | no | m | 5,3 | c.1987C>T | p.R663C | Pathogenic | no | yes | yes | yes | 11133230  15358028 |
| HCM | no | f | 0,7 | c.677C>T | p.A226V | Likely pathogenic | no | yes | N/A | N/A | ClinVar |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |

HTX - heart transplantation, N/A - not available, individuals †≤18 years and ‡>18 years with PubMed ID of study; ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)