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**Supplemental Methods**

**Patient inclusion/exclusion criteria and guidelines followed for clinical diagnostic**

The inclusion criteria were childhood-onset cardiomyopathy with or without other organ systems involvement, submitted to our center for inotrope support, invasive hemodynamic examinations, and/or pretransplantation evaluation. All the invasive and pretransplantation evaluations are centralized to Helsinki in Finland. Exclusion criteria were congenital heart disease, arrhythmia-induced cardiac dysfunction, Kawasaki´s disease, ALCAPA (anomalous origin of left coronary arising from pulmonary artery), secondary cardiac dysfunction resulting from abnormalities in other organs, and maternal diabetes for infants who presented with septal hypertrophy before four weeks of age.

The assignment of patients to a diagnostic category followed the statement of the European Society Working Group on myocardial and pericardial diseases ([1](#_ENREF_1)). Further, one patient received a diagnosis of histiocytoid cardiomyopathy according to autopsy findings.

Dilated cardiomyopathy (DCM) was defined by reduced ventricular systolic function (fractional shortening <25%) and left ventricular dilatation (>2 SD above the mean). Hypertrophic cardiomyopathy (HCM) was defined by otherwise unexplained septal hypertrophy, left ventricular free wall hypertrophy (wall thickness >2 SD above the mean), or both ([2](#_ENREF_2)). Restrictive cardiomyopathy (RCM) was defined as restrictive ventricular physiology in the presence of normal or reduced diastolic volumes, normal or reduced systolic volumes, and normal ventricular wall thickness. Catheterization confirmed the RCM diagnosis in the patients. Left ventricular noncompaction (LVNC) was diagnosed by echocardiography or MRI when prominent left ventricular trabeculae and deep inter-trabecular recess were observed ([3](#_ENREF_3)). No patient with arrhythmogenic right ventricular cardiomyopathy was detected.

Cardiac examinations including echocardiography were performed for all first degree relatives, as well as for all participants included in the pedigrees. The biological samples were collected from patients and their families for diagnostic purposes. The patients (when >10 years) and/or parents gave written informed consent (when patients were <10 years). The ethics committee of Helsinki and Uusimaa Region Hospital approved the study plan.

**Bioinformatic analysis**

The data obtained with whole-exome sequencing and with CMP-custom Haloplex panel were processed using the bioinformatic pipeline of the Institute for Molecular Medicine Finland: ([4](#_ENREF_4)) quality filtering of reads, alignment to reference genome (hg19) using Burrows-Wheeler Algorithm (BWA) ([5](#_ENREF_5)), duplicate removal, Single Nucleotide Variant (SNV) identification and annotation with SAMtools pileup ([6](#_ENREF_6)) and an in-house algorithm, detection of insertions-deletions with Pindel ([7](#_ENREF_7)).

The data from Pan Cardiomyopathy panel were analyzed as follows: quality trimming of the raw paired-end reads and adapter sequence removal using Trimmomatic ([8](#_ENREF_8)), mapping of the high-quality reads to the reference genome (hg19) with BWA ([5](#_ENREF_5)), exclusion of PCR duplicates, GATK software for variant calling across all samples simultaneously ([9](#_ENREF_9)), annotation of variants with Ensembl Variant Effect Predictor ([10](#_ENREF_10)).

The filtering steps and prioritization of SNVs and indels (Figure S1B) to identify the likely pathogenic variants included: 1) selection of rare variants with a frequency less or equal to 0.01 in public databases (gnomAD <http://gnomad.broadinstitute.org/>, ExAC (<http://exac.broadinstitute.org/>), SISu, <http://www.sisuproject.fi/>); 2) for dominant inheritance, occurrence was not allowed in public databases, while for recessive inheritance only heterozygotes were permitted; 3) prediction of variants’ deleteriousness based on CADD C-score ([11](#_ENREF_11)), SIFT [13](#_ENREF_13), [14](#_ENREF_14), and PolyPhen-2 ([12](#_ENREF_12)); 4) further prioritization considered the protein function in relation to patient’s phenotype and the amino acid conservation in species; 5) the confirmation included segregation analysis, molecular modeling, and functional studies.

**SDS-PAGE and Western blotting**

Cultured fibroblasts of patient P1 and his brother, as well as three control fibroblast lines were used for protein extraction. The cells were collected by trypsinization, total cellular proteins extracted in RIPA buffer (50 mM Tris-Cl pH8, 150 mM NaCl, 1% TritonX 100, 0.5% sodium deoxycholate, 0.1% SDS) containing protease and phosphatase inhibitors (Thermo Fisher Scientific), and quantified by the Bradford method (Protein Assay, Bio-Rad).

The proteins were separated on 4-20% Mini-Protean® TGX Stain-FreeTM gels (Bio-Rad), and transferred to 0.2 μm PVDF (polyvinylidene fluoride) membranes (Trans-Blot® TurboTM Transfer System, Bio-Rad). The blots were blocked in 5% milk in TBS-Tween 20 (0.1%), incubated overnight with primary antibody, followed by one-hour incubation the next day with HRP conjugated secondary antibody. Primary antibodies were against: PPA2 (Abcam, ab177935), TOM20 (Santa Cruz, sc-11415), MTCO1 (Abcam, ab14705), MTCO2 (Abcam, ab110258), and SDHA (Abcam, ab14715). Secondary antibodies used: goat anti-mouse HRP (Jackson Immunoresearch, 115-035-146) and goat anti-rabbit HRP (Jackson Immunoresearch, 115-035-144). The chemiluminescent reaction (ClarityTM Western ECL substrate, Bio-Rad) was used to develop the signal which was captured with Bio-Rad ChemiDoc™ XRS+ imager.

**Blue native-PAGE and Western blotting**

The samples were prepared for blue native-PAGE as previously described ([13](#_ENREF_13)) from fibroblasts of patient P1, his brother, and three controls. The samples were separated at 4°C on 4-16% Bis-Tris Native Page gels (Invitrogen). Western blotting was carried out as above with the following primary antibodies: NDUFA9 (Abcam, ab14713), ATP5A (Total OXPHOS Cocktail, Abcam, ab110413), TOM40 (Santa Cruz, sc-11414), MTCO2 (Abcam, ab110258), SDHA (Abcam, ab14715).

**RNA extraction, cDNA synthesis, and quantitative real-time PCR**

Total RNA was extracted from heart biopsies of P20 and three controls with RNeasy® Mini Kit (Qiagen), quantified by Nanodrop and its integrity checked on agarose gel. About 360 ng of total RNA were further used for cDNA synthesis (Maxima First Strand Synthesis Kit, Thermo Fisher Scientific). NRAP cDNA was amplified (part of exons 13-14) to verify if the patient’s NRAP mRNA is degraded by nonsense-mediated decay. Further, qPCR reactions were performed in triplicate using IQ SYBR Green qPCR kit (Bio-Rad) on CFX96 Touch qPCR System (Bio-Rad), to quantify the NRAP mRNA level against ACTB (Beta-Actin). All primers are presented in Online Table 3.

**Molecular modeling**

We modeled the consequences of PPA2 and TBX20 variants using the software Discovery Studio 4.5 (Biovia). As templates we used the homology models of human T-box domain of TBX20 (based on DNA-bound T-box domain of human TBX1, PDB id 4A04), and of human PPA2 (based on *Saccharomyces cerevisiae* PPA2 structure, PDB id 1WGI). The homology models were retrieved from Swiss-Model databank.

**Supplemental Case Reports**

**P5, HCM with liver involvement without renal dysfunction, compound heterozygous *NEK8*, Serine/threonine-protein kinase 8, c.644T>C, p.(I215T), and c.1055G>T, p.(R352L).** Patient P6,the daughter of non-consanguineous healthy parents, presented with congenital cholestasis and liver cirrhosis of unknown etiology at the age of 4 months, leading to liver transplantation at two years of age. Her growth was delayed. HCM with left ventricular outflow obstruction was diagnosed at the age of three years, one year after liver transplantation, during exposure to tacrolimus medication. After changing tacrolimus to cyclosporine, HCM and left ventricular obstruction were diminished, but not resolved. The patient is currently 9 years old. Exome sequencing identified two highly conserved novel recessive variants in *NEK8* (Serine/threonine-protein kinase 8, NM\_178170, NP\_835464, with compound heterozygous c.644T>C, p.(I215T), and c.1055G>T, p.(R352L)). *NEK8* defects impair the function of the primary cilium on the cell surface involved in development, thereby leading to severe multi-organ anomalies and nephronophtisis, sometimes associated with cardiomegaly and paucity of bile ducts ([14-17](#_ENREF_14)). The variant p.(I215T) occurs in the kinase region of the enzyme, while p.(R352L) locates near the RCC1-1 domain, both are conserved and cluster with previously described pathogenic variants (Online Figure 2A). Our finding of *NEK8* underlying HCM with congenital cholestasis, short stature and liver cirrhosis expands the clinical phenotypes associated with the gene. It also suggests that *NEK8* has postnatal functions, and indicates that *NEK8* mutations should be considered in infants with CMP and liver involvement, even without renal dysfunction. Based on resemblance to previous clinical phenotypes, segregation in family and high conservation of the amino acids we judged the variants pathogenic in syndromic HCM.

**P6, infantile DCM with rapid progression, *de novo* haploinsufficiency of *TAB2*, TGF-beta-activating kinase 2, c.1168delT, p.(S390Qfs\*37).** Patient P7, a girl, was born by Cesarean section indicated by breech presentation and had mild hypotonia and hypermobile joints. She manifested with DCM at the age of 8 months. Left ventricular assist device was used for bridging to transplantation, which was performed at the age of 10 months. The child died suddenly one year after the heart transplantation. We identified a *de novo* truncating variant in *TAB2* (TGF-beta-activating kinase 2, NM\_015093, NP\_055908, c.1168delT, p.(S390Qfs\*37)), which encodes a developmentally-regulated gene. Our patient presented a frameshift variant introducing a premature stop codon (Online Figure 2B), indicating loss of function of the allele. We report that *de novo* *TAB2* haploinsufficiency, previously involved in congenital heart disease ([18](#_ENREF_18)), can cause fatal progressive infantile DCM.

**P7, rapidly progressive HCM, likely *de novo* variant in *PTPN11*, Protein tyrosine phosphatase non-receptor type 11, c.1528C>G, p.(Q510E).** The patient manifested with rapidly progressive HCM and early death. *PTPN11*(NM\_002834, NP\_002825) **is** a component of Ras/MAPK pathway, which regulates cell growth and differentiation. We found a recurrent *de novo* mutation, p.(Q510E). Parental samples were unavailable, but this variant is known to occur *de novo* and to cause a severe disease. Three mutation clusters exist PTPN11 and the variant p.(Q510E) is localized to one of them, at the end of the tyrosine-protein phosphatase domain (Online Figure 2B).

**P8 and P9, HCM and Noonan syndrome,likely *de novo* variants in *RAF1* proto-oncogene.** Both patients presented with significant HCM and Noonan phenotype soon after birth. **P8, c.770C>T, p.(S257L):** HCM (9 mm, Z + 9) and thickening of mitral and pulmonary valves. Due to progressive symptomatic left ventricular obstruction, the patient was operated at the age of 2 months (Konno operation). Operative complications led to his death a month later.

**P9, c.782C>G, p.(P261R):** HCM (IVS 9 mm, Z + 9). Progressive hypertrophy and restrictive physiology indicated a heart transplantation, performed at the age of 3 years.

*RAF1* (NM\_002880, NP\_002871) contributes to Ras/MAPK pathway, and the two affected amino acids in our patients are known hot spots for *de novo* mutations: p.(S257L) has been reported, and p.(P261R) is novel, but other pathogenic variants are known to affect the same amino acid. These amino acid changes are clustered within CR2 domain, which is the area harboring most often disease-causing variants (Online Figure 2B).

**P12, long-QT, LVNC, previously described *de novo* variant in *CACNA1C*, Voltage-dependent L-type calcium channel subunit alpha-1, c.1216G>A, p.(G406R).** The patient manifested with 2:1 heart block and LVNC before birth. Further detection of long QT syndrome occurred postnatally. Dual chamber pacemaker was implanted, but the patient died suddenly at home at the age of 3 months. *CACNA1C* (NM\_001129830, NP\_001123302) mediates the influx of calcium into cardiomyocytes. In the patient we identified the known pathogenic *de novo* variant p.(G406R). Exons 8 and 8A of *CACNA1C* are alternatively spliced, mutually exclusive, both disease-associated, and the patient’s *de novo* variant resides in exon 8. At protein level, the variant resides in the 6th transmembrane domain of this calcium channel, which together with the cytoplasmic loop between the 6th and the 7th transmembrane domains form an area enriched in pathogenic variation (Online Figure 3A).

**P3, progressive RCM, *de novo* variant in *BAG3*, BCL2-associated athanogene 3, c.626C>T, p.(P209L).** The patient received the diagnosis of RCM, asymptomatic first degree AV-block and normal QTc at the age of 13 years. Her disease progressed with myopathy, demyelinating polyneuropathy, ataxia and scoliosis. The patient died at the age of 18 years.

*BAG3* (NM\_004281, NP\_004272) encodes a co-chaperone of Z-disk in muscle cells. BCL2-associated athanogene 3 is also involved in apoptosis, autophagy, and mitochondrial quality control. The identified pathogenicvariant p.(P209L) is affecting the IPV 2 motif involved in the interaction with HSPB8 and HSPB6, and is a well-known site of *de novo* occurrence. Most mutations are located to the BAG domain involved in HSP70 binding. However, another pathogenic variant has been described in the vicinity of P209 (Online Figure 3B).

**P13, progressive RCM, *de novo* variant in *TNNC1*,Troponin C, c.91G>T, p.(A31S).** The diagnosis of RCM and atrial septum defect was made at the age of 6.5 years, prompted by decreased exercise capacity (work load 65 % of the expected value). Transpulmonary gradient was 5 mmHg, PVRI 1.8, mean pulmonary pressure 23 mmHg, and cardiac-index 3.4. The patient received a heart transplant at the age of 7 years, but died suddenly two years later. *TNNC1* (NM\_003280, NP\_003271) encodes the calcium-sensing subunit of troponin complex which regulates the interaction between myosin and actin in response to changes in calcium concentration. Troponin C harbors disease-causing variants along its entire length, including EF-hand domains. The p.(A31S) variant locates within the inactive EF-hand 1 domain (Online Figure 4), modifying its properties.

**P14, HCM, autosomal dominant pathogenic variant in *TNNI3*, Troponin I, c.526G>A, p.(V176M).** The patient was resuscitated at school after exercise at the age of 13 years and found to have significant HCM (IVS 24 mm, Z + 9), but no myocardial bridging. His treatment included an ICD implant and shock therapy for ventricular fibrillation. Despite beta-blocker treatment, the follow-up investigations detected ventricular tachycardia. His mother presents the same variant as well as significant HCM, and had an ICD implant. The maternal grandmother died suddenly at 40 years of age while jogging. *TNNI3* (NM\_000363, NP\_000354) encodes the inhibitory component of the troponin complex. The variant p.(V176M) is localized to the C-terminal mobile domain of the protein, enriched in pathogenic variants (Online Figure 4).

**P15 and P16**, **DCM, with pathogenic variants in Alpha-cardiac actin, *ACTC1*.**

**P15, c.524A>G, p.(H175R):** The patient received DCM diagnosis at 3 years of age. Her disease has stabilized with heart failure treatment and now she is 20 years old. Her mother manifested with severe DCM during peripartum period and required a left ventricular assist device, but she died for thromboembolic complications during the device treatment. We have identified in the patient the variant p.(H175R) in *ACTC1* as the most likely cause of her disease.

**P16, c.658T>C, p.(Y220H):** The patient was diagnosed at the age of 13 years to have DCM and received heart transplant at the age of 14 years. Currently he is 28 years old. None of the family members had cardiomyopathy. We identified a variant in *ACTC1*, p.(Y220H), as presumptive cause of patient’s disorder. Parental samples were unavailable to test its likely *de novo* status.

Alpha-cardiac actin(*ACTC1*, NM\_005159, NP\_005150) forms the thin filament of the sarcomere in cardiomyocytes. Figure S3B shows the diagram of the protein. Based on the structure of filamentous actin ([19](#_ENREF_19)), a change at H175 likely affects interaction between actin monomers, while Y220 is located at the edge of ATP-binding pocket and in the vicinity of tropomyosin filament.

**P17 with stabilizing HCM, P18 with stabilizing DCM, P19 with progressive DCM, and pathogenic variants in *MYH7* (NM\_000257, NP\_000248),Myosin heavy chain 7.**

**P17, c.2526T>A, p.(S842R):** Decreased exercise capacity prompted medical investigations, which showed HCM (IVS 16 mm, Z + 7) with mild left ventricular outflow obstruction of 25 mmHg, at the age of 5 years. At the time of the diagnosis left ventricular end diastolic pressure was 18 mmHg, PVRI 2.6, and mean pulmonary pressure 22 mmHg. Beta-blocker treatment was started. During 6 years follow-up, there were no changes in hemodynamics. The patient has normal development. We identified the *de novo* variant in *MYH7* p.(S842R) as the underlying cause of patient’s disease.

**P18, c.2773A>G, p.(R925G):** The family presented an autosomal dominant inheritance and all the affected family members were diagnosed with dilated cardiomyopathy in infancy (the index patient, his half-brother, and their father). All received heart failure treatment since infancy. Their cardiac findings have stabilized at the level of mild dysfunction. The autosomal dominant mutation p.(R925G) in *MYH7* segregated with disease in the family.

**P19, c.5635A>G, p.(K1879E):** The patient presented a severe progressive infantile DCM, with onset at 4 months of age and cardiac transplantation at the age of 13 months. Afterwards, his development has been normal and now he is 13 years of age. We identified the *MYH7* *de novo* variant p.(K1879E) as the most likely cause of patient’s disease.

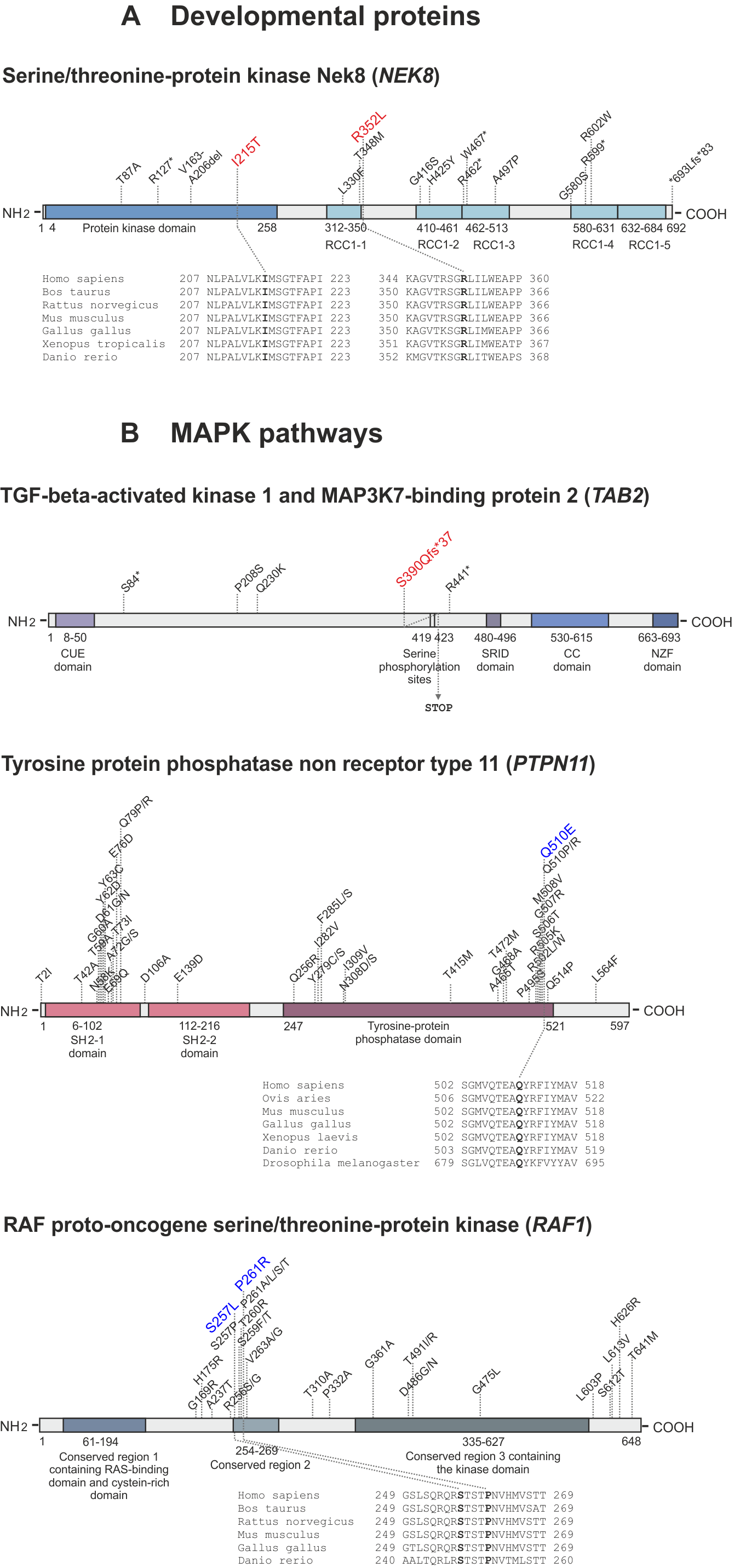
*MYH7* variants are a major cause of cardiomyopathy and have been reported across the entire length of the protein, although enriched in the motor domain which presents binding sites for ATP, actin, and essential or regulatory light chains. The variants discovered in our study, p.(S842R), p.(R925G), and p.(K1879E), are novel and locate in the beginning and at the end of the rod domain, and are predicted to alter the regularity of the coiled-coiled structure (Online Table 4, Online Figure 4).

**Supplemental Figures**

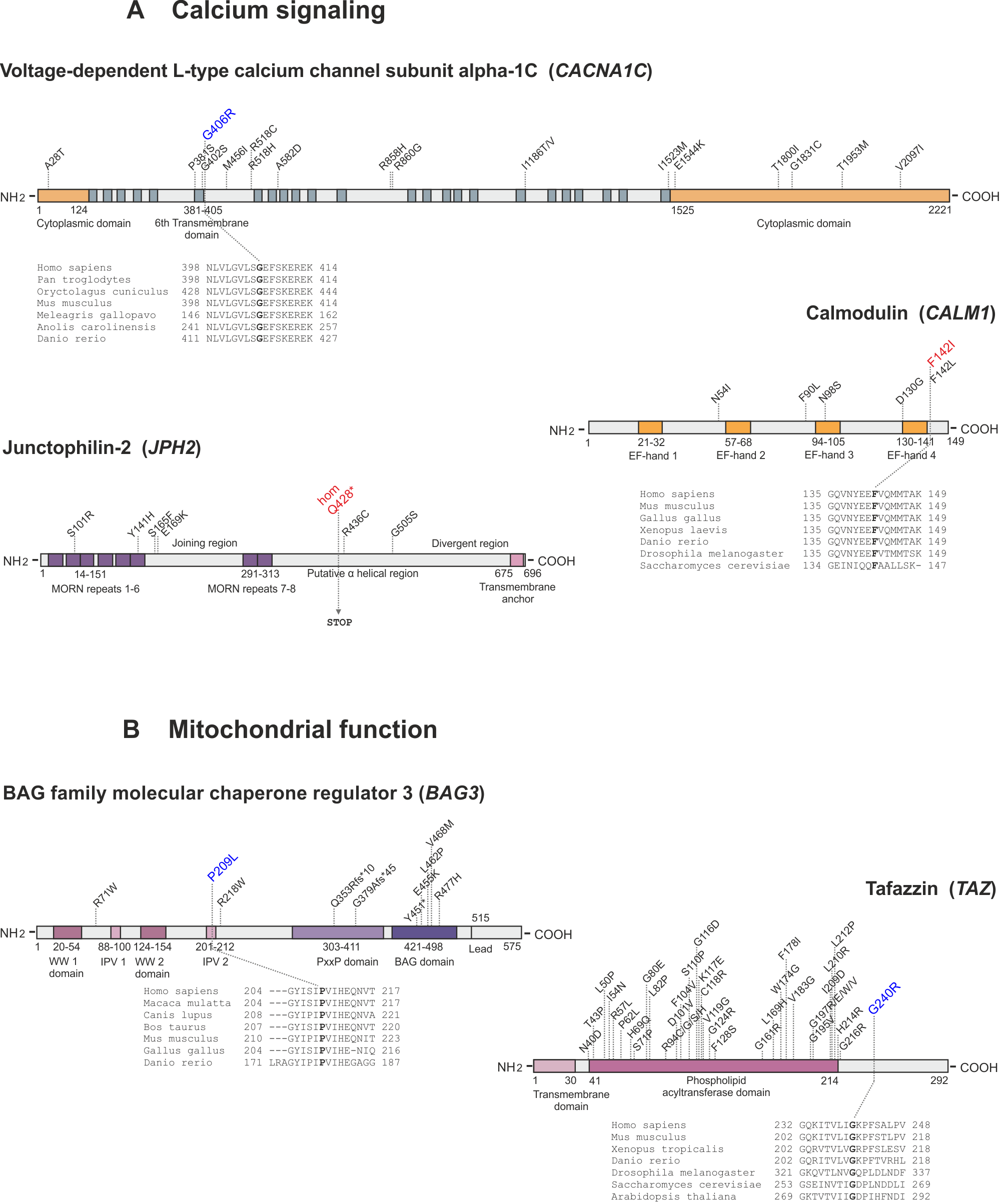
\\ATKK\home\v\vasilesc\Desktop\AJHG\Jacc\Online Figure 1_Panels_Filtering.tif

**Online Figure 1. Comparison of the NGS panels and variant filtering strategy.**

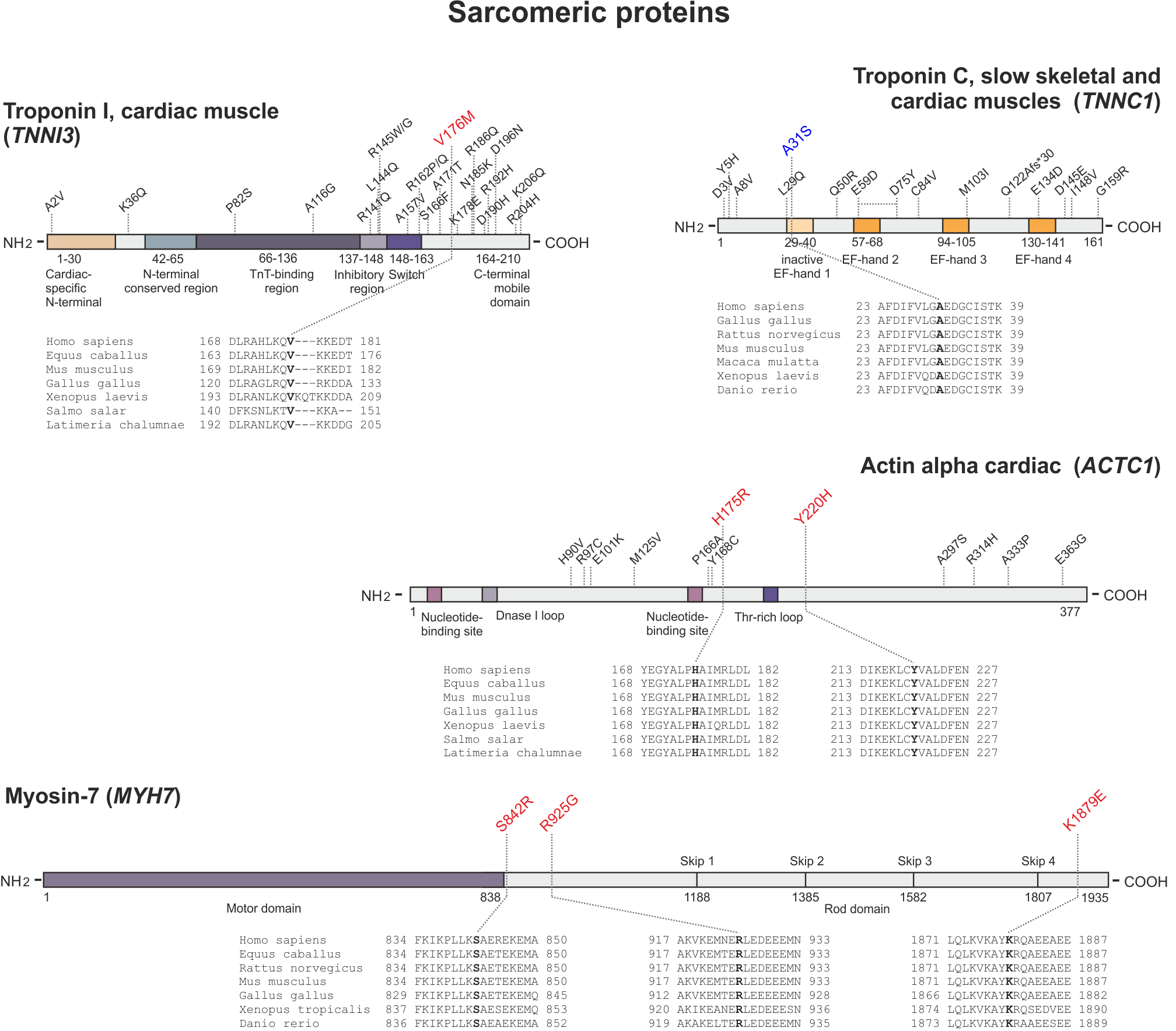
(**A**) Comparison of gene composition of the CMP-Custom HaloPlex and Pan Cardiomyopathy v.1.0 panels. (**B**) Steps in NGS data filtering and the validation of likely pathogenic variants to identify the causative ones. SNV (single nucleotide variant) and indel (insertion-deletion) analyses, for all the methods, included a sequence of filtering steps to retrieve only rare and likely pathogenic variants. First were selected the rare variants with a frequency less or equal to 0.01 (gnomAD, ExAC, SISu). Second, predictions of variants’ effect on the protein were accomplished using CADD, SIFT, and PolyPhen-2. Further prioritization was based on the relevance of protein function to the disease and on the amino acid conservation in species. Segregation analysis in the family, molecular modeling, and functional studies were applied to validate the causative pathogenic variants.



**Online Figure 2. Proteins with identified variants involved in development and MAPK pathways.** Causative variants in this study: red-novel, blue-known. (**A**) Developmental proteins. *NEK8* defects impair the function of the primary cilium on the cell surface. It contains a protein kinase domain and five RCC1 domains. The recessive changes identified in our patient, p.(I215T) and p.(R352L), locate in regions where other pathogenic variants have been reported, and lead to HCM with liver cirrhosis. (**B**) MAPK signaling. We found that *de novo* *TAB2* haploinsufficiency, previously involved in congenital heart disease, can cause fatal progressive infantile DCM. *PTPN11* and *RAF1* signal through Ras/MAPK pathway. The changes in these proteins are known hot spots for *de novo* pathogenic variation, inducing gain-of-function effects.



**Online Figure 3. Proteins with identified variants affecting calcium handling and mitochondrial function.** Causative variants in this study: red-novel, blue-known. (**A**) Calcium signaling. *CACNA1C* encodes the alpha-1 subunit of a voltage-dependent calcium channel which mediates the influx of calcium into cardiomyocytes. The *de novo* p.(G406R) (c.1216G>A in exon 8), was found in a patient with LVNC and infantile death, the second described in the literature. The 6th transmembrane domain of this channel subunit is enriched in pathogenic variation. Junctophilin-2 (*JPH2*) is crucial for calcium signaling in cardiomyocytes. The homozygous p.(Q428\*) in *JPH2* links this gene to childhood-onset CMP. Calmodulin (*CALM1*), a conserved second messenger of calcium, contains four EF-hand (calcium-binding) domains where pathogenic variants occur. Our patient’s change p.(F142I) is novel, affects EF-hand 4, and locates at the same amino acid position with a reported pathogenic variant ([20](#_ENREF_20)). (**B**) Mitochondrial function. *BAG3* affects mitochondrial quality control and chaperone-mediated autophagy. The variant p.(209L) is a recurrent *de novo* change found in multiple patients worldwide. It occurs in the IVP motif 2, described to mediate the interaction with HSPB8 and HSPB6, proteins involved in protein quality control. Tafazzin (*TAZ*), a mitochondrial protein involved in cardiolipin remodeling. The variant we found in *TAZ* has been described before ([21](#_ENREF_21)) but is atypically located toward the carboxy-terminal end of the protein, whereas all other known variants are within or adjacent to the phospholipid acyltransferase domain. This difference may reflect the recuperative clinical course in our patients.



**Online Figure 4. Sarcomeric proteins with disease-causing variants in this study.**

Causative variants in this study: red-novel, blue-known. The troponin complex regulates the interaction between myosin and actin in response to changes in calcium concentration. Troponin C (*TNNC1*) is the calcium-sensing subunit, harboring three active and one silenced calcium-binding domains (EF-hands). We identified a known *de novo* pathogenic variant, p.(A31S), in a patient with RCM. This variant is predicted to partially activate the silenced EF-hand1. Pathogenic variants in this protein are not restricted to EF-hand domains, occurring throughout its entire length. Troponin I (*TNNI3*) is the inhibitory component of the complex. In a patient with familial HCM we identified a novel variant, p.(V176M), localized to the C-terminal mobile domain, enriched in disease-causing variants. The primary structural proteins of the sarcomere are myosin and actin, major components of the thick and thin filaments respectively. Alpha cardiac actin (*ACTC1*) showed two novel amino acid changes in our cohort: p.(H175R), predicted to affect the interaction between actin monomers, and p.(Y220H), which locates at the edge of ATP-binding pocket and in the vicinity of tropomyosin filament. Myosin-7 (*MYH7*) is a major cause of cardiomyopathy, with pathogenic variants reported across the entire length of the protein, although enriched in the motor domain. The pathogenic changes discovered in this study reside in the beginning and at the end of the rod domain, and are predicted to alter its repetitive structure. The previously reported variants in *MYH7* have been omitted from the figure due to their increased number.

**Supplemental Tables**

**Online Table 1. CMP-Custom HaloPlex Panel.** The panel includes 117 genes, of which 85 are cardiomyopathy-related, 29 cause heart rhythm disorders, and three putative CMP genes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No** | **Gene** | **Ensembl ID** | **HGNC description** | **Disease involvement** |
| 1 | ABCC9 | ENSG00000069431 | ATP-binding cassette, sub-family C (CFTR/MRP), member 9 | DCM, AF |
| 2 | ACADVL | ENSG00000072778 | acyl-CoA dehydrogenase, very long chain | sHCM, sDCM |
| 3 | ACTC1 | ENSG00000159251 | actin, alpha, cardiac muscle 1 | HCM, DCM, LVNC, RCM, ASD |
| 4 | ACTN2 | ENSG00000077522 | actinin, alpha 2 | HCM, DCM |
| 5 | ANKRD1 | ENSG00000148677 | ankyrin repeat domain 1 (cardiac muscle) | HCM, DCM |
| 6 | BAG3 | ENSG00000151929 | BCL2-associated athanogene 3 | DCM |
| 7 | CALR3 | ENSG00000141979 | calreticulin 3 | HCM |
| 8 | CASQ2 | ENSG00000118729 | calsequestrin 2 (cardiac muscle) | LVNC, CPVT |
| 9 | CAV3 | ENSG00000182533 | caveolin 3 | HCM, sHCM |
| 10 | CPT2 | ENSG00000157184 | carnitine palmitoyltransferase 2 | sDCM |
| 11 | CRYAB | ENSG00000109846 | crystallin, alpha B | HCM, DCM |
| 12 | CSRP3 | ENSG00000129170 | cysteine and glycine-rich protein 3 (cardiac LIM protein) | HCM, DCM |
| 13 | CTF1 | ENSG00000150281 | cardiotrophin 1 | DCM |
| 14 | CTNNA3 | ENSG00000183230 | catenin (cadherin-associated protein), alpha 3 | ARVC |
| 15 | DES | ENSG00000175084 | desmin | HCM, DCM, ARVC, RCM |
| 16 | DMD | ENSG00000198947 | dystrophin | DCM, sDCM |
| 17 | DNAJC19 | ENSG00000205981 | DnaJ (Hsp40) homolog, subfamily C, member 19 | DCM, LVNC |
| 18 | DNM1L | ENSG00000087470 | dynamin 1-like | sDCM |
| 19 | DOLK | ENSG00000175283 | dolichol kinase | sDCM |
| 20 | DSC2 | ENSG00000134755 | desmocollin 2 | DCM, ARVC |
| 21 | DSG2 | ENSG00000046604 | desmoglein 2 | DCM, ARVC |
| 22 | DSP | ENSG00000096696 | desmoplakin | DCM, sDCM, ARVC |
| 23 | DTNA | ENSG00000134769 | dystrobrevin, alpha | LVNC |
| 24 | EMD | ENSG00000102119 | emerin | DCM, sDCM |
| 25 | EYA4 | ENSG00000112319 | eyes absent homolog 4 (Drosophila) | DCM |
| 26 | FHL2 | ENSG00000115641 | four and a half LIM domains 2 | HCM, DCM |
| 27 | FKTN | ENSG00000106692 | fukutin | DCM, sDCM |
| 28 | FXN | ENSG00000165060 | frataxin | sHCM |
| 29 | GAA | ENSG00000171298 | glucosidase, alpha; acid | sHCM |
| 30 | GATAD1 | ENSG00000157259 | GATA zinc finger domain containing 1 | DCM |
| 31 | GJA5 | ENSG00000143140 | gap junction protein, alpha 5, 40kDa | Atrial CM with heart block |
| 32 | GLA | ENSG00000102393 | galactosidase, alpha | sHCM |
| 33 | HADHA | ENSG00000084754 | hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit | sDCM |
| 34 | HRAS | ENSG00000174775 | v-Ha-ras Harvey rat sarcoma viral oncogene homolog | sHCM |
| 35 | ILK | ENSG00000166333 | integrin-linked kinase | DCM |
| 36 | JPH2 | ENSG00000149596 | junctophilin 2 | HCM |
| 37 | JUP | ENSG00000173801 | junction plakoglobin | DCM, ARVC |
| 38 | KRAS | ENSG00000133703 | v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog | sHCM |
| 39 | LAMA4 | ENSG00000112769 | laminin, alpha 4 | DCM |
| 40 | LAMP2 | ENSG00000005893 | lysosomal-associated membrane protein 2 | sHCM, sDCM |
| 41 | LDB3 | ENSG00000122367 | LIM domain binding 3 | HCM, DCM, ARVC, LVNC |
| 42 | LMNA | ENSG00000160789 | lamin A/C | HCM, DCM, sDCM, ARVC, LVNC |
| 43 | MURC | ENSG00000170681 | Muscle-restricted coiled-coil | DCM |
| 44 | MYBPC3 | ENSG00000134571 | myosin binding protein C, cardiac | HCM, DCM, LVNC |
| 45 | MYH6 | ENSG00000197616 | myosin, heavy chain 6, cardiac muscle, alpha | HCM, DCM, ASD |
| 46 | MYH7 | ENSG00000092054 | myosin, heavy chain 7, cardiac muscle, beta | HCM, DCM, sDCM, LVNC, RCM |
| 47 | MYL2 | ENSG00000111245 | myosin, light chain 2, regulatory, cardiac, slow | HCM, RCM |
| 48 | MYL3 | ENSG00000160808 | myosin, light chain 3, alkali; ventricular, skeletal, slow | HCM, RCM |
| 49 | MYLK2 | ENSG00000101306 | myosin light chain kinase 2 | HCM |
| 50 | MYO6 | ENSG00000196586 | myosin VI | sHCM |
| 51 | MYOM2 | ENSG00000036448 | myomesin 2 | ARVC |
| 52 | MYOZ2 | ENSG00000172399 | myozenin 2 | HCM |
| 53 | MYPN | ENSG00000138347 | myopalladin | HCM, DCM, LVNC, RCM |
| 54 | NEBL | ENSG00000078114 | nebulette | DCM |
| 55 | NEXN | ENSG00000162614 | nexilin (F actin binding protein) | HCM, DCM |
| 56 | PKP2 | ENSG00000057294 | plakophilin 2 | HCM, DCM, ARVC |
| 57 | PLN | ENSG00000198523 | phospholamban | HCM, DCM, ARVC |
| 58 | PRKAG2 | ENSG00000106617 | protein kinase, AMP-activated, gamma 2 non-catalytic subunit | HCM, WPW Syndrome |
| 59 | PSEN1 | ENSG00000080815 | presenilin 1 | DCM |
| 60 | PSEN2 | ENSG00000143801 | presenilin 2 (Alzheimer disease 4) | DCM |
| 61 | RAF1 | ENSG00000132155 | v-raf-1 murine leukemia viral oncogene homolog 1 | sHCM, DCM |
| 62 | RBM20 | ENSG00000203867 | RNA binding motif protein 20 | DCM |
| 63 | RYR2 | ENSG00000198626 | ryanodine receptor 2 (cardiac) | DCM, ARVC, CPVT |
| 64 | SCN5A | ENSG00000183873 | sodium channel, voltage-gated, type V, alpha subunit | DCM, CPVT, VF, LQTS, Brugada syndrome |
| 65 | SDHA | ENSG00000073578 | succinate dehydrogenase complex, subunit A, flavoprotein (Fp) | DCM, sDCM, LVNC |
| 66 | SGCB | ENSG00000163069 | sarcoglycan, beta (43kDa dystrophin-associated glycoprotein) | DCM |
| 67 | SGCD | ENSG00000170624 | sarcoglycan, delta (35kDa dystrophin-associated glycoprotein) | DCM, sDCM |
| 68 | SLC22A5 | ENSG00000197375 | solute carrier family 22 (organic cation/carnitine transporter), member 5 | sHCM, sDCM |
| 69 | SLC25A4 | ENSG00000151729 | solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4 | HCM |
| 70 | SLC25A20 | ENSG00000178537 | solute carrier family 25 (carnitine/acylcarnitine translocase), member 20 | sHCM |
| 71 | SOS1 | ENSG00000115904 | son of sevenless homolog 1 (Drosophila) | sHCM |
| 72 | TAZ | ENSG00000102125 | tafazzin | sHCM, DCM, sDCM, LVNC |
| 73 | TCAP | ENSG00000173991 | titin-cap (telethonin) | HCM, DCM |
| 74 | TGFB3 | ENSG00000119699 | transforming growth factor, beta 3 | ARVC |
| 75 | TMEM43 | ENSG00000170876 | transmembrane protein 43 | ARVC |
| 76 | TMEM70 | ENSG00000175606 | transmembrane protein 70 | sHCM |
| 77 | TMPO | ENSG00000120802 | thymopoietin | DCM |
| 78 | TNNC1 | ENSG00000114854 | troponin C type 1 (slow) | HCM, DCM |
| 79 | TNNI3 | ENSG00000129991 | troponin I type 3 (cardiac) | HCM, DCM, RCM |
| 80 | TNNT2 | ENSG00000118194 | troponin T type 2 (cardiac) | HCM, DCM, LVNC, RCM |
| 81 | TPM1 | ENSG00000140416 | tropomyosin 1 (alpha) | HCM, DCM, LVNC, RCM |
| 82 | TSFM | ENSG00000123297 | Ts translation elongation factor, mitochondrial | sHCM |
| 83 | TTN | ENSG00000155657 | titin | HCM, DCM, sDCM, ARVC |
| 84 | TTR | ENSG00000118271 | transthyretin | FAC |
| 85 | VCL | ENSG00000035403 | vinculin | HCM, DCM |
| 86 | CALM1 | ENSG00000198668 | calmodulin 1 (phosphorylase kinase, delta) | CPVT, VF, cardiac arrest |
| 87 | CALM2 | ENSG00000143933 | calmodulin 2 (phosphorylase kinase, delta) | VF, cardiac arrest |
| 88 | CALM3 | ENSG00000160014 | calmodulin 3 (phosphorylase kinase, delta) |  |
| 89 | UTS2R | ENSG00000181408 | urotensin 2 receptor |  |
| 90 | MMGT1 | ENSG00000169446 | membrane magnesium transporter 1 |  |
| 91 | KCNE1 | ENSG00000180509 | potassium voltage-gated channel, Isk-related family, member 1 | LQTS |
| 92 | KCNE2 | ENSG00000159197 | potassium voltage-gated channel, Isk-related family, member 2 | LQTS, AF |
| 93 | KCNH2 | ENSG00000055118 | potassium voltage-gated channel, subfamily H (eag-related), member 2 | SQTS, LQTS, AF |
| 94 | KCNQ1 | ENSG00000053918 | potassium voltage-gated channel, KQT-like subfamily, member 1 | SQTS, LQTS, AF |
| 95 | KCNJ8 | ENSG00000121361 | potassium inwardly-rectifying channel, subfamily J, member 8 | VF |
| 96 | KCNJ2 | ENSG00000123700 | potassium inwardly-rectifying channel, subfamily J, member 2 | SQTS, LQTS, AF |
| 97 | GPD1L | ENSG00000152642 | glycerol-3-phosphate dehydrogenase 1-like | Brugada syndrome |
| 98 | SCN1B | ENSG00000105711 | sodium channel, voltage-gated, type I, beta subunit | Brugada syndrome, AF |
| 99 | SCN2B | ENSG00000149575 | sodium channel, voltage-gated, type II, beta subunit | AF |
| 100 | SCN3B | ENSG00000166257 | sodium channel, voltage-gated, type III, beta subunit | Brugada syndrome, AF |
| 101 | KCNE3 | ENSG00000175538 | potassium voltage-gated channel, Isk-related family, member 3 | Brugada syndrome, AF |
| 102 | KCND3 | ENSG00000171385 | potassium voltage-gated channel, Shal-related subfamily, member 3 | Brugada syndrome |
| 103 | CACNA1C | ENSG00000151067 | calcium channel, voltage-dependent, L type, alpha 1C subunit | Brugada syndrome, Timothy syndrome |
| 104 | CACNB2 | ENSG00000165995 | calcium channel, voltage-dependent, beta 2 subunit | Brugada syndrome |
| 105 | CACNA2D1 | ENSG00000153956 | calcium channel, voltage-dependent, alpha 2/delta subunit 1 | Brugada syndrome |
| 106 | KCNE1L | ENSG00000176076 | KCNE1-like | AF |
| 107 | KCNA5 | ENSG00000130037 | potassium voltage-gated channel, shaker-related subfamily, member 5 | AF |
| 108 | GJA5 | ENSG00000143140 | gap junction protein, alpha 5, 40kDa | AF |
| 109 | NPPA | ENSG00000175206 | natriuretic peptide A | AF |
| 110 | NUP155 | ENSG00000113569 | nucleoporin 155kDa | AF |
| 111 | GJA1 | ENSG00000152661 | gap junction protein, alpha 1, 43kDa | AVSD |
| 112 | ANK2 | ENSG00000145362 | ankyrin 2, neuronal | LQTS |
| 113 | SCN4B | ENSG00000177098 | sodium channel, voltage-gated, type IV, beta subunit | LQTS, AF |
| 114 | AKAP9 | ENSG00000127914 | A kinase (PRKA) anchor protein (yotiao) 9 | LQTS |
| 115 | SNTA1 | ENSG00000101400 | syntrophin, alpha 1 | LQTS |
| 116 | KCNJ5 | ENSG00000120457 | potassium inwardly-rectifying channel, subfamily J, member 5 | LQTS |
| 117 | DPP6 | ENSG00000130226 | dipeptidyl-peptidase 6 | SCD |

(s)DCM = (syndromic) dilated cardiomyopathy, (s)HCM = (syndromic) hypertrophic cardiomyopathy, LVNC = left ventricular noncompaction, RCM = restrictive cardiomyopathy, HICM = histiocytoid cardiomyopathy, WPW Syndrome = Wolff-Parkinson-White Syndrome, ARVC = arrhythmogenic right ventricular tachycardia, FAC= familial amyloid cardiomyopathy, CPVT = catecholaminergic polymorphic ventricular tachycardia, AF = atrial fibrillation, VF = ventricular fibrillation, AVSD = atrioventricular septal defects, LQTS = long QT syndrome, SQTS = short QT syndrome, SCD = sudden cardiac death

**Online Table 2. Pan Cardiomyopathy v.1.0 Panel.** The panel consists of 101 cardiomyopathy-related genes (Blueprint Genetics).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No** | **Gene** | **Ensembl ID** | **HGNC description** | **Disease involvement** |
| 1 | ABCC9 | ENSG00000069431 | ATP-binding cassette, sub-family C (CFTR/MRP), member 9 | DCM, AF |
| 2 | ACADVL | ENSG00000072778 | acyl-CoA dehydrogenase, very long chain | sHCM, sDCM |
| 3 | ACTC1 | ENSG00000159251 | actin, alpha, cardiac muscle 1 | HCM, DCM, LVNC, RCM, ASD |
| 4 | ACTN2 | ENSG00000077522 | actinin, alpha 2 | HCM, DCM |
| 5 | AGL | ENSG00000162688 | amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase | sHCM |
| 6 | ANKRD1 | ENSG00000148677 | ankyrin repeat domain 1 (cardiac muscle) | HCM, DCM |
| 7 | ATP5E | ENSG00000124172 | ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit | sHCM |
| 8 | BAG3 | ENSG00000151929 | BCL2-associated athanogene 3 | DCM |
| 9 | BRAF | ENSG00000157764 | v-raf murine sarcoma viral oncogene homolog B | sHCM |
| 10 | CALR3 | ENSG00000269058 | calreticulin 3 | HCM |
| 11 | CASQ2 | ENSG00000118729 | calsequestrin 2 (cardiac muscle) | LVNC, CPVT |
| 12 | CAV3 | ENSG00000182533 | caveolin 3 | HCM, sHCM |
| 13 | CBL | ENSG00000110395 | Cbl proto-oncogene, E3 ubiquitin protein ligase |  |
| 14 | COA5 | ENSG00000183513 | cytochrome c oxidase assembly factor 5 | sHCM |
| 15 | CRYAB | ENSG00000109846 | crystallin, alpha B | HCM, DCM |
| 16 | CSRP3 | ENSG00000129170 | cysteine and glycine-rich protein 3 (cardiac LIM protein) | HCM, DCM |
| 17 | CTF1 | ENSG00000150281 | cardiotrophin 1 | DCM |
| 18 | CTNNA3 | ENSG00000183230 | catenin (cadherin-associated protein), alpha 3 | ARVC |
| 19 | DES | ENSG00000175084 | desmin | HCM, DCM, ARVC, RCM |
| 20 | DMD | ENSG00000198947 | dystrophin | DCM, sDCM |
| 21 | DMPK | ENSG00000104936 | dystrophia myotonica-protein kinase | sDCM |
| 22 | DNAJC19 | ENSG00000205981 | DnaJ (Hsp40) homolog, subfamily C, member 19 | DCM, LVNC |
| 23 | DNM1L | ENSG00000087470 | dynamin 1-like | sDCM |
| 24 | DOLK | ENSG00000175283 | dolichol kinase | sDCM |
| 25 | DSC2 | ENSG00000134755 | desmocollin 2 | DCM, ARVC |
| 26 | DSG2 | ENSG00000046604 | desmoglein 2 | DCM, ARVC |
| 27 | DSP | ENSG00000096696 | desmoplakin | DCM, sDCM, ARVC |
| 28 | DTNA | ENSG00000134769 | dystrobrevin, alpha | LVNC |
| 29 | EMD | ENSG00000102119 | emerin | DCM, sDCM |
| 30 | EYA4 | ENSG00000112319 | eyes absent homolog 4 (Drosophila) | DCM |
| 31 | FHL1 | ENSG00000022267 | four and a half LIM domains 1 | HCM, sHCM |
| 32 | FHL2 | ENSG00000115641 | four and a half LIM domains 2 | DCM, HCM |
| 33 | FKTN | ENSG00000106692 | fukutin | DCM, sDCM |
| 34 | FOXRED1 | ENSG00000110074 | FAD-dependent oxidoreductase domain containing 1 | sHCM |
| 35 | FXN | ENSG00000165060 | frataxin | sHCM |
| 36 | GAA | ENSG00000171298 | glucosidase, alpha; acid | sHCM |
| 37 | GATAD1 | ENSG00000157259 | GATA zinc finger domain containing 1 | DCM |
| 38 | GLA | ENSG00000102393 | galactosidase, alpha | sHCM |
| 39 | GLB1 | ENSG00000170266 | galactosidase, beta 1 | sHCM, sDCM |
| 40 | GUSB | ENSG00000169919 | glucuronidase, beta | sHCM |
| 41 | HFE | ENSG00000010704 | hemochromatosis | sDCM |
| 42 | HRAS | ENSG00000174775 | Harvey rat sarcoma viral oncogene homolog | sHCM |
| 43 | ILK | ENSG00000166333 | integrin-linked kinase | DCM |
| 44 | JPH2 | ENSG00000149596 | junctophilin 2 | HCM |
| 45 | JUP | ENSG00000173801 | junction plakoglobin | DCM, ARVC |
| 46 | KRAS | ENSG00000133703 | Kirsten rat sarcoma viral oncogene homolog | sHCM |
| 47 | LAMA4 | ENSG00000112769 | laminin, alpha 4 | DCM |
| 48 | LAMP2 | ENSG00000005893 | lysosomal-associated membrane protein 2 | sHCM, sDCM |
| 49 | LDB3 | ENSG00000122367 | LIM domain binding 3 | HCM, DCM, ARVC, LVNC |
| 50 | LMNA | ENSG00000160789 | lamin A/C | HCM, DCM, sDCM, ARVC, LVNC |
| 51 | MAP2K1 | ENSG00000169032 | mitogen-activated protein kinase kinase 1 | sHCM |
| 52 | MAP2K2 | ENSG00000126934 | mitogen-activated protein kinase kinase 2 | sHCM |
| 53 | MRPL3 | ENSG00000114686 | mitochondrial ribosomal protein L3 | sHCM |
| 54 | MYBPC3 | ENSG00000134571 | myosin binding protein C, cardiac | HCM, DCM, LVNC |
| 55 | MYH6 | ENSG00000197616 | myosin, heavy chain 6, cardiac muscle, alpha | HCM, DCM, ASD |
| 56 | MYH7 | ENSG00000092054 | myosin, heavy chain 7, cardiac muscle, beta | HCM, DCM, sDCM, LVNC, RCM |
| 57 | MYL2 | ENSG00000111245 | myosin, light chain 2, regulatory, cardiac, slow | HCM, RCM |
| 58 | MYL3 | ENSG00000160808 | myosin, light chain 3, alkali; ventricular, skeletal, slow | HCM, RCM |
| 59 | MYLK2 | ENSG00000101306 | myosin light chain kinase 2 | HCM |
| 60 | MYOM1 | ENSG00000101605 | myomesin 1 | HCM |
| 61 | MYOZ2 | ENSG00000172399 | myozenin 2 | HCM |
| 62 | MYPN | ENSG00000138347 | myopalladin | HCM, DCM, LVNC, RCM |
| 63 | NEBL | ENSG00000078114 | nebulette | DCM |
| 64 | NEXN | ENSG00000162614 | nexilin (F actin binding protein) | HCM, DCM |
| 65 | NRAS | ENSG00000213281 | neuroblastoma RAS viral (v-ras) oncogene homolog | sHCM |
| 66 | PDLIM3 | ENSG00000154553 | PDZ and LIM domain 3 | HCM, DCM |
| 67 | PKP2 | ENSG00000057294 | plakophilin 2 | HCM, DCM, ARVC |
| 68 | PLN | ENSG00000198523 | phospholamban | HCM, DCM, ARVC |
| 69 | PRKAG2 | ENSG00000106617 | protein kinase, AMP-activated, gamma 2 non-catalytic subunit | HCM, WPW Syndrome |
| 70 | PSEN1 | ENSG00000080815 | presenilin 1 | DCM |
| 71 | PSEN2 | ENSG00000143801 | presenilin 2 (Alzheimer disease 4) | DCM |
| 72 | PTPN11 | ENSG00000179295 | protein tyrosine phosphatase, non-receptor type 11 | sHCM |
| 73 | RAF1 | ENSG00000132155 | v-raf-1 murine leukemia viral oncogene homolog 1 | sHCM, DCM |
| 74 | RBM20 | ENSG00000203867 | RNA binding motif protein 20 | DCM |
| 75 | RYR2 | ENSG00000198626 | ryanodine receptor 2 (cardiac) | ARVC |
| 76 | SCN5A | ENSG00000183873 | sodium channel, voltage-gated, type V, alpha subunit | DCM |
| 77 | SCO2 | ENSG00000130489 | SCO2 cytochrome c oxidase assembly protein | sHCM |
| 78 | SDHA | ENSG00000073578 | succinate dehydrogenase complex, subunit A, flavoprotein (Fp) | DCM, sDCM, LVNC |
| 79 | SGCD | ENSG00000170624 | sarcoglycan, delta (35kDa dystrophin-associated glycoprotein) | DCM, sDCM |
| 80 | SHOC2 | ENSG00000108061 | soc-2 suppressor of clear homolog (C. elegans) | sHCM |
| 81 | SLC25A3 | ENSG00000075415 | solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3 | sHCM |
| 82 | SOS1 | ENSG00000115904 | son of sevenless homolog 1 (Drosophila) | sHCM |
| 83 | SPRED1 | ENSG00000166068 | sprouty-related, EVH1 domain containing 1 |  |
| 84 | SYNE1 | ENSG00000131018 | spectrin repeat containing, nuclear envelope 1 | DCM, sDCM |
| 85 | SYNE2 | ENSG00000054654 | spectrin repeat containing, nuclear envelope 2 | sDCM |
| 86 | TAZ | ENSG00000102125 | tafazzin | sHCM, DCM, sDCM, LVNC |
| 87 | TCAP | ENSG00000173991 | titin-cap | HCM, DCM |
| 88 | TGFB3 | ENSG00000119699 | transforming growth factor, beta 3 | ARVC |
| 89 | TMEM43 | ENSG00000170876 | transmembrane protein 43 | ARVC |
| 90 | TMEM70 | ENSG00000175606 | transmembrane protein 70 | sHCM |
| 91 | TMPO | ENSG00000120802 | thymopoietin | DCM |
| 92 | TNNC1 | ENSG00000114854 | troponin C type 1 (slow) | HCM, DCM |
| 93 | TNNI3 | ENSG00000129991 | troponin I type 3 (cardiac) | HCM, DCM, RCM |
| 94 | TNNT2 | ENSG00000118194 | troponin T type 2 (cardiac) | HCM, DCM, LVNC, RCM |
| 95 | TPM1 | ENSG00000140416 | tropomyosin 1 (alpha) | HCM, DCM, LVNC, RCM |
| 96 | TSFM | ENSG00000123297 | Ts translation elongation factor, mitochondrial | sHCM |
| 97 | TTN | ENSG00000155657 | titin | HCM, DCM, sDCM, ARVC |
| 98 | TTR | ENSG00000118271 | transthyretin | FAC |
| 99 | TXNRD2 | ENSG00000184470 | thioredoxin reductase 2 | DCM |
| 100 | VCL | ENSG00000035403 | vinculin | HCM, DCM |
| 101 | XK | ENSG00000047597 | X-linked Kx blood group (McLeod syndrome) | sDCM |

Abbreviations as in Online Table 2

**Online Table 3**. Primers used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Genomic DNA Sequencing** | | | | |
| **Gene** | **Variant** | **Primer** | **Sequence 5´-3´** | **Patient** |
| *PPA2* | c.514G>A, p.(E172K) | F | aaaactttgtgattatgtggaaaga | P1 |
| R | gaaatcaataggcttgaactctga |
| c.556G>A, p.(V186M) | F | ttgaggctttgttacatttcagtc |
| R | ccaatgacacactaatttgctttc |
| *TAZ* | c.718G>A, p.(G240R) | F | ccaggaccaggaccttgttt | P2 |
| R | gaggcaccctgctggaag |
| *BAG3* | c.626C>T, p.(P209L) | F | aagccaggggagtcatttgt | P3 |
| R | ctggacagatgacctgaacg |
| *TBX20* | c.670A>G, p.(M224V) | F | tgcagttgttttggtaaaatgtg | P4 |
| R | tgcaggaactaaattgtgaactg |
| *NEK8* | c.644T>C, p.(I215T) | F | tgagggctctgttctgttcc | P5 |
| R | GTCGGTGTGGAGGTTGAGG |
| c.1055G>T, p.(R352L) | F | GGCTGCCAATGCTCAACA |
| R | AGGAAACGCGAGATGAACTG |
| *TAB2* | c.1168delT, p.S390Qfs\*37 | F | CGTTCTTCTGGACCTCGAAC | P6 |
| R | GACTTTTGGGAGGATGGTGA |
| *PTPN11* | c.1528C>G, p.(Q510E) | F | ctgtagccattgcaacatgc | P7 |
| R | cctgtcctcctgctcaaaag |
| *JPH2* | hom c.1282C>T, p.(Q428\*) | F | cttctgccacattgtctcca | P10 |
| R | ggcatccaggaaaccactt |
| *CALM1* | c.424T>A, p.(F142I) | F | tgatggagacggacaagtca | P11 |
| R | tgcagattttgtgtgtgtgg |
| *CACNA1C* | c.1216G>A, p.(G406R) | F | ctgcgtgggtcagtgtctc | P12 |
| R | tctcctccattgtccaccac |
| *TNNC1* | c.91G>T, p.(A31S) | F | agaggtctccgagggaggt | P13 |
| R | agctggggttcttctggag |
| *TNNI3* | c.526G>A, p.(V176M) | F | agtacccaccccctcgttt | P14 |
| R | gccctaatgcacttcctgtc |
| *ACTC1* | c.524A>G, p.(H175R) | F | atgcccattgctagcatctc | P15 |
| R | atagggaggtaggcggattc |
| c.658T>C, p.(Y220H) | F | agcgtggctactcctttgtc | P16 |
| R | ctctgcaccagaccctacaa |
| *MYH7* | c.2526T>A, p.(S842R) | F | gtgggagggatgaaggaaat | P17 |
| R | gggtggaagagccaacagta |
| c.2773A>G, p.(R925G) | F | CCCTCCTATTTGAGTGATGTGC | P18 |
| R | TGGTCTGAGAGTCCTGATGAGA |
| c.5635A>G, p.(K1879E) | F | gggtcaggatatcagatgaagc | P19 |
| R | ggaacttggacaggttggtg |
| *NRAP* | hom c.1344T>A, p.(Y448\*) | F | tccttgaaggtgatgaaactga | P20 |
| R | aggaaggaagcgacaagtga |
| **cDNA Amplification and Sequencing** | | | | |
| **Gene** | **Variant** | **Primer** | **Sequence 5´-3´** | **Patient** |
| *NRAP* | hom c.1344T>A, p.(Y448\*) | F | TATGGACAGACGCACTCTGC | P20 |
| R | TTGGCTTGAACAATCTGTGG |
| **qPCR** | | | | |
| **Gene** | | **Primer** | **Sequence 5´-3´** | **Patient** |
| *NRAP* | | F | CCGGAGCTTGGAAGATGATA | P20 |
|  | | R | GAACTGCGCCTTTGAGTGTT |
| *ACTB* | | F | cctggcacccagcacaat |
|  | | R | gggccggactcgtcatac |
| **Fingerprinting** | | | | |
| **Microsatellite Marker** | | **Primer** | **Sequence 5´-3´** | **Patient** |
| D8S1179 | | F | 6Fam-GTATCGTATCCCATTGCGTG | P3, P11, P12, P13, P17, P19 |
| R | CGCCTTTGCCTGAGTTTTG |
| D21S11 | | F | 6Fam-TGTGAGTCAATTCCCCAAGTG |
| R | CACTGAGAAGGGAGAAACACTG |
| D16S538 | | F | 6Fam-GTTCCCATTTTTATATGGGAGC |
| R | TTTACGTTTGTGTGTGCATCTG |
| D2S1338 | | F | 6Fam-GAAGCCAGTGGATTTGGAAAC |
| R | TCCTACCAGAATGCCAGTCC |
| D18S51 | | F | 6Fam-CATGCCACTGCACTTCACTC |
| R | AAGGTGGACATGTTGGCTTC |
| VWA | | F | 6Fam-CCCTAGTGGATGATAAGAATAATCAGTATG |
| R | GGACAGATGATAAATACATAGGATGGATGG |
| FGA | | F | 6Fam-TGCCCCATAGGTTTTGAAC |
| R | CTTTGCGCTTCAGGACTTC |

**Online Table 4. Consequences of variants at protein level.** Summary of biochemical and predicted functional consequences of the variants, based on the literature and our protein analyses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Protein name** | **Variant** | **Effects at protein level** | **Evidence** |
| *PPA2* | Inorganic pyrophosphatase 2, mitochondrial | p.(E172K)  p.(V186M) | Protein instability induced by altered interactions inside the hydrophobic core of the protein. | Molecular modeling.  Western blotting. |
| *TAZ* | Tafazzin | p.(G240R) | May affect protein stability. | *In silico* modeling of tafazzin variants. Hijikata *et al* ([22](#_ENREF_22)) |
| *BAG3* | BAG3 family molecular chaperone regulator 3 | p.(P209L) | The variant occurs in IVP motif 2, described to mediate the interaction with HSPB8 and HSPB6. | Fuchs *et al* ([23](#_ENREF_23)) |
| *TBX20* | T-box transcription factor 20 | p.(M224V) | Likely affects DNA binding in the major groove. | Molecular modeling. |
| *NEK8* | Serine/threonine-protein kinase Nek8 | p.(I215T)  p.(R352L) | Unknown. Other pathogenic variants lead to altered Hippo signaling. | Grampa *et al* ([15](#_ENREF_15)) |
| *TAB2* | TGF-beta-activated kinase 1 and MAP3K7-binding protein 2 | p.(S390Qfs\*37) | Haploinsufficiency. | Early stop codon. |
| *PTPN11* | Tyrosine-protein phosphatase non-receptor type 11 | p.(Q510E) | Gain of function: although the altered protein presents decreased phosphatase activity, this feature is counteracted by decreased autoinhibition, whereby the protein more easily adopts an open, active conformation. | Activity measurement and crystal structure determination of the altered protein harboring p.(Q510E). Yu *et al* ([24](#_ENREF_24)) |
| *RAF1* | RAF proto-oncogene serine/threonine-protein kinase | p.(S257L) | Increased kinase activity and enhanced ERK activation. | Pandit *et al* ([25](#_ENREF_25))  Razzaque *et al* ([26](#_ENREF_26)) |
| p.(P261R) | Similar variants, p.(P261A), p.(P261L), and p.(P261S), show greater kinase activity and enhanced MAPK1 activation. | Pandit *et al* ([25](#_ENREF_25))  Razzaque *et al* ([26](#_ENREF_26)) |
| *JPH2* | Junctophilin-2 | p.(Q428\*) | The variant likely causes a complete loss of protein function. | Position of the premature stop codon. |
| *CALM1* | Calmodulin | p.(F142I) | F142 is adjacent to the fourth EF-hand (calcium-binding) domain.  By similarity with p.(F142L), the variant probably reduces the affinity for calcium in the C-terminal domain. This may alter the ability of the protein to transduce calcium signals. | Position in the protein.  Experimental testing of a similar variant, p.(F142L).  Crotti *et al* ([20](#_ENREF_20))  Rocchetti *et al* ([27](#_ENREF_27)) |
| *CACNA1C* | Voltage-dependent L-type calcium channel subunit alpha-1 | p.(G406R) | Reduced channel inactivation resulting in maintained depolarizing L-type calcium currents. | Splawski *et al* ([28](#_ENREF_28)) |
| *TNNC1* | Troponin C, slow skeletal and cardiac muscles | p.(A31S) | Partial activation of a silenced calcium-binding domain, EF-hand 1. Increased affinity for calcium. | Paravatiyar *et al* ([29](#_ENREF_29)) |
| *TNNI3* | Troponin I, cardiac muscle | p.(V176M) | Not clear. The change occurs in the α-helix 4 of the C-terminal mobile domain. More data is needed regarding the interaction with other sarcomeric/cytoskeletal proteins and about the dynamics of the complex during contraction cycle. | Crystal structure of cardiac troponin.  Takeda *et al* ([30](#_ENREF_30)) |
| *ACTC1* | Actin, alpha cardiac muscle 1 | p.(H175R) | May affect interaction between actin monomers. | Cryo-EM structure of a human cytoplasmic actomyosin complex.  von der Ecken *et al* ([19](#_ENREF_19)) |
| p.(Y220H) | The affected amino acid is localized at the edge of ATP-binding pocket and in the vicinity of tropomyosin filament. | Cryo-EM structure of a human cytoplasmic actomyosin complex.  von der Ecken *et al* ([19](#_ENREF_19)) |
| *MYH7* | Myosin-7 | p.(S842R) | Substitution between amino acids with different properties in the beginning of the rod domain: a small neutral serine is replaced by a large positive arginine.  Removal of a predicted phosphorylation site. | MyoMAPR <http://bmf.colorado.edu/myomapr>  phosphosite.org |
| p.(R925G) | Amino acids with different properties.  Size: large arginine – small glycine.  Charge: transition from + to 0 in position g of the rod domain repeat 3.  By analogy with p.(E924K), may influence the binding affinity of MyBP-C. | MyoMAPR <http://bmf.colorado.edu/myomapr>  Experimental evidence for p.(E924K).  Gruen *et al* ([31](#_ENREF_31)) |
| p.(K1879E) | Amino acid charge transition from + to - in position e of the rod domain repeat 37. | MyoMAPR <http://bmf.colorado.edu/myomapr> |
| *NRAP* | Nebulin-related-anchoring protein | hom p.(Y448\*) | The variant likely causes a complete loss of protein function. We showed that NRAP mRNA harboring the premature stop codon is not degraded in patient’s heart biopsy. | Position of the premature stop codon. |

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