

## Supplemental Materials

**Table S1.** The 72 genes in our NGS panel and their associated general and clinical features

Gene	Protein	Role of protein in cardiomyocytes	Inheritance	HC M	DC M	RCM	ARVC	LVNC
<i>ABCC9</i>	Sulfonylurea receptor 2 (SUR2)	Involving in the formation of potassium channels	AD		X			
<i>ACTA1</i>	Actin alpha 1, skeletal muscle	The core of the thin filament of the sarcomere in adult skeletal muscle	AD, AR	X				
<i>ACTC1</i>	Actin alpha 1, cardiac muscle	The core of the thin filament of the sarcomere in adult cardiac muscle	AD	X	X	X		X
<i>ACTN2</i>	Actinin alpha 2	Structural and functional role in the sarcomeres	AD	X	X			
<i>ALPK3</i>	Alpha protein kinase 3	Transcriptional regulator which phosphorylates cardiac transcriptional factors; involving in cardiac muscle cell development	AR	X				
<i>ANK2</i>	Ankyrin B	Interacting with ion channels, ion transporters and signalling molecules across the cell membranes	AD					
<i>ANKRD1</i>	Ankyrin repeat protein	Z-disc component	N/A	X	X			
<i>BAG3</i>	BCL2 associated athanogene 3	Co-chaperone with members of the heat-shock protein family; inhibiting apoptosis; Z-disc structural role	AD	X	X	X		
<i>CACNA1C</i>	Calcium voltage-gated channel subunit alpha 1	Involving in the formation of calcium channels	AD					
<i>CACNB2</i>	Calcium voltage-gated channel subunit beta 2	Involving in the formation of calcium channels	AD					

<i>CASQ2</i>	Calsequestrin 2	Involving in the storage and transport of calcium ions through the sarcoplasmic reticulum	AR					X
<i>CAV3</i>	Caveolin 3	Component of the plasma membrane of muscle cells; regulating ion channels; playing role in signal transduction	AD, DD	X	X			
<i>CRYAB</i>	Crystallin alpha B	Acting as a molecular chaperone	AD, AR	X	X			
<i>CSRP3</i>	Cysteine and glycine rich protein 3	Z-disc structural role; implicating in transcriptional regulation, cytoskeleton organization, cell adhesion and signal transduction	AD	X	X			
<i>DES</i>	Desmin	Structural and mechanical integrity of the contractile apparatus	AD, AR		X	X	X	
<i>DMD</i>	Dystrophin	Part of the dystrophin-glycoprotein complex, which bridges the cytoskeleton and the extracellular matrix	XLR, XL		X			X
<i>DSC2</i>	Desmocollin 2	Component of the desmosome, which provides adhesion between cardiac cells	AD, AR		X		X	
<i>DSG2</i>	Desmoglein 2	Component of the desmosome, which provides adhesion between cardiac cells	AD		X		X	
<i>DSP</i>	Desmoplakin	Linking the desmosomes to intermediate filaments	AD, AR		X		X	
<i>DTNA</i>	Dystrobrevin alpha	Part of the dystrophin-glycoprotein complex, which bridges the cytoskeleton and the extracellular matrix	AD					X
<i>EMD</i>	Emerin	Component of the nuclear envelope	XLR		X			
<i>EYA4</i>	EYA transcriptional coactivator and phosphatase 4	Acting as a transcriptional activator	AD		X			

<i>FKTN</i>	Fukutin	Ribitol 5-phosphate transferase in the cis-Golgi compartment involving in the glycosylation of alpha dystroglycan	AR		X			
<i>FLNC</i>	Filamin C	Crosslinking actin filaments and anchoring the plasma membrane proteins to the cytoskeleton	AD	X		X		
<i>GLA</i>	Galactosidase alpha	Hydrolysing the terminal alpha-galactose groups from glycoproteins and glycolipids in lysosomes	XL	X		X		X
<i>HCN4</i>	Hyperpolarization activated cyclic nucleotide gated potassium channel 4	Transporting ions into cells of the sino-atrial node, which are necessary for the generation of electrical impulses that start each heartbeat	AD					
<i>JPH2</i>	Junctophilin 2	Component of the junctional membrane complexes which connect the plasma membrane and the sarcoplasmic reticulum	AD	X				
<i>JUP</i>	Junction Plakoglobin	Component of the desmosome, which provides adhesion between cardiac cells	AD, AR				X	
<i>KCNE1</i>	Potassium voltage-gated channel subfamily E regulatory subunit 1	Binding to the potassium channels and regulating their activity	AD, AR					
<i>KCNE2</i>	Potassium voltage-gated channel subfamily E regulatory subunit 2	Binding to the potassium channels and regulating their activity	AD					
<i>KCNH2</i>	Potassium voltage-gated channel subfamily H member 2	Making up potassium channels	AD					
<i>KCNJ2</i>	Potassium voltage-gated channel subfamily J member 2	Making up potassium channels	AD					
<i>KCNQ1</i>	Potassium voltage-gated channel subfamily Q member 1	Making up potassium channels	AD					

<i>LAMA4</i>	Laminin subunit alpha 4	The major noncollagenous component of the basement membrane; implicating in cell adhesion, differentiation, migration, signalling and metastasis	AD		X			
<i>LAMP2</i>	Lysosomal-associated membrane protein 2	Involving in the process of the autophagosome maturation in lysosomes	XLD	X	X			
<i>LDB3</i>	LIM domain binding 3	Providing structural stability to the Z-disc	AD	X	X			
<i>LMNA</i>	Lamin A/C	Component of the nuclear envelope; implicating in cell cycle regulation, chromatin organization and DNA damage response	AD, AR		X		X	X
<i>MYBPC3</i>	Myosin-binding protein C	Linking the thin and thick filaments of the sarcomeres	AD, AR	X	X			X
<i>MYH6</i>	Alpha myosin heavy chain 6	Component of the cardiac myosin, which makes up the thick filaments of the sarcomeres	AD	X	X			
<i>MYH7</i>	Beta myosin heavy chain 7	Major component of the cardiac myosin, which makes up the thick filaments of the sarcomeres	AD, DD, AR	X	X	X		X
<i>MYL2</i>	Myosin regulatory light chain 2	Essential for the activation of the cardiac myosin during muscle contraction	AD	X		X		
<i>MYL3</i>	Myosin essential light chain 3	Stabilizing the alpha-helical neck of the cardiac myosin head	AD, AR	X		X		
<i>MYLK2</i>	Myosin light chain kinase 2	Phosphorylating the light chains of myosin, thereby regulating the actin-myosin interaction	AD, DD	X				
<i>MYOM1</i>	Myomesin 1	Structural and functional role in the M-band of the sarcomeres	N/A	X				
<i>MYOZ2</i>	Myozenin 2	Tethering calcineurin to alpha-actinin of the sarcomeres, thus it	AD	X				

		is important for calcineurin signaling						
<i>MYPN</i>	Myopalladin	Part of the alpha-actinin-myopalladin-nebulette complex that tethers titin and actin to the Z-disc of the sarcomeres	AD, AR		X	X		
<i>NEBL</i>	Nebulette	Aligning the thin filaments of the sarcomeres and linking them to the Z-disc	N/A		X			
<i>NEXN</i>	Nexilin	Involving in Z-disc stability	AD	X	X			
<i>NKX2.5</i>	NK2 homeobox 5	Homeobox-containing transcription factor, which is important for normal cardiac development	AD	X				
<i>PDLIM3</i>	PDZ and LIM domain protein 3	Involving in cytoskeletal assembly	N/A		X			
<i>PKP2</i>	Plakophilin 2	Component of the desmosome, which provides adhesion between cardiac cells	AD		X		X	
<i>PLN</i>	Phospholamban	Playing key role in the release and storage of calcium in the sarcoplasmic reticulum	AD	X	X		X	
<i>PRDM16</i>	PR domain containing 16	Transcription factor, which is important for normal cardiac development	AD		X			X
<i>PRKAG2</i>	AMP-activated protein kinase, non-catalytic subunit gamma 2	Involving in cellular ATP metabolic regulation	AD	X				
<i>PTPN11</i>	Protein tyrosine phosphatase SHP2	Involving in the regulation of RAS/MAPK signalling pathway, which regulates important cell functions (proliferation, differentiation, migration, apoptosis); critical in normal cardiac development	AD	X				

<i>RAF1</i>	Raf-1 proto-oncogene, serine/threonine kinase	Part of the RAS/MAPK signalling pathway, which regulates important cell functions (proliferation, differentiation, migration, apoptosis)	AD	X				
<i>RBM20</i>	RNA-binding motif protein 20	Binding RNA and regulating mRNA splicing of genes involved in cardiac development	AD		X			
<i>RYR2</i>	Ryanodine receptor 2	Making up calcium channels, which control the flow of calcium ions through the sarcoplasmic reticulum	AD	X			X	
<i>SCN5A</i>	Voltage-gated sodium channel subunit alpha Nav1.5	Making up sodium channels, which control the flow of sodium ions into cardiomyocytes	AD, AR		X		X	
<i>SGCD</i>	Sarcoglycan delta	Component of the sarcoglycan complex, which is a member of dystrophin-glycoprotein complex, which bridges the cytoskeleton and the extracellular matrix	AD?, AR		X			
<i>TAZ</i>	Tafazzin	Involving in the metabolism of cardiolipin, the main phospholipid of the mitochondrial inner membrane	XLR	X	X			X
<i>TCAP</i>	Titin-cap protein	Involving in sarcomere development and stability	AD, AR	X	X			
<i>TGFB3</i>	Transforming growth factor beta 3	Binding to receptor proteins on the surface of cells and triggering the cell signalling within the cells, which regulates important cell functions (proliferation, differentiation, migration, apoptosis)	AD				X	
<i>TMEM43</i>	Transmembrane protein 43	Involving in the organization of the nuclear membrane	AD				X	

<i>TMPO</i>	Thymopoietin	Maintaining the structural organization of the nuclear envelope	N/A		X			
<i>TNNC1</i>	Troponin C type 1	Acting as a myofilament calcium sensor; the binding of calcium to troponin C allows the interaction of actin with myosin for the generation of contractile force	AD	X	X			
<i>TNNI3</i>	Troponin I type 3	The inhibitory protein of the troponin complex; blocking the actin-myosin interaction in response to the reduction in the intracellular calcium concentration, leading to the muscle relaxation	AD, AR	X	X	X		
<i>TNNT2</i>	Troponin T type 2	The tropomyosin-binding subunit of the troponin complex, which regulates the muscle contraction in response to the changes of the intracellular calcium concentration	AD	X	X	X		X
<i>TPM1</i>	Tropomyosin 1	Maintaining the stability of the thin filament of the sarcomeres; regulating the calcium-dependent actin-myosin interaction	AD	X	X	X		X
<i>TTN</i>	Titin	Maintaining sarcomere structural integrity	AD, AR	X	X	X	X	
<i>TTR</i>	Transthyretin	Involving in the transportation of hormone thyroxine and retinol (vitamin A) throughout the body	AD	X	X	X		X
<i>VCL</i>	Vinculin	Involving in the anchoring of F-actin to the sarcolemma, thus it is important for cell-cell and cell-extracellular matrix junctions	AD	X	X			X

AD: Autosomal Dominant, AR: Autosomal Recessive, DD: Digenic Dominant, XL: X-linked, XLD: X-linked Dominant, XLR: X-linked Recessive

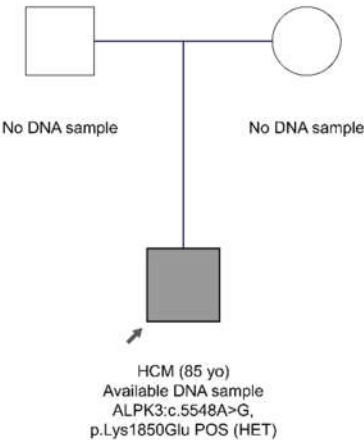


**Table S2:** Variants which based on our data were upgraded to Likely Pathogenic and description of the relevant ACMG criteria.

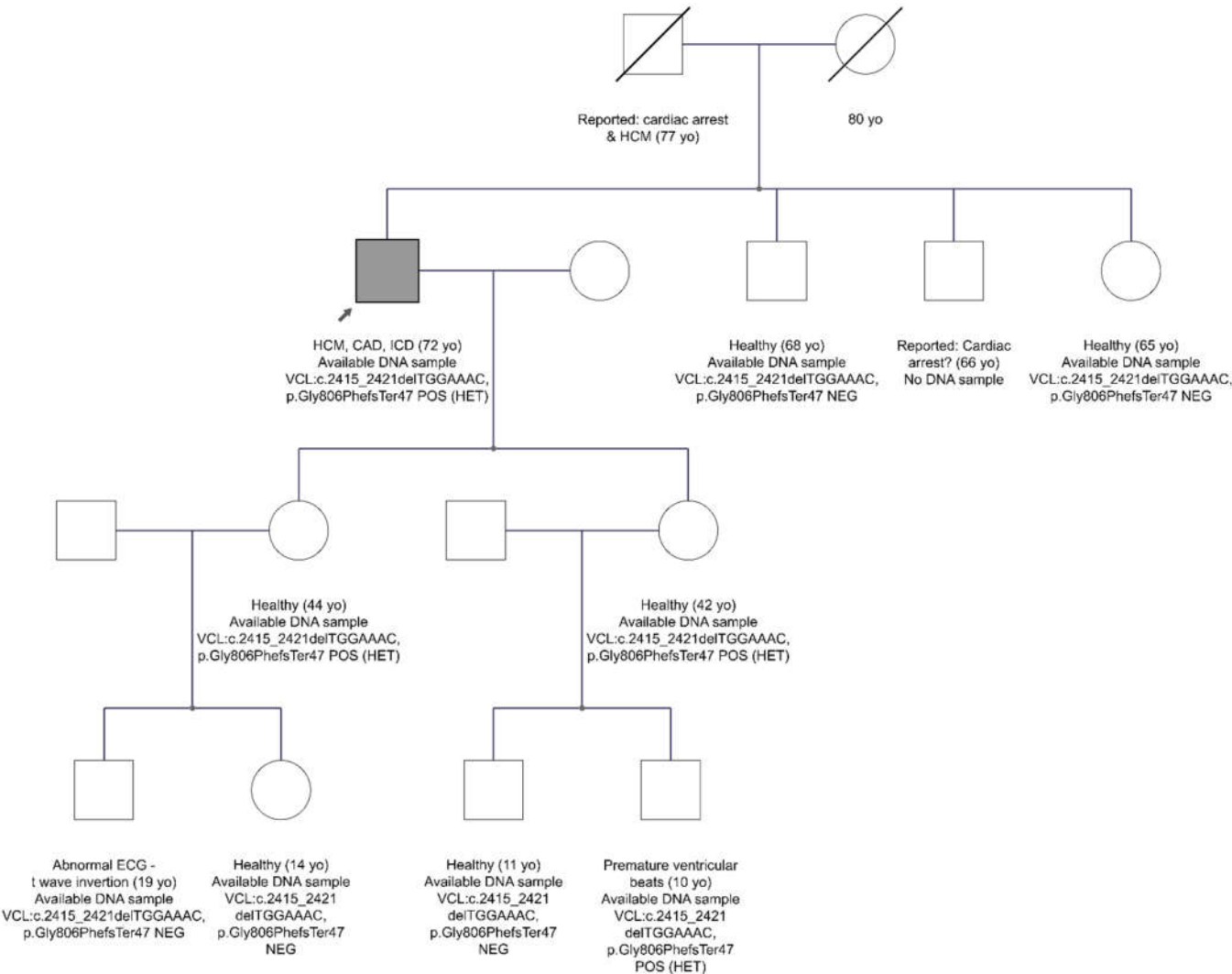
Family	Gene-Exon	Chromosome position; Transcript	Coding	Protein	Franklin classification	Cosegregation data	Our classification
FAM03 (HOCM)	<i>TNNI3</i> (Exon 7)	chr19:55665519; NM_000363.4	c.428C>A	p.Thr143Asn	VUS to likely pathogenic (PM1: Pathogenic Moderate, PP2: Pathogenic Supporting, PM2: Pathogenic Moderate)	Proband with HOCM carries the variant, whereas his healthy son does not	Likely pathogenic
FAM10 (HOCM)	<i>PLN</i> (Exon 2)	chr6:118880229; NM_002667.4	c.145G>A	p.Val49Met	VUS to likely pathogenic (PM2: Pathogenic Moderate, PP3: Pathogenic Moderate)	Proband with HOCM carries the variant, whereas the healthy family members do not. His mother and his grandmother, who have milder symptoms, carry also the variant.	Likely pathogenic
FAM24 (ARVC)	<i>DSC2</i> (Exon 8)	chr18:28662978; NM_024422.4	c.991C>A	p.Gln331Lys	VUS (PM2: Pathogenic Moderate)	Proband with ARVC carries the variant as well as his brothers with ARVC. His father, who is healthy, does not carry the variant.	Likely pathogenic

CAD: Coronary Artery Disease, HOCM: Hypertrophic Obstructive Cardiomyopathy, ICD: Implantable Cardioverter Defibrillator, ISH: Isolated Septal Hypertrophy

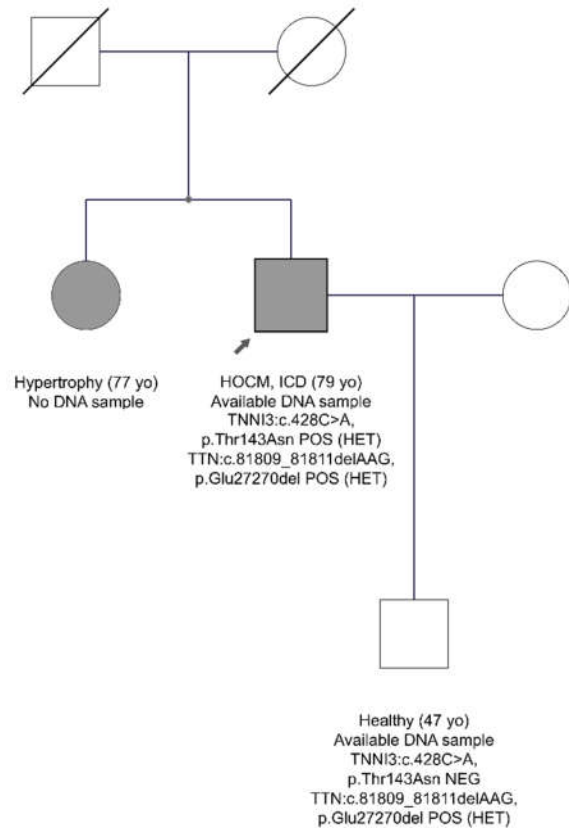
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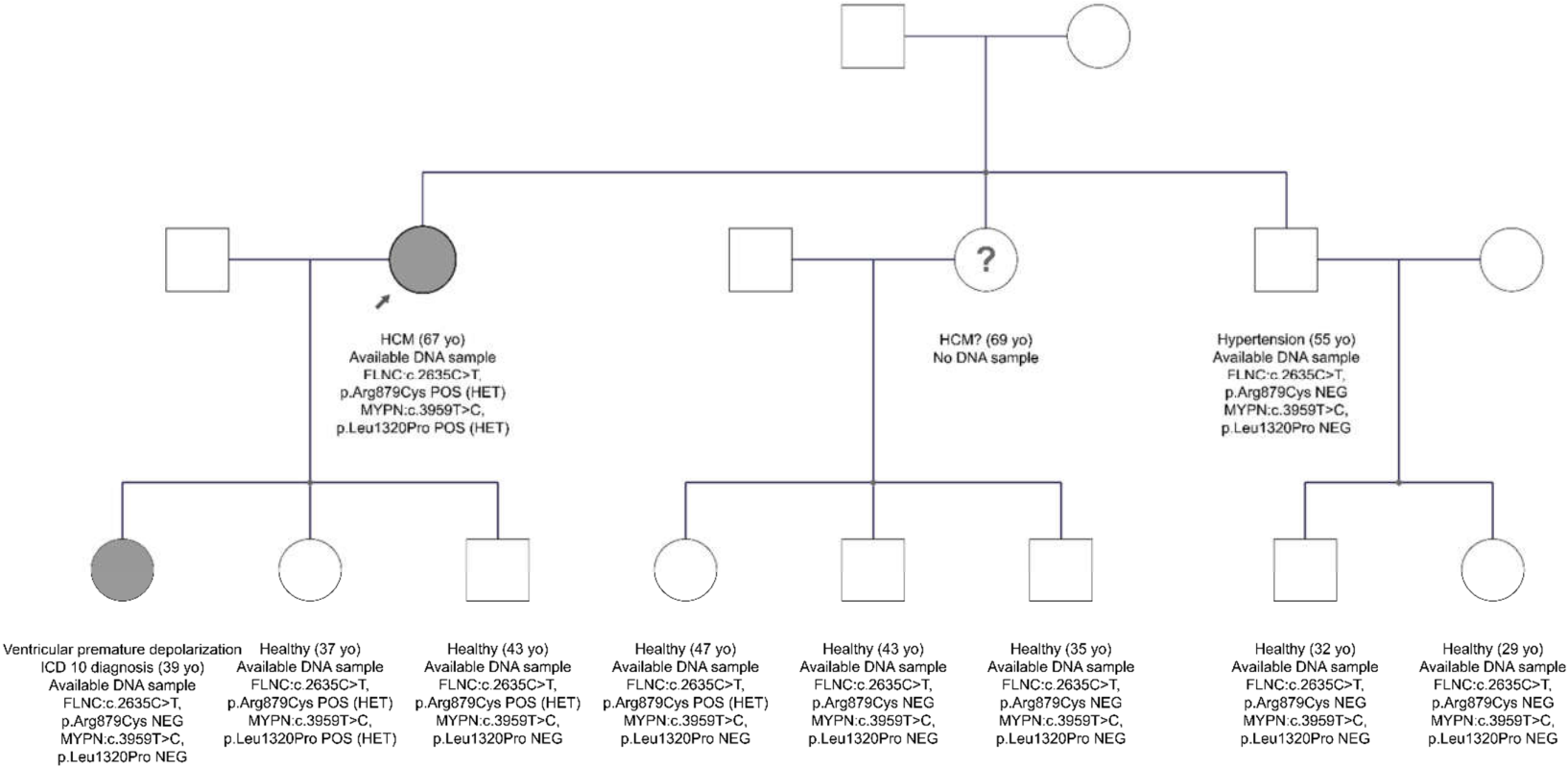
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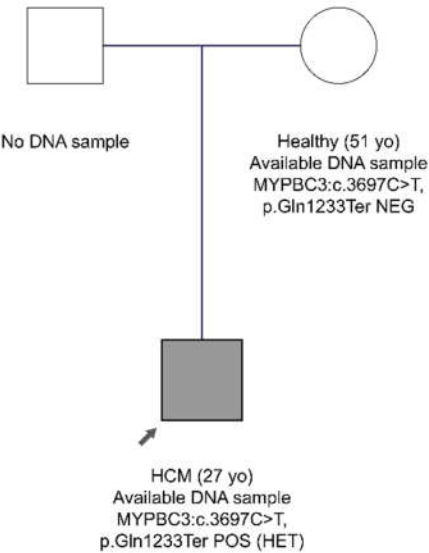
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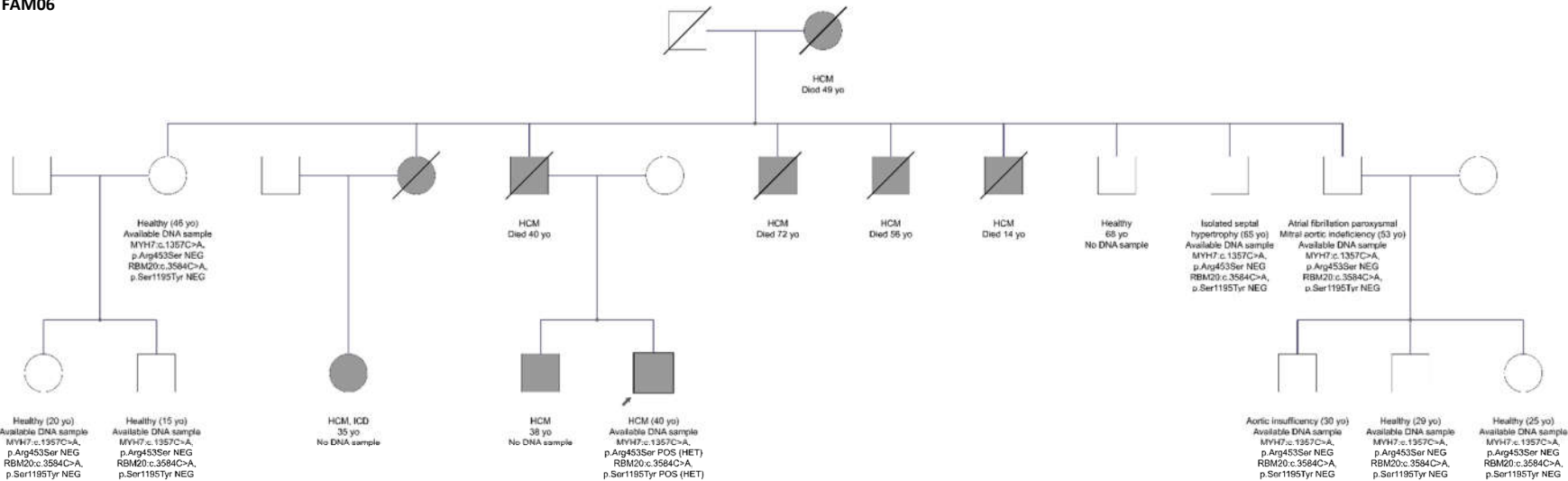
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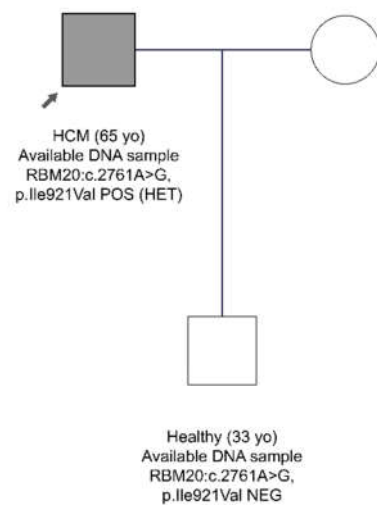
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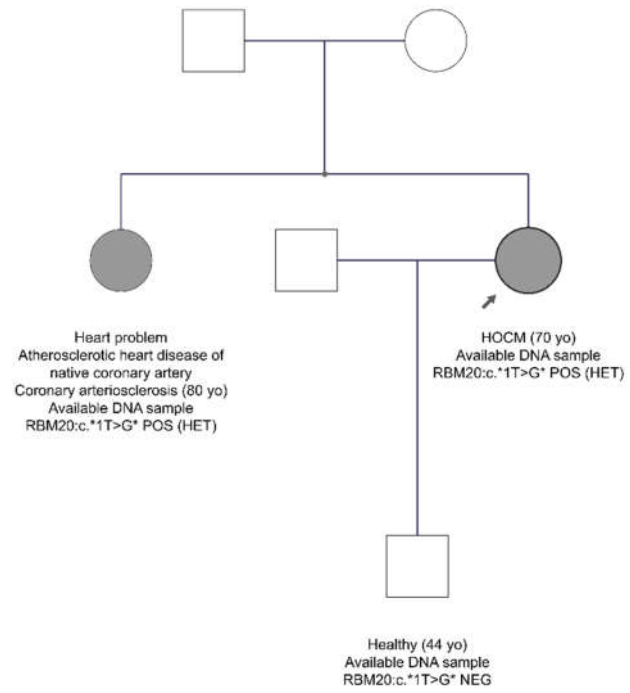
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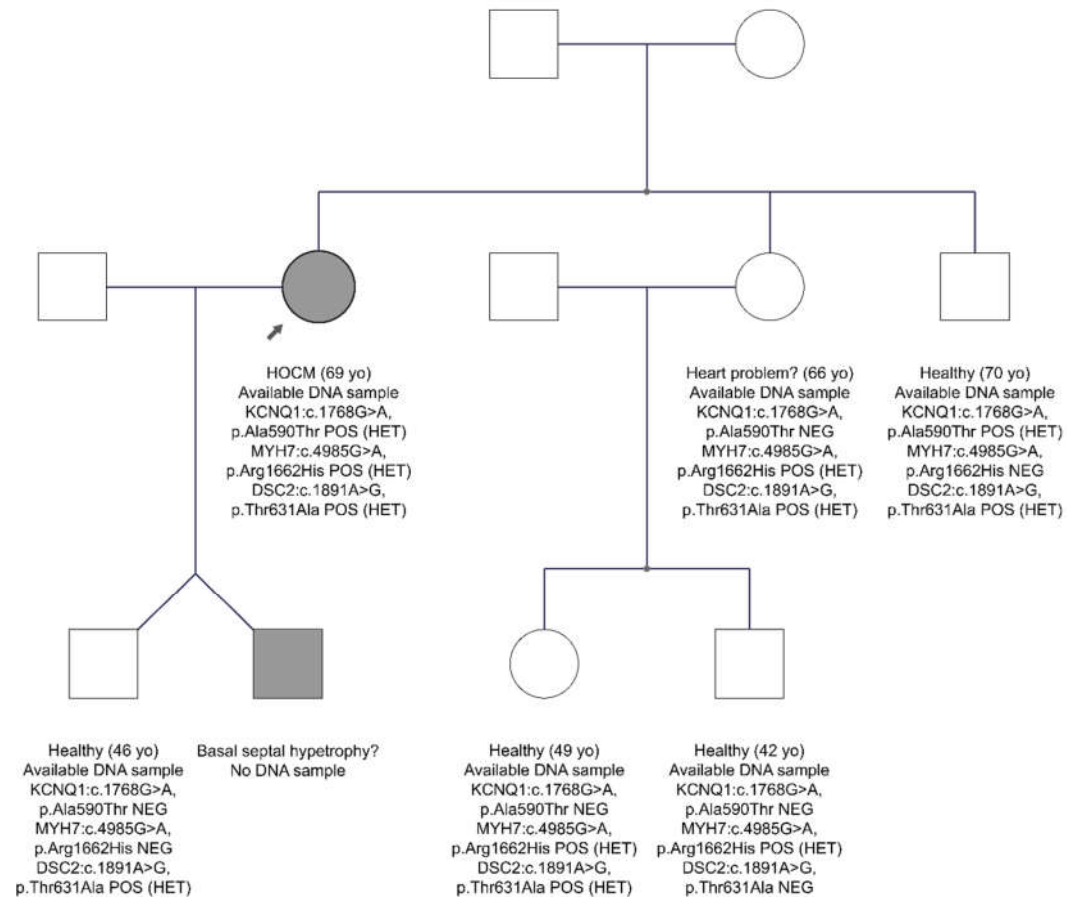
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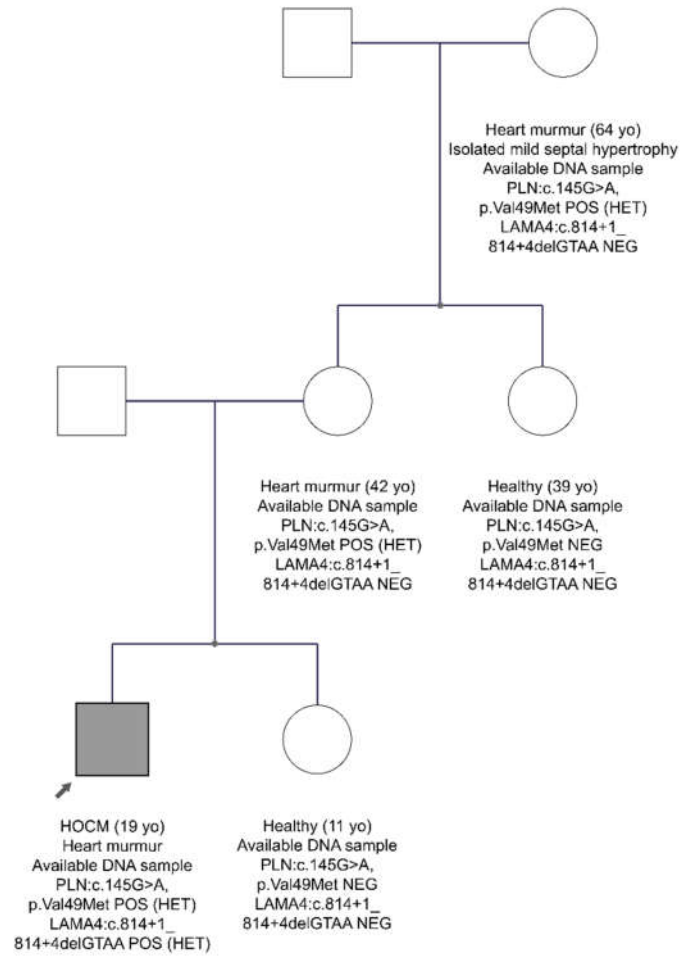
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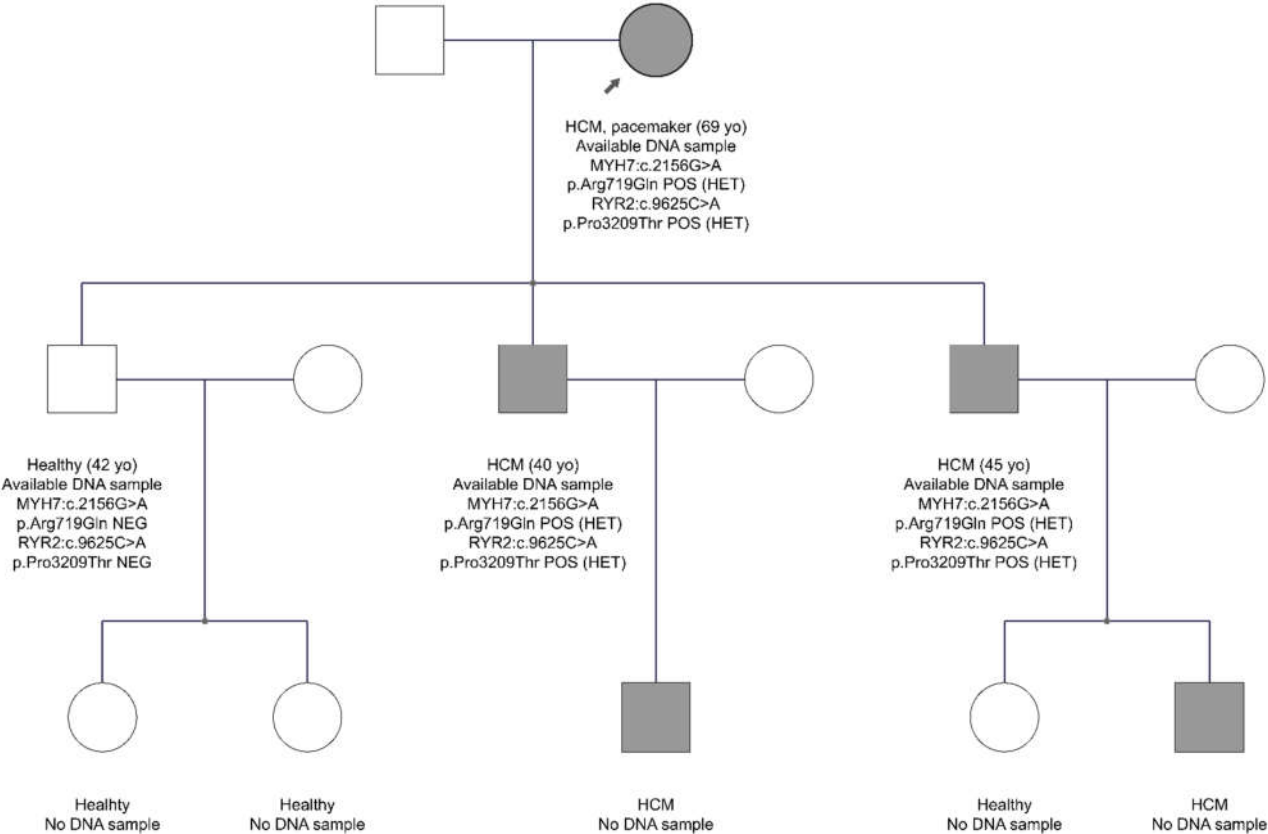
FAM09



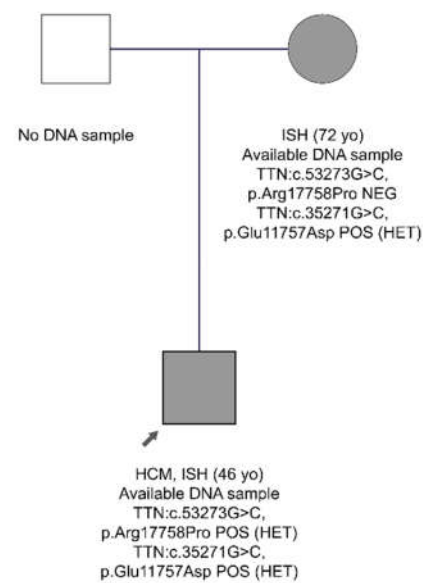
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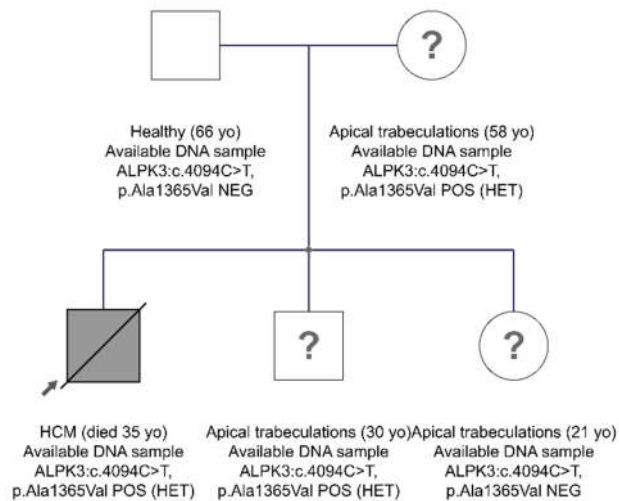
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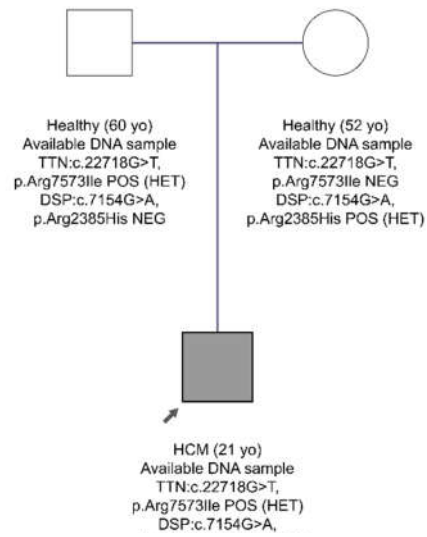
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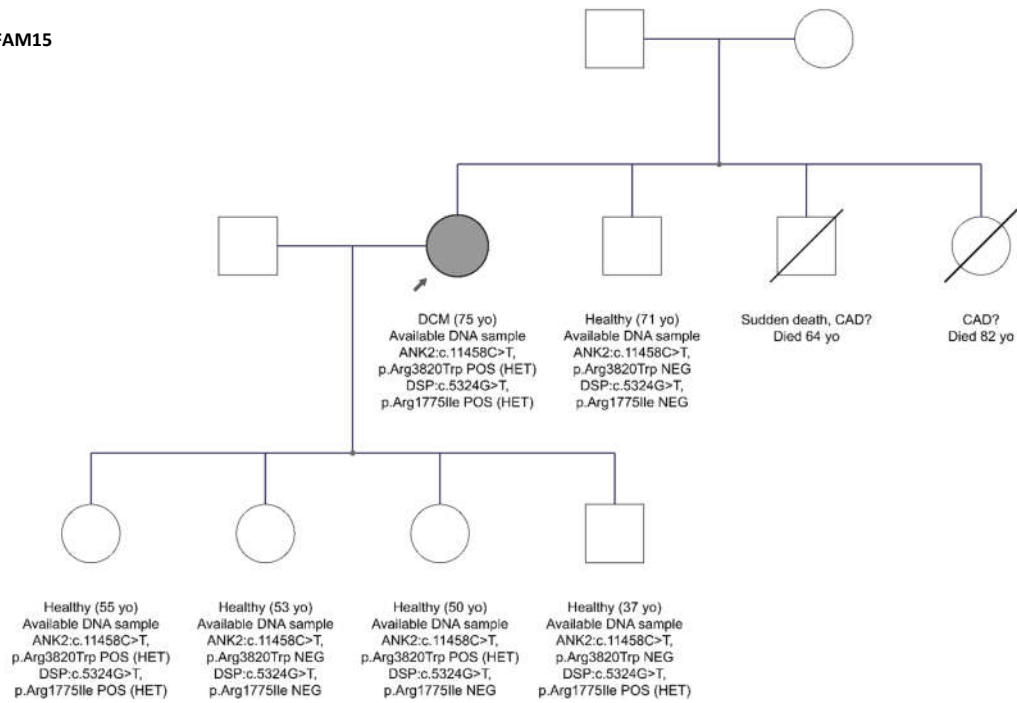
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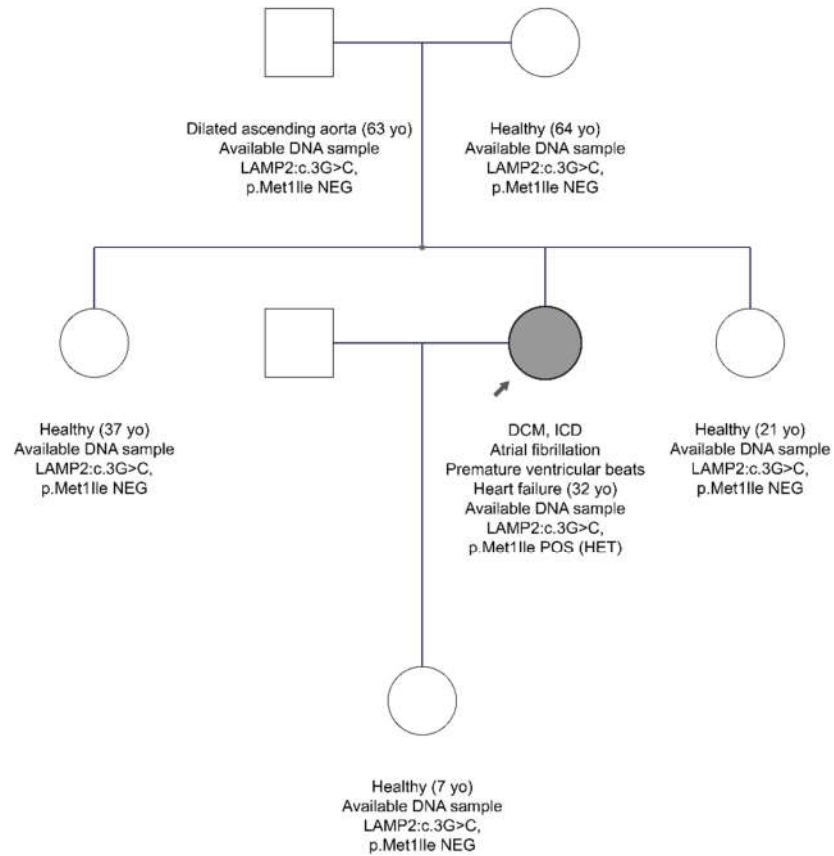
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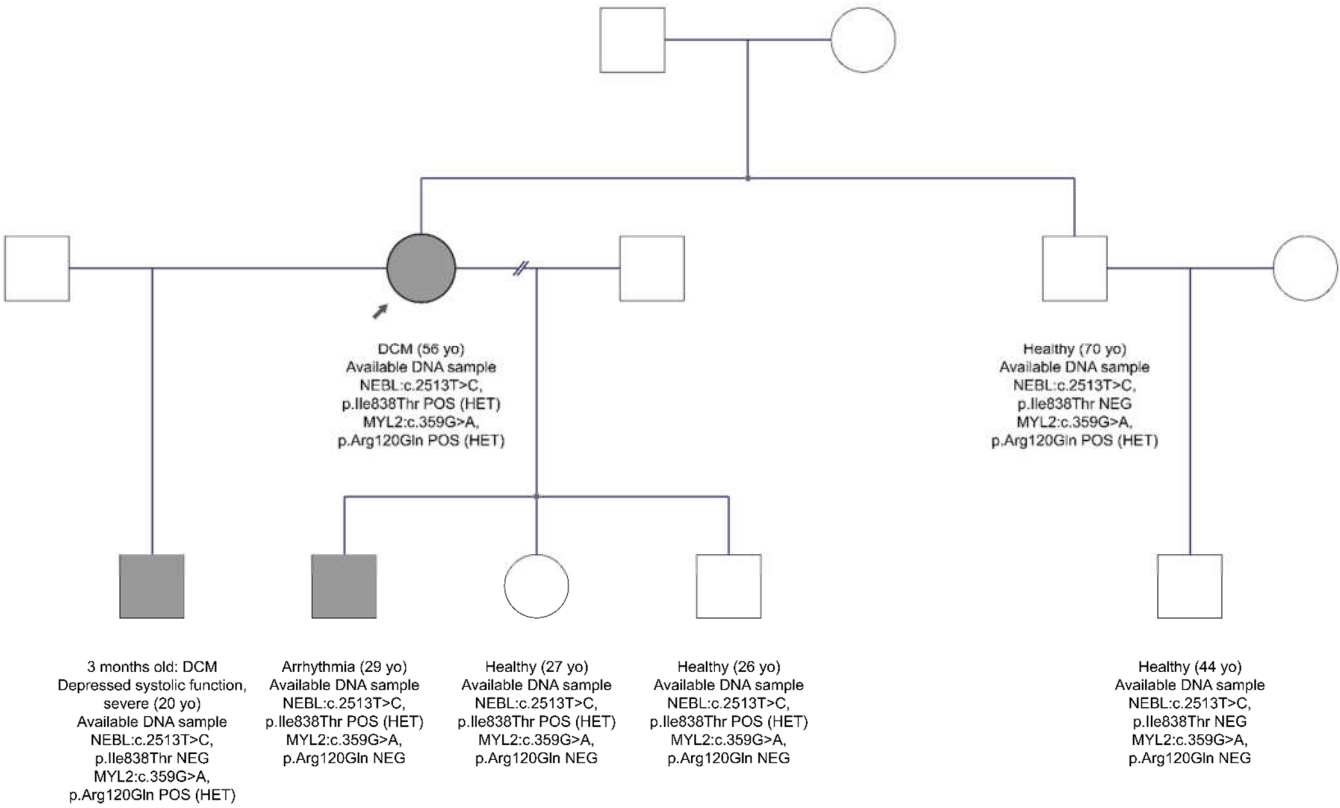
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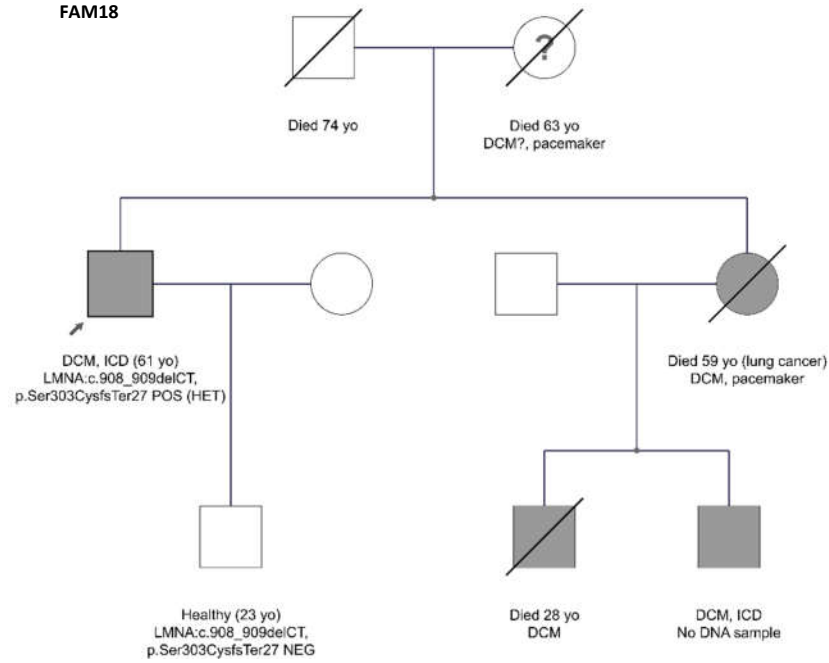
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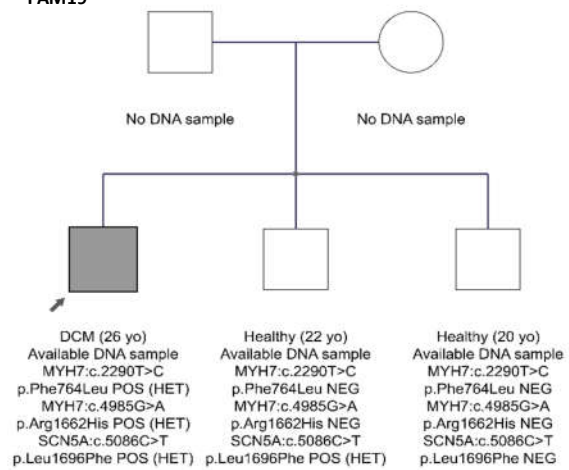
FAM17



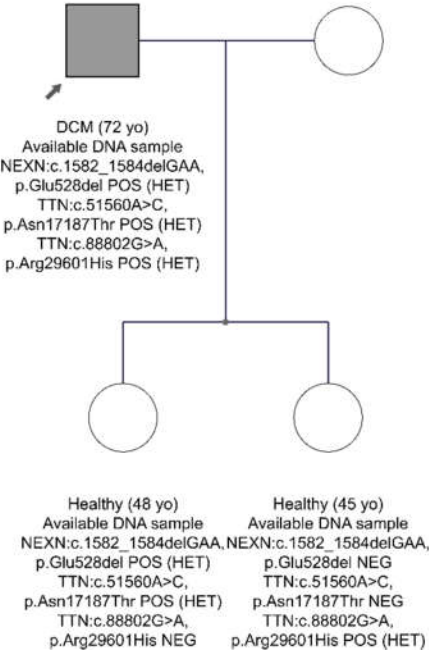
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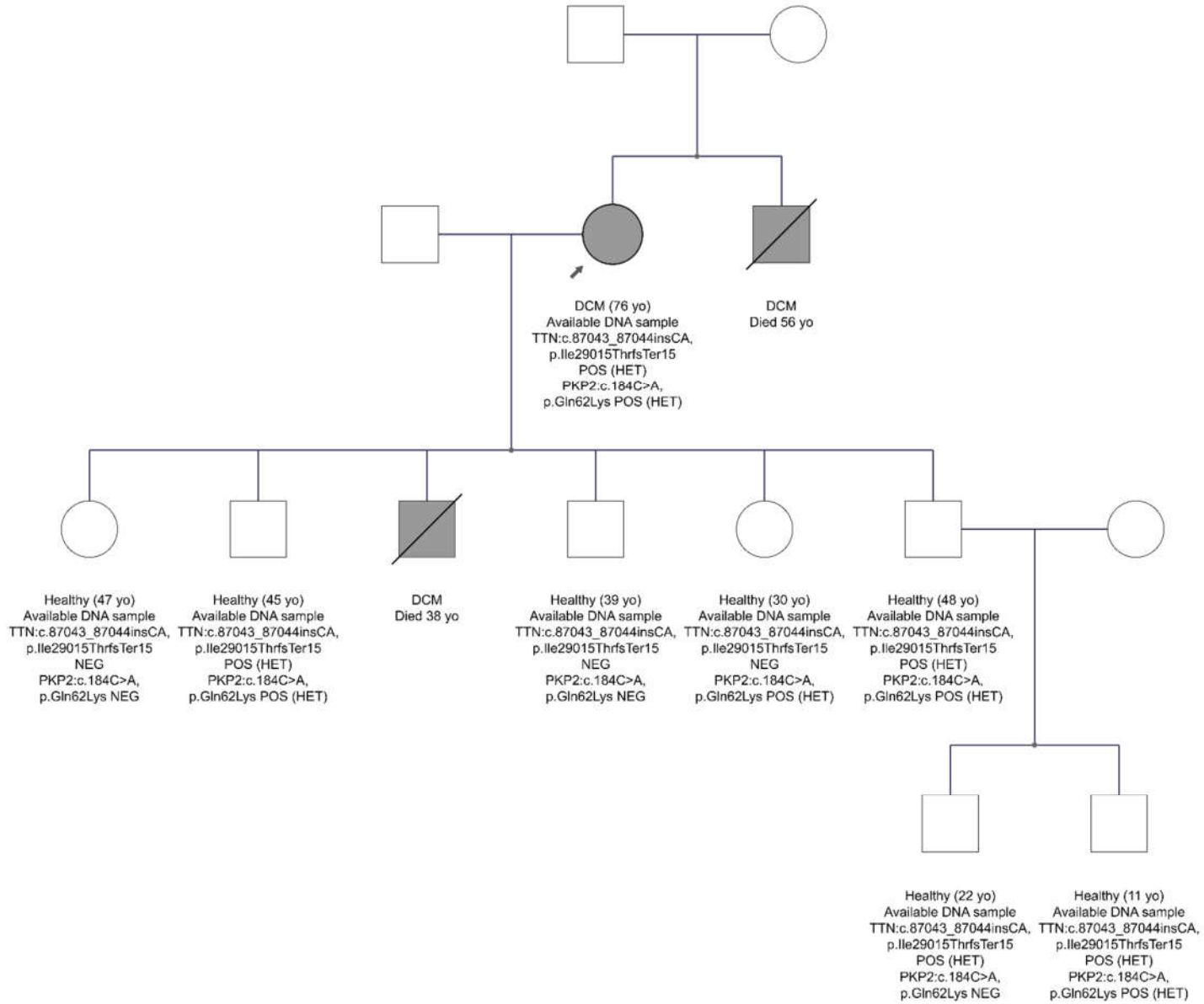
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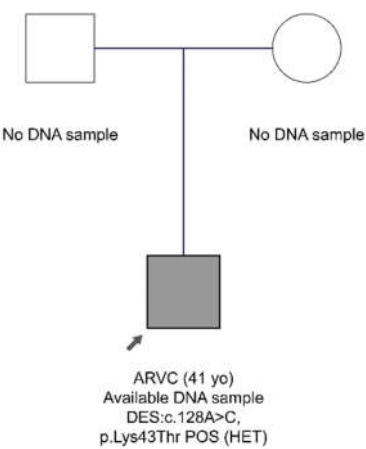
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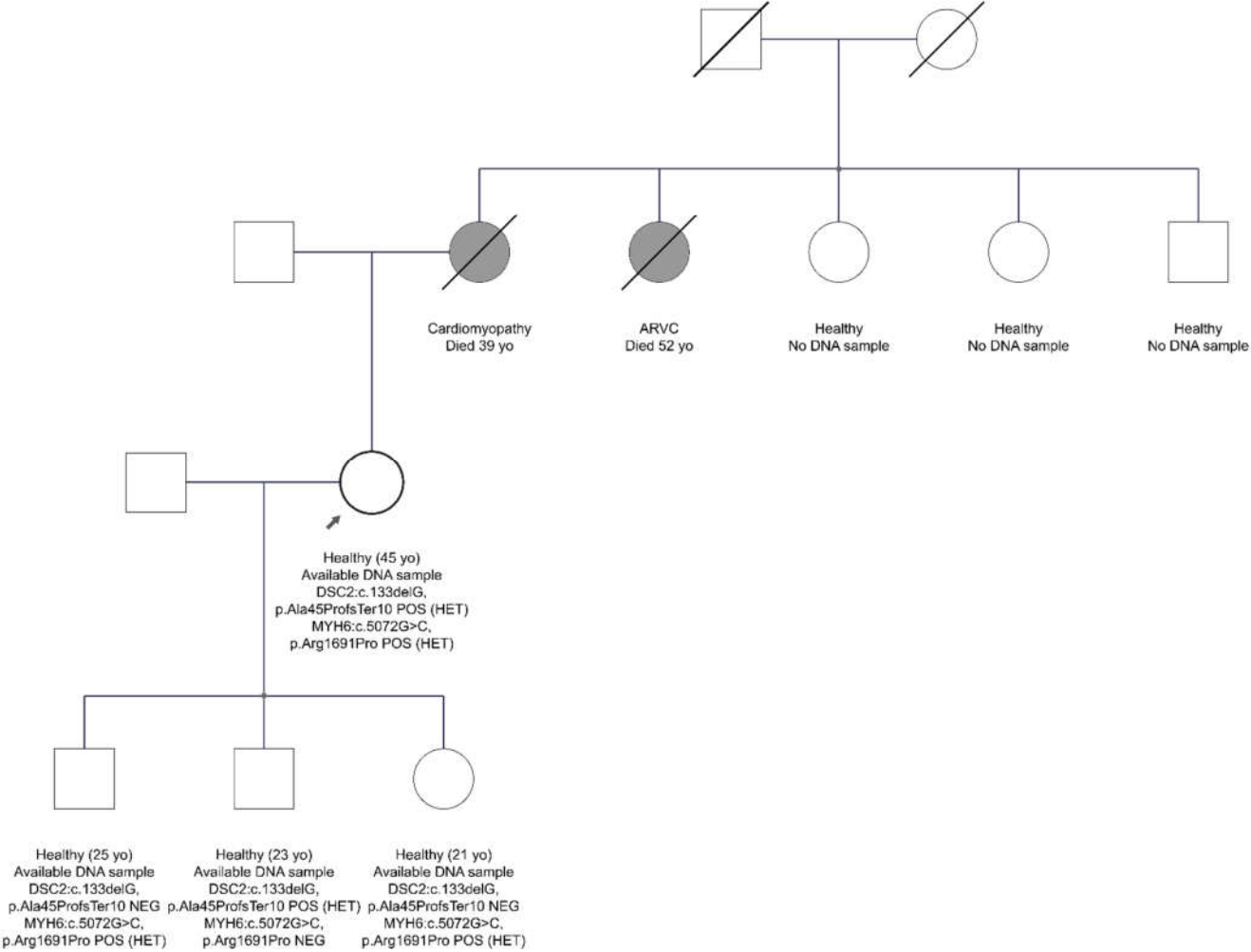
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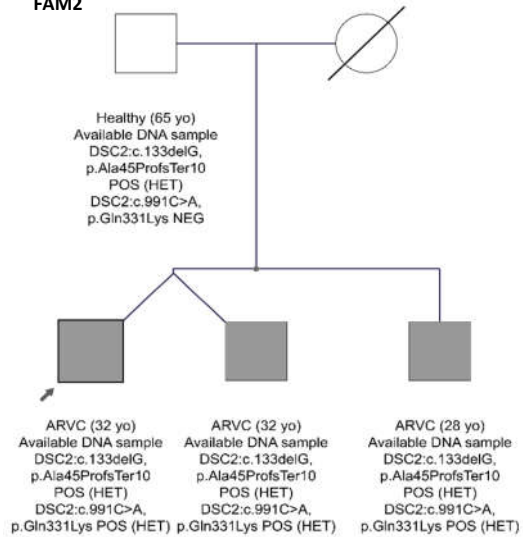
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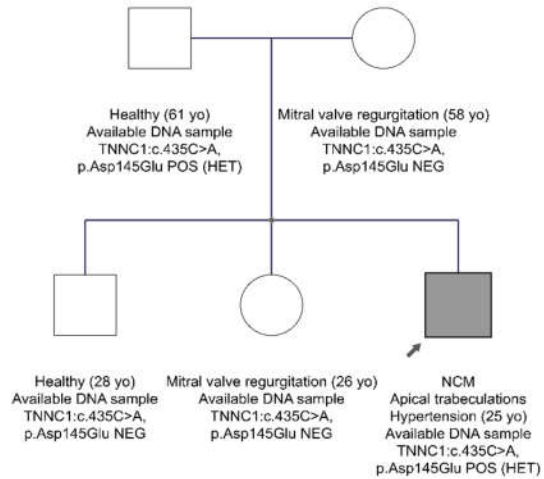
FAM23



## FAM2



## FAM25



**Figure S1:** Pedigrees of FAM01-25 described in the paper. The proband is indicated by the black arrow. Information provided below each subject is the clinical data, age, the gene and variant(s) found.