The Phenotypic Variability of R249Q MYH7 Mutation In Familial Cardiomyopathy

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The authors claim no conflict of interests with the topics presented in this presentation

Definitions and Proposed Contemporary Classification (2006)- AHA statement

Cardiomyopathies *are a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilatation and are due to a variety of causes that* ***frequently are genetic****.*

Maron et al, Circulation 2006; 113: 1807-16

Restrictive Cardiomyopathy

- Increased stiffness ventricles.
- Inflammatory, infiltrative or storage disease .
- Accelerated severe HF symptoms .
- High mortality rate
- Mutations in sarcomeric genes which are also associated with HCM, DCM .

Classification of the Cardiomyopathies European Society of Cardiology Statement

"Restrictive ventricular physiology in the presence of normal or reduced diastolic volumes (of one or both ventricles), normal or reduced systolic volumes, and normal ventricular wall thickness."

Keren , European Heart Journal, 2007

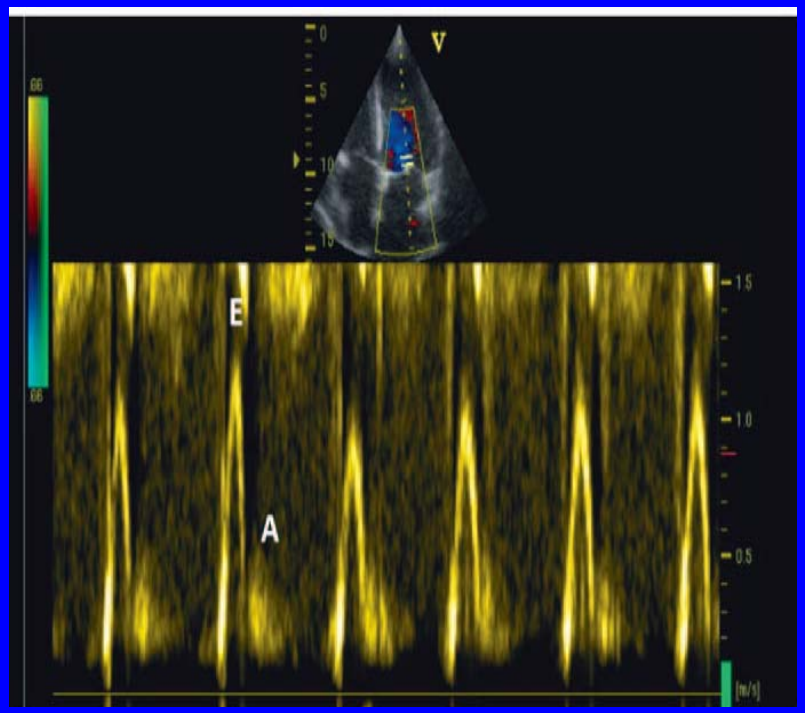
Aim

We report a novel mutation in the myosin gene in a family with RCM inherited as an autosomal dominant trait.

The same mutation was previously implicated in HCM and in DCM.

32y/o, severe CHF
A. Fibrillation
CVA, LAThrombus
Big Atria
Normal Ventricles
LVWT 9-10

32 F y/o HF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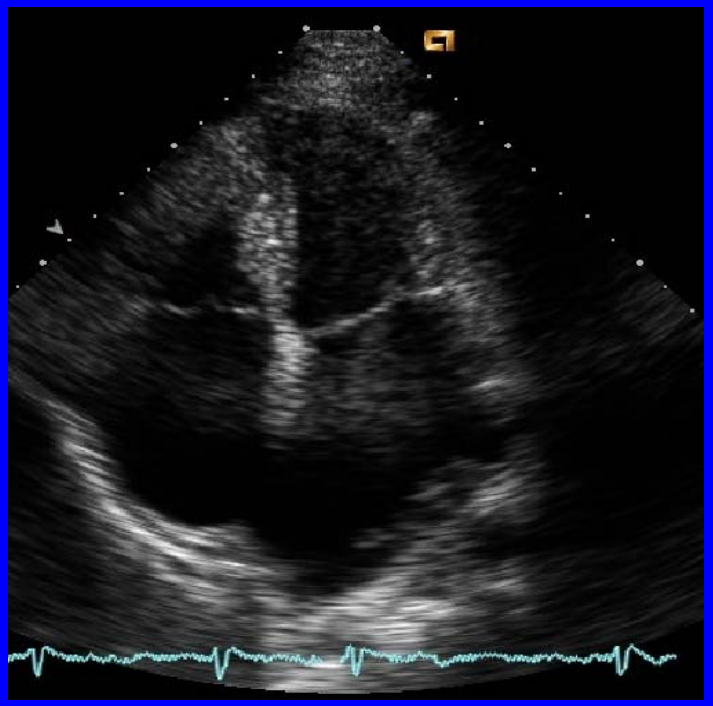

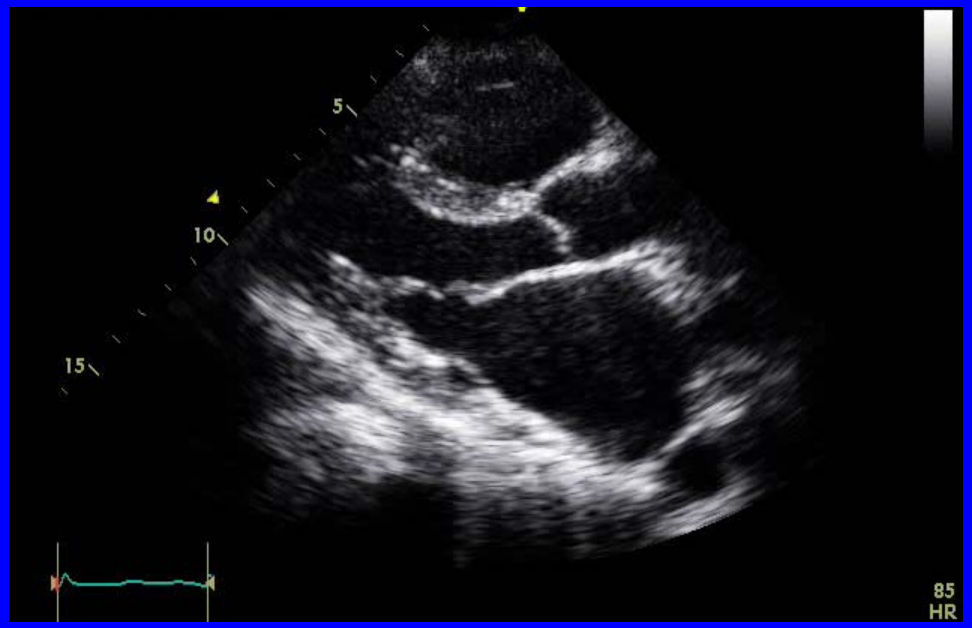
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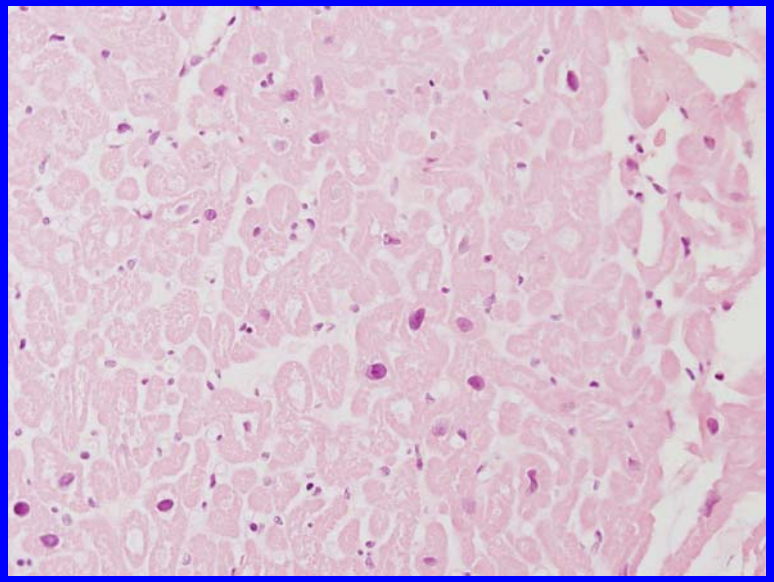
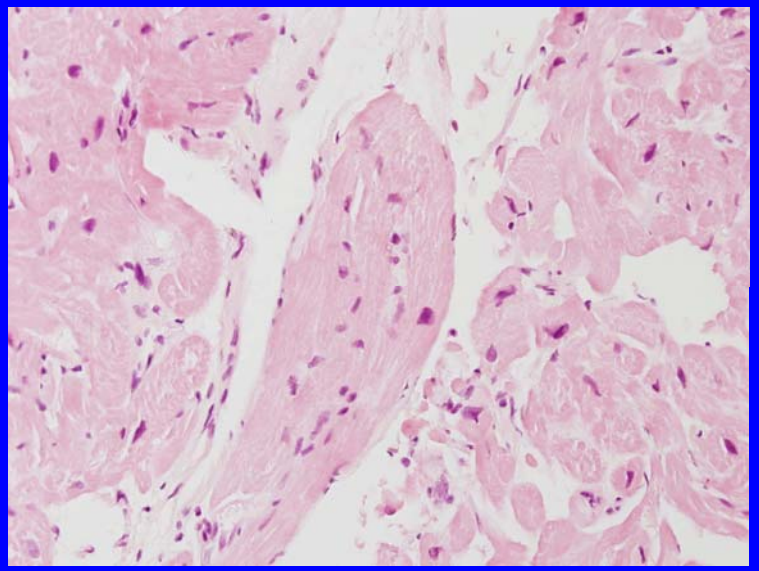
Myocardial Biopsy



Hematoxylin and eosin stain



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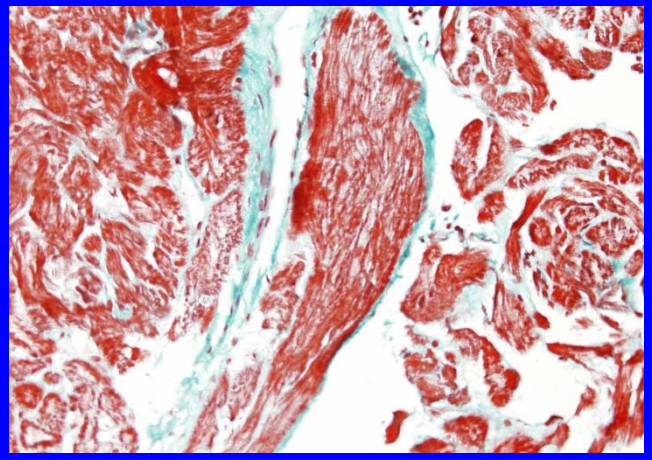

Masson's stain

Methods

- Family screening : physical examination, ECG, echo, biomarkers.
- DNA was extracted from peripheral venous blood
- Candidate genes analysis was done using Next Generation Sequencing.

Family Pedigree

32y/o, severe CHF
A. Fibrillation
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Normal Ventricles
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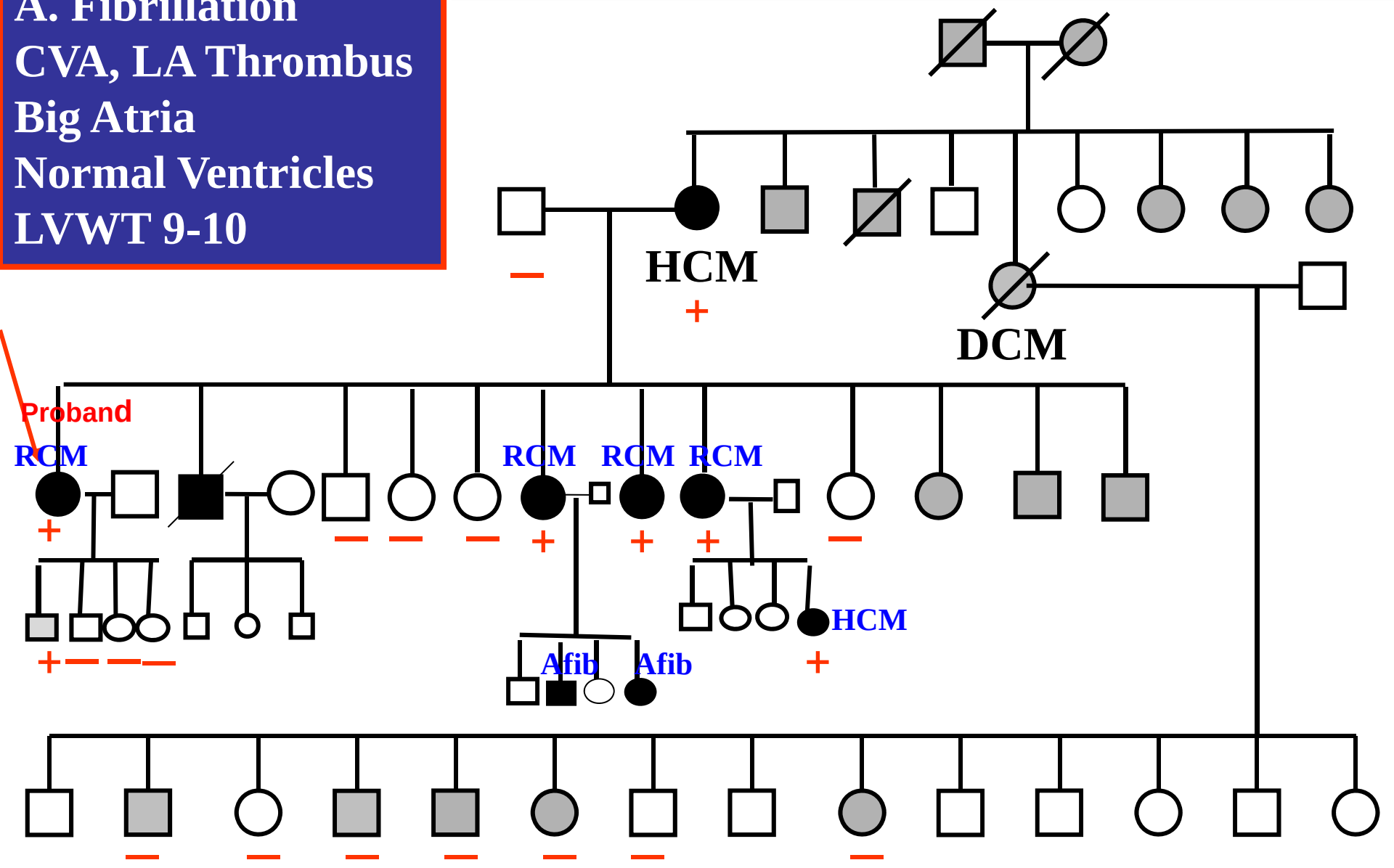


Genetic Analysis

- Candidate genes analysis was done using Next Generation Sequencing. The sensibility and specificity of this test is >95%.
- Confirmation by direct sequencing (forward and reverse) of the exon 9 and flanking intronic regions of MYH7 Gene (Encoding the protein β–Myosin), were performed in this patient's sample.
- Gene sequencing identified a heterozygous missense mutation within beta-myosin heavy chain gene

Exclusion of Candidate Genes

The following candidate genes have been excluded, including genes responsible for glycogen storage diseases

ABCC9, ACTC1, ACTN2, ADRB1, ADRB2, ADRB3, AGL, AKAP9, ANK2, ANKRD1, BAG3, BMPR2, BRAF, CACNA1B,CACNA1C, CACNA1D, CACNA2D1, CACNB2, CALR3, CASQ2, CAV3, CRYAB, CSRP3, CTF1 , DES, DMD, DSC2, DSG2, DSP, DTNA, ELN, EMD, EYA4, FHL1, FHL2, FKTN, FLNC, FXN, GAA, GATA4, GJA1, GJA5, GLA, GPD1L, HCN1, HCN4, HRAS, JAG1, JPH2 , JUP, KCNA5, KCND3, KCNE1, KCNE1L, KCNE2, KCNE3, KCNE4, KCNH2, KCNJ11, KCNJ12, KCNJ2, KCNJ3, KCNJ5, KCNJ8, KCNQ1, KCNQ2, KRAS, LAMA4, LAMP2, LBD3, LMNA, LRP6, MAP2K1, MAP2K2 , MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOT, MYOZ2, MYPN, NEXN, NKX2-5, NPPA, NRAS, PDLIM3, PKP2, PKP4, PLEC, PLN, PNN, PRKAG2, PSEN1, PSEN2, PTPN11, RAF1 , RANGRF, RBM20, RYR2, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SCNN1B, SCNN1G, SGCD, SHOC2, SLC25A4, SNTA1, SOS1, TAZ, TBX20, TCAP, TGFB3, TMEM43, WX, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, VCL

Gene: MYH7 (Encoding the protein Myosin-7)

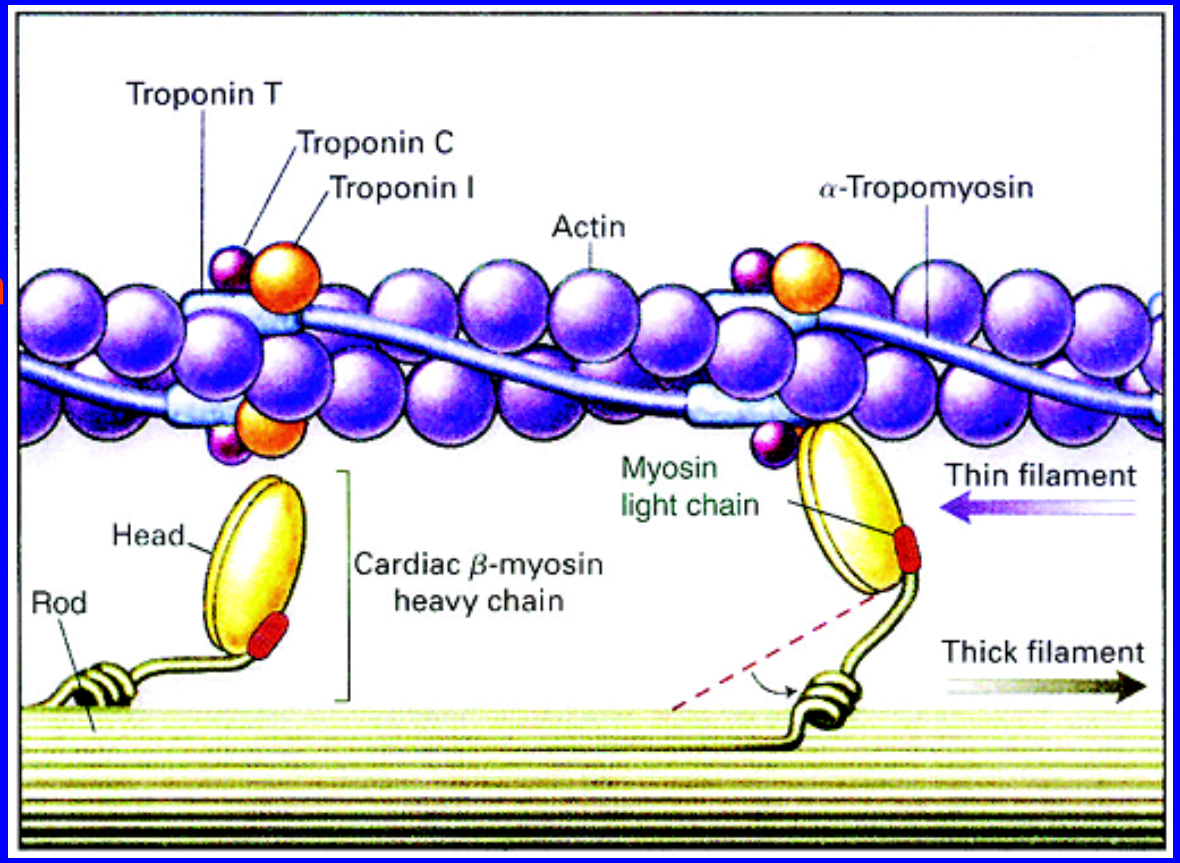
- A point mutation in heterozygosis has been identified

  NM_000257.2:c.746G>A Resulting in the replacement of arginine by glutamine – R249Q

- This mutation affects an arginine residue that has been stringently conserved throughout evolution and is invariant in all muscle myosins characterized to date
- Family members were genotyped by BamHi restriction digestion (mutation abolishes the restriction site)

Schematic Diagram of Sarcomere Organisation & Contraction Process

The thin filament is made up of actin, troponin complex (T,C and I) & tropomyosin

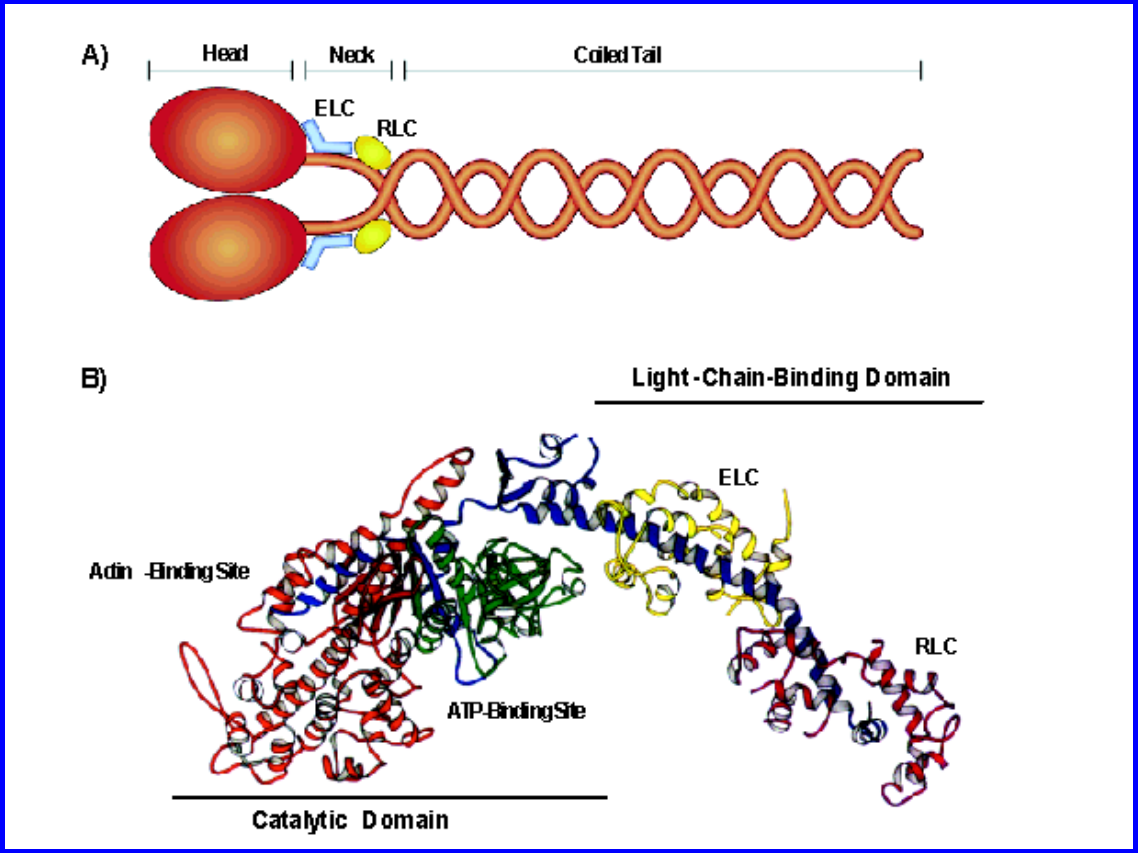


The thick filament is composed of myosin heavy and light chains.

The sarcomere produces muscle contraction by sliding of myofilaments: the myosin heads interact with actin and pull it towards the center of the sarcomere resulting in shortening of the sarcomere.

A) Schematic representation of a myosin molecule constituting the thick filaments of the sarcomere

B) Three-dimensional (crystal) structure of a chicken skeletal myosin head.



Myosin is called "molecular motor" of the sarcomere due to its ability to hydrolyse ATP and thereby to transfer chemical energy into contraction force and motion.

Each myosin molecule is made up of two myosin heavy chains ,two pairs of light chains and two regulatory light chains

CGA→CAA	Arg249Gln	-1	Active site

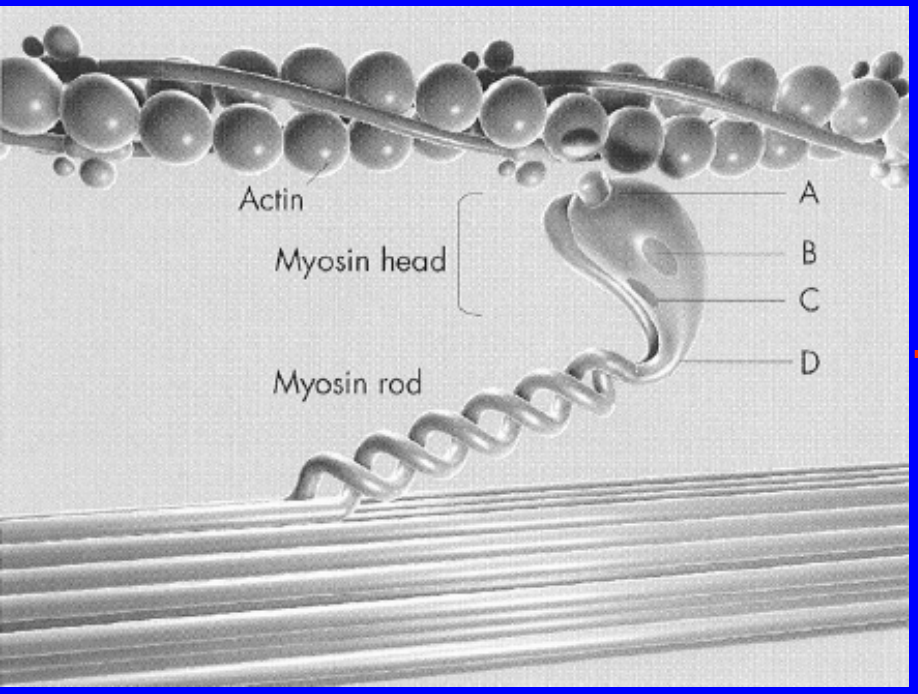
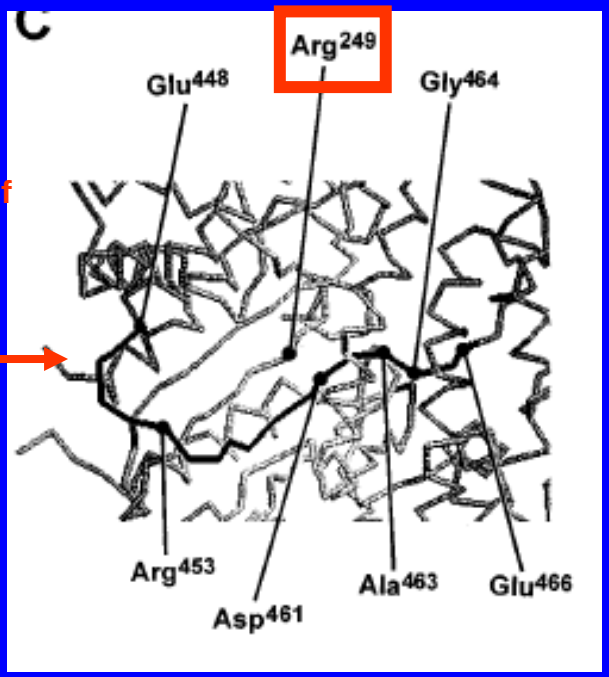


Diagram of the functional domains of ß myosin heavy chain

Close up stereoview of ATP binding pocket



R249Q mutation identified in this family is located to the active site (hydrolyses of ATP)

The replaced arginine residue is stringently conserved throughout evolution and is invariant in all muscle myosins characterized to date

Previously associated with HCM and DCM but for the first time we describe it in RCM

Information related to the mutation

- Beta myosin heavy chains with this mutation were expressed in insect cells and resulted in decreased actin translocating activity (61% of the wild type) with decreased intrinsec ATPase activity .

  Sata et al. J Clin Invest 1996

- This mutation affects the active ATP binding site and produces a change in the charge of the substituted amino acid (change of -1 in the net charge of substituted amino acid, based on charges of the amino acid at pH 7).

  Fananapazir L. JAMA 1999;281:1746-1752.

AVAILABLE INFORMATION ABOUT IDENTIFIED MUTATIONS

- The mutation has been associated with HCM and DCM **but not with RCM**

- This mutation has been described in **15 families**. Information is available about 56 mutation carriers (39 patients with HCM, 8 patients with possible HCM, 1 patient with possible DCM, 5 not affected or healthy, 3 without phenotypic study).

- R249Q mutation in MYH7 gene has been previously associated with hypertrophic and dilated cardiomyopathy, but for the first time we describe it in a family with restrictive cardiomyopathy.

- Unique to the family descreibed - these findings demonstrate the remarkable phenotypic variability in families with cardiomyopathy

Family Pedigree

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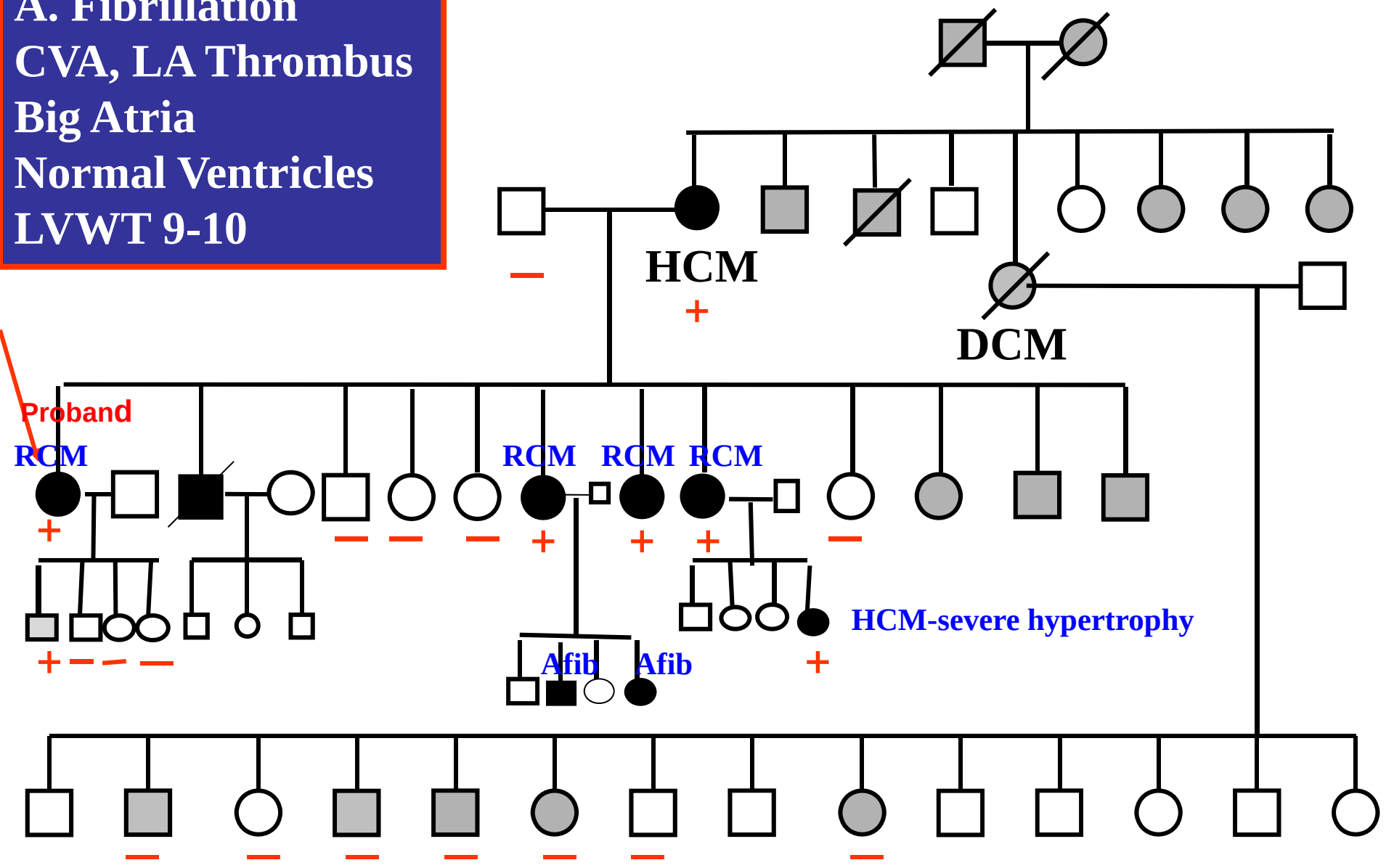

Double Dose Gene Mutation

Over 200 different mutations in at least 11 genes have been identified in HCM. In most cases, HCM is caused by single heterozygote mutations in genes encoding sarcomeric proteins.

Most recently, it has been reported that approximately 5% of HCM patients carry more than one disease causing gene mutation, leading to a double or compound heterozygote genotype. It appears that these patients may develop a more severe clinical phenotype because of a ‘‘double dose’’ gene mutation effect

Ingles et al. J Med Genet 2005

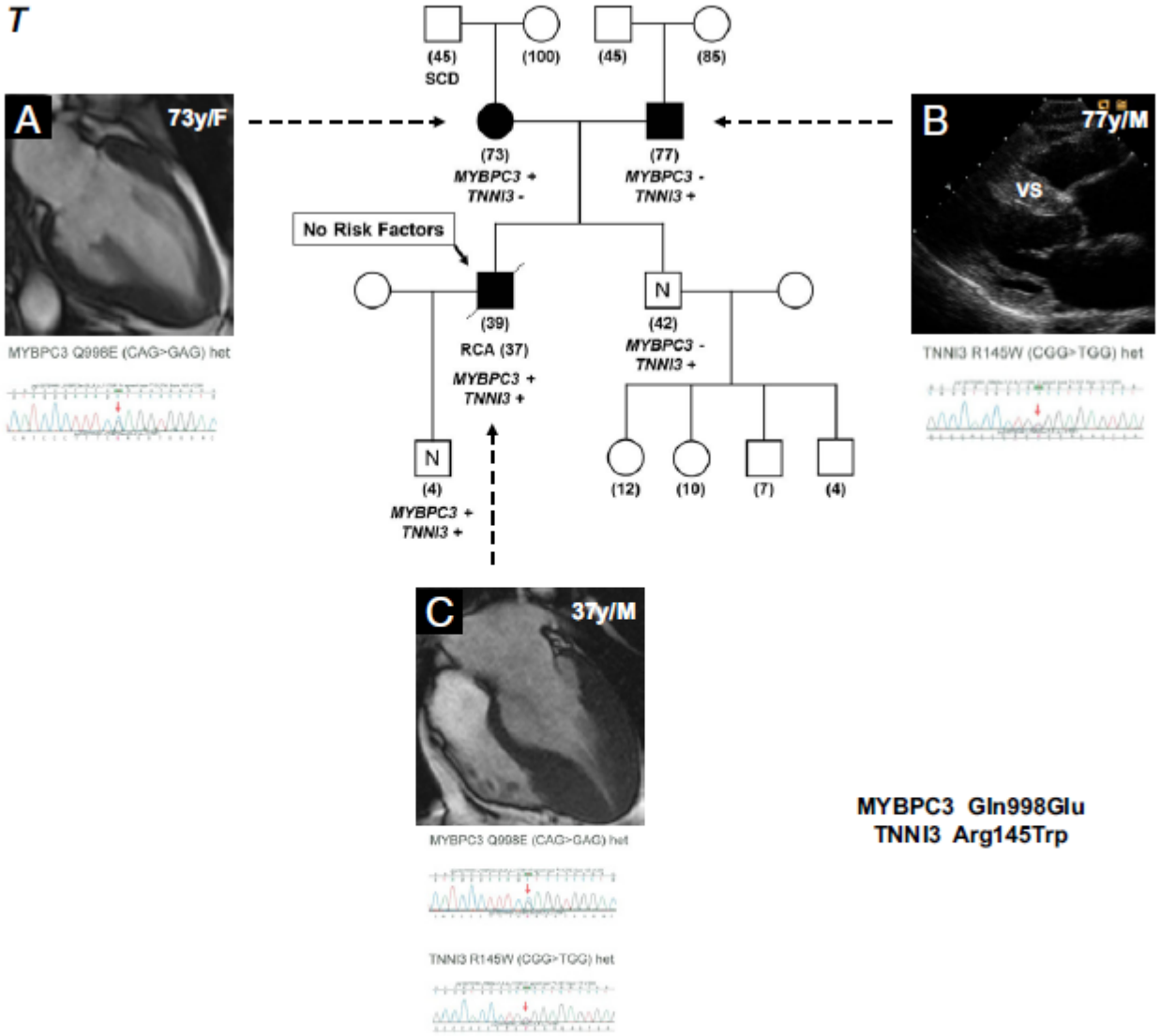

Figure 8 Double Mutations as a Potential Risk Factor in HCM

Asymptomatic proband **(arrow)** with 2 disease-causing mutations, *MYBPC3* and *TNN13*, experienced resuscitated cardiac arrest (RCA) at age 37 years, but without conventional risk factors. Both parents have HCM, and each contributed 1 mutation to their offspring. Electropherograms show identified mutations. From Maron et al. (51), with permission of the Heart Rhythm Society. **Solid symbols** are those clinically affected with HCM. N = normal on clinical screening with imaging; + = heterozygote for mutation; − = without mutation; other symbols and abbreviations as in Figure 3.

Maron *et al.*

JACC Vol. 60, No. 8, 2012

CONCLUSIONS

R249Q mutation in MYH7 gene has been previously associated with HCM and DCM

For the first time we describe it in a family with restrictive cardiomyopathy.

These findings demonstrate the remarkable phenotypic variability in families with cardiomyopathy.

The genetic and environmental modifiers responsible for different modes of cardiac remodeling remain to be identified.

Thank You

Next-generation methods

- The high demand for low-cost sequencing has driven the development of high-throughput sequencing (or next-generation sequencing) technologies that parallelize the sequencing process, producing thousands or millions of sequences at once. High-throughput sequencing technologies are intended to lower the cost of DNA sequencing beyond what is possible with standard dye-terminator methods. In ultra-high-throughput sequencing as many as 500,000 sequencing-by-synthesis operations may be run in parallel.

Next-generation methods

Method	Single-molecule real-time sequencing (Pacific (Bio	Ion semiconductor (Ion Torrent (sequencing	Pyrosequenc (ing (454	Sequencing by synthesis ((Illumina	Sequencing by ligation (SOLiD (sequencing	Chain termination ((Sanger sequencing
Read length	[38]bp average 2900	bp 200	bp 700	to 250 bp 50	or 50+50 50+35 bp	to 900 bp 400
Accuracy	read length) 87% mode), 99% ((accuracy mode	98%	99.9%	98%	99.9%	99.9%
Reads per run	[39] thousand 75–35	up to 5 million	million 1	up to 3 billion	to 1.4 billion 1.2	N/A
Time per run	minutes to 2 30 [40] hours	hours 2	hours 24	to 10 days, depending upon 1 sequencer and specified read [41]length	to 2 weeks 1	minutes to 3 hours 20
Cost per 1 million bases (in ($US	2$	1$	10$	to $0.15 0.05$	0.13$	2400$
Advantage s	Longest read length. Fast. Detects 4mC, [42].5mC, 6mA	Less expensive equipment. .Fast	Long read .size. Fast	Potential for high sequence yield, depending upon sequencer model .and desired application	Low cost per .base	Long individual reads. Useful for many .applications
Disadvant ages	Low yield at high accuracy. Equipment can be very .expensive	Homopolymer .errors	Runs are expensive. Homopolymer .errors	.Equipment can be very expensive	Slower than .other methods	More expensive and impractical for larger .sequencing projects

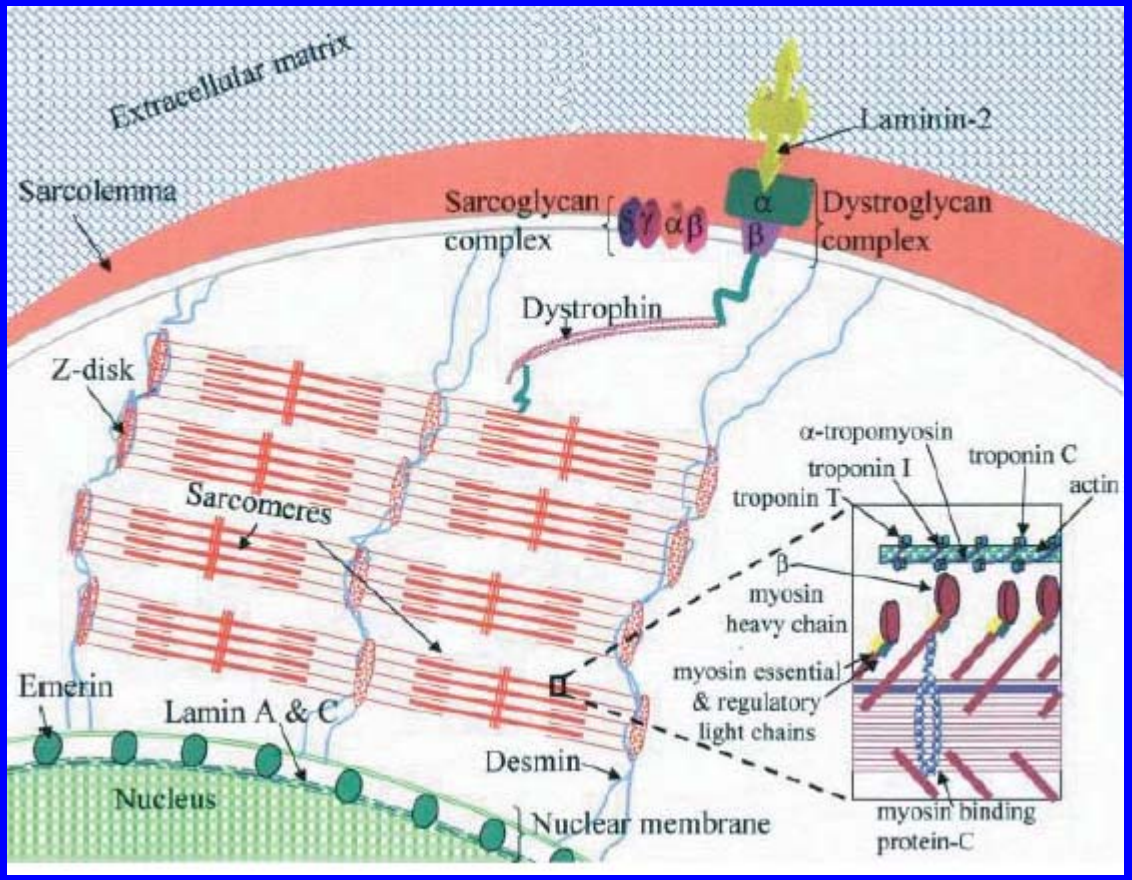

Fig. 1. Cellular localisation and interactions of proteins involved in HCM and DCM. Schematic representation of a section through part of a cardiac myocyte, illustrating the position and interactions of many of the various proteins that have been implicated in HCM and/or DCM.