The impact of multiple gene mutations in determining severity of cardiomyopathy and heart failure

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Summary

1. Familial hypertrophic cardiomyopathy (FHC) is a primary cardiac disorder, characterised by myocardial hypertrophy, which demonstrates substantial diversity in both genetic causes and clinical manifestations.

2. Clinical heterogeneity can be explained by the causative gene (at least 13 are identified to date), the position of the amino acid residue affected by a mutation within the protein (over 450 mutations have been reported to date) and modifying genetic and environmental factors.

3. Multiple mutations are found in up to 5% of human FHC cases, who typically present with a more severe phenotype compared to single-mutation carriers, *i.e.* earlier onset of disease, greater left ventricular hypertrophy, and a higher incidence of sudden cardiac death events.

4. Multiple mutations usually involve MYH7, MYBPC3 and, to a lesser extent, TNNI2, reflecting the higher contribution of mutations in these genes to FHC.

5. Multiple mutation mouse models appear to mimic the human multiple mutation phenotype, and thus will help to improve our understanding of disease pathogenesis. The models provide a tool for future studies of disease mechanisms and signalling pathways in FHC, and its sequelae, heart failure and sudden death, thereby allowing identification of novel targets for potential therapies and disease prevention strategies.

Introduction

Familial hypertrophic cardiomyopathy (FHC) is a primary cardiac disorder characterised by myocardial hypertrophy, usually affecting the left ventricle, in the absence of loading conditions such as hypertension. FHC demonstrates substantial diversity in both genetic causes and clinical manifestations. Heterogeneous autosomal dominant mutations, primarily in genes which encode sarcomere proteins including cardiac β-myosin heavy chain (MYH7), cardiac myosin-binding protein C (MYBPC3), cardiac troponin T (TNNT2), cardiac troponin I (TNNI3), essential myosin light chain (MYL3), regulatory myosin light chain (MYL2), α-tropomyosin (TPM1) and cardiac actin (ACTC), have been found to cause FHC, with a few cases attributed to mutations in other genes. Currently more than 450 different mutations in at least 13 genes have been reported.[1,2] FHC typically presents in early adulthood with a wide range of clinical severity, from asymptomatic individuals and those with very mild symptoms such as palpitations and dyspnea, to patients experiencing major complications including syncope, ventricular arrhythmias, congestive heart failure and sudden death.

Based on current knowledge, the phenotypic heterogeneity seen in FHC can be explained by at least three important factors. First, the causative gene, *i.e.* mutations in MYH7 and TNNT2 genes appear to be associated with poor prognosis while mutations in MYBPC3 are associated with a relatively late onset and benign symptoms.[1,3] Second, the position of the amino acid residue affected by a mutation within the protein, *e.g.* the Arg403Gln and Arg719Trp replacements in MYH7 are associated with particularly severe hypertrophy and predispose to sudden death and heart failure, whereas the Gly256Glu, Phe513Cys and Leu908Val replacements cause less severe phenotypes.[4] Third, modifying factors, which include environmental influences such as exercise[5-7] and diet,[8] and genetic factors such as mutations in other genes,[9] are likely to play an important role in explaining the clinical heterogeneity observed in FHC. The focus of this review is to explore the effects of second disease-causing mutations on clinical outcome in FHC and to highlight the challenges that multiple mutations pose both in understanding disease pathogenesis, as well as in diagnosis and counselling in families with FHC.

Multiple mutations in human FHC

We have recently shown that in up to 5% of human FHC cases, two disease-causing mutations exist in affected individuals.[10] Genetic screening of seven FHC genes (MYH7, MYBPC3, TNNT2, TNNI3, MYL3, MYL2 and ACTC) was undertaken in 80 unrelated probands and multiple gene mutations were identified in four families. One family had a double mutation affecting MYH7 and MYBPC3 and three had compound mutations in MYBPC3. This finding is consistent with a number of recent studies. Erdmann *et al.*[11] reported 1% multiple mutations (108 unrelated probands) in 6 genes; Richard *et al.*,[12] 4.5% (197 unrelated probands) in 9 genes; van Driest *et al.*[13] 2.5% (389 unrelated probands) in MYBPC3; and Mohiddin *et al.*[14] 2% (100 unrelated probands) in MYH7.

Table 1 summarises all the multiple mutations reported in FHC families to date. Compared to individuals with single FHC gene mutations, those with homozygous and double or compound heterozygous mutations typically present with more severe left ventricular hypertrophy, and a higher incidence of sudden cardiac death events among

Table 1. Multiple mutations in human FHC. *H – homozygous; DH – double heterozygous; CH – compound heterozygous; ASH – asymmetric septal hypertrophy; LAD – left atrial dilation; CHF – congestive heart failure; HT – heart transplant; SD – sudden death; FHC – familial hypertrophic cadiomyopathy; LVD – left ventricular dilation; ALVH – asymmetric left ventricular hypertrophy; VT – ventricular tachycardia; RVH – right ventricular hypertrophy; LVH – left ventricle hypertrophy; ICD – implantable cardioverter defibrillator; CA – cardiac arrest; LVOTO – left ventricular outflow tract obstruction; BVH – biventricular hypertrophy; IVS – interventricular septum*

Gene1	Mutation1	Gene2	Mutation2	Geno-type	Initial Symptoms and Clinical Progression	IVS (age)	References
MYH7	Lys207Gln	MYH7	Lys207Gln	H	ASH, LAD, CHF	21mm (64y)	14, 56
MYH7	Arg403Trp	MYH7	Arg403Trp	H	LAD, HT	20mm (38y)	57
MYH7	Asp778Glu	MYH7	Asp778Glu	H	SD	19mm	12
MYH7	Arg869Gly	MYH7	Arg869Gly	H	Early onset FHC, LAD	35mm (29y)	58, 12
MYH7	Glu935Lys	MYH7	Glu935Lys	H	ASH, LVD, CHF 31y	13mm (25y)	16
MYBPC3	Gln76Ter	MYBPC3	Gln76Ter	H	CHF	16mm (<1y)	12
MYBPC3	Ala627Val	MYBPC3	Ala627Val	H	ALVH, 16y	28mm (47y)	59
MYBPC3	Arg810His	MYBPC3	Arg810His	H	Dyspnoea	32mm (32y)	17
MYBPC3	Pro873His	MYBPC3	Pro873His	H	VT	27mm (27y)	17
MYBPC3	Asp1064fsX38	MYBPC3	Asp1064fsX38	H	Early onset FHC, SD	n/a	18
TNNT2	Phe110Ile	TNNT2	Phe110Ile	H	RVH	21mm (49y)	20
TNNT2	Ser179Phe	TNNT2	Ser179Phe	H	SD	25mm (17y)	19
MYH7	Ala355Thr	MYBPC3	Val896Met	DH	Early onset LVH	n/a	12
MYH7	Glu483Lys	MYBPC3	Glu1096Ter	DH	LVH	28-32mm	21, 12
MYH7	Arg694Cys	MYBPC3	Gln791fsX40	DH	n/a	n/a	13
MYH7	Arg719Gln	MYBPC3	Arg273His	DH	LVD, LAD, HT	17mm (35y)	10
MYH7	Glu894Gly	MYBPC3	Asp605Asn	DH	n/a	n/a	13
MYH7	Arg453Cys	TNNT2	Gln191del	DH	n/a	n/a	13
MYH7	Arg453Ser	TNNI3	Pro82Ser	DH	LAD, Syncope, ICD	15mm (19y)	22
MYH7	Cys905Phe	TNNI3	Ser166Phe	DH	n/a	14mm (39y)	11
MYH7	Arg787Cys	ACTC	Arg97Cys	DH	Early onset FHC		15
MYBPC3	Val256Ile	TNNT2	Arg92Trp	DH	n/a	n/a	13
MYBPC3	Ala833Thr	TNNT2	Arg286His	DH	n/a	n/a	13
MYBPC3	Arg495Gln	TNNI3	Arg141Gln	DH	Early onset FHC		15
MYBPC3	Arg943Ter	TNNI3	Ser166Phe	DH	n/a	n/a	13
MYBPC3	Phe1113Ile	TPM1	Ile172Thr	DH	n/a	n/a	13
MYH7	Val39Met	MYH7	Arg723Cys	CH	n/a	20mm	12
MYH7	Arg54Ter	MYH7	Arg870His	CH	ASH	20mm (16y)	26
MYH7	Pro211Leu	MYH7	Arg663His	CH	ASH, CHF	20mm (65y)	14
MYH7	Met349Thr	MYH7	Arg719Trp	CH	LVH, CA 6.5 y	18mm (8.5y)	27
MYH7	Arg663His	MYH7	Val763Met	CH	Early onset FHC		15
MYH7	Arg719Gln	MYH7	Thr1513Ser	CH	n/a	n/a	13
MYH7	Asp906Gly	MYH7	Leu908Val	CH	LAD	n/a	56
MYBPC3	Gly5Arg	MYBPC3	Arg502Trp	CH	n/a	n/a	13
MYBPC3	Gln76Ter	MYBPC3	His257Pro	CH	Mild HCM	18mm	12
MYBPC3	Ile154Thr	MYBPC3	Asp605del	CH	Early onset FHC		15
MYBPC3	Glu258Lys	MYBPC3	Ala954fsX94	CH	n/a	n/a	13
MYBPC3	Arg502Trp	MYBPC3	Ser858Asn	CH	Early onset FHC		15
MYBPC3	Glu542Gln	MYBPC3	Ala851Val	CH	Dyspnoea, LVOTO	34mm (34y)	10
MYBPC3	Asp745Gly	MYBPC3	Pro873His	CH	Syncope, ICD	30mm (29y)	10
MYBPC3	Trp792fsX17	MYBPC3	IVS15+1G>A	CH	Early onset LVD, SD	11mm (<1y)	29
MYBPC3	Arg810His	MYBPC3	Arg820Gln	CH	Dyspnoea	23mm (53y)	17
MYBPC3	Arg943Ter	MYBPC3	Glu1096fsX92	CH	BVH, SD	n/a	29
MYBPC3	Thr1028Ser	MYBPC3	IVS31+2T>G	CH	Early onset FHC		15
MYBPC3	Gln1233Ter	MYBPC3	Arg326Gln	CH	Dyspnoea	28mm (53y)	10

family members.[10] Moreover, patients with double mutations are significantly younger at diagnosis[13] and more commonly present with childhood-onset hypertrophy.[10,15] Multiple mutations usually involve MYH7, MYBPC3 and to a lesser extent TNNI2, reflecting the higher contribution of mutations in these genes to FHC (Table 1).

Homozygous FHC mutations

A double mutation in FHC was first reported in two brothers homozygous for a Lys935Glu amino acid substitution in MYH7, *i.e.* they had no normal cardiac β-myosin heavy chain protein.[16] Both brothers presented

with severe left ventricular hypertrophy and a clinical course culminating in congestive heart failure and sudden death at ages 31 and 34 years, respectively. Analysis of family members revealed that both parents were heterozygous for Lys935Glu, and while both had left ventricular hypertrophy, neither showed any clinical symptoms. Furthermore, a sister also heterozygous for Lys935Glu showed no ventricular hypertrophy on her echocardiogram. Four additional homozygous mutations in MYH7 have since been reported, each with a severe clinical course culminating in a dilated form of cardiomyopathy, heart failure, sudden death or a heart transplant (Table 1). Homozygous amino acid substitutions in MYBPC3 tend to show less severe clinical symptoms than those occurring in MYH7, which is in accordance with the milder phenotypes of single mutations in MYBPC3 compared to MYH7. Nevertheless, individuals homozygous for Arg810His and Pro873His substitutions in MYBPC3 have more severe disease than heterozygous individuals.[17]

FHC does not usually present in young children, however, two homozygous mutations that severely truncate the MYBPC3 protein have been reported as causing neonatal FHC resulting in sudden cardiac death within the first year of life. In one case, homozygosity for a Gln76Ter nonsense mutation in MYBPC3 led to a severe FHC phenotype with heart failure and death at age 6 months.[12] In a second report, a single mutation segregated in three Amish families with mild FHC, however, each family had a homozygous child who died of heart failure before age 1 year.[18] The intronic mutation identified was shown to cause skipping of exon 30 leading to a premature translation stop codon 211 amino acid residues from the carboxyl-terminal end, and thus removing the major myosin-binding domain of MYBPC3. Strikingly, in the three Amish families, heterozygous members had mild FHC. No cases of MYH7 homozygous null alleles have been reported and given the more severe phenotype of mutations in MYH7 compared to MYBPC3, it is possible that such cases may result in an embryonic lethal phenotype.

Homozygous Phe110Ile and Ser179Phe substitutions in TNNT2 have been reported in two large families with FHC. Both mutations cause severe FHC characterized by biventricular hypertrophy, reverse septal curvature and a high incidence of sudden death.[19,20] Each mutation was not fully penetrant as some heterozygous family members were asymptomatic, while others had mild left ventricular hypertrophy. The left ventricular septal wall thickness for two homozygous Phe110Ile patients (19.9 ± 1.1mm) was greater than family members heterozygous for this mutation (13.8 ± 5.1mm), suggesting that one normal TNNT2 allele can largely compensate for the disruption caused by the Phe110Ile allele.[20] Collectively, the above cases demonstrate that homozygous FHC mutations cause an earlier onset with more severe symptoms than heterozygous mutations and suggest a mutation dosage effect.

Double heterozygous FHC mutations

FHC patients having single mutations in two different genes were first reported in a family with a Glu1096Ter nonsense mutation in MYBPC3 segregating in 7 members and a Glu483Lys substitution in MYH7 segregating in 6 members, of which two also had the MYBPC3 nonsense mutation and were therefore double heterozygous.[12,21] There was no difference in the degree or extent of hypertrophy between patients with single mutations in MYH7 and MYBPC3 (intraventricular septum 15 mm to 25 mm), but the extent of hypertrophy was significantly higher in the two double heterozygous patients (intraventricular septum 28 and 32mm). Thus, both mutations are responsible for FHC and have an additive effect on hypertrophy when found together in the same patient.

We recently reported on a double heterozygous proband having an Arg719Gln and Arg273His substitution in MYH7 and MYBPC3, respectively.[10] The proband, (IV:4 in Figure 1) passed on both mutations to her son (V:1), who at 15 years is clinically affected, whereas his 13 year old brother (V:2), who inherited only the MYBPC3 mutation is clinically normal. Double heterozygous mutations in FHC have also been reported involving mutations in MYH7 together with MYBPC3, TNNT2, TNNI3, and ACTC, and mutations in MYBPC3 together with TNNT2, TNNI3 and TPM1 (Table 1). Double mutations affecting MYH7 plus ACTC, and MYBPC3 plus TNNI3 are associated with severe childhood onset hypertrophy.[15] However, each mutation in the single heterozygous state causes mild FHC. Fraizer *et al.*[22] reported a family with history of FHC and sudden cardiac death involving a novel Arg453Ser substitution in MYH7 together with a Pro82Ser substitution in TNNI3. In this family, the mother was double heterozygous and had a severe clinical course with an ICD implanted. Her daughter and son were heterozygous for the substitution in MYH7 and TNNI3, respectively. Arg453 residue was previously reported as an FHC mutation when substituted for cysteine, histidine and leucine[23] and the Arg453Ser heterozygous daughter has presented with FHC. The Pro82Ser substitution in TNNI3 was previously identified as disease causing in elderly patients with FHC[24] and has been inherited by the currently asymptomatic son. This variant may be a polymorphism or disease modifier, as it is present in the healthy Afro-Caribbean population.[25]

Compound heterozygous FHC mutations

A compound heterozygous mutation, where two mutations are identified in the same gene, was first reported in a proband with a nonsense mutation and missense mutation on different MYH7 alleles, and only those family members heterozygous for the missense mutation were affected.[26] The nonsense mutation did not show a dominant phenotype as it might not be translated into a protein and incorporated into the sarcomere. In another case, a boy aged 6.5 years suffered a cardiac arrest and showed marked left ventricular hypertrophy with a wall thickness 18mm.[27] Genetic analysis revealed a *de novo* Arg719Trp substitution on the paternally inherited MYH7 allele and a Met349Thr substitution on the maternally inherited MYH7 allele. The former mutation has been reported as causing severe FHC,

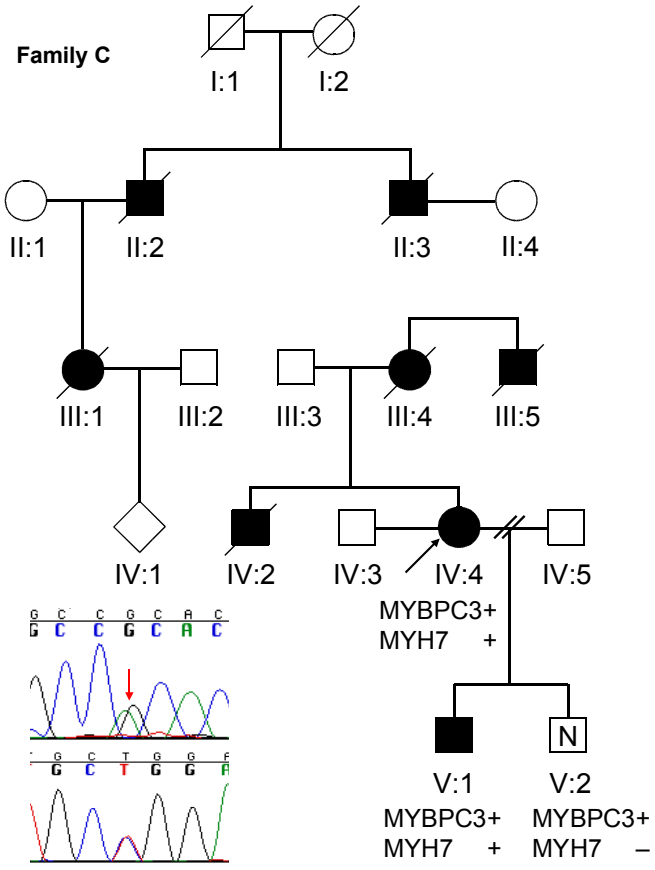

Figure 1. Double heterozygous mutations in FHC family. *Squares represent males, circles represent females, black arrow indicates proband, black symbols represent clinically affected individuals, crossed symbols represent deceased individuals, N – clinically unaffected. The genotype is shown below the individual, "+" – individual has a mutation in the indicated gene, "–" – individual does not have a mutation in the indicated gene. Arrows indicate position of the mutation on sequence chromatograms (top chromatogram – MYBPC3 Arg273His, bottom chromatogram – MYH7 Arg719Gln). (Modified from Ingles* et al., *2005* J. Med. Genet.*[10])*

including sudden death in young adults, with Kaplan-Meier survival analysis suggesting 50% of carriers of this mutation die before the age of 38 years.[28] The latter mutation was present in five asymptomatic family members and was postulated to add to the phenotype by aggravating symptoms in this unusually young patient, since no normal β-myosin heavy chain protein exists. Met349Thr variant in MYH7 is reported as a polymorphic variant in the normal population.[23] In this family only the MYH7 gene was screened for mutations leaving open the possibility of a second mutation in another FHC gene.

Lekanne Deprez *et al.*[29] reported a compound heterozygous mutation in MYBPC3 in a patient with severe hypertrophy who died suddenly at age 5 weeks. The normal mother had a single base insertion causing a reading frame shift, leading to a premature translation stop, whereas the father, who had mild FHC, had an intronic mutation in an invariant splice donor (AG) signal sequence, which predicts skipping of exon 15 in the mRNA. The authors further report on a second unrelated patient who died at age 6 weeks and also had two MYBPC3 mutations that caused premature termination of translation. Both of the above patients lacked an intact MYBPC3 protein, accounting for the neonatal FHC seen. This phenotype is similar to that of patients with homozygous MYBPC3 nonsense mutations mentioned previously. Parents heterozygous for the mutations have one intact functional allele, which can largely compensate for the MYBPC3 haploinsufficiency.

Diagnosis and counselling

Homozygous and compound or double heterozygous mutations occur in an appreciable proportion of FHC patients. The most accurate measures of the frequency of multiple mutations in FHC are with studies of all relevant genes in an unbiased FHC cohort, using a screening method that approaches 100% efficiency. Such studies indicate a frequency of up to 5%.[10] Such cases produce challenges to traditional methods of linkage analysis, as families with double mutations may not show segregation of a single marker with disease,[21] therefore, a systematic screen for mutations in all FHC genes would be required, even when a mutation is found. The genetic councillor must be aware that two mutations may segregate in families, particularly with an allele having incomplete penetrance, which may not be under strong negative selection pressure and thus persist in the family. This suggests that some inherited cases of FHC may be more complex than a simple autosomal dominant trait, with a second mutation acting in a recessive manner, causing no symptoms on its own, but having a marked additive influence on the severity and clinical progression. Furthermore, the phase of compound heterozygous mutations, *i.e.* whether they are on the same or on different chromosomes, will alter the chance of inheriting a mutation, from 50% to 100% respectively.

The question of whether a variant is a pathogenic mutation, rather than a neutral polymorphism remains. In many of the cases described above, a variant was silent on its own but enhanced the phenotype of a second FHC mutation. Typically, indirect evidence can be suggestive of a pathogenic mutation, including co-segregation with the phenotype in a family or arising *de novo* in sporadic hypertrophic cardiomyopathy, conservation of the nucleotide or amino acid residue across species, absence in a genetically relevant control population and molecular modelling of the mutation on the X-ray crystal structure of the protein. Recapitulating the phenotype in an animal model by introducing the specific mutation is a very strong indicator of a pathogenic mutation.

Animal models of multiple mutations in FHC

Animal models have been invaluable in elucidating the pathogenesis of cardiac disease and advances in technology during the last 15 years have made it possible to

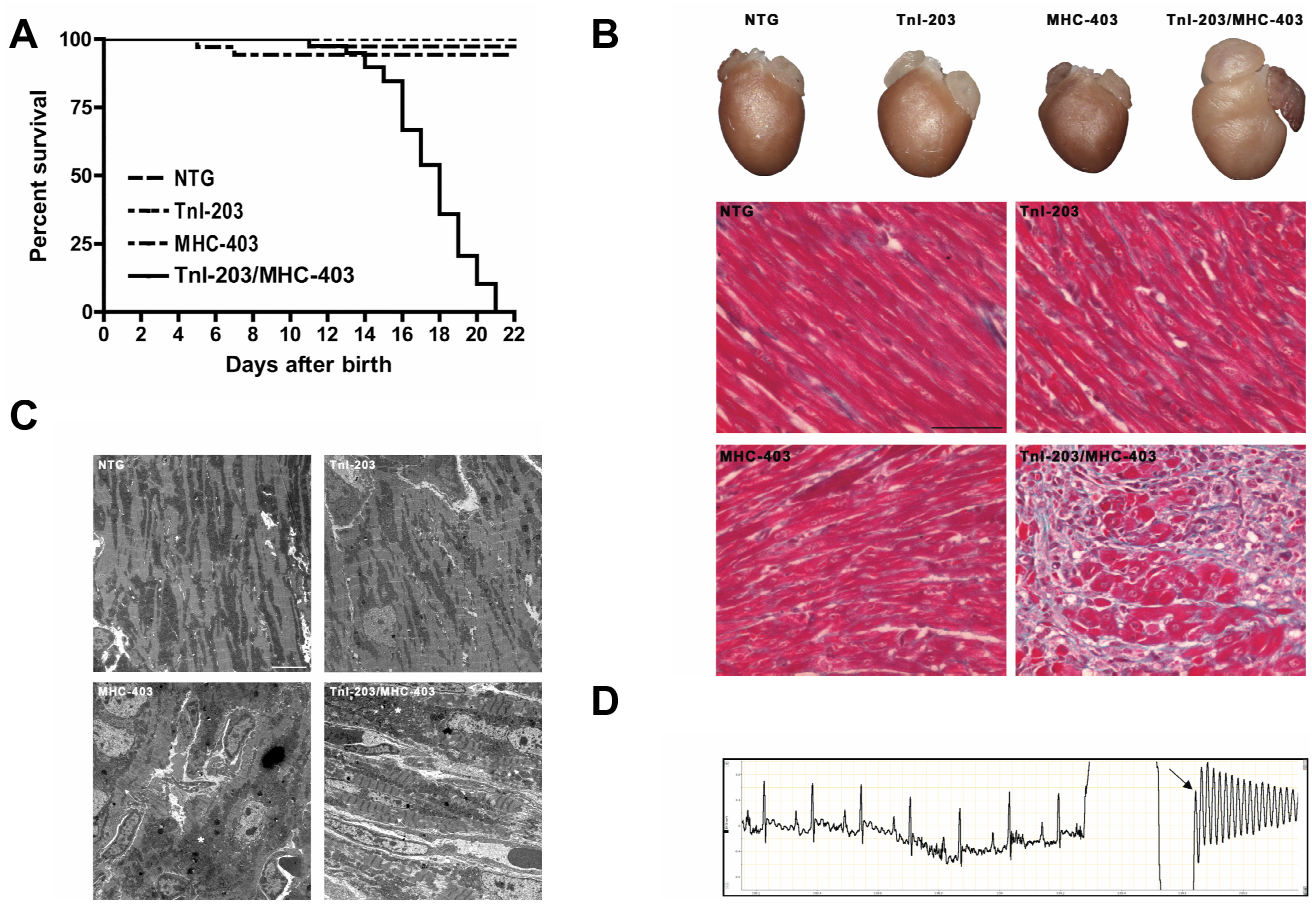

Figure 2. TnI-203/MHC-403 double-mutant mouse model of severe FHC. A *Kaplan-Meier survival curves of double-mutant TnI-203/MHC-403 mice.* ***B*** *Whole hearts at age 14 days, with corresponding light microscopy of myocardial sections stained with Milligan's trichrome. Myocytes stained red, collagenous tissue stained blue.* ***C*** *Transmission electron micrographs. Arrows indicate misalignment of Z-disks, asterisks indicate clusters of mitochondria.* ***D*** *Inducibility of ventricular tachycardia in TnI-203/MHC-403 mice (arrow indicates onset of ventricular tachycardia). (Modified from Tsoutsman* et al., *2008 Circulation*)

create virtually any model with cardiac specific loss- or over-expression of proteins of interest. Two major methodologies exist for creating genetically-modified animal models, *i.e.* transgenesis and gene-targeting *via* homologous recombination.[30] Both methodologies have advantages and limitations[30,31] and are widely used to confirm necessity, sufficiency and causality of specific proteins in cardiac pathology. Currently *Circulation Research* collection lists more then 4800 papers describing animal models of human disease.[32]

The mouse is the most widely used animal model due to its small size, low maintenance cost, rapid gestational period, large litter size and extensive knowledge of the mouse genome. A number of mouse models have been reported targeting the role of single mutations known to cause human FHC and are reviewed elsewhere.[33-36] The single-mutant mouse models demonstrate histopathological features of FHC including interstitial fibrosis and myocyte disarray, but the phenotypic hallmark of FHC, *i.e.* cardiac hypertrophy, is often mild or absent, which could be considered as one of the limitations of animal models.

TnI-203/MHC-403 double-mutation mouse model

To explore if introduction of a second mutation has an effect on the development of single-mutant phenotype in terms of severity as seen in humans, we recently developed a double-mutant model of FHC.[37] Arg403Gln α-myosin heavy chain (MHC-403) knock-out/knock-in mouse,[38] the first and the best characterised mouse model of FHC, was crossbred with a Gly203Ser cardiac troponin I (TnI-203) transgenic mouse model, which we previously generated in our laboratory.[39] α-myosin heavy chain (Myh6) is the predominant isoform of myosin expressed in the adult mouse heart corresponding to β-myosin heavy chain (MYH7), the predominant isoform expressed in the adult human heart. Both single-mutant mice develop characteristic features of FHC such as myocyte disarray, interstitial fibrosis and left ventricular hypertrophy by age 20-30 weeks, but each have been shown to have a normal life span (Table 2).[39,40] Both of the mutations have been

described to cause FHC in humans.[41,42] In complete contrast, the double-mutant mice, designated TnI-203/MHC-403, develop very severe disease, with 100% mortality by the age of 21 days (Figure 2). At age 14 days TnI-203/MHC-403 mice develop a significantly increased heart:body weight ratio, marked interstitial myocardial fibrosis, and increased expression of the hypertrophy-related genes atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP), compared to non-transgenic, TnI-203, and MHC-403 littermates. In addition, double-mutant mice demonstrate significantly altered alignment of myofibrils and several ultrastructural abnormalities including myocyte atrophy and fragmentation, altered distribution of mitochondria between myofibrils, myofibril disarray and discontinuity, degeneration and loss of myocyte structure. By age 16-18 days, TnI-203/MHC-403 mice rapidly develop a severe dilated cardiomyopathy with significant enlargement of left ventricular cardiac chambers and a reduction of fractional shortening, heart failure with inducibility of ventricular arrhythmias, leading to death by 21 days. Marked down-regulation of mRNA levels of key regulators of intracellular Ca^{2+} homeostasis in TnI-203/MHC-403 mice is also observed.

Clearly introduction of the second mutation has led to earlier onset of the disease, with severe heart failure and premature mortality, compared to either of the single-mutant FHC models. These findings demonstrate the similar effect double FHC mutations have on human disease caused by multiple mutations (Table 3).

Homozygous mutation mouse models

The TnI-203/MHC-403 mouse model is the first double-mutation model developed having two FHC-causing mutations located on different sarcomere genes. Previously, only homozygous models have been described, including Myh6 Arg403Gln[43,44] (heterozygous mouse have been used for the generation of TnI-203/MHC-403 double-mutant mouse) myosin-binding protein C (Mybpc3) mutants expressing truncated protein.[45,46] Although the homozygous mouse models differ from each other with respect to the timing of the phenotype development, both of them, unlike corresponding FHC heterozygous models, progress to a dilated cardiomyopathy (Table 2) as we observed in our double-mutant model. Homozygous Myh6 Arg403Gln mice develop left ventricular dilation, wall thinning and reduced systolic contraction between postnatal days 4 and 6 leading to 100% mortality by postnatal day 8. Myocardial necrosis with calcification was found on histopathology, the ultrastructure demonstrated normal architecture intermixed with focal myofibrillar disarray.[44]

The phenotype caused by homozygous truncated Mybpc3, which lacks the myosin and titin binding regions, is more mild as mice are viable and fertile and live normal life span, but onset of the disease, as in the case of homozygous Myh6 Arg403Gln, is neonatal with left ventricular dilation and reduced contractile function. Histopathology is characterised by myocyte hypertrophy, myofibrillar disarray, fibrosis and calcification by 8-12 weeks of life. In addition, markers of cardiac remodelling such as BNP, α-skeletal actin and β-MHC are up-regulated compared to the wild type mice.

Multiple mutation models: Insights into disease pathogenesis

The few multiple mutation mouse models described to date raise interesting issues related to our understanding of disease pathogenesis in FHC, including transition to heart failure. All three models described demonstrate an increased severity in phenotype compared to single-mutant models, which parallels our understanding of multiple mutations in humans with FHC, who similarly develop severe clinical phenotypes. The Myh6 Arg403Gln mutation in heterozygous mice results in the development of FHC by the age of 30 weeks, while truncated Mybpc3 does not precipitate the phenotype in heterozygous mice until they are over 2 years old. Accordingly, Myh6 Arg403Gln homozygous mice demonstrate neonatal lethality; Mybpc3 homozygous mice although present with the disease at the neonatal stage, live a normal life span. Double mutations in Myh6 and Troponin I (Tnni3) (phenotype in single-mutants by 20-30 weeks of life) also result in neonatal lethality.

These unique models also provide an opportunity to understand the underlying mechanisms as to how the two mutations in TnI-203/MHC-403 model which cause a mild phenotype if present alone, can result in such a rapidly developing phenotype if introduced together. One possibility is that the two gene mutations may each contribute to the phenotype. Indeed, both myosin and TnI play vital yet distinct roles in the sarcomere organisation and function. Myosin is the main component of the thick filament and directly involved in the force generation, while TnI is the main switch molecule in the sarcomere responsible for the phase of contraction or relaxation in a Ca^{2+} dependent manner. From the Myh6 homozygous model we gain information that duplication of the Arg403Gln mutation results in early lethality, with the implication that abnormal mutant Myh6 performance cannot be compensated for by any other component of the sarcomere. We do not know what will be the outcome of duplicating the Tnni3 Gly203Ser, but the Tnni3 knockout model[47] demonstrates that abolishing cardiac Tnni3 expression also cannot be compensated for, and results in mortality from heart failure by postnatal day 19. Two human FHC cases have been reported where mutations in MYH7 and mutation/sequence variant in TNNI3 were identified (Table 1).

Why, in the case of Mybpc3, a truncated protein in the presence of the second mutation does not lead to increased mortality of the homozygous mice, as seen in MHC homozygous or TnI-203/MHC-403 double-mutant mice or in isolated human cases (Table 1), remains unknown. There are a number of possible explanations. Myosin-binding protein C has been assigned roles in both the structure assembly and stability of the sarcomere, as well as in the modulation of contraction.[48] The sarcomere organisation of homozygous mice expressing a truncated

Table 2. Effect of the presence of second mutations in mouse models of FHC. *MD – myocyte disarray; IF – interstitial fibrosis; ↑ – increased; ↓ – decreased; LVWT – left ventricular weight thickness; LVEDD – left ventricular end diastolic diameter; LVESD – left ventricular end diastolic diameter; FS – fractional shortening; LV – left ventricular; ECG – electrocardiogram; ANF – atrial natriuretic factor; BNP – brain natriuretic peptide; SR – sarcoplasmic reticulum; CSQ – calsequestrin, RyR – ryanodin receptor; Trdn – triadin; PR – PR interval on ECG; $[Ca^{2+}]i$, - Ca^{2+} transients; HW – heart weight; BW – body weight, LTCC – L-type calcium channel; SERCA – sarco/endoplasmic reticulum calcium transporting ATPase; PLB – phospholamban; DCM – dilated cardiomyopathy; ΔMyBP-C - truncated form of myosin-binding protein C; LVW – left ventricular weight; RV – right ventricular; TEM – transmission electron microscopy; VT – ventricular tachycardia*

Mouse model	Phenotype	Age of phenotype development	Life span	References
αMHC Arg403Gln heterozygous **single mutant**	MD, IF, ↑LVWT, ↓LVEDD and LVESD, ↑FS, altered LV systolic and diastolic kinetics, ↑time of relaxation, ↓cardiac output, normal ECG, ↑repolarisation and sinus node recovery time, inducible ventricular ectopy, inducible arrhythmias, ↑ANF, BNP, α skeletal actin, ↓SR $[Ca^{2+}]$, ↓SR proteins CSQ, RyR2, Trdn, junctin, ↑RyR2 phosphorylation	30 weeks	Normal	38, 60, 61, 62, 40, 63, 64
TnI Gly203Ser hemizygous **single mutant**	MD, IF, ↑LVWT, ↓LVEDD, ↑PR, ↑decay of $[Ca^{2+}]i$, ↓decay of caffeine induced $[Ca^{2+}]i$, ↑ANF and BNP	21 weeks	Normal	39
TnI Gly203Ser/ αMHC Arg403Gln **double mutant**	MD, IF, ↑HW:BW, ↑LVEDD and LVESD, ↓FS, ↑PR, ↑ANF and BNP, abnormal ultrastructure, ↓SR mRNA LTCC, RyR2, SERCA2a and PLB	Neonatal	100% mortality by 21 day	37
αMHC Arg403Gln **homozygous**	DCM lethal phenotype by postnatal day 8, ↑of both atria, ↑LVESD, ↓FS, ↓LVWT, myocardial necrosis, normal ultrastrucure with focal MD, ↑force generation	Neonatal	100% mortality by 8 days	44, 43
ΔMyBP-C heterozygous **single mutant**	↑atria mass, ↑LVWT at 50 wk in 30% animals, normal cardiac function, normal ECG at 30-55 weeks, ↑ANF, BNP and α skeletal actin at >125 weeks	>125 weeks	Normal	40, 45
ΔMyBP-C **homozygous**	MD, IF, ↑HW:BW and LVW:BW, ↑LVWT, ↑LVESD and LVEDD, ↓systolic contractility with diastolic dysfunction at 8-12 weeks, LV and RV calcification, no M-line on TEM, ↑BNP and α skeletal actin, normal ECG at 30-55 weeks, significant risk of inducible nonsustained VT	Neonatal	Normal	46, 45

protein however remains mainly intact, as confirmed by electron microscopy. It has been suggested that other proteins such as Mybph can compensate for the truncated Mybpc3.[46] Although a role in modulation of contraction has been suggested, Mybpc3 does not directly participate in force generation, and so may not be required for sarcomere performance. Further, while the homozygous mice lack the C-terminal region of myosin binding, other low affinity binding sites in the C1-C2 domain and possibly C0-C1 domain,[49] can potentially compensate for the absence of the C-terminal region. Additional evidence that Mybpc3 may not be essential for filament assembly and function is that two homozygous Mybpc3 knockout models[50,51] do develop classical phenotypic features of FHC but survive into adulthood. In contrast two human cases of MYBPC3 knockouts discussed above[29] demonstrate severe neonatal phenotype and suggest that there could be other modifying factors which lead to more severe disease in humans.

Perhaps the most interesting insight gained from multiple mutation mouse models is the fact that in all three cases, an FHC mutation coupled with a second sarcomere mutation (either the same one or different) results in progression to DCM. The exact mechanism is unclear, but it is likely that the severity of sarcomere dysfunction is the central signal redirecting the compensatory myocyte growth towards uncompensated heart failure. In human FHC, up to

Table 3. Comparison of multiple mutation phenotype in humans and mice.

Clinical feature	Human FHC patients with multiple mutations	Double-mutant TnI203/MHC403 mice
Survival	Decreased (often < 50 years)	Decreased (21 day)
Early onset	Yes	Yes
Ventricular tachycardia	Present	Present
Left ventricular hypertrophy	Yes	No
Progression to dilation	Yes	Yes
Increased incidence of sudden death	Yes	Yes
More severe phenotype compared to single-mutant carriers	Yes	Yes

10% of patients progress to a DCM-like phenotype associated with left ventricular chamber dilation, wall thinning and systolic dysfunction.[52] Multiple mutations may be responsible for some of those cases (Table 1 – homozygous MYH7, compound MYH7 and MYBPC3[37]). Interestingly, mutations in all three genes have been reported to cause DCM and FHC in humans.[53,54] A recent study[43] investigated the functional consequences of different FHC and DCM mutations in the cardiac myosin gene in homozygous mice. A DCM phenotype caused by the Arg403Gln FHC mutation appears to be more severe than that caused by the Ser532Pro DCM mutation (homozygous Ser532Pro mice survive >1 year).[55] Although phenotypically both of the mutations in the homozygous state cause a dilated phenotype, the mechanism of disease development most likely involves different pathways, which start with molecular mechanical alterations in myosin. The Arg403Gln mutation results in enhanced molecular motor function while Ser532Pro mutation results in depressed function.[43,55] Clearly, increased force generation in FHC homozygous mice leads to decompensated cardiac function, heart failure and death while decreased force generation in DCM homozygous mice does not affect the normal life span. Collectively, these findings are beginning to explore the potential mechanistic links between FHC and DCM.

Future developments

The discovery of multiple disease causing mutations present in an individual has changed the long standing paradigm of FHC as being a monogenic disorder caused by a single gene mutation and may explain, at least in part, the clinical heterogeneity seen in families with FHC. The coexistence of two mutations results in enhanced severity of FHC including earlier onset, increased left ventricular hypertrophy, increased incidence of sudden cardiac death and, in some cases, progression to a dilated cardiac phenotype and heart failure. The multiple mutation genotype in FHC also has significant clinical implications, and highlights the importance of full panel FHC gene screening, difficulties in clinical prognosis, and challenges in genetic counselling of families. Multiple mutation mouse models appear to mimic the human disease, and thus will help to improve our understanding of disease pathogenesis. The models provide a tool for studies of disease mechanisms and signalling pathways in FHC, and its sequelae, heart failure and sudden death, thereby allowing identification of novel targets for potential therapies and disease prevention strategies.

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