CASE REPORT

Homozygous missense *MYBPC3* Pro873His mutation associated with increased risk for heart failure development in hypertrophic cardiomyopathy

Antheia Kissopoulou[1,2]*, Cecilia Trinks[3], Anna Green[3], Jan-Erik Karlsson[1,2], Jon Jonasson[3] and Cecilia Gunnarsson[3,4]

[1]*Department of Internal Medicine, County Council of Jönköping, Jönköping, Sweden;* [2]*Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Linköping, Sweden;* [3]*Department of Clinical Genetics, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden;* [4]*Centre for Rare Diseases in South East Region of Sweden, Linköping University, Linköping, Sweden*

Abstract

Hypertrophic cardiomyopathy (HCM) is a primary autosomal-dominant disorder of the myocardium with variable expressivity and penetrance. Occasionally, homozygous sarcomere genetic variants emerge while genotyping HCM patients. In these cases, a more severe HCM phenotype is generally seen. Here, we report a case of HCM that was diagnosed clinically at 39 years of age. Initial symptoms were shortness of breath during exertion. Successively, he developed a wide array of severe clinical manifestations, which progressed to an ominous end-stage heart failure that resulted in heart transplantation. Genotype analysis revealed a missense *MYBPC3* variant NM_000256.3:c.2618C>A,p.(Pro873His) that presented in the homozygous form. Conflicting interpretations of pathogenicity have been reported for the Pro873His *MYBPC3* variant described here. Our patient, presenting with two copies of the variant and devoid of a normal allele, progressed to end-stage heart failure, which supports the notion of a deleterious effect of this variant in the homozygous form.

Introduction

Although most patients with hypertrophic cardiomyopathy (HCM) have a good prognosis, a significant number suffer from life-threatening complications, primarily sudden cardiac death and end-stage heart failure.[1–3] The heterogeneous phenotypic expression of HCM has been related to its diverse genetic profile. In larger series, ~3–5% of adult HCM patients prove to be compound or double heterozygotes for two disease-causing variants in the same or different sarcomeric protein genes.[4–7] Although data are still limited, indications are that patients with more than one sarcomere genetic variant, either in a compound heterozygote state or as a homozygous variant, have a more profound clinical profile of HCM.[4,5,8–13]

Case report

A 39-year-old man presented in 1998 with shortness of breath during exertion. Nine years earlier, in 1989, he was treated for myocarditis after an upper respiratory tract infection presumably due to a self-limited virus infection. No test was performed then for any specific viruses. The echocardiogram (ECHO) showed a mildly reduced septal movement of the left ventricle. A year later, during follow-up, a new ECHO showed an entirely ordinary function of the left ventricle, but the left ventricular wall was at the upper reference thickness limit. In the following 8 years, he did not have any heart problems. There was no family history of heart disease.

In 1998, the index patient experienced heart symptoms including breathlessness and therefore electrocardiography (ECG) (*Figure 1*) and ECHO (*Figure 2*) were performed. He had a regular sinus rhythm. Auscultation revealed a systolic murmur, heard best at the left parasternal region. The blood pressure was normal. The ECHO showed a thick interventricular septum at about 26 mm, and the posterior wall of the left ventricle was more than 16 mm thick. There were signs of outflow obstruction with a maximal flow velocity of 2.8 m/s at rest. An exercise test, where he cycled up to 130 W (55% of reference), was stopped because of a hypotension episode. He was initially treated with beta-blockers. As no positive effect had been observed, he had a pacemaker (PM) implanted in 1999 with short A-V interval. He was feeling better after PM implantation, and objectively, he was in a better condition.

He experienced several problems with PM pocket infections that resulted in surgical extraction of the whole PM system in 2010. His clinical condition remained stable until 2013. Then he was admitted because of severe retrosternal chest pain that was due to a heart attack. Primary coronary angiography showed that the third marginal branch of the circumflex artery was occluded. The left anterior descending artery showed only slight atherosclerosis. The coronary circulation was 'right dominant' as the posterior descending artery was supplied by the right coronary artery, without any essential narrowing. He was treated with a drug-eluting stent with a good result. As regards risk factors, he did not have any history of coronary artery disease and had no known thrombophilia. He was not obese, but he was smoking, which he stopped doing after the heart attack. He had slightly elevated cholesterol levels: total cholesterol of 6.8 mmol/L and LDL of 4.6 mmol/L. The ECHO showed a vigorous hypertrophied left ventricle with a septum thickness of 27 mm and at least moderate systolic dysfunction with a generally restrained wall motion. The worst abnormal movement was seen apically. No outflow obstruction was seen. The left atrium was moderately dilated. The pressure in the lung circulation was about 50 mmHg. The heart rate monitoring revealed short episodes of non-sustained ventricular tachycardia, and this, together with the hypertrophied septum, prompted an implantable cardioverter defibrillator

Figure 1 The electrocardiography (ECG) of the index patient taken in 1998, when he was seeking medical advice for breathlessness. The ECG figure showed sinus rhythm with increased R-wave amplitude in the left-sided ECG leads (V4–V6) and increased S-wave depth in the right-sided leads (V1–V3), which are typical signs of hypertrophy of the left ventricle.
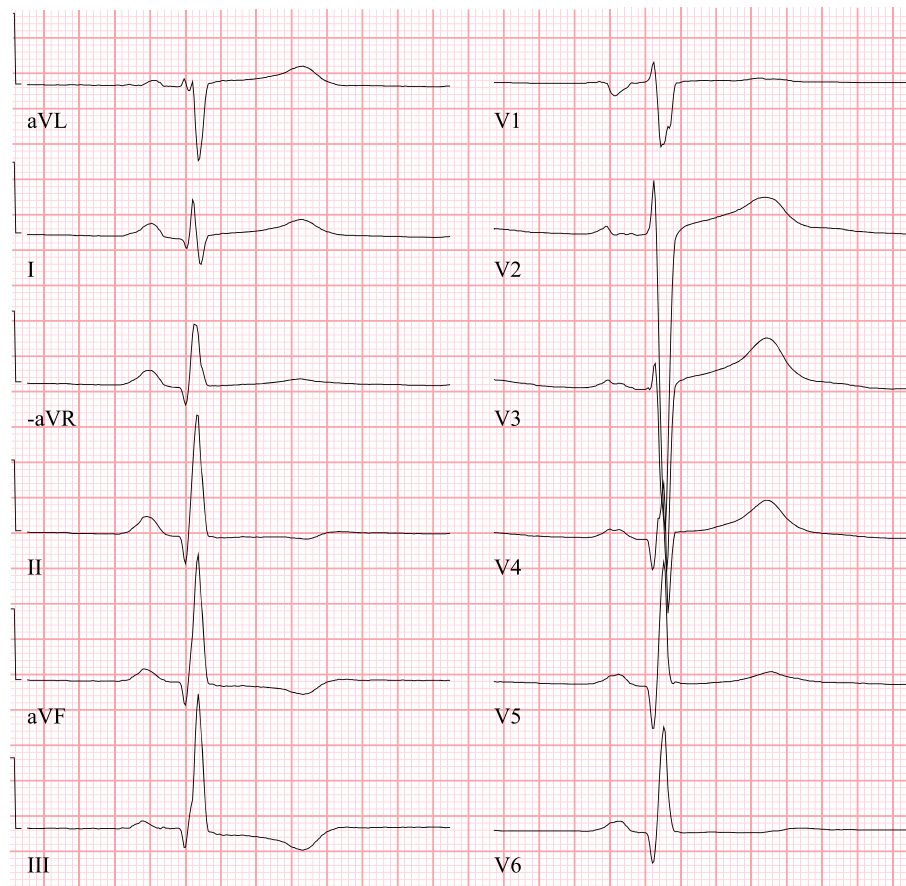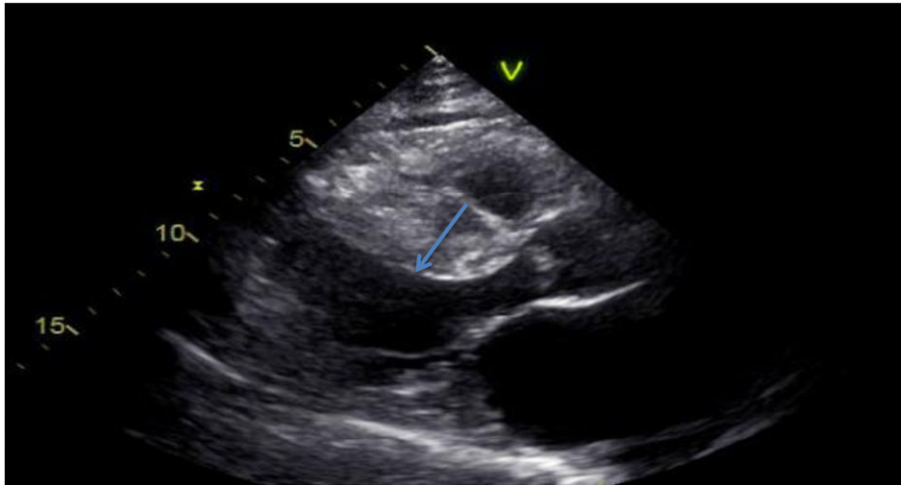
Figure 2 Echocardiogram of the index patient performed in 1998. The echocardiogram showed a thick septum (arrow) at about 26 mm, and the posterior wall of the left ventricle was more than 16 mm thick.

implantation. A year later, he suffered from atrial fibrillation attacks. During these episodes, he was extremely limited in his physical condition with severe dyspnoea, fatigue, and lack of energy, precluding any activity. A spirometry was also performed, showing a normal FEV1/VC ratio of 81% and normal total lung capacity. He had an optimal medical heart failure therapy with full-dose beta-blockers, angiotensin-converting enzyme inhibitor and aldosterone antagonist. He was not a candidate for cardiac resynchronization therapy as he did not have a width-increased QRS complex. Ablation of his atrial fibrillation would not have had any profound effect because of the severe impairment of the ventricle systolic function.

In 2015, ECHO showed a slightly dilated (about 80 mL/m^2), asymmetrically hypertrophied left ventricle with severe impaired systolic and diastolic functions. VO$_2$ max cycling in 2016 showed that he had a severely reduced maximum oxygen uptake of 1.2 L/min, equal to 14 mL/kg/min. A heart catheterization was performed. At rest, there was hypokinetic circulation with a cardiac output of 3.6 L/min and a systolic system pressure around 105 mmHg. End-diastolic left ventricular pressure was moderately elevated to 24 mmHg with a corresponding rise in the pulmonary capillary wedge pressure, and secondary to this, there was a moderate pressure increase in the pulmonary circulation (pulmonary pressure: 56/27 mmHg). Pulmonary vascular resistance was slightly higher, 2.7 mmHg/L/min. The filling pressure of the left ventricle rose even when he lifted his feet to the bike pedals at rest. During the exercise test load, the end-diastolic left ventricular pressure was severely elevated up to 38 mmHg, corresponding to a rise in pulmonary capillary wedge pressure and secondary elevated pressure in the pulmonary circulation with systolic levels of 70 mmHg. Magnetic resonance imaging of the heart was not performed,

but the patient underwent a myocardial biopsy that showed moderate interstitial fibrosis, focal cell hypertrophy, and even atrophy, with no signs of storage/infiltrative disease. His severe clinical deterioration as well as the ominous results of the above examinations prompted a new heart to be transplanted, and he has recently undergone the operation well.

Genetic method

Next-generation sequencing with a heart cardiomyopathy gene panel, including 84 genes (*Table 1*), was performed. The index patient was found to be homozygous for the *MYBPC3* variant NM_000256.3:c.2618C>A,p.(Pro873His). Multiplex ligation-dependent probe amplification was used to confirm that the *MYBPC3* c.2618 G>A substitution was in true homozygous state. With American College of Medical Genetics and Genomics criteria[14] for predicting pathogenicity of genetic variants, *MYBPC3* c.2618 C>A was predicted to be 'likely pathogenic' (Class 2) in the heterozygous state.

Clinical and genetic follow-up of relatives

Cascade genetic screening using Sanger sequencing was offered to family members, primarily to first-degree relatives (*Figures 3* and *4*). The patient's parents were not alive and could not be genetically tested. There was no known consanguinity. So far, 12 family members have been analysed for the *MYBPC3* c.2618 C>A variant. One brother to our index patient also had the mutation in the homozygous form and seven first-degree or second-degree relatives were shown to be heterozygous carriers of the variant. All the relatives

Table 1 Table of the genes included in the panel, as well as coverage and reference sequences used for testing the proband

Gene	NCBI reference sequence	Coverage (%)
ABCC9	NM_005691.3	99.18
ACTA2	NM_001613.2	100.00
ACTC1	NM_005159.4	97.18
ACTN2	NM_001103.2	100.00
AKAP9	NM_005751.4	99.35
ANK2	NM_001148.4	99.97
ANKRD1	NM_014391.2	100.00
APOB	NM_000384.2	100.00
BAG3	NM_004281.3	100.00
CACNA1C	NM_000719.6	100.00
CACNA2D1	NM_000722.2	97.59
CACNB2	NM_201590.2	97.31
CASQ2	NM_001232.3	96.17
CAV3	NM_033337.2	100.00
CBS	NM_001178009.1	100.00
COL3A1	NM_000090.3	99.39
COL5A1	NM_000093.3	100.00
COL5A2	NM_000393.3	99.33
CSRP3	NM_003476.4	100.00
DES	NM_001927.3	100.00
DMD	NM_004006.2	99.37
DSC2	NM_024422.3	100.00
DSG2	NM_001943.3	99.26
DSP	NM_004415.2	99.92
EMD	NM_000117.2	100.00
FBN1	NM_000138.4	100.00
FBN2	NM_001999.3	99.95
FHL1	NM_001449.4	100.00
FHL2	NM_201555.1	100.00
GLA	NM_000169.2	99.69
GPD1L	NM_015141.3	100.00
JUP	NM_002230.2	100.00
KCNE1	NM_000219.4	100.00
KCNE2	NM_172201.1	100.00
KCNE3	NM_005472.4	100.00
KCNH2	NM_000238.3	99.86
KCNJ2	NM_000891.2	100.00
KCNJ5	NM_000890.3	100.00
KCNJ8	NM_004982.3	100.00
KCNQ1	NM_000218.2	98.52
LAMP2	NM_002294.2	99.19
LDB3	NM_001080116.1	100.00
LDLR	NM_000527.4	100.00
LDLRAP1	NM_015627.2	100.00
LMNA	NM_170707.3	100.00
MYBPC3	NM_000256.3	100.00
MYH11	NM_002474.2	99.53
MYH6	NM_002471.3	99.5
MYH7	NM_000257.2	100.00
MYL2	NM_000432.3	91.42
MYL3	NM_000258.2	100.00
MYLK	NM_053025.3	99.86
MYLK2	NM_033118.3	100.00
MYOZ2	NM_016599.4	100.00
NEBL	NM_006393.2	100.00
NEXN	NM_144573.3	100.00
PCSK9	NM_174936.3	99.62
PKP2	NM_004572.3	100.00
PLN	NM_002667.3	100.00
PRKAG2	NM_016203.3	100.00
RBM20	NM_001134363.1	99.46
RYR2	NM_001035.2	99.46
SCN1B	NM_001037.4	100.00
SCN3B	NM_018400.3	100.00
SCN4B	NM_174934.3	100.00

(*Continues*)

Table 1 (continued)

| Gene	NCBI reference sequence	Coverage (%)
SCN5A	NM_198056.2	100.00
SLC2A10	NM_030777.3	100.00
SMAD3	NM_005902.3	99.77
SNTA1	NM_003098.2	100.00
TAZ	NM_000116.3	100.00
TCAP	NM_003673.3	100.00
TGFB2	NM_001135599.2	100.00
TGFB3	NM_003239.2	100.00
TGFBR1	NM_004612.2	96.83
TGFBR2	NM_003242.5	100.00
TMEM43	NM_024334.2	100.00
TMPO	NM_003276.2	100.00
TNNC1	NM_003280.2	100.00
TNNI3	NM_000363.4	100.00
TNNT2	NM_001001430.2	99.31
TPM1	NM_001018005.1	99.53
TTN	NM_133378.4	99.86
TTR	NM_000371.3	100.00
VCL	NM_014000.2	100.00

who have the variant in heterozygous form have a normal ECG, a normal ECHO, and no heart problems so far.

Our patient (III: 2 in the pedigree) has three children, one daughter and two sons. The youngest son (IV: 3 in the pedigree) has not been properly evaluated yet. The eldest son (IV: 1 in the pedigree), who is 29 years old, has the variant in heterozygous form. He has a normal ECG, a normal ECHO, and no heart problems. Our patient's daughter (IV: 2 in the pedigree) is 25 years old. She also has the variant in heterozygous form. She does not have any cardiopulmonary symptoms either. ECHO in 2016 showed a normal-sized left ventricle with normal systolic and diastolic functions.

The patient's sister (III: 4 in the pedigree), who is 43 years old, has the variant in heterozygous form. She does not experience any heart problems. Her ECG shows sinus tachycardia, with 105 b.p.m. and slight ST-T changes inferiorly. The ultra-sonography of the heart has been completely normal without any signs of cardiomyopathy. She has normal wall thickness of the left ventricle with normal systolic and diastolic functions. A cardiac stress test showed normal work capacity; she cycled up to 143 W (88% of reference) and stopped because of general fatigue. She has three children, two sons who are 16 and 10 years old (IV: 8 and 9 in the pedigree) and an 18-year-old daughter (IV: 7 in the pedigree). All of them have been offered genetic testing, and they are still waiting for the results. None of them have experienced any heart symptoms.

The patient's 54-year-old brother (III: 3 in the pedigree) who has the variant in homozygous form cycled 220 W (corresponding to 95% of reference) during an exercise test. No arrhythmia and no evidence of underlying coronary insufficiency were seen. He can sometimes experience a chest discomfort even at rest but no pain, dizziness, fainting, or

Figure 3 Pedigree analysis of the family in this study. Circles in the pedigree denote female, squares male. A crossed-over symbol indicates that this particular individual has died. The arrow points out the index patient. The genetic variant *MYBPC3* c.2618 C>A is indicated +/+ if homozygous and +/− if heterozygous. The individuals 7, 8, and 9 in the IV generation are also being offered genetic testing, but the results are not yet known.
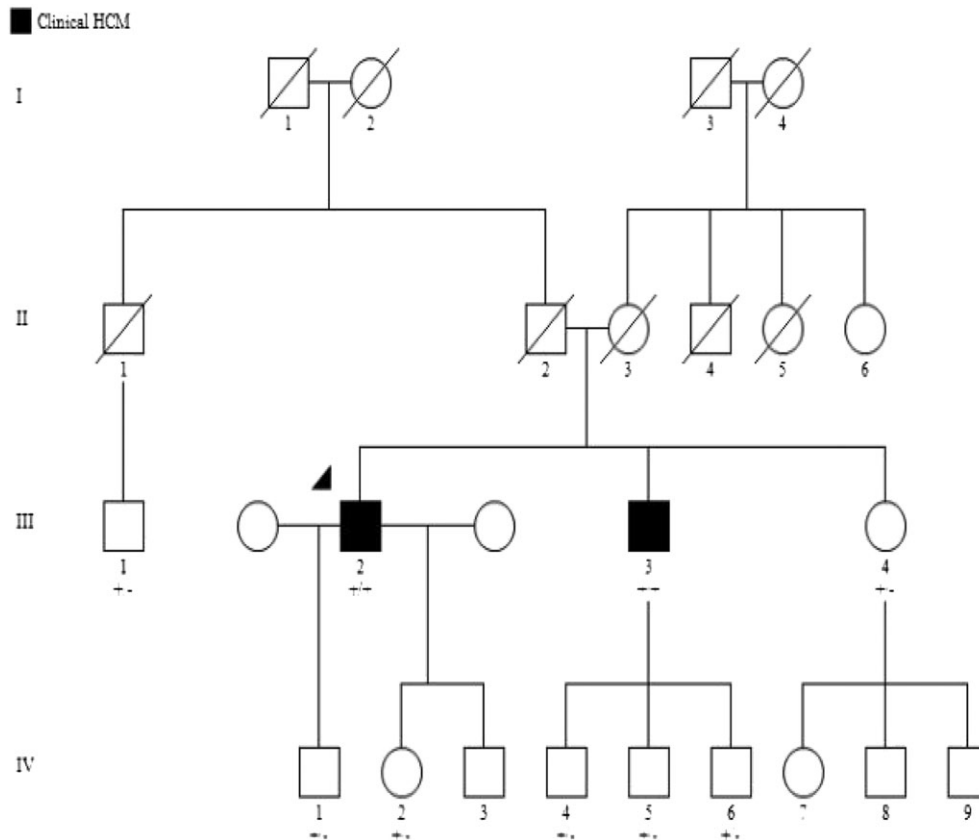

shortness of breath. The ECHO showed a normal-sized left ventricle, regionally increased wall thickness, moderately increased septum (16 mm), slightly increased over the posterior wall (13 mm) and a normal systolic function (ejection fraction of about 56%). The ECG showed sinus rhythm with increased R-wave amplitude in the left-sided ECG leads, as seen in ventricular hypertrophy. This man has three sons (10, 16, and 20 years old; IV: 4, 5, and 6 in the pedigree). All of them are heterozygous for the variant and have normal ECGs and ECHOs. A cousin to our index patient (III: 1 in the pedigree) who is 52 years old also carries the variant in heterozygous form. He also has a normal ECHO and ECG and does not have any cardiac symptoms.

Discussion

In the majority of patients with familial cardiomyopathy owing to a variant in one of the genes encoding sarcomeric proteins, a single autosomal-dominant pathogenic mutation is found. *MYBPC3* encodes for the thick filament-associated protein cardiac myosin-binding protein C (cMyBP-C), a signalling node in cardiac myocytes that contributes to the maintenance of sarcomeric structure and regulation of contraction and relaxation. *MYBPC3* is the most frequently mutated gene in HCM, representing 40–50% of all HCM mutations. The majority of *MYBPC3*-associated HCM mutations are heterozygous, and patients often have a late disease onset with a benign disease progression.[15] Studies have also reported that 70% of the mutations in *MYBPC3* are truncating variants, which cause a more severe HCM phenotype than those associated with missense and in-frame deletions.[16] In contrast to heterozygous pathogenic mutations, homozygous or compound heterozygous truncating pathogenic *MYBPC3* variants cause severe neonatal cardiomyopathy, which leads to heart failure and death within the first year of life.[13] Furthermore, several studies have demonstrated that HCM patients carrying multiple pathogenic gene variants have poorer prognosis in terms of earlier disease onset, increased left ventricular hypertrophy, and increased frequency of heart failure and sudden cardiac death, when compared with those carrying a

A. IV:4 Heterozygote NM_000256.3:c.2618C>A,p.(Pro873His)

B. III:3 Homozygote NM_000256.3:c.2618C>A,p.(Pro873His)
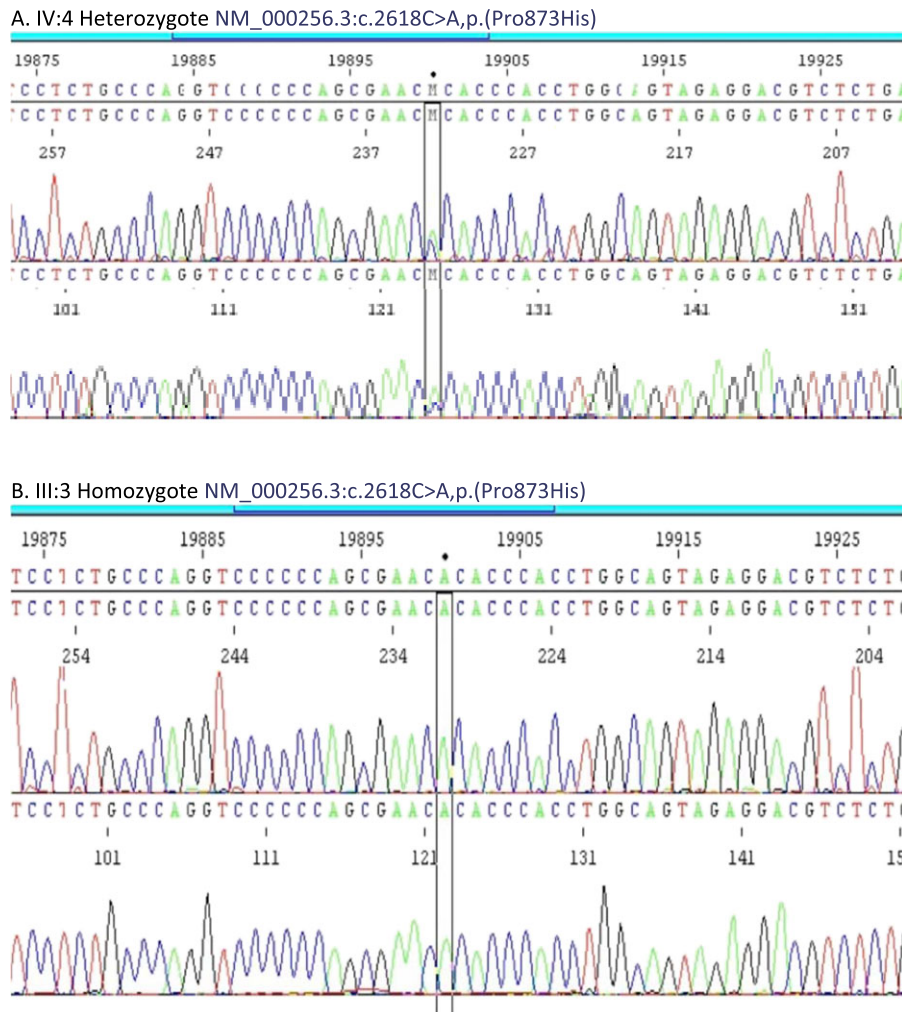
single mutation.[5,6,11,16] Thus, the present case supports previous claims that rare homozygous variants may aggravate the clinical severity of HCM. Our HCM patient with a homozygous missense variant in *MYPBC3* needed a heart transplantation, while his brother who also has the variant in homozygosis and the clinical phenotype of HCM will require specific attention at follow-up as there is the possibility of more adverse manifestations of HCM later. None of the heterozygotes in the family has so far demonstrated any sign of HCM.

The *MYBPC3* Pro873His variant described here has been reported in the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/), at a frequency of 0.00006781 (14/206 446 in the entire studied population, 13/97 288 in the European non-Finnish population).

One fundamental question pertains to the nature of the gene product derived from the mutant allele. Certain mutant proteins may be more or less stable or incorporate more or less efficiently into the sarcomere. Wijnker *et al.*[17] compared the pathomechanisms of a truncating mutation and a missense mutation in *MYBPC3* in engineered heart tissues. The widely accepted hypothesis is that truncating *MYBPC3* mutations causes haploinsufficiency, as opposed to missense mutations, which incorporate into the sarcomere and act in a dominant-negative fashion. Some studies[18,19] provided evidence that truncated cMyBP-Cs are not detectable in human patient samples, as they seem to be susceptible to degradation by nonsense-mediated RNA decay. In contrast to truncating mutations, missense mutations lead to stable mutant cMyBP-Cs that exert a more potent effect in disrupting sarcomere function.[16] The *MYBPC3* variant described here presumptively leads to the substitution of a non-polar residue (Pro) with a positively charged residue (His). It occurs in the C7 domain of the protein and could interfere with the protein incorporation in the A-band of the sarcomere.

The 'poison peptide' hypothesis proposes that mutant sarcomeric proteins incorporate into myofibrils and act as dominant-negative proteins.[20]

Evidently, conflicting interpretations of pathogenicity have been obtained for heterozygous missense and compound heterozygous missense *MYBPC3* variants. For example, another case was presented with the *MYBPC3* Pro873His variant associated with HCM where one additional *MYBPC3* (Asp745Gly) variant of uncertain significance was also observed. The patient was diagnosed with HCM at 18 years of age. He had asymmetric hypertrophy with a maximal wall thickness of 30 mm, and he had received appropriate therapy from his implantable cardioverter defibrillator.[10] Predictions from *in silico* tools were supportive of a deleterious effect. However, because of co-occurrence with an additional *MYBPC3* variant, limited familial data, and insufficient evidence, the *MYBPC3* Pro873His variant found in that patient must be classified as one of 'uncertain significance' according to American College of Medical Genetic and Genomics criteria.[14]

In addition, the *MYBPC3* Pro873His variant has been identified previously in an HCM individual who was also homozygous for the variant.[21] In that study, while trying to identify genes that are consistently associated to HCM progressing to dilated cardiomyopathy, the authors found a homozygous *MYBPC3* Pro873His mutation in a man from a gipsy family, where consanguinity between the parents was very likely. That male patient was clinically evaluated at the age of 27. He had a moderate/severe hypertrophy (interventricular septum: 27 mm; left ventricular outflow tract obstruction: 50 mmHg) and showed a non-sustained ventricular tachycardia at Holter monitoring. As in our case, the patient's hypertrophied heart progressed to a slightly dilated left ventricle with severe systolic impairment.

The question of whether the myocarditis event that our index patient had experienced before he was clinically diagnosed with HCM might have contributed, as an external factor, to the clinical appearance of the disease, by triggering the underlying genetic substrate, arises. It is also possible that the heart attack that he faced might have worsened his ventricular function. However, the occlusion of the branch of the circumflex artery cannot explain the severe impairment of the systolic function. Thus, homozygosity of the *MYBPC3* Pro873His variant seems to have caused a cumulative deleterious effect on the clinical course of our HCM patient who progressed to end-stage heart failure. Given the important clinical issue of risk stratification for determining which HCM patients are at highest risk of heart failure and sudden cardiac death, the presence of bi-allelic *MYBPC3* missense variants in an individual may be a major factor in this cardiac event risk algorithm.

In conclusion, allelic balance may influence genotype–phenotype correlations and should be an important consideration in the risk stratification of patients with HCM and their family members. Our data show that homozygosity for the *MYBPC3* Pro873His variant very likely increases the risk of HCM pathogenesis and may contribute a cumulative effect, leading to earlier disease onset and adverse cardiac events. Interestingly, none of the heterozygote carriers in the present study have so far developed any hypertrophic features, suggesting that this variant as a heterozygote trait has a low penetrance and might be insufficient to cause a strong HCM phenotype. Therefore, homozygous and compound heterozygous *MYBPC3* variants should prove to be clinically useful prognostic markers. However, the current evidence is preliminary, and more studies and case reports are required before the potential for translating the basic genetic information into clinical decision making concerning longitudinal evaluation and monitoring of HCM patients can be fully assessed.

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Conflict of interest

None declared.

Funding

Ethics approval

Regional Ethics Committee in Linköping, Dnr 2016/389-31. Participants in the present study have signed an informed consent to participate in the study.

Data accessibility

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request. The individuals in the present study have not given their permission for publishing data on an individual basis.

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