

## ARTICLE

# Compound heterozygous or homozygous truncating *MYBPC3* mutations cause lethal cardiomyopathy with features of noncompaction and septal defects

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Familial hypertrophic cardiomyopathy (HCM) is usually caused by autosomal dominant pathogenic mutations in genes encoding sarcomeric or sarcomere-associated cardiac muscle proteins. The disease mainly affects adults, although young children with severe HCM have also been reported. We describe four unrelated neonates with lethal cardiomyopathy, and performed molecular studies to identify the genetic defect. We also present a literature overview of reported patients with compound heterozygous or homozygous pathogenic *MYBPC3* mutations and describe their clinical characteristics. All four children presented with feeding difficulties, failure to thrive, and dyspnea. They died from cardiac failure before age 13 weeks. Features of left ventricular noncompaction were diagnosed in three patients. In the fourth, hypertrabeculation was not a clear feature, but could not be excluded. All of them had septal defects. Two patients were compound heterozygotes for the pathogenic c.2373dup p.(Trp792fs) and c.2827C>T p.(Arg943\*) mutations, and two were homozygous for the c.2373dup and c.2827C>T mutations. All patients with biallelic truncating pathogenic mutations in *MYBPC3* reported so far ( $n=21$ ) were diagnosed with severe cardiomyopathy and/or died within the first few months of life. In 62% (13/21), septal defects or a patent ductus arteriosus accompanied cardiomyopathy. In contrast to heterozygous pathogenic mutations, homozygous or compound heterozygous truncating pathogenic *MYBPC3* mutations cause severe neonatal cardiomyopathy with features of left ventricular noncompaction and septal defects in approximately 60% of patients.

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## INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a major cause of sudden cardiac death in people younger than age 35 years under physical stress, and a major cause of mortality and morbidity in the elderly. It has an estimated prevalence of 1 in 500 individuals. In ~60% of cases, a pathogenic variant in one of the sarcomeric contractile protein genes is found.<sup>1,2</sup>

Familial HCM is usually an autosomal dominant disorder that is transmitted due to heterozygous pathogenic gene mutations with incomplete penetrance. Variants in genes encoding proteins involved in the sarcomere, cytoskeleton and Z-disk, in calcium handling, and in mitochondrial and lysosomal functions have been associated with HCM.<sup>3</sup> To date, >1000 different pathogenic mutations have been found in genes that encode sarcomeric proteins, such as  $\beta$ -cardiac myosin heavy chain (*MYH7*) and cardiac myosin-binding protein-C (*MYBPC3*).<sup>4</sup>

Interfamilial and intrafamilial clinical variability in HCM is high, and it is difficult to establish genotype–phenotype correlations.<sup>4</sup> In addition to the primary genetic defect, the effects of modifier genes or

additional sarcomeric gene variants may contribute to the phenotypic expression of HCM.<sup>5</sup> Childhood-onset cardiac hypertrophy is also genetically determined in the majority of cases, and two-thirds of familial cases of childhood-onset cardiac hypertrophy are caused by a pathogenic mutation in one of the sarcomeric protein genes.<sup>6</sup>

We describe four unrelated children with severe cardiomyopathy, features of ventricular noncompaction, and septal defects due to compound heterozygosity or homozygosity for truncating pathogenic mutations in *MYBPC3*, resulting in early death.

## MATERIALS AND METHODS

### Clinical diagnosis

Four unrelated families with an index patient with severe neonatal cardiomyopathy were studied, after obtaining informed consent. Clinical evaluation included clinical history and physical examination, electrocardiography (ECG) and 2D and M-mode echocardiography. The clinical diagnosis of HCM is made when there is a hypertrophied (often asymmetric), non-dilated left ventricle on echocardiography (LVW  $\geq 15$  mm) in the absence of other cardiac or systemic diseases.<sup>1</sup> In children, diagnosis is made on the basis of left ventricular wall thickness two or more standard deviations above the normal population mean

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for body surface area.<sup>7</sup> Noncompaction of the left ventricle was diagnosed based on three echocardiographic criteria defined by Jenni *et al*,<sup>8</sup> including (1) a thick noncompacted (NC) endocardial layer in the end systole on parasternal short-axis views (ratio NC/C > 2) with numerous, excessively prominent trabeculations and deep intertrabecular recesses, (2) these recesses were perfused on color Doppler studies, and (3) a predominantly apical localization.

### Pathologic studies

Microscopic examination and electron microscopy of cardiac septal autopsy material of patient 1 were performed with standard techniques (hematoxylin and eosin staining, magnification × 100).

### Molecular analysis

Genomic DNA of the patients was isolated from blood samples or fibroblasts. All coding regions and intron–exon boundaries of the *MYBPC3* gene were analyzed by direct sequencing analysis as described.<sup>9</sup> In addition, MLPA analysis of the *MYBPC3* gene was performed (SALSA MLPA kit P100, MRC Holland, Amsterdam, the Netherlands) to detect possible genomic rearrangements. (*MYBPC3* exon numbering according to NG\_007667.1, DNA variants were numbered according to reference sequence LRG\_386 (identical to reference sequence NM\_000256.3) using HGVS nomenclature (www.HGVS.org)). Variants have been submitted to the Leiden Open Variation Database (http://databases.lovd.nl/shared/genes/MYBPC3 (patient IDs: 00019603–00019606).

### Literature review

We searched the literature for clinical and molecular data of patients with neonatal cardiomyopathy and biallelic *MYBPC3* variants, focusing on English-language articles published in PubMed and EMBASE between 1995 and January

2013. We identified articles in which the title/abstract included one of the following terms: double *MYBPC3* mutations, biallelic *MYBPC3* mutations, homozygous or compound heterozygous *MYBPC3* mutations or neonatal hypertrophic cardiomyopathy. We then assessed all the references cited by these articles. In our analysis, we only used reports if pathogenic homozygous or compound heterozygous *MYBPC3* variants were identified.

## RESULTS

Table 1 summarizes the characteristics of the patients and their parents.

### Patient 1

Patient 1 was hospitalized at age 5 weeks because of feeding problems and cyanosis. X-thorax showed a grossly enlarged heart. Echocardiography revealed a moderately dilated left ventricle with severe systolic dysfunction. The apical wall of the left ventricle was excessively thickened with prominent hypertrabeculation. The left and right atria showed mild dilatation. A small secundum atrial septal defect (ASD) was also present. Within 2 weeks the X-thorax and echocardiography revealed further enlargement of the heart (heart–thorax ratio ± 0.7) with severe left ventricular hypertrophy and deep trabeculations in the apex (interventricular septum in diastole (IVSd) 12 mm, Z+10; left ventricular posterior wall in diastole (LVPWd) 10 mm, Z+10) and a fractional shortening (FS) of the left ventricle of 10% (left ventricular internal dimension (LVID) 23/26). The child died from cardiac failure at age 12 weeks. Macroscopic examination of the heart revealed severe cardiomegaly and dilatation with a total weight of 115 g (normal

**Table 1** Clinical and molecular characteristics of the four patients and their parents

Patient	1	2	3	4
Gender	Male	Male	Female	Female
Gestational age (weeks)	40	38	42	40
Birth weight (g)	3110	3345	3750	2950
Age of diagnosis (weeks)	5	4	7	6
Noncompaction	LV	LV	Not evident	LV
Structural defect	ASDII	VSD	OFO	ASDII
Pathology	Myofibrillar disarray LVNC	Not performed	Not performed	Not performed
Viral serology	Normal	Normal	Normal	Normal
Metabolic screening	Normal	Normal	Normal	Normal
Age of death (weeks)	12	12	12	7
<i>MYBPC3</i> genotype	<i>c.[2373dup];[2827C&gt;T]</i> <i>p.[(Trp792fs)];[(Arg943*)]</i>	<i>c.[2373dup];[2827C&gt;T]</i> <i>p.[(Trp792fs)];[(Arg943*)]</i>	<i>c.[2373dup];[2373dup]</i> <i>p.[(Trp792fs)];[(Trp792fs)]</i>	<i>c.[2827C&gt;T];[2827C&gt;T]</i> <i>p.[(Arg943*)];[(Arg943*)]</i>
Family history (SCD, HCM)	Positive	Unknown	Positive	Positive
Parental consanguinity	No	No	Yes	Yes
Father genotype	<i>c.[2827C&gt;T];[=]</i> <i>p.[(Arg943*)];[(=)]</i>	Not performed	<i>c.[2373dup];[=]</i> <i>p.[(Trp972fs)];[(=)]</i>	<i>c.[2827C&gt;T];[=]</i> <i>p.[(Arg943*)];[(=)]</i>
Phenotype				
Echocardiography	Normal (age 31 years)	Not performed	Normal	Normal
ECG	Repolarization abnormal	Not performed	Normal	Incomplete RBBB
Follow-up	IVS 12 mm (age 38 years)	Not performed	Low ECG voltages (age 38 years)	Unknown
Mother genotype	<i>c.[2373dup];[=]</i> <i>p.[(Trp972fs)];[(=)]</i>	Not performed	<i>c.[2373dup];[=]</i> <i>p.[(Trp972fs)];[(=)]</i>	<i>c.[2827C&gt;T];[=]</i> <i>p.[(Arg943*)];[(=)]</i>
Phenotype				
Echocardiography	Normal (age 32 years)	Not performed	Normal	HCM (age 27 years)
ECG	Normal (age 32 years)	Not performed	Normal	L VH, abnormal repolarization
Follow-up	IVS 14 mm (age 39 years)	Not performed	IVS 11 mm (age 41 years)	Unknown

Abbreviations: ASD, atrial septal defect; ECG, electrocardiography; HCM, hypertrophic cardiomyopathy; IVS, intraventricular septum; LV, left ventricle; LVNC, left ventricular noncompaction; OFO, open foramen ovale; SCD, sudden cardiac death; VSD, ventricular septal defect.

weight at this age, 30 g). Right ventricular thickening was noted, especially of the posterior wall. The anterior wall of the left ventricle was severely thickened and showed abnormal trabeculation and multiple intertrabecular recesses as seen in noncompaction cardiomyopathy. Secundum ASD was confirmed. No other congenital malformations were found. Microscopic examination of cardiac tissue (Figure 1a) showed myofibrillar disarray in both the ventricular septum and the left ventricular wall. Hypertrophic myocytes with a diameter of 20–30  $\mu\text{m}$  (normal 12  $\mu\text{m}$ ) and multiple vacuoles on electron microscopy suggested glycogenosis (Figure 1b). As the echocardiographic images and ECG did not suggest Pompe disease and urine oligosaccharide analysis was normal, no  $\alpha$ -glucosidase enzyme or molecular assay was performed.

*MYBPC3* mutation analysis revealed compound heterozygosity for the pathogenic mutations c.2373dup p.(Trp792fs) and c.2827C>T p.(Arg943\*). Both pathogenic mutations are known founder mutations within the Dutch HCM population.<sup>10,11</sup> Neither parent had cardiac symptoms. Initial echocardiography revealed no abnormalities in the 32-year-old mother, who was heterozygous for c.2373dup, nor in the 31-year-old father, heterozygous for c.2827C>T. ECG in the father showed mild repolarization abnormalities (ST elevation of 0.5 mm in V1, followed by a negative T in V1–V4). Re-evaluation after 7 years revealed moderate septal hypertrophy (HCM) with an interventricular septum of 14 mm in the mother, and interventricular septal measurements at the upper limit of the normal range (12 mm) in the father. The family history of both parents was positive for HCM and sudden death (Figure 2a).

#### Patient 2

Patient 2 had feeding problems in the first weeks of life and dyspnea and mild hypotonia were noticed at age 4 weeks. A grossly enlarged heart was observed on the X-thorax (heart–thorax ratio 0.8%) and low oxygen saturation blood levels were found. Echocardiography revealed moderately dilated ventricles with severe diastolic and systolic dysfunction. Hypertrabeculation with flow perfused intertrabecular recesses was present in both ventricles, including the apical walls (Figure 3). An apical muscular ventricular septal defect (VSD) was visualized, although deep recessal flow complicated interpretation of the images. Unfortunately, owing to the critically ill status of the patient, there are no reliable M-mode measurements of the left ventricle. A muscle computed tomography scan showed no signs of atrophy, and a muscular biopsy revealed no congenital myopathy. His condition deteriorated at age 12 weeks and he died.

In this case too, we identified compound heterozygosity for the pathogenic c.2373dup p.(Trp792fs) and c.2827C>T p.(Arg943\*) mutations in the *MYBPC3* gene. The parents agreed neither to an autopsy on the child nor to cardiologic evaluation for themselves or pedigree analysis.

#### Patient 3

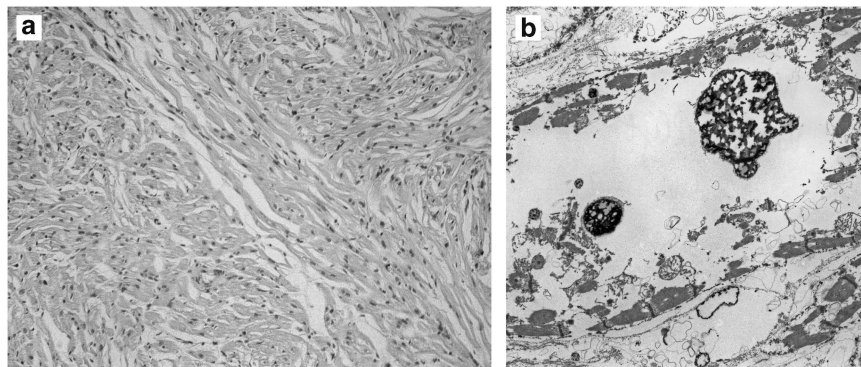
The third proband presented with severe feeding problems and signs of cardiac decompensation at age 7 weeks. Cardiomegaly was seen on chest X-ray, and echocardiography showed HCM with dilated ventricles. M-Mode measurements were unavailable. Noncompaction was not a clear feature, but could not be excluded. There was a small foramen ovale and mitral valve insufficiency. The condition of the patient deteriorated owing to a viral infection and she died at age 12 weeks. The parents did not give permission for autopsy and, at that time, molecular analysis of genes involved in cardiomyopathy was not available. Cardiac analyses of first-degree relatives were normal.

Eleven years later, a cousin of the father presented with cardiac arrest due to HCM. He had a heterozygous pathogenic c.2373dup p.(Trp792fs) mutation in *MYBPC3*. We subsequently confirmed homozygosity for the c.2373dup variant in DNA isolated from stored fibroblasts from the deceased proband. Both parents of the proband were heterozygous for this truncating variant (Figure 2b). Cardiac re-evaluation showed only slightly reduced ECG voltages in the father (age 38) and borderline hypertrophy (septal thickness 11 mm) in the mother (age 41).

#### Patient 4

Patient 4 presented with dyspnea, hypotension, feeding difficulties, and failure to thrive at age 6 weeks. Echocardiography showed severe HCM with features of noncompaction, and severe diastolic and systolic left ventricular dysfunction (IVSd 11 mm, Z+10; LVPWd 10 mm, Z+10, FS 13% (LVID 17/20) and left ventricular mass 255 g/m<sup>2</sup>). A secundum ASD with a left–right shunt was also observed. One week later she died after an unsuccessful resuscitation. Homozygosity for the c.2827C>T p.(Arg943\*) nonsense mutation in *MYBPC3* was found. Both parents were heterozygous for this pathogenic mutation.

Her mother was diagnosed with postpartum HCM with diastolic dysfunction, confirmed by cardiac MRI, at age 26. Echocardiography of the father was normal; ECG showed a right bundle branch block. A paternal uncle of patient 4 had died aged 3 months of unknown cause (Figure 2c).



**Figure 1** (a) Microscopic postmortem examination of heart muscle from patient 1. Hypertrophic myocytes with myofibrillar disarray typical of HCM due to sarcomeric protein variations were present, albeit without a significant amount of interstitial fibrosis (hematoxylin and eosin staining, magnification  $\times 100$ ). (b) Electron micrograph showing a large, irregular vacuole.

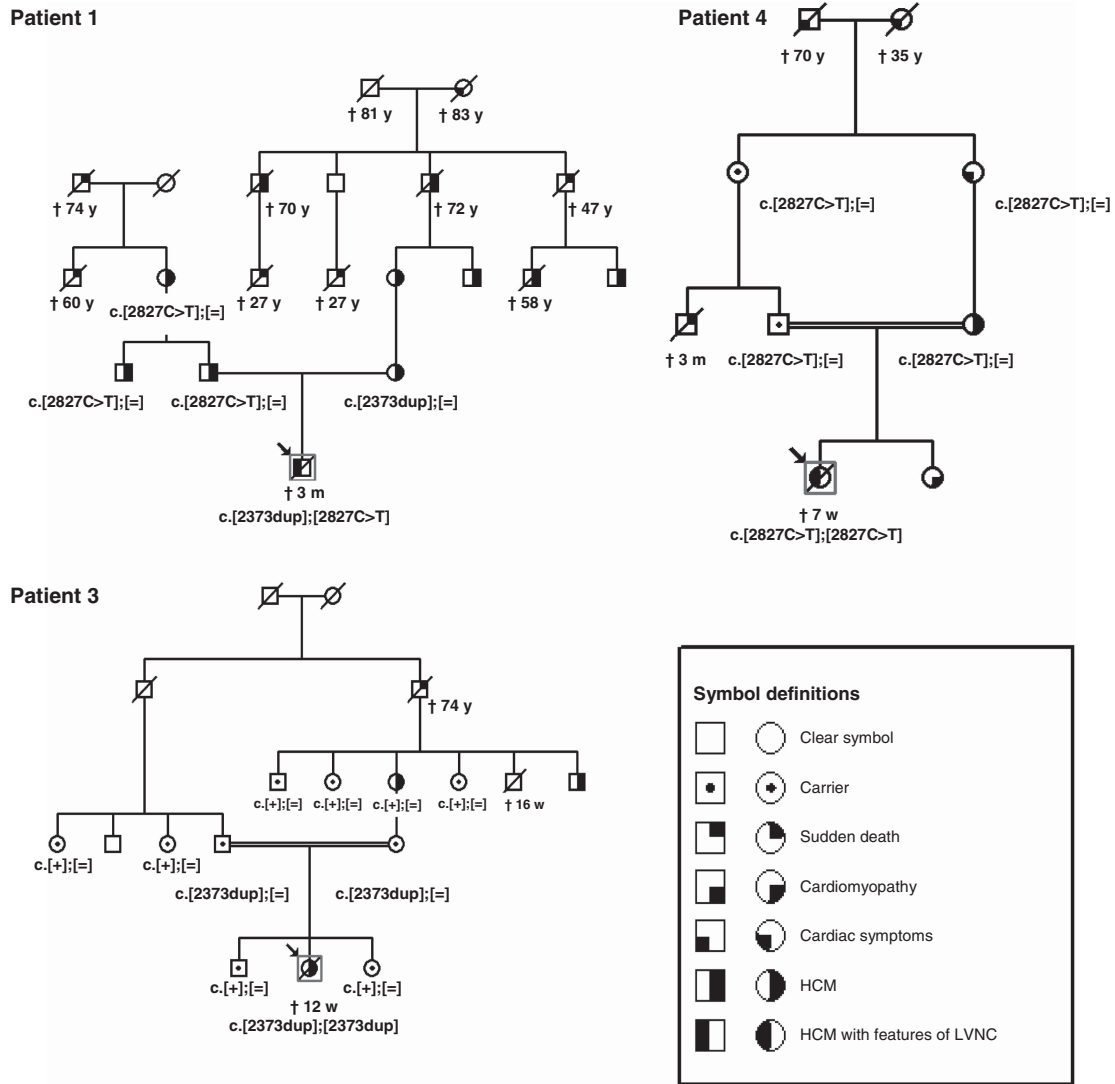


Figure 2 Pedigrees of patients 1, 3, and 4.

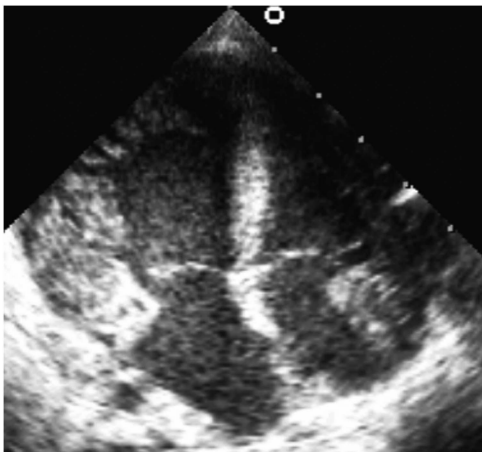


Figure 3 Echocardiographic studies in patient 2 showing cardiomyopathy with LVNC. The four-chamber view shows excessive trabeculation (arrows) at the apical right ventricular wall, whereas the left ventricular wall also showed numerous deep trabeculae and recesses.

### Literature review

The results of our literature review are summarized in Table 2, showing 21 cases (including our four cases) of double-truncating pathogenic mutations, all presented neonatally or in early childhood. Fifteen died before the age of 1 year; the others had either undergone transplantations or were severely affected. Structural defects were detected in 13 cases, but neither hypertrabecularization nor deep intertrabecular recesses were described in any of the previously reported cases.

### DISCUSSION

We describe four unrelated newborns with severe cardiomyopathy due to compound heterozygosity or homozygosity for pathogenic truncating mutations in the *MYBPC3* gene. All of them died soon after birth. Although initially their cardiomyopathies were described as severe atypical HCM, HCM with features of left ventricular noncompaction (LVNC) was considered after re-evaluation of serial ultrasounds and pathologic examination in three of them. Like HCM, LVNC is genetically heterogeneous.<sup>9,12</sup> Recently, LVNC was shown to be mainly caused by heterozygous variants in genes encoding sarcomeric



**Table 2** Patients with cardiomyopathy due to 2 variants in the MYBPC3 gene<sup>a</sup>

MYBPC3/variant 1	MYBPC3/variant 2	Phenotype	Structural	Severity	Age at first study	Reference
<i>Compound heterozygous or homozygous variants in MYBPC3</i>						
c.2618C>A p.(Pro873His)	c.2618C>A p.(Pro873His)	HCM		Moderate/severe	27 years	Nanni <i>et al</i> <sup>21</sup>
c.2429G>A p.(Arg810His)	c.2429G>A p.(Arg810His)	HCM		Severe	39 years	Nanni <i>et al</i> <sup>21</sup>
c.1504C>T p.(Arg502Trp)	c.13G>C p.(Gly5Arg) <sup>b</sup>	HCM		c	d	Van Driest <i>et al</i> <sup>15</sup>
c.2864_2865del p.(Pro955fs)	c.772G>A p.(Glu258Lys)	HCM		c	d	Van Driest <i>et al</i> <sup>15</sup>
c.1880C>T p.(Ala627Val)	c.1880C>T p.(Ala627Val)	HCM		Severe	47 years	Garcia-Castro <i>et al</i> <sup>22</sup>
c.2618C>A p.(Pro873His)	c.2234A>G p.(Asp745Gly)	HCM		Severe	29 years	Ingles <i>et al</i> <sup>23</sup>
c.1624G>C p.(Glu542Gln)	c.2552C>T p.(Ala851Val)	HCM		Severe	34 years	Ingles <i>et al</i> <sup>23</sup>
c.226C>T p.(Gln76*)	c.770A>C p.(His257Pro)	HCM		Mild symptoms	24 years	Richard <i>et al</i> <sup>14</sup>
c.226C>T p.(Gln76*)	c.770A>C p.(His257Pro)	Childhood HCM		Mild symptoms	14 years	Richard <i>et al</i> <sup>14</sup>
c.3083C>G p.(Thr1028Ser)	c.3490+2T>G	Childhood HCM			Before 15 years	Morita <i>et al</i> <sup>f</sup>
c.1504C>T p.(Arg502Trp)	c.2573G>A p.(Ser858Asn)	Childhood HCM			Before 15 years	Morita <i>et al</i> <sup>f</sup>
c.461T>C p.(Ile154Thr)	c.1814_1816del p.(Asp605del)	Childhood HCM			Before 15 years	Morita <i>et al</i> <sup>f</sup>
c.1091+1G>T	c.2504G>T p.(Arg835Leu) <sup>e</sup>	HCM		Moderate	43 years	Otsuka <i>et al</i> <sup>24</sup>
c.1777del p.(Ser593fs)	c.3370T>C p.(Cys1124Arg) <sup>e</sup>	Childhood HCM		Severe	17 years	Otsuka <i>et al</i> <sup>24</sup>
c.3776del p.(Gln1259fs)	c.3599T>C p.(Leu1200Pro)	Neonatal HCM/LVNC		9 weeks <sup>†</sup>	11 days	Dellefave <i>et al</i> <sup>25</sup>
c.2234A>G p.(Asp745Gly)	c.2618C>A p.(Pro873His) <sup>e</sup>	HCM		Severe	35 years	Maron <i>et al</i> <sup>20</sup>
c.1624G>C p.(Glu542Gln)	c.2552C>T p.(Ala851Val) <sup>e</sup>	HCM		Severe	41 years	Maron <i>et al</i> <sup>20</sup>
c.2234A>G p.(Asp745Gly)	c.2618C>A p.(Pro873His) <sup>e</sup>	None			43 years	Maron <i>et al</i> <sup>20</sup>
c.3794A>T p.(Glu1265Val) <sup>d</sup>	c.3796T>C p.(Cys1266Arg) <sup>e</sup>	HCM		Severe	46 years	Maron <i>et al</i> <sup>20</sup>
c.2905C>T p.(Gln969*)	c.2003G>A p.(Arg668His) <sup>f</sup>	HCM		Moderate	48 years	Girolami <i>et al</i> <sup>26</sup>
c.772G>A p.(Glu258Lys)	c.805-1G>A	Neonatal HCM		1 month <sup>†</sup>	2 days	Marziliano <i>et al</i> <sup>27</sup>
c.772G4A p.(Glu258Lys)	c.805-1G4A	Neonatal HCM		2 days <sup>†</sup>	2 days	Marziliano <i>et al</i> <sup>27</sup>
c.1880C>T p.(Ala627Val)	c.1880C>T p.(Ala627Val)	Childhood HCM		Severe	16 years	Garcia-Castro <i>et al</i> <sup>28</sup>
c.1504C>T p.(Arg502Trp)	c.2573G>A p.(Ser858Asn)	Childhood HCM		Moderate	6 years	Saltzman <i>et al</i> <sup>29</sup>
c.1504C>T p.(Arg502Trp)	c.1624G>C p.(Glu542Gln)	Childhood HCM		Severe, SCD	5 years	Saltzman <i>et al</i> <sup>29</sup>
c.1504C>T p.(Arg502Trp)	c.1624G>C p.(Glu542Gln)	Childhood HCM		Severe	2 years	Saltzman <i>et al</i> <sup>29</sup>
c.1504C>T p.(Arg502Trp)	c.442G>A p.(Gly148Arg)	Childhood HCM		Severe	12 years	Saltzman <i>et al</i> <sup>29</sup>
c.1504C>T p.(Arg502Trp)	c.442G>A p.(Gly148Arg)	Childhood HCM		10 years <sup>†</sup>	6 years	Saltzman <i>et al</i> <sup>29</sup>
c.1504C>T p.(Arg502Trp)	c.442G>A p.(Gly148Arg)	Childhood HCM		Moderate	8 years	Saltzman <i>et al</i> <sup>29</sup>
c.3628-41_3628-17del	c.3656T>C p.(Leu1219Pro)	HCM		Moderate	68 years	Bashyam <i>et al</i> <sup>30</sup>
c.456del p.(Ile154fs)	c.2128G>A p.(Glu710Lys)	HCM		Moderate	40 years	Bashyam <i>et al</i> <sup>30</sup>
c.772+5G>A <sup>g</sup>	c.772+5G>A <sup>g</sup>	HCM		Severe	28 years	Ortiz <i>et al</i> <sup>34</sup>
c.772+5G>A <sup>g</sup>	c.772+5G>A <sup>g</sup>	HCM		Severe	34 years	Ortiz <i>et al</i> <sup>34</sup>
<i>Double-truncating mutations in MYBPC3</i>						
c.226C>T p.(Gln76*)	c.226C>T p.(Gln76*)	Neonatal HCM		9 months <sup>†</sup>	?	Richard <i>et al</i> <sup>14</sup>
c.1624+1G>A	c.2373dup p.(Trp792fs)	Neonatal HCM		5 weeks <sup>†</sup>	3 days	Lekanne Deprez <i>et al</i> <sup>32</sup>
c.2827C>T p.(Arg943*)	c.3288del p.(Glu1096fs)	Neonatal HCM	VSD	6 weeks <sup>†</sup>	2 weeks	Lekanne Deprez <i>et al</i> <sup>32</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM	VSD	6 weeks <sup>†</sup>	<4 weeks	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM		7 months <sup>†</sup>	?	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM	VSD	Severe	Newborn	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	HCM		Severe	3 months	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM	VSD	5 weeks <sup>†</sup>	Newborn	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	HCM		Severe	5 months	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	HCM		Severe	2 months	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM		6 months <sup>†</sup>	3 weeks	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM	PDA, ASD	4 months <sup>†</sup>	2 weeks	Zahka <i>et al</i> <sup>33</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM	VSD	Severe	Newborn	Zahka <i>et al</i> <sup>33</sup>
c.2827C>T p.(Arg943*)	c.2827C>T p.(Arg943*)	HCM/skeletal myopathy		Severe	8 weeks	Tajsharghi <i>et al</i> <sup>35</sup>
c.3330+2T>G	c.3330+2T>G	Neonatal HCM	VSD	1 weeks-10 <sup>†</sup> months	Newborn-3 weeks	Xin <i>et al</i> <sup>31</sup> (3 patients)
c.2827C>T p.(Arg943*)	c.2373dup p.(Trp792fs)	HCM	ASD	12 weeks <sup>†</sup>	5 weeks	This study
c.2827C>T p.(Arg943*)	c.2373dup p.(Trp792fs)	Neonatal HCM	VSD	12 weeks <sup>†</sup>	4 weeks	This study
c.2373dup p.(Trp792fs)	c.2373dup p.(Trp792fs)	HCM	OFO	12 weeks <sup>†</sup>	7 weeks	This study
c.2827C>T p.(Arg943*)	c.2827C>T p.(Arg943*)	HCM	ASD	7 weeks <sup>†</sup>	6 weeks	This study

Abbreviations: ASD, atrial septal defect; OFO, open foramen ovale; PDA, patent ductus arteriosus; SCD, sudden cardiac death; VSD, ventricular septal defect. Mutations in italic are functional O-alleles.  
<sup>a</sup>The pathogenicity of some of the missense variants listed here is uncertain.

<sup>b</sup>The mean allele frequency of this variant is 0.1%; therefore, this is probably a polymorphism.

<sup>c</sup>More severe HCM and a higher incidence of myectomy compared with patients with single pathogenic MYBPC3 mutations.<sup>15</sup>

<sup>d</sup>Diagnosis at a younger age (between 0.2 and 37.4 yrs) compared with patients with single pathogenic MYBPC3 mutations.<sup>15</sup>

<sup>e</sup>Not clear whether these variants are in *cis* or in *trans*.

<sup>f</sup>A third variant in MYH7 was found.

<sup>g</sup>c.772+5G>A (IVS6+5G>A) is an intronic variant not affecting the canonical consensus splice site. Given the later age of onset, this is probably not a truncating mutation, but a hypomorphic allele leading to less efficient normal splicing. Consistent with this notion both patients had no septal defect.

<sup>†</sup>Deceased.

proteins, including *MYH7*, *ACTC1*, and *TNNT2*.<sup>9,12</sup> This study confirms our previous results demonstrating that pathogenic variants in another sarcomeric protein gene, *MYBPC3*, can also lead to LVNC (OMIM number #615396, LVNC10).<sup>9,13</sup> *MYBPC3* mutations in HCM lead to an altered primary contractile function. Whether contractile dysfunction is the mechanism that links mutant sarcomere protein to the morphologic features of LVNC is still uncertain.<sup>13</sup>

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. In contrast, our four patients were compound heterozygotes (two patients) or homozygous (two patients) for two truncating *MYBPC3* variants, suggesting a cumulative effect. Pathogenic mutations in the *MYBPC3* gene are one of the most common genetic causes of HCM in many populations, found in 20–40% of individuals with HCM.<sup>14,15</sup> Autosomal dominant variants in the *MYBPC3* gene, which are mostly truncating pathogenic mutations and sometimes missense variants, give rise to HCM with an age of onset after the third decade, moderate left ventricular hypertrophy, and a favorable prognosis.<sup>16</sup> As variants in *MYBPC3* (and *MYH7*) are the most common genetic cause of familial HCM,<sup>6</sup> compound heterozygous or homozygous variants should be considered in a neonate who presents with severe HCM or LVNC, even in the absence of symptoms in family members. This is illustrated by patients 1 and 2 who were compound heterozygotes for the two most common Dutch pathogenic founder mutations for HCM: c.2373dup p.(Trp792fs) and c.2827C>T p.(Arg943\*).<sup>10,11</sup> Patients 3 and 4 were homozygous for the c.2373dup p.(Trp792fs) and c.2827C>T p.(Arg943\*) mutations, respectively.

The pathogenic c.2373dup p.(Trp792fs) mutation accounts for approximately one-fifth of all HCM cases in the Netherlands. It is an important founder mutation in the Dutch HCM population and is also present in other populations.<sup>10,11,14</sup> It creates a new aberrant splice donor site leading to skipping of exon 24, resulting in a frameshift after p.Gln791 and a premature stop codon.<sup>17</sup> No truncated protein product from the c.2373dup p.(Trp792fs) allele could be detected in the sarcomere-using antibodies, suggesting that the truncated protein was unstable, or the aberrant transcript was degraded by cell surveillance mechanisms such as nonsense-mediated decay.<sup>18</sup>

The c.2827C>T p.(Arg943\*) nonsense mutation was found in three of our patients and is located in exon 27, leading to a premature stop codon and protein truncation beyond domain C7 of *MYBPC3*. It is thought to lead to reduction in *MYBPC3* owing to protein instability and/or loss of the C-terminus of *MYBPC3* that binds myosin thick filaments and titin, which is required to incorporate *MYBPC3* normally into the A-band of the sarcomere.<sup>19</sup> Consequently, the *MYBPC3* protein was not expected to be incorporated into the sarcomere in any of these four patients. This may explain the early and severe presentation of their cardiomyopathy.

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.<sup>14,15</sup> However, most of these are missense variants and their pathogenic nature is not always easy to establish. In these adult cases, a more severe HCM phenotype is generally seen, characterized by an earlier age of onset around the second decade or during childhood (Table 2).<sup>6,14,15,20–30</sup> In a recent study on sarcomeric protein gene variants in childhood-onset HCM, 6 out of 84 children (7%) had compound variants.<sup>6</sup> This suggests that a gene–dosage effect might be responsible for manifestations at a younger age. As Table 2 shows, all patients with two functional null alleles died within the first year of life. Patients with a

missense variant and a truncating pathogenic mutation, or patients with two missense variants in *MYBPC3*, had a milder phenotype and an onset of cardiomyopathy at a more advanced age. This might be explained by some residual activity of the *MYBPC3* protein.

All cases with biallelic truncating *MYBPC3* mutations present as neonates. Nevertheless, we do expect prenatal onset of HCM in these patients. One of the reasons that prenatal presentation has not yet been reported, could be that prenatal ultrasounds in the Netherlands, as well as in most other European countries, are not performed at a regular basis after 22 weeks of gestation. Also, mutation analysis of sarcomeric genes is not always performed in pregnancies of fetuses with cardiomyopathy, and some of these fetuses might die before a molecular diagnosis can be made. In addition, prenatal circulation is different from postnatal circulation, after the fetus is disconnected from the umbilical vessels. Owing to the loss of tremendous blood flow through the placenta, the systemic vascular resistance at birth doubles. As resistance rises, aortic and left ventricular pressure increase. The neonatal heart adapts (remodels) to sudden increased systolic pressures following birth, by increasing ventricular wall thickness and stiffness (ie tensile strength). This is a result of a twofold increase in the number of fibroblasts and the formation, compaction, and alignment of collagen fibrils that envelop myocytes. We hypothesize that absence of *MYBPC3* protein would impair this process, leading to HCM with features of noncompaction in neonates.

Few neonatal cases with severe cardiomyopathy owing to homozygous or compound heterozygous truncating pathogenic mutations in *MYBPC3* have been described.<sup>14,31–35</sup> A homozygous truncating pathogenic splice site variant c.3330+2T>G in *MYBPC3* was reported in three neonates with severe HCM who all died at an average age of 3–4 months in a consanguineous Old Order Amish pedigree with severe HCM.<sup>31</sup> This variant was also reported in another cohort of 10 neonates with severe infantile HCM of Old Order Amish descent, suggesting a founder effect.<sup>33</sup> Of note, several of the affected Amish neonates with homozygous *MYBPC3* truncating variants also presented with septal defects including apical muscular VSD, ASD, and patent ductus arteriosus. Septal defects were also present in the neonates with severe HCM owing to compound heterozygous truncating pathogenic mutations described by Lekanne *et al*<sup>32</sup> and in three of our cases. Different congenital heart malformations (septal defects, patent ductus arteriosus, aortic aneurysm, and Ebstein anomaly) have been reported in families with pathogenic mutations in sarcomeric protein genes, including *MYBPC3*, *MYH6*, *MYH7*, *MYH11*, and *ACTC1*.<sup>31,36–38</sup> In children with noncompaction cardiomyopathy, Tsai *et al*<sup>39</sup> showed that 78% had a congenital heart defect. These data suggest that sarcomeric cardiac muscle proteins are not only involved in cardiomyopathies but also in congenital heart malformations.<sup>40</sup> Table 2 shows that only patients with double-truncating pathogenic mutations have a septal defect, suggesting that functional *MYBPC3* protein has a crucial role in septal development. Septal defects have not been reported in patients with missense variants, or with a missense variant in combination with a truncating variant. In conclusion, the absence of functional *MYBPC3* from the sarcomere can lead to a phenotype of severe HCM with features of ventricular noncompaction and septal defects, which appears to be lethal in the postnatal period.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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- 1 Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE: Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. *Circulation* 1995; **92**: 785–789.
- 2 Alcalai R, Seidman JG, Seidman CE: Genetic basis of hypertrophic cardiomyopathy: from bench to the clinics. *J Cardiovasc Electrophys* 2008; **19**: 104–110.
- 3 Bos JM, Ommen SR, Ackerman MJ: Genetics of hypertrophic cardiomyopathy: one, two, or more diseases?. *Curr Opin Cardiol* 2007; **22**: 193–199.
- 4 Lopes LR, Rahman MS, Elliott PM: A systematic review and meta-analysis of genotype-phenotype associations in patients with hypertrophic cardiomyopathy caused by sarcomeric protein mutations. *Heart* 2013; **99**: 1800–1811.
- 5 Daw EW, Chen SN, Czernuszewicz G *et al*: Genome-wide mapping of modifier chromosomal loci for human hypertrophic cardiomyopathy. *Hum Molec Genet* 2007; **16**: 3463–3471.
- 6 Morita H, Rehm HL, Menesses A *et al*: Shared genetic causes of cardiac hypertrophy in children and adults. *N Engl J Med* 2008; **358**: 1899–1908.
- 7 Grenier MA, Osganian SK, Cox GF *et al*: Design and implementation of the North American Pediatric Cardiomyopathy Registry. *Am Heart J* 2000; **139**: S86–S95.
- 8 Jenni R, Oechslin EN, van der Loo B: Isolated ventricular non-compaction of the myocardium in adults. *Heart* 2007; **93**: 11–15.
- 9 Hoedemaekers YM, Caliskan K, Michels M *et al*: The importance of genetic counseling, DNA diagnostics, and cardiologic family screening in left ventricular noncompaction cardiomyopathy. *Circ Cardiovasc Genet* 2010; **3**: 232–239.
- 10 Alders M, Jongbloed R, Deelen W *et al*: The 2373insG mutation in the MYBPC3 gene is a founder mutation, which accounts for nearly one-fourth of the HCM cases in the Netherlands. *Eur Heart J* 2003; **24**: 1848–1853.
- 11 Christiaans I, Nannenbergh EA, Dooijes D *et al*: Founder mutations in hypertrophic cardiomyopathy patients in the Netherlands. *Neth Heart J* 2010; **18**: 248–254.
- 12 Klaassen S, Probst S, Oechslin E *et al*: Mutations in sarcomere protein genes in left ventricular noncompaction. *Circulation* 2008; **117**: 2893–2901.
- 13 Probst S, Oechslin E, Schuler P *et al*: Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. *Circ Cardiovasc Genet* 2011; **4**: 367–374.
- 14 Richard P, Charron P, Carrier L *et al*: Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003; **107**: 2227–2232.
- 15 Van Driest SL, Vasile VC, Ommen SR *et al*: Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2004; **44**: 1903–1910.
- 16 Niimura H, Bachinski LL, Sangwatanaroj S *et al*: Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. *N Engl J Med* 1998; **338**: 1248–1257.
- 17 Moolman JA, Reith S, Uhl K *et al*: A newly created splice donor site in exon 25 of the MyBP-C gene is responsible for inherited hypertrophic cardiomyopathy with incomplete disease penetrance. *Circulation* 2000; **101**: 1396–1402.
- 18 van Dijk SJ, Dooijes D, dos Remedios C *et al*: Cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy: haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. *Circulation* 2009; **119**: 1473–1483.
- 19 Rottbauer W, Gautel M, Zehelein J *et al*: Novel splice donor site mutation in the cardiac myosin-binding protein-C gene in familial hypertrophic cardiomyopathy. Characterization Of cardiac transcript and protein. *J Clin Invest* 1997; **100**: 475–482.
- 20 Maron BJ, Maron MS, Semsarian C: Double or compound sarcomere mutations in hypertrophic cardiomyopathy: a potential link to sudden death in the absence of conventional risk factors. *Heart Rhythm* 2012; **9**: 57–63.
- 21 Nanni L, Pieroni M, Chimenti C *et al*: Hypertrophic cardiomyopathy: two homozygous cases with "typical" hypertrophic cardiomyopathy and three new mutations in cases with progression to dilated cardiomyopathy. *Biochem Biophys Res Comm* 2003; **309**: 391–398.
- 22 Garcia-Castro M, Reguero JR, Alvarez V *et al*: Hypertrophic cardiomyopathy linked to homozygosity for a new mutation in the myosin-binding protein C gene (A627V) suggests a dosage effect. *Int J Cardiol* 2005; **102**: 501–507.
- 23 Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, Semsarian C: Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. *J Med Genet* 2005; **42**: e59.
- 24 Otsuka H, Arimura T, Abe T *et al*: Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy. *Circ J* 2012; **76**: 453–461.
- 25 Dellefave LM, Pytel P, Mewborn S *et al*: Sarcomere mutations in cardiomyopathy with left ventricular hypertrabeculation. *Circ Cardiovasc Genet* 2009; **2**: 442–449.
- 26 Girolami F, Ho CY, Semsarian C *et al*: Clinical features and outcome of hypertrophic cardiomyopathy associated with triple sarcomere protein gene mutations. *J Am Coll Cardiol* 2010; **55**: 1444–1453.
- 27 Marziliano N, Merlini PA, Vignati G *et al*: A case of compound mutations in the MYBPC3 gene associated with biventricular hypertrophy and neonatal death. *Neonatology* 2012; **102**: 254–258.
- 28 Garcia-Castro M, Coto E, Reguero JR *et al*: [Mutations in sarcomeric genes MYH7, MYBPC3, TNNT2, TNNI3, and TPM1 in patients with hypertrophic cardiomyopathy]. *Rev Esp Cardiol* 2009; **62**: 48–56.
- 29 Saltzman AJ, Mancini-DiNardo D, Li C *et al*: Short communication: the cardiac myosin binding protein C Arg502Trp mutation: a common cause of hypertrophic cardiomyopathy. *Circ Res* 2010; **106**: 1549–1552.
- 30 Bashyam MD, Purushotham G, Chaudhary AK *et al*: A low prevalence of MYH7/MYBPC3 mutations among familial hypertrophic cardiomyopathy patients in India. *Molec Cell Biochem* 2012; **360**: 373–382.
- 31 Xin B, Puffenberger E, Tumbush J, Bockoven JR, Wang H: Homozygosity for a novel splice site mutation in the cardiac myosin-binding protein C gene causes severe neonatal hypertrophic cardiomyopathy. *Am J Med Genet A* 2007; **143A**: 2662–2667.
- 32 Lekanne Deprez RH, Muurling-Vlietman JJ, Hruza J *et al*: Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the MYBPC3 gene. *J Med Genet* 2006; **43**: 829–832.
- 33 Zahka K, Kalidas K, Simpson MA *et al*: Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency amongst the Amish. *Heart* 2008; **94**: 1326–1330.
- 34 Ortiz MF, Rodriguez-Garcia MI, Hermida-Prieto M *et al*: A homozygous MYBPC3 gene mutation associated with a severe phenotype and a high risk of sudden death in a family with hypertrophic cardiomyopathy. *Rev Esp Cardiol* 2009; **62**: 572–575.
- 35 Tajsharghi H, Leren TP, Abdul-Hussein S *et al*: Unexpected myopathy associated with a mutation in MYBPC3 and misplacement of the cardiac myosin binding protein C. *J Med Genet* 2010; **47**: 575–577.
- 36 Ching YH, Ghosh TK, Cross SJ *et al*: Mutation in myosin heavy chain 6 causes atrial septal defect. *Nat Genet* 2005; **37**: 423–428.
- 37 Zhu L, Vranckx R, Khau Van Kien P *et al*: Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. *Nat Genet* 2006; **38**: 343–349.
- 38 Matsson H, Eason J, Bookwalter CS *et al*: Alpha-cardiac actin mutations produce atrial septal defects. *Hum Molec Genet* 2008; **17**: 256–265.
- 39 Tsai SF, Ebenroth ES, Hurwitz RA, Cordes TM, Schamberger MS, Batra AS: Is left ventricular noncompaction in children truly an isolated lesion?. *Pediatr Cardiol* 2009; **30**: 597–602.
- 40 Wessels MW, Willems PJ: Mutations in sarcomeric protein genes not only lead to cardiomyopathy but also to congenital cardiovascular malformations. *Clin Genet* 2008; **74**: 16–19.