A Case of Compound Mutations in the *MYBPC3* Gene Associated with Biventricular Hypertrophy and Neonatal Death

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Key Words

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Abstract

Hypertrophic cardiomyopathy (HCM) is a familial, genetically determined, primary cardiomyopathy caused by mutations in genes coding for proteins of the sarcomere, or, less frequently, genes involved in storage diseases. In pediatric settings, pure HCM has an estimated incidence of 4.7 per million children. The disease is often sub-clinical and goes unrecognized mainly because most patients with HCM have only mild symptoms, if any. However, sudden cardiac death, the most dramatic clinical occurrence and the primary concern for patients and physicians alike, may be the first manifestation of the disease. We describe a case of compound heterozygosity in the *MYBPC3* gene (p.Glu258Lys and IVS25-1G>A) associated with biventricular hypertrophy, atrial enlargement and subsequent neonatal death 33 days postpartum. Other studies have reported compound and/or double heterozygosity in the same or different sarcomeric genes during childhood and adulthood, and neonatal presentations

have also been described. Our observations show that the combination of a missense (p.Glu258Lys) and a splice-site mutation (IVS25-1G>A) profoundly affects the clinical course. In families in which parental mutations are known, preimplantation (where ethically and legally feasible) or prenatal genetic screening should be adopted because: (1) neonatal HCM in genetic heterozygosity is potentially lethal and (2) heart disease is the most common developmental malformation and the leading cause of neonatal mortality and morbidity.

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Hypertrophic cardiomyopathy (HCM) is a familial, genetically determined, primary cardiomyopathy (CMH1 to CMH15; www.ncbi.nlm.nih.gov/omim) caused by mutations in genes coding for proteins of the sarcomere, or, less frequently, genes involved in storage diseases (e.g. Anderson-Fabry's, Pompe's and Hurler's disease), or even mtDNA [1]. In pediatric settings, pure HCM has an estimated incidence of 4.7 per million children [2]. The disease is often sub-clinical and goes unrecognized mainly because most patients with HCM have only mild symp-

Fig. 1. a Family pedigree and genotypes of the index case (II:3; red arrow). Top left: the missense mutation p.Glu258Lys (E258K) spanning exons 7 and 8. Top right: the mutation affecting the splice site across intron 25 and exon 26 (IVS25-1G>A). **b** Echocardiogram of II:3 showing massive hypertrophy of the LV. **c** ECG showing sinus rhythm, normal A-V and intraventricular conduc-

tion, and biventricular hypertrophy (high QRS voltages with negative T on precordial leads and positive T on V1). **d** Left to right: explanted heart (autopsy) showing massive left (1), right (2) and septum hypertrophy (*; 13 mm); hematoxylin-eosin (EE) staining did not reveal any cardiomyocyte disarray. ×10 (color only in online version).

toms if any. However, sudden cardiac death (SCD), the most dramatic clinical occurrence and the primary concern for patients and physicians alike, may be the first manifestation of the disease. HCM is the most common discernible cause of SCD in competitive athletes younger than 35 years of age, but, in absolute terms, SCD is rare in HCM patients and annual cardiac mortality is as low as 1% per year [3].

Classic (sarcomeric) HCM is inherited in an autosomal dominant manner, although recessive inheritance has been documented [4]. Mutations in the *MYBPC3* gene are among the most common causes of HCM, and often lead to milder phenotypes with presentation in adult life [3]. Neonatal presentations of pure HCM are very rare and often associated with other syndromic features (such as Noonan's syndrome). Moreover, the prev-

Table 1. Clinical features of the proband (II:3) and her relatives

Individual	Sex	Relation to index case	Genotype		Age at diagnosis	Echocardiographic features						
			1	2		evidence of HCM	maximum LV thickness mm	site	ECG findings	symptoms	other	events/outcome
II:3 ^b	F	–	+/-	+/-	2 days	yes	6	septum	LV hypertrophy	respiratory distress, LV hypokinesia and trigonocephaly		death
I:1	M	father	+/-	-/-	24 years	yes	18	septum	diffuse T wave	NYHA II	competitive sports	none
I:2	F	mother	-/-	+/-	23 years	yes	16	septum/ LVPW	atrial conduction delay	NYHA II	competitive sports	none
II:1	F	sister	+/-	-/-	–	no	–	–	–	–	–	none
II:2	M	brother	+/- ^a	+/- ^a	2 days	yes	7	septum	giant P waves	cyanosis	–	death

Genotype 1: p.Glu258Lys (E258K). Genotype 2: IVS25-1G>A. LVPW = LV posterior wall; NYHA = New York Heart Association classification of the clinical symptoms (II equals to mild shortness of breath and/or angina and slight limitation during ordinary activity).

^a Molecular diagnosis conducted postmortem on formalin-fixed paraffin-embedded specimens.

^b At the autopsy of the probanda (II:3), we did not identify any additional extra-cardiac features except for the metopic sutures, particularly: cardiofaciocutaneous, Di George, Holt-Oram, Noonan, Hurler (MPS I H), Hurler/Scheie (MPS I H/S) and San Filippo (MPS III A, B, C and D) syndromes were excluded. Furthermore, by means of routine mass spectrometry screening, storage diseases were also excluded.

Table 2. Neonatal presentation of HCM due to compound heterozygosity of the *MYBPC3* gene

Patient(s)	Mutation 1	Mutation 2	Age at onset	Age at death days	Phenotype	Reference
23	c.3330+2T>C (IVS30+2T>C)	c.3330+2T>C (IVS30+2T>C)	at birth	7–319	no significant dysmorphic features (3 out of 23 showed premature metopic crest fusion); out of 23 patients, 1/3 died of respiratory distress, the remaining 2/3 presented to primary care during the first 3 weeks of life with poor feeding, excessive sweating during feeding, lethargy, difficulty with breathing, intermittent perioral cyanosis, cardiomegaly on X-rays and ECG revealed nonobstructive HCM with ventricular dysfunction in the RV or LV or in both	Xin et al. [9]
1	c.1624+1G>A (p.Trp486X?)	c.2373_2374insG (p.Trp792fsX17)	at birth	35	poor feeding, cyanosis, difficulty with breathing, an enlarged liver at the physical examination and no dysmorphic features, ECG showed abnormal right superior frontal axis deviation, AVB I degree, deep QS complexes in the left precordial leads associated with T waves abnormalities and LVPW 36 mm, IVS 11.8 mm	Lekanne Deprez et al. [7]
1	c.2872C>T (p.Arg943X)	c.3288delA (p.Glu1096fsX92)	at birth	42	poor circulation, soft first heart sound and grade II systolic murmur at the apex, enlarged liver, cardiomegaly on X-rays, ECG showed an axis of +150, giant P waves, flattened T waves and revealed enlarged right atrium, HCM of both ventricles with diminished contractility and mild shunting of the foramen ovale	Lekanne Deprez et al. [7]
1	c.3775delA (p.Asp1259fs)	c.3599T>C (p.Leu1200Pro)	at birth	42	he was delivered by an emergent cesarean section for low heart rate, ECG showed fractional shortening ranging from 6.5 to 22.8%, LV noncompaction, an LV free wall thickness of 14 mm, giant atria and biventricular HCM	Dellefave et al. [10]

Data are from a review of the literature. The number of reported patients, type of mutation, age at onset and outcomes, clinical features and references are given. LVPW = LV posterior wall; RV = right ventricle; IVS = interventricular septum.

absence of compound/double heterozygosity in sarcomeric genes can be as high as 8% (depending on the studied cohorts) [5]. We here describe a case of compound heterozygosity in the *MYBPC3* gene (p.Glu258Lys and IVS25-1G>A) associated with biventricular hypertrophy, atrial enlargement and subsequent neonatal death 33 days postpartum.

The proband (II:3 in fig. 1a) was the third daughter of a young couple, both of whom are affected by HCM: the father carries the p.Glu258Lys mutation and the mother carries IVS25-1G>A. The proband was born in gestational week 38 due to placenta previa and, soon after delivery, showed ventilatory distress that resolved spontaneously. The cardiological workup showed biatrial enlargement, left ventricular (LV) hypertrophy (posterior wall and interventricular septum 6 mm; fig. 1b) with diffuse hypokinesia (ejection fraction 37%), which improved with carvedilol (0.1 mg/kg). Holter monitoring did not reveal any arrhythmia. The patient also showed dysmorphic features (i.e. the presence of prominent metopic crests leading to trigonocephaly). Upon cardiological follow-up, the LV hypertrophy was unchanged and the ejection fraction mildly depressed (40–43%). At the age of 4 weeks, she was hospitalized urgently because of a low-output syndrome secondary to pulmonary infection, and died immediately after admission.

An older sister (II:1) aged 18 months carries the paternal mutation, and a brother (II:2) carrying both mutations (detected at the postmortem examination) presented neonatal HCM (interventricular septum 7 mm and giant P waves) and died of severe respiratory distress 2 days postpartum. The clinical characteristics of the entire family are given in table 1.

Other studies have reported compound and/or double heterozygosity in the same or different sarcomeric genes during childhood and adulthood, with the severity of the phenotype varying with the genetic defects [6], and neonatal presentations have also been described [7]. Our observations show that the combination of a missense (p.Glu258Lys) and a splice-site mutation (IVS25-1G>A) profoundly affects the clinical course: although reported as a missense mutation, the paternal mutation (E258K) spans a splice site affecting exon 7, whereas the maternal mutation is a putative splice acceptor mutation that has not been previously reported and is not included in the public databases. Unfortunately, neither RNA nor protein was available from the patient, but the splicesitefinder (www.genet.sickkid-s.on.ca) study predicted a premature termination codon leading to an insertion of 10 amino acids in the cardio-specific phosphorylation do-

main of the protein (p.G867insX10?). Both mutations probably lead to a condition in which no functional copy of the genes is expressed (null mutations), thus resembling a neonatal phenotype which has been described by Lekanne Deprez et al. [7]. Moreover, we could also hypothesize the presence of an additional mutation in the probanda (II:3) in our study, due to the atypical presentation of the HCM phenotype (i.e. the lack of myocyte disarray at the histology and atrioventricular conduction disease).

Despite the fact that HCM due to a single gene mutation may show phenotypical variability, patients with compound heterozygosity have a neonatal presentation associated with severe phenotypes and poor cardiological outcomes (reviewed in table 2). In families in which parental mutations are known, preimplantation (where ethically and legally feasible) or prenatal genetic screening should be adopted because: (1) neonatal HCM in genetic heterozygosity is potentially lethal and (2) heart disease is the most common developmental malformation and the leading cause of neonatal mortality and morbidity [8].

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References

- 1 Watkins H, Ashrafian H, Redwood C: Inherited cardiomyopathies. *N Engl J Med* 2011; 364:1643–1656.
- 2 Colan SD, Lipshultz SE, Lowe AM, Sleeper LA, Messere J, Cox GF, Lurie PR, Orav EJ, Towbin JA: Epidemiology and cause-specific outcome of hypertrophic cardiomyopathy in children: findings from the Pediatric Cardiomyopathy Registry. *Circulation* 2007;115: 773–781.
- 3 Maron BJ, Casey SA, Hauser RG, Aeppli DM: Clinical course of hypertrophic cardiomyopathy with survival to advanced age. *J Am Coll Cardiol* 2003;42:882–888.
- 4 Zahka K, Kalidas K, Simpson MA, Cross H, Keller BB, Galambos C, Gurtz K, Patton MA, Crosby AH: Homozygous mutation of *MYBPC3* associated with severe infantile hypertrophic cardiomyopathy at high frequency among the Amish. *Heart* 2008;94:1326–1330.

- 5 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.
- 6 Girolami F, Ho CY, Semsarian C, Baldi M, Will ML, Baldini K, Torricelli F, Yeates L, Cecchi F, Ackerman MJ, Olivetto I: Clinical features and outcome of hypertrophic cardiomyopathy associated with triple sarcomere protein gene mutations. *J Am Coll Cardiol* 2010;55:1444–1453.
- 7 Lekanne Deprez RH, Muurling-Vlietman JJ, Hruda J, Baars MJ, Wijnaendts LC, Stolte-Dijkstra I, Alders M, van Hagen JM: Two cases of severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the *MYBPC3* gene. *J Med Genet* 2006;43:829–832.
- 8 Chung ML, Lee BS, Kim EA, Kim KS, Pi SY, Oh YM, Park IS, Seo DM, Won HS: Impact of fetal echocardiography on trends in disease patterns and outcomes of congenital heart disease in a neonatal intensive care unit. *Neonatology* 2010;98:41–46.
- 9 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* 2007;143:2662–2667.
- 10 Dellefave LM, Pytel P, Mewborn S, Mora B, Guris DL, Fedson S, Waggoner D, Moskowitz I, McNally EM: Sarcomere mutations in cardiomyopathy with left ventricular hypertrabeculation. *Circ Cardiovasc Genet* 2009; 2:442–449.