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*Case report*

# Myopathy and hypertrophic cardiomyopathy with selective lysis of thick filaments

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**Abstract.** We present a undescribed condition in a girl who died at 8 years of hypertrophic cardiomyopathy. Muscle and endomyocardial biopsies disclosed a selective loss of thick filaments ultrastructurally. In muscle biopsy histochemical abnormalities of myofibrillar ATPase were confined to type 1 fibres. Gel electrophoresis of muscle homogenate showed no qualitative abnormalities of slow and fast myosin heavy chains (MHC) and light chains, and the amount of the different myosin isozymes was in agreement with histochemical myofibrillar ATPase findings. The pathogenetic mechanisms have not been elucidated in this case but we suspect an abnormality of the $\beta$-cardiac MHC gene, the only gene expressed in the heart and in type 1 skeletal muscle fibres.

**Key words:** Myopathy – Cardiomyopathy – Slow myosin heavy chain

## Introduction

Selective lysis of thick filaments in skeletal muscle has rarely been reported. It has been described occasionally in the muscle biopsy of patients with childhood dermatomyositis (Carpenter et al. 1976), in a child affected by benign congenital hypotonia (Yarom and Shapira 1977), in an adult patient in whom associated factors were shock, hypoxia and acidosis (Sher et al. 1979), and in some patients following status asthmaticus and therapy with steroids (Waclawik et al. 1990; Danon and Carpenter 1991; Hirano et al. 1991).

Here, we present a previously undescribed condition in which selective lysis of thick filaments was observed ultrastructurally in skeletal muscle and myocardium of a child with fatal cardiomyopathy (CMP).

## Case report

This girl was admitted to the Cardiological Department at the age of 8 years with severe signs of cardiac failure. She was the first child, born following a normal pregnancy and by a normal delivery. Motor and intellectual performances were reportedly normal. The parents were not related and were healthy. There was no family history of CMP.

Two months before admission she started to feel precordial pain, malaise and several days later she developed an acute left hemiparesis. The girl was admitted to a paediatric hospital where a diagnosis of severe cardiac failure, hypertrophic CMP and left hemiparesis was made. The stroke was attributed to a thromboembolic process of cardiac origin. Cranial CT scan showed an ischaemic lesion in the right internal capsule. At admission the child showed weight loss (21 kg) and poor general condition with signs of severe cardiac failure: dyspnoea, tachycardia, oedema of the legs and hepatomegaly. Cardiac auscultation demonstrated a gallop rhythm, and a 2/6 systolic murmur on the precordium.

Neurological examination showed an alert and suffering child with a left hemiparesis and left Babinski sign. There was generalized weakness and muscle wasting and standing position was impaired. EMG of the right quadriceps femoris muscle showed a myopathic pattern. Tendon jerks were normally evocable and were increased on the left side. Moderate hepatomegaly was present. Radiography of the thorax showed cardiomegaly.

Echocardiography showed severe enlargment of both atria, moderate concentric hypertrophy (the end-diastolic thickness of interventricular septum and posterior left ventricular wall was 14 mm) and diffuse hypocontractility of the left ventricle (ejection fraction, 45%). Cardiac catheterization showed a low cardiac output (3.2 l/min per $m^2$) with mild pulmonary hypertension (38/20 mmHg and a mean of 26 mmHg) and marked elevation of the early and late diastolic pressure in both ventricles (6–13 mmHg in the right and 13–23 mmHG in the left). Left ventriculogram showed a hypertrophic and hypocontractile left ventricle with no mitral regurgitation.

An extensive virological investigation for adenovirus influenza A and B, parainfluenza 1, 2 and 3, syncytial respiratory viruses, myocoplasma, hepatitis A and B, coxsackie B1, B2, B3, B4, B5, B6, herpes simplex, herpes zoster, and Epstein-Barr viruses showed no significant elevation of antibody levels. Study of lymphocyte subpopulations (OKT3, OKT4, OKT8, NK, B7 and OKT4/OKT8) gave normal results. Serum creatine kinase, lactate dehydrogenase glutamic oxaloacetic acid, lactate and pyruvate were in normal ranges.

A muscle biopsy was performed on the left quadriceps femoris and a endomyocardial biopsy was performed during catheterization.

The girl died 1 month after admission. Consent for autopsy was not obtained.

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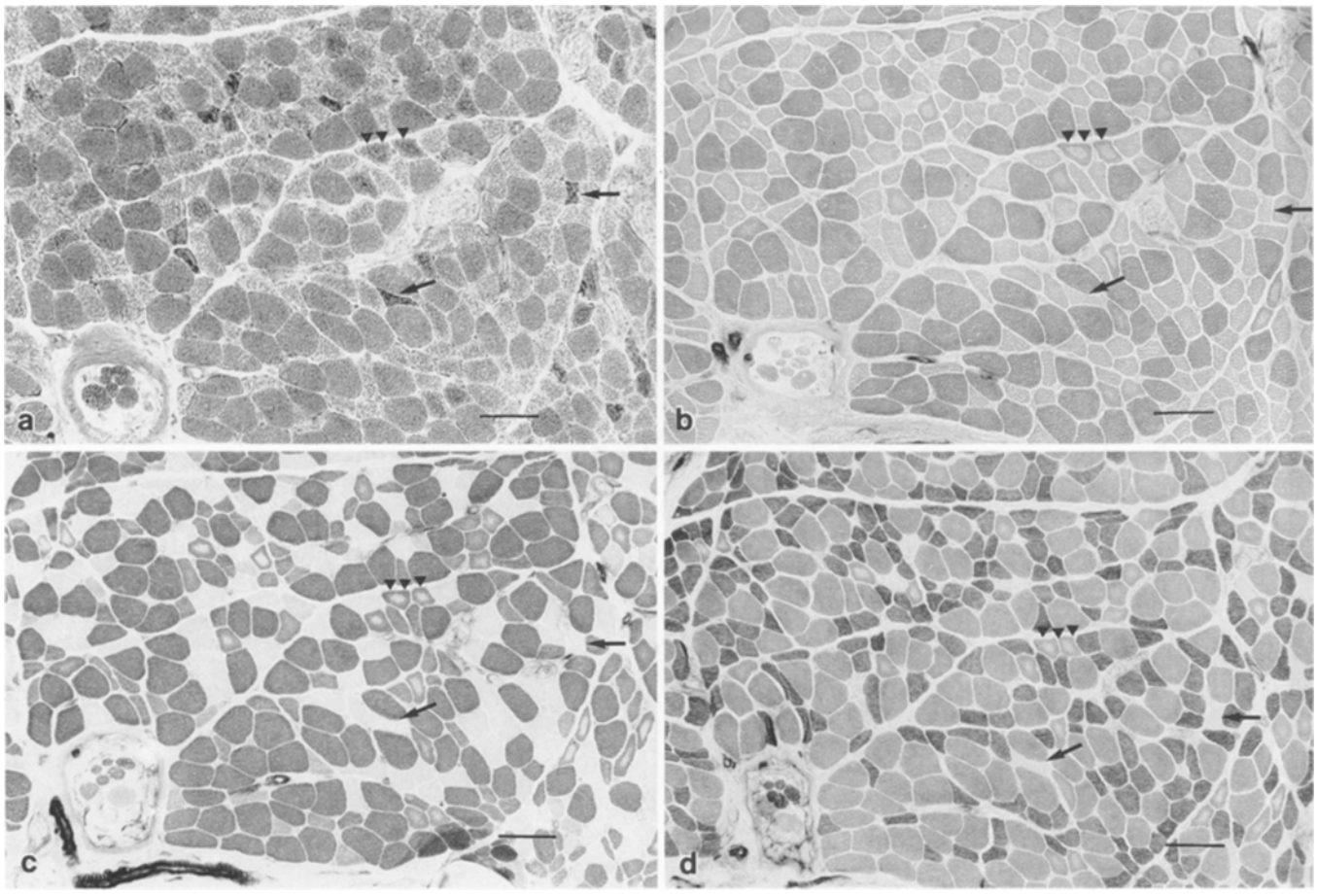


**Fig. 1a–d.** Serial sections of the left quadriceps femoris muscle biopsy. ×126 (original magnification). **a**, **b** Atrophic angulated fibres stain intensively for oxidative enzymes (**a**). These fibres are negative for myofibrillar ATPase pre-incubated at all pH conditions (**b–d**) (arrow). Histochemistry for myofibrillar ATPase at pH 4.3, counterstained with eosin (**b**), pH 4.6 (**c**) and pH 9.6 (**d**) shows that some type 1 fibres do not stain at the centre (*arrowheads*) and correspond to dark fibres in oxidative enzyme histochemistry

## Materials and methods

The muscle biopsy and the endomyocardial biopsy were divided into several parts. One sample of both was quickly frozen in liquid nitrogen-cooled isopentane and 7-μm cryostat sections were stained with standard techniques (Dubowitz 1985); another portion of the muscle specimen was frozen and preserved at −80° C for biochemical studies and the myocardium was fixed in formalin and embedded in paraffin.

Other specimens of heart and skeletal muscle were fixed in phosphate-buffered 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in Epon, counterstained by lead citrate and uranyl acetate and observed with a Zeiss EM10 C electron microscope.

One- and two-dimensional SDS-gel electrophoresis were carried out as previously described (Salviati et al. 1983). Muscle samples of about 10 mg were homogenized with 20 volumes of either SDS (sodium dodecyl sulphate) solution (one-dimensional electrophoresis) or urea solution (two-dimensional electrophoresis). Myosin heavy chains (MHC) were separated on 6% polyacrylamide gels. Total myofibrillar protein and myosin light chains (MLC) were analysed by one-dimensional gel electrophoresis on 10–20% polyacrylamide linear gradient. MLC were stained with silver (6% polyacrylamide gels) or Coomassie briliant blue. Densitometry was performed on one-dimensional SDS gel by using a Shimatzu densitometer.

## Results

Haematoxylin and eosin-stained, 7-μm-thick cryostat sections of the muscle biopsy showed no signs of inflammation, necrosis or fibrosis. There were scattered atrophic angulated fibres with a granular cytoplasm that stained dark purple by modified Gomori's trichrome. These atrophic fibres were strongly PAS-positive and showed high oxidative enzyme activity (Fig. 1a). In other fibres, similar changes were found in the centre.

**Table 1.** Correlation of morphological and biochemical findings

| | [a] Total area (%) | [b] MHC (%) | [b] MLC (%) |
|---|---|---|---|
| Type 1 fibres | 74.7 | 79 | 67.9 |
| Type 2 fibres | 25.3 | 21 | 32.1 |

[a] Area of type 1 and type 2 fibres were measured on ATPase sections at pH 4.3–4.5 and 9.4

[b] Densitometric measurements were done on one-dimensional electrophoretic gels: MHC, myosin heavy chain; MLC, myosin light chain

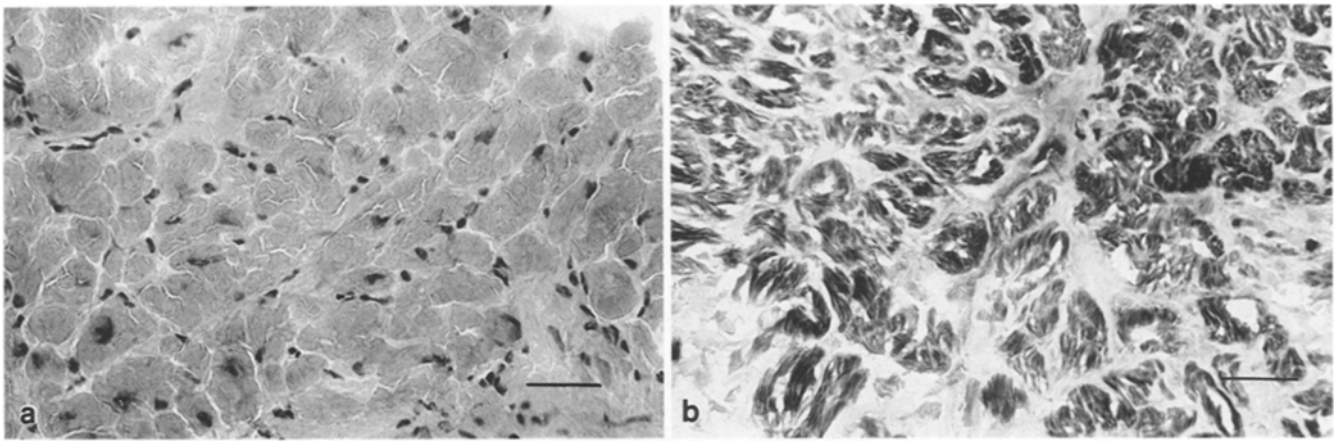


**Fig. 2. a** Endomyocardial biopsy that shows only fibre hypertrophy. Haematoxylin and eosin. **b** Myofibrillar ATPase is normally present in myocytes. ×250 (original magnification)

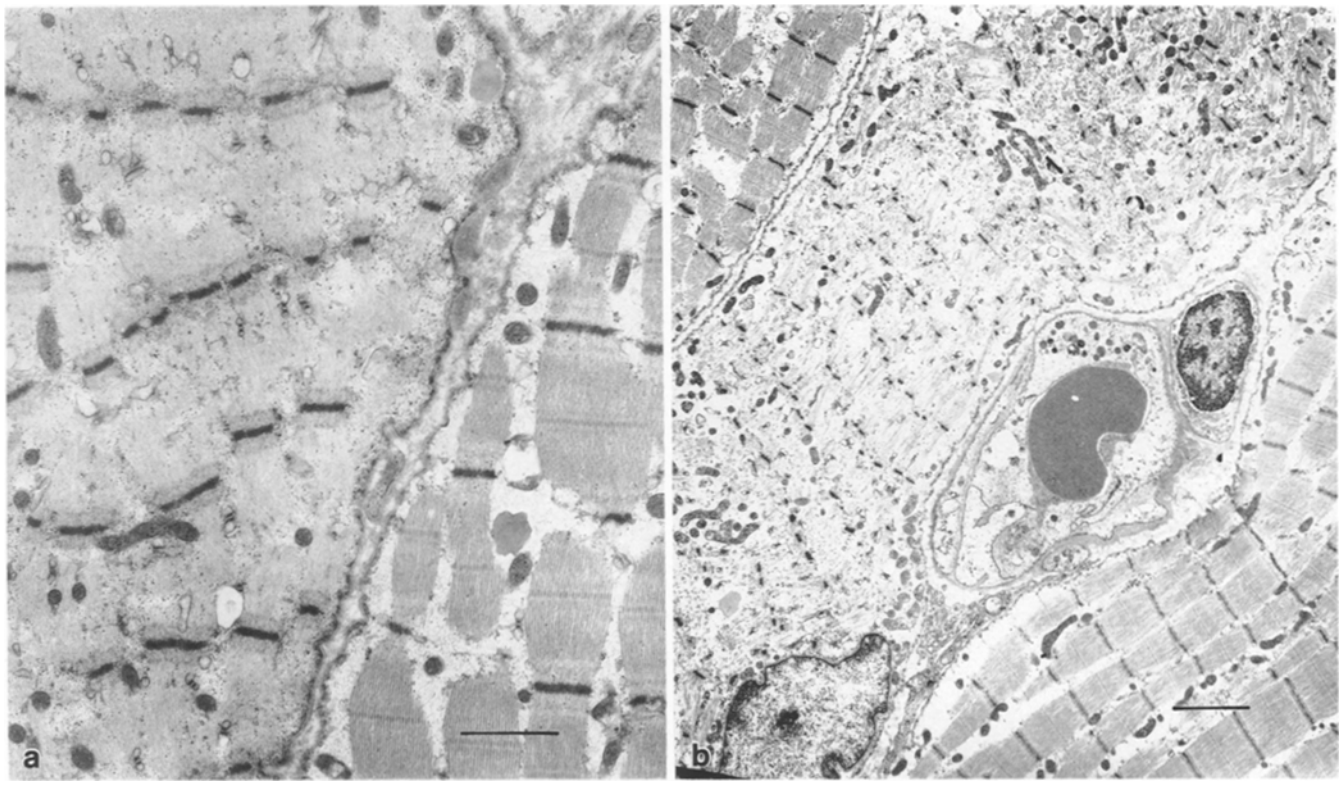


**Fig. 3a, b.** Electron microscopy of skeletal muscle showing selective lysis of thick filaments in one fibre with no appreciable changes in I and Z bands. Compare with a normal fibre on the other side (**a**), ×17800. Some fibres were more severely involved showing atrophy, severe disruption of sarcomers, and increased density of mitochondria and triads (**b**), ×7150

Myofibrillar ATPase staining at different pH conditions showed that type 2 fibres were relatively atrophic due to prolonged immobilization, that some very atrophic fibres were completely unreactive and that 11% of type 1 fibres were pale only at the centre (Fig. 1b–d). As shown in Table 1, total area of type 1 fibres was markedly prevalent (79.7%) compared to type 2 fibres with a relative small calibre.

The myocardium showed hypertrophic fibres with no signs of necrosis, endomyocardial fibrosis or interstitial inflammation (Fig. 2a). Myocytes showed normal myofibrillar ATPase stain at acid and alkaline pH (Fig. 2b) and strong staining for oxidative enzyme activity.

Ultrastructurally, some skeletal muscle fibres showed selective lysis of thick filaments with disappearance of A bands with no appreciable changes of Z lines and I bands (Fig. 3a). Other atrophic fibres showed severe disruption of sarcomeres with increased density of mitochondria and sarcoplasmic reticulum (Fig. 3b). Most of the cardiac myocytes showed loss of thick filaments, myofibrillar disorganization and impressive proliferation of mitochondria (Fig. 4a, b).

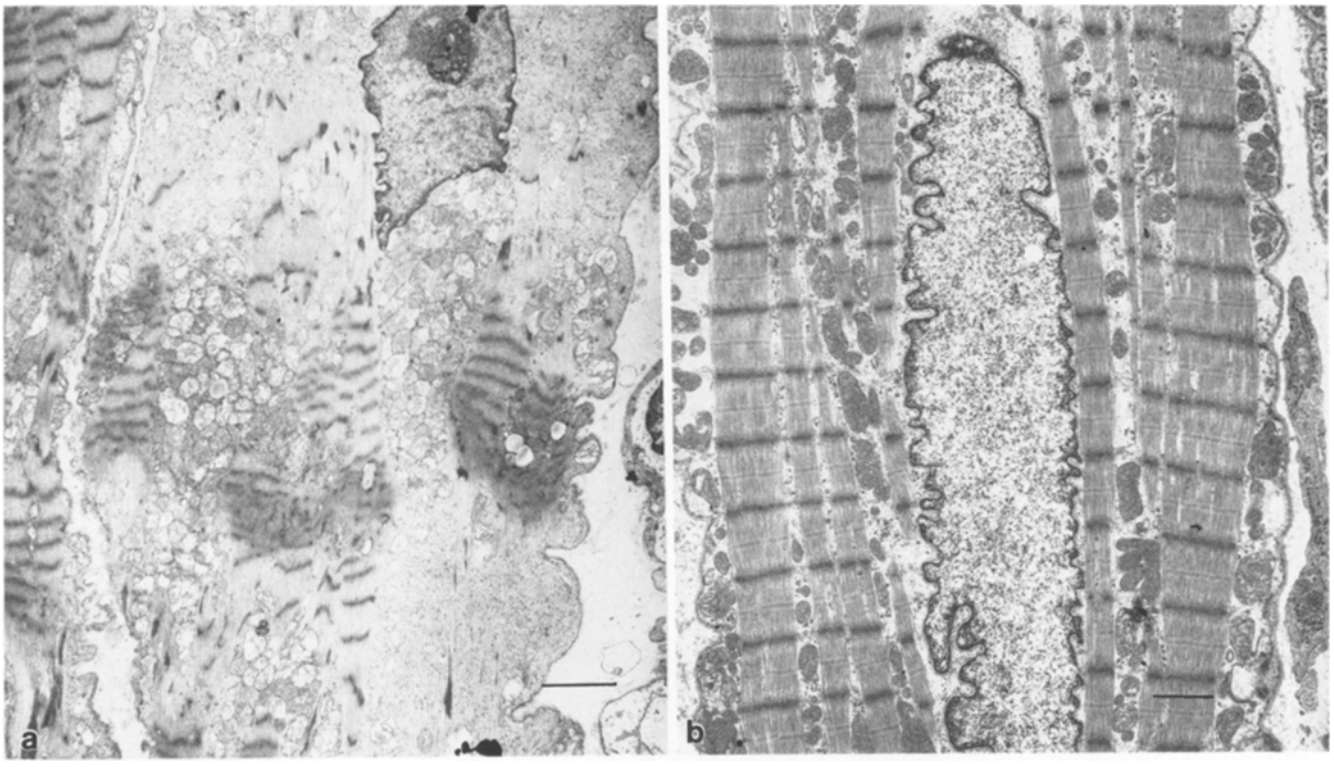


**Fig. 4a, b.** Electron microscopy of endomyocardial biopsy of the patient (**a**; ×7150) and control (**b**; ×11300). Marked proliferation of mitochondria and loss of thick filaments (A bands) in all myocardial fibrils (**a**)

The pattern of MHC in the homogenates from the patient's muscle is shown in Fig. 5. As expected from the results of myofibrillar ATPase histochemistry, three isoforms of MHC (types 1, 2A and 2b) were found in the muscle. Densitometric measurements on six separate lanes at different amounts of protein loaded showed that the percentage of type 1 MHC was 79% and that type 2 (A+B) MHC was 21% of total MHC protein (Table 1). This is in agreement with the results of the histochemical myofibrillar ATPase, which showed a prevalence of type 1 fibres. The area of type 1 fibres was in fact 75% of total muscle cross-sectional area (Table 1). Furthermore, when the pattern of patient muscle homogenate was investigated by two-dimensional SDS-gel electrophoresis (Fig. 5) a prevalence of the slow type of MLC was also found. Densitometry of one-dimensional SDS-gels of the same homogenates showed that the percentage of slow MLC was 68% of total MLC material, in very good agreement with histochemical results (Table 1).

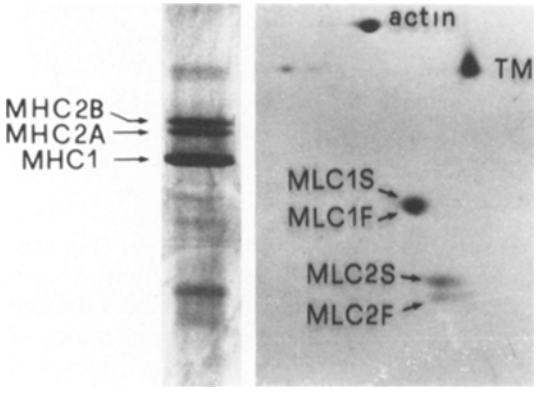


**Fig. 5.** One and two-dimensional electrophoresis (2D-ETF) of myosin proteins in patient. The lane on the left corresponds to one-dimensional electrophoresis and shows the three isoforms of MHC. The column on the right is a 2D-ETF and shows separation of MLC. *s*, Slow; *F*, fast; *1S*, *1F*, *2S*, *2F*, light chain fragments; *MHC*, myosin heavy chain; *MLC*, myosin light chain

## Discussion

Our patient was affected by hypertrophic CMP with characteristic morphological changes in both skeletal muscle and myocardium.

Morphological features included an abnormal reaction for myofibrillar ATPase that was confined to type 1 skeletal muscle fibres and, ultrastructurally, in a selective loss of thick filaments in skeletal muscle fibres and myocytes. In skeletal muscle, selective loss of myosin filaments has been associated with different pathological conditions. In 1976, Carpenter et al. described similar morphological abnormalities in two patients with childhood dermatomyositis. Since then, thick filament degeneration has been reported in congenital myopathies (Yarom and Shapira 1977; Fidzianzka et al. 1981), in an adult patient with acute-onset reversible disease characterized by macular rash, respiratory failure, generalized muscle weakness, stupor, acidosis, and hypoxia as well

as in a few patients affected by status asthmaticus who were treated with high doses of steroids (Waclawik et al. 1990; Danon and Carpenter 1991; Hirano et al. 1991). CMP has never been associated with these conditions.

We were unable to identify aetiological factors in our patient. The disease seemed to have an acute onset at age 8 years and the leading clinical manifestation was severe heart failure due to CMP. Pathogenetic mechanisms such as ischaemia or hypoxia, and experimental models of focal loss of contractile skeletal muscle proteins do not provoke a selective loss of myosin filaments (Sher et al. 1979) but are accompanied by focal loss of all components of the myofibril. Specific lysosomal protease-sensitive regions have been demonstrated for type 1 as well as for type 2 skeletal muscle myosins (Dufour et al. 1989). Consequently, selective loss of myosin filaments in type 1 fibres could be due to a selective activation of certain lysosomal proteases against both slow skeletal muscle myosin and myocardial myosin. However, to our knowledge, lysosomal proteases sensitive regions common for skeletal muscle and myocardial myosins have not been reported.

We suggest that our patient may be affected by an inherited disease involving type 1 MHC of both skeletal muscle and myocardium. Human $\alpha$- and $\beta$-cardiac MHC genes are located on chromosome 14. $\beta$-MHC gene is expressed in both heart and skeletal muscle while human $\alpha$-cardiac MHC gene is expressed only in cardiac tissue (Saez and Leinwand 1986; Saez et al. 1987). Skeletal muscle type 2 MHC genes are located on chromosome 17 (17p11-17pter) and are not expressed in the heart (Edwards et al. 1985) and thus a mutation in the $\beta$-cardiac MHC might cause abnormalities both in the heart and skeletal muscle without affecting other skeletal muscle genes. A $\beta$-cardiac MHC gene mutation has been reported in familial hypertrophic CMP but skeletal muscle biopsy was not performed in this study (Geisterfer-Lowrance et al. 1990; Tanigawa et al. 1990). Type 1 fibre abnormalities have been found in patients with hypertrophic CMP and subclinical myopathy. Muscle biopsy showed non-specific changes such as type 1 fibre atrophy or increased lipid droplets (Caforio et al. 1989) but selective lysis of myosin filaments was not reported.

In our case the absence of myofibrillar ATPase staining was either complete in the very atrophic angulated fibres or confined to the centre of many type 1 fibres. This histochemical pattern was not apparent in the myocardium although, ultrastructurally, loss of thick filaments was also evident in myocardial fibres.

We have no clear explanation for the abnormal myofibrillar ATPase staining in muscle fibres. MHC has two functional domains consisting of two globular heads attached to a long $\alpha$-helical, rod-like tail (Lowey 1986). Both globular heads contain the MgATPase activity and other regions that bind to MLC and actin. A possible explanation for the persistence of histochemical myofibrillar ATPase stain in cardiomyocytes could be that it is known that $\alpha$-cardiac MHC polypeptide, which is supposed to be spared in this disease, exhibits greater ATPase activity than the $\beta$-cardiac MHC (Tanigawa et al. 1990).

Slow MHC in the present case shows no gross abnormalities in physical characteristics by gel electrophoresis. The abnormality may be present at the level of the MgATPase activity of the globular heads. Morphological abnormalities probably do not completely reflect the abnormality of myosin but only show the more severely affected areas.

Molecular genetic studies are in progress in this patient, to demonstrate a possible mutation of the $\beta$-MHC gene.

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