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# Usefulness of the ultrastructural and immunohistochemical analysis of cardiac biopsy in affected heart

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**Abstract**

*In the last few years endomyocardial biopsy becomes a useful diagnostic tool for the investigation of idiopathic dilated cardiomyopathy. The aim of our current study was to try to identify ultrastructural and immunohistochemical specificity of truncated cardiac proteins in affected heart. The focal loss of plasma membrane continuity together with the lack of dystrophin activity in affected myocytes facilitated to find mutation in dystrophin gene. The accumulation of granulofilamentous desmin-positive material in cytoplasm of myocytes was the main indicator of presented mutation in the desmin gene. Nuclear structure remodeling, concomitantly with loss of lamin A/C activity, contributed to identify mutation in lamin A/C gene. Analysis of hypertrophic heart with disarray of sarcomeres and lack of I-Z-I bands suggested embryonic faiulure in titin activity. All this findings indicate that endomyocardial biopsy reperesent a useful method for a correct diagnosis of heart dysfunction.*

*Key words: cardiac truncated proteins, sarcolemmopathy, sarcomeropathy, laminopathy.*

## Introduction

In the last decade, molecular analysis was established to play a pivotal role in the diagnosis of idiopathic cardiomyopathies. Advancing knowledge on myocyte structural proteins coupled with an increasing availability of a broad diversity of antibodies against these proteins are promising tools to bring the diagnosis. Ultrastructural and immunohistochemical analysis may be useful for both identification of normal antigenic constituents of myocyte proteins and the evaluation of their loss, accumulation or maldistribution in analysed myocytes. Since the initial discovery of a mutation in the beta myosin heavy chain gene [7] a growing number of mutations has been found in components of cardiac architecture including sarcolemmal [18], cytoskeletal [17] and nuclear [16] proteins. In our study, immunohistochemical and ultrastructural approach has been used to demonstrate that such analysis facilitates the molecular study of idiopathic cardiomyopathies.

## Material and methods

An endomyocardial biopsy was performed in the right ventricle of three affected patients with the

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**Table I.** Short clinical data of three patients with DCM and one boy with HCM

| Patient | Age/Sex | Inheritance | Diagnosis | Clinical events | Mutation |
|---|---|---|---|---|---|
| GR | 25/M | X-linked | DCM | Sudden death | Dystrophin ex 45-49 |
| BR | 44/M | AD | DCM | Alive | Desmin R355P (5) |
| BS | 26/M | AD | DCM | Alive | Lamin A/C D192G (4) |
| LS | 6/M | S | HCM | Sudden death | – |

diagnosis of DCM. In addition, samples obtained during an autopsy of the heart of a boy with congenital hypertrophic cardiomyopathy (HCM) were analysed. The diagnosis was established on the basis of clinical history, physical examination, chest radiography and electrocardiography (Table I). For indirect immmunofluorescence examination, cryostat sections were stained using monoclonal antisera against desmin, dystrophin (Novocastra) and four epitopes of lamin A/C as was previously reported [4].

For electron microscopy, specimens were fixed in 1% osmium tetroxide in the same buffer, following that, they were dehydrated and embedded in Spurr-resin. Thin sections double stained with uranyl acetate and lead citrate were examined with a JEM II electron microscope.

## Results

Abnormal myocytes with a prominent loss of myofibrils located in fibrotic areas were observed in the first case of DCM. Narrowed myofibrils were separated by markedly widened spaces (Fig. 1A). Loss of myofibrils was also observed in subsarcolemmal spaces. The plasma membrane overlying the myocytes was either disrupted or indicated focal loss of continuity (Fig. 1B), while the basement membrane was always preserved. In order to identify the defect in sarcolemmal proteins, the activity of dystrophin was investigated. The absence of rod domain of dystrophin with the presence of N and C-terminal domains (Figs. 1C and D) in the affected myocytes facilitated molecular analysis (Table I). Overproduction of cytoskeletal protein was observed in the second analysed case of DCM. Abnormal dense osmophilic material (Fig. 2A) was observed in intermyofibrillar as well as subsarcolemmal spaces, at the level of Z-disc as well as around the intercalated discs (Fig. 2B). Such accumulation of truncated protein leads to disorganization and destruction of myocyte architecture. To determine the nature of this accumulated material, we used monoclonal anti-desmin antibody. Intensive desmin staining of the affected myocytes (Fig. 2C) allowed us to suspect a desmin defect in the investigated heart (Table I). An abnormal architecture of myocyte nuclei was observed in the case third of DCM. In contrast to the normal ovoid nuclei seen in normal myocytes, the nuclei in the affected case exhibited a convoluted appearance and nuclear matrix reorganization (Fig. 3A).

The specific hallmark of such nuclei was the focal or total lack of nuclear membrane and penetration of sarcoplasmic components into the nuclear interior (Fig. 3B). The nuclear membrane defect and changes in chromatin organization suggested failure of lamin A/C activity. Indirect immunoflurescence analysis showed lack of lamin A (Figs. 3C and D) facilitating molecular analysis (Table I).

The most surprising abnormality which we found in the heart of a boy with congenital HCM was pronounced sarcomeric disarray of myocyte myofibrils.

"Naked" A bands with well preserved architecture showing various position were incorporated into myofibrils. Sometimes A-bands formed long tapes oriented longitudinally along the myocytes (Fig. 4A). Incompletely organized sarcomeres were found in numerous myocytes. Such truncated sarcomeres were devoid of I-Z-I lines with the appearance of an empty space between A bands (Fig. 4B). These structural abnormalities indicated that the assembly of sarcomeres was quite incomplete and that unstable sarcomeres were formed in the affected hypertrophic heart.

## Discussion

In this study, we present the usefulness of the immunohistochemical and ultrastructural analysis of the heart biopsy material in the diagnosis of some idiopathic cardiomyopathies. The absence of dys-

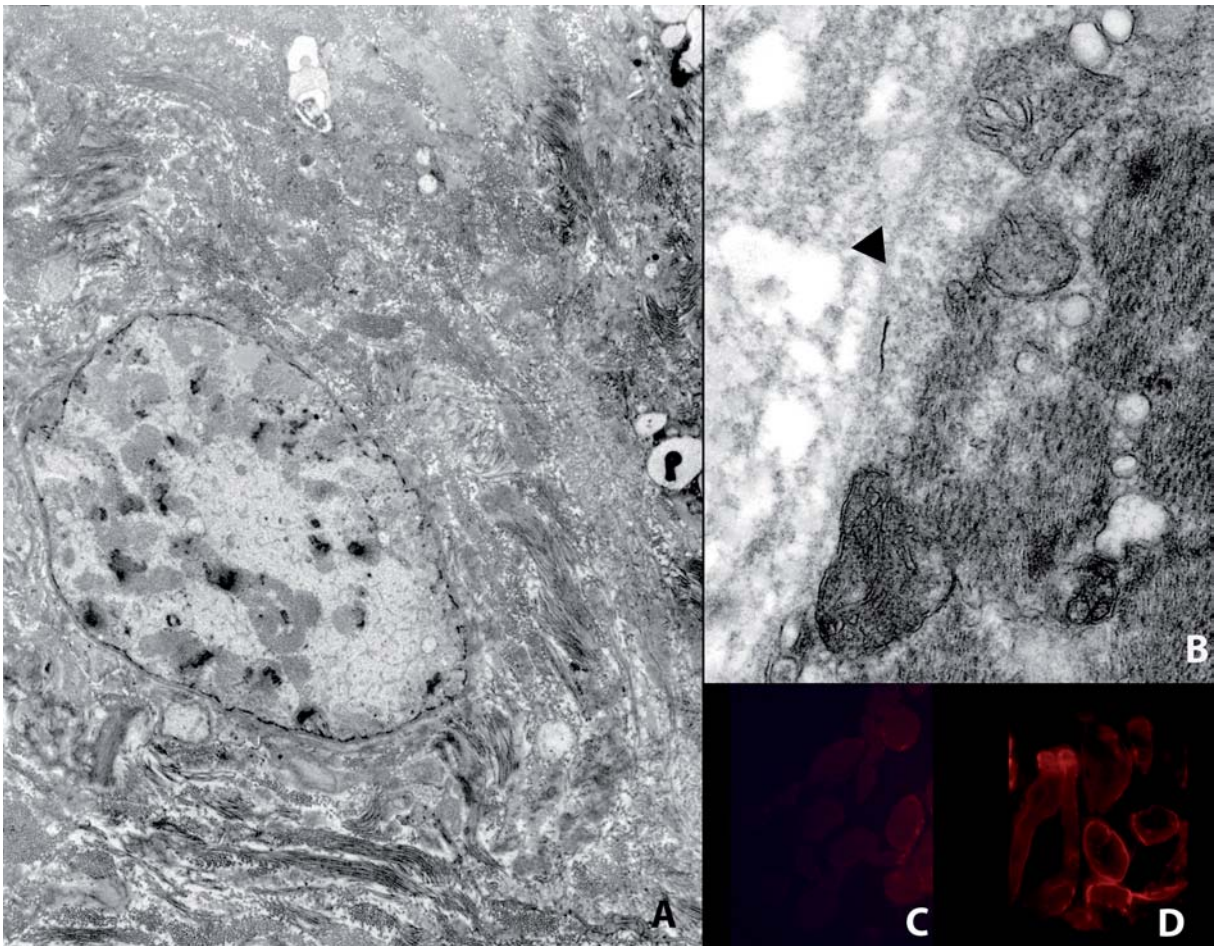


**Fig. 1.** Dilated cardiomyopathy. A) The myocyte surrounding by collagen fibrils. Note that myofibrils are separated by markedly widened space. x 12 000. B) Focal loss of sarcolemmal continuity (arrowhead) x 75 000. C) The lack of dystrophin activity. Antidystrophin 30 kDa antibody x 480. D) Dystrophin 60 kDa decorates plasma membrane of myocytes. x 480.

trophin or abnormal dystrophin expression weakens the physical link between the extracellular matrix and the skeleton [19]. Dystrophin is one of the constituent protein of the sarcolemma which interacts with both cytoskeletal actin and dystrophin associated glycoproteins complex [22]. Disruption of the glycoprotein complex caused by a mutation in the gene encoding dystrophin produces Duchenne muscular dystrophy (DMD), the milder Becker dystrophy (BMD), and X-linked dilated cardiomyopathy [13]. Dilated cardiomyopathy is frequent in both DMD and BMD while subjects with XLDM differ in that they have little or no affected skeletal muscle [12]. Our study revealed that dystrophin deficiency in the myocyte membrane leads to the sarcolemma fragility, loss of membrane integrity resulting in degeneration of myocytes, and tissue fibrosis. An abnormal accumulation of immunostaining material observed in myocytes of the affected case with DCM suggested genetic defect in a cytoskeletal protein – desmin. Desmin is 53kDa cytoskeletal protein that forms an intracytoplasmic network and maintains spatial relationship between the contractile apparatus and other structural elements of the cell [2]. Abnormal accumulation of desmin within the muscle cell was originally described as the morphological hallmark of desmin related myopathy [8]. Most of these disorders are caused by a mutation in the desmin gene [2] while another form is associated with a mutation in the alphaB crystalin gene [21]. Accumulation of mutant desmin impares myocyte activity and destroys myocyte architecture. Diagnostic difficulties regard-

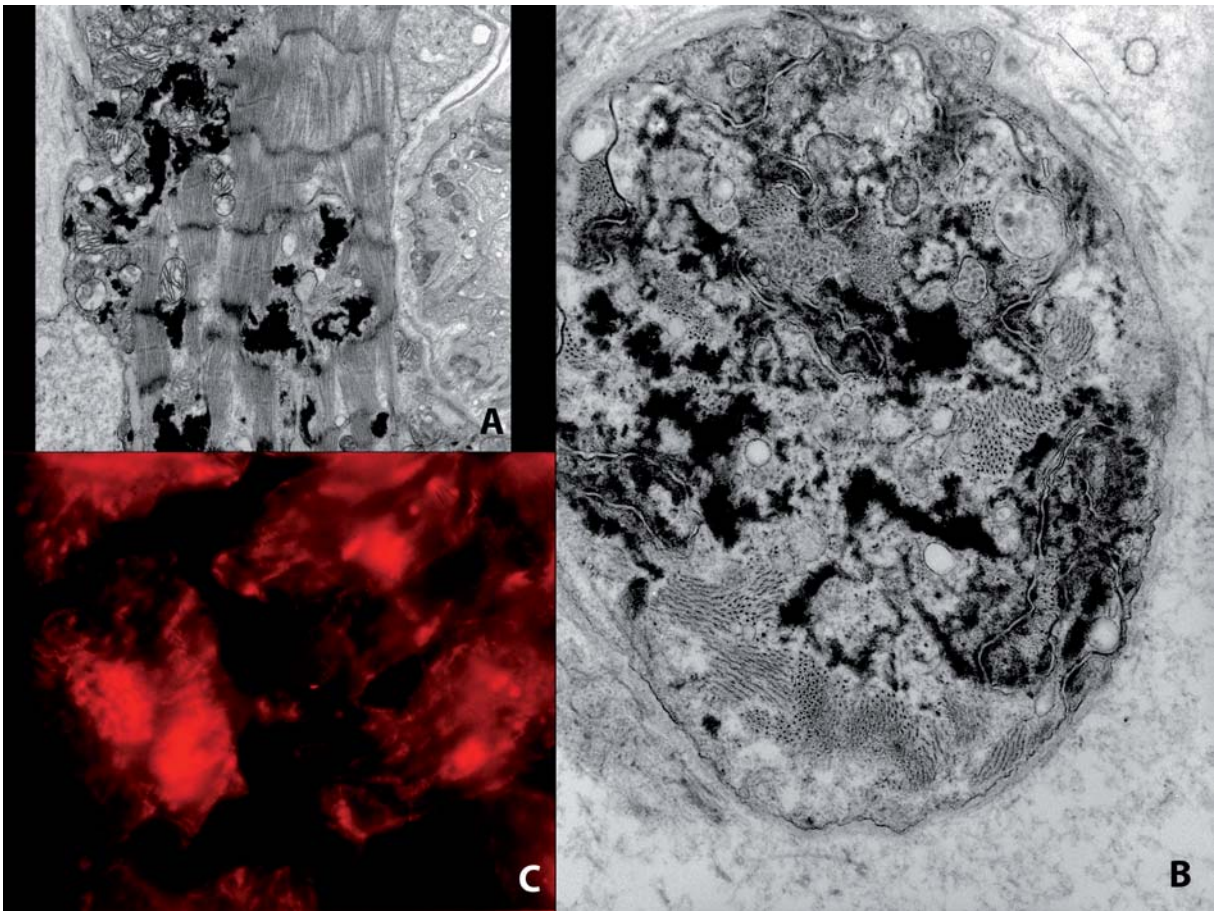


**Fig. 2.** Dilated cardiomyopathy. A) An abnormal dense material seen in intramyofibrilar spaces. x 21 000. B) Dense osmophilic deposits located around of inercalated disc. x 45 000. C) Cardiac myocytes with desmin storage. Anti-desmin antibody. x 4800.

ing desminopathies arise from the fact that this disease is extremely heterogeneous and in some cases it manifests as a progressive skeletal myopathy [2] while in other cases cardiomyopathy is the leading [9] or even exclusive manifestation [15]. The combination of familial skeletal myopathy and cardiomyopathy [5] suggests that truncated protein may play a major recognized role in cardiomyopathies.

An important point of this study is the fact that the deficiency of nuclear lamin may have participated in the appearance of DCM. The nuclear lamina, a dense fibrillar network composed of A/C and B lamins [20] is located between the inner nuclear membrane and the peripheral chromatin [1]. Lamins interact directly with chromatin and several nuclear proteins. Lamin A/C is the major product of the LMNA gene located at the chromosome 1q21. During the past few years, mutations of lamin A/C were found to cause a wide range of human disorders, among of them DCM playing an important role. The lack of lamin A activity in cardiomyocyte nuclei causes myocyte modification, altered nuclear shape, distorted nuclear membrane continuity, heterochromatin reorganization, and myocyte remodeling. The entry of mitochondria into the nuclear interior seen in myocyte nuclei, denuded of lamin A [5, 6] is a morphological hallmark of the lamin deficiency in the cardiac tissue. Presented morphological alterations are not surprising because nuclear lamins play an important role in the nuclear assembly, organization, and nuclear shape.

A failure of the sarcomere organization observed in the case with fatal hypertrophic cardiomyopathy is a rarely observed phenomenon. Sarcomeres are

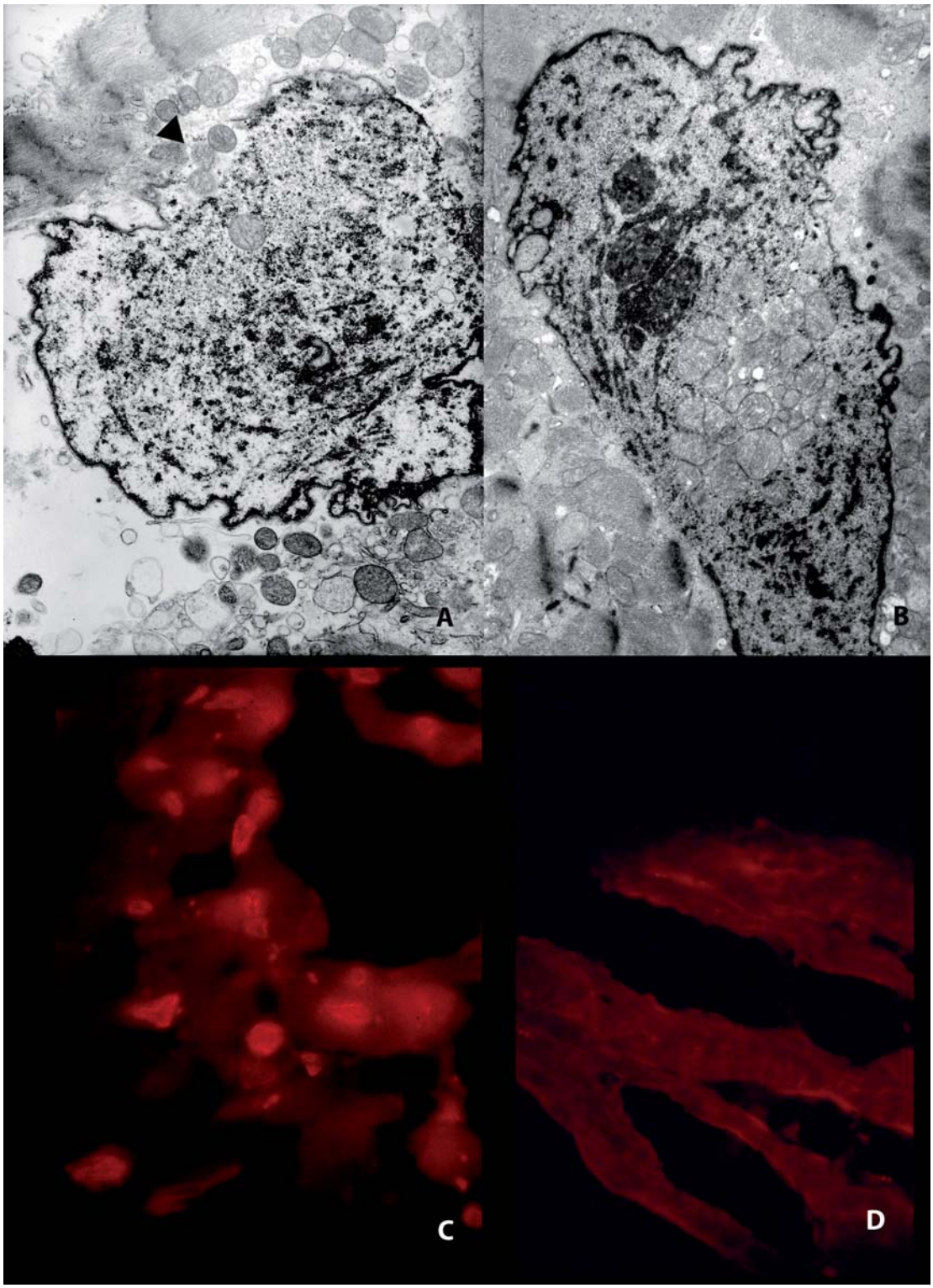


**Fig. 3.** Dilated cardiomyopathy. The nuclear matrix reorganization. A) Note the focal loss of nuclear membrane and the penetration of mitochondria into nuclear matrix (arrowhead). x 21 000. B) The appearance of mitochondria within nuclear matrix. x 18 000. C) Nuclei of myocytes with intensive activity of lamin A/C2. x 480. D) The lack of lamin A4 activity. x 480.

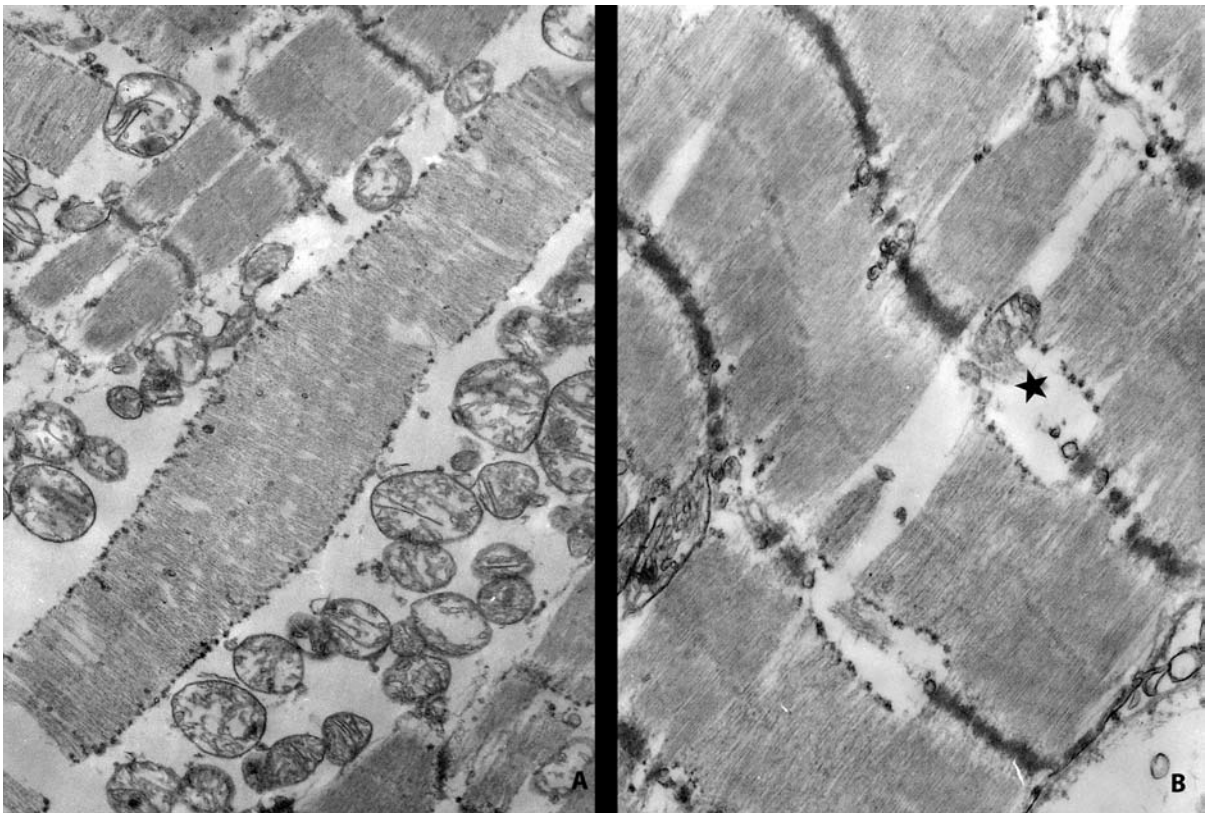


**Fig. 4.** Congenital hypertrophic cardiomyopathy. A) An abnormal position of A-band forming long tape longitudinally oriented along the myocyte. x 30 000. B) An incompletely organized sarcomeres devoid of I-Z-I lines (asterisk) x 45 000.

assembled into myofibrils during the early embryonic stage. The process of myofibril assembly requires both spatial and temporal coordination of protein interactions. In cardiac tissue, two molecular proteins titin and nebulette play an integral role in the sarcomere formation. Titin, the largest sarcomeric protein, spans a half of the sarcomere from the Z-disc to the M-line [14]. Nebulette molecules in the cardiac tissue play a critical role in the formation of the thin-filament Z-disc complex [3,11]. A lack of one of these proteins may affect sarcomere organization. The gradual sarcomeric disassembly was demonstrated in a mouse expressing mutant titin [10]. The presence of truncated sarcomeres in the case of fatal congenital hypertrophic cardiomyopathy suggests that impaired sarcomere formation might be implicated in the early stage of cardiac development. Although molecular analysis was not done in this case, considering data from the cardiac biopsy findings may speculate that a mutation in one of the sarcomeric genes titin or nebulette might have been responsible for hypertrophic cardiomyopathy in our case.

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