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Case Report

Autopsy of a patient with restrictive cardiomyopathy with and *MYH7* mutation

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ABSTRACT

Restrictive cardiomyopathy (RCM) is a rare type of primary myocardial disease, and its pathological features remain unclear. We report the case of a 78-year-old Japanese woman with RCM and *MHY7* mutation who died of heart failure 13 years after the diagnosis. Upon autopsy, focal myocyte amorphous degeneration positive for ubiquitin was revealed, as well as myocardial disarrangement and interstitial fibrosis. Electron microscope demonstrated electron-dense structure in the cardiac myocytes. These may be one of the pathological features of RCM.

Introduction

Restrictive cardiomyopathy (RCM) is a rare type of primary myocardial disease characterized by diastolic dysfunction caused by increased myocardial stiffness that results in impaired ventricular filling, although the diastolic volume and ventricular wall thickness remain normal [1]. This condition has multiple types, that is, idiopathic, familiar, infiltrative, storage, and endomyocardial [1,2].

Primary (or idiopathic) RCM without other diseases has been reported [3,4], and it may be a genetic disease [5]. However, there have been a few autopsy reports that showed the precise histopathological features of primary RCM [3,6–8].

Here, we aimed to describe the focal myocyte amorphous degeneration with positive for ubiquitin and electron-dense structure in a patient with primary RCM with *MYH7* mutation at autopsy.

Case report

A 78-year-old Japanese woman was admitted due to severe heart failure and cardiogenic shock. She was clinically diagnosed with restrictive cardiomyopathy (RCM) at the age of 65 years; her younger brother was also diagnosed with RCM. Later, genetic analysis of blood samples revealed an R870C missense mutation in the β-myosin heavy chain (β-MHC) gene in both the proband and her younger brother. Hence, the patient was diagnosed with familial primary RCM with *MYH7* mutation (R870C). One year later, endomyocardial biopsy was performed from the left ventricle, and it showed myocardial disarrangement and interstitial fibrosis. However, it could not reach the diagnosis of RCM at that time.

The patient was treated with angiotensin-converting enzyme inhibitor lisinopril (5 mg/day), furosemide (60 mg/day), aldosterone (25 mg/day), and digoxin (0.25 mg/day). However, she was admitted after experiencing three episodes of congestive heart failure exacerbation.

Electrocardiography showed atrial fibrillation, mild ST-segment depression, and inverted T waves in the II, III, aVF, and V4–6 leads, while chest radiography showed cardiomegaly (cardiothoracic ratio, 80%) with bilateral pleural effusion. Transthoracic echocardiography showed biatrial enlargement, normal left ventricular end-diastolic dimension (40 mm), and mild diffuse hypokinetic left ventricle with a left ventricular ejection fraction of 52%. Results of laboratory test results

Abbreviations: β-MHC, β-myosin heavy chain; cMyBP-C, cardiac myosin binding protein c; RCM, restrictive cardiomyopathy; UPS, ubiquitin-proteasome system.

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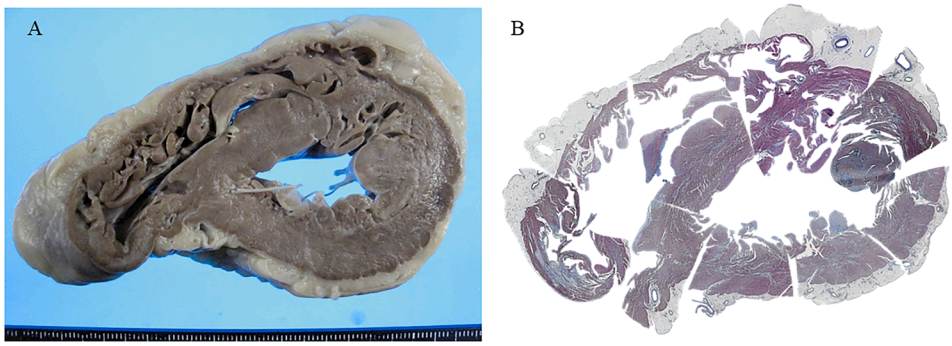

Fig. 1. Macrophotography of autopsied heart and loupe statue. The left ventricular wall thickness was 10 mm (A), and mild diffuse interstitial fibrosis was observed in the ventricles (B, Azan stain).

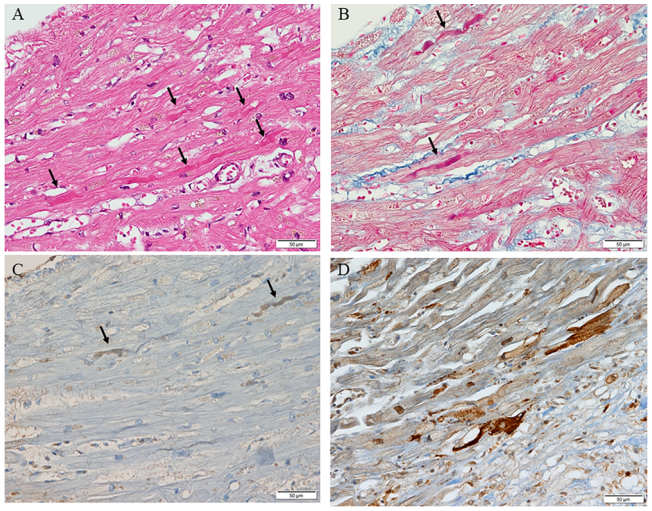

Fig. 2. Microphotographs of the ventricle. Focal myocyte degeneration (arrows) and lipofuscin were found in the myocytes (A, hematoxylin and eosin stain; B, Azan stain). These degenerations were positive for ubiquitin (arrows) (C, immunostaining), and p62-positive cardiac myocytes were observed (D).

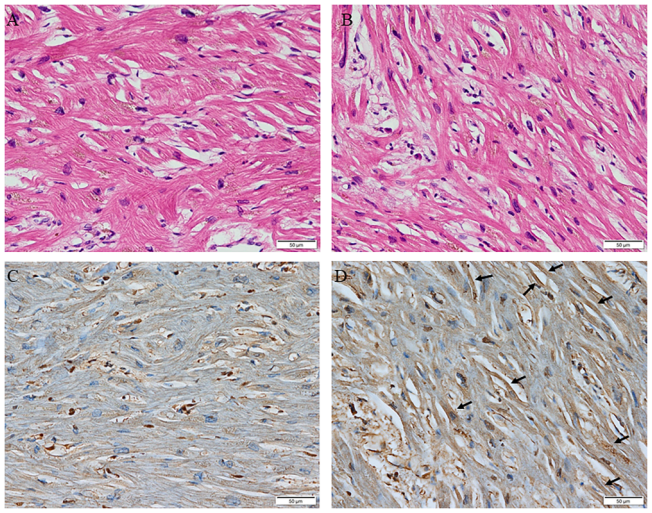

Fig. 3. Microphotographs of the ventricle. Myocardial disarrangement is observed in the ventricles (A, B; hematoxylin and eosin stain). Some of myocardium show hypertrophy (A), while others did not (B). Although few hypertrophic myocytes with disarrangement were positive for ubiquitin (C), this protein was also observed in non-hypertrophic myocytes with disarrangement (arrows) (D).

showed total bilirubin level of 2.2 mg/dL, direct bilirubin level of 1.7 mg/dL, glutamic oxaloacetic transaminase level of 50 IU/L, glutamate pyruvate transaminase level of 20 IU/L, lactate dehydrogenase level of 561 IU/L, creatine kinase level of 338 IU/L, blood urea nitrogen level of 33.3 mg/dL, and creatinine level of 1.7 mg/dL, indicating liver congestion and renal dysfunction.

After admission, intravenous infusion of furosemide, dopamine, and dobutamine was initiated. However, the patient died 9 days after admission, and an autopsy was performed after obtaining consent from her family.

The patient had a heart weight of 345 g and a left ventricular wall thickness of 10 mm (Fig. 1A) and showed signs of biatrial enlargement. No significant coronary artery stenosis was noted. Mild diffuse interstitial fibrosis was observed in the ventricles (Fig. 1B). Focal long and narrow-shaped myocyte amorphous structure, presence of lipofuscin in the myocytes, and mild interstitial fibrosis were observed (Fig. 2A, B).

Immunohistochemical staining demonstrated long and narrow-shaped accumulation of ubiquitin (Fig. 2C) and p62-positive inclusion (Fig. 2D) in some cardiomyocytes.

Myocardial disarrangement was also observed in the ventricular walls (Fig. 3). Some of myocardium showed hypertrophy (Fig. 3 A), while others showed absence of hypertrophy (Fig. 3B). Although few hypertrophic myocytes with disarrangement were positive for ubiquitin (Fig. 3C), this protein was also found in non-hypertrophic myocytes with disarrangement (Fig. 3D).

Electron microscopy revealed the presence of focal long and narrow-shaped electron-dense structures in the myocardium (Fig. 4A). These structures connected adjacent myocytes with Z bands (Fig. 4B, C), and they contained intercalated discs (Fig. 4D) and myofilaments (Fig. 4E). Those indicated myofilament streaming but not necrosis. Moreover, there were many tubular structures in some myocyte nuclei, and pseudo-inclusion bodies with indentation of nuclear membranes (Fig. 4F, G). Autophagic vacuoles were not seen in our specimen on electron microscopy.

Discussion

Four case reports have shown the precise autopsy or pathological examination of a recipient's heart with primary RCM [3,6–8]. These reports showed myocardial fibrosis and disarray in RCM [3,6] although in two reports of infant primary RCM with *MHY7* mutation, one had no evidence of fibrosis and disarray [7], while the other showed minimal myocardial disarray and replacement fibrosis caused by ischemia due to stenosis of the left anterior descending branch vessels [8]. Myocardial fibrosis and disarrangement shown in previous reports was comparable to that in the present case.

We also demonstrated focal long and narrow-shaped myocyte amorphous degeneration positive for ubiquitin. Ubiquitin-proteasome system (UPS) is indispensable for degradation of most proteins

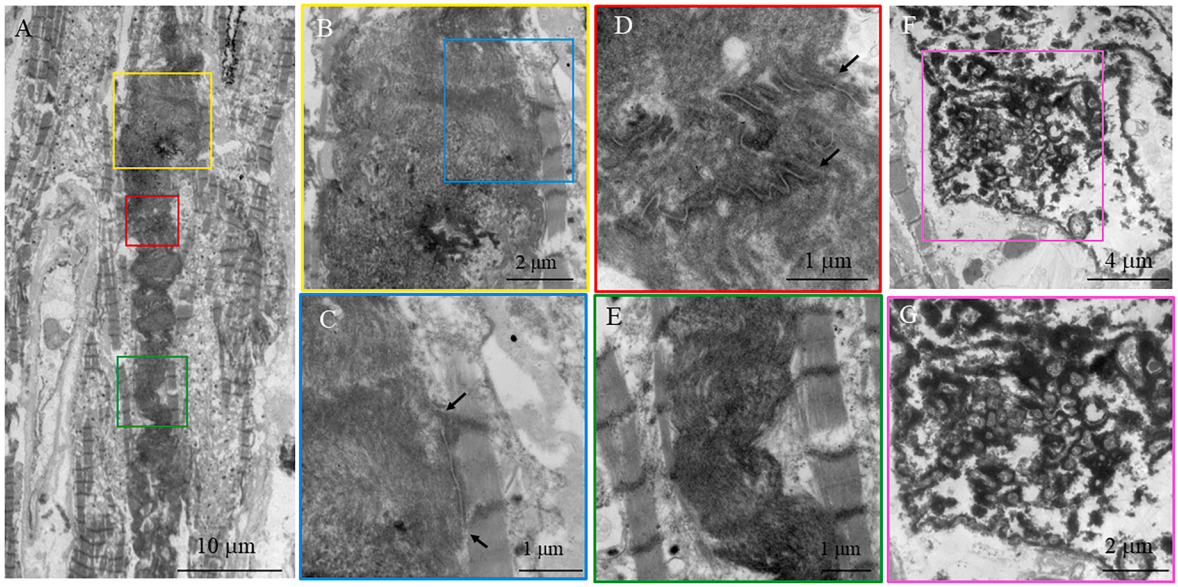

Fig. 4. Electron micrographs. Electron microscopy reveals the presence of focal long and narrow-shaped electron-dense structures in the myocardium (A). These structures connect adjacent myocytes with Z bands (arrows) (B, C), and contain intercalated discs (arrows) (D) and myofilaments (E). There are many tubular structures in some myocyte nuclei, and pseudo-inclusion bodies with indentation of nuclear membranes (F, G).

including myofibrillar proteins, and ubiquitination of target proteins is first required for the degradation by UPS [9]. Sarikas et al. [10] showed that human truncated cardiac myosin binding protein c (cMyBP-C) mutants in hypertrophic cardiomyopathy were not incorporated into the sarcomere, form ubiquitin-positive aggregates. And, sarcomere protein mutants may be resistant to degradation, and clog the proteasome, preventing entry other proteasome substrates leading to proteasome dysfunction. Therefore, these mechanisms may be related to the presence of focal myocyte amorphous degeneration positive for ubiquitin in our RCM patient.

Kostin et al. [11] reported that cysotolic ubiquitin-positive aggregates linked to increased levels of autophagy have been observed in dilated cardiomyopathy. In the present patient with RCM, electron microscope showed no increased autophagy, the similar shaped streaming of myofibrils with electron-dense structure to focal myocyte amorphous degeneration positive for ubiquitin, tubular structures and pseudo-inclusion in some myocyte nuclei. This suggests that focal myocyte amorphous degeneration positive for ubiquitin may be identical to streaming of myofibrils with electron-dense structure but not related to increased autophagy, and that the nuclear abnormality may suggest the nuclear dysfunction related to these myofibril lesions.

Moreover, p62-positive cardiac myocytes were observed in the present patient. P62 is essential for the clearance of ubiquitinated or non-ubiquitinated proteins by autophagy. P62 itself is degraded during autophagy, and suppression of autophagy leads to accumulation of p62 [12]. Therefore, suppression of autophagy may be another possible mechanism of the focal myocyte amorphous degeneration with ubiquitin-positive proteins.

Finally, we demonstrated that non-hypertrophic myocytes with disarrangement were positive for ubiquitin although this protein was found in few hypertrophic myocytes with disarrangement. Thus, ubiquitin-positive protein or myofibril streaming due to abnormal sarcomere protein might be related to suppression of myocyte hypertrophy with disarrangement. Immune electron microscope for ubiquitin may be useful for precise investigation although we could not perform it.

In conclusion, focal myocyte amorphous degeneration positive for ubiquitin with electron-dense material was seen in RCM, and it may be related to the pathogenesis of RCM as well as fibrosis and disarrangement.

Ethics Statement including Patient Consent Statement

This project (2020-6-37) was approved by the ethics committee of Nagasaki University Hospital. For human subjects, the investigation was conducted in accordance with the Declaration of Helsinki of 1975. We also had patient consent statement from her family.

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Author Contributions

Conception and design of the study, Hiroaki Kawano; acquisition and analysis of data, Munetake Kanda, Muneo Tanigawa, Mitsuaki Ishijima, Yuji Matsumoto, Koji Maemura; drafting the manuscript or figures, Hiroaki Kawano, Koichi Kawamura, Nozomi Ueki, Masahiro Nakashima.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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