Images and Case Reports in Heart Failure

Long-Term Pathological Follow-Up of Myocardium in a Carrier of Duchenne Muscular Dystrophy with Dilated Cardiomyopathy

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Background

Duchenne muscular dystrophy (DMD) is a fatal X-linked disorder, with an incidence of approximately 1 in 3600–6000 male births. DMD is caused by mutations in the dystrophin gene located at Xp21.2 and is clinically characterized by progressive muscle degeneration and dilated cardiomyopathy (DCM). Some female DMD carriers show a variety of clinical manifestations, ranging from creatine kinase (CK) elevation to severe muscle weakness. DCM has been reported in 8–18% of female DMD carriers and sometimes results in a lethal course. Here, we describe long-term follow-up observations of myocardial changes in a DMD carrier with DCM.

Case

A 29-year-old female presented with progressive shortness of breath, increasing ankle edema, and orthopnea after a full-term normal delivery. A chest X-ray showed cardiomegaly and bilateral pleural effusions, and echocardiography showed a severely reduced left ventricular ejection fraction (LVEF) of 24%, with a markedly increased left ventricular end-diastolic diameter (LVDd) of 71 mm. She was admitted for heart failure and her symptoms improved with furosemide and inotropes. There were no symptoms to suggest myopathy and blood analysis revealed no elevation of CK (60 U/L). Her newborn boy showed extreme elevation of CK (105,868 U/L) and was diagnosed with DMD by genetic testing. Dystrophin immunostaining of

her right ventricular endomyocardial biopsy showed a mosaic pattern of dystrophin-negative and dystrophin-positive cardiomyocytes stained with antidystrophin monoclonal antibodies (**Figure**), findings compatible with DMD carrier status. When the patient was 35 years of age, she was admitted again because of heart failure exacerbation induced by a second pregnancy and did not improve after delivery. Echocardiography revealed severe mitral and tricuspid regurgitation; LVEF decreased to 13% and LVDd increased to 90 mm. The patient underwent mitral valve replacement with a Medtronic Mosaic 29 mm porcine prosthesis (Medtronic Inc., Minneapolis, MN, USA) and tricuspid annuloplasty with an Edwards MC3 annuloplasty system (Edwards LifeScience, Irvine, CA, USA). A specimen from the left ventricular free wall myocardium was obtained during surgery. At the age of 44 years, she showed progressively worsening dyspnea (New York Heart Association functional class IV) and had repeated hospitalizations for heart failure in spite of taking an angiotensin-converting enzyme inhibitor, a beta blocker, and an aldosterone blocker. Echocardiography indicated LVEF 13% and LVDd 90 mm. She was referred to our hospital and an endomyocardial biopsy was performed as part of the evaluation for heart transplantation. Myocardial sampling was performed three times in 15 years of follow-up.

Pathological images are shown in the Figure. Hematoxylin-eosin staining revealed a variable cardiomyocyte diameter and cardiomyocyte hypertrophy but no signs of myocardial inflammation. Mean cardiomyocyte diameter increased from 21 μm (29 years) to 37 μm (35

years), and did not change significantly at 44 years (34 μm). The myocardial fibrotic area assessed with picrosirius red staining progressively increased (29 years old, 3%; 35 years old, 14%; 44 years old, 28%). Immunohistochemical staining was performed on paraffin sections using antidystrophin monoclonal antibodies for the rod domain, Dy4/6D3 (DYS1). The primary antibody was diluted 1:20, followed by the secondary antibody and avidin–biotin complex (Vectastain Elite ABC system; Vector Laboratories Inc., Burlingame, CA, USA). Dystrophin immunostaining showed an impressive mosaic staining pattern consisting of dystrophin-negative and dystrophin-positive cardiomyocytes. Interestingly, the percentage of dystrophin-negative cardiomyocytes did not change significantly over time (28 years old, 52%; 35 years old, 53%; 44 years old, 60%).

Discussion

This is the first reported longitudinal observations of pathological myocardial changes in a DMD carrier with DCM. The findings indicated that the degree of myocardial fibrosis increased while the proportion of dystrophin-negative cardiomyocytes remained nearly constant, even with heart failure progression. Our results suggest that both dystrophin-negative and dystrophin-positive cardiomyocytes may be injured during the course of heart failure progression. In a female heterozygote mdx mouse model of DMD, Karpati et al. reported a

marked gradual decrease of dystrophin-negative fibers in skeletal muscle, but persistence of a large number of dystrophin-negative segments in cardiac muscle with aging.[1] In addition, approximately 50% of the myocardial cardiomyocytes in female carriers of DMD are reportedly destrophin-negative.[2,3] These results are consistent with those in our case report.

Multiplex ligation-dependent probe amplification, a screening genetic test for DMD and DMD carriers, can detect only 65% of cases caused by deletions and duplications of the DMD gene. In addition, dystrophin analysis with western blotting shows normal or near normal results because of the presence of normal dystrophin fibers. As a result, genetic testing and western blotting sometimes fail to diagnose DMD carriers.[2,3] In contrast, immunohistochemical analysis was demonstrated to have high specificity for the diagnosis and a low risk for a false-negative result.[2] Our results indicate that dystrophin-negative cardiomyocytes persist for a long duration, suggesting that immunohistochemical analysis is warranted for the detection of DMD carriers at any age, and may be superior to genetic testing in terms of sensitivity and specificity.

Nelson et al. recently demonstrated that genome editing modified the dystrophin gene and led to partial recovery of functional dystrophin protein in skeletal and cardiac muscle in the mdx mouse.[4] Dystrophin-negative cardiomyocytes did not disappear in our case, suggesting the potential for improvement of abnormal dystrophin-absent cardiomyocytes at any stage of heart failure. Cardiac involvement is sometimes the only clinical manifestation in female DMD

carriers. Because of the potential for treatment of DMD carriers in the future, immunohistochemical staining for dystrophin in endomyocardial biopsies should be considered in women affected by apparently isolated DCM, even when family history of DMD is absent.

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Disclosure

None

Reference

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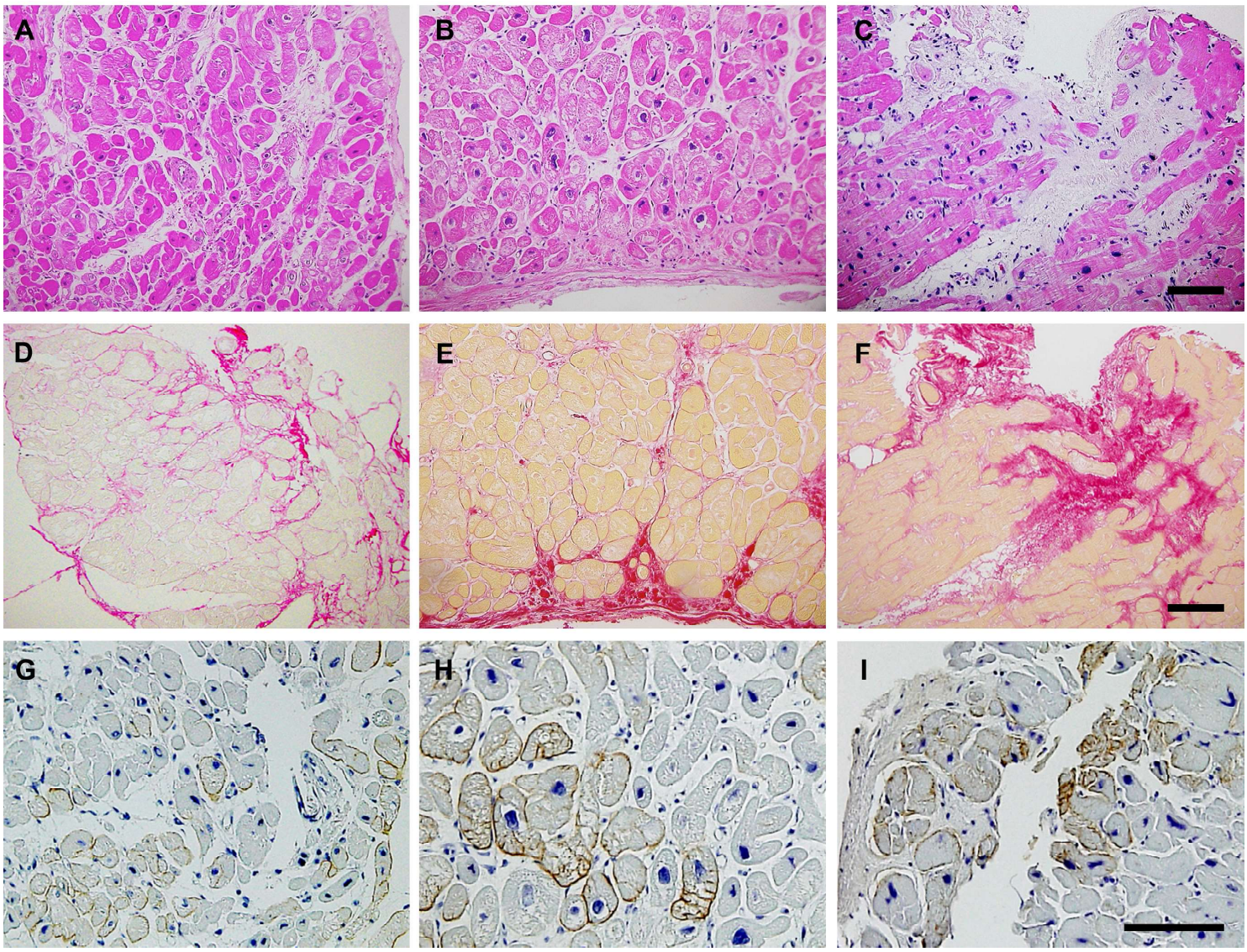
Figure legend

Left panels show the right ventricular endomyocardial biopsy taken at the age of 29 years, middle panels show the left ventricular free wall specimen at the age of 35 years, and right panels show the right ventricular endomyocardial biopsy at the age of 44 years. A, B, C: Hematoxylin-eosin stain. D, E, F: Picrosirius red stain. G, H, I: Dystrophin immunostain. Bars, 100 μm.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