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Uniform low-level dystrophin expression in the heart partially preserved cardiac function in an aged mouse model of Duchenne cardiomyopathy

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ABSTRACT

Dystrophin deficiency results in Duchenne cardiomyopathy, a primary cause of death in Duchenne muscular dystrophy (DMD). Gene therapy has shown great promise in ameliorating the cardiac phenotype in mouse models of DMD. However, it is not completely clear how much dystrophin is required to treat dystrophic heart disease. We and others have shown that mosaic dystrophin expression at the wild-type level, depending on the percentage of dystrophin positive cardiomyocytes, can either delay the onset of or fully prevent cardiomyopathy in dystrophin-null mdx mice. Many gene therapy strategies will unlikely restore dystrophin to the wild-type level in a cardiomyocyte. To determine whether low-level dystrophin expression can reduce the cardiac manifestations in DMD, we examined heart histology, ECG and hemodynamics in 21-m-old normal BL6 and two strains of BL6-background dystrophin-deficient mice. Mdx3cv mice show uniform low-level expression of a near full-length dystrophin protein in every myofiber while mdx4cv mice have no dystrophin expression. Immunostaining and western blot confirmed marginal level dystrophin expression in the heart of mdx3cv mice. Although low-level expression did not reduce myocardial histopathology, it significantly ameliorated QRS prolongation and normalized diastolic hemodynamic deficiencies. Our study demonstrates for the first time that low-level dystrophin can partially preserve heart function.

1. Introduction

Deficiency of cytoskeletal protein dystrophin leads to Duchenne muscular dystrophy (DMD) [1,2]. Skeletal muscle related symptoms (such as limited ambulation and respiratory restriction) are observed early on in young DMD patients [3]. While cardiac involvement appears at the later stage of the disease, all patients eventually develop cardiac dysfunction and heart failure causes up to 40% of death [4–6]. Currently, only palliative treatments are available for symptom management. Restoration of dystrophin expression using adeno-associated virus (AAV)-mediated micro/mini-dystrophin gene transfer, exon-skipping and genome editing are promising new approaches to treat DMD [7,8]. However, these therapies may not restore dystrophin expression to the normal level in patients. An important issue is whether low-level dystrophin expression is therapeutically relevant.

Numerous studies have investigated the amount of dystrophin required for treating skeletal muscle disease in mouse models of DMD and in human patients. These studies suggest that homogenous dystrophin expression at 20–30% of the wild-type level in every myofiber can significantly enhance muscle function and reduce muscle pathology [9–13]. Recent studies further suggest that uniform low-level dystrophin expression at even 5% of the normal level can still improve clinical outcome in dystrophic mice [14–17]. In the case of mosaic expression, approximately 50% myofibers have to express dystrophin in order to achieve a mild phenotype in skeletal muscle [18–20].

In contrast to the abundant information on low-level dystrophin expression in skeletal muscle, little is known about the dystrophin level needed for correcting heart disease in DMD. A study in genetically modified mice suggests that expression in 3 to 5% of cardiomyocytes at the wild-type level (in every dystrophin positive cell) may delay the onset of heart disease [21]. In a different study, Wu et al. found that 5% dystrophin positive cells in the heart of adult mdx mice did not improve cardiac histology/baseline function although mice tolerated dobutamine stress better [22]. We examined female carrier mice and found that normal level dystrophin expression in half of heart cells is sufficient to completely prevent dystrophic cardiomyopathy [23,24]. While these results have provided critical insight on the percentage of dystrophin positive cells needed for treating cardiac manifestations, it should be noted

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that in all these studies dystrophin is expressed at the wild-type level in every positive cardiomyocyte. It remains unclear whether sub-physiological expression in a cardiomyocyte can benefit the heart.

We and others have previously shown that mdx3cv mice express marginal level dystrophin in skeletal muscle [14,15,25]. This residual level expression significantly enhanced skeletal muscle function although it did not improve histopathology [14,26]. Mdx3cv mice were generated by Chapmen et al. using N-ethyl-N-nitrosourea mutagenesis [27]. A point mutation in intron 65 aborts full-length dystrophin expression. However, a slightly truncated Δ65/66 transcript is generated (Supplementary Fig. 1). This results in the production of a near full-length dystrophin protein at ~5% of the wild-type level [15,25].

To study the impact of low-level uniform dystrophin expression in the heart, we compared the cardiac phenotype among C57Bl/6 (BL6), mdx3cv and mdx4cv mice. All three strains are on the BL6 background. BL6 and mdx4cv mice are normal and dystrophin-null controls, respectively. The characteristic heart presentation in DMD is dilated cardiomyopathy. We have previously shown that dystrophin-deficient mice do not develop dilated cardiomyopathy until they reach 21 months of age [24,28]. For this reason, we intentionally conducted our study in aged

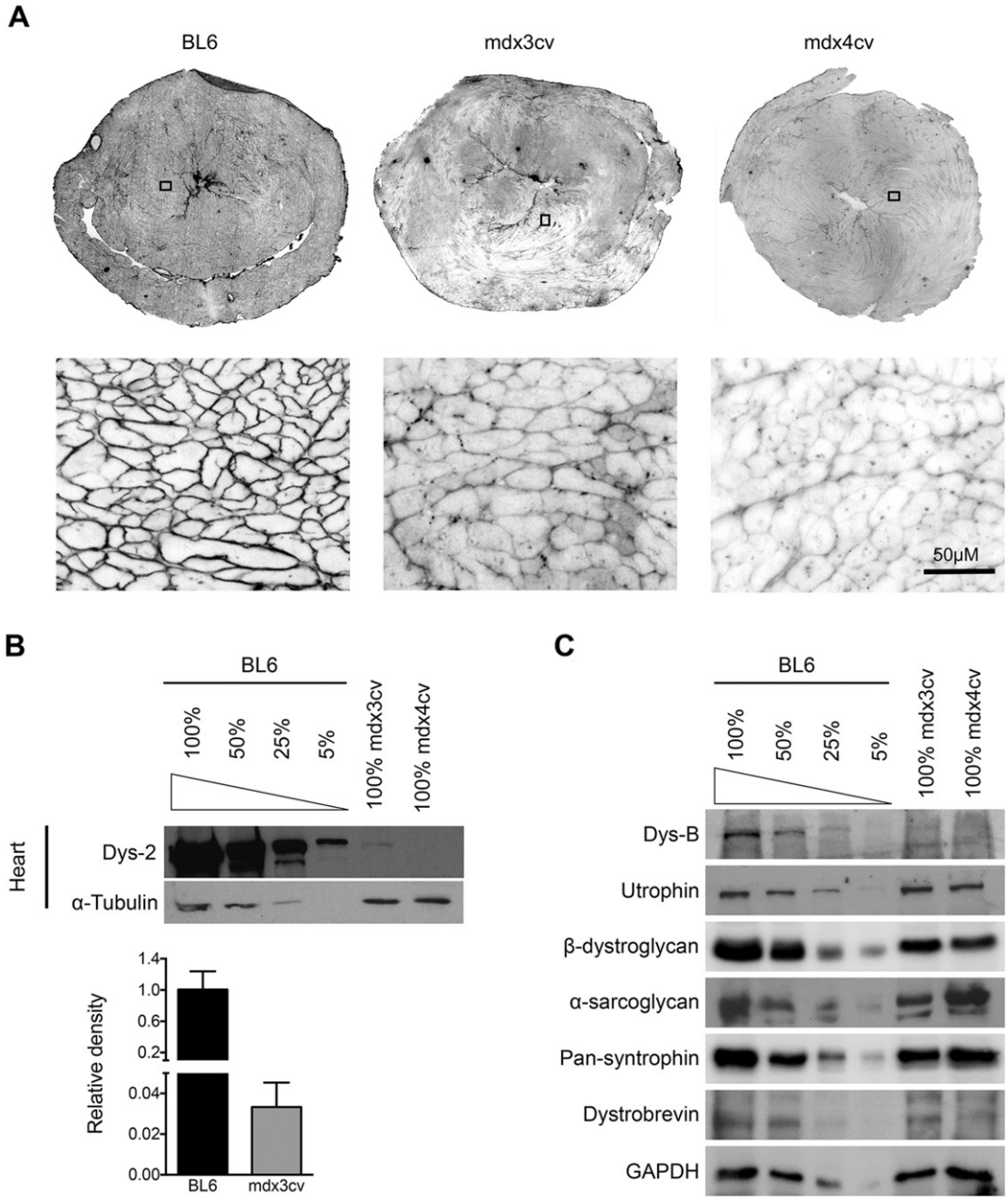

Fig. 1. Mdx3cv mouse heart expressed low-level dystrophin. A, Representative photomicrographs of dystrophin immunofluorescence staining in BL6, mdx3cv and mdx4cv heart. Upper panel shows the whole heart view and the lower panel shows a higher magnification of the corresponding boxed region in the whole heart view. B, Top panel, Representative heart western blot from BL6, mdx3cv and mdx4cv mice. The BL6 heart lysate was loaded at 100%, 50%, 25% and 5%. The mdx3cv and mdx4cv heart lysate was loaded at 100%; Bottom panel, Densitometry quantification of cardiac dystrophin expression ($N = 3$ for each group). Dys-2, a monoclonal antibody against the dystrophin C-terminal domain. The heart of mdx3cv mice showed uniform dystrophin expression at approximately 3.3% of the wild-type level. C, Representative cardiac western blots for utrophin and selected components of dystrophin-associated glycoprotein complex (β-dystroglycan, α-sarcoglycan, syntrophin and dystrobrevin). DysB, a monoclonal antibody against the dystrophin exons 10–12; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.