

Skeletal muscle and cardiac function in B10-mdx mice

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Introduction

Duchenne muscular dystrophy (DMD) is a disease characterized by progressive muscle weakness, eventually leading to heart failure. B10-mdx mice, which lack dystrophin, are widely used as a model of DMD¹⁾. However, the decline in muscle and cardiac functions is known to be mild²⁾, and it is thought that the compensatory upregulation of utrophin is one of the contributing factors³⁾. In this study, the pathological progression by aging of the muscle and cardiac functions was investigated in B10-mdx mice over time. Furthermore, we investigated whether the reduction of utrophin, in addition to dystrophin deficient, exacerbates the pathology.

Highlight of this study

- ✓ The decline in muscle function was detected by the grip test and treadmill test, but not by the rotarod test.
- ✓ Regarding cardiac function, diastolic function declined first, followed by a mild decline in systolic function.
- ✓ *Dmd* ex52 hemi knockout/*Utn* hetero knockout mice were generated.
- ✓ The decline in muscle and cardiac functions was comparable between mdx52 mice and mdx52/*Utn*^{+/-} mice.

Methods

Treadmill

- Running speed started at 6 cm/sec, then speed was increased 2 cm/sec every minute until exhaustion.

Grip strength

- Forelimb grip strength was measured 3 times for each mice.
- Maximum grip strength and force-decline rate* were calculated.

* (3rd measurement / 1st measurement) × 100 (%)

Rotarod

- The rotarod started at 4 rpm and accelerates to 40 rpm over 300 seconds.
- Latency to fall was determined (300 sec at maximum).

Echocardiography

- During the measurement, the heart rate was adjusted to approximately 500 bpm under isoflurane anesthesia.
- Data were obtained by long-axis B-mode, short axis M-mode, PW-doppler, and tissue doppler.



Aging effects of muscle and cardiac function in B-10 mdx mice

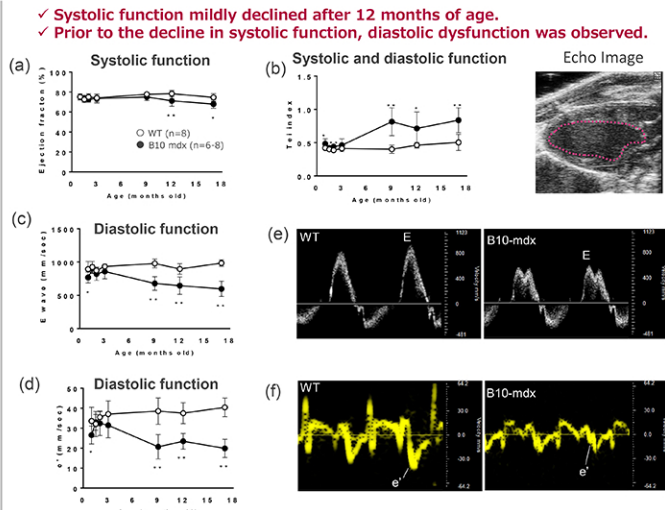
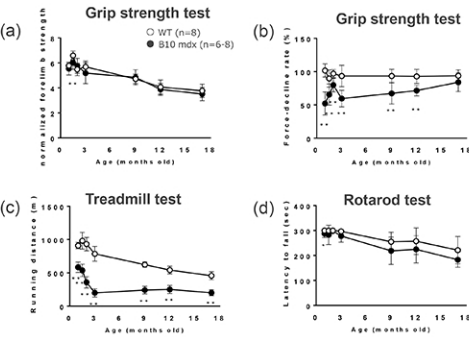
- ✓ The decline in muscle function was detected by the grip test and treadmill test, but not by the rotarod test.

Animals

C57BL/10 Jic (WT)
C57BL/10-mdx (B10-mdx)

Timepoint

1, 1.5, 2, 3, 9, 12 and 17 months of age



Comparison of muscle and cardiac function between mdx52 mice and mdx52/*Utn*^{+/-} mice

- ✓ To expect more severe and early onset of the disease, we generated *Dmd* ex52 hemi knockout/*Utn* hetero knockout mice.

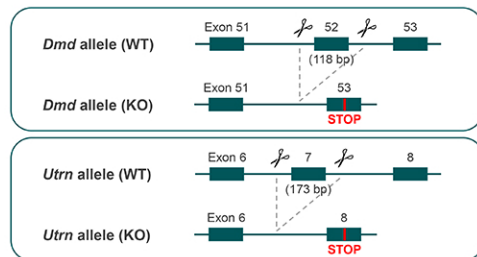


Fig.3 Generation of mdx52 mice and mdx52/*Utn*^{+/-} mice. Mdx52 knockout (KO) mice were generated using CRISPR/Cas9 technology. Guide RNAs (gRNAs) were designed to flank *Dmd* exon 52, and the resulting frameshift mutation creates a stop codon at exon 53, leading to the knockout of the *Dmd* gene. *Utn* KO mice were also generated using CRISPR/Cas9 technology. gRNAs were designed to flank *Utn* exon 7, and the resulting frameshift mutation creates a stop codon at exon 8, leading to the knockout of the *Utn* gene. Mdx52/*Utn*^{+/-} mice were obtained by crossing female heterozygous mdx52 mice with male heterozygous *Utn* KO mice.

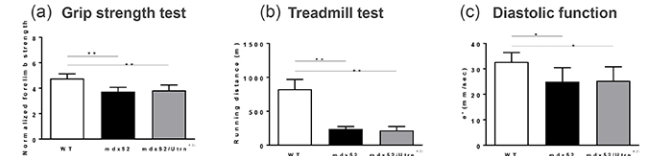
- ✓ Muscle function and cardiac function at 13-week-old mdx52 and mdx52/*Utn*^{+/-} mice were evaluated.
- ✓ Both mdx52 and mdx52/*Utn*^{+/-} mice exhibited a decline in muscle function and diastolic function.
- ✓ No significant difference were noted between mdx52 and mdx52/*Utn*^{+/-} mice

Animals

Wild type (WT)
Dmd ex52 hemi knockout (mdx52)
Dmd ex52 hemi knockout/*Utn* hetero knockout (mdx52/*Utn*^{+/-})

Timepoint

3 months of age



We might generate *Dmd* ex52 hemi knockout/*Utn* homo knockout mice to aim for a more severe disease model.

Conclusion

We evaluated the muscle and cardiac functions of B10-mdx mice over time and were able to detect functional decline. To expect more severe and early onset of the disease, we generated *Dmd* ex52 hemi knockout/*Utn* hetero knockout mice and evaluated the function similarly. Unfortunately, the severity of functional decline in mdx52/*Utn*^{+/-} mice was comparable to that in mdx52 mice. We might generate *Dmd* ex52 hemi knockout/*Utn* homo knockout mice to aim for a more severe disease model.

Reference

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- Gisela Gaina, et al., *Exp Ther Med*. 2021 Jun;21(6):610
- R M Grady, et al., *Cell*. 1997 Aug 22;90(4):729-38

COI disclosure information:

We have no financial relationship to disclose for our presentation contents.

If you are interested in the evaluation of cardiac function, muscle function, and dystrophin-utrophin double knockout, please contact the following.

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