Early cardiac dysfunction in mdx52/Utrn$^{-/-}$ mice as a severe preclinical model for Duchenne muscular dystrophy

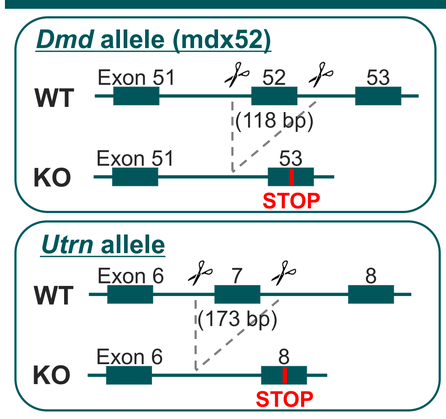
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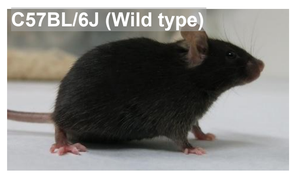
Introduction

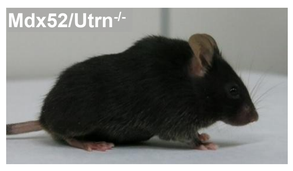
- Duchenne muscular dystrophy (DMD) is an X-linked disease characterized by progressive muscle weakness and cardiomyopathy, which is the leading cause of death.
- Conventional B10-mdx mice show mild pathology and lack early-onset cardiac dysfunction, limiting their utility for evaluating therapies targeting DMD-associated cardiomyopathy.
- We evaluated mdx52/Utrn$^{-/-}$ mice, which lack both dystrophin and utrophin, as a severe model that recapitulates key features of DMD, with a focus on cardiac function.

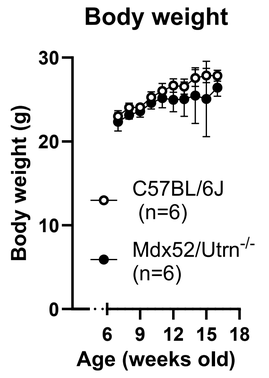
Aim: To characterize cardiac dysfunction in mdx52/Utrn$^{-/-}$ mice and evaluate their utility as a preclinical model of DMD.

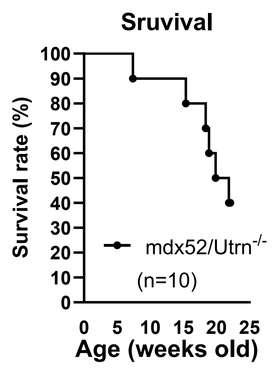
mdx52/Utrn$^{-/-}$ mice







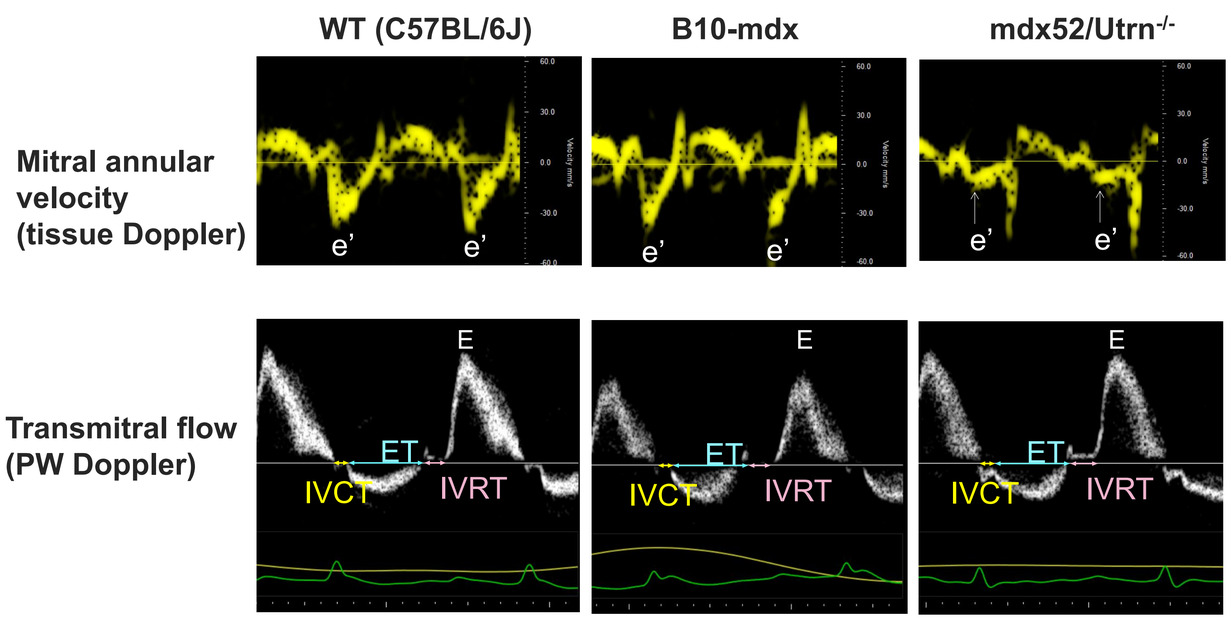




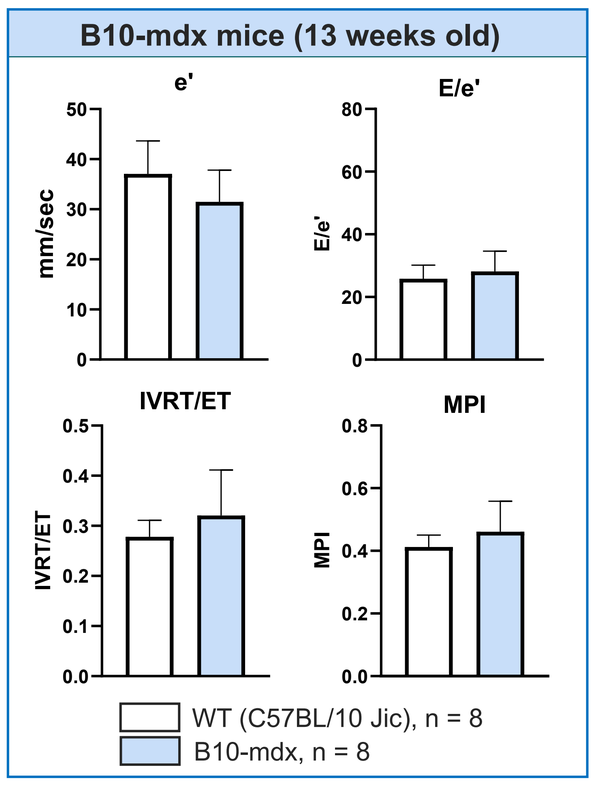
mdx52 and Utrn KO mice were generated by CRISPR/Cas9-mediated deletion of Dmd exon 52 and Utrn exon 7, respectively. mdx52/Utrn$^{-/-}$ mice were generated by a breeding colony and identified by genotyping.

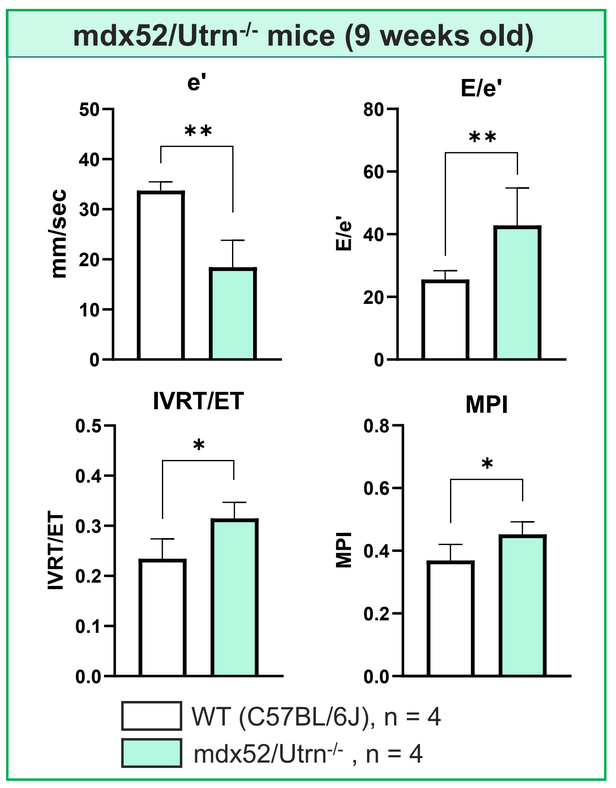
Results 1. Cardiac phenotypes in B10-mdx mice and mdx52/Utrn$^{-/-}$ mice

A Representative echocardiographic images



B Quantitative echocardiographic parameters



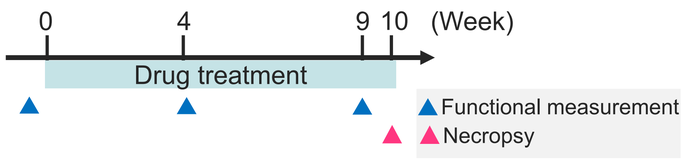


✓ **Diastolic dysfunction was evident in mdx52/Utrn$^{-/-}$ mice as early as 9 weeks, but not in B10-mdx mice at 13 weeks.**

Fig.1 Cardiac function in B10-mdx and mdx52/Utrn$^{-/-}$ mice. (A) Representative tissue Doppler and transmitral flow images from WT (C57BL/6J), B10-mdx, and mdx52/Utrn$^{-/-}$ mice. (B) Quantitative echocardiographic parameters in B10-mdx at 13 weeks (left, blue) and mdx52/Utrn$^{-/-}$ mice at 9 weeks (right, green). Data are expressed as mean + SD. *p < 0.05, **p < 0.01 vs. WT by Student's t-test. e', early diastolic mitral annular velocity; IVRT/ET, isovolumic relaxation time/ejection time; MPI, myocardial performance index.

Results 2. Time course and drug treatment in mdx52/Utrn$^{-/-}$ mice

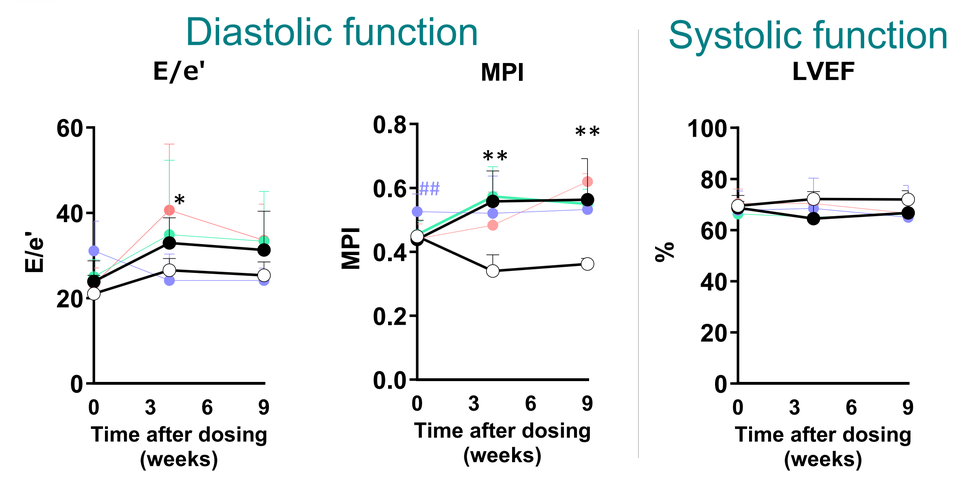
A Experimental design



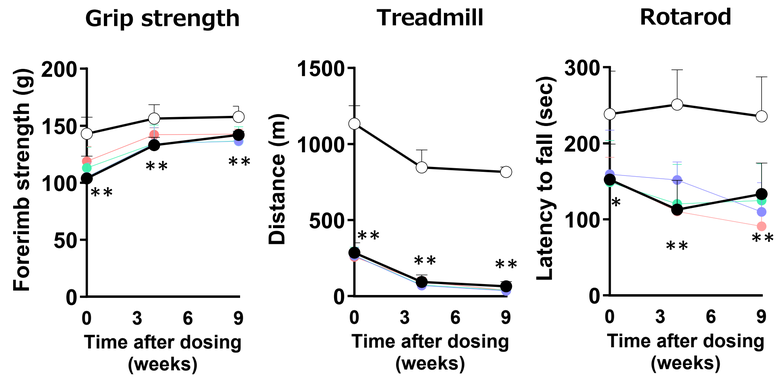
Animal	Drug	Dose (mg/kg/day)	Route	N
C57BL/6J	Vehicle	–	p.o.	6
mdx52/Utrn$^{-/-}$	Vehicle	–	p.o.	6
mdx52/Utrn$^{-/-}$	Roflumilast (PDE4i)	3	p.o.	6
mdx52/Utrn$^{-/-}$	Sildenafil (PDE5i)	80	p.o.	6
mdx52/Utrn$^{-/-}$	Empagliflozin (SGLT2i)	10	p.o.	6

WT (C57BL/6J)
mdx52/Utrn$^{-/-}$_Control
mdx52/Utrn$^{-/-}$_Roflumilast
mdx52/Utrn$^{-/-}$_Sildenafil
mdx52/Utrn$^{-/-}$_Empagliflozin

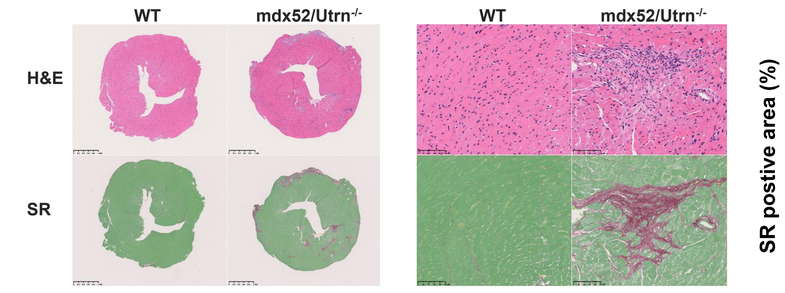
B Cardiac function



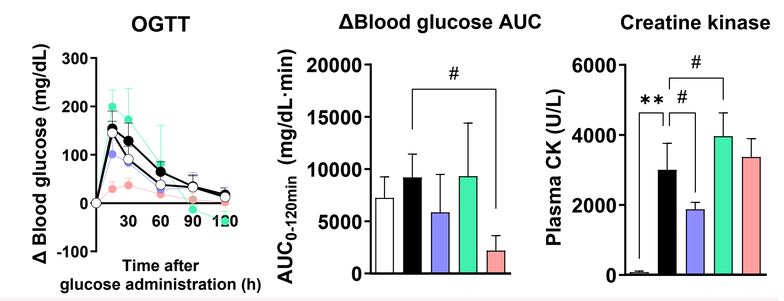
C Muscle function



D Cardiac fibrosis



E Pharmacodynamic effects



✓ **Diastolic dysfunction developed while systolic function was relatively preserved.**
✓ **Despite clear pharmacodynamic responses, none of the tested compounds significantly improved cardiac or muscle function under the conditions examined.**

Fig.2 Time course and drug treatment in mdx52/Utrn$^{-/-}$ mice. (A) Experimental design and treatment groups. (B) Cardiac diastolic function (E/e' and MPI) and systolic function (LVEF). (C) Muscle function (grip strength, treadmill and rotarod). (D) Representative H&E- and Sirius Red-stained cardiac sections and quantification of fibrotic area. (E) Pharmacodynamic effects. Data are expressed as mean + SD (n = 4–6 per group, depending on time point and group due to interim mortality). *p < 0.05, **p < 0.01 vs. WT by Student's t-test, #p < 0.05, ##p<0.01 vs. mdx52/Utrn$^{-/-}$ control by Dunnett's test. LVEF, left ventricular ejection fraction; OGTT, oral glucose tolerance test.

Conclusion

✓ **mdx52/Utrn$^{-/-}$ mice develop early-onset diastolic dysfunction, while systolic function is relatively preserved.**
✓ **This early diastolic dysfunction is consistent with the cardiac disease progression observed in patients with DMD.**
✓ **These findings support the utility of mdx52/Utrn$^{-/-}$ mice as a translationally relevant preclinical model for evaluating cardiac dysfunction in DMD.**

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Conflict of interest disclosure
All authors are employees of Axcelead Drug Discovery Partners, Inc.