# Silencing of Myotonic Dystrophy Protein Kinase (DMPK) Does Not Affect Cardiac or Muscle Function in Mice.

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## Background

- Myotonic dystrophy type 1 (DM1) results from a CTG repeat expansion in *DMPK,* and transcripts with a CUG expansion have toxic effects (RNA toxicity).
- Antisense oligonucleotides (ASOs) designed to reduce levels of toxic RNA are in clinical trails for DM1, but are capable of silencing wild-type *DMPK.*
- DMPK protein levels are already reduced by 50% in DM1 patients.
- Mice with *Dmpk* deletion reported to show cardiac conduction slowing (in heterozygotes) and muscle weakness and myopathy (in homozygotes).

Rationale: As antisense drugs may aggravate DMPK deficiency, this work explores the effect of *Dmpk* deletion and post-developmental *Dmpk* silencing on cardiac and skeletal muscle physiologic function in mice.

## SubQ injection of Dmpk-targeting ASO leads to silencing of *Dmpk* in heart and skeletal muscle

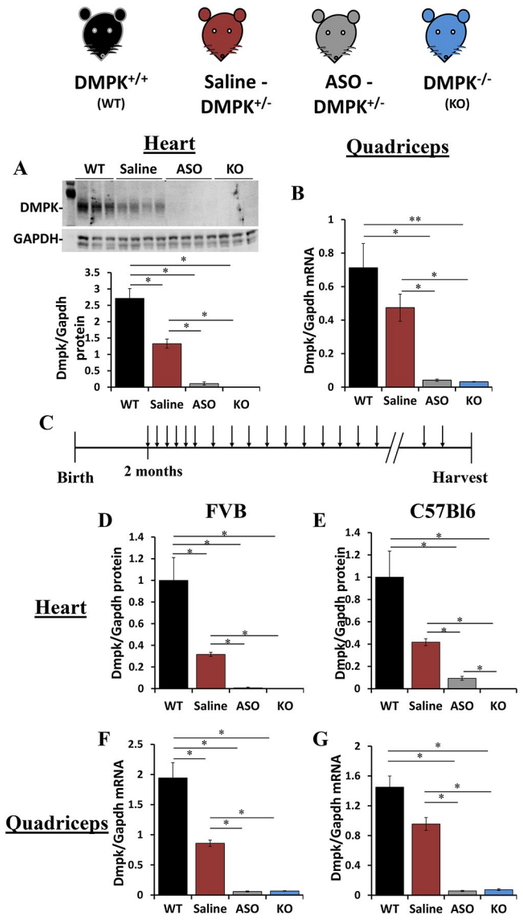



**Figure 1.** Dmpk heterozygous knockout mice were given subcutaneous injections of ASO 486178 or saline at 50 mg/kg. (A) Western blot and quantification of Dmpk protein levels in the heart (FVB) following 4 weeks of injections, with wild-type and Dmpk homozygous knockout controls. (B) Dmpk mRNA levels in quadriceps muscle (FVB) after 4 weeks of injections. (C) Long-term dosing regimen of weekly 50 mg/kg for 6 weeks, then biweekly. Dmpk protein levels in the heart (D and E) and Dmpk mRNA in quadriceps muscles (F and G) of FVB and C57Bl6 mice, respectively, following 8-10 months of ASO injections. * = ANOVA p < 0.05 and T-test p < 0.0084.

## *Dmpk* knockdown does not slow atrioventricular conduction in mice

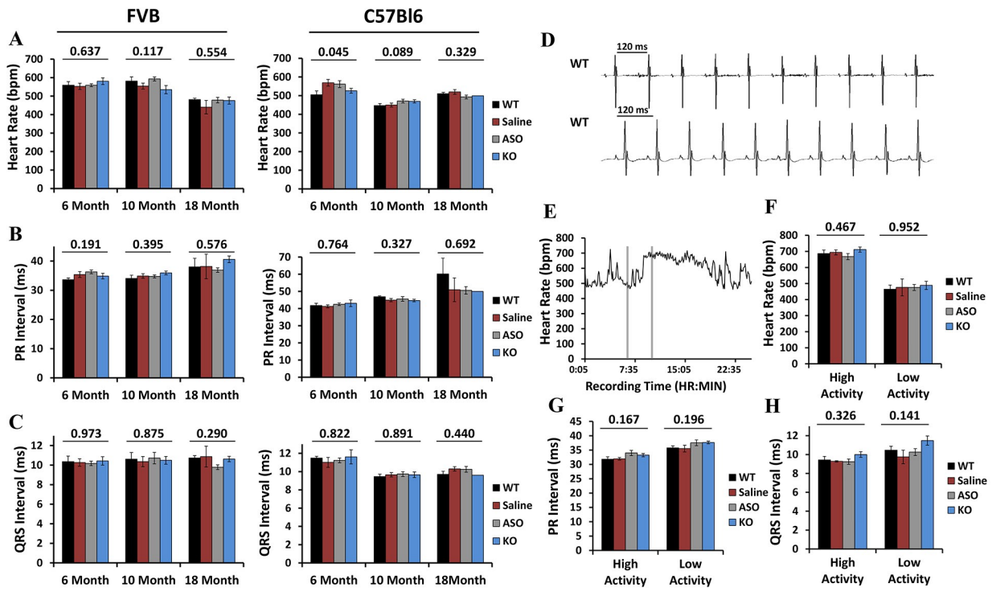



**Figure 2.** (A-C) Signal-averaged surface electrocardiography (ECG) measurements performed under light isoflurane anesthesia in 6-, 10-, and 18-month old FVB and C57Bl6 mice (n = 9-12 per group, gender-balanced at 6 and 10 months; n = 2-8 per group, gender-balanced at 18 months). (D) Representative ECG traces from surface recording (top) and implantable radio-telemeters (bottom). Conduction intervals were measured from an averaged waveform of 3-4 minutes of recording for surface ECG; 15 minutes of recording for radiotelemetry (high and low heart rate). (E) Representative plot of heart rate over 24 hour period for one mouse, demonstrating how high and low heart rate intervals were chosen. (F-H) Radiotelemetry ECG average heart rate and conduction intervals in 11-12 month old FVB mice. ANOVA p-value above each data set.

## *Dmpk* knockdown does not effect heart or skeletal muscle performance

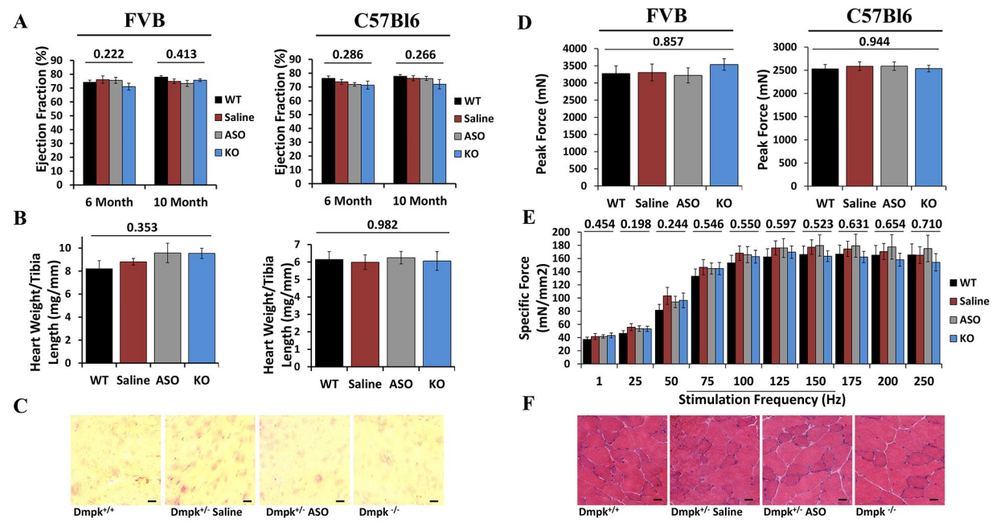
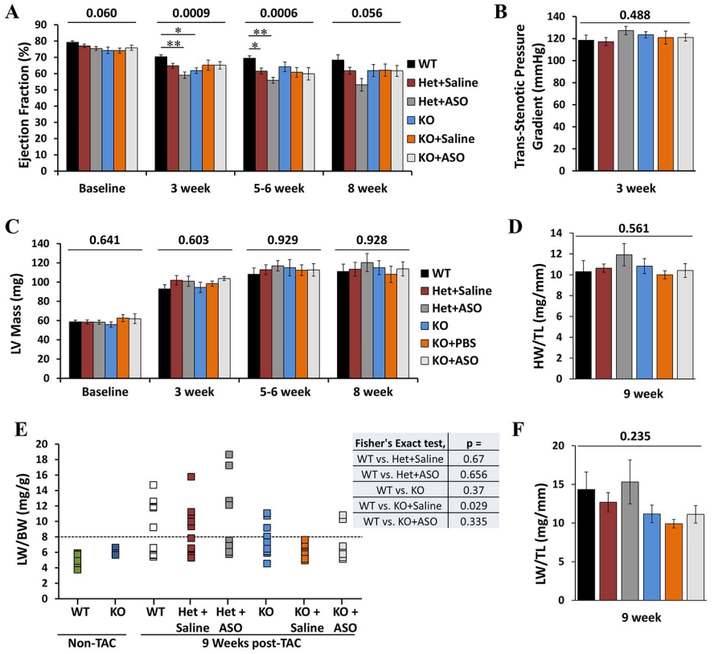



**Figure 3.** (A) Ejection fraction determined by M-mode echocardiography performed under light isoflurane anesthesia in FVB and C57Bl6 mice at 6 and 10 months of age (n = 9-12 per group, gender-balanced). (B) Average heart weight of FVB (n = 5-6) and C57Bl6 (n = 4-5) mice at 12 and 10 months, respectively. (C) Representative images of myocardial sections stained with picrosirious red for collagen from 18 month old FVB mice. Scale bar is 50 µm (D) Four-limb grip strength measurements in 8-9 month old mice (n = 9-12 per group). (E) Ex-vivo force measurements of *extensor digitorum longus* (EDL) muscles from 11-12 month old C57Bl6 mice (n = 3-4 mice; 5-8 muscles per group). (F) Representative images of quadriceps muscle stained with H&E from 18 month old FVB mice. Scale bar is 25 µm. ANOVA p-value above each data set..

## *Dmpk* knockdown has minimal effect in heart and skeletal muscle with superimposed stress




| Fisher's Exact test, | p = |
|---|---|
| WT vs. Het+Saline | 0.67 |
| WT vs. Het+ASO | 0.656 |
| WT vs. KO | 0.37 |
| WT vs. KO+Saline | 0.029 |
| WT vs. KO+ASO | 0.335 |

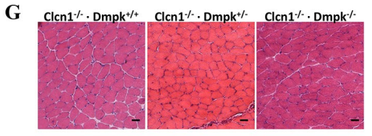



**Figure 4.** (A) Ejection fraction in FVB *Dmpk* mice before and following transverse aortic constriction (TAC) banding. Mice began dosing at 2 months of age and TAC was performed at 3 months of age (n = 17-19 for WT, Het-Saline, and Het-ASO at baseline thru 5-6 weeks. n = 8-11 for KO, KO+Saline, and KO+ASO and all groups at 8 weeks.) (B) Average stenosis pressure gradient as determined by peak velocity of flow, measured by pulsed-wave doppler echocardiography, groups same as in A. (C) Average left ventricular mass (Echo estimate) before and after TAC banding, group numbers same as in A. (D) Average heart weight of TAC groups 9 weeks post-TAC (n = 8-12). (E) Lung weight normalized to body weight, demonstrating frequency of decompensated heart failure (LW/BW > 8) for all groups 9 weeks post-TAC banding, with non-TAC controls, and (F) average lung weights normalized to tibia length, same groups as in D. (G) Representative images of cross-sections from gastrocnemius muscle stained with H&E from mice with generalized myotonia (*Clcn1* knockout mice) crossed with Dmpk knockouts (FVB). ANOVA p-value above each data set. * = T-test p < 0.003, ** = T-test p < 0.00005.

## *Conclusions*

- High-dose antisense oligonucleotide treatment can silence Dmpk mRNA in the mouse heart.
- Deletion or ASO-mediated long-term knockdown of *Dmpk* does not cause:
  - Cardiac conduction slowing
  - Cardiac contractile dysfunction
  - Cardiac fibrosis
  - Skeletal muscle contractile dysfunction (*in vivo* and *ex vivo*)
  - Skeletal myopathy
- Mice lacking Dmpk compensate to extra-physiologic stress similar to wild-type mice.

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