

Evaluation of MRI as an outcome measure for therapeutic trials in muscular dystrophy mice

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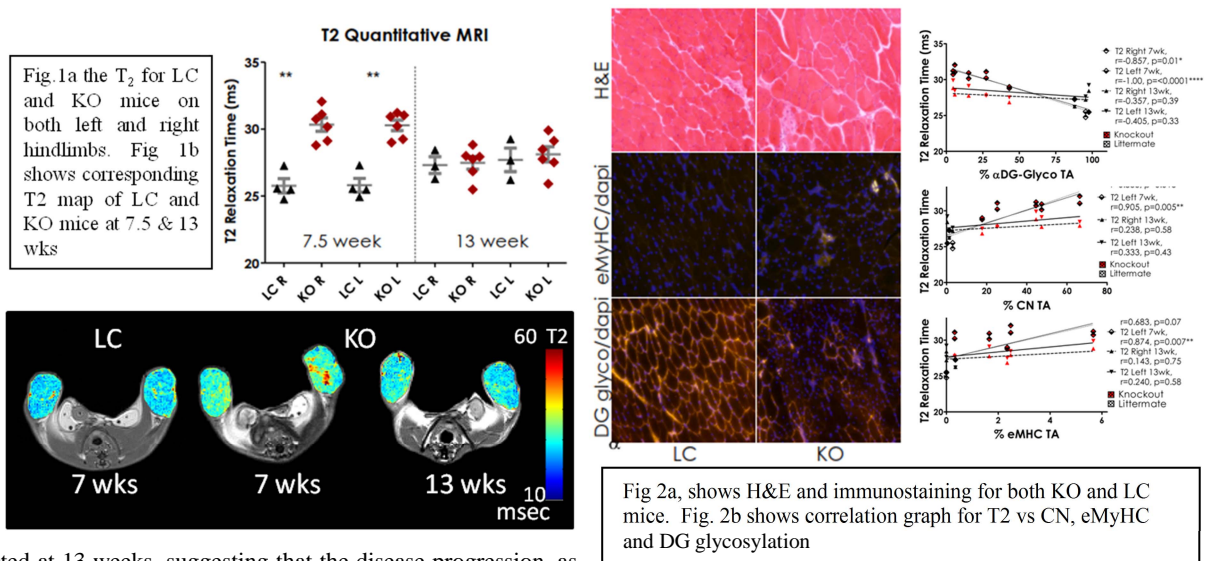
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Introduction: “Dystroglycanopathy” type muscular dystrophy occurs due to mutations in genes involved in dystroglycan (DG) glycosylation. Dystroglycanopathy patients and our model KO mice (ref) develop moderate to severe muscle disease. This variability in phenotype complicates therapeutic testing. Our primary goal is to identify reliable imaging tools to quantify disease phenotype before and after drug treatment for better intra-animal assessment of drug effect to overcome issues of inter-animal variation. Muscle MR imaging of dystrophic patients is becoming more prevalent (ref), so a similar biomarker in mouse drug trials, if reliable, could more easily be translated to clinical trials.

Materials and Methods:

Mice and MRI: Conditional dystroglycanopathy *Fktn* knockout (KO) (n=6) and littermate (LC) mice (n=3-4) at 7.5 and 13 wks were anesthetized and scanned by 7T Agilent small animal magnet using 38 mm ID birdcage coil. Quantitative T₂ relaxation measurements of mouse hindlimb were obtained using multislice spin echo (FOV=30mm, generated in Varian software by mono exponential, non-linear least squares fit of measured data at each pixel to the canonical T₂ signal equation. The mean T₂ of all hindlimb muscle pixels was calculated per slice and averaged from 5 to 6 slices to obtain the T₂ signal for each animal. Muscles were cryopreserved after the final scan and processed for histology. **Histological Analyses:** Muscle cryosections from KO and LC muscle were stained with H&E or processed for immunofluorescence. Central nucleation (CN), regeneration (embryonic myosin heavy chain, eMyHC, expression), and residual glycosylation were measured. Statistical measurements were performed using spearman (two tailed) and Kruskal-wallis ANOVA.

Results and Discussion: The KO mice scanned at 7.5 weeks showed edema-like “dystrophic pathology”- in all slices in the muscle with an elevated mean T₂ of 30.53±0.81 msec, whereas 13 week KO mice showed a T₂ of 28.89±0.8 msec (Fig 1a). The T₂ relaxation time of LC mice was 25.91±1.22. Severely dystrophic mice are weak and underweight so body weight is an indicator of disease in our mouse model. We found a strong correlation between body weight and T₂ at 7.5 weeks indicating that T₂, at certain stages of disease, may be a predictor of phenotype. There was clear distinction between the LC and KO mice histologically. DG glycosylation and regeneration (eMyHC, CN) profiles of all muscles tested (quadriceps; tibia anterior, TA; iliopsoas) were different between LC and KO mice (fig 2). We analyzed T₂ relaxation time versus histology in the TA. At 7.5 wks, there is a significant correlation between relaxation time and CN (r=0.905, p=0.005*), DG glycosylation (r=0.874, p=0.007*) and eMyHC (r=1, p<0.0001*) in our population of KO and LC mice (Fig 2b). However, unexpectedly, there was no correlation for T₂ collected at 13 weeks, suggesting that the disease progression, as measured by T₂, is not linearly progressive.



Conclusions: Our data suggest that quantitative T₂ MRI may be useful for prescreening mice for drug trials, but is limited by the stage of disease progression. Applying additional MR techniques, such as diffusion or ¹H MRI spectroscopy, may be able to answer why there was reduction of T₂ at 13 weeks in order for better understanding disease pathology.

References:

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