

AAV6-mediated delivery of a U7 exon skipping construct improves regional cardiac function in Golden Retriever muscular dystrophy dogs

S. C. Forbes¹, L. T. Bish², M. M. Sleeper³, W. Mai⁴, H. Sweeney², and G. A. Walter⁵

¹Department of Physical Therapy, University of Florida, Gainesville, FL, United States, ²Department of Physiology, University of Pennsylvania, Philadelphia, PA,

³Department of clinical studies, University of Pennsylvania, Philadelphia, PA, ⁴School of Veterinary Medicine, University of Florida, Philadelphia, PA, ⁵Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL

Introduction

Duchenne muscular dystrophy (DMD) is an x-linked genetic disorder that affects one in 3,500 boys, and is due to a deficiency in the protein dystrophin on the cytoplasmic membrane of muscle fibers (Hoffman et al. 1987). Cardiac dysfunction is prevalent in DMD, with heart failure a common cause of death (Finsterer & Stollberger 2003). Unfortunately, there is no cure or effective treatment for DMD; however, one promising intervention is exon skipping, which enables correcting the disrupted reading frame resulting in partial restoration and function of dystrophin. This study implements a novel approach of exon skipping by using viral mediated delivery of a modified U7 snRNA carrying antisense sequence to correct the disrupted reading frame in large animals. In order to evaluate the effects of this treatment on cardiac function we acquired cardiac magnetic resonance (CMR) left ventricular circumferential strain measures. Previous studies have shown CMR strain measures as an effective means of monitoring disease progression in *mdx* mice (Li et al. 2009) and children with DMD (Hor et al. 2010), and it has been suggested that this is a more sensitive measure to monitor disease progression in dystrophy than global cardiac functional measures, such as ejection fraction (Ashford et al. 2005; Hor et al. 2009). Thus, in this study we acquired CMR strain measures in treated and untreated Golden Retriever muscular dystrophy (GRMD) dogs. GRMD dogs are characterized by rapidly progressing myocardial involvement that appear to closely match that of children with DMD (Moise et al. 1991; Chetboul et al. 2004).

Methods

Percutaneous transendocardial delivery of AAV6-U7-ESE6/8 was employed to induce exon skipping in four GRMD dogs (9-10 months old, 14-19 kg). These dogs were treated with either low-dose AAV6 (n=2) or high-dose AAV6 (n=2). Various CMR sequences were implemented throughout treatment including gadolinium enhanced T1-weighted imaging and cine. Tagged cardiac images for strain analysis were acquired during the final time point after 13 months of treatment and were compared to three age-matched untreated GRMD dogs (age 23-25 month, 14-20 kg). During the CMR scans dogs were induced and maintained with intravenous infusion of propofol and fentanyl via the cephalic vein. Dogs were placed in the dorsal position in the bore of the magnet of a GE 1.5Telsa Signa MR system (GE Healthcare, Milwaukee, Wisconsin) and a torso array receive-only coil was positioned over the thoracic region. Cardiac imaging was performed with retrospective ECG gating and data were acquired in apnea by turning off the ventilator, along with infusion of a bolus of cisatracurium. Cardiac-gated tagged images were acquired using a fast spoiled gradient recalled (FSGR) sequence (FOV 24X24 cm; Acquisition Matrix 256X128; TR 9.2 ms; TE 5.5 ms; Flip angle 20°; Grid spacing 7mm). Images were acquired in the short-axis trans-ventricular view (4-5 slices, 8mm slice thickness, no slice gap). Positioning of the short-axis images was achieved using sagittal localizers and long axis four-chamber images of the heart (Mai et al. 2010). Triggering utilized a single cardiac phase cycle with minimum trigger delay and 16 frames per cardiac cycle. Analysis of the tagged images was performed using harmonic phase analysis (HARP; Diagnosoft, Palo Alto, California) for peak circumferential myocardial strain (Ecc) (Osman et al. 1999; Hor et al. 2010). The short-axis slice in the mid-papillary region of the left ventricle was chosen for analysis, with the average Ecc from each of six segments (Figs 2a and 2b) being calculated using the mid-wall with Eulerian algorithms (Osman et al. 2000).

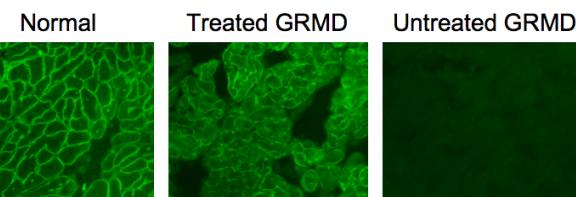


Fig. 1 Immunofluorescence showing dystrophin expression in normal, treated, and untreated GRMD dogs. Dystrophin was detected in all treated hearts, but was expressed at higher levels and in a greater number of regions throughout the heart in those treated with high-dose AAV.

Results

Exon skipping by viral-mediated delivery of an antisense sequence linked to U7 small nuclear RNA in GRMD dogs resulted in restoration of cardiac dystrophin expression at 13 months post-treatment (Fig.1). This dystrophin expression in the treated dogs was accompanied by a greater ($p<0.05$) average peak circumferential strain (Ecc) in the lateral left ventricular free wall region of the heart compared to untreated GRMD dogs (Fig 2c). Other regions of the heart showed more variability in Ecc among the treated dogs, with some dogs demonstrating greater strain than untreated dogs and other treated dogs showing similar values as the untreated dogs. Overall, there was general agreement between dystrophin expression and Ecc. For example, the treated dog with the lowest expression of dystrophin was also the dog that showed the lowest average Ecc of the treated dogs. Furthermore, when this dog was omitted from the comparison between groups due to the lower dystrophin expression levels in the heart, the overall average peak circumferential strain was greater ($p=0.027$) in treated ($-12.2 \pm 0.8\%$) than untreated ($-9.1 \pm 0.4\%$) GRMD dogs.

Conclusions

AAV6-mediated delivery of a U7 exon skipping construct enhanced dystrophin expression and resulted in greater left ventricular circumferential strain values in the lateral free wall segment than untreated dogs. Thus, the exon skipping treatment implemented in this study seemed to be effective in reducing regional cardiac dysfunction in dystrophic dogs. This treatment shows promise as a potential intervention in children with DMD.

References

- Ashford et al. Circulation 112 (16), 2462-2467, 2005.
- Chetboul et al. Eur Heart J 25 (21), 1934-1939, 2004.
- Finsterer J & Stollberger C. Cardiology 99 (1), 1-19, 2003.
- Hoffman et al. Cell. 51 (6), 919-928, 1987.
- Hor et al. JACC Cardiovasc Imaging. 3 (2), 144-151, 2010.
- Hor et al. J Am Coll Cardiol 53 (14), 1204-1210, 2009.
- Li et al. J Cardiovasc Magn Reson. 11 (1), 40, 2009.
- Mai et al. Vet Radiol Ultrasound. 51 (4), 428-435, 2010.
- Moise et al. J Am Coll Cardiol. 17 (3), 812-820, 1991.
- Osman et al. Magn Reson Med 42 (6), 1048-1060, 1999.
- Osman et al. IEEE Trans Med Imaging 19 (3), 186-202, 2000.

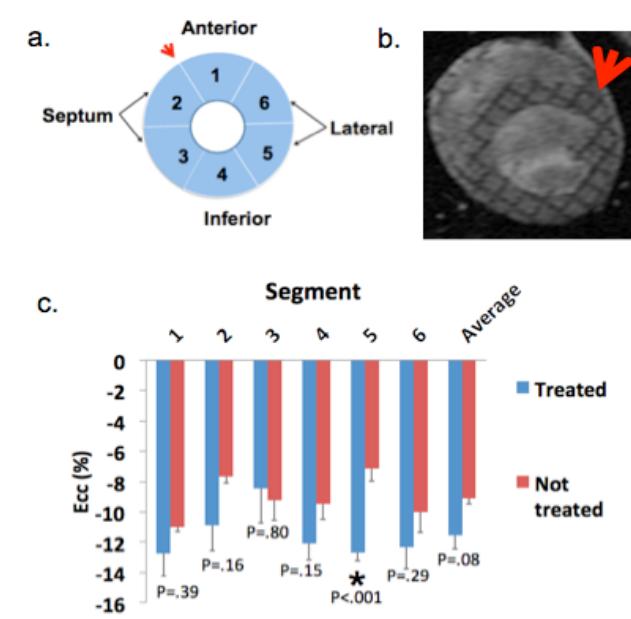


Fig. 2 Left ventricle myocardium in the short-axis view was divided into six equal segments with the first segment placed in the anterior region (a). The slice analyzed was in the mid-papillary region (b) and average peak circumferential strain (Ecc) in each region was calculated in the treated and untreated GRMD dogs (c).