**INTRODUCTION** Duchenne muscular dystrophy (DMD) is a most common and lethal genetic muscle disorder which lacks efficacious treatments. We have used AAV-human-mini-dystrophin genes to prevent and alleviate the pathologies of DMD in the mdx mouse model (5). However, the large animal model, the golden retriever muscular dystrophy (GRMD) dog, is clinically and biologically superior to the mdx model. This advantage is mainly because the disease characteristics observed in the GRMD model are more similar to human DMD patients, and as a large animal model it will be more relevant to assessing proper therapeutic doses. Due to limited availability of the GRMD dogs, we have first tested the biological functions of the AAV-mediated canine-mini-dystrophin in the mdx mouse model.

**MATERIALS AND METHODS**  
**Construction of Canine Mini-dystrophin and AAV Vector Production:** A canine version of the mini-dystrophin gene was constructed via RT-PCR from normal dog muscle. The N-terminus, 5 central rod domains, and the cysteine-rich domain were spliced together, generating the canine mini-dystrophin gene of 3.8 kb. This gene was subsequently cloned into the AAV vector driven by the CMV promoter (5). The serotype I AAV vectors were purified using the previously published protocol (6).

**Mice and AAV Vector Administration:** For young (10-day-old pups) and adult (2 months of age) male mdx mice, the AAV1-CMV-canine-mini-dystrophin vector was administered to one side of the gastrocnemius (GAS) muscle at the volume of 3 µl (1.25x10^11 VGP-particles) and 100 µl (2.5 x 10^11 VGP-particles), respectively, while the untreated contralateral side served as a negative control. The vector-treated four young and four adult mdx mice were analyzed 1 and 3 months post-treatment, respectively. The age-matched C57/BL10 served as a wild-type control. Evan’s Blue dye (EBD) was used to test the myofiber integrity according to previously described methods (5).

**RESULTS**  
We show highly efficient mini-dystrophin expression in muscles of both young and adult mdx mice after AAV1-mediated intramuscular gene delivery. In addition, histology of the canine-mini-dystrophin vector-treated muscle revealed a lack of fibrosis and fat infiltration, when compared to the contralateral untreated muscle (Fig. 1)

The results obtained in mdx mice demonstrated that AAV1-mediated canine mini-dystrophin gene transfer in mdx mice ameliorates dystrophic pathology and protects membrane integrity in both young and adult mdx mice. In the sarcolemma, nNOS regulates the homeostasis of reactive free radical species (Fig. 6A). The loss of nNOS due to DGC destabilization may contribute to the oxidative damage to muscles in DMD, since it has been shown that nNOS is dramatically reduced in mdx mice and DMD patients (1). Our canine mini-dystrophin construct lacking exons 45 ~ 48 and 71 ~ 79, was also able to restore nNOS to the sarcolemma, which was in agreement with previous study (2). We hypothesis that nNOS-synthrophin was recruited to the sarcolemma of the muscle cells mainly through dystrobrevin binding sites, not the dystrophin C terminal binding sites (Fig. 6B). Thus, the results obtained in these mdx mice pave the way for further testing of the canine mini-dystrophin vector in the GRMD model.

**DISCUSSION**  
The “mini- or micro- dystrophins” improve muscle pathology and protect myofiber membranes in the mdx mouse model (3-5, 7). We wish to build on their success with human mini-dystrophin vector into the dystrophin model. Unfortunately, our initial attempts were met with little success (data not shown). It is our hypothesis that the human mini-dystrophin gene might elicit an immune response in the GRMD dog, which in turn affects the efficacy of the vector delivery.

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**REFERENCES**  