Genetic reduction of an extracellular matrix gene improves contractile function in dystrophic *mdx* mice

D.R. McCulloch,[1] N.L. McRae,[1] R. Kelly,[1] L. Forgan[2] and N. Stupka ,[1] [1]School of Medicine, Deakin University, Warun Ponds, VIC 3216, Australia and [2]School of Life and Environmental Sciences, Deakin University, Warun Ponds, VIC 3216, Australia.

Versican is a chondroitin sulphate proteoglycan (CSPG) that serves as a transitional scaffold for extracellular matrix (ECM) deposition. Remodeling of versican by ADAMTS proteoglycanases regulates a plethora of cell behaviors associated with developmental morphogenesis. In skeletal muscle, expression of V0/V1 versican isoforms is increased during myogenesis and clearance of a versican rich pericellular matrix by ADAMTS5 and ADAMTS15 is necessary for efficient myoblast fusion (Stupka *et al.*, 2013). Versican is also a mediator of cellular inflammatory processes, including macrophage polarization (Chang *et al.*, 2012).

Duchenne muscular dystrophy (DMD) is a fatal hereditary disease caused by a mutation in the dystrophin gene rendering skeletal muscles highly susceptible to contraction-induced injury. In DMD, there is an inevitable expansion of the ECM and chronic inflammation leading to loss of contractile tissue. Versican protein levels are significantly elevated in skeletal muscle biopsies from patients with DMD, but absent in healthy, control muscle. In diaphragm and hindlimb muscles of dystrophin deficient *mdx* mice versican expression is also increased, with greater expression in the more severely affected diaphragm. Therefore, we hypothesised that the genetic reduction of versican will attenuate the pathology of dystrophic *mdx* mouse muscles.

All experiments were carried out with approval of Deakin University's Animal Ethics Committee in accordance with NH&MRC guidelines. Female *mdx* mice were crossed with male mice heterozygous for a transgene insertional mutation in the versican gene (*hdf* – heart defect mice; Mjaatvedt *et al.*, 1998). The resultant F1 male dystrophic pups, *mdx-hdf* and *mdx* control littermates, were confirmed by genotyping and Q-RT-PCR. At 6 weeks of age, prior to contractile function testing, mouse body composition was assessed (ESF-005, EchoMRI). Then the mice were anaesthetized *via* i.p. injection of medetomidine (0.6 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg) such that they were unresponsive to tactile stimuli. Fast twitch *extensor digitorum longus* (EDL) and the slow twitch *soleus* muscles were surgically excised, and muscle force production, endurance and recovery from fatigue were assessed *in vitro* (1300A Whole Mouse Test System, Aurora Scientific; N = 6). Following functional testing, anaesthetized mice were humanely euthanized by cervical dislocation and the heart and hindlimb muscles were collected for molecular and histological analysis.

Hindlimb muscles, specifically the fast twitch tibialis anterior muscle, of *mdx-hdf* mice had a ˜60% reduction in versican (*vcan*) mRNA transcripts (P=0.04; independent t-test). Body weight, lean mass, fat mass, EDL and *soleus* muscle mass were similar in *mdx-hdf* mice and control *mdx* littermates. Twitch force (P_t), maximal force (P_o) and specific force (sP_o) were similar in EDL muscles from *mdx-hdf* mice and *mdx* littermates. In *soleus* muscles, P_t and sP_o were also not different between *mdx-hdf* mice and control *mdx* littermates; however, a trend towards an increase in P_o was observed in the *mdx-hdf* mice (P=0.054; independent t-test). To assess muscle endurance and force recovery, EDL and *soleus* muscles were stimulated submaximally at 60 Hz every 5 s for 4 min, and then again at 2 min, 5 min and 10 min post fatigue. During 4 min of contractile activity, fast twitch EDL muscles from *mdx-hdf* mice fatigued less than those from *mdx* littermates (P<0.001; main effect - general linear model ANOVA). Furthermore, EDL force recovery following fatigue was 50-70% greater in *mdx-hdf* mice (P=0.003; main effect - general linear model ANOVA).Whereas in soleus muscles of *mdx-hdf* mice, endurance and force recovery following fatigue were similar as compared to *mdx* littermates. Our data indicate that a genetic reduction of versican levels significantly improves muscle function on an *mdx* background. Follow up studies are ongoing to assess the effects of versican reduction on skeletal muscle fibre type and markers of muscle damage, fibrosis and regeneration.

Chang MY, Chan CK, Braun KR, Green PS, O'Brien KD, Chait A, Day AJ, Wight TN. (2012). Monocyte-to-macrophage differentiation: synthesis and secretion of a complex extracellular matrix. *Journal of Biological Chemistry,* **287,** 14122-14135.

Mjaatvedt CH, Yamamura H, Capehart AA, Turner D, Markwald RR. (1998). The Cspg2 gene, disrupted in the hdf mutant, is required for right cardiac chamber and endocardial cushion formation. *Developmental Biology,* **202,** 56-66.

Stupka N , Kintakas C, White JD, Fraser FW, Hanciu M, Aramaki-Hattori N, Martin S, Coles C, Collier F, Ward AC, Apte SS, McCulloch DR. (2013). Versican processing by a disintegrin-like and metalloproteinase domain with thrombospondin-1 repeats proteinases-5 and -15 facilitates myoblast fusion. *Journal of Biological Chemistry,* **88**, 1907-1917.