Genetic reduction of the extracellular matrix protein versican modulates the pathology and function of dystrophic *mdx* mouse hindlimb muscles

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Expression of V0/V1 versican is increased during myogenesis, and clearance of a versican rich pericellular matrix by ADAMTS5 and ADAMTS15 facilitates efficient myoblast fusion (Stupka *et al.*, 2013). Versican also regulates inflammation, including macrophage polarisation and cytokine bioavailability. Versican expression is tightly regulated and overexpression is associated with disease pathology, including Duchenne muscular dystrophy (DMD), where the loss of dystrophin leads to increased muscle damage, inflammation and fibrosis. Versican is a major constituent of this fibrosis, as versican protein levels are elevated in muscle biopsies from patients with DMD. In diaphragm and hindlimb muscles of dystrophin deficient *mdx* mice, versican and vesikine (cleaved versican) expression is also increased. Thus, we wanted to investigate the effect of versican reduction on the pathology of *mdx* mouse muscles.

All experiments were carried out with approval of the Deakin University 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* (Mjaatvedt *et al.*, 1998)). The resultant F1 male dystrophic pups, *mdx-hdf* and *mdx*, were confirmed by genotyping and immunohistochemistry to assess versican and versikine protein levels. Experiments were conducted on 6 week and 26 week old *mdx-hdf* and *mdx* mice. Between 3 and 6 weeks, hindlimb muscles of *mdx* mice undergo a spontaneous bout of degeneration and regeneration, whereas by 26 weeks there is additional time for progressive degenerative changes to occur.

At 26 weeks, *mdx-hdf* and *mdx* were placed in metabolic cages for 25 h (Columbus Instruments). Body composition was assessed at 6 and 26 weeks (EchoMRI). Mice were then anaesthetized *via* IP injection of medetomidine (0.5 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg), such that they were unresponsive to tactile stimuli. EDL and *soleus* muscles were surgically excised, and muscle force production (determined from a force frequency curve) and fatigability were assessed *in vitro* (Aurora Scientific; N = 8-11 mice per group). Anaesthetised mice were humanely euthanised by cervical dislocation and tissues were collected for histological and molecular analysis.

At 6 and 26 weeks, body weight, lean mass, fat mass and EDL muscle mass were similar in *mdx-hdf* and *mdx* mice. Muscle force production (P_o) was also not different between *mdx-hdf* and *mdx* mice. During 4 min of intermittent contractile activity (60 Hz stimulation every 5 s), 6 week old *mdx*-hdf mice fatigued less than mdx littermates and force recovery (at 2, 5 and 10 min post) was also greater ($P < 0.001$, main effect genotype - GLM ANOVA). By 26 weeks, the fatiguablity of EDL muscles from *mdx* and *mdx*-hdf mice was no longer different. Although, spontaneous physical activity was increased in *mdx*-hdf mice by ~20% ($P = 0.024$; independent T-test).

Muscle morphology and dystrophic pathology was assessed using H&E staining. At 6 weeks, the total number of muscle fibres/mm^2 ($P = 0.01$) and the number of undamaged muscle fibres/mm^2 ($P = 0.01$) was greater in EDL muscles of *mdx* mice compared to *mdx-hdf* mice, whilst the number of centrally nucleated fibres was not different. By 26 weeks, there was no difference in the total number of muscle fibres/mm^2 between *mdx* and *mdx-hdf* mice, however, the *mdx-hdf* mice had more undamaged fibres/mm^2 ($P = 0.01$). This was associated with a trend towards reduced degeneration and mononuclear infiltration (P=0.07) and reduced gene expression of the macrophage marker F4/80 ($P = 0.003$) in EDL muscles from 26 week old *mdx-hdf* mice.

We hypothesize that regeneration is altered in 6 week old *mdx-hdf* mice, given the decreased number of muscle fibres and improved fatigability, possibly mediated by the effects of versican reduction on satellite cell activation or proliferation (Velleman *et al.*, 2012). By 26 weeks, the EDL muscle morphology of *mdx-hdf* mice is improved, perhaps due to attenuated inflammation and degeneration, and physical activity is also increased. Together these initial findings indicate that the genetic reduction of versican modulates the dystrophic pathology of *mdx* mice and analysis is ongoing to comprehensively characterize the effects of versican reduction on regeneration and inflammation.

Mjaatvedt CH, Yamamura H, Capehart AA, Turner D, Markwald RR. (1998). *Dev Biol* **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). *J Biol Chem* **288,** 1907-1917.
Velleman SG, Sporer KR, Ernst CW, Reed KM, Strasburg GM. (2012). *Poult Sci* **91**, 1964-1973.