The consequences of the genetic reduction of the extracellular protein versican on hindlimb muscle function and structure depend on muscle fibre type and age in dystrophic *mdx* mice

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Duchenne muscular dystrophy (DMD) is characterized by muscle degeneration and ineffective repair. Accumulation of extracellular matrix (ECM) is a consequence of the disease and exacerbates the pathology. Versican, a transitional ECM proteoglycan, is upregulated in fibrotic lesions of dystrophic muscles. Versican is remodelled by A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS) versicanases to generate the bioactive DPEAAE versikine fragment. In cultured C2C12 muscle cells, versican expression is down-regulated by glucocorticoids, the only treatment with clinical efficacy in DMD. Thus, we hypothesized that the genetic reduction of versican would ameliorate the pathology of dystrophic hindlimb muscles from *mdx* mice. Hence, 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)). All experiments were carried out in accordance with NHMRC guidelines with approval from the Animal Ethics Committee at Deakin University (A79-2011; G06-2015). At 6 or 26 weeks of age, F1 *mdx* and *mdx*-hdf male pups were anesthetized *via* intraperitoneal injection of medetomidine (0.6 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg) until unresponsive to tactile stimuli. Fast twitch *extensor digitorum longus* (EDL), and slow twitch *soleus* muscles were surgically excised, and then muscle strength, fatiguability and recovery from fatigue were assessed *in vitro* (1300A Whole Mouse Test System, Aurora Scientific). Mice were euthanized by cervical dislocation and tissues collected for molecular and histological analysis.

To confirm the genetic reduction of versican, immunohistochemical staining and specific antibodies for versican (anti-GAGβ domain; Merk Millipore) and versikine (versican V0, V1 neo antibody; ThermoFisher Scientific) were used. Versican and versikine protein levels were greater in dystrophic hindlimb muscle at 6 *versus* 26 weeks of age. Versikine, but not versican, protein levels were decreased 51% (P=0.01) in EDL muscles of *mdx*-hdf mice. Whereas, in *soleus* muscles of 6 week old *mdx*-hdf mice, versican expression was decreased by 91% (P=0.004) with similar versikine expression. By 26 weeks of age, this decrease in versican expression was no longer apparent.

Bodyweight, EDL and *soleus* muscle mass were similar in *mdx*-hdf and *mdx* mice. The genetic reduction of versican had no effect on EDL muscle strength normalized to muscle size (sP_o) at 6 and 26 weeks of age. Following 4 min of intermittent, submaximal (60 Hz) stimulation, EDL muscles from 6 week old *mdx*-hdf mice fatigued less (P<0.001; main effect GLM ANOVA) and recovered force better (P<0.001; main effect GLM ANOVA) than those from *mdx* littermates. By 26 weeks, this improvement in EDL muscle endurance was lost. Soleus muscles from 6 week old *mdx*-hdf mice were stronger than those from *mdx* mice, as indicated by an upward shift of the force (sP_o) frequency curve (P=0.02; main effect GLM ANOVA). However by 26 weeks, *soleus* muscles from *mdx*-hdf mice produced less force (P<0.001; main effect GLM ANOVA). Fatigability was reduced in *soleus* muscles from *mdx*-hdf mice at 6 (P<0.03, main effect GLM ANOVA) and 26 weeks (P<0.02, main effect GLM ANOVA), with no change in force recovery.

The genetic reduction of versican had distinct effects on muscle pathology depending on fibre type and age. At 6 weeks, EDL and *soleus* muscles from *mdx*-hdf mice had fewer muscle fibres/mm^2 of tissue (P=0.01 and P=0.04, respectively, t-test). In *soleus* muscles, this was associated with a trend for an increase in fibre size (P=0.07), which may contribute to the increase in muscle strength. Whilst at 26 weeks, the number of muscle fibres/mm^2 was similar in EDL and *soleus* muscles from *mdx*-hdf and *mdx* mice. Muscle fibre cross-sectional area was reduced in *soleus*, but not EDL, muscles from *mdx*-hdf mice. Only in *soleus* muscles, was an increase in mRNA transcript abundance of genes associated with ECM synthesis and remodelling observed; including, TGF-β1 (P=0.02), collagen a1 (P=0.05) and a trend in ADAMTS5 (P=0.07). All together, these changes may account for the decrease in *soleus* muscle strength in *mdx*-hdf mice at 26 weeks of age.

Overall, our data demonstrate that the genetic reduction of versican has distinct effects on muscle structure and function depending on the muscle fibre type and age, specifically postnatal growth. These factors need to be considered when developing preclinical strategies to target fibrosis and ECM remodelling in dystrophic muscles.

Mjaatvedt CH, Yamamura H, Capehart AA, Turner D, Markwald RR. (1998). *Dev Biol* **202**, 56-66.