

# Blocking CCN2 Reduces Established Palmar Neuromuscular Fibrosis and Improves Function following Repetitive Overuse Injury

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**INTRODUCTION:** Overuse injuries are a leading cause of long-term musculoskeletal pain and disability, and result in significant lost work time for patients and increased health care expenditures annually. We have developed a volitional rat model of overuse injuries in which subjects perform a reaching and lever-bar pulling task. Rats engaged in long-term performance of a lever pulling task at high repetition high force (HRHF) levels develop changes in neuromuscular tissues of the forelimb, including muscle fibrosis, epitendon thickening, and extraneural fibrosis. These changes in forelimb tissues are accompanied by functional declines. We have identified the matricellular protein connective tissue growth (CTGF/CCN2) as critical to the early progression of chronic forelimb tissue fibrosis in this model.<sup>1</sup> We have previously shown that inhibition of CCN2 using the anti-CCN2 monoclonal antibody, FG-3019 (Pamrevlumab), reduces established forelimb muscle fibrosis, nerve fibrosis, and improves functional declines.<sup>2,3</sup> Here, we tested whether fibro-degenerative changes also occur in forepaws following HRHF task performance and if anti-CCN2 antibody treatment reduces them and corresponds with improvements functional declines.

**METHODS:** All experiments were approved by the Institutional Animal Care and Use Committee in compliance with NIH guidelines. Adult female rats (3 mo at onset) performed a high repetition high force (HRHF) task at 4 reaches/min, at 55% maximum pulling force (1.22 N) for 2 hr/d, in four 30 min sessions/d, with 1.5 hr between sessions, 3 d/wk, for 18 wks. One subset of HRHF rats was euthanized after 18 wks of task performance (HRHF-Untreated, n=10). Two subsets were provided 6 wks of rest after task cessation, with concurrent treatment with anti-CCN2 monoclonal antibody (FG-3019, gift from FibroGen, Inc; 40 mg/kg body wt, i.p.; 2x per wk; HRHF-Rest/FG-3019 group, n=6), or non-immune human IgG as vehicle control (HRHF-Rest/IgG group, n=5). Results were compared to control rats administered vehicle IgG (Control+IgG, n=10). Grip strength, forepaw mechanical allodynia (using monofilament testing), and median nerve conduction (Natus system) were assayed. Forepaw and wrist tissues were collected. Fibrosis was quantified as % area with Masson's trichrome blue stained pixels. After fast green/Safranin O staining, entheses were scored in a binary fashion on six domains: tidemark changes, underlying bone remodeling, fissuring, void space, vascular invasion, and attachment site holes.

**RESULTS:** Muscle fibrosis in forepaw muscles (including thenar, hypothenar, and intrinsic) was increased in HRHF-Untreated and HRHF-Rest/IgG rats, but not HRHF-Rest/anti-CCN2, compared to Controls (Fig. 1A-B). Extraneural fibrosis was also increased in HRHF-Untreated and HRHF-Rest/IgG rats, but not HRHF-Rest/anti-CCN2, compared to Controls (Fig. 1E). Enteseal damage was increased in HRHF-Untreated rats, compared to Controls and HRHF-Rest/anti-CCN2 (Fig. 1C-D). Grip strength declines observed in HRHF-Untreated improved in HRHF-Rest groups, more so in HRHF-Rest/anti-CCN2 (Fig. 1F). Mechanical sensitivity seen in HRHF-Untreated improved with rest (Fig. 1G). We found an inverse correlation between grip strength and muscle fibrosis  $r = -0.41$ , Fig. 1H), enteseal damage, and extraneural fibrosis ( $r = -0.40$ ). We found a positive relationship between grip strength and nerve conduction velocity ( $r = 0.62$ , Fig. 1I), and extraneural fibrosis and mechanical sensitivity ( $r = 0.55$ , Fig. 1J).

**DISCUSSION:** This study demonstrates for the first time that anti-CCN2 treatment reduces established forepaw muscle and extraneural fibrosis and enteseal damage following overuse injury and that these changes correlate with improvements in function. These results are highly encouraging for use of FG-3019 (Pamrevlumab) for therapeutic treatment of neuromuscular fibrosis to help restore upper extremity function following chronic overuse.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Repetitive overuse is a major contributor to musculoskeletal injuries that are leading causes of physical disability worldwide. The use of FG-3019 (Pamrevlumab), a drug already granted Orphan Drug Designation in Idiopathic Pulmonary Fibrosis and Duchenne Muscular Dystrophy, could provide great therapeutic benefit in these patients.

**REFERENCES:** <sup>1</sup>Barbe et al, J Orthop Res. 2019 Sep; 37(9):2004-2018. <sup>2</sup>Barbe et al, FASEB J. 2020 May; 34(5):6554-6569. <sup>3</sup>Barbe et al, J Orthop Res. 2020 Nov; 38(11):2396-2408.

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