The Vitamin D Activator, CYP27B1, is a Novel Muscle Biomarker of ALS Disease Progression

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Objective: To validate CYP27B1 as a novel muscle biomarker in amyotrophic lateral sclerosis (ALS).

Background: Pathological changes in skeletal muscle, including the neuromuscular junction, occur at the earliest stages of ALS and progress in parallel with clinical disease. Molecular changes that underlie skeletal muscle pathology in ALS may therefore serve as biomarkers that can assist with diagnosis and/or monitoring of disease progression. We previously performed RNA sequencing on muscle biopsies from ALS patients and identified an elevation of the vitamin D activator, CYP27B1.

Design/Methods: RNA was extracted from muscle biopsy samples from patients with definite or probable ALS (n = 29), myopathy (n = 8), neuropathy (n = 12), or no neuromuscular disease (n = 10) and assessed by qPCR for CYP27B1. For some of the biopsies, CYP27B1 protein expression was assessed by western blot and immunohistochemistry. To evaluate disease progression, muscle samples from the $SOD1^{G93A}$ ALS mouse were collected at different ages and assayed for CYP27B1 expression.

Results: CYP27B1mRNA and protein levels were significantly increased in human ALS muscle samples versus control and myopathy samples. Neuropathy-associated samples also had increased levels, but to a lesser extent than the ALS group. In the $SOD1^{G93A}$ mouse, mRNA and protein levels were similarly elevated and showed progressive increases with clinical progression, beginning in the pre-symptomatic stage up to end-stage. Immunohistochemistry showed intense CYP27B1 staining of atrophied muscle fibers in both human and mouse ALS muscle.

Conclusions: These data validate CYP27B1 as a muscle biomarker in ALS and indicate its potential as a marker of ALS disease progression. These findings also suggest a perturbation of vitamin D signaling in the denervated myofiber. Further characterization of this pathway may provide insight into underlying molecular processes linked to muscle denervation.