Montana State University Billings

An Elongator Knockout Mouse Model for ALS

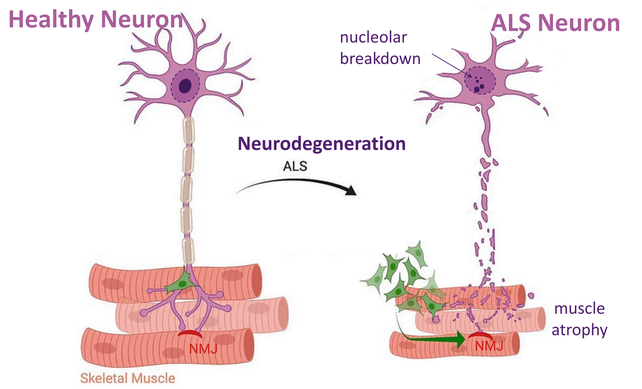
Magge Snow[1], Sara Snyder[1], Rachel Trudell[1], Renzie Pond[1], BreAnna Cameron[1], Lynn George[1]

[1]Montana State University Billings, Billings Montana, USA

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that results in the death of motor neurons. As a consequence of motor neuron death, the muscles they innervate atrophy, causing patients to lose their ability to walk, talk, eat, and eventually breath, such that patients typically die within 4 years of diagnosis. Worldwide, ALS is the most common motor neuron disease. Fifteen people are diagnosed with ALS every day and importantly, the number of cases is projected to increase 69% by the year 2040. The George Lab studies a molecular complex called Elongator, and specific mutations in genes encoding Elongator subunits are associated with ALS. To determine whether motor neurons express Elongator, we used a genetically engineered reporter mouse that "reports" the expression of *Elp1,* encoding the scaffolding subunit for Elongator. Our results indicate that *Elp1* is in fact expressed by alpha motor neurons, a subpopulation of motor neurons in the spinal cord that is most impacted in ALS. To investigate Elongator's specific function in this cell type, we then generated a conditional knockout (CKO) mouse, where *Elp1* is selectively ablated in motor neurons. These mice exhibit reduced motor function, as evidenced by PaGE testing, motor fasciculations, diminished muscle mass and overall body weight (~ ½ the weight of their littermate controls), and a shortened life span (averaging only 3 months). All of these symptoms are hallmark features of ALS. We hypothesized that the phenotype of our CKO mice is due to the death of motor neurons. To investigate this question, the number of alpha motor neurons in the lumbar enlargement was quantified in control and CKO mice using immunohistochemistry and Image J software. Alpha motor neuron numbers were found to be significantly decreased in the CKO. In conclusion, these data demonstrate that Elongator function is essential for the function and survival of motor neurons. Additionally, our *Chat-Cre; Elp1LoxP/LoxP* mice represent a new Elongator mouse model for studying the cellular and molecular mechanisms that contribute to ALS.

Amyotrophic Lateral Sclerosis



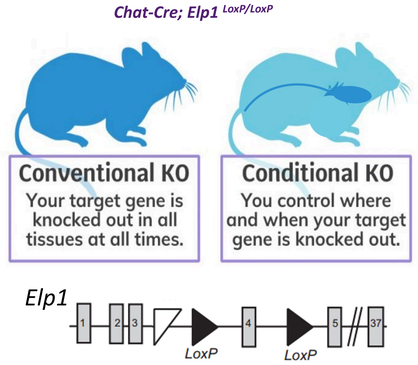
METHODS

***Elp1* reporter mice and beta galactosidase staining:** In *Elp1:BetaGal* mice, the enzyme beta galactosidase (beta gal) is selectively expressed in *Elp1* expressing cells (the beta gal gene is regulated by the same regulatory elements that regulate *Elp1* expression). In the presence of the substrate for beta galactosidase (X-gal), a blue pigment is produced, allowing the visualization of *Elp1* expression.

25 um frozen cross sections from the thoracic spinal cord of adult *Elp1:BetaGal ; Chat-EGFP* mice were collected using a Leica CM1860 cryostat. Sections were incubated in a staining solution containing 1mg/ml X-gal for 3 hours, followed by immunohistochemistry (IHC) using primary antibody anti-GFP (Abcam 13970; 1:1000) and a fluorescent secondary antibody (ThermoFisher Alexafluor 488; 1:2000) to visualize motor neurons. Sections were imaged at 10X (Fig. 1B) and 40X (Fig. 1C) using a Nikon TE200 inverted microscope and were captured with a QImaging QICAM 12-bit Mono Fast 1394 cooled camera and SPOT software. Images were overlaid using Photoshop.

***Elp1* conditional knockout mice.** In *Chat-Cre; Elp1$^{LoxP/LoxP}$* conditional knockout (CKO) mice, *Elp1* is selectively ablated in motor neurons. This is accomplished by Cre recombinase recognizing *LoxP* sites and excising the intervening sequence from the *Elp1* gene.

Spinal cords from control and CKO mice were sectioned at 16µm, collecting every third section, through the lumbar enlargement, beginning at the rostral end of each spinal cord segment. IHC was preformed using primary antibody anti-GFP followed by a fluorescent secondary antibody as described above. Images of every fifth section were collected at 10X magnification, and every 20th section at 40X magnification. For quantification of motor neurons, a single spinal cord hemisphere from the six sections containing the most alpha motor neurons was analyzed. Image J was used to outline and measure each motor neuron. Motor neurons measuring ≥ 500µm2 were classified as alpha motor neurons, and those measuring ≤ 300µm2 were classified as gamma motor neurons. Neurons measuring less than 150µm2 were omitted.



FUNDING

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RESULTS

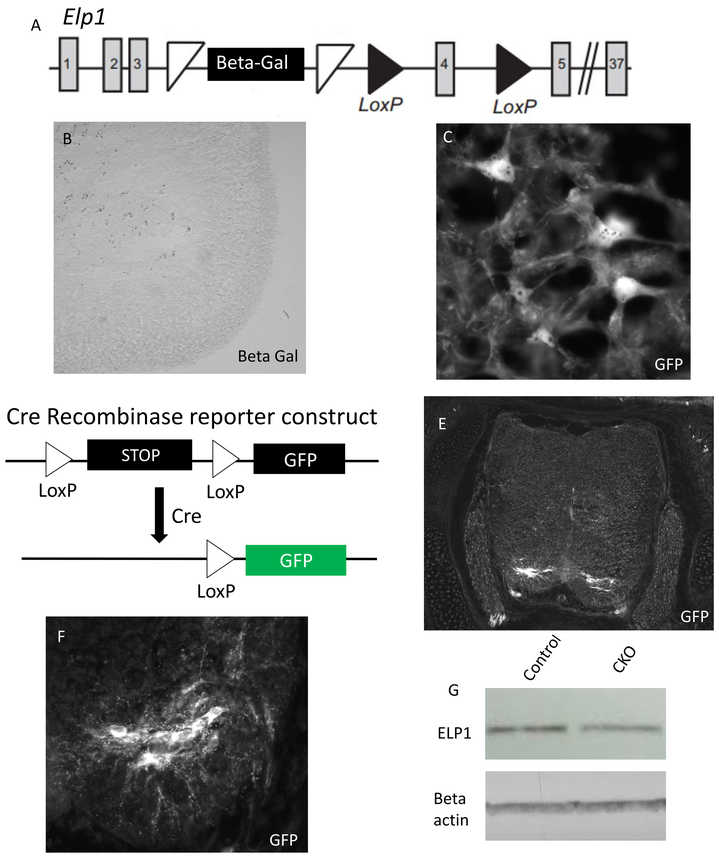

Figure 1. Control Experiments. A) Genetic construct carried by *Elp1:Beta Gal* reporter mice. B) *Elp1:Beta Gal* expression in the ventral horn of the spinal cord. C) Beta Gal staining (black puncta) and *Chat-GFP* expression co-localize in motor neurons of the spinal cord of *Elp1:Beta Gal; Chat-GFP* mice D) Cre recombinase reporter construct shows *LoxP* sites flanking a stop codon. In the presence of Cre recombinase, the stop codon is removed and GFP is expressed, "reporting" the activity of Cre. E,F) P0. GFP expression in the spinal cord of *Chat-Cre; Cre* recombinase reporter mice shows that recombinase activity is specific to motor neurons. E = 10X; F = 40X. G) Western blots using an anti-ELP1 antibody show that ELP1 protein levels are reduced in spinal cords of *Chat-Cre; Elp1$^{LoxP/LoxP}$* CKO mice. Residual ELP1 in the CKO lane corresponds to intact *Elp1* expression in spinal cord cell types other than MNs.

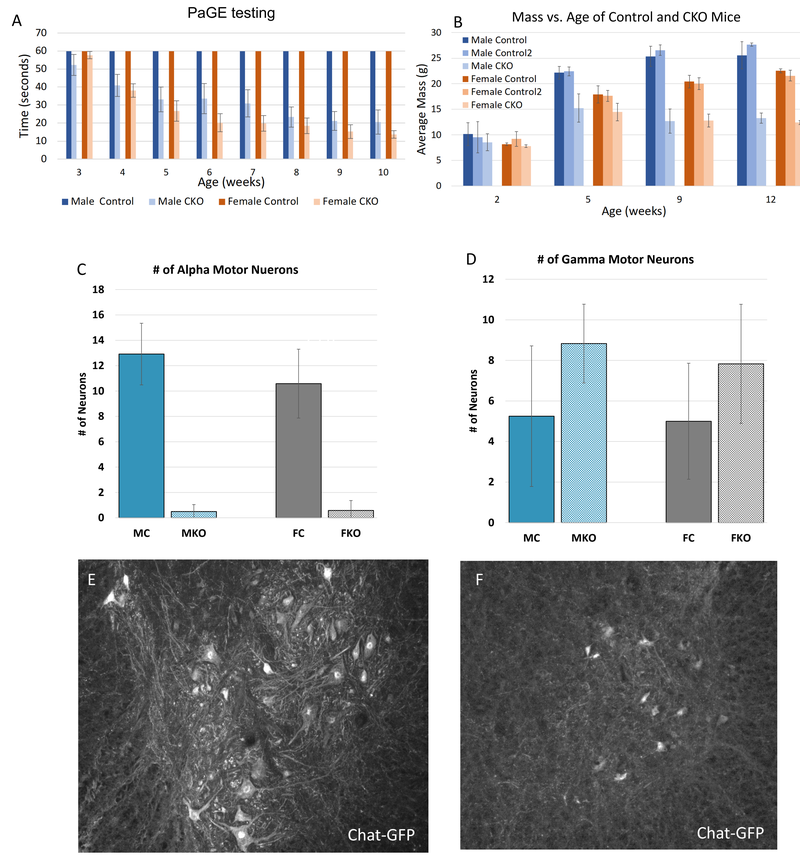

Figure 2. Impact of *Elp1* ablation in Motor Neurons. A) Paw grip endurance testing (PaGE) shows that grip strength deteriorates in *Chat-Cre; Elp1$^{LoxP/LoxP}$* CKO mice as they age. B) Although CKO mice exhibit normal weights during their first few weeks of life, they reach their maximum weight at 5 weeks. This weight loss corresponds to decreased muscle mass evident upon autopsy. C,D) Alpha and gamma motor neuron quantification in Control and CKO mice, respectively. E, F) 10X, representative images of the ventral horn in Control and CKO mice (E=female control 73R; F=female CKO 72 R).

Discussion

These data demonstrate that a selective loss of Elongator in motor neurons leads to their deterioration and a gradual loss of motor function, similar to human disease progression in ALS. Elongator functions in tRNA modification and the translation of AA and AG-ending codons for the amino acids lysine, glutamine, and glutamic acid. As such, its loss primarily impacts genes that exhibit a biased usage of AA or AG-ending codons. A primary candidate for being mis-regulated in the absence of Elongator is *TTN,* encoding titin. *TTN* is an AA-biased gene whose protein product functions in the elasticity of striated muscle. The George lab recently discovered that titin is also present in the nucleolus of motor neurons. Since decreased expression of titin is associated with the severity of ALS progression, our hypothesis is that mutations in Elongator contribute to ALS via the mis-regulation of *TTN*. The next step in this study is to compare the levels of titin and other proteins encoded by codon-biased genes in control and CKO motor neurons. These experiments will help elucidate the Elongator targets that mediate neurodegeneration in ALS, providing new targets for therapeutic intervention.