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**PAD2 Dysregulation and Abnormal Protein citrullination in ALS disease models**

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### Abstract

Amyotrophic lateral sclerosis (ALS) is a deadly neurodegenerative disease characterized by loss of motor neurons, paralysis and eventual death. The mechanism of ALS is still incompletely understood, and the disease is to date without an effective remedy. Protein arginine deiminase 2 (PAD2) converts peptidyl-Arg into peptidyl-Citrulline, a post-translational modification responsible for citrullination. Aberrant expression of PAD2 and protein citrullination are increased in several neurodegenerative conditions. Whether this increase is involved in ALS is unknown. In this study, we investigated PAD2 and protein citrullination in two genetic mouse models of ALS expressing human mutant SOD1G93A andPFN1C171G, and in human ALS spinal cord. We show that PAD2 gene expressions and protein citrullination are increased along ALS progression. These changes occur in areas with the most severe motor neuron degeneration including the spinal cord, and brainstem.

We show that the increase in PAD2 and citrullinated proteins occur specifically in astrocytes, while decreasing in neurons. Citrullinated proteins also form non-astrocyte aggregate patterns; and are dominantly expressed in insoluble protein fractions. Furthermore, the ALS mice spinal cord shows altered citrullinome. Finally, knocking out PAD2 prevented protein citrullination in SOD1G93A mice, quicken disease onset, while slowing disease progression. These results demonstrate that aberration of PAD2 protein citrullination alters protein functions, our results suggest that PAD2 and protein citrullination play a role in astroglial and astrocytic toxicity in ALS and other neurodegenerative conditions.

### Protein citrullination is increased in SOD1G93A and PFN1C171G ALS mouse models

![Image](https://example.com/protein_citrullination_schematic)

**Fig. 1. A and B.** Quantitative real-time PCR of PAD2 mRNA from the lumbar spinal cords of mice expressing mutant SOD1G93A and mutant PFN1C171G, respectively. C and D. Western blot and Quantification of PAD2 protein from the lumbar spinal cords of mice expressing mutant SOD1G93A and mutant PFN1C171G, respectively. P values are from student t-test comparing transgenic mice with their age matched controls. * = p<0.05, ** = p<0.01, *** = p<0.001.

### The increase in PAD2 and protein citrullination correlates spatially with neurodegeneration in ALS mouse models

![Image](https://example.com/protein_citrullination_distribution)

**Fig. 2. A and B.** Western blot and quantification of citrullinated proteins using the anti-modified citrulline (AMC) antibody detection method in SOD1G93A mouse model, and C-D in PFN1C171G mouse model. P values are from student t-test comparing transgenic mice with their age matched controls. ** = p<0.01, *** = p<0.001.

### Citrullinated proteins forms non-astrocyte aggregate patterns in SOD1G93A and PFN1C171G ALS mouse models

![Image](https://example.com/protein_aggregate_patterns)

**Fig. 3.** A and B. Western blot and quantification of citrullinated proteins using the anti-modified citrulline (AMC) antibody detection method in SOD1G93A mouse model, and C-D in PFN1C171G mouse model. P values are from student t-test comparing transgenic mice with their age matched controls. ** = p<0.01, *** = p<0.001.

### Citrullinated proteins increase in insoluble fractions in SOD1G93A and PFN1C171G ALS mouse models

![Image](https://example.com/protein_insoluble_fraction)

**Fig. 4.** A and B. Double immunofluorescence staining of spinal cord sections for GFAP and citrullinated proteins from different disease stages in SOD1G93A and PFN1C171G, respectively.

### PAD2 knock out prevents protein citrullination in SOD1G93A mouse model.

![Image](https://example.com/pad2_knockout_effect)

**Fig. 5.** A. PAD2 Knockout does not affect protein expression of PAD3 and 4 in SOD1G93A ALS Mice. B. PAD2 Knockout prevents protein citrullination in SOD1G93A and ALS Mice. C. PAD2 knock out accelerated disease onset but slowed down the disease progression in SOD1G93A ALS mice. The plots are averages of 3-6 animals that have been monitored throughout their lifespan. Peak body weight before the decline are pointed by arrows.

### PAD2 and Protein citrullination increase in Human ALS spinal cord

![Image](https://example.com/human_als_citrullination)

**Fig. 6.** A and B. Western blot and quantification of citrullinated proteins in human ALS spinal cord, respectively. C and D. Western blot and quantification of citrullinated proteins in human ALS spinal cord, respectively. P values are from comparing ALS patients and non-ALS controls. ** = p<0.01.

### Summary

Our results show that PAD2 expression and protein citrullination increase as the disease progresses in two different genetic mouse models of ALS expressing mutant SOD1 and PFN1; and in ALS patients. This increase particularly occurs in the astrocytes, while decreasing in neurons. The alteration of PAD2 and protein citrullination seen in these mouse models coincides with areas showing motor neuron degeneration in ALS. The ALS mice spinal cord citrullinome shows a significant alteration in a wide range of protein classes and functional pathways. In addition, citrullinated proteins form non-astrocyte aggregate patterns; and are dominantly expressed in insoluble protein fractions, suggesting citrullination may possibly drive protein insolubility. Finally, PAD2 knockout prevented protein citrullination in ALS mice, quickens disease onset, while slowing disease progression. These results suggest that dysregulation of PAD2 and protein citrullination contribute to the pathogenesis of ALS.