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PFN1 phosphorylation marks protein aggregation and white matter pathology in ALS

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Et al.

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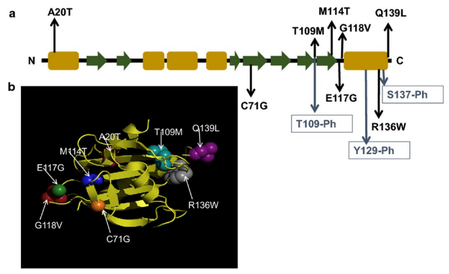
Background

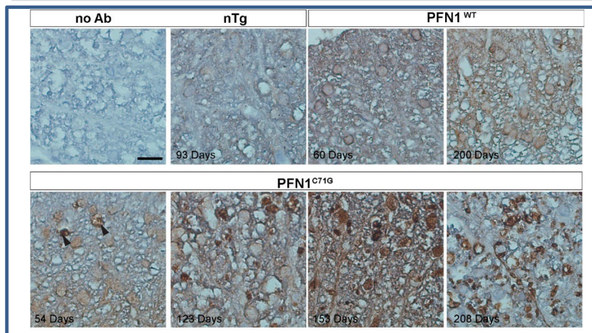
Amyotrophic lateral sclerosis (ALS) is a progressive, paralytic disorder characterized by degeneration of motor neurons in the brain and spinal cord. Common feature in cases of both familial and sporadic ALS is aggregation of cytoplasmic proteins, prominently but not exclusively in motor neurons. One of the well-characterized animal models in our lab is expressing an ALS related mutation, C71G, in Profilin (PFN1), a recognized actin-binding protein. Mutations in the *pfn1* gene were previously shown to result in protein aggregation and insolubility of PFN1 in mouse ALS model; however, the impact and mechanism of PFN1 aggregation remain not well understood.



Profilin1 protein structure and ALS-linked mutations

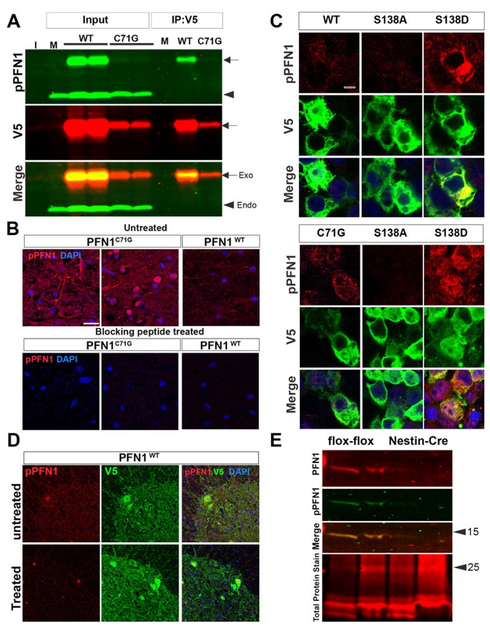
Post-Translational modification (PTMs) of neural proteins is one of the frequent observations in most neurodegenerative conditions related to protein aggregation. I hypothesize that under-characterized PFN1 protein post-translational modification, such as phosphorylation, contributes to the pathogenesis of ALS by accelerating PFN1 aggregation.

1. pPFN1 antibody marks a pathology specific aggregate pattern in ALS mice model white matter



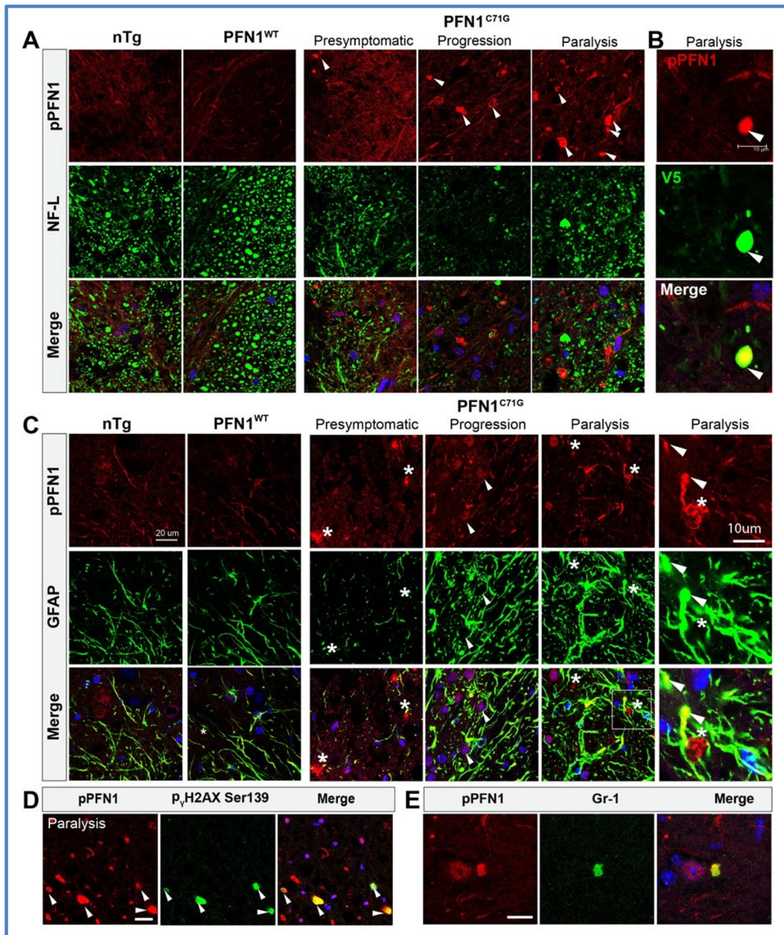
Paraffin embedded Lumbar spinal cord sections from PFN1 WT and non-transgenic animals co-stained with pPFN1 antibody and hematoxylin post antigen retrieval show a gradual increase of pPFN1 intense staining in white matter.

pPFN1 antibody selectively reacts with phosphorylated PFN1



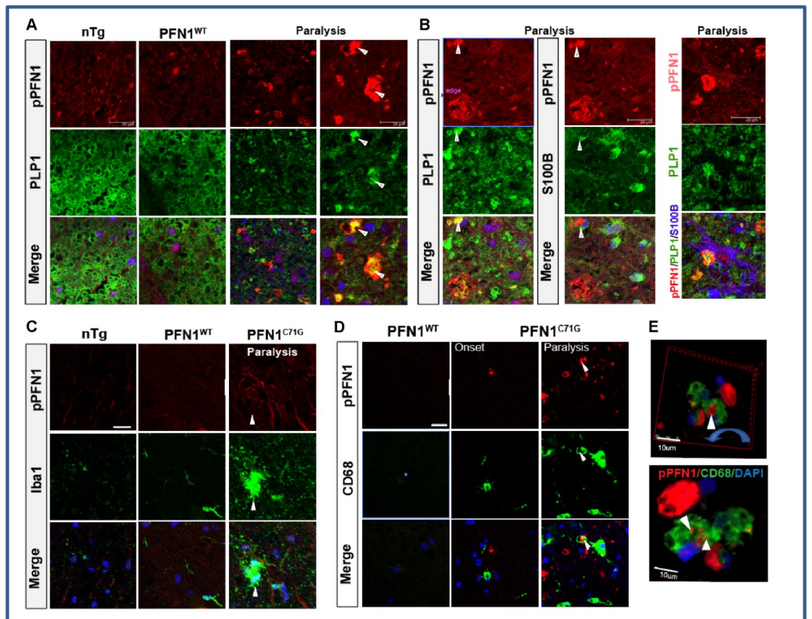
A. pPFN1 antibody recognizes IPed PFN1 in soluble fraction
Western blot using pPFN1 and PFN1 antibodies on the whole-cell extract of NSC-34 cells transfected with PFN1wt (WT) and PFN1C71G (C71G) constructs exposed to immunoprecipitation using V5 antibody (marking exogenously expressed PFN1). Mouse IgG (I) and lysate transfected with an empty plasmid (M) were used as controls. Mutant is marked with small arrow and endogenous protein is marked with arrowhead.
B. pPFN1 staining with peptide absorption. Lumbar spinal cord section stained with the pPFN1 antibody with and without the presence of its commercial blocking peptide is shown. A PFN1C71G symptomatic stage section is compared to an age matched PFN1wt (scale bar=20um)
C. Specificity of pPFN1 in immunofluorescence staining on NSC34 cells expressing phospho mutant(S138A) and phospho mimics (S138D) PFN1 constructs visualized with confocal microscopy. pPFN1 signal is increased in presence of mutant PFN1 and its phosphomimic but not in phosphomutant condition (scale bar=5um)
D. A PFNWT expressing mice spinal cord section treated with lambda phosphatase for 1hr compared to a non-treated control
E. PFN1 and pPFN1 antibodies validation on a conditional PFN1 knockout spinal cord protein lysate

2. pPFN1 aggregates are found in the white matter



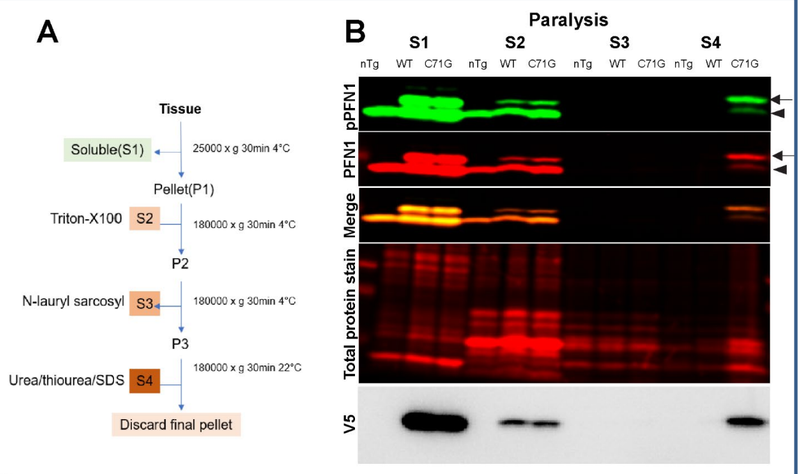
A. Endogenous pPFN1 aggregates differentially found in PFN1C71G white matter. Mouse spinal cord sections were stained for pPFN1 and Neurofilament-L antibodies and visualized with confocal microscopy. (scale bar = 20 um)
B. Mutant PFN1 aggregates double positive for V5 and pPFN1 found in spinal cord white matter and show a distinctive morphology. (scale bar=10um) C. Double staining with GFAP and pPFN1 antibodies confirms the abundant presence of PFN1 phosphorylation in astrocytes. D. Some aggregates are colocalized with DNA-damaged cells. E. Few of aggregates are colocalized with neutrophils positive for Gr-1(scale bar=10um)

3. pPFN1 marked myelin colocalized aggregates interacting with microglia in the white matter



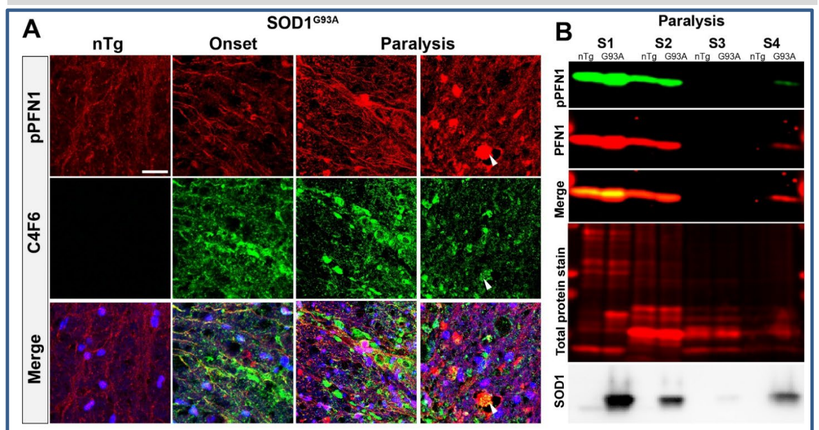
A. Double staining with pPFN1 and PLP1 myelin marker
B. Increased Ppfn1 in PLP1+ S100B+ cells
C. Double staining with pPFN1 and Iba1. pPFN1 signal does not overlap with the Iba1 signal (arrowhead) (Scale bar=20um)
D. CD68 positive glial cells often found in proximity of pPFN1 positive areas but do not colocalize with pPFN1 signal. Arrowhead indicate an aggregate positive area that is engulfed by a CD68 positive cell (Scale bar=20um). E. The magnified panel in the right establishes the relationship of a pPFN1 stain positive area with CD68 expressing cells from two different angles in a confocal 3D reconstruction image (Scale bar=10um).

4. Endogenous pPFN1/PFN1 is found in both soluble and insoluble fractions in PFN1^{C71G}



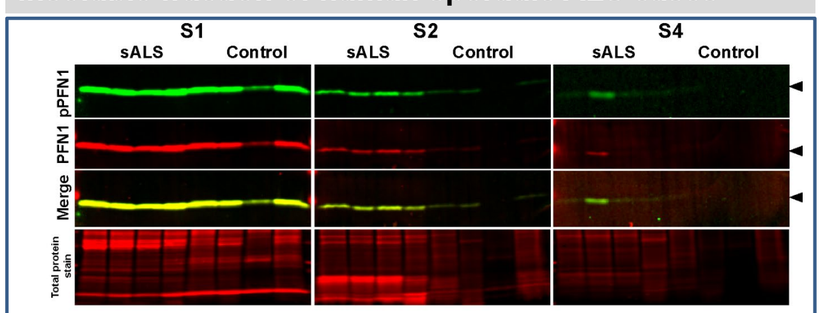
A. Schematic showing the summary of protocol used for fractionation of brainstem tissue explained in detail in material methods section.
B. Representative blot of age matched nTg, PFN1 WT and PFN1 C71G (paralyzed) showing the PFN1 and its phosphorylation in the soluble (S1), Triton-x100(S2), sarcosyl(S3) and Urea/SDS(S4) fractions respectively. The total protein stain is indicating the amount of total fluorescent protein present in each lane.

5. pPFN1 also marks aggregates in mutant SOD1 ALS model



A. Double staining with pPFN1 and misfolded SOD1 antibody. pPFN1 is partially overlapping with SOD1 positive aggregates marked with arrowheads (Scale bar=20um). All panels are same magnification and obtained after maximum projection of pPFN1 staining across z-stacks in white matter.
B. Brainstem biochemical fractionation of nTg matched with a paralyzed mouse showing the presence of endogenous pfn1 aggregation in SOD1 G93A mice in Urea/SDS fraction. Soluble (S1), Triton-x100(S2), sarcosyl(S3) and Urea/SDS(S4)

6. Both PFN1 and its phosphorylation is present in insoluble fraction of human sporadic ALS cases



Biochemical fractionation of spinal cord postmortem frozen samples to Soluble (S1), Triton-x100 (S2) and Urea/SDS (S4) fractions respectively. Sarcosyl soluble (S3) fraction has not been shown for simplicity since signal was below detection in this fraction.

Conclusion

pPFN1 antibody marks phosphorylation of PFN1 which can be found in aggregates. White matter pathology resulting from potentially endogenously expressed PFN1 phosphorylation in both ALS mice models independent of mutation type. Overall, our results suggest for the first time a role for phosphorylation of PFN1 in protein aggregation and white matter pathology in ALS that will shed more light on the mechanism of disease and developing potential therapeutics in near future