A Harmonized Multisite Digital TDP-43 Pathology Resource Enables Automated Quantitative Analysis Across ALS and FTD

Mayisha Rahman[1], Marina Selenica[2], Laura Dugom[3], Daniel Weatherill[2], Brent Harris[1], Galam Khan[1], Carlo Tornatore[2], Ambria Manuel[2], Katherine Schwetye[2], Richard J. Perrin[3], Cindy V. Ly[4], John Ravits[5], Cole Ferguson[2], Anita Hermann[2], Benjamin N. Hoover[6], Jiyoon Hwang[2], Vlad Korben[2], Matthew Harms[2], Neil Shneider[2], Karina McDade[2], Colin Smith[6], Holly Fahning[7], Jennifer Eschbacher[7], Nadine Bakkar[2], Robert Bowser[2], Steve Finkbeiner[8], Ruth Kassahun[8], Amy Easton[1]

1. Department of Neurology and Pathology, Georgetown University, Washington, DC, USA
2. Target ALS, New York, NY, USA
3. Department of Neurology, Washington University St. Louis, St. Louis, MO, USA
4. Department of Neurosciences, University of California, San Diego, CA, USA
5. Department of Neurology, Columbia University Medical Center, New York, NY, USA
6. Centre for Clinical Brain Sciences, University of Edinburgh, Scotland, UK
7. Departments of Translational Neuroscience and Neurology, Barrow Neurological Institute, Phoenix, AZ, USA
8. Gladstone Institutes, University of California, San Francisco, CA, USA

ABSTRACT

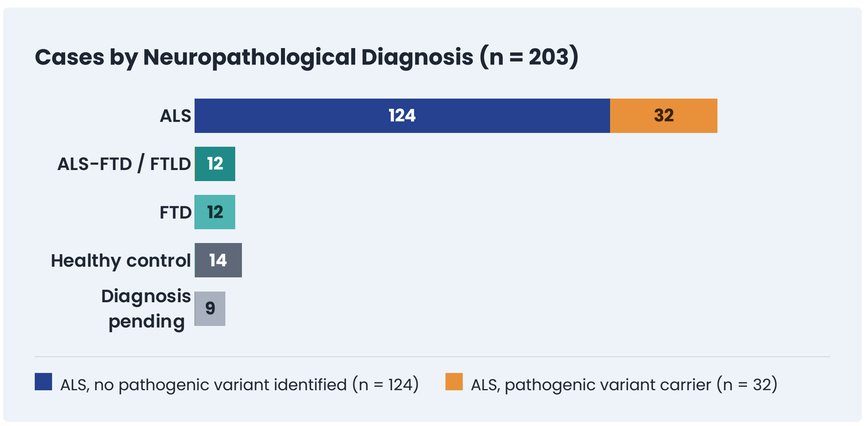
Background: Phosphorylated TDP-43 (pTDP-43) pathology is the defining neuropathologic hallmark of amyotrophic lateral sclerosis (ALS) and many cases of frontotemporal dementia (FTD), yet quantitative analyses have largely been limited to single-center cohorts using heterogeneous tissue processing and staining methods. The Target ALS Postmortem Tissue Core (PMTC) was established to accelerate ALS therapeutic development by generating high-quality, extensively annotated, and uniformly collected postmortem biospecimens across a six-site international network, collecting brain and spinal cord tissue from individuals with ALS, ALS-FTD, FTD, and non-neurological healthy controls. Here, we describe the development of a centralized digital pathology resource of standardized pTDP-43 whole-slide images integrated into the Target ALS Data Engine to enable quantitative neuropathologic analyses and AI-based biomarker discovery.

Methods: Postmortem brain and spinal cord tissue from more than 300 cases collected across six Target ALS PMTC sites underwent centralized immunohistochemical staining at Georgetown University using a harmonized protocol and a phosphorylated TDP-43 (pTDP-43) antibody developed by Leonard Petrucelli. Whole-slide images were generated from an average of 8–10 neuroanatomic regions per case, including motor cortex, hippocampus, entorhinal cortex, medulla, cervical, thoracic, and lumbosacral spinal cord, cerebellum, and amygdala. Following digitization, images underwent quality control, were linked using a universal case identifier, and were integrated into the Target ALS Data Engine to create a standardized, searchable digital pathology resource for quantitative analysis.

Results: To date, 203 cases have been processed and integrated into the Target ALS Data Engine, creating one of the largest harmonized digital pTDP-43 pathology resources for ALS and FTD research. The cohort includes 156 ALS cases, 12 ALS-FTD/FTLD cases, 12 FTD cases, 14 neurologically healthy controls, and 9 cases pending final diagnosis; 17 ALS cases had concurrent Alzheimer's disease (7) or another neurological disorder (10) recorded. Of 38 pathogenic variant carriers, 32 had ALS, 5 had ALS-FTD/FTLD (all *C9orf72*), and 1 was pending diagnosis (*ANXA11*). Variants were identified in *C9orf72* (22), *SOD1* (6), *ATXN2* (4), *ANG* (2), and single cases with *SQSTM1*, *OPTN*, *NEK1*, *VCP*, *ANXA11*, and *TBK1*; two cases carried two variants. Together, these harmonized whole-slide images provide a standardized digital pathology resource spanning multiple neuroanatomic regions and genetically defined ALS subtypes, enabling quantitative comparisons of pTDP-43 pathology across ALS with and without a known pathogenic variant. Across cases, standardized whole-slide images encompass multiple neuroanatomic regions and provide an AI-ready resource for quantitative analyses of regional pathology, genotype-pathology relationships, and future biomarker discovery.

Conclusion: This resource provides a foundation for scalable and reproducible analysis of pTDP-43 pathology. In collaboration with Steve Finkbeiner, Brent Harris, and Cole Ferguson, a deep learning model developed by the Finkbeiner group at the Gladstone Institutes and UCSF is being applied directly to whole-slide images. The model is designed to quantify aggregate density and morphology within defined neuroanatomic regions. It will also distinguish neuronal from glial pTDP-43 inclusions, characterize the differential morphologic variability of aggregates, and identify potential differences between sporadic and genetic ALS. By making harmonized whole-slide images available through the Target ALS Data Engine, this resource enables reproducible biomarker discovery and AI-driven neuropathology research.

DIGITIZED pTDP-43 COHORT



203 cases imaged as of Oct 13, 2026; >300 projected

>300 cases stained centrally

6 PMTC sites

8–10 regions imaged per case

HARMONIZED IMAGING WORKFLOW

1. **Collection** — Brain & spinal cord from >300 cases at 6 PMTC sites
2. **Central staining** — Harmonized pTDP-43 IHC at Georgetown (Petrucelli antibody)
3. **Digitization** — Whole-slide images of 8–10 regions per case
4. **Quality control** — Every whole-slide image reviewed before release
5. **Linkage** — Universal case identifier joins images to case data
6. **Target ALS Data Engine** — Searchable, AI-ready digital pathology resource

SITES & REGIONS IMAGED

PMTC Site	Location
Georgetown University	Washington, DC, USA
Barrow Neurological Institute	Phoenix, AZ, USA
Columbia University	New York, NY, USA
University of California, San Diego	San Diego, CA, USA
University of Edinburgh	Edinburgh, Scotland, UK
Washington University in St. Louis	St. Louis, MO, USA

Neuroanatomic regions in the whole-slide image set

BRAIN

Motor cortex · Hippocampus · Entorhinal cortex · Amygdala · Cerebellum · Medulla

SPINAL CORD

Cervical · Thoracic · Lumbosacral

An average of 8–10 regions imaged per case.

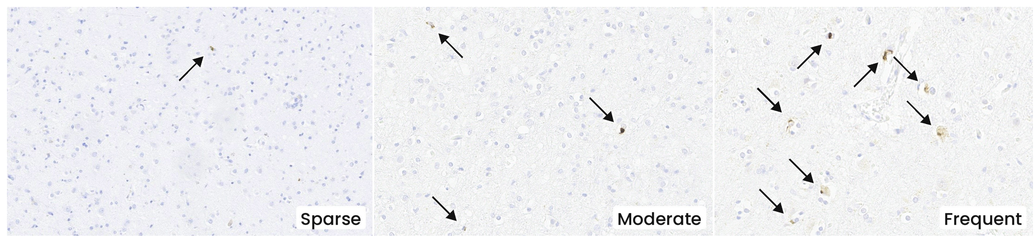
IMPACT ON ALS RESEARCH

- **Harmonized across sites:** one protocol and one pTDP-43 antibody remove staining variability across six sites.
- **Scale:** 203 cases imaged as of October 13, 2026, with more than 300 projected, one of the largest harmonized digital pTDP-43 resources for ALS and FTD.
- **Genotype–pathology:** 38 pathogenic variant carriers across 10 genes enable comparison with ALS without a known variant, FTD and controls.
- **Regional mapping:** 8–10 brain and spinal cord regions per case support quantification across the neuraxis.
- **AI-ready:** >2000 standardized QC'd whole-slide images with universal case IDs for training automated algorithms.
- **Open access:** images shared through the Target ALS Data Engine for reproducible biomarker discovery.

References

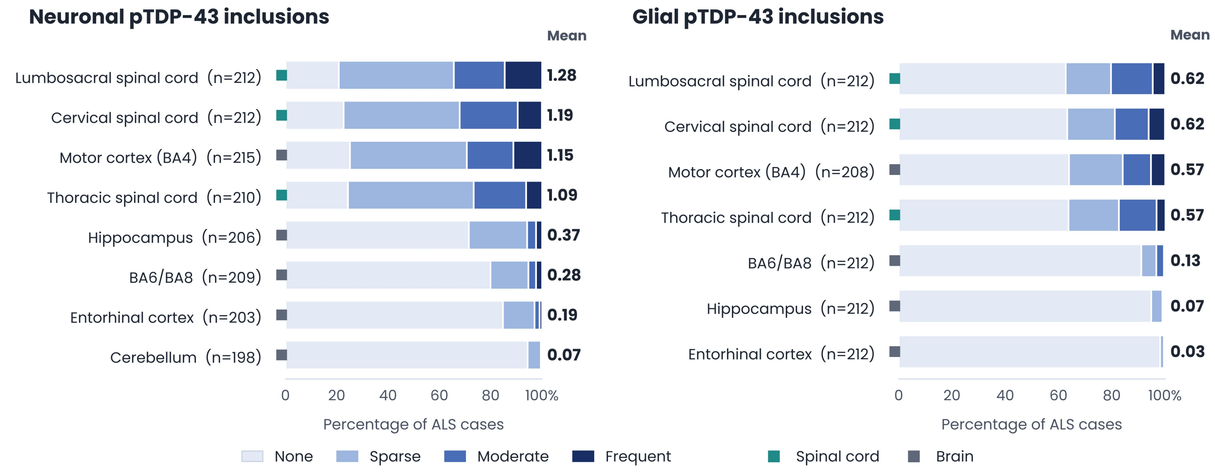
1. Neumann M, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006;314(5796):130–133.
2. Brettschneider J, et al. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann Neurol*. 2013;74(1):20–38.

TDP-43 HISTOPATHOLOGY AND SEMI-QUANTITATIVE SCORING



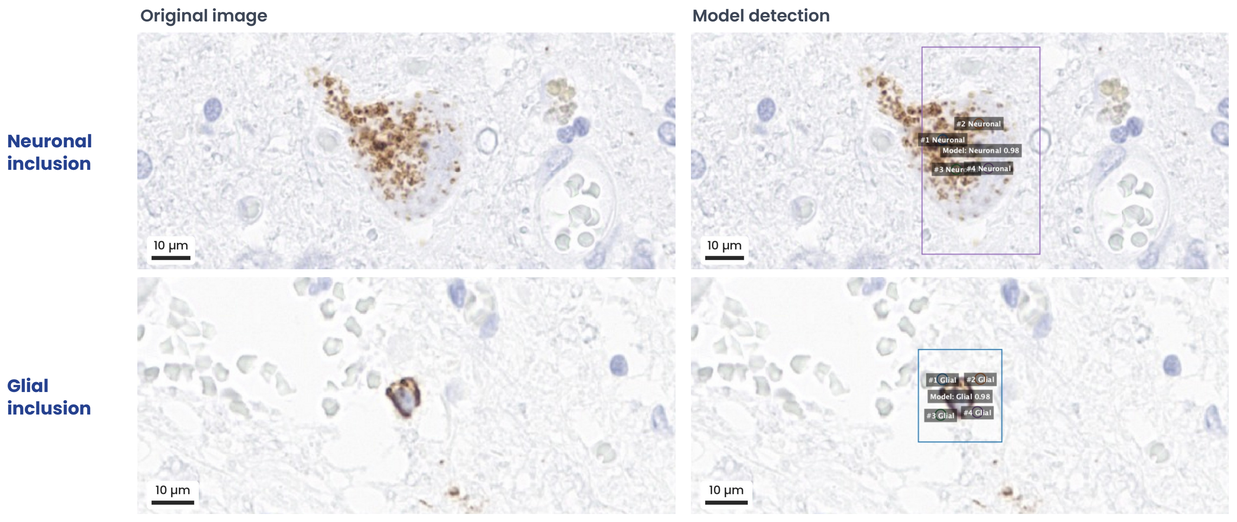
Neuronal and Glial Inclusions of Phosphorylated TAR DNA-Binding Protein 43 (TDP-43)

NB: Score based on the quantification per high power field not intensity of expression.



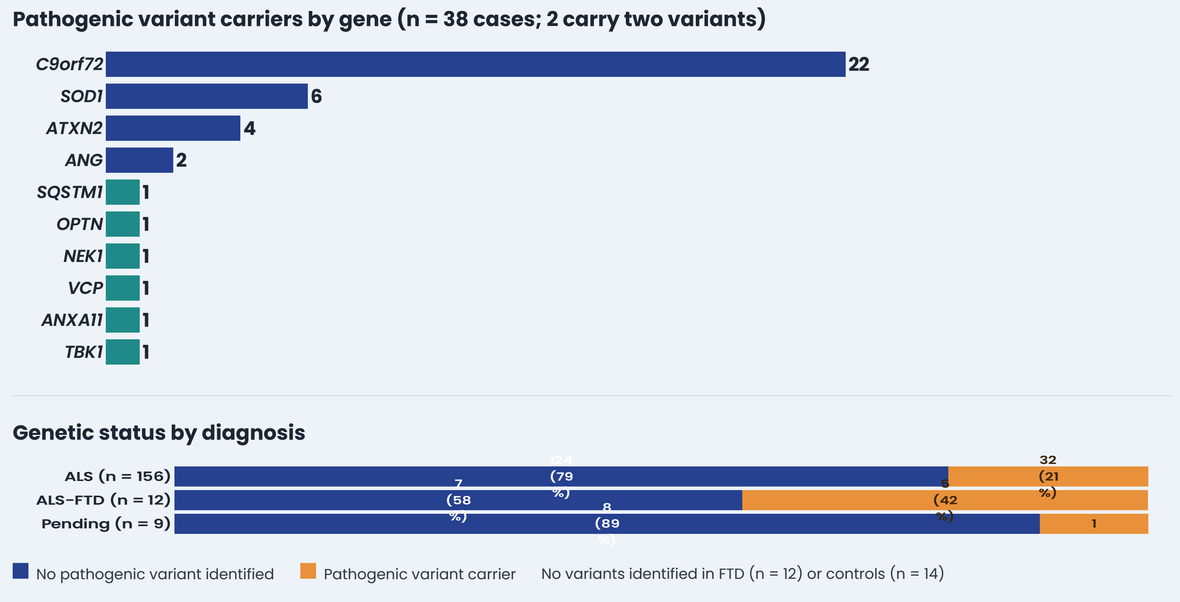
Percentage of ALS cases (ALS-FTD excluded) at each inclusion severity level by region; mean score at right. Lumbosacral cord had the most abundant neuronal and glial inclusions; neuronal lumbosacral scores were significantly higher than every other region (paired Wilcoxon, FDR-adjusted q of 0.02 or less). Frontal, limbic and cerebellar regions had little pathology (Friedman test across regions, $p < 10^{-60}$).

AUTOMATED DETECTION OF pTDP-43 STAINING USING MACHINE LEARNING



Example classifications from a deep learning model (Finkbeiner lab, Gladstone Institutes/UCSF) that detects pTDP-43 aggregates directly in whole-slide images; numbers indicate model confidence.

GENETIC LANDSCAPE



CONCLUSION & NEXT STEPS

Harmonized whole-slide images in the Target ALS Data Engine provide a foundation for scalable, reproducible analysis of pTDP-43 pathology. With the Finkbeiner group (Gladstone Institutes/UCSF) and the Target ALS PMTC pathologists, we are developing a deep learning model that analyzes whole-slide images directly. It is designed to:

- **Quantify** aggregate density and morphology within defined neuroanatomic regions
- **Distinguish** neuronal from glial pTDP-43 inclusions
- **Characterize** differential morphologic variability of aggregates
- **Compare** ALS with and without a known pathogenic variant

- Whole-slide images and data available upon request: www.targetals.org/resource/human-postmortem-tissue-core/
- We thank the patients and families who donated to this collection, and the neuropathologists and coordinators at all six PMTC sites.