



Constitutive neuronal expression and disease-associated upregulation of chitinases in amyotrophic lateral sclerosis

Nayana Gaur,^{1,†} Christin Angerer,^{2,†} Zeynep I. Gunes,³ Mihai Ancau,^{2,4,5} Mengzhe Wang,² Henrick Riemenschneider,^{5,6} Charlene-Annett Hurler,⁷ Simon Mungwa,⁸ Patrick Lüningschrör,⁸ Anxhela Zhiti,² Robert Steinbach,¹ Michael Briesse,⁸ Mugdha Srivastava,⁹ Mario Plaas,¹⁰ Andreas Hermann,^{11,12} Michael Sendtner,⁸ Sarah Jäkel,^{5,7,13} Dieter Edbauer,^{5,6} Jochen Herms,^{5,6,14} Sabine Liebscher,^{3,5,15,16} Julian Grosskreutz^{17,18,†} and Monika S. Brill^{2,5,†}

^{†,‡}These authors contributed equally to this work.

Chitinases are hydrolytic enzymes responsible for degrading chitin and have been evolutionarily conserved across various species. Although their signalling pathways are not fully understood, the chitinases are considered active immunomodulators across several cell types. Specific isoforms, including chitotriosidase-1 (CHIT1), chitinase-3-like protein 1 (CHI3L1), and human-specific chitinase-3-like protein 2 (CHI3L2), have emerged as markers of inflammation across the neurodegenerative spectrum, including amyotrophic lateral sclerosis (ALS).

ALS is a fatal neuromuscular condition, and therapeutic development has been severely hindered by phenotypic heterogeneity and an incomplete understanding of aetiology. Although several overlapping disease mechanisms can contribute to neuronal death, inflammation can exacerbate pathology. Prior studies have reported that CHIT1, CHI3L1 and CHI3L2 levels are elevated in the CSF of ALS patients and associated with disease aggressiveness. Nevertheless, several open questions critical to our understanding of the role of chitinases in ALS disease burden remain: namely, (i) which cell types in the CNS are chitinase sources under physiological conditions; (ii) which of these display chitinase upregulation in ALS; and (iii) what is the diagnostic utility of the chitinases relative to established biomarkers. Here, we utilize preclinical models and post-mortem human tissue to demonstrate at both the transcriptomic and protein level that neurons are a primary source of chitinases; furthermore, neuronal chitinase expression is conserved across species. Under physiological conditions, CHI3L1 is more abundant and widely expressed across various cell types, whereas CHIT1 is predominantly expressed in neurons. Additionally, utilizing symptomatic mice from three familial ALS models, we demonstrate isoform-specific expression profiles, with astroglial and microglial upregulation of CHI3L1, and neuronal and microglial upregulation of CHIT1. Differing expression dynamics and diagnostic utility were also noted in our clinical cohort: CSF CHIT1 and CHI3L2 levels had more discriminatory power when distinguishing between ALS versus non-ALS controls, while CHI3L1 was more closely associated with inflammation and ageing across the neurodegenerative spectrum. Although the chitinases did not diagnostically outperform the neurofilament proteins, we posit that their expression profiles can inform the optimization of biomarker-guided trial design.

Taken together, we demonstrate that chitinase upregulation in ALS is evident in various CNS cell types and that its neuronal expression may provide new insights into its role in disease activity.

Received September 20, 2024. Revised January 01, 2026. Accepted January 30, 2026. Advance access publication February 28, 2026

© The Author(s) 2026. Published by Oxford University Press on behalf of the Guarantors of Brain.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

- 1 Department of Neurology, Jena University Hospital, Jena 07740, Germany
- 2 Institute of Neuronal Cell Biology, School of Medicine and Health, Technical University of Munich, Munich 80802, Germany
- 3 Institute of Clinical Neuroimmunology, Klinikum der Universität München, Ludwig-Maximilians University Munich, Martinsried 82152, Germany
- 4 Department of Neurology, Klinikum Rechts der Isar, School of Medicine and Health, Technical University of Munich, Munich 81675, Germany
- 5 Munich Cluster for Systems Neurology (SyNergy), Munich 81377, Germany
- 6 German Centre for Neurodegenerative Diseases (DZNE), Munich 81377, Germany
- 7 Institute for Stroke and Dementia Research (ISD), LMU University Hospital, LMU Medizin, LMU Munich, Munich 81377, Germany
- 8 Institute of Clinical Neurobiology, University Hospital Wuerzburg, Wuerzburg 97078, Germany
- 9 Core Unit Systems Medicine, University of Wuerzburg, Wuerzburg 97078, Germany
- 10 Institute of Biomedicine and Translational Medicine, Laboratory Animal Centre, University of Tartu, Tartu 50411, Estonia
- 11 Translational Neurodegeneration Section 'Albrecht Kossel', Department of Neurology, University Medical Center Rostock, Rostock 18147, Germany
- 12 German Center for Neurodegenerative Diseases (DZNE), Greifswald 17489 and Rostock 18147, Germany
- 13 Department of Psychiatry and Psychotherapy, Klinikum Rechts der Isar, TUM School of Medicine and Health, Technical University of Munich, Munich 81675, Germany
- 14 Centre for Neuropathology and Prion Research, Ludwig-Maximilians-University Munich, Munich 81377, Germany
- 15 Biomedical Center, Ludwig-Maximilians University Munich, Martinsried 82152, Germany
- 16 Institute of Systems Neuroscience, Medical University of Innsbruck, Innsbruck 6020, Austria
- 17 Cluster for Precision Medicine in Inflammation, Universities of Kiel and Lübeck, Kiel 24118, Germany
- 18 Precision Neurology of Neuromuscular and Motor Neuron Diseases, University of Lübeck, Lübeck 23538, Germany

Correspondence to: Monika S. Brill

Institute of Neuronal Cell Biology, School of Medicine and Health

Technical University of Munich, Biedersteiner Str. 29, Munich D-80802, Germany

E-mail: monika.leischner-brill@tum.de

Keywords: chitinase; CHI3L1; CHIT1; CHI3L2; amyotrophic lateral sclerosis; neuron

Introduction

The chitinases belong to glycosyl hydrolase family 18 and cleave chitin, a natural polysaccharide found in the coating of various pathogens.¹ Mammals possess both 'true' chitinases with enzymatic activity and chitinase-like proteins (CLPs), which bind chitin with high affinity but are inactive. Humans have three active chitinases: chitotriosidase (CHIT1), acidic mammalian chitinase (AMCase), and di-N-acetylchitinase; and several CLPs, including chitinase 3-like-1 (CHI3L1) and chitinase 3-like-2 (CHI3L2). The majority of these genes are located on chromosome 1, adjacent to the major histocompatibility complex (MHC), with this synteny likely indicating an organizational relationship between the chitinases and the immune system.² Indeed, the chitinases participate in various processes, including tissue remodelling, T-helper 2 inflammatory responses and chemotactic signalling,^{1,3} suggesting that their role extends beyond innate immunity against chitin to active immunomodulation. Chitinase expression is differentially regulated by various immunogenic stimulants and cytokines, including TNF- α , IFN- γ and members of the interleukin family 4–6; in turn, the chitinases themselves can regulate cytokine production via autocrine and/or paracrine signalling.^{4,5}

Unsurprisingly, they are dysregulated in both systemic and neurodegenerative conditions characterized by chronic inflammation (e.g. diabetes, frontotemporal dementia, Alzheimer's disease and Parkinson's disease).^{6–9} The chitinases have also been reported as surrogate markers of neuroinflammation in amyotrophic lateral

sclerosis (ALS), a rapidly progressive neurodegenerative disease with a median survival of 2–4 years from symptom onset.^{10–12}

Although over 30 ALS-linked genes have been identified, no disease-associated variant can be identified for close to 70% of cases, and available animal models do not fully recapitulate the phenotypic variability observed in patients.^{13,14} While several therapeutic agents targeting different pathological processes are being investigated, trials are significantly hampered because ALS is a multisystemic and heterogeneous condition.¹¹ Several pathogenic mechanisms, including oxidative stress, excitotoxicity, axonal transport defects, protein metabolism and neuroinflammation, converge on the final irreversible outcome of motor neuron degeneration.^{15–17} Independent studies have shown that CSF levels of CHIT1, CHI3L1 and CHI3L2 are elevated in ALS patients^{18–20} and correlative with a decline in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score, disease aggressiveness and overall survival.^{19–22} Nevertheless, the cellular origins of elevated chitinase levels remain to be fully characterized; this is crucial to understanding how chitinase upregulation is triggered and how it influences disease burden and progression. The existing literature suggests that multiple sources, including peripheral immune cells, contribute to the baseline secretion of CHIT1 and CHI3L1, as monocyte-derived macrophages from ALS patients exhibit higher levels of CHIT1 and CHI3L1 secretion than those from healthy donors.²³ Within the CNS, both microglial and astroglial expression of chitinases have been reported; however, analyses have been limited to post-mortem tissue at presumably advanced disease stages.^{18,24}

Here, we show that CSF CHIT1, CHI3L1 and CHI3L2 levels are elevated in a cohort of ALS patients and that, unexpectedly, neurons express these very isoforms. We use three ALS mouse models and post-mortem patient tissue to show that neuronal expression is evident at both the transcriptomic and protein levels. By further characterizing chitinase levels in the spinal cord and cortex, we show that all major CNS populations, including neurons, are chitinase sources that are vulnerable to dysregulation in ALS.

Materials and methods

Participant recruitment and case classification for biomarker analyses

This study was performed in accordance with regulations stipulated by the local ethics committee (3633-11/12) and the Declaration of Helsinki. All participants were recruited from the regional registry at the Department of Neurology, University Hospital Jena, between 2015 and 2019, and biofluids were collected during routine clinical workups. The final cross-sectional study cohort comprised 39 patients with either definite, probable or laboratory-supported probable ALS (as per the revised El Escorial Criteria),²⁵ 13 individuals with other neurodegenerative diseases (neurodegenerative disease controls, NDEGs) and 11 individuals with non-neurodegenerative conditions (non-neurodegenerative disease controls, NNDs). Participants formed a convenience series to ensure biofluid availability, confirm diagnoses and capture high- and low-disease aggressiveness phenotypes (as determined by on-site D50 modelling²²). Diagnoses for NDEG and NND cohorts are provided in [Supplementary Table 1](#). Primary exclusion criteria constituted current intake of any immunomodulatory therapy, recent surgical interventions and the presence of comorbid frontotemporal dementia (FTD) within the ALS cohort (as assessed according to the revised Strong criteria²⁶).

Biofluid collection and laboratory marker analyses

CSF was obtained by lumbar puncture, and venous blood was drawn in EDTA Monovettes immediately after. All CSF samples were visually inspected for evidence of a traumatic puncture. CSF and plasma samples were centrifuged (5000g, 10 min, 4°C) within a maximum of 1 h of collection and stored at –80°C until further use. Commercially validated sandwich ELISA kits were used to assay all analytes: CHIT1 (CY-8074, MBL Life Science), CHI3L1 (DY2599, R and D Systems), CHI3L2 (CY-8087, MBL Life Science), pNfH (EQ 6561-9601, EuroImmun Diagnostics) and NfL (UD5100, IBL International). All samples and standards were assayed in duplicate per manufacturer instructions, and mean intra- and inter-assay coefficients of variation for all assays were <10% and <20%, respectively. Absorbance was measured at 450 nm (wavelength correction at 540 nm, or 635 nm for pNfH) using a TECAN multi-mode plate reader. Sample concentrations were extrapolated using four-parameter logistic regression analysis.

Mouse lines and husbandry

All animal experiments conform to the regulations of the local authorities (e.g. the Government of Upper Bavaria). Experimental animals were kept with littermates, and all animals were housed in individually ventilated cages with *ad libitum* access to food and water. Mice of both sexes were included in all experiments.

Immunostaining experiments included the following models: Thy1-(GA)₁₄₉-CFP²⁷ (abbreviated to C9 GA-CFP), rNLS8-hTDP-43²⁸ (abbreviated to hTDP-43) and SOD1-G93A²⁹ [Tg(SOD1)2Gur/J, abbreviated to SOD1]. Heterozygous transgenic SOD1 mice (Jackson, Stock No. 002726) and heterozygous C9 GA-CFP mice were crossbred to C57BL6J mice to generate negative littermate controls and transgenic experimental animals. Transgenic C9 GA-CFP mice express codon-modified GA₍₁₄₉₎ [fused to cyan fluorescent protein (CFP)] at levels comparable to those observed in patients.²⁷ Intercrossing of the two monogenic lines B6;C3-Tg(NEFH-tTA)8Vle/J (NEFH-tTA line 8, Stock No. 025397) and B6;C3-Tg(tetO-TARDBP)4Vle/J (tetO-hTDP-43ΔNLS, line 4, Stock No. 014650; both lines obtained from Jackson), yielded bigenic (NEFH-tTA +/-; tetO-hTDP-43ΔNLS +/-) animals that were kept on doxycycline-containing (DOX) chow to suppress transgene expression; the disease phenotype was induced by switching to DOX-free chow. Expression can be reversibly induced in neurons by withdrawing doxycycline from the diet, with widespread hTDP-43 expression and motor symptom onset evident at 1 and 2 weeks, respectively, after doxycycline withdrawal.²⁸ Monogenic (NEFH-tTA -/-; tetO-hTDP-43ΔNLS +/-) mice, which do not develop TDP-43 pathology upon DOX removal, were used as controls. Ten-month-old adult Cx3cr1-GFP (Jackson, Stock No. 005582) and Aldh1l1-EGFP (MMRRC, Stock No. 011015-UCD) were used to assess chitinase expression in neurons, as well as GFP-expressing microglial and astroglial cells, respectively.

Transcriptomic experiments included 2- to 12-month-old male and female Aldh1l1-EGFP mice (n = 15); these animals were previously used for a second unrelated study and were either untreated (n = 3) or sham-treated (recombinant control IgG ICOS-5-2, 6 µg/ml, with 20% healthy donor serum, diluted in artificial CSF). These animals also had a 4 mm cranial window above the right somatosensory cortex for *in vivo* imaging.

Genotyping was performed according to standard protocols as previously described.^{30,31} Primers and expected products are available upon request. No specific inclusion/exclusion criteria were set, and no data were excluded from analysis. All analyses were performed with the experimenter blinded to genotypes during experimental workflows.

Transcriptomic and RNA sequencing analyses

Mouse tissue

Superficial cortical tissue from the right main somatosensory area of Aldh1l1-EGFP mice was harvested and fresh-frozen (n = 15, disk width 4 mm, depth 250–500 µm). Total nuclear RNA was extracted on ice as described in the ‘Frankenstein protocol for nuclei isolation from fresh and frozen tissue’.³² Briefly, tissue was homogenized in 1 ml of Nuclei EZ Prep Lysis Buffer (Sigma-Aldrich) using pestle homogenizers for 1.5/2 ml Eppendorf microtubes (Eppendorf) for 5 min, filtered using a 70 µm strainer mesh (Miltenyi Biotech) and then centrifuged (4°C, 500g, 5 min). The resulting pellet was resuspended in 1.5 ml of EZ Lysis buffer supplemented with Protector RNase-Inhibitor (Roche) and subjected to two additional centrifugation–resuspension cycles. Finally, the pellet was resuspended in 500 µl of buffer and filtered using 30 µm cell strainers (Miltenyi Biotech). The isolated nuclei were stained using NeuN (Abcam, Cat. No. EPR12763, 1:500). Subsequently, fluorescence-activated cell sorting, employing forward- and side-scatter-gating, was used to isolate cellular nuclei from both neuronal and non-neuronal populations. A stringent isolation step was applied, excluding 90% of neurons (as indicated by the NeuN+ signal), ensuring

a robust statistical analysis of both neuronal and non-neuronal cells. Following cell sorting, samples were loaded onto 10x chips (10x Genomics) for cDNA library preparation, conducted according to the manufacturer's protocol. Single-nucleus RNA sequencing (snRNA-seq) libraries were generated using the 10x Genomics Single Cell 3' (v3) RNA-seq technology combined with nucleus hashing. Single-index library preparation was performed according to the manufacturer's guidelines. The libraries were sequenced by Novogene using Illumina NovaSeq. Bioinformatics analyses were performed using R version 4.2.2 within RStudio 2023.06.0. For downstream analysis, the following packages were used: Seurat (version 4.3.0), ggplot2 (version 3.4.1) and UMAP (version 0.2.10.0).

Human tissue

Post-mortem brain tissue samples were provided by the Neurobiobank Munich in accordance with the Ethics Committee guidelines of Ludwig-Maximilians University Munich. All cases were collected with informed consent, and the cohort included ALS patients ($n=9$) and neurologically healthy controls ($n=5$). For nuclei isolation from post-mortem snap-frozen human cortical tissue, we utilized an optimized version of the 'Frankenstein protocol for nuclei isolation from fresh and frozen tissue'.³² Snap-frozen cortical tissue samples were collected and homogenized in Nuclei EZ Lysis Buffer (Sigma-Aldrich) with the help of pestles. To remove impurities, nuclei were filtered sequentially with 70 μm and 30 μm strainers (Miltenyi Biotech). To further purify the homogenate, nuclei were stained with DAPI and fluorescence-activated nuclei sorting was performed on DAPI+ nuclei. Sorted nuclei were used to generate cDNA libraries using the 10x Chromium 3' platform (v3.1, single index) according to the manufacturer's instructions (10x Genomics). cDNA libraries were sequenced at the Institute of Clinical Molecular Biology (IKMB) at the University of Kiel, Germany (NovaSeq6000, S4 500M reads/sample, 2×150 bp). The Cell Ranger Single Cell Software Suite (version 5.0.1, 10x Genomics, Pleasanton, CA) was employed to obtain a unique molecular identifier (UMI) count matrix. The resulting gene expression count matrix was analysed by the 'R' package 'Seurat' (v4.0.2) for single-cell RNA sequencing, using the default 'RNA' assay.³³

Motor neuron cultures

Primary mouse motor neuron cultures were derived from embryonic Day (E) 12.5 embryos and cultured for 7 days *in vitro* as previously described.³⁴ Induced pluripotent stem cell (iPSC)-derived motor neuron cultures were established from a patient with familial ALS (harbouring pathogenic mutation S393L in TDP43, female, 83 years of age, positive family history of ALS) and a neurologically healthy control (female, 48 years of age, negative for most common ALS-causing mutations, including C9, SOD1, FUS and TDP43) as previously described.³⁵ iPSC differentiation was performed as previously described³⁶ and based on established protocols.^{37,38} Briefly, iPSCs were differentiated into expandable neural progenitor cells (NPCs), followed by differentiation into motor neurons. For motor neuron maturation and subsequent RNA isolation, NPCs were further differentiated for 9 days before dissociation, and 500 000 cells were plated into polyornithine/laminin-coated Xona microfluidic chambers. Motor neurons were processed for RNA isolation 3 weeks after plating; analytical details are provided in the [Supplementary material](#).

Immunostaining analyses

Mouse tissue

Animals were lethally anaesthetized with isoflurane (CP Pharma) and transcardially perfused with a pre-flush of PBS followed by 4% paraformaldehyde (PFA) in PBS. Brains and spinal cords were post-fixed in 4% PFA at 4°C for 24–48 h. Free-floating vibratome sections were cut at a thickness of 50 μm (VT1200S, Leica). Antigen retrieval was performed before immunostaining for 15 min at 90°C in sodium citrate buffer (10 mM, pH 6.0). Sections were incubated for a maximum of two nights at 4°C with primary antibodies diluted in blocking solution [0.5% Triton, 5% donkey serum and 5% bovine serum albumin (BSA, Sigma-Aldrich in PBS)] and washed in PBS-T (PBS with 0.05% Tween-20).

Primary antibodies included anti-CHIT1 (Invitrogen, Cat. No. PA5-76778, 1:400), anti-CHI3L1 (Invitrogen, Cat. No. PA5-95897, 1:400), anti-NeuN (Millipore, Cat. No. MAB377, 1:500), anti-NeuN conjugated to Alexa Fluor 488 (Abcam, Cat. No. ab190195), anti-Iba-1 conjugated to SPICA Dye568 (Wako, Cat. No. 015-28011, 1:500), anti-GFAP (Invitrogen, Cat. No. A-21282, 1:500), anti-GFAP (Sigma-Aldrich, Cat. No. G3893), anti-GFP (Abcam, Cat. No. ab13970, 1:1000; Invitrogen, Cat. No. A10262) and ChAT antibody (Sigma-Aldrich, Cat. No. AB144P, 1:60). Sections were incubated with suitable secondary antibodies coupled to Alexa Fluor 488, Alexa Fluor 568, Alexa Fluor 594 or Alexa Fluor 647 (Invitrogen: anti-rabbit: Cat. No. A-21246, Cat. No. A32795; anti-mouse: Cat. No. A-11005, Cat. No. A21203; anti-chicken: Cat. No. A-11042; anti-goat: Cat. No. A11057 and Jackson Immuno: anti-chicken Cat. No. 703-545-155) for 1.5 h at room temperature, counterstained with DAPI (ThermoFisher, Cat. No. 62248) and mounted using Fluoromount-G (Southern Biotech) or Vectashield Antifade Mounting Medium (H-1000-10, Vector Laboratories). Notably, initial optimization experiments revealed that the simultaneous application of primary antibodies anti-CHI3L1 (Abcam, Cat. No. ab180569) and anti-GFAP (Encor Biotech, Cat. No. CPCA-GFAP) resulted in cross-reaction and complete co-localization of these antigens. We therefore used an anti-CHI3L1 antibody from Invitrogen. Negative controls omitting the primary antibodies were also performed.

Human tissue

Immunostaining was performed on fresh-frozen, formalin-fixed, paraffin-embedded motor cortex samples from the snRNA sequencing cohort described above. The immunostaining cohort included 6/9 ALS patients and 3/5 neurologically healthy controls (individuals highlighted in [Supplementary Table 1](#)). Paraffin-embedded sections of 4 μm thickness were first deparaffinized using 100% RotiHistol ($2 \times$, 5 min) and then rehydrated along an ethanol gradient (100% twice, 96%, 80%, 70%; 1 min each). Antigen retrieval was performed by boiling for 10 min in preheated antigen unmasking solution (Vector Laboratories, H-3300, 1:100). Sections were briefly washed in distilled water, and autofluorescence was quenched (EMD Millipore, 2160, 1 min); following this, sections were incubated in freshly prepared 3% H_2O_2 for 10 min. Sections were then washed in TBS-T (TBS pH 7.6; 0.001% Triton X-100) and blocked for 1 h in blocking solution (PBS, 0.5% Triton X-100, 10% heat-inactivated horse serum). Primary antibodies were diluted in blocking solution and incubated overnight at 4°C. Antibodies included anti-NeuN (chicken, ABN91, Sigma-Aldrich, 1:500); anti-CHIT1 (rabbit, PA5-76778, Invitrogen, 1:100); anti-CHI3L1 (rabbit, PA5-95897, Invitrogen, 1:200) and anti-CHI3L2 (rabbit, PA5-112921, Invitrogen, 1:100). After TBS-T washes, sections were

incubated with corresponding peroxidase-conjugated secondary antibodies for 2 h at room temperature: horse anti-rabbit (MP-7401) ImmPRESS IgG Polymer Kit, Peroxidase, Vector Laboratories; goat anti-chicken IgY (H + L) Secondary Antibody, horseradish peroxidase (A16054, Thermo Fisher Scientific, 1:500). After TBS-T washes, sections were incubated with fluorophore diluted 1:500 in freshly prepared borate buffer (100 mM borate buffer, 0.003% H₂O₂) for 10 min. The fluorophores used were TSA Vivid Fluorophore 520 and 570 (7523/1, 7526/1, Bio-Techne). Multiplexed staining was performed sequentially: primary antibody incubations were performed separately overnight, following repetition of antigen retrieval (boiling for 3 min and then keeping in hot buffer for 20 min). Nuclei were counterstained with DAPI (1:1000, in PBS, 3 min), and slides were mounted with Fluoromount-G (0100-01, Southern Biotech).

Image acquisition, analyses and representation

Z-stack images were acquired using a confocal microscope (LSM 710, Zeiss, or FV3000, Olympus) with 20×/N.A. 0.8; 40×/N.A. 1.3 and 63×/N.A. 1.4 oil objectives. Acquisition parameters were held constant for each specimen, allowing comparisons of immunostaining intensities between experimental and control mice; intensities were quantified using the ‘mean gray value’ parameter in ImageJ.³⁹ CHIT1 and CHI3L1 immunoreactivity was measured inside marker-positive (NeuN, Iba-1, GFAP) cells. Custom in-house macros defined marker-positive (NeuN+, Iba-1+, GFAP+) cells. Images were pre-processed to develop an automated mask that could be applied to the original image for accurate segmentation. Macro accuracy was visually inspected and verified on several stainings. For NeuN, preprocessing included the application of a pseudo-flat field correction, rolling ball background subtraction (200 pixels), the ‘Median’ filter and finally the ‘Huang 2’ auto-threshold. For Iba-1, preprocessing included the application of a rolling ball background subtraction (200 pixels), the ‘Maximum’ and ‘Minimum’ filters sequentially, the ‘Li’ auto-threshold and exclusion of all objects <500 pixels using the ‘Analyse Particles’ function. For GFAP, preprocessing included the application of a rolling ball background subtraction (50 pixels), sequential use of the ‘Minimum’ and ‘Maximum’ filters, an ‘Unsharp’ mask and finally the ‘Huang 2’ auto-threshold. These objects were then superimposed on the corresponding chitinase channel, allowing for the measurement of mean grey values within specific cell populations. Chitinase quantification within neuronal and glial populations was performed on single optical sections and sum projections (10 slices), respectively.

For microglial count around NeuN-positive objects (‘Sholl analysis’), chitinase intensity in NeuN-positive objects was measured to select the ‘highest’ and ‘lowest’ chitinase-expressing neurons (six regions of interest each). Preprocessing included the area selection around each NeuN-positive object by drawing six concentric circles [macro including $n(\text{Circles}) = 6$ and $\text{radius}(\text{Step}) = 50$ pixels] around the selected region of interest. For total Iba-1-positive objects, the preprocessing (on sum projection) included the ‘Triangle White’ auto-threshold, a selection of objects with $\text{radius} = 2.2$, ‘Skeletonize’ filter and exclusion of all objects <20.5 pixels using the ‘Analyse Particles’ function. Single optical sections are presented for all figures; channels were merged in pseudocolour using the ‘screen’ function in Adobe Photoshop.

Statistical analyses

All analyses were performed using SPSS Statistics (v26.0), GraphPad Prism (V10.4) and MedCalc® (v20.023) software packages. Normality was assessed using the Shapiro–Wilk test, and all bioanalyte levels

were log-transformed. Differences in age and gender between diagnostic cohorts and ALS sub-cohorts were assessed using one-way ANOVA and Fisher’s exact test. Correlations were assessed using Pearson’s r . Between-group differences were assessed using a one-way analysis of covariance (ANCOVA), followed by the Bonferroni correction for *post hoc* tests. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the classifier’s performance in distinguishing between ALS and non-ALS cases. Optimal cut-off values were determined using the Youden index, and all percentages are rounded to the nearest whole value. Between-group differences in immunostaining experiments were assessed using an unpaired t -test with (with Welch’s correction for unequal standard deviations) following log-transformation; data are reported as the mean and the standard error for the mean difference. For reporting immunostaining intensity, mean grey values from patients and experimental mice were normalized to the average of respective data set controls (set to 1), resulting in an x -fold expression index. Differential gene expression testing was performed using the non-parametric Wilcoxon rank sum test. Two-tailed statistical significance was set at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

CSF chitinase levels are substantially increased in ALS patients

We first assessed if chitinase upregulation is a specific feature of ALS by analysing chitinase levels in the plasma and CSF of the three following cohorts: NND controls, $n = 11$; NDEG controls, $n = 13$ (see [Supplementary Table 1](#) for all diagnoses); and ALS patients [$n = 39$, onset type (bulbar:limb) 15:24]. C9orf72 mutations were confirmed in three ALS patients, and 37/39 patients were on Riluzole treatment at the time of sampling. Age was similar between NDEGs [median = 69.8 (61.7–76.9) years] and ALS patients [median = 67.4 (61.3–75.4) years]; however, both these cohorts were significantly older than the NND cohort [median = 56.9 (44.5–70) years; $P = 0.015$]. The gender distribution did not significantly differ between diagnostic cohorts [female %, NND = 27%, sex ratio (male:female) 8:3; NDEG = 53%, sex ratio (male:female) 6:7; ALS = 48%, sex ratio (male:female) 20:19]. Given that peripheral immune cells, including macrophages and neutrophils, are chitinase sources,⁹ we first quantified chitinase levels in plasma, where concentrations of CHI3L1 ([Fig. 1A](#)), CHIT1 ([Fig. 1B](#)) and CHI3L2 ([Fig. 1C](#)) were found to be similar across groups. In contrast, in CSF, CHI3L1 ([Fig. 1D](#)), CHIT1 ([Fig. 1E](#)) and CHI3L2 ([Fig. 1F](#)) levels showed significant inter-group differences. All three chitinases were significantly elevated in the CSF of ALS patients relative to NND controls ([Fig. 1D–F](#)), while NND and NDEG controls exhibited similar chitinase concentrations ([Fig. 1D–F](#)). Notably, CSF levels of CHIT1 and CHI3L2 ([Fig. 1E and F](#)), but not CHI3L1 ([Fig. 1D](#)), were also significantly higher in ALS patients compared with NDEG individuals. This suggests that upregulation of these two isoforms is reflective of an ALS-specific signature,⁹ while CHI3L1 upregulation appears to be a more general feature of neurodegenerative conditions. This is largely consistent with previous reports of age-related increases and their potential role as an indicator of the broader ‘inflammaging’ processes.⁴⁰

Glial chitinase expression is upregulated in symptomatic ALS mice

Given that our CSF data confirmed elevated chitinase levels in the CNS and that glial chitinase expression has been previously

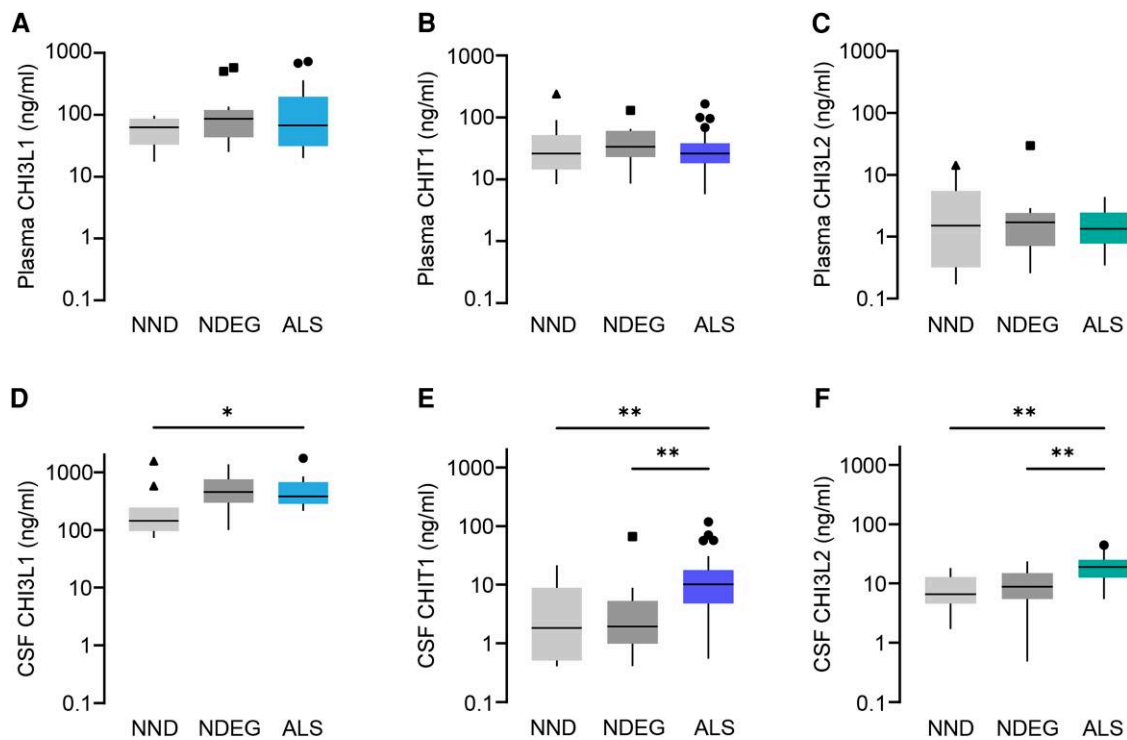


Figure 1 Cross-sectional analyses of chitinase levels in plasma and CSF. Chitinase concentrations (ng/ml) in the plasma (A–C) and CSF (D–F) of NNDs ($n = 11$), NDEGs ($n = 13$) and ALS patients ($n = 39$). (A) Plasma, CHI3L1; (B) plasma, CHIT1; (C) plasma, CHI3L2; (D) CSF, CHI3L1; (E) CSF, CHIT1; (F) CSF, CHI3L2. Between-group differences were assessed using a one-way ANCOVA with age included as a covariate. Data are presented as box plots with whiskers demarcating 95% confidence intervals. Statistical significance, * $P < 0.05$ and ** $P < 0.01$. All axes are displayed in log₁₀ scale. ALS = amyotrophic lateral sclerosis; NDEGs = neurodegenerative disease controls; NNDs = non-neurodegenerative disease controls.

reported,^{18,24,41} we next sought to characterize both potential cellular chitinase sources and disease-associated expression dynamics in the murine spinal cord. To this end, we employed the Thy1-(GA)₁₄₉-CFP (abbreviated as C9 GA-CFP),²⁷ SOD1-G93A (abbreviated as SOD1) and rNLS8-hTDP-43 (abbreviated as hTDP-43) ALS mouse models. C9 GA-CFP⁺ mice develop aggregates of poly-GA dipeptide repeat proteins in neurons, which are implicated in C9orf72-mediated pathology, and exhibit microgliosis and moderate TDP-43 phosphorylation, but no overt neuronal loss.²⁷ Transgenic SOD1 mice express large amounts of human SOD1 with the disease-causing G93A mutation and rapidly display gliosis and motor neuron loss, leading to paralysis at approximately 3 months of age.²⁹ We also qualitatively inspected the hTDP-43 model, which expresses a human TDP-43 protein lacking the nuclear localization leading to rapid ALS pathology, including TDP-43 pathology, motor neuron loss and reduced survival.²⁸ Overall, the hTDP-43 and SOD1 models present with a more aggressive disease phenotype than the C9 GA-CFP model.

First, we assessed CHI3L1 and CHIT1 expression and protein levels within GFAP⁺ astrocytes in the ventral horn of the lumbar spinal cord, as this is a key site of ALS pathology. CHI3L2 was not assessed, as this isoform is only present in humans. Immunostaining was performed on sections from symptomatic C9 GA-CFP⁺ ($n = 5$), SOD1⁺ ($n = 4$) and hTDP-43 ($n = 2$) mice and their respective control littermates ($n = 5$, $n = 3$ and $n = 2$; Fig. 2 and Supplementary Fig. 1). Approximately half of all GFAP⁺ astrocytes in control mice were CHI3L1⁺, while this proportion was 20% higher in transgenic C9 GA-CFP⁺ and SOD1⁺ mice (Fig. 2A–F). Moreover, astrocytic CHI3L1 immunostaining intensity was 1.5-fold higher in transgenic mice (Fig. 2A–F), and a similar trend was observed in the hTDP-43 mouse

model (Supplementary Fig. 1A–D). In contrast, both the proportion of CHIT1⁺ astrocytes and expression levels were similar between transgenic and control mice in the C9 GA-CFP and SOD1 models (Fig. 2G–I) and only appeared qualitatively increased in hTDP-43⁺ mice (Supplementary Fig. 1E–H).

Next, we analysed chitinase expression in Iba1⁺ microglia, as they have been described as chitinase sources and play a key role in neuroinflammation and disease progression.⁹ As observed with astrocytes, transgenic C9 GA-CFP⁺ and SOD1⁺ mice had a 20% higher proportion of CHI3L1⁺ microglia relative to their littermate controls (Fig. 2M–Q), with an approximately 1.5-fold increase in CHI3L1 immunostaining intensity in transgenic C9 GA-CFP⁺ mice (Fig. 2O). Transgenic mice in both models also presented with an increased proportion of CHIT1⁺ microglia (~15%) and approximately 1.3-fold higher microglial CHIT1 levels relative to control mice (Fig. 2S–X). This was also qualitatively confirmed in transgenic hTDP-43⁺ mice (Supplementary Fig. 1I–P).

Collectively, these findings demonstrate consistent CHIT1 and CHI3L1 upregulation in spinal cord glia across three ALS mouse models, underscoring the link between chitinases and neuroinflammatory burden. Notably, CHIT1 upregulation was restricted to microglia, whereas CHI3L1 was upregulated in both astro- and microglia.

Neuronal expression of CHI3L1 and CHIT1 and dysregulation in ALS mouse models

Given that our initial experiments indicated chitinase expression in structures reminiscent of neuronal somata, we also performed multiplex CHIT1 or CHI3L1 immunostaining on spinal cord sections

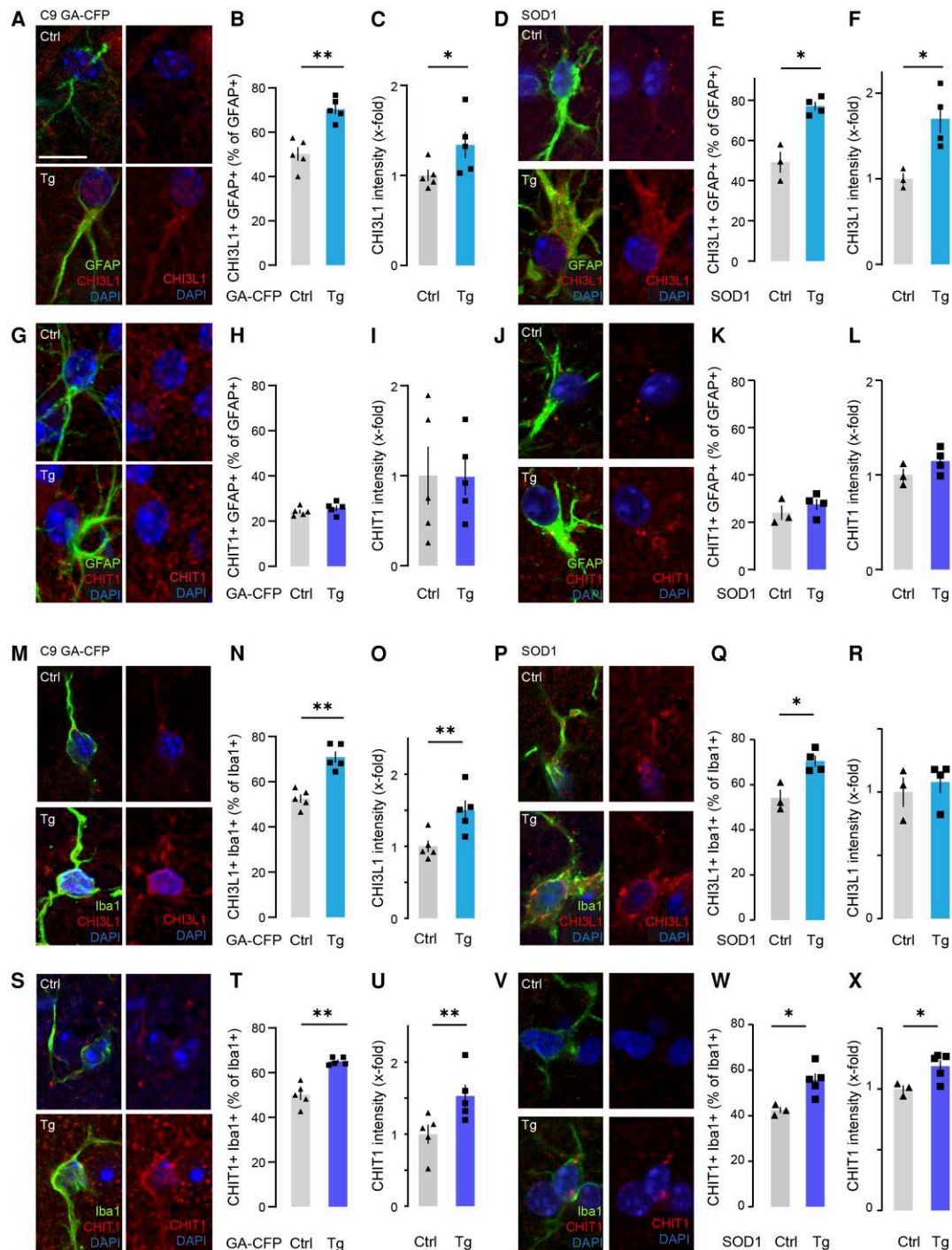


Figure 2 Glial chitinase expression in the spinal cord of the C9 GA-CFP and SOD1 ALS mouse models. (A, D, G and J) Representative confocal images of quantitative immunostaining for (A and D) CHI3L1 or (G and J) CHIT1 (red), GFAP (green) and DAPI (nuclei, blue) in spinal cord ventral horn. Right: CHI3L1 or CHIT1 (red), DAPI (blue) (A and G) C9 GA-CFP[−] littermate controls (top, Ctrl) and symptomatic C9 GA-CFP⁺ mice (bottom, Tg). (D and J) SOD1[−] littermate controls (top, Ctrl) and symptomatic SOD1⁺ mice (bottom, Tg). (B, E, H and K) Quantification of CHI3L1- (B and E) or CHIT1-expressing (H and K) GFAP⁺ astrocytes in Ctrl versus Tg mice in the C9 GA-CFP and SOD1 models (CHI3L1, C9 GA-CFP $P = 0.007$, SOD1 $P = 0.02$; CHIT1, C9 GA-CFP $P = 0.001$, SOD1 $P = 0.002$). (C, F, I and L) Quantification of CHI3L1 (C and F) or CHIT1 (I and L) immunostaining intensity in GFAP⁺ astrocytes in Ctrl versus Tg mice in the C9 GA-CFP and SOD1 models (CHI3L1, C9 GA-CFP $P = 0.03$, SOD1 $P = 0.01$; CHIT1, C9 GA-CFP and SOD1 $P > 0.05$). (M, P, S and V) Representative confocal images of quantitative immunostaining for (M and P) CHI3L1 or (S and V) CHIT1 (red), Iba-1 (green) and DAPI (nuclei, blue) in the spinal cord ventral horn. Right: CHI3L1 or CHIT1 (red) and DAPI (blue). (M and S) C9 GA-CFP[−] littermate controls (top, Ctrl) and symptomatic C9 GA-CFP⁺ mice (bottom, Tg). (P and V) SOD1[−] littermate controls (top, Ctrl) and symptomatic SOD1⁺ mice (bottom, Tg). (N, Q, T and W) Quantification of CHI3L1- (N and Q) or CHIT1-expressing (T and W) Iba-1⁺ microglia in Ctrl versus Tg mice in the C9 GA-CFP and SOD1 models (CHI3L1, C9 GA-CFP $P = 0.005$, SOD1 $P = 0.01$; CHIT1, C9 GA-CFP and SOD1 $P > 0.05$). (O, R, U and X) Quantification of CHI3L1 (O and R) or CHIT1 (U and X) immunostaining intensity in Iba-1⁺ microglia in Ctrl versus Tg mice in the C9 GA-CFP and SOD1 models (CHI3L1, C9 GA-CFP $P = 0.01$, SOD1 $P = 0.60$; CHIT1, C9 GA-CFP $P = 0.03$, SOD1 $P = 0.02$). Immunostaining intensity reported as x-fold index. Data are presented as mean $\pm$ standard error of the mean; statistical significance, * $P < 0.05$ and ** $P < 0.01$, unpaired t-test. Scale bar = 10 μ m in A, applies to all images. C9 GA-CFP model: five Ctrl, five Tg mice. SOD1 model: three Ctrl, four Tg mice. Cells quantified/animal: Iba1⁺ microglia $n \geq 50$, GFAP⁺ astrocytes $n \geq 50$. Ctrl = control; Tg = transgenic.

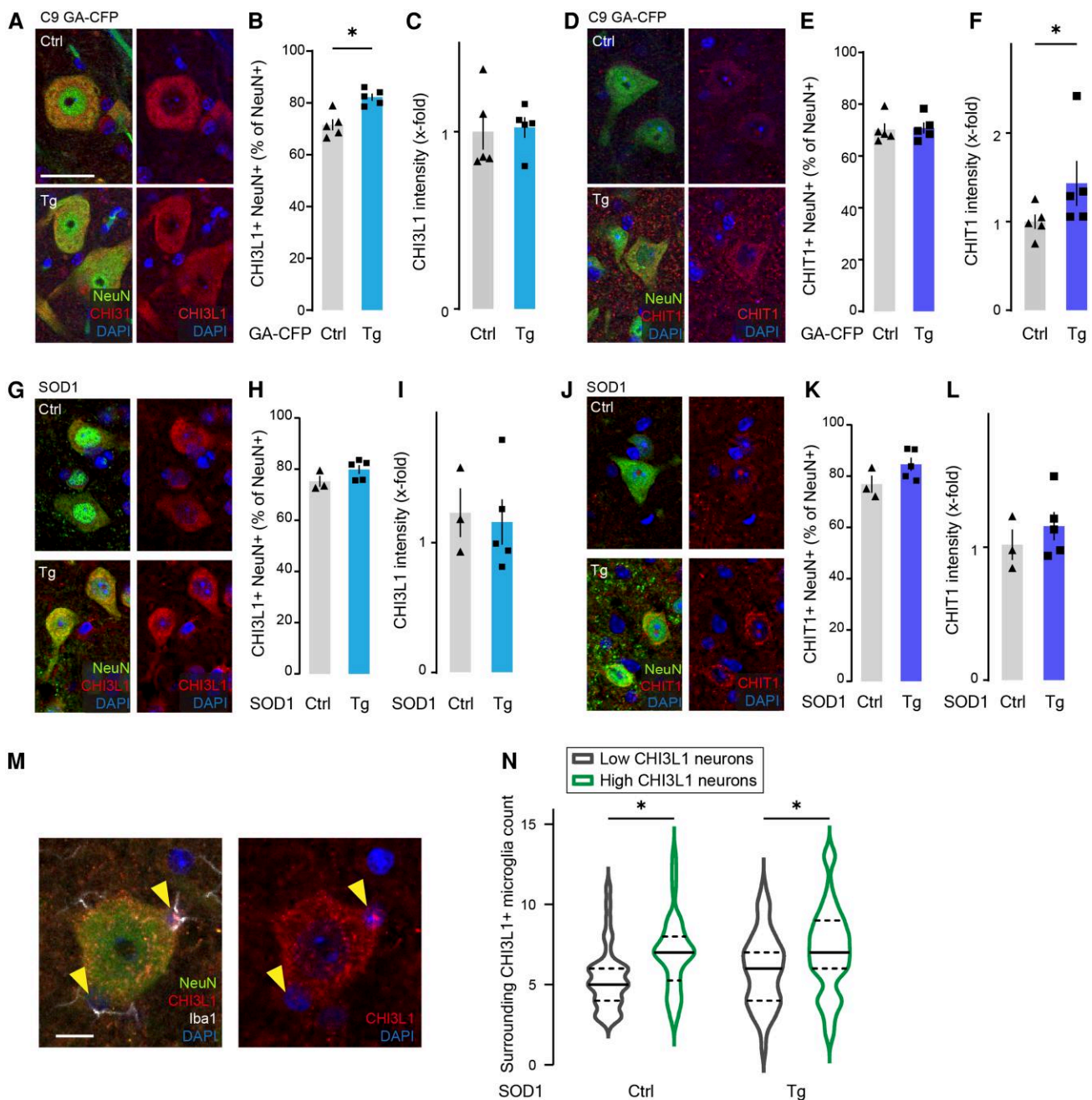


Figure 3 Neuronal chitinase expression in the spinal cord of the C9 GA-CFP and SOD1 ALS mouse models. (A, D, G and J) Representative confocal images of quantitative immunostaining for (A and G) CHI3L1 or (D and J) CHIT1 (red), the neuronal marker NeuN (green) and DAPI (nuclei, blue) in the spinal cord ventral horn. Right: CHI3L1 or CHIT1 (red) and DAPI (blue). (A and D) C9 GA-CFP⁺ littermate controls (top, Ctrl) and symptomatic C9 GA-CFP⁺ mice (bottom, Tg). (G and J) SOD1⁺ littermate controls (top, Ctrl) and symptomatic SOD1⁺ mice (bottom, Tg). (B and E) CHI3L1⁺ or (E and K) CHIT1-expressing NeuN⁺ cells in (B and E) C9 GA-CFP⁺ versus C9 GA-CFP⁻ (CHI3L1 $P = 0.01$, CHIT1 $P > 0.05$) or (H and K) SOD1⁺ versus SOD1⁻ mice (CHI3L1 and CHIT1 $P > 0.05$). (C, F, I and L) Quantification of (C and F) CHI3L1 or (F and L) CHIT1 immunostaining intensity in NeuN⁺ cells in (C and F) C9 GA-CFP⁺ versus C9 GA-CFP⁻ (CHI3L1 $P > 0.05$, CHIT1 $P = 0.03$) or (I and L) SOD1⁺ versus SOD1⁻ mice (CHI3L1 and CHIT1 $P > 0.05$). (M) Representative confocal images of quantitative immunostaining for NeuN (green), CHI3L1 (red), Iba1 (white) and DAPI (nuclei, blue) in the spinal cord ventral horn. Right: CHI3L1 (red) and DAPI (blue). Yellow arrows indicate CHI3L1⁺ Iba1⁺ microglia surrounding a CHI3L1⁺ neuron. (N) Quantification of the number of CHI3L1⁺ microglia surrounding high (green) or low (grey) expressing CHI3L1⁺ neurons in SOD1⁺ versus SOD1⁻ mice—at least $n \geq 50$ cells/animal. Immunostaining intensity reported as x-fold index. Data are presented as mean $\pm$ standard error of the mean; statistical significance, * $P < 0.05$ and ** $P < 0.01$, unpaired t-test. Scale bar = 10 μ m in A, applies to all images. C9 GA-CFP model: five Ctrl, five Tg mice. SOD1 model: five Ctrl, five Tg mice. Cells quantified/animal: NeuN⁺ neurons $n \geq 50$. Ctrl = control; Tg = transgenic.

with NeuN, a general neuronal marker. Both chitinases showed clear and robust cytoplasmic localization in NeuN⁺ neurons in the ventral horn of transgenic C9 GA-CFP⁺, SOD1⁺ and hTDP-43⁺ mice, as well as their littermate controls (Fig. 3 and Supplementary Fig.

1Q–X). Neuronal chitinase immunostaining was also validated with a second CHI3L1 antibody (Supplementary Fig. 2). C9 GA-CFP⁺ mice exhibited an approximately 10% increase in CHI3L1⁺ neurons (Fig. 3A–C) and a 1.4-fold elevation in CHIT1

intensity relative to controls (Fig. 3D–F), whereas neither CHIT1 nor CHI3L1 proportion or intensity were altered in the SOD1 ALS model (Fig. 3G–I). Although judged qualitatively, immunostainings revealed a similar disease-associated neuronal chitinase upregulation in the hTDP-43 mouse model (Supplementary Fig. 1Q–X). Together, these data show that neurons are a physiological chitinase source, and their role in disease-associated chitinase upregulation has been underappreciated. We further hypothesized that CHI3L1⁺ neurons may recruit microglia, given that CHI3L1 is a secreted glycoprotein and our observation of several CHI3L1⁺ microglia adjacent to CHI3L1⁺ neurons in the SOD1 model (Fig. 3M). To this end, we employed Sholl analyses to quantify Iba1⁺ cells within a 50 µm radius of neurons with high versus low CHI3L1 levels in transgenic SOD1⁺ and control mice. Indeed, neurons with higher CHI3L1 levels were surrounded by significantly more CHI3L1⁺ microglia in both control and transgenic mice, suggesting chemotactic signalling (Fig. 3N).

Given our observation of neuronal chitinase expression in the ventral horn and the selective vulnerability of motor neurons in ALS, we examined chitinase expression directly in motor neurons *in vivo* and *in vitro*. First, we performed multiplexed staining for CHIT1/CHI3L1 and choline acetyltransferase (ChAT), a marker specific to motor neurons in the ventral horn of the lumbar spinal cord. Both control and transgenic mice from the C9 GA-CFP and SOD1 models showed similar proportions (~70%–80%) of chitinase-expressing ChAT⁺ motor neurons (Supplementary Fig. 3). Intriguingly, RNA-seq analysis of primary murine motor neuron cultures indicated minimal chitinase expression (Supplementary Fig. 3). In line with this, compartmentalized RNA-seq analysis of iPSC-derived motor neurons differentiated from an ALS patient and a matched healthy control showed similarly low chitinase transcript abundance (Supplementary Fig. 3). In summary, adult motor neurons from both control and ALS transgenic mice exhibit robust chitinase expression *in vivo*, a phenomenon absent in cultured neurons.

As neuronal chitinase expression has not been previously reported, we examined another CNS region using 10x snRNA-seq of somatosensory cortical grey matter from Aldh1l1-EGFP mice (*n* = 15). This generated 36 000 nuclear transcriptomes (Fig. 4A) and, after unbiased clustering, 30 cellular populations comprising neurons (30%), oligodendrocytes (34%), oligodendrocyte precursor cells (OPCs, 3.5%), astrocytes (15%), microglia (16%) and minor cell types (3.5%; Fig. 4B). CHI3L1 transcripts were detected mainly in glia but also in neurons (astrocytes 5.5%; microglia 2.8%; neurons 0.8%; oligodendrocytes 0.4%; OPCs 1.25%; Fig. 4C–F). In contrast, CHIT1 expression was lower overall and relatively enriched in neurons, with minimal detection in glial populations (astrocytes 0.03%; microglia 0%; neurons 0.16%; oligodendrocytes 0.02%; OPCs 0.08%; Fig. 4C–F). Neuronal cluster mapping revealed that chitinase-positive neurons aligned primarily with excitatory glutamatergic and pyramidal cell identities, with smaller contributions from bipolar neurons (Fig. 4G–I). To validate these transcriptomic findings, we performed immunostaining in adult wild-type mice (*n* = 4). Both CHI3L1 and CHIT1 showed robust expression in NeuN⁺ neurons in cortical layers V (Fig. 5A–D) and II/III (Supplementary Fig. 4). Quantification of immunostaining intensity demonstrated considerable intrinsic variation in CHI3L1 (Fig. 5E) and CHIT1 (Fig. 5F) expression levels across the neuronal population, as predicted by the snRNA-seq dataset. Next, we assessed glial chitinase expression by immunostaining cortical sections from Aldh1l1-EGFP and Cx3cr1-EGFP mice, which provide transgenic labelling of astrocytes and microglia, respectively. CHI3L1 protein co-localized with both

Aldh1l1-EGFP⁺ astrocytes and Cx3cr1-EGFP⁺ microglia, whereas CHIT1 expression was low in both glial populations (Fig. 5G–N), which aligns with our data from the spinal cord. Overall, transcriptomic expression patterns in the cortex were broadly similar to those observed at the protein level by immunostaining. In the absence of neurodegenerative stimuli, CHIT1 expression is lower and predominantly neuronal, while CHI3L1 is both more abundant and broadly expressed across all major CNS populations.

Neuronal chitinase expression in post-mortem human motor cortex

To determine whether neuronal chitinase expression is conserved in humans, we performed snRNA-seq on cortical nuclei isolated from the motor cortex of five neurologically healthy controls (HCs) and nine ALS patients, including cases with familial C9orf72 (*n* = 5) and sporadic cases (*n* = 4; Supplementary Table 2). All ALS cases were neuropathologically confirmed by the presence of intracytoplasmic phospho-TDP43 inclusions in the motor cortex and spinal cord, corticospinal tract degeneration with myelin loss and microglial activation. We assessed transcript abundance of CHI3L1, CHIT1 and the human-specific isoform CHI3L2 across major CNS cell types, including excitatory and inhibitory neurons, astrocytes, oligodendrocytes and microglia (Fig. 6A–C). Of all the cellular clusters defined by Uniform Manifold Approximation and Projection (UMAP) plots of the cortex of healthy individuals (*n* = 98, 522 cells), CHI3L1 expression was most abundant in astrocytes, but also observed in excitatory neurons. In ALS patients, both the proportion of CHI3L1⁺ astrocytes and CHI3L1 transcript levels were significantly elevated (Fig. 6D and E), mirroring observations in ALS mouse models. Similarly, CHIT1⁺ and CHI3L1⁺ microglia were more abundant in ALS patients, accompanied by higher microglial CHI3L1 transcript levels (Fig. 6D and E). Oligodendrocytes expressed all three chitinases in healthy individuals, with a modest expansion of the CHI3L1⁺ fraction in ALS in comparison to healthy individuals, but no significant change in overall transcript levels (Fig. 6D and E). Taken together, these data identify astrocytes as the primary glial source of CHI3L1 and indicate an ALS-associated upregulation of CHI3L1 in both astrocytes and microglia. We next analysed neurons by excitatory and inhibitory cluster identity (Fig. 6A); CHI3L1 and CHIT1 neuronal transcripts were observed in both control and ALS patient samples, whereas CHI3L2 was barely detectable (Fig. 6F and G). Consistent with our transcriptomic mouse data, chitinase expression was predominantly observed in excitatory neurons, particularly cluster 7 (Fig. 6G and Supplementary Fig. 5A–E). This subcluster was further characterized by positive expression of a combination of markers, including ARSJ (arylsulfatase family member J), TLL1 (tolloid-like 1) and TRABD2A (TraB domain containing 2A) (Supplementary Fig. 5F and G). Furthermore, cluster 7 neurons express upper cortical layer markers like CUX2 (cut like homeobox 2) and RORB (RAR related orphan receptor B), pointing to a potential localized reduction of chitinase expression in layers II/III of the motor cortex in ALS (Supplementary Fig. 6F). Additionally, CHI3L1 expression was significantly elevated in a subset of interneurons in ALS (*P* < 0.05; Fig. 6F–H), whereas CHIT1 levels were unchanged. Finally, comparative analysis revealed no significant differences in chitinase expression across CNS populations in sporadic versus familial ALS. These findings expand our understanding of neuronal chitinase biology, revealing isoform- and cell-type-specific regulation in the human cortex.

To corroborate the human neuronal transcriptomic data, we performed immunostainings for the three chitinase isoforms in

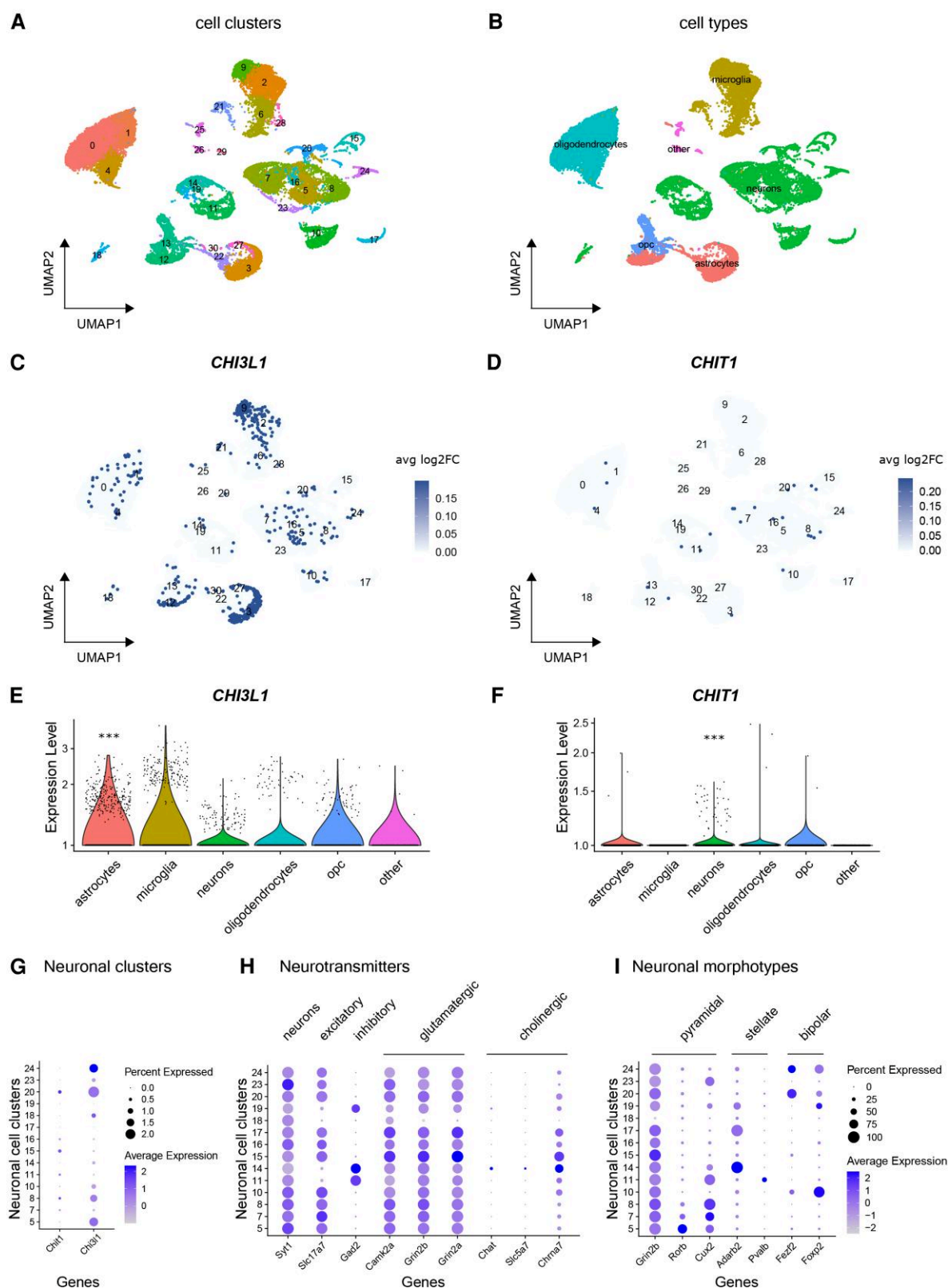


Figure 4 Single-nuclei RNA sequencing of murine cortex confirms neuronal *CHIT1* and *CHI3L1* expression. (A and B) Uniform Manifold Approximation and Projection (UMAP) of six cell types and 30 cell-type specific subclusters from $n = 36\,000$ cells from the somatosensory cortex (Aldh1l1-EGFP reporter mice, $n = 15$), mapping on main broad cortical cell types. (C and D) UMAP plots highlight the distribution of (C) *CHI3L1* and (D) *CHIT1* expression across cortical cell clusters. (E and F) Violin plots comparing (E) *CHI3L1* and (F) *CHIT1* expression levels between clusters. (G) Dot plot of average gene expression (DEG) of *CHI3L1* and *CHIT1* across all neuronal cell clusters; cluster identifiers match those in B. (H and I) Mapping of neuronal clusters from G onto canonical neurotransmitter H and morphology I markers. Differences in gene expression levels calculated using the Wilcoxon rank sum test; statistical significance, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Axes for E and F are displayed in $\log_{10}$ scale. OPC = oligodendrocyte precursor cells.

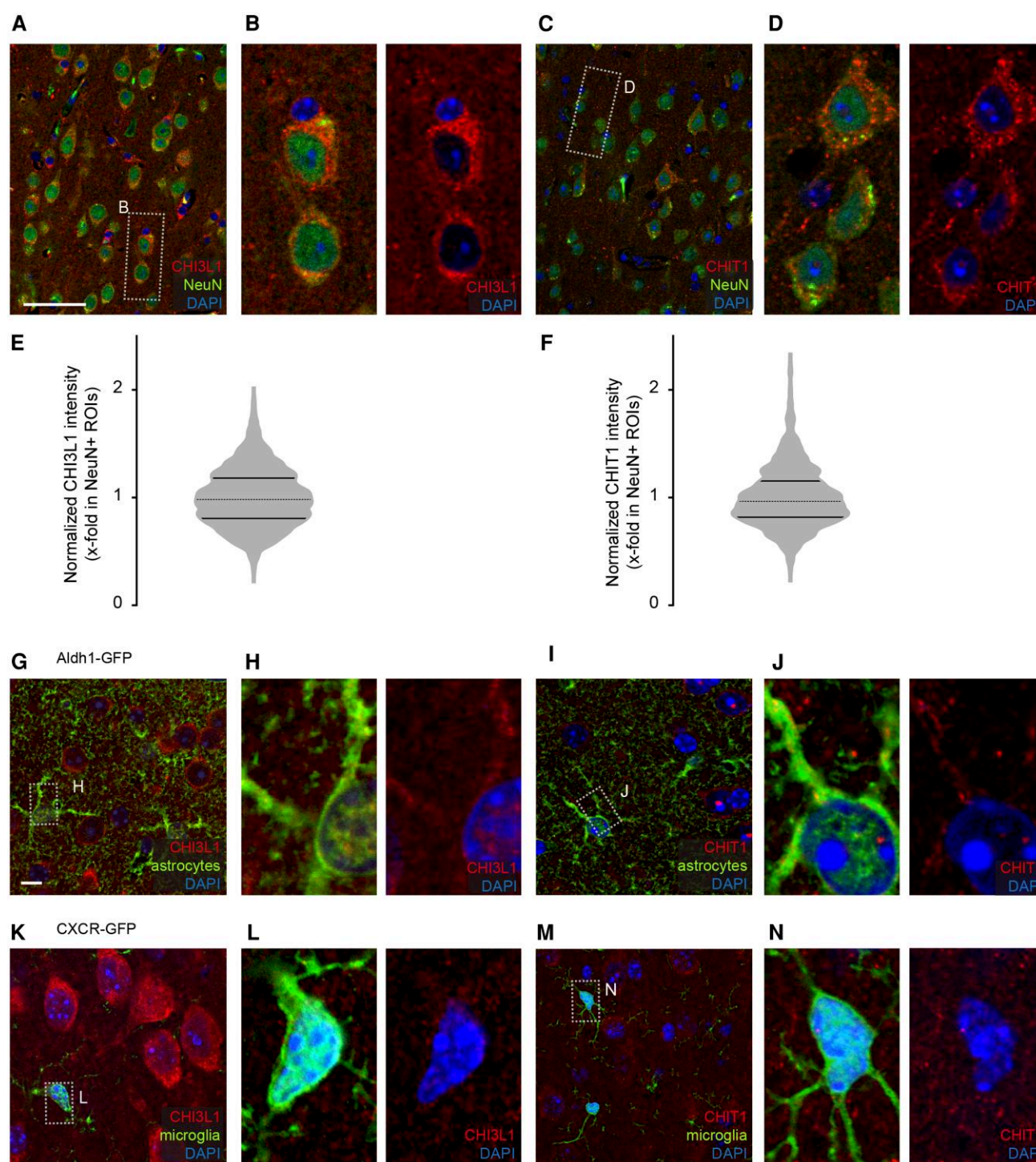


Figure 5 CHIT1 and CHI3L1 protein expression in the mouse motor cortex. (A–D) Representative confocal images of cortical layer V in a healthy adult mouse immunostained for (A and B) CHI3L1 (red) or (C and D) CHIT1 (red), NeuN (green) and DAPI (nuclei, blue). (B and D) Enlarged boxed insets in merged pseudocolour for (B) CHI3L1 and (D) CHIT1 and DAPI (nuclei, blue) and corresponding chitinase-only panels to the right. (E and F) Violin plots depicting intrinsic variation in (E) CHI3L1 and (F) CHIT1 intensity in NeuN⁺ neurons after normalization to group mean intensity ($n = 4$ Aldh1l1-EGFP mice, ≥ 250 NeuN⁺ cells/animal); dashed line = median, black lines = quartile positions. (G–J) Representative confocal images of cortical layers II/III in an adult Aldh1l1-EGFP mouse immunostained for (G and H) CHI3L1 (red) or (I and J) CHIT1 (red), astrocytes (green) and DAPI (nuclei, blue). (H and J) Enlarged boxed insets for (H) CHI3L1 and (J) CHIT1 and DAPI (nuclei, blue) in merged pseudocolour and corresponding chitinase only panels to the right. (K–N) Representative confocal images of cortical layers II/III in an adult Cx3cr1-GFP mouse immunostained for (K and L) CHI3L1 (red) or (M and N) CHIT1 (red), microglia (green) and DAPI (nuclei, blue). (L and N) Enlarged boxed insets for (L) CHI3L1 and (N) CHIT1 and DAPI (nuclei, blue) in merged pseudocolour corresponding to chitinase-only panels to the right. Scale bar = 10 μ m in A applies to A–D. Scale bar = 10 μ m in G applies to G–N.

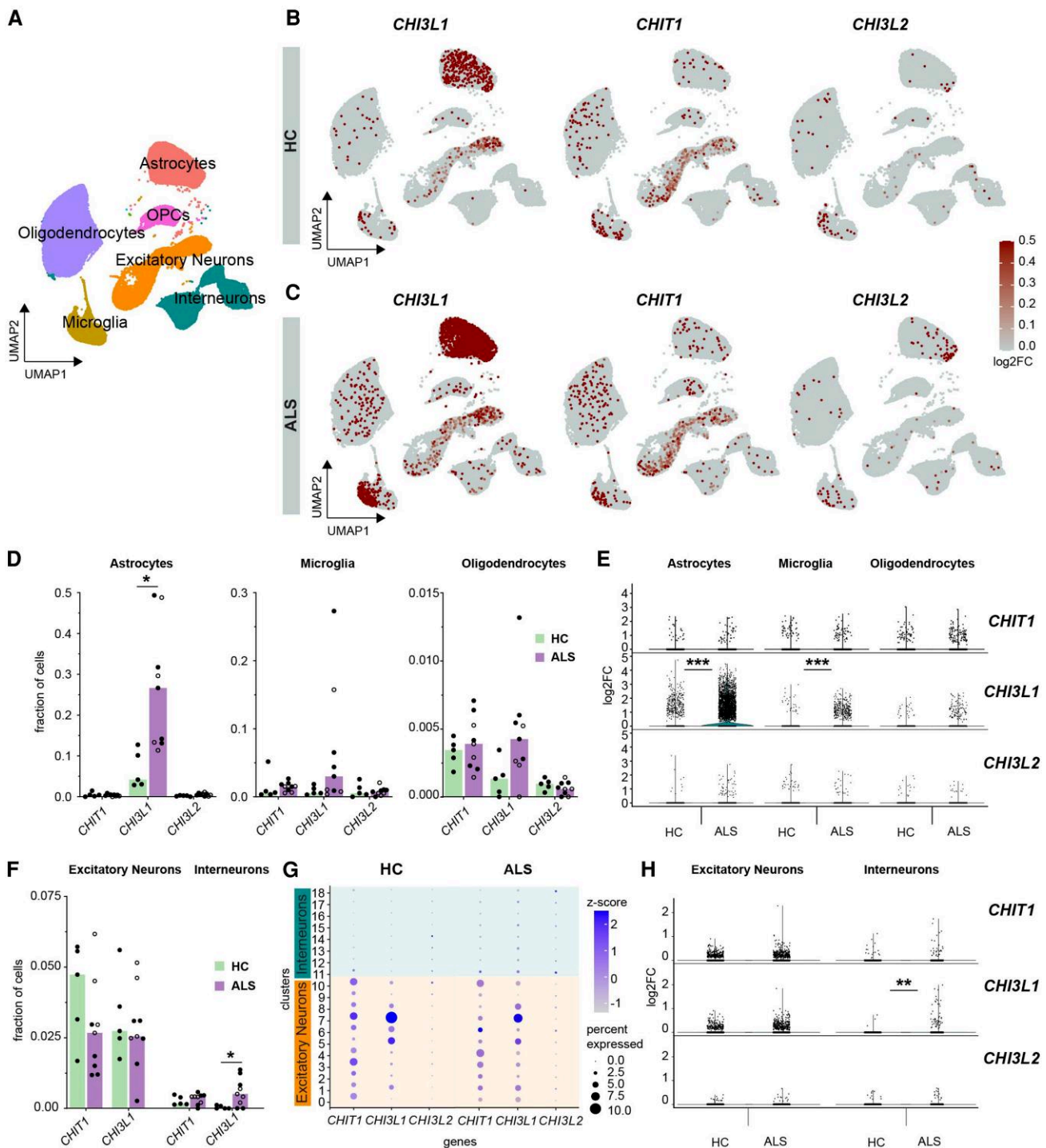


Figure 6 Chitinase expression in healthy controls and ALS patients based on single-nuclei RNA sequencing of human primary motor cortex. Single-nuclei RNA sequencing in the primary motor cortex in a cohort of healthy controls (HCs, $n = 5$) and patients with sporadic ($n = 4$) or familial (C9orf72, $n = 5$) ALS. (A) Uniform Manifold Approximation and Projection (UMAP) of main glial and neuronal clusters displaying six main cell types: oligodendrocytes (violet, $n = 41\,910$), astrocytes (light red, $n = 13\,979$), microglia (ochre, $n = 7234$), OPC (pink, $n = 6138$), excitatory neurons (orange, $n = 14\,705$) and interneurons (green, $n = 14\,556$). (B and C) UMAP plots highlight the distribution of log-normalized gene expression ($\log_2$ -fold change, $\log_2FC$) levels of *CHI3L1*, *CHIT1* and *CHI3L2* in (B) HCs and (C) ALS patients. (D) Fraction of astrocytes (left), microglia (middle) and oligodendrocytes (right) expressing *CHIT1*, *CHI3L1* and *CHI3L2*. (E) Expression levels of *CHIT1*, *CHI3L1* or *CHI3L2* in astrocytes, microglia and oligodendrocytes in HCs and ALS. (F) Fraction of excitatory and inhibitory interneurons expressing *CHIT1* or *CHI3L1* in HCs and ALS. (G) Dot plot of z-scored gene expression of *CHIT1*, *CHI3L1* and *CHI3L2*, and the fraction of cells expressing them per cluster in HCs and ALS patients. (H) Expression levels of *CHIT1*, *CHI3L1* or *CHI3L2* in excitatory neurons and interneurons in HCs and ALS patients. Data in (D and F) correspond to the median plus individual data (open circles, sporadic ALS; closed circles, C9orf72 familial ALS); groups were compared using two-tailed, unpaired t-tests. * $P < 0.05$. In E and H, significance was determined by a two-tailed Wilcoxon rank sum test. ** $P < 0.01$. ALS = amyotrophic lateral sclerosis; OPC = oligodendrocyte precursor cells.

motor cortex sections from the same individuals. Neuronal expression was evident for CHI3L1 (Fig. 7A–C), CHIT1 (Fig. 7D–F) and CHI3L2 (Fig. 7G–I) in cortical layers V and II/III (Supplementary Fig. 6) for both controls and patients with ALS. Quantitative analysis of neuronal chitinase immunoreactivity revealed no significant between-group differences, although substantial inter-individual variability was noted (Fig. 7C, F and I and Supplementary Fig. 6). Finally, chitinase expression levels were similar between patients with sporadic versus familial ALS, as observed in the snRNA-seq experiment.

In summary, we show that neurons are a major physiological source of chitinases in both the mouse and human cortex and, like glia, show isoform-specific dysregulation of chitinases in ALS.

Chitinases in CSF are correlated with but do not diagnostically outperform the neurofilament proteins

Given the neuronal origin of the chitinases, we wanted to examine how they relate to the neurofilament polypeptides, as the latter are well-established biomarkers for ALS.^{16,42,43} The neurofilaments are heteropolymers that are essential components of the neuronal cytoskeleton and comprise several subunits, including neurofilament light (NfL), medium and heavy (NfH) chains.⁴⁴ Elevated NfL and phosphorylated NfH (pNfH) levels in the CSF and plasma of ALS patients have been consistently reported and are believed to reflect neuro-axonal injury.^{45,46} As expected, both CSF NfL and pNfH levels were significantly higher in the ALS cohort relative to both the NDEG and NND cohorts, where baseline levels were very low (Fig. 8A and B). Notably, CSF neurofilament levels also significantly correlated with levels of three chitinases (Fig. 8C and D), underscoring the link between neuronal damage and increased CSF chitinase levels. Discriminatory power was assessed by combining the NDEG and NND groups and using ROC curve analysis to distinguish between 'ALS' versus 'non-ALS' (Fig. 8E and Supplementary Table 3). The strongest classifier performance was noted for pNfH, which had the largest area under the curve (AUC). CHI3L1 showed no significant discriminatory power, in line with the lack of significant difference observed between the ALS and NDEG cohorts, whereas CHIT1 and CHI3L2 were able to discriminate well between groups (Fig. 1E).

Discussion

Here, we show that key chitinase family members, previously considered exclusively glial in origin, are constitutively expressed by cortical and spinal neurons in both preclinical disease models and human tissue. Neurons, in addition to astrocytes and microglia, present with chitinase upregulation in ALS. These data, combined with the strong association with neurofilament levels, suggest that elevated CSF chitinase levels could be recruited as a metric of neural injury rather than inflammation alone. Consequently, our work adds chitinases to the biomarker repertoire and provides a new lens through which to interpret the role of the chitinases in ALS and exploit them to expedite therapeutic development.

We observed that CHIT1 and CHI3L2 were significantly elevated in ALS CSF compared with both NDEG and NND groups.^{19,24,47} CHI3L1 was increased only relative to NNDs and failed to distinguish ALS from non-ALS controls, which fits with its broad CNS expression and upregulation in other neurodegenerative disorders, including Alzheimer's⁴⁸ and Parkinson's disease.⁴⁹ Among the

chitinases, CHI3L2 showed the highest AUC and strongest correlation with pNfH—the most robust diagnostic marker overall.^{46,50} The differing diagnostic potential of chitinases supports the idea that varying expression reflects subtle differences in the underlying pathology, as seen in ALS and FTD, which exhibit overlapping but divergent chitinase profiles. CHIT1 levels predict ALS progression⁵¹ and motor impairment,^{19,52,53} and CHIT1/CHI3L2 can differentiate C9orf72-ALS from C9orf72-FTD.⁵⁴ Conversely, CHI3L1 CSF levels are higher in FTD compared to ALS⁵² and associated with increased cognitive deficits.^{19,52,55} Our results underscore that despite being strongly intercorrelated, individual chitinases display distinct expression profiles and cannot be used interchangeably. pNfH, CHIT1 and CHI3L2 are more reflective of ALS pathology and therefore better suited for diagnostic refinement, while NfL and CHI3L1 are highly sensitive to disease aggressiveness and more appropriate for prognostic assessment.²² We did not detect between-group differences for CHIT1, CHI3L1 or CHI3L2 in plasma, confirming that chitinase upregulation is predominantly a feature of the CNS.^{18,24}

Our study uses preclinical models to quantitatively recapitulate chitinase upregulation in the CSF of ALS patients at the cellular level. We observed CHIT1 upregulation in microglia, thus corroborating previous findings,^{18,56} and CHI3L1 expression in both astrocytes and microglia,^{18,41,57} contrary to prior reports of it being exclusively astroglial in ALS.²⁴ A recent study of human iPSC-derived microglia confirmed enriched CHI3L1 expression and secretion,⁴¹ while RNA-seq of motor cortex tissue from ALS patients revealed that CHI3L1 and CHI3L2 upregulation was likely driven by an overrepresentation of microglia.⁵⁸ Similarly, in brain autopsies from patients with multiple sclerosis, CHI3L1 expression predominated in CD68⁺ macrophages/microglia in the early disease stages but subsequently spread to astrocytes in later stages.⁵⁶ Interestingly, neurotoxic astrocytes in ALS are induced by classically activated microglia via the secretion of inflammatory factors⁵⁹ and exposure to conditioned macrophage media induced CHI3L1 expression in astrocytes.⁶⁰ Increased glial CHI3L1 expression in ALS may therefore follow as a sequela of neuronal damage.

Using two independent methods, we identify neurons as a previously unrecognized chitinase source, a finding that supports the idea of a pro-inflammatory cascade in ALS pathology. Progressive neuronal loss might be accompanied by elevated intraneuronal chitinase levels and increased extracellular secretion, possibly as an acute response to cell death and aggregate formation. Secreted chitinases can act on glia, which are themselves chitinase sources, by triggering the production of pro-inflammatory cytokines and increasing their migratory capacity.^{18,57,60,61} Degenerating neurons may create a damage-associated chemotactic axis, thus recruiting neighbouring glia, as we observed in the SOD1 model, where CHI3L1⁺ neurons were surrounded by CHI3L1⁺ microglia. An analogous 'neuronal-glial' inflammatory 'shift' has been reported in Alzheimer's disease, wherein pro-inflammatory cytokines were first evident in neurons and subsequently triggered the activation of neighbouring microglia.⁶²

Moreover, high chitinase concentrations are neurotoxic *in vitro* and *in vivo* following intrathecal injections.^{61,63} This, combined with the autocrine and paracrine effects of the chitinases, can set up a feed-forward pathological cascade, ultimately contributing to the chronic neuroinflammatory milieu characteristic of ALS. This hypothetical cascade also aligns with observations from longitudinal studies showing that chitinase elevation is a feature of late presymptomatic disease: levels rise sharply in mutation carriers at the time of phenoconversion, possibly in response to neuronal loss, and then stay relatively stable over time.^{22,24,64} Although

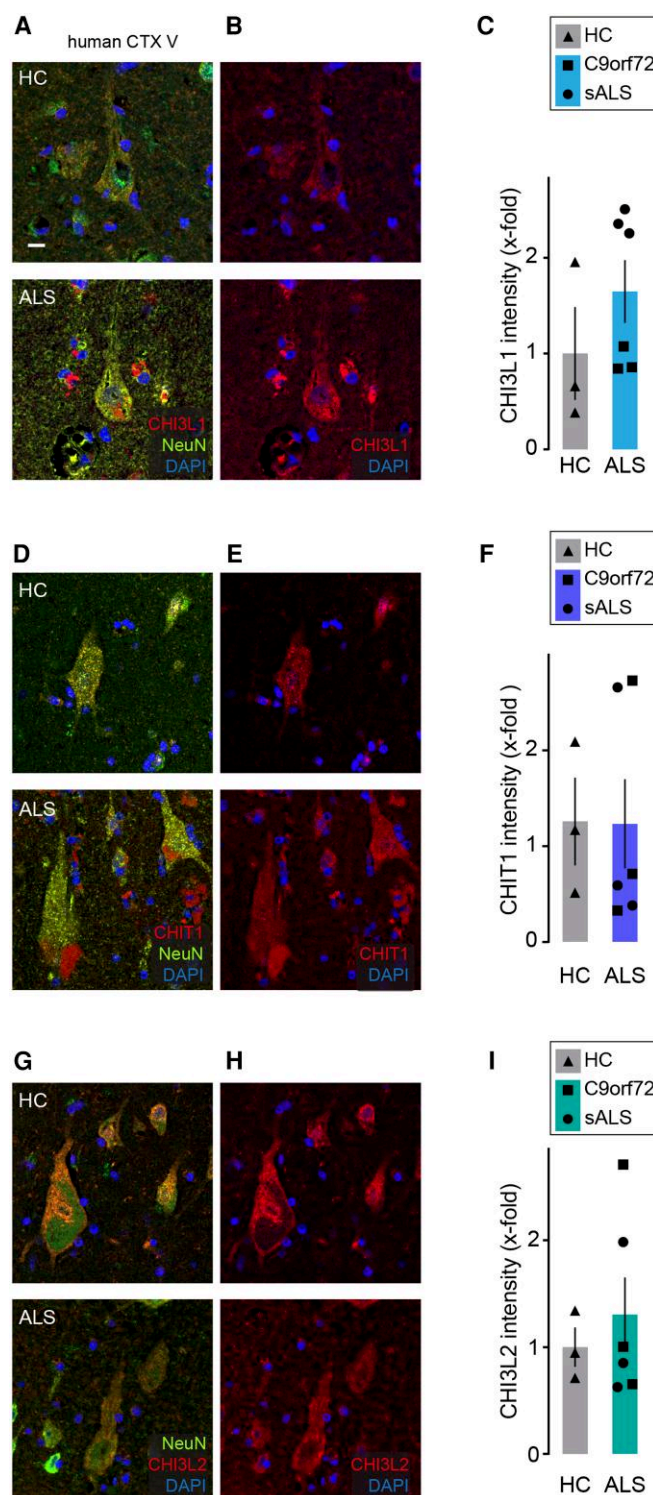


Figure 7 Immunostaining of human motor cortex confirms neuronal chitinase expression in healthy controls and ALS patients. Chitinase immunostaining in cortical layer V of the primary motor cortex (M1) in a cohort of healthy controls (HCs, $n = 3$), patients with sporadic ALS (sALS, $n = 3$) and patients with familial ALS (C9orf72, $n = 3$). (A–C) Representative confocal images immunostained for CHI3L1 (red), NeuN (green) and DAPI (nuclei, blue) from a HC and an ALS patient. (B) Insets on the right depict CHI3L1 (red) and DAPI (blue). (C) Quantification of CHI3L1 intensity in NeuN⁺ regions of interest normalized to the mean intensity of HCs. (D–F) Representative confocal images immunostained for CHIT1 (red), NeuN (green) and DAPI (nuclei, blue) from a HC and an ALS patient. (E) Insets on the right depict CHIT1 (red) and DAPI (blue). (F) Quantification of CHIT1 intensity in NeuN⁺ regions of interest normalized to the mean intensity of HCs. (G–I) Representative confocal images immunostained for CHI3L2 (red), NeuN (green) and DAPI (nuclei, blue) from a HC and an ALS patient. (H) Insets on the right depict CHI3L2 (red) and DAPI (blue). (I) Quantification of CHI3L2 intensity in NeuN⁺ areas normalized to the mean intensity of HCs. Data in graphs C, F and I are presented as fold changes relative to the HC mean, lines indicate the mean, and data points indicate individual patients (triangles, HC; squares, C9orf72; circles, sALS). Scale bar = 10 μ m in A, applies to all images. Cells quantified/individual $n \geq 50$ NeuN⁺ neurons. ALS = amyotrophic lateral sclerosis.

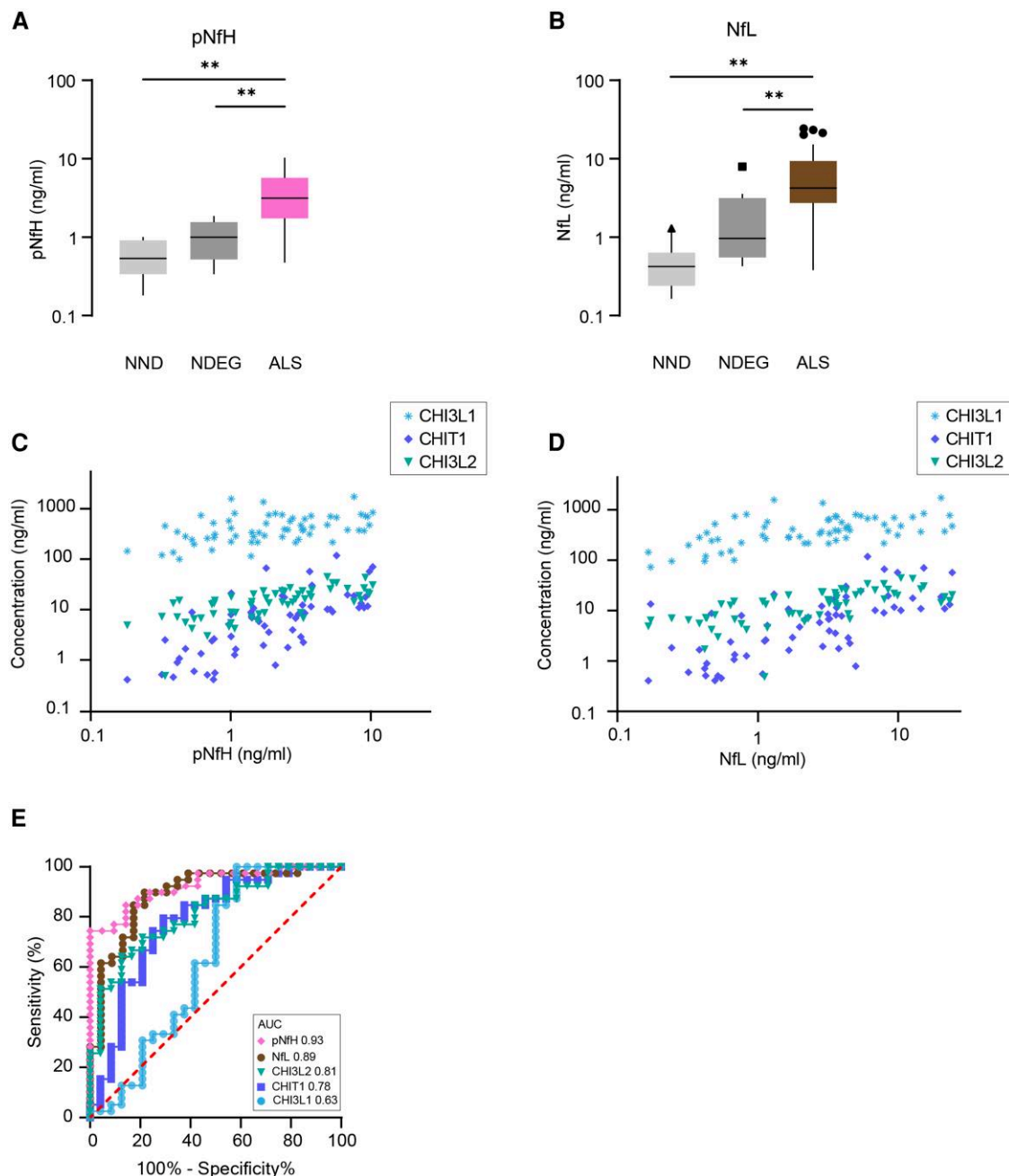


Figure 8 Cross-sectional analyses of neurofilament levels in CSF. (A) pNfH and (B) NfL concentrations (ng/ml) in the CSF of NNDs ($n = 11$), NDEGs ($n = 13$) and ALS patients ($n = 39$), i.e. the same cohort as in Fig. 1. (C and D) Correlation analyses for chitinases CHI3L1 (stars), CHIT1 (diamonds) and CHI3L2 (inverted triangles) with (C) pNfH and (D) NfL. Data are displayed as combined scatter plots. Individual rho values for correlations with pNfH: CHI3L2 $r = 0.75$; CHIT1 $r = 0.74$; CHI3L1 $r = 0.47$ and NfL: CHI3L2 $r = 0.75$; CHIT1 $r = 0.71$; CHI3L1 $r = 0.56$. (E) ROC curve analysis with sensitivity and specificity. NDEG and NND cohorts were combined to create a 'non-ALS' cohort to assess if analytes can distinguish between ALS versus non-ALS. Values are displayed in Supplementary Table 3. Axes for A–D are displayed in log₁₀ scale; statistical significance * $P < 0.05$ and ** $P < 0.01$. ALS = amyotrophic lateral sclerosis; NDEG = neurodegenerative disease controls; NND = non-neurodegenerative disease controls; ROC = receiver operating characteristic.

qualitative, our observation of more pronounced chitinase upregulation in the phenotypically severe hTDP-43 mouse model suggests a link to overall disease aggressiveness in ALS. This is supported by the recent finding that chitinase upregulation in spinal cord samples from ALS patients positively correlated with increased expression and activity of RIPK1, a key regulator of the necrotic cell death pathway.⁶⁵ We have also previously used the D50 model to show that CSF CHIT1 and CHI3L1 levels are significantly higher in

patients with increased disease aggressiveness, independent of disease phase.²²

Transcriptomic analyses revealed that neuronal chitinase expression was predominantly associated with glutamatergic neurons—a notable finding, given the established role of glutamatergic excitotoxicity in ALS and that Riluzole, one of the few approved treatments, is thought to act partly by modulating glutamatergic transmission. While the therapeutic targeting of chitinases

remains unexplored, their biomarker potential is clear: they predict disease aggressiveness,²² improve the prognostic utility of the neurofilament proteins²² and reflect the primary degenerative process in ALS. The recent approval of Tofersen was based on its ability to reduce plasma NfL levels rather than meeting the primary clinical end point of slowing ALSFRS-R decline,⁶⁶ underscoring the critical need for validated biomarkers to demonstrate therapeutic effect and target engagement.

Data availability

The data supporting the conclusions of this article will be made available by the authors upon reasonable request.

Acknowledgements

We are grateful to Ina Ingrisch, Claudia Sommer, Kristina Wullmann, Yvonne Hufnagel, Daniela Steinmetz and Nebahat Budak for excellent technical assistance and animal husbandry. We also thank Marie Dreger, Beatrice Stubendorff and Benjamin Vlad for their help with cohort building. Finally, we extend our deepest gratitude to the clinical staff at the UKJ and the community of ALS patients and their families. We thank the Core Unit SysMed at the University of Würzburg for excellent technical support, RNA-seq data generation and analysis. The IZKF supported this work at the University of Würzburg (project Z-6). NGS analyses were carried out at the Competence Centre for Genomic Analysis (Kiel). We thank Dr Thomas Arzberger for his invaluable guidance on the selection and analysis of post-mortem tissue samples. Finally, we thank Prof. Dr Thomas Misgeld for infrastructural and funding support.

Funding

J.G. was supported by the German Ministry for Education and Research (Bundesministerium für Bildung und Forschung, BMBF) through the EU Joint-Programme-Neurodegenerative Disease Research (JPND) grants SOPHIA (01ED1202B) and ONWebDUALS (01ED15511A), the E-RARE grant PYRAMID (01GM1304), the Deutsche Forschungsgemeinschaft (DFG Grant GR 1578/6-1) and the Boris Canessa Foundation. M.S.B. is the recipient of a Deutsche Forschungsgemeinschaft research grant (ID 450131873). M.S.B., D.E. and S.L. are funded by the Deutsche Forschungsgemeinschaft—Excellence Cluster SyNergy (EXC 2145—ID 390857198). S.L., J.G. and M.S.B. are also supported by the Deutsche Gesellschaft für Muskelkranke (DGM). S.L. also received support from the Frick Foundation, the Milken-Tambourine Philanthropies (ALS Breakthrough Research Fund), the Agence Nationale de la Recherche-Deutsche Forschungsgemeinschaft (ANR-21-CE37-0033-01) and the Emmy Noether Program of the German Research Foundation. N.G. was supported by a doctoral scholarship (Landesgraduiertenstipendium) from the Graduate Academy of Friedrich Schiller University, Jena, Germany, and the state of Thuringia, and by a postdoctoral grant from the Estonian Research Council (SJD90). M.P. is funded by the Estonian Research Council (Grant PSRG3114). R.S. was supported by the Deutsche Forschungsgemeinschaft (DFG) with a clinician scientist program grant [413668513] and is presently supported with an advanced clinician scientist programme (ACSP 14) by the Interdisciplinary Centre of Clinical Research of the Medical Faculty Jena. M.A. was supported by the Transregional

Collaborative Research Centre (TRR) 274/1 2020 and the DFG project 408885537 (B03 and Z01). Z.G. was supported by The Graduate School of Systemic Neurosciences (GSN). A.H. and M.S. are supported by the Hermann and Lilly Schilling Stiftung für medizinische Forschung. High-resolution microscopy was supported via a DFG instrumentation grant (INST95/1755-1 FUGG, ID 518284373). This work was supported by the DFG Research Infrastructure NGS_CC (project 407495230) as part of the Next Generation Sequencing Competence Network (project 423957469).

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at [Brain](#) online.

References

1. Ziatabar S, Zepf J, Rich S, Danielson BT, Bollyky PI, Stern R. Chitin, chitinases, and chitin lectins: Emerging roles in human pathophysiology. *Pathophysiology*. 2018;25:253-262.
2. Funkhouser JD, Aronson NN Jr. Chitinase family GH18: Evolutionary insights from the genomic history of a diverse protein family. *BMC Evol Biol*. 2007;7:96.
3. Elias JA, Homer RJ, Hamid Q, Lee CG. Chitinases and chitinase-like proteins in T(H)2 inflammation and asthma. *J Allergy Clin Immunol*. 2005;116:497-500.
4. Correale J, Fiol M. Chitinase effects on immune cell response in neuromyelitis optica and multiple sclerosis. *Mult Scler*. 2011;17:521-531.
5. Hartl D, He CH, Koller B, et al. Acidic mammalian chitinase is secreted via an ADAM17/epidermal growth factor receptor-dependent pathway and stimulates chemokine production by pulmonary epithelial cells. *J Biol Chem*. 2008;283:33472-33482.
6. Gomes LC, Galhoz A, Jain G, et al. Multi-omic landscaping of human midbrains identifies disease-relevant molecular targets and pathways in advanced-stage Parkinson's disease. *Clin Transl Med*. 2022;12:e692.
7. Kanneganti M, Kamba A, Mizoguchi E. Role of chitotriosidase (chitinase 1) under normal and disease conditions. *J Epithel Biol Pharmacol*. 2012;5:1-9.
8. Kawada M, Hachiya Y, Arihiro A, Mizoguchi E. Role of mammalian chitinases in inflammatory conditions. *Keio J Med*. 2007;56:21-27.
9. Pintea R, Montalban X, Comabella M. Chitinases and chitinase-like proteins as biomarkers in neurologic disorders. *Neurol Neuroimmunol Neuroinflamm*. 2021;8:e921.
10. Gaur N, Perner C, Witte OW, Grosskreutz J. The chitinases as biomarkers for amyotrophic lateral sclerosis: Signals from the CNS and beyond. *Front Neurol*. 2020;11:377.
11. Grossman M. Amyotrophic lateral sclerosis—A multisystem neurodegenerative disorder. *Nat Rev Neurol*. 2019;15:5-6.
12. Swash M. Chitinases, neuroinflammation and biomarkers in ALS. *J Neurol Neurosurg Psychiatry*. 2020;91:338.
13. Ruf WP, Boros M, Freischmidt A, et al. Spectrum and frequency of genetic variants in sporadic amyotrophic lateral sclerosis. *Brain Commun*. 2023;5:fcad152.
14. Akcimen F, Lopez ER, Landers JE, et al. Amyotrophic lateral sclerosis: Translating genetic discoveries into therapies. *Nat Rev Genet*. 2023;24:642-658.
15. van Es MA, Hardiman O, Chio A, et al. Amyotrophic lateral sclerosis. *Lancet*. 2017;390:2084-2098.

16. Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. *Nat Rev Dis Primers*. 2017;3:17071.
17. Burk K, Pasterkamp RJ. Disrupted neuronal trafficking in amyotrophic lateral sclerosis. *Acta Neuropathol*. 2019;137:859–877.
18. Steinacker P, Verde F, Fang L, et al. Chitotriosidase (CHIT1) is increased in microglia and macrophages in spinal cord of amyotrophic lateral sclerosis and cerebrospinal fluid levels correlate with disease severity and progression. *J Neurol Neurosurg Psychiatry*. 2018;89:239–247.
19. Thompson AG, Gray E, Bampton A, Raciborska D, Talbot K, Turner MR. CSF chitinase proteins in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*. 2019;90:1215–1220.
20. Costa J, Gromicho M, Pronto-Laborinho A, et al. Cerebrospinal fluid chitinases as biomarkers for amyotrophic lateral sclerosis. *Diagnostics (Basel)*. 2021;11:1210.
21. Gille B, De Schaepdryver M, Dedeene L, et al. Inflammatory markers in cerebrospinal fluid: Independent prognostic biomarkers in amyotrophic lateral sclerosis? *J Neurol Neurosurg Psychiatry*. 2019;90:1338–1346.
22. Gaur N, Steinbach R, Plaas M, Witte OW, Brill MS, Grosskreutz J. Chitinase dysregulation predicts disease aggressiveness in ALS: Insights from the D50 progression model. *J Neurol Neurosurg Psychiatry*. 2023;94:585–588.
23. Gaur N, Huss E, Prell T, et al. Monocyte-derived macrophages contribute to chitinase dysregulation in amyotrophic lateral sclerosis: A pilot study. *Front Neurol*. 2021;12:629332.
24. Vu L, An J, Kovalik T, Gendron T, Petrucelli L, Bowser R. Cross-sectional and longitudinal measures of chitinase proteins in amyotrophic lateral sclerosis and expression of CHI3L1 in activated astrocytes. *J Neurol Neurosurg Psychiatry*. 2020;91:350–358.
25. Ludolph A, Drory V, Hardiman O, et al. A revision of the El Escorial criteria—2015. *Amyotroph Lateral Scler Frontotemporal Degener*. 2015;16:291–292.
26. Strong MJ, Abrahams S, Goldstein LH, et al. Amyotrophic lateral sclerosis—Frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotroph Lateral Scler Frontotemporal Degener*. 2017;18:153–174.
27. Schludi MH, Becker L, Garrett L, et al. Spinal poly-GA inclusions in a C9orf72 mouse model trigger motor deficits and inflammation without neuron loss. *Acta Neuropathol*. 2017;134:241–254.
28. Walker AK, Spiller KJ, Ge G, et al. Functional recovery in new mouse models of ALS/FTLD after clearance of pathological cytoplasmic TDP-43. *Acta Neuropathol*. 2015;130:643–660.
29. Tu PH, Raju P, Robinson KA, Gurney ME, Trojanowski JQ, Lee VM. Transgenic mice carrying a human mutant superoxide dismutase transgene develop neuronal cytoskeletal pathology resembling human amyotrophic lateral sclerosis lesions. *Proc Natl Acad Sci U S A*. 1996;93:3155–3160.
30. Wang M, Kleele T, Xiao Y, et al. Completion of neuronal remodeling prompts myelination along developing motor axon branches. *J Cell Biol*. 2021;220:e201911114.
31. Brill MS, Kleele T, Ruschkies L, et al. Branch-specific microtubule destabilization mediates axon branch loss during neuromuscular synapse elimination. *Neuron*. 2016;92:845–856.
32. Martelotto LG. ‘Frankenstein’ protocol for nuclei isolation from fresh and frozen tissue for snRNAseq V.2. protocols.io. Published 18 March 2020. Accessed 28 January 2021. doi:10.17504/protocols.io.3fkgjkw
33. Hao Y, Hao S, Andersen-Nissen E, et al. Integrated analysis of multimodal single-cell data. *Cell*. 2021;184:3573–3587.e29.
34. Briese M, Saal L, Appenzeller S, Moradi M, Baluapuri A, Sendtner M. Whole transcriptome profiling reveals the RNA content of motor axons. *Nucleic Acids Res*. 2016;44:e33.
35. Kreiter N, Pal A, Lojewski X, et al. Age-dependent neurodegeneration and organelle transport deficiencies in mutant TDP43 patient-derived neurons are independent of TDP43 aggregation. *Neurobiol Dis*. 2018;115:167–181.
36. Massih B, Veh A, Schenke M, et al. A 3D cell culture system for bioengineering human neuromuscular junctions to model ALS. *Front Cell Dev Biol*. 2023;11:996952.
37. Naumann M, Pal A, Goswami A, et al. Impaired DNA damage response signaling by FUS-NLS mutations leads to neurodegeneration and FUS aggregate formation. *Nat Commun*. 2018;9:335.
38. Pal A, Glass H, Naumann M, et al. High content organelle trafficking enables disease state profiling as powerful tool for disease modelling. *Sci Data*. 2018;5:180241.
39. Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. The ImageJ ecosystem: An open platform for biomedical image analysis. *Mol Reprod Dev*. 2015;82:518–529.
40. Sanfilippo C, Castrogiovanni P, Imbesi R, et al. Sex difference in CHI3L1 expression levels in human brain aging and in Alzheimer’s disease. *Brain Res*. 2019;1720:146305.
41. Vahsen BF, Gray E, Candalija A, et al. Human iPSC co-culture model to investigate the interaction between microglia and motor neurons. *Sci Rep*. 2022;12:12606.
42. Poesen K, De Schaepdryver M, Stubendorff B, et al. Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease. *Neurology*. 2017;88:2302–2309.
43. Dreger M, Steinbach R, Otto M, Turner MR, Grosskreutz J. Cerebrospinal fluid biomarkers of disease activity and progression in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*. 2022;93:422–435.
44. Yuan A, Rao MV, Veeranna, Nixon RA. Neurofilaments and neurofilament proteins in health and disease. *Cold Spring Harb Perspect Biol*. 2017;9:a018309.
45. Dreger M, Steinbach R, Gaur N, et al. Cerebrospinal fluid neurofilament light chain (NfL) predicts disease aggressiveness in amyotrophic lateral sclerosis: An application of the D50 disease progression model. *Front Neurosci*. 2021;15:651651.
46. Feneberg E, Oeckl P, Steinacker P, et al. Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. *Neurology*. 2018;90:e22–e30.
47. Thompson AG, Gray E, Thezenas ML, et al. Cerebrospinal fluid macrophage biomarkers in amyotrophic lateral sclerosis. *Ann Neurol*. 2018;83:258–268.
48. Hellwig K, Kvartsberg H, Portelius E, et al. Neurogranin and YKL-40: Independent markers of synaptic degeneration and neuroinflammation in Alzheimer’s disease. *Alzheimers Res Ther*. 2015;7:74.
49. Hall S, Surova Y, Ohrfelt A, et al. Longitudinal measurements of cerebrospinal fluid biomarkers in Parkinson’s disease. *Mov Disord*. 2016;31:898–905.
50. Behzadi A, Pujol-Calderon F, Tjust AE, et al. Neurofilaments can differentiate ALS subgroups and ALS from common diagnostic mimics. *Sci Rep*. 2021;11:22128.
51. Steinacker P, Feneberg E, Halbigbauer S, et al. Chitotriosidase as biomarker for early stage amyotrophic lateral sclerosis: A multicenter study. *Amyotroph Lateral Scler Frontotemporal Degener*. 2021;22:276–286.
52. Oeckl P, Weydt P, Steinacker P, et al. Different neuroinflammatory profile in amyotrophic lateral sclerosis and frontotemporal dementia is linked to the clinical phase. *J Neurol Neurosurg Psychiatry*. 2019;90:4–10.
53. Verde F, Zaina G, Bodio C, et al. Cerebrospinal fluid phosphorylated neurofilament heavy chain and chitotriosidase in

- primary lateral sclerosis. *J Neurol Neurosurg Psychiatry*. 2021;92:221-223.
54. Barschke P, Oeckl P, Steinacker P, et al. Different CSF protein profiles in amyotrophic lateral sclerosis and frontotemporal dementia with C9orf72 hexanucleotide repeat expansion. *J Neurol Neurosurg Psychiatry*. 2020;91:503-511.
 55. Craig-Schapiro R, Perrin RJ, Roe CM, et al. YKL-40: A novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry*. 2010;68:903-912.
 56. Cubas-Nunez L, Gil-Perotin S, Castillo-Villalba J, et al. Potential role of CHI3L1+ astrocytes in progression in MS. *Neurol Neuroimmunol Neuroinflamm*. 2021;8:e972.
 57. Varghese AM, Sharma A, Mishra P, et al. Chitotriosidase—A putative biomarker for sporadic amyotrophic lateral sclerosis. *Clin Proteomics*. 2013;10:19.
 58. Dols-Icardo O, Montal V, Sirisi S, et al. Motor cortex transcriptome reveals microglial key events in amyotrophic lateral sclerosis. *Neurol Neuroimmunol Neuroinflamm*. 2020;7:e829.
 59. Liddel SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017;541:481-487.
 60. Bonne-Barkay D, Bissel SJ, Kofler J, Starkey A, Wang G, Wiley CA. Astrocyte and macrophage regulation of YKL-40 expression and cellular response in neuroinflammation. *Brain Pathol*. 2012;22:530-546.
 61. Varghese AM, Ghosh M, Bhagat SK, et al. Chitotriosidase, a biomarker of amyotrophic lateral sclerosis, accentuates neurodegeneration in spinal motor neurons through neuroinflammation. *J Neuroinflammation*. 2020;17:232.
 62. Welikovitsh LA, Do Carmo S, Magloczky Z, et al. Early intraneuronal amyloid triggers neuron-derived inflammatory signaling in APP transgenic rats and human brain. *Proc Natl Acad Sci U S A*. 2020;117:6844-6854.
 63. Matute-Blanch C, Calvo-Barreiro L, Carballo-Carbajal I, et al. Chitinase 3-like 1 is neurotoxic in primary cultured neurons. *Sci Rep*. 2020;10:7118.
 64. Gray E, Thompson AG, Wu J, et al. CSF chitinases before and after symptom onset in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol*. 2020;7:1296-1306.
 65. Zelic M, Blazier A, Pontarelli F, et al. Single-cell transcriptomic and functional studies identify glial state changes and a role for inflammatory RIPK1 signaling in ALS pathogenesis. *Immunity*. 2025;58:961-979.
 66. Miller TM, Cudkowicz ME, Genge A, et al. Trial of antisense oligonucleotide tofersen for SOD1 ALS. *N Engl J Med*. 2022;387:1099-1110.