

TMEM119-positive microglia were increased in the brains of patients with amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that has been reported to be affected by inflammatory cells, such as microglia and macrophages, through the concept of non-cell autonomous neuronal death. Resident microglia in the human brain and monocyte-derived macrophages (MoDM) infiltrating in tissues are difficult to distinguish. Therefore, the effects of microglia and MoDMs in ALS remain poorly understood. This study aimed to investigate the role of resident microglia and MoDMs in the pathogenesis of ALS using postmortem brain and spinal cord samples. The samples used for immunohistochemical analysis included 11 cases of sporadic ALS and 11 age-matched controls. We stained the cells with TMEM119 to detect resident microglia and CCR2 to detect MoDMs. In ALS cases, TMEM119-immunopositive resident microglia were abundant in the motor cortex and subcortical white matter (SWM) of the motor area, whereas CCR2-immunopositive MoDM was similar to control cases. In addition, the mean density of CD68-immunopositive cells in the SWM significantly correlated with the mean density of pTDP-43-positive GCIs. These results suggest that resident microglial activation plays an important role in the cerebral pathogenesis of ALS and may provide novel therapeutic strategies to target excessive activation of resident microglia in ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the death of motor neurons; however, the cause of ALS is not fully understood. According to the concept of non-cell-autonomous neuronal death, glial cells, such as microglia, are related to motor neuron death in ALS [1]. The activation of resident microglia is important in ALS [2]; however, the contribution of resident microglia to ALS has not been fully elucidated. Resident microglia in the brain originate from primitive macrophages in the yolk sac, which are distinct from the monocyte-derived macrophages (MoDM) infiltrating the tissues, which originate in the bone marrow [3]. It has been suggested that microglia and MoDMs have different functions. It has been reported that myelin phagocytosis is greater in microglia than in MoDM [4]. There were no useful markers for distinguishing between resident microglia and MoDM, which made distinguishing them difficult.

Recently, it has become possible to classify microglia and MoDM by combining multiple new markers such as TMEM119 and CCR2. TMEM119 is primarily used as a marker for detecting resident microglia [5], and CCR2 is primarily used as a marker for detecting MoDMs [6].

Both microglia and MoDMs are involved in central nervous system inflammation in neurological diseases. For example, TMEM119-immunopositive microglial activation has been reported to be associated with Parkinson's disease [7]. In ALS mouse models, infiltration of CCR2-positive monocytes into the lumbar spinal cord increases during disease progression suggesting the involvement of CCR2-positive macrophages [8].

The relationship between microglia and MoDM activation in patients with ALS is not fully understood. Hence, we investigated the classification of microglia and MoDMs in the human motor cortex and cervical spinal cord of patients with ALS.

Abbreviations: ALS, amyotrophic lateral sclerosis; MoDM, monocyte-derived macrophages; MC, motor cortex; SWM, subcortical white matter; VH, ventral horn; LF, lateral funiculus; DAB, 3, 3'-diaminobenzidine; GCIs, glial cytoplasmic inclusions.

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2. Materials and methods

2.1. Subjects

We used formalin-fixed human motor area tissue samples from 11 patients with sporadic ALS (51–82 years old) and 11 patients with age-matched controls (51–86 years old), and cervical spinal cord tissue samples from five patients with sporadic ALS (aged 51–71 years) and three controls (aged 75–86 years) from the same patient group. Individuals with significant TDP-43-related diseases or a clinical history of ALS were not included but other neurodegenerative diseases were included as age-matched controls. Diagnoses were established based on clinical information and pathological examination (Table 1). This study was approved by the Ethics Committee of Kyoto University (approval number: R1038).

2.2. Single immunohistochemistry staining

The primary antibodies used were listed in the [supplementary methods](#).

Paraffin sections (6 μ m) were pretreated with Histofine deparaffinizing antigen retrieval buffer, pH 6 (product code 415281, Nichirei Biosciences, Tokyo, Japan), at 120 °C for 20 min, then incubated overnight at 4 °C with the primary antibodies in phosphate-buffered saline containing 3 % bovine serum albumin. The antibody reaction was visualized using a peroxidase-polymer-based method using a Histofine Simple Stain MAX-PO kit (Nichirei, Tokyo, Japan) with diaminobenzidine (DAB Substrate Kit, SK4100; Vector Laboratory, Burlingame, CA, USA) as the chromogen, followed by counterstaining with hematoxylin.

2.3. Double immunohistochemical staining

The primary antibodies used were listed in the [supplementary methods](#).

We carried out double immunohistochemical staining of pTDP-43

and CD68, TMEM119, GFAP, Oligo2, or NeuN in one ALS case. The labeling of pTDP-43 (mouse and rabbit) using the universal immunoperoxidase polymer method. The other labeling (pTDP-43 and CD68, TMEM119, GFAP, Oligo2, or NeuN) was visualized using diaminobenzidine (DAB Substrate Kit, SK4100; Vector Laboratory, Burlingame, CA, USA). In the incubation step with peroxidase-conjugated anti-IgG, we used an appropriate host-type-specific anti-IgG that was different for the secondary staining. We immersed the slides in the solution provided by the DAB substrate kit containing nickel and then incubated the slides with secondary primary antibodies in phosphate-buffered saline containing 3 % bovine serum albumin overnight at 4 °C. Secondly, we incubated the slides with an alkaline phosphatase-conjugated anti-IgG antibody. Staining was visualized using Fast Red Substrate Kit II (catalog number 415261, Nichirei) according to the manufacturer's instructions. We used the following enzyme-linked IgG antibodies: anti-mouse IgG (Histofine Simple Stain Max-PO (M), Nichirei), anti-rabbit IgG (Histofine Simple Stain Max-PO (R), Nichirei) for the peroxidase polymer-based method, anti-mouse IgG (Histofine Simple Stain AP (R), Nichirei), and anti-rabbit IgG (Histofine Simple Stain AP (R), Nichirei) for the universal immunoperoxidase polymer method.

2.4. Computer-assisted quantitative image analyses

Slides were viewed using a BX43 microscope (Olympus, Tokyo, Japan) with a 10x objective. However, Fig. 2A–D, 3A–D, S1A–D and S3A–D show image data taken with a 20x objective. Three images of the subcortical white matter (SWM) and motor cortex (MC), and two images of the ventral horn (VH) and lateral funiculus (LF) were obtained for each case. Quantitative analysis of the immunostaining-positive areas was performed using open-source image processing software FIJI (ImageJ ver. 1.53c; National Institutes of Health, Bethesda, MD, USA). Briefly, we separated hematoxylin staining and 3, 3'-diaminobenzidine (DAB) staining by using Color Deconvolution [9]. The obtained DAB signal image was converted into a black-and-white binary image. Subsequently, we measured the total and the DAB-immunopositive area

Table 1
Case material studied.

Case	Diagnosis	Age	Sex	Brain weight (g)	Duration of disease (year)	Postmortem interval (hours)
1	SALS	56	M	1130	NA	5.5
2	SALS	56	F	1030	1.7	10.5
3	SALS	58	F	NA	2.9	3.4
4	SALS	54	F	1240	0.9	3.3
5	SALS	71	F	1280	1.7	3.3
6	SALS	55	M	1110	1.4	14
7	SALS	64	M	1470	2.0	3.1
8	SALS	62	M	645	1.2	1.3
9	SALS	82	M	1210	1.2	1.4
10	SALS	65	M	1465	1.9	1.6
11	SALS	57	M	NA	8.8	4.4
Sub-total†		61.8 $\pm$ 8.5		1175.6 $\pm$ 248.9		4.7 $\pm$ 4.0
12	DLB	69	F	NA		1.0
13	PD	76	M	NA		1.3
14	multiple CI, DLB	84	M	1160		3.0
15	SCD	66	F	1020		1.5
16	PD	76	F	1170		3.5
17	CI	75	M	NA		0.9
18	CI	86	M	NA		4.7
19	SCD	78	M	1100		1.8
20	myotonic dystrophy	53	F	1200		5.9
21	Epilepsy	51	F	1390		11.4
22	Olivopontocerebellar atrophy	66	M	1360		3.5
Sub-total†		70.9 $\pm$ 11.3		1200 $\pm$ 133.3		3.5 $\pm$ 3.1
Total†		66.4 $\pm$ 10.8		1186.3 $\pm$ 200.8		4.1 $\pm$ 3.5

† Average $\pm$ standard deviation.

SALS, sporadic amyotrophic lateral sclerosis; DLB, dementia with Lewy bodies; PD, Parkinson's disease; SCD, spinocerebellar degeneration; CI, cerebral infarction; NA, not available.

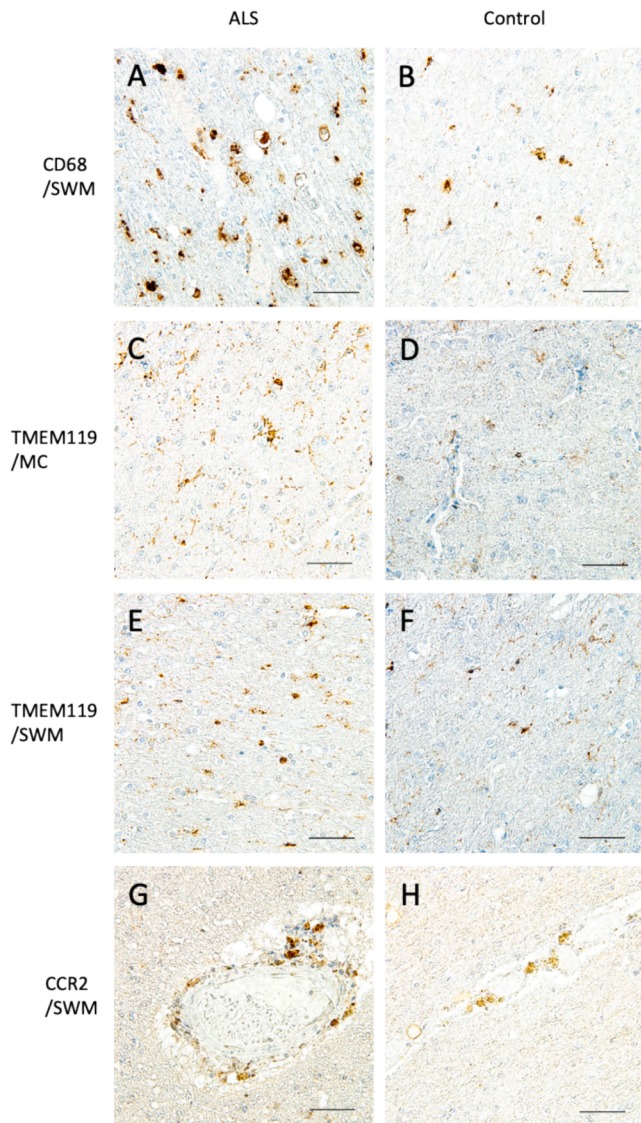


Fig. 1. Immunohistochemical staining results in ALS and control cases. In the SWM, abundant CD68-immunopositive cells mainly stained in the cell bodies and processes, which are morphologically macrophages, were observed in (A) patients with ALS, and some were observed in the (B) control cases. More TMEM119-immunopositive cells which were microglia that stained mainly the thin cell bodies and thin processes, were observed in the (C) MC of ALS than (D) control cases. A similar trend was observed (E), (F) in SWM. A small number of CCR2-immunopositive cells mainly stained the cell bodies, were observed, which were morphologically monocytes, in the cerebellar perivascular region of (G) patients with ALS and (H) controls. The scale bar in all images is 50 μ m. ALS, amyotrophic lateral sclerosis; SWM, subcortical white matter; MC, motor cortex.

(μ m²). Finally, the mean density of each immunostain was determined by dividing the positive area of the DAB signal images by the total area and calculating the average value for each case. Our quantitative analysis of pTDP-43 included positive structures (neuronal cytoplasmic inclusions, dystrophic neurites, neuronal intranuclear inclusions, and glial cytoplasmic inclusions (GCI)).

2.5. Statistical analysis

The Wilcoxon rank-sum test was used to evaluate the mean density of DAB-positive. The correlation was assessed using Spearman rank correlation. Differences were considered significant if the p-value was <

0.05.

3. Results

CD68-immunopositive cells were consistently observed in the SWM of ALS cases (Fig. 1A), whereas some were also observed in the SWM of control patients (Fig. 1B). CD68 predominantly stained the macrophage cell bodies and processes.

TMEM119-immunopositive cells were consistently observed in the MC of patients with ALS (Fig. 1C) and in small numbers in the MC of the control cases (Fig. 1D). A similar trend was observed in SWM (Fig. 1E, 1F). TMEM119 mainly stained rod microglial cell bodies and processes.

CCR2-immunopositive cells were few in all cases (Fig. 1G, 1H). CCR2 stained mainly in the monocyte cell bodies and was observed around the perivascular region.

CD68-immunopositive cells were abundant in the SWM of ALS cases (Fig. S1A). In the MC (Fig. S1B), fewer CD68-immunopositive cells were observed compared to SWM. In the VH (Fig. S1C) and LF (Fig. S1D), a large number of CD68-immunopositive cells were observed in all cases. In the SWM, the mean density of CD68-immunopositive cells was significantly higher in patients with ALS than controls (Fig. S1E, Table S1), whereas in other areas no significant difference was observed (Fig. S1F, S1G, S1H).

There were profuse TMEM119-immunopositive cells in the SWM (Fig. 2A) and the MC (Fig. 2B) of ALS. In the cervical spinal cords, approximately similar levels of TMEM119-immunopositive cells were observed in ALS and control cases (Fig. 2C, 2D). The mean density of TMEM119-immunopositive cells in the SWM (Fig. 2E) and the MC (Fig. 2F) of ALS cases was significantly higher than that of control patients (Table S1), whereas no significant difference was observed in the cervical spinal cords (Fig. 2G, 2H).

All areas showed a small number of CCR2-immunopositive cells in ALS and control cases (Fig. 3A-D) and the mean density of CCR2-immunopositive cells did not differ significantly between patients with ALS and control patients (Fig. 3E-H).

The mean density of pTDP43-positive GCIs was similar in the MC and SWM of patients with ALS (Fig. S2A). In contrast, the mean density of CD68-immunopositive cells was significantly higher in the SWM than in the MC (Fig. S2B, Table S1). There was no significant difference in the mean density of TMEM119- (Fig. S2C) and CCR2-immunopositive cells (Fig. S2D).

There was abnormal aggregation of pTDP-43 in the SWM (Fig. S3A), MC (Fig. S3B), VH (Fig. S3C), and LF (Fig. S3D) of patients with ALS, whereas most of the control cases had few abnormal pTDP-43-positive depositions.

The mean density of pTDP-43-positive GCIs in the SWM (Fig. S3E), MC (Fig. S3F), VH (Fig. S3G), and LF (Fig. S3H) of patients with ALS was significantly higher than control cases (Table S1).

There was a significant correlation between the mean density of pTDP-43-positive GCIs and CD68-immunopositive cells in the SWM (Fig. S4A) but not in the MC (Fig. S4B) (Spearman rank correlation, $p < 0.05$). However, there was no correlation between pTDP-43-positive GCIs and TMEM 119-immunopositive cells (Fig. S4C, S4D), or CCR2-immunopositive cells (Fig. S4E, S4F).

There was no correlation between NeuN-immunopositive cells and pTDP-43-positive GCIs (Fig. S5A) and TMEM-119-immunopositive cells (Fig. S5B) in MC.

CD68- (Fig. S6A), TMEM119- (Fig. S6B), and Oligo2-immunopositive cells (Fig. S6C) were present in close proximity to pTDP-43-positive GCIs. GFAP- (Fig. S6D) and NeuN-immunopositive cells (Fig. S6E) were present separately from pTDP-43-positive GCIs.

4. Discussion

The present study examined microglia and MoDMs in the cerebrum of patients with ALS using TMEM119 and CCR2 markers, respectively.

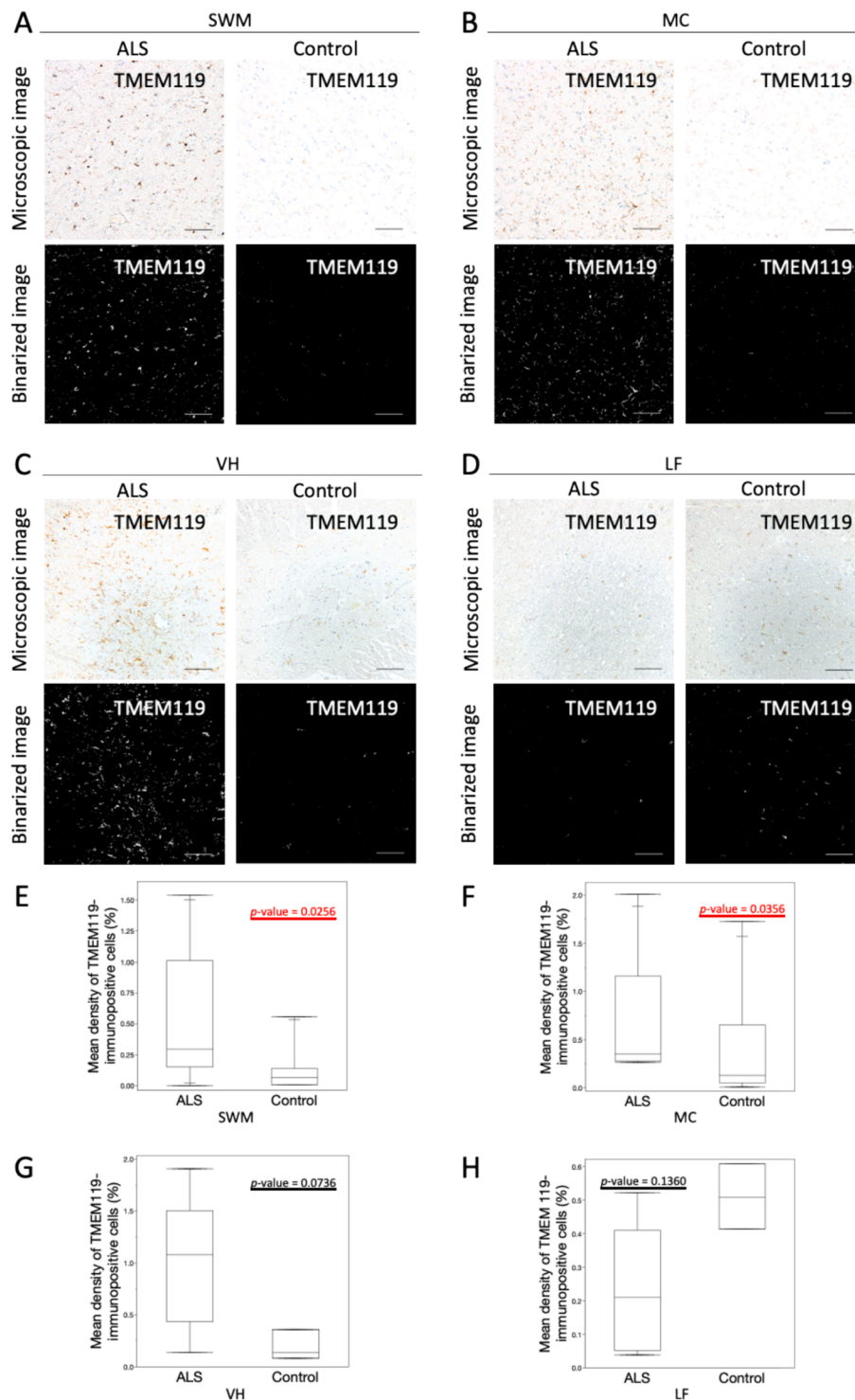


Fig. 2. ALS compared to control cases stained with TMEM119. Images of immunohistochemical staining with TMEM119 and binarized images in (A) the SWM, (B) MC, (C) VH, and (D) LF. In (E) the SWM and (F) MC, the mean density of TMEM119-immunopositive cells was significantly higher in patients with ALS than controls. In (G) the VH and (H) LF, there was no significant difference. The scale bar in all images is 100 μ m. ALS, amyotrophic lateral sclerosis; SWM, subcortical white matter; MC, motor cortex; VH, ventral horn; LF, lateral funiculus.

TMEM119-immunopositive cells, indicative of microglia, exhibited similar morphology in ALS and control patients. Additionally, rod microglia were prevalent in patients with ALS. The density of TMEM119-immunopositive cells in the SWM and MC of patients with ALS was higher than in controls, with no significant difference between MC and SWM in patients with ALS. Regarding MoDMs, CCR2-immunopositive cells displayed monocyte-like characteristics around

perivascular areas in both patients with ALS and controls, with comparable densities among them in the cerebrum and spinal cord. MoDMs may have less impact than microglia in ALS pathology. Moreover, CD68-immunopositive cell density was higher in the SWM than in the MC in patients with ALS. Additionally, pTDP-43-positive GCIs were more abundant in the cerebrum and spinal cord of patients with ALS compared to controls, with CD68-immunopositive cell density in the

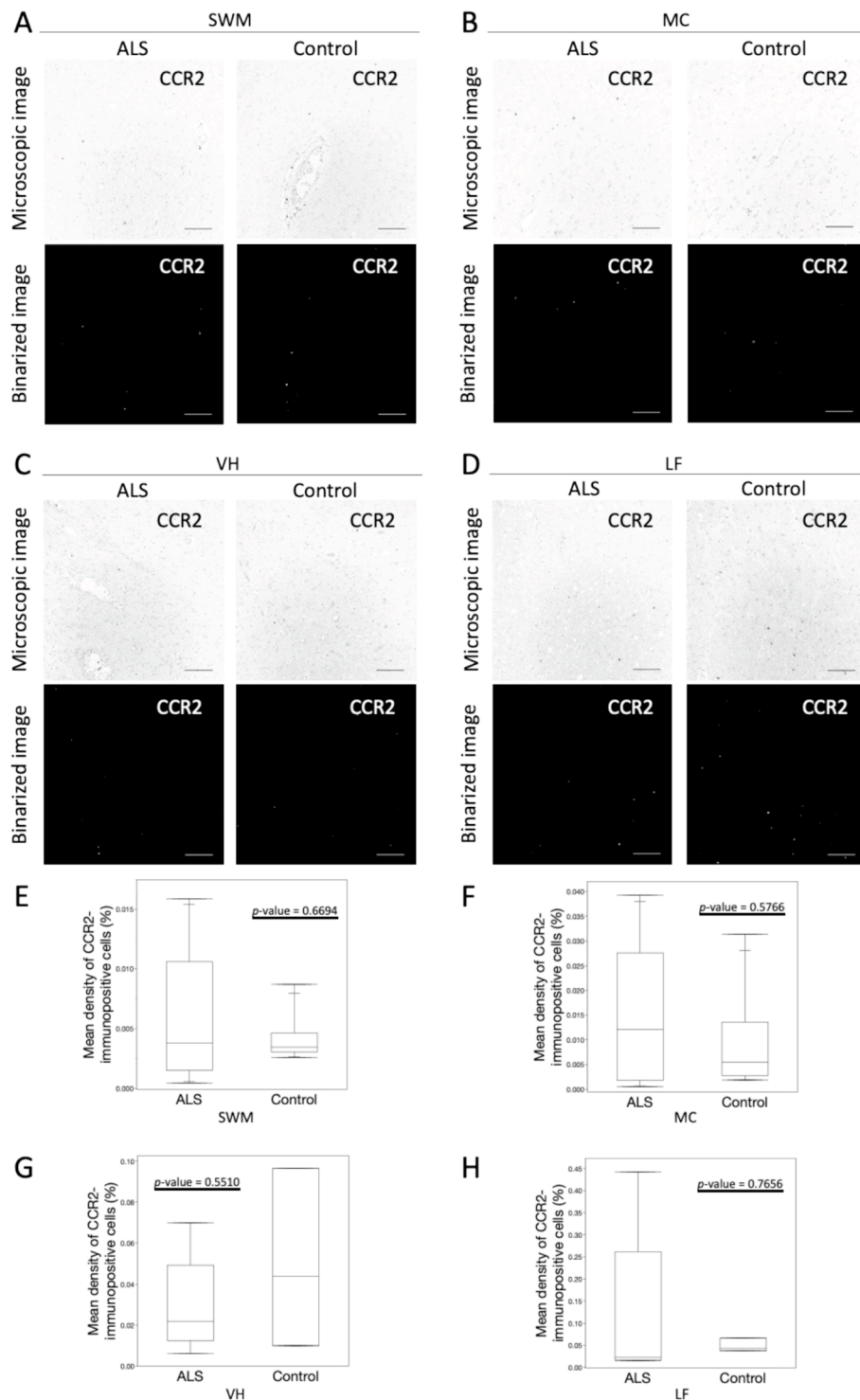


Fig. 3. ALS compared to control cases stained with CCR2. Images of immunohistochemical staining with CCR2 and binarized images in (A) the SWM, (B) MC, (C) VH, and (D) LF. In (E) the SWM, (F) MC, (G) VH and (H) LF, the mean density of CCR2-immunopositive cells was not significantly different between patients with ALS and controls. The scale bar in all images is 100 μ m. ALS, amyotrophic lateral sclerosis; SWM, subcortical white matter; MC, motor cortex; VH, ventral horn; LF, lateral funiculus.

SWM significantly correlated with pTDP-43-positive GCI density.

Recent studies have highlighted the significant role of microglia in the progression of ALS and motor neuron death [1]. In mouse models of mutant SOD1 ALS, microglia are increased and activated in the spinal cord prior to disease onset [10]. Suppression of SOD1 in microglia has been shown to slow disease progression and extend survival in ALS mouse models [1]. However, our present findings found no correlation

between NeuN-immunopositive cells and pTDP-43-positive GCIs or TMEM-119-immunopositive cells in the motor cortex, possibly influenced by differences in disease duration.

Microglia also impact the progression of other neurodegenerative diseases. In a mouse model of Parkinson's disease, administration of minocycline inhibited microglial activation and protected dopaminergic neurons [11]. Furthermore, microglial involvement has been observed

in the early stages of neurodegenerative diseases. A pathological study of Parkinson's disease patients transplanted with healthy embryonic dopamine neurons reported that TMEM119-immunopositive microglial activation in graft deposits was observed long before accumulation of α -synuclein, suggesting active involvement of microglia in disease progression during early stages [7].

Previous research has indicated that microglia can have both neuroprotective and neurotoxic effects [12], yet their role in ALS, whether neurotoxic or neuroprotective, remains contentious [13]. Our discussion primarily focuses on the interplay between TDP-43, a major contributor to ALS neurodegeneration [14], and microglia. A recent study demonstrated increased microglial CD68 expression in the motor cortex of patients with ALS, correlating significantly with pTDP-43 pathology load [15], suggesting microglia exacerbate ALS pathology as TDP-43 pathology increases. Our findings reveal a correlation between pTDP-43-positive GCIs and CD68-immunopositive cells in the subcortical white matter, supporting the notion that neuroinflammation aggravates ALS pathology. Although we observed elevated TMEM119-immunopositive cell density in the subcortical white matter and motor cortex of patients with ALS compared to controls, no correlation was found between pTDP-43-positive GCIs and TMEM119-immunopositive cells. Additionally, CD68 staining, while not specific to microglia, correlated with pTDP-43-positive GCIs, implying an association with the overall macrophage count, encompassing both microglia and MoDMs. However, these results may have been influenced by differences in sample size and microglial staining methods.

Previous evidence suggests that CCR2-positive cells might hasten ALS progression in mouse models [8], but no correlation was found between pTDP-43-positive GCIs and CCR2-immunopositive cells, possibly due to differences between mouse models and human autopsy cases. Our present study observed increased microglia in the cerebrum of patients with ALS compared to controls. While no correlation was found between pTDP-43-positive GCIs and microglia, CD68- and TMEM119-immunopositive cells were found near GCIs, indicating a potential influence of microglia on ALS pathology. Our findings suggest inhibiting microglia to halt ALS progression, though there are limitations, due to the observational nature of the study. Animal model experiments are necessary to confirm microglia's role in ALS progression. Previous research has reported that TMEM119 immunoreactivity decreases when microglia are activated [16]. In our study, some cases included advanced-stage ALS, therefore microglia may have completed the activation phase in cases that have already passed the peak stage of the disease. No significant differences were observed in the mean density of CCR2-immunopositive cells. Based on this finding, it is possible that MoDM could not be sufficiently detected due to antibody sensitivity. Further research, particularly on antibody testing for various macrophage types, is deemed necessary.

5. Conclusions

Our results suggest that microglia are associated with abnormal pTDP-43 deposition and motor neuron death in the MC and SWM of patients with ALS. These findings may provide novel therapeutic strategies to target excessive activation of microglia in ALS.

Ethical statement

All procedures performed in the study were approved by the Ethics Committee of Kyoto University Hospital.

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CRedit authorship contribution statement

Natsuko Togawa: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Takashi Ayaki:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Daisuke Yoshii:** Validation, Methodology. **Takakuni Maki:** Supervision, Resources, Funding acquisition. **Nobukatsu Sawamoto:** Supervision, Funding acquisition. **Ryosuke Takahashi:** Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neulet.2024.137829>.

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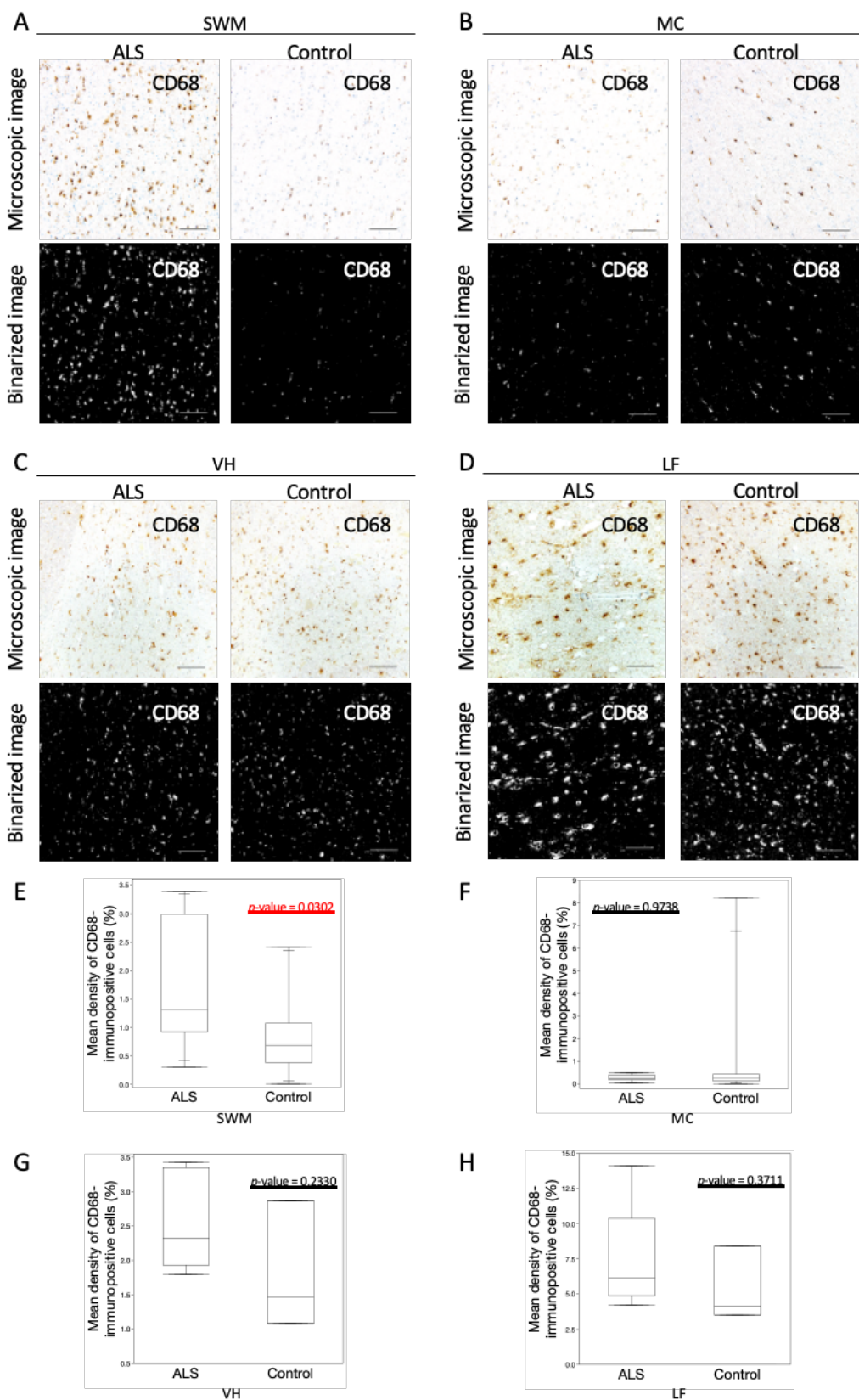


Figure S1. ALS compared to control cases stained with CD68.

Images of immunohistochemical staining with CD68 and binarized images in (A) the SWM, (B) MC, (C) VH, and (D) LF. In (E) the SWM, the mean density of CD68-immunopositive cells was significantly higher in patients with ALS than controls. In (F) the MC, (G) VH, and (H) LF, there was no significant difference. The scale bar in all images is 100 μm . ALS, amyotrophic lateral sclerosis; SWM, subcortical white matter; MC, motor cortex; VH, ventral horn; LF, lateral funiculus.

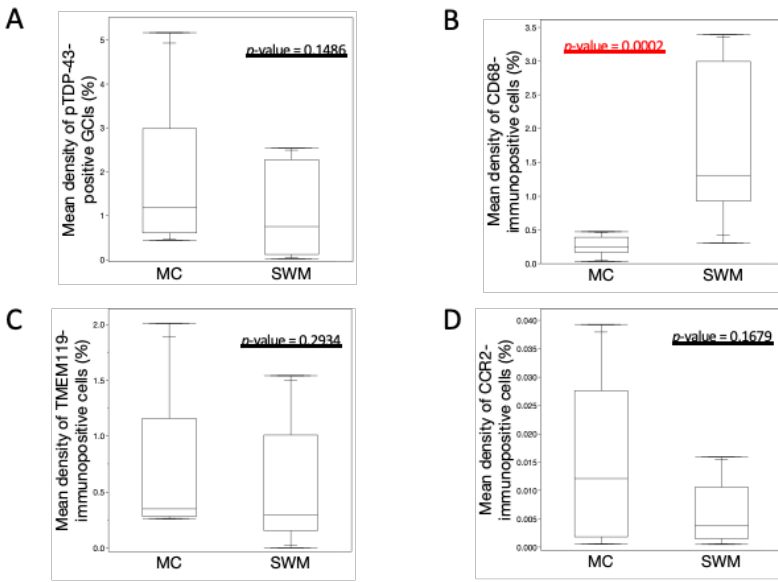


Figure S2. The SWM compared to the MC in ALS cases.

Comparison of the mean densities of immunohistochemical staining with (A) pTDP-43, (B) CD68, (C) TMEM119, and (D) CCR2 in SWM and MC in patients with ALS. The mean density of (B) CD68-immunopositive cells in the SWM of ALS was significantly increased. The mean densities of (A) pTDP43-positive GCIs, (C) TMEM119-, and (D) CCR2-immunopositive cells did not differ. SWM, subcortical white matter; MC, motor cortex; ALS, amyotrophic lateral sclerosis; GCIs, glial cytoplasmic inclusions.

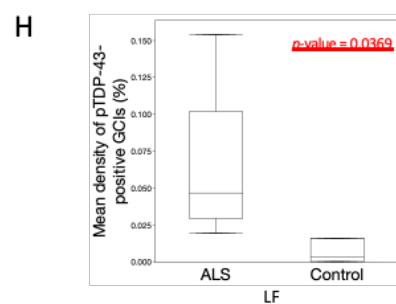
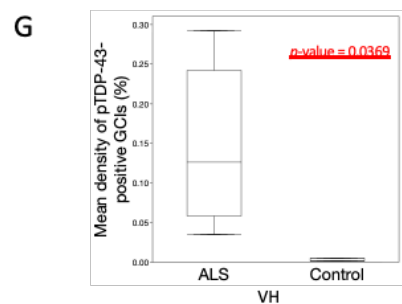
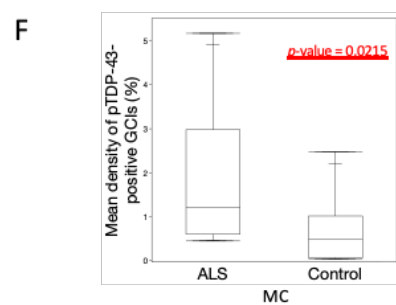
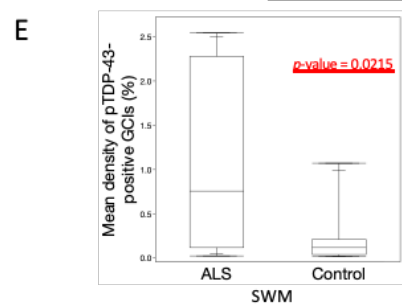
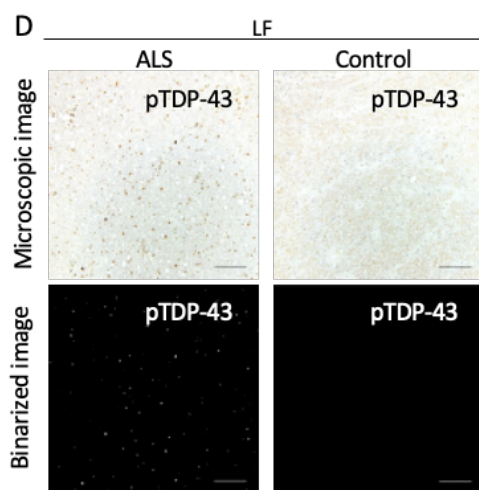
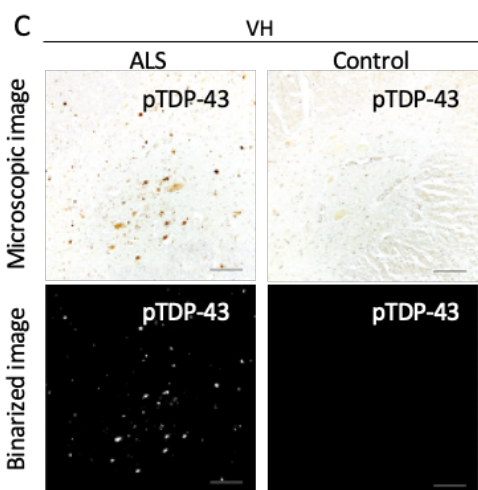
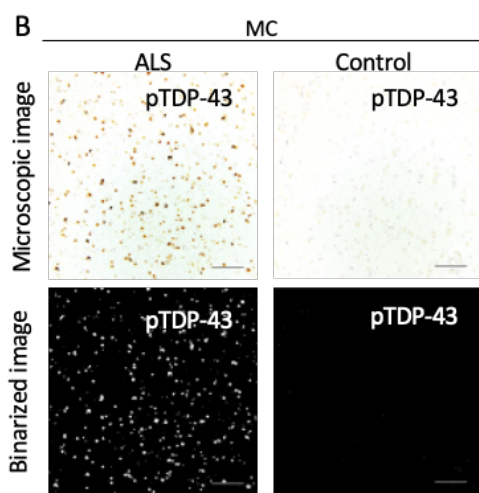
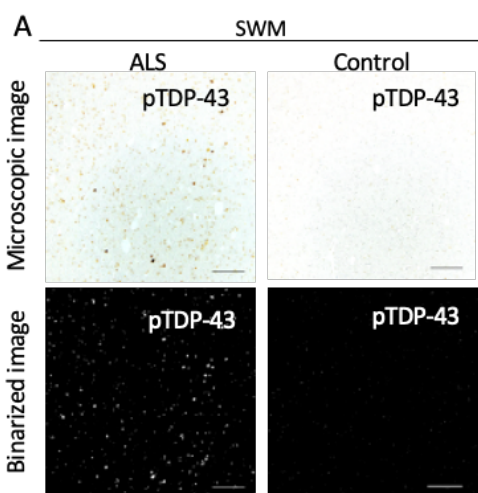
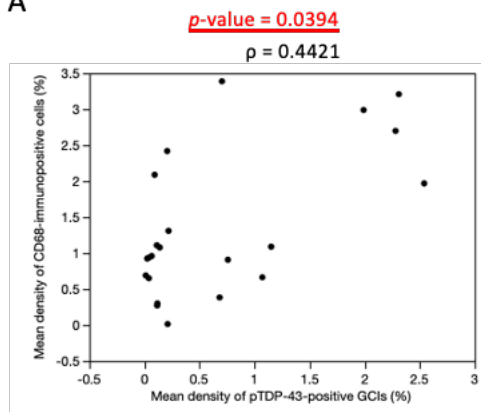


Figure S3. ALS compared to control cases stained with pTDP-43.

Images of immunohistochemical staining with pTDP-43 and binarized images in (A) the SWM, (B) MC, (C) VH, and (D) LF. In (E) the SWM, (F) MC, (G) VH and (H) LF, the mean density of pTDP-43-positive GCIs was significantly higher in patients with ALS than controls. The scale bar in all images is 100 μ m. ALS, amyotrophic lateral sclerosis; SWM, subcortical white matter; MC, motor cortex; VH, ventral horn; LF, lateral funiculus; GCIs, glial cytoplasmic inclusions.

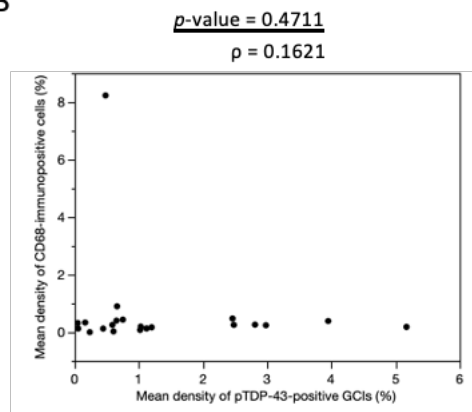
SWM

A

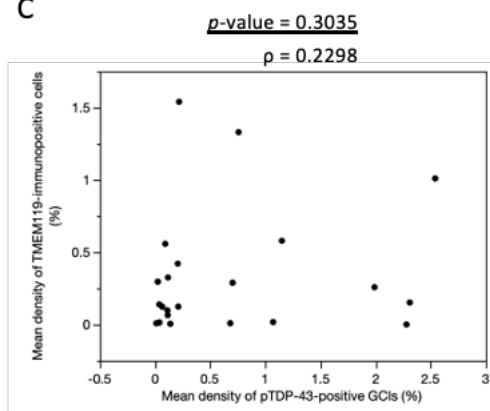


MC

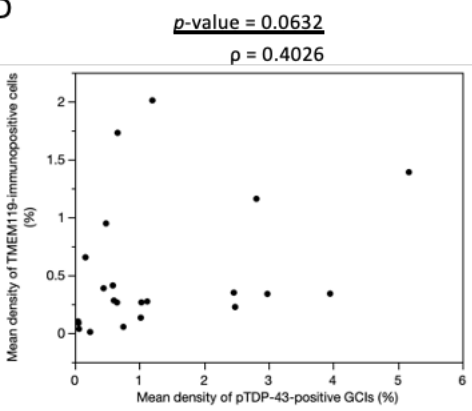
B



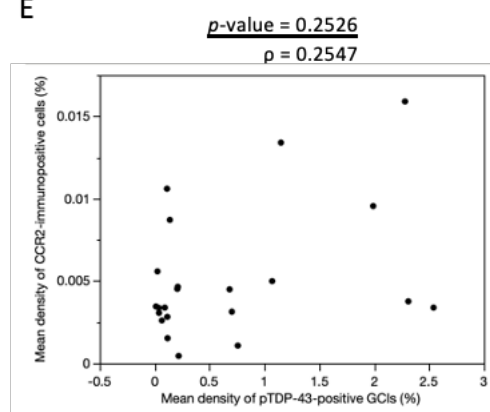
C



D



E



F

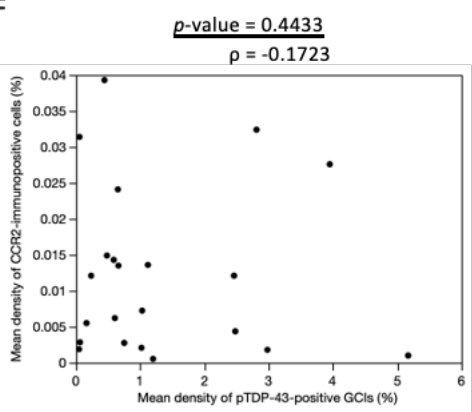


Figure S4. Correlation between pTDP-43-positive GCIs and immune cells.

The mean density of CD68-, TMEM119-, and CCR2-immunopositive cells was plotted as a function of the mean density of pTDP-43-positive GCIs in all cases. There was a significant correlation between the mean density of pTDP-43-positive GCIs and CD68-immunopositive cells in (A) the SWM (Spearman rank correlation, $p < 0.05$) but not in (B) the MC. There was no correlation between the mean density of pTDP43-positive GCIs and (C), (D) TMEM119-immunopositive cells or (E), (F) CCR2-immunopositive cells. ρ , coefficient of Spearman; p-value, value of significance; GCIs, glial cytoplasmic inclusions; SWM, subcortical white matter; MC, motor cortex.

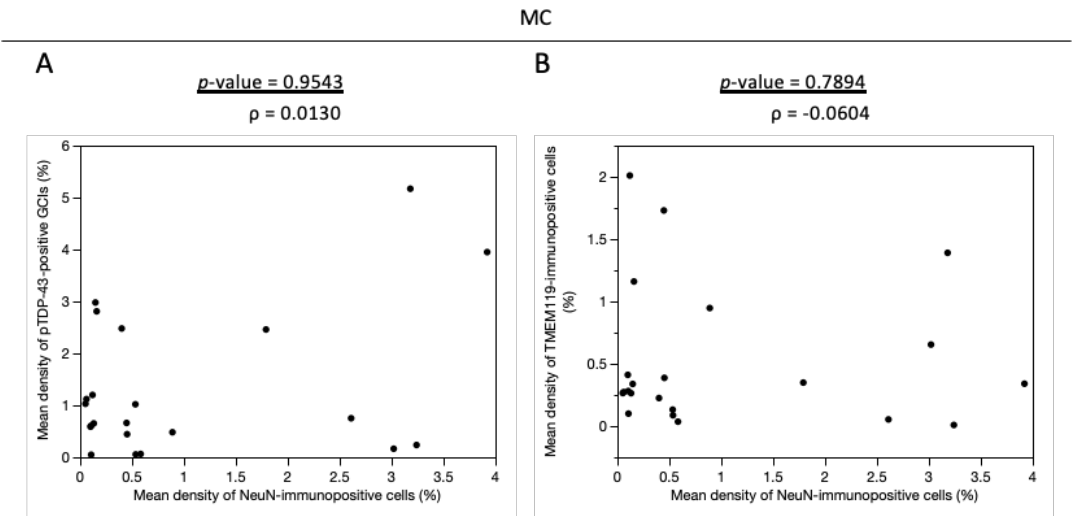


Figure S5. Correlation between NeuN-immunopositive cells and pTDP-43-positive GCIs or TMEM119-immunopositive cells.

The mean density of (A) pTDP-43-positive GCIs and (B) TMEM119-immunopositive cells was plotted as a function of the mean density of NeuN-immunopositive cells in the MC in all cases. There was no correlation between the mean density of (A) pTDP43-positive GCIs or (B) TMEM119-immunopositive cells and NeuN-immunopositive cells in the MC. ρ , coefficient of Spearman; p-value, value of significance; GCIs, glial cytoplasmic inclusions; MC, motor cortex.

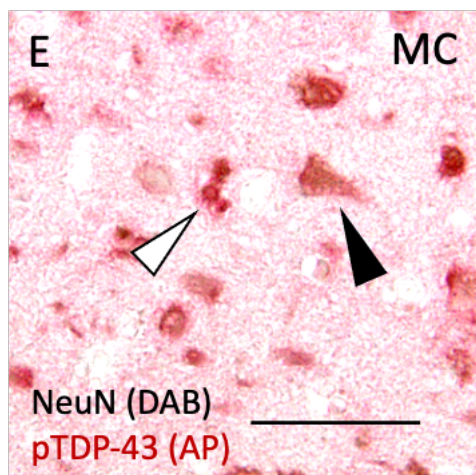
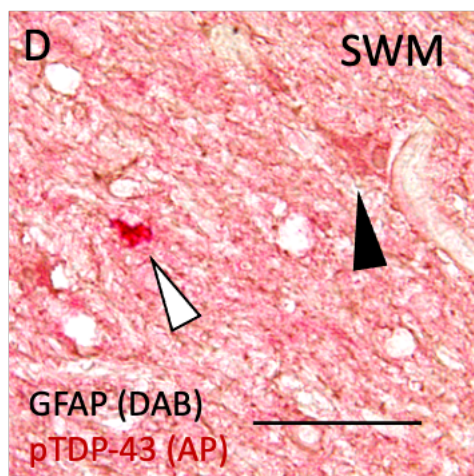
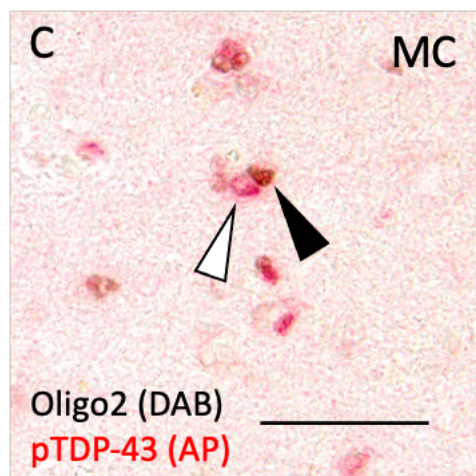
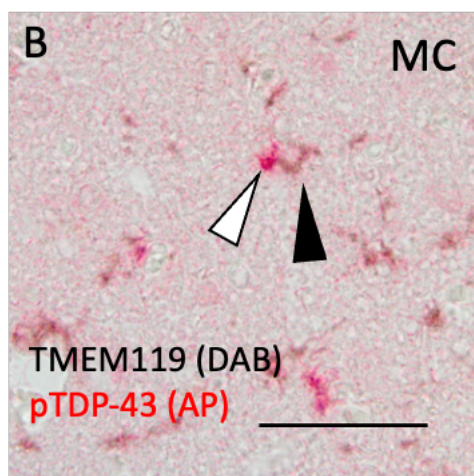
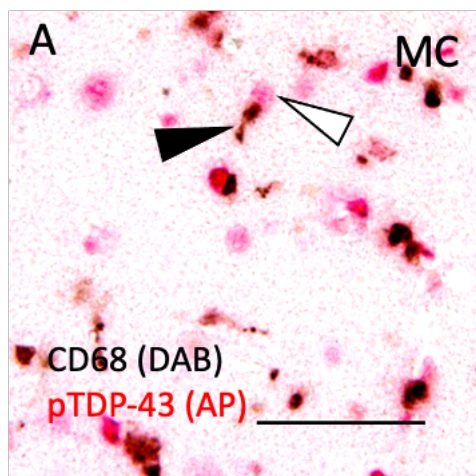


Figure S6. Double immunohistochemical staining results in one ALS case.
 In the MC of patients with ALS, (A) CD68- (blown by DAB, black arrowhead), (B) TMEM119- (blown by DAB, black arrowhead), or (C) Oligo2-immunopositive cells (black by DAB, black arrowhead) were observed close to pTDP-43-positive GCIs (red by AP, white arrowhead). (D) GFAP-immunopositive cell (blown by DAB, black arrowhead) in the SWM of patients with ALS or (E) NeuN-immunopositive cell (blown by DAB, black arrowhead) in the MC of patients with ALS were observed existed apart from pTDP-43-positive GCIs (red by AP, white arrowhead). The scale bar in all images is 50 μ m. ALS, amyotrophic lateral sclerosis; MC, motor cortex; DAB, 3, 3'-diaminobenzidine; AP, alkaline phosphatase; GCIs, glial cytoplasmic inclusions; SWM, subcortical white matter.

Mean density of CD68-immunopositive cell (%)			
	ALS†	Control†	p-value
SWM	1.31 [0.926, 2.99]	0.691 [0.385, 1.08]	0.0302‡

Mean density of TMEM119-immunopositive cell (%)			
	ALS†	Control†	p-value
SWM	0.296 [0.152, 1.01]	0.0646 [0.009, 0.139]	0.0256‡
MC	0.349 [0.281, 1.16]	0.132 [0.0536, 0.654]	0.0356‡

Mean density of pTDP-43-immunopositive cell (%)			
	ALS†	Control†	p-value
SWM	0.757 [0.117, 2.28]	0.115 [0.0389, 0.21]	0.0215‡
MC	1.2 [0.605, 2.98]	0.482 [0.0635, 1.02]	0.0215‡
VH	0.126 [0.0584, 0.242]	0.00163 [0.00133,0.00473]	0.0369‡
LF	0.0466 [0.02935, 0.10165]	0.00323 [0, 0.0161]	0.0369‡

Mean density of immunopositive cell (%)			
	MC†	SWM†	p-value
CD68	0.241 [0.17, 0.389]	1.31 [0.926, 2.99]	0.0002‡

Table S1. Wilcoxon rank sum test results with significant differences.

† median [first quartile, third quartile]

‡ p-value < 0.05

ALS, amyotrophic lateral sclerosis; SWM, subcortical white matter; MC, motor cortex; VH, ventral horn; LF, lateral funiculus.

The primary antibodies list

Antibody	Host	Source [catalog number]	Dilution
pTDP-43	rabbit polyclonal	Cosmo Bio, Tokyo, Japan; [Cat No. TIP-PTD-P03]	1:2000
pTDP-43	mouse monoclonal	Cosmo Bio, Tokyo, Japan; [Cat No. TIP-PTD-M01]	1:2000
CD68	mouse monoclonal	Dako, Glostrup, Denmark; [Cat No. M0814]	1:200
TMEM119	rabbit polyclonal	Atlas Antibodies, Stockholm, Sweden; [Cat No. HPA051870];	1:1000
CCR2	mouse monoclonal	R&D systems, Minneapolis, MN, USA; [Cat No. Clone48607]	1:400
NeuN	mouse monoclonal	Millipore, CA, USA; [Cat No. MAB377]	1:200
NeuN	rabbit polyclonal	Millipore, CA, USA; [Cat No. ABN78]	1:200
GFAP	rabbit polyclonal	Abcam, Cambridge, UK; [Cat No. ab7260]	1:200
Olig2 Rabbit	rabbit polyclonal	IBL, Gumma, Japan; [Cat No. 18953]	1:200