

The stimulator of interferon genes (STING) pathway is upregulated in striatal astrocytes of patients with multiple system atrophy

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ABSTRACT

Multiple system atrophy (MSA) is a progressive neurodegenerative disorder characterized by the accumulation of pathogenic phosphorylated α -synuclein in oligodendrocytes. In brains affected by MSA, severe astrogliosis is also observed, but its precise role in MSA pathogenesis remains largely unknown. Recently, the stimulator of interferon genes (STING) pathway and type I interferons, its downstream molecules, have been reported to be involved in the neurodegenerative process and to be activated in astrocytes. This study aimed to investigate the role of the STING pathway in the pathogenesis of MSA using postmortem brains. Samples used for immunohistochemical analysis included 6 cases of MSA parkinsonism type (MSA-P), 6 cases of MSA cerebellar type (MSA-C), and 7 age-matched controls. In MSA-P cases, astrocytes immunopositive for STING and TANK-binding kinase 1 (TBK1), its downstream molecule, were abundantly observed in the putamen and the substantia nigra. Moreover, these molecules colocalized with glial fibrillary acidic protein (GFAP) in reactive astrocytes, and the density of STING-positive astrocytes correlated with that of GFAP-positive reactive astrocytes in the brains of patients with MSA-P. These results suggest that the upregulated expression of STING pathway-related proteins in astrocytes and the subsequent inflammation may contribute to the pathogenesis in MSA-P and could provide novel therapeutic targets for the treatment of MSA.

1. Introduction

Multiple system atrophy (MSA) is a neurodegenerative disorder characterized by varying degrees of parkinsonism, cerebellar syndrome, and autonomic dysfunction. MSA can be categorized into two subtypes according to the predominant clinical manifestation: the parkinsonian type (MSA-P) and the cerebellar type (MSA-C). Patients with MSA-P show more symptoms of parkinsonism, whereas those with MSA-C commonly exhibit cerebellar symptoms. Pathologically, MSA is characterized by glial cytoplasmic inclusions (GCIs) in the oligodendrocyte. The GCI consists of the accumulation of misfolded phosphorylated α -synuclein (pSyn) [1,2]. Neural loss and demyelination with GCIs mainly affect specific regions, including the basal ganglia, cerebellum, pons, inferior olivary nuclei, and spinal cord. The predominance of the affected region reflects the clinical subtype. Parkinsonism, one of the main manifestations of MSA-P, is refractory to dopamine replacement therapy treatment because of the degeneration of postsynaptic neurons

in the putamen. The neurons receive projective fibers from the substantia nigra (S. nigra) [1–3]. Astrogliosis in the putamen is one of the most important aspects of MSA pathogenesis. Researchers have attempted to clarify the relationship between α -synuclein and astrocyte pathology in MSA [4]. In an in vitro primary culture model, α -synuclein induces astrocyte activation in an ERK/MAPKK-dependent manner [4]. However, the precise mechanism of astrogliosis in MSA remains unknown.

Innate immune activation in the central nervous system (CNS) is thought to be the main pathomechanism of acute diseases such as viral infection and stroke [5]. Innate immune activation is initiated by pathogen-associated molecular patterns from bacterial and viral infections or damage-associated molecular patterns from damaged neuronal and glial cells, leading to the release of cytokines and interferon, as well as the upregulation of interferon-stimulated genes in several pathways [6]. Recent studies have identified that innate inflammatory processes also cause the deterioration in chronic

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Table 1

Case material studied.

Case	Diagnosis	Age	Sex	Brain weight (g)	Duration of disease (years)	PMI (hours)
1	MSA-P	60	f	1190	11	7
2	MSA-P	69	f	880	7	12
3	MSA-P	72	f	1205	12	4
4	MSA-P	56	m	1105	2	3
5	MSA-P	88	f	1160	6	13
6	MSA-P	52	f	1300	4	2.5
Sub-total*		66.2 ± 13.1		1140.0 ± 142.5	7.0 ± 3.9	6.9 ± 4.6
7	MSA-C	71	f	1180	4	8
8	MSA-C	72	f	990	NA	2
9	MSA-C	66	m	1360	3	3.5
10	MSA-C	78	m	1100	2	2
11	MSA-C	60	m	940	6	3
12	MSA-C	66	f	1020	7	1.5
Sub-total*		68.8 ± 6.2		1098.3 ± 153.7	4.4 ± 2.1	3.3 ± 2.4
13	FTLD-TDP	66	f	900		3
14	ALS	63	f	930		1.5
15	CIDP	75	m	1110		6
16	CI	86	f	940		4
17	FTLD-TDP	69	m	1140		2.5
18	ALS	62	m	1290		1
19	GBS	62	m	1380		3
Sub-total*		69.0 ± 8.8		1098.6 ± 187.4		3.0 ± 1.7
Total*		68.1 ± 9.3		1111.6 ± 155.9		4.3 ± 3.4

* Average ± standard deviation. Abbreviations: PMI, postmortem interval; MSA-C, multiple system atrophy-cerebellar; MSA-P, multiple system atrophy-parkinsonian; FTLD, frontotemporal lobar degeneration; ALS, amyotrophic lateral sclerosis; CIDP, chronic inflammatory demyelinating polyneuropathy; CI, cerebral infarction; GBS, Guillain-Barré syndrome.

neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease (AD), amyotrophic lateral sclerosis [5], and MSA [7]. In the neuronal immune system, the importance of type I interferons (IFNs) in the progression of neuronal diseases [8], including prion disease [8], AD [9], and relapsing-remitting multiple sclerosis [10], has been highlighted. Type I IFNs act upstream of pro-inflammatory cytokines, which are involved in the initiation and regulation of the pro-inflammatory response [11]. Type I IFNs are produced in pathways involving toll-like receptors (TLR3, TLR7, and TLR9), retinoic acid-inducible gene-I (RIGI), mitochondrial antiviral signaling (MAVS), and stimulator of interferon genes (STING), among others [6].

STING is a pattern recognition receptor in response to cytoplasmic DNA [6]. STING is activated by several DNA sensors such as cyclic-GMP-AMP (c-GAMP) synthase (cGAS) [12], DEAD-box helicase 41 (DDX41) [13], among others [6]. For example, in the presence of DNA released by bacteria, viruses, or injured nuclei or mitochondria, cGAS catalyzes cytosolic DNA into c-GAMP, leading to activation of TANK-binding kinase 1 (TBK1) and STING [12]. Activated STING induces the secretion of type I IFNs [14]. In Parkinson's disease, the relationship between the STING pathway and neuronal loss was pointed out [15], and a role for astrocytes in several diseases associated with the STING pathway has been reported [16,17]. However, the relationship between the STING pathway and astrogliosis in MSA has not been studied in detail.

Here, we report that in MSA-P, many glial fibrillary acidic protein (GFAP)-positive astrocytes in the putamen have strong immunoreactivity for STING and TBK1. We propose that the STING pathway is activated by astrocytes contributes to the exacerbation of the MSA-P pathology in the putamen.

2. Materials and methods

2.1. Subjects

Formalin-fixed human brain tissue samples from 6 patients with MSA-P (52–88 years old), six patients with MSA-C (60–78 years old), and seven age-matched controls (62–86 years old) were used in this study. Age-matched controls did not include individuals with significant α -synuclein disease and a clinical history of parkinsonism. However, this

group did have individuals with another neurodegenerative disease, specifically amyotrophic lateral sclerosis.

The diagnoses were determined 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. Immunohistochemistry

The primary antibodies used in this study were as follows: stimulator of interferon genes/transmembrane protein 173 (STING/TMEM173, rabbit monoclonal [Cat No. NBP2–24683], Novus Biologicals, Colorado, USA; 1:200), TANK-binding kinase 1/NF- κ B activating kinase (TBK1/NAK, rabbit monoclonal [Cat No. ab40676], Abcam, Cambridge, UK; 1:400), GFAP (mouse monoclonal [Cat No. M0761], DAKO, Glostrup, Denmark; 1:200), cluster of differentiation 68 (CD68, mouse monoclonal [Cat No. M0814], DAKO; 1:200), and pSyn (mouse monoclonal [Cat No. 01,525,191], WAKO, Tokyo, Japan; 1:1000).

2.2.1. Single immunohistochemical staining

For single-labeling immunohistochemistry, 6- μ m sections were deparaffinized, and antigens were retrieved by autoclaving for 20 min at 120 °C using the Histofine deparaffinization antigen retrieval buffer, pH 6 (Cat No. 415,281; Nichirei, Tokyo, Japan), followed by overnight incubation at 4 °C with primary antibodies in phosphate-buffered saline containing 3% bovine serum albumin. Immunohistochemistry was performed with primary antibodies as mentioned above and conducted with the peroxidase polymer-based method using the Histofine Simple Stain MAX-PO MULTI (Cat No. 424,154; Nichirei) and DAB Substrate Kit (Cat No. SK 4100; Vector Laboratories, Burlingame, CA).

2.2.2. Double immunohistochemical staining

Double immunohistochemical stainings of STING and GFAP or TBK1 and GFAP were performed in all cases. The labeling of STING and CD68 or the labeling of STING and pSyn were performed for some cases, employing the universal immuno-alkaline phosphatase polymer method. At the stage of the incubation with peroxidase-conjugated anti-IgG, an appropriate host-type-specific anti-IgG was used that differed for the secondary staining. After immersing slides in the solution provided by the DAB substrate kit with nickel, overnight incubation at 4 °C with

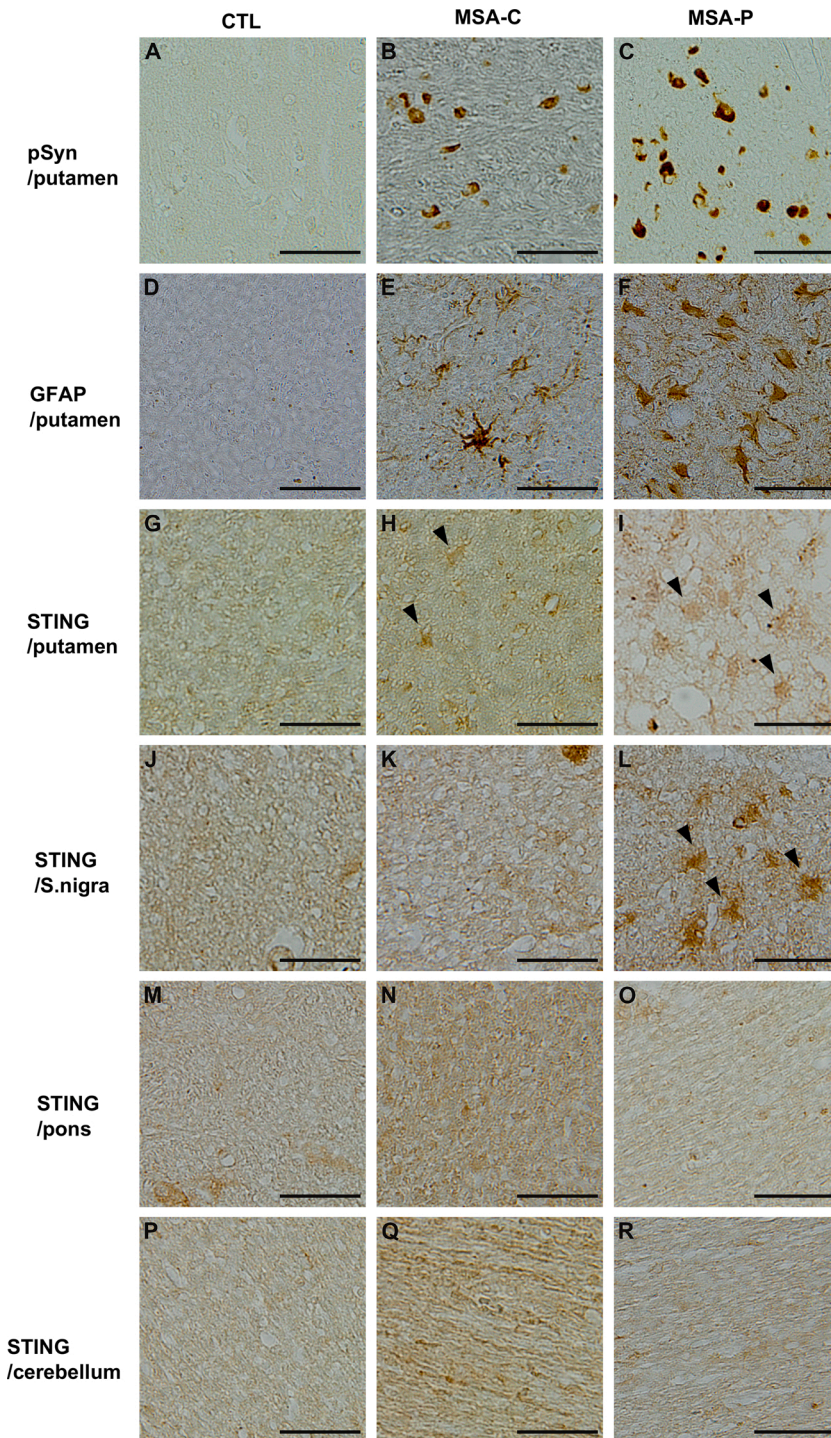


Fig. 1. Representative neuropathological findings in age-matched controls (CTL), multiple system atrophy in parkinsonism (MSA-P), and multiple system atrophy in cerebellar ataxia (MSA-C). In the putamen, no phosphorylated α -synuclein (pSyn)-positive deposition and glial fibrillary acidic protein (GFAP)-positive astrocytes in a CTL case (A, D), while both of them were observed in MSA cases. The degree of immunopositive findings in MSA-P is stronger than MSA-C (B, C, E, F). Moreover, in the putamen, many stimulators of interferon genes (STING)-positive astrocytes morphologically were found in MSA-P cases (arrowhead, I). A small number of them were found in MSA-C cases as well (arrowhead, H). In the substantia nigra (S.nigra), the amount of them is less than in the putamen. But the same tendency was confirmed in MSA cases (K), (arrowhead, L), while there were no findings in CTL (G, J). In pons, a very small number of them were found in a part of MSA-C cases (N). In CTL and MSA-P cases, there were no findings (M, O) in the cerebellum. There were no immunopositive astrocytes in all cases (P, Q, R). Scale bars: 50 μ m.

secondary primary antibodies in phosphate-buffered saline containing 3% bovine serum albumin followed. Then, slides were incubated with an alkaline phosphatase-conjugated anti-IgG antibody. Staining was visualized with Fast Red Substrate KitII (Cat No. 415261, Nichirei) according to the manufacturer's instructions. The enzyme-conjugated IgG antibodies used were as follows: 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 immuno-alkaline phosphatase polymer method.

2.2.3. Double-Labeling immunofluorescence staining

Sections from an MSA and Control case were selected for the double-labeling immunofluorescence method. The sections were simultaneously incubated with primary antibodies against GFAP and STING, GFAP, and TBK1 in 0.01 M PBS at 4°C overnight. After washing with 0.01 M PBS, the sections were reacted with secondary antibodies (Alexa Fluor 488-labeled goat anti-mouse or rabbit IgG and Alexa Fluor 546-labeled goat anti-mouse or rabbit IgG, 1:200; Molecular Probes, Eugene, OR). After rinsing with 0.01 M PBS, the slides were coverslipped with Vectashield containing DAPI (Vector Laboratories) and were then viewed with a FLUOVIEW FV-1000 confocal laser scanning microscope (Olympus, Tokyo, Japan).

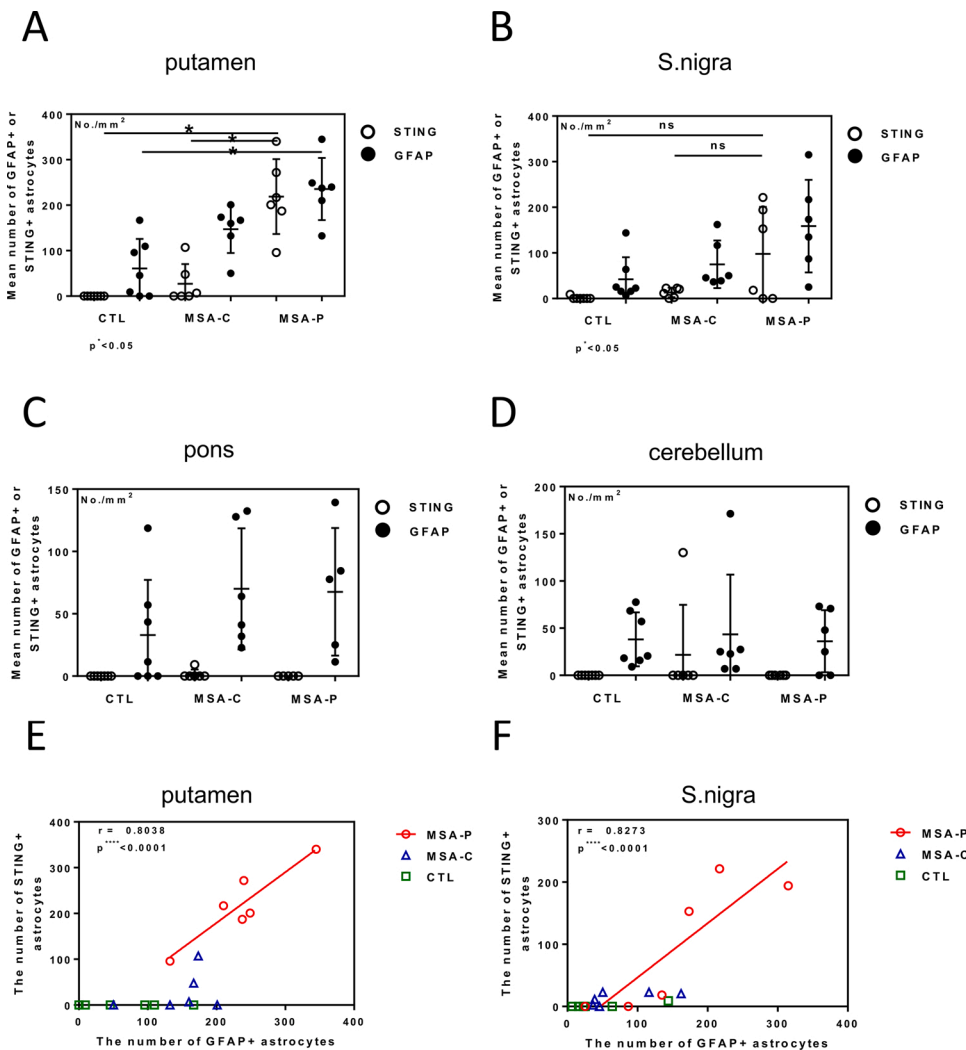


Fig. 2. Mean numbers of STING-positive astrocytes or GFAP-positive astrocytes in every region of three groups; control (CTL) group ($n = 7$), multiple system atrophy in parkinsonism (MSA-P) group (pons $n = 5$, others $n = 6$), and multiple system atrophy in cerebellar ataxia (MSA-C) group ($n = 6$). In the putamen (A), in the S. nigra (B), in the pons (C), in the cerebellum (D). $p^* < 0.05$. p values were calculated by the Kruskal-Wallis test and post hoc Dunn's multiple comparisons test. (E) Astrocytes with STING-positive count versus astrocytes with GFAP-positive count in the putamen. (F) Astrocytes with STING-positive count versus astrocytes with GFAP-positive count in the S. nigra. $\square$, age-matched control (CTL) group; $\circ$, multiple system atrophy in parkinsonism (MSA-P); $\triangle$, multiple system atrophy in cerebellar ataxia (MSA-C); $p^{****} < 0.0001$. p -values were calculated with Pearson correlation coefficient analysis.

2.3. Quantitative analysis of immunolabeled cells and data analysis

To determine the exact densities of pathological changes of GFAP, STING, and TBK1, we took from each case three pictures using light microscopy with $40\times$ magnification for each slide in four regions: the putamen, S. nigra, pons, and cerebellum. In each picture, the number of immunopositive cells was counted manually by two experienced pathologists who were blinded to the case diagnosis. The mean density of pathological changes was calculated per square millimeter as the average of the three pictures. For double-stainings, we calculated the mean ratio of double-immunopositive astrocytes to GFAP-positive astrocytes as the average of the three pictures.

Data are expressed as the mean $\pm$ standard deviation (SD). The results were analyzed using the Kruskal-Wallis test and post hoc Dunn's multiple comparisons test. The Pearson correlation coefficient was used to assess the correlation between the density of STING-positive cells and that of GFAP-positive cells. Prism 6 software was used for all statistical analyses. Significance was considered at $p < 0.05$ unless noted otherwise.

3. Results

pSyn-immunopositive GCIs were abundantly observed in the putamen, S. nigra, pons, and cerebellum of MSA-C (Fig. 1B) and MSA-P (Fig. 1C) cases, but not in control cases (Fig. 1A). Regarding astrogliosis, numerous reactive astrocytes with hypertrophic cell bodies were

observed in the dorsal part of the putamen in MSA-P cases (Fig. 1F), whereas only moderate reactive astrogliosis was observed in MSA-C cases (Fig. 1E). By contrast, in the putamen of control cases, there were sparse inactive astrocytes with weak immunostaining for GFAP (Fig. 1D). STING-positive cells were not observed in the putamen of control cases (Fig. 1G) but were detected in samples of MSA-C cases (Fig. 1H) and were most abundant in MSA-P cases (Fig. 1I). These STING-positive cells were identified as astrocytes based on their morphology, distribution, and double immunohistochemistry with GFAP. Although STING-positive astrocytes were rarely observed in the S. nigra of control (Fig. 1J) and MSA-C cases (Fig. 1K), they were frequently observed in the S. nigra of MSA-P cases (Fig. 1L). In the pons and cerebellum, where the MSA-C pathology mainly localizes, a small number of STING-positive cells were observed in all groups, including the control (Fig. 1M, P), MSA-C (Fig. 1N, Q), and MSA-P cases (Fig. 1O, R).

To quantitatively analyze the four regions, including the dorsal putamen, S. nigra, pons, and the cerebellum, we determined the numbers of GFAP- and STING-immunopositive astrocytes per square millimeter. In the putamen, the mean density of STING-positive cells in MSA-P cases was significantly higher than that in the other two groups, and the mean density of GFAP-positive astrocytes in the MSA-P group was also significantly higher than that in the control cases (Fig. 2A). The density of STING-positive cells in MSA-C cases was relatively low and much lower than that in MSA-P cases (Fig. 2A). The density of GFAP-positive astrocytes tended to be higher in the MSA-C group than in the control

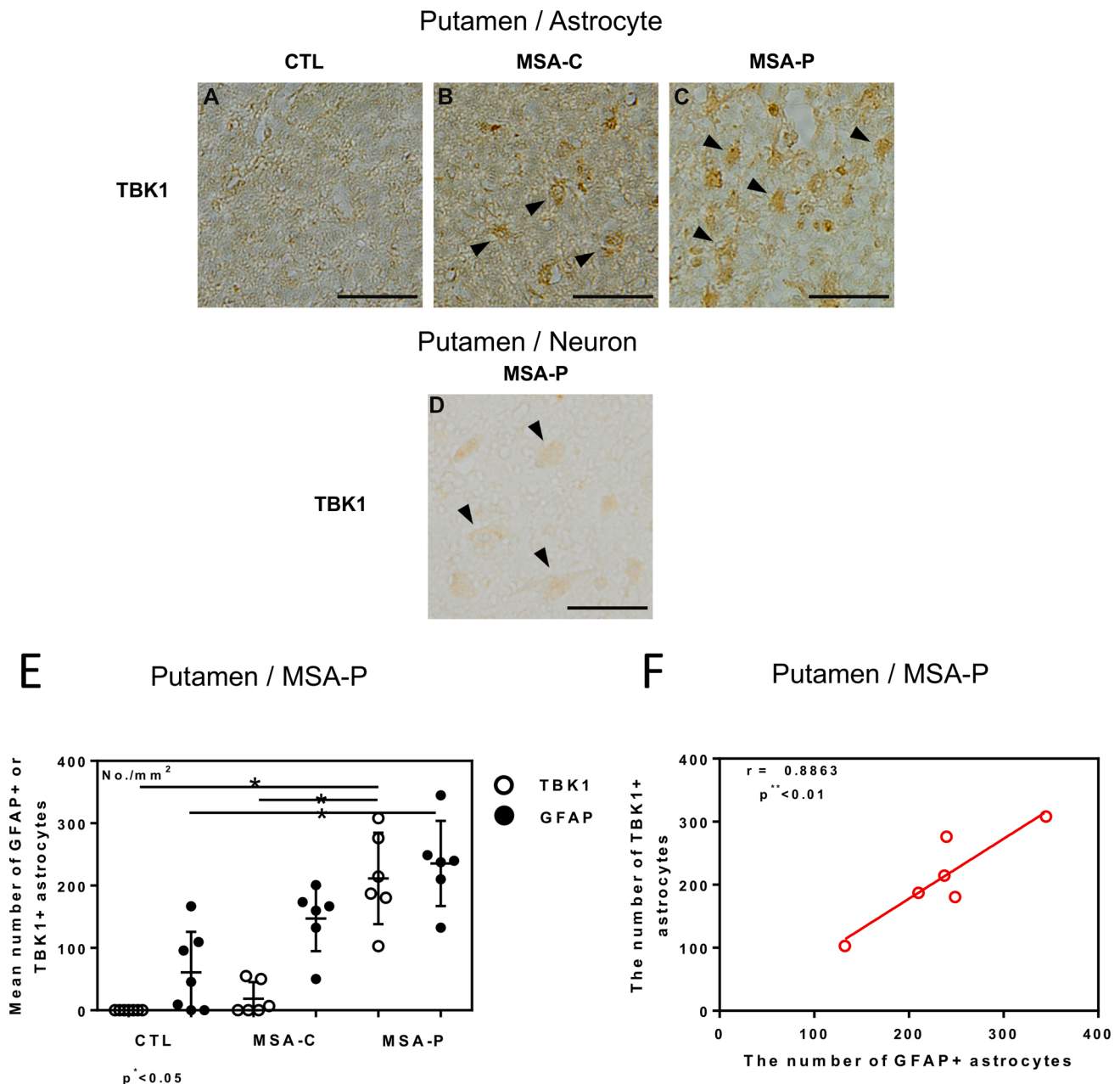


Fig. 3. Representative immunohistochemical findings of astrocytes stained with TANK-binding kinase 1 (TBK1) antibodies. In MSA-P cases, many TBK1-positive astrocytes were found (arrowhead, C). In MSA-C, a small number of astrocytes existed as well (arrowhead, B). There were no findings in CTL (A). Scale bars: 50 μ m. In MSA-P, the neurons were faintly stained for TBK1 on the two samples (D). Mean numbers of TBK1-positive astrocytes or GFAP-positive astrocytes (using the data of Fig. 2A) in the putamen of three groups; control (CTL) group ($n = 7$), multiple system atrophy in parkinsonism (MSA-P) group ($n = 5$), and multiple system atrophy in cerebellar ataxia (MSA-C) group ($n = 6$) (DE). $p^* < 0.05$. p values were calculated by the Kruskal-Wallis test and post hoc Dunn's multiple comparisons test. (F) Astrocytes with TBK1-positive count versus astrocytes with GFAP-positive count in the putamen in MSA-P cases. ○, multiple system atrophy in parkinsonism (MSA-P); △, multiple system atrophy in cerebellar ataxia (MSA-C); $p^{**} < 0.01$. p -values were calculated with Pearson correlation coefficient analysis.

group, but the difference was not statistically significant (Fig. 2A). These results indicate that STING, in addition to GFAP, is specifically up-regulated in MSA-P cases. In the *S. nigra*, the mean density of GFAP-positive and STING-positive astrocytes in MSA cases tended to be higher than that in control cases. This density was the highest in MSA-P cases, but the difference was not statistically significant (Fig. 2B). In the pons and cerebellum, STING-positive cells were rarely observed (Fig. 2C, D).

Next, we analyzed the correlations between the mean densities of STING-positive and GFAP-positive cells in both the putamen and the *S. nigra* in each group. In MSA-P cases, we confirmed strong positive correlations of mean cell densities in the putamen and a slight

correlation in the *S. nigra* (Fig. 2E, F). This result suggests that the positivity of STING immunoreactivity in astrocytes correlates with the severity of reactive astrogliosis.

We also stained the putamen sections of all cases with an antibody against TBK1, a downstream molecule of the STING pathway. We found that the number of TBK1-positive cells was increased in MSA cases (Fig. 3B, C), and this increase was substantial, specifically in MSA-P cases (Fig. 3E). Most of these TBK1-positive cells were morphologically identifiable as astrocytes. But in some of the samples in two MSA-P cases, faint immunostaining for TBK1 was observed in a few neurons (Fig. 3D). Similar to the result of STING staining (Fig. 2A), the density of TBK1-positive cells was almost the same as that of GFAP-positive cells in

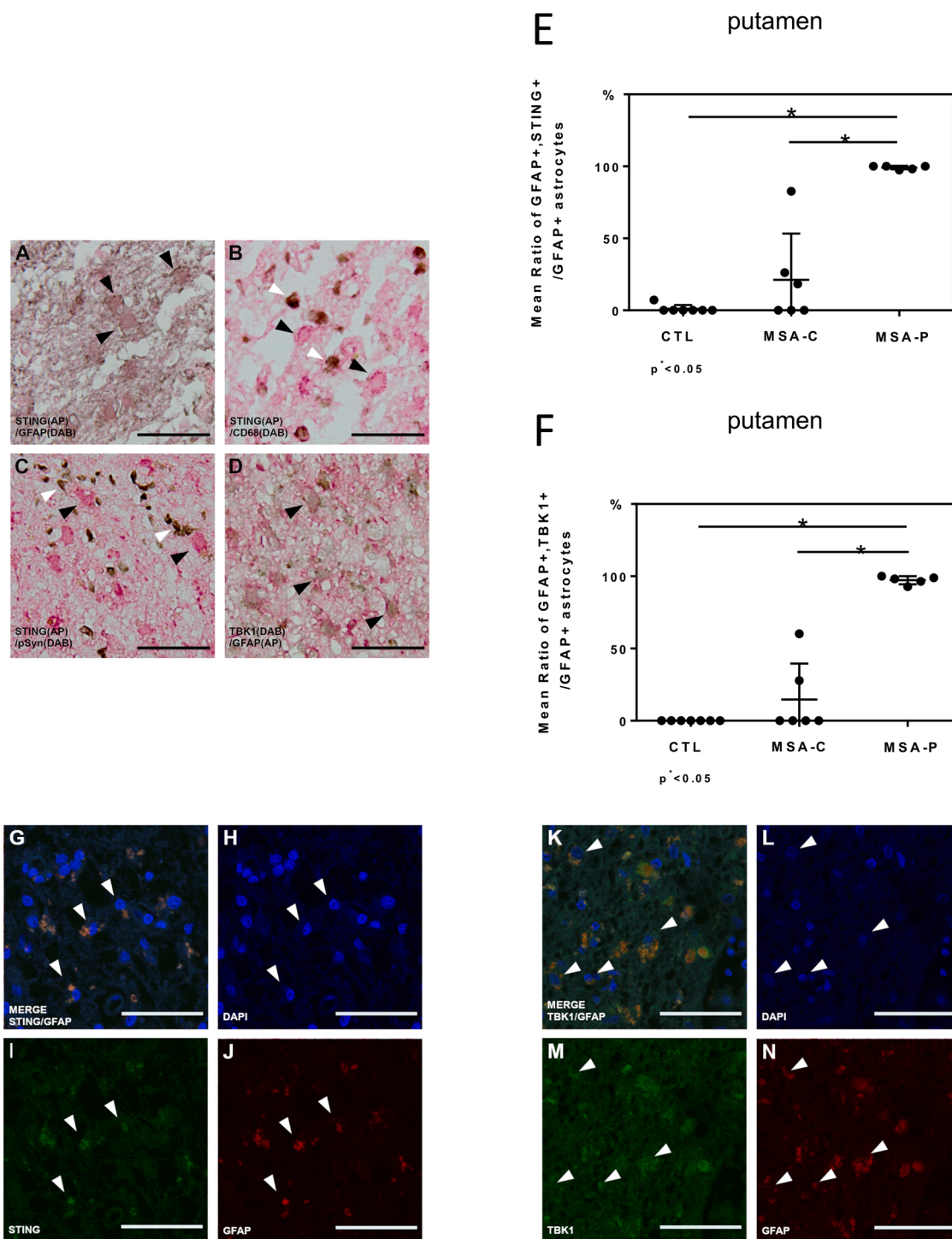


Fig. 4. Representative immunohistochemical findings of co-immuno-positive astrocytes with GFAP antibodies (blown by DAB) and STING antibodies (red by AP) in the putamen in MSA-P (black arrowhead, A). On the other hand, STING-positive astrocytes (black arrowhead, red by AP) were not merged Cluster of Differentiation 68 (CD68)-positive cells (white arrowhead, brown by DAB) (B) or pSyn-positive cells (white arrowhead, brown by DAB) (C). GFAP-positive astrocytes (red by AP) in the putamen in MSA-P also were stained with TBK1 (brown by DAB) (black arrowhead, D). Scale bars: 50 μ m. Mean ratio of double immunopositive (STING and GFAP) astrocytes to GFAP-positive astrocytes in the putamen of three groups (E). Mean ratio of double immunopositive (TBK1 and GFAP) astrocytes to GFAP-positive astrocytes in the putamen of three groups (F); control (CTL) group (n = 7), multiple system atrophy in parkinsonism (MSA-P) group (n = 5), and multiple system atrophy in cerebellar ataxia (MSA-C) group (n = 6). $p^* < 0.05$. p values were calculated by the Kruskal-Wallis test and post hoc Dunn's multiple comparisons test. Confocal microscopy and double immunofluorescence staining using antibodies to STING(I), TBK1(M), and GFAP(J, N) with nuclear staining by DAPI(H, L). The merged picture shows the overlap of green and red fluorescence, indicating co-localization of STING or TBK1 and GFAP in the astrocytes in the MSA-P cases (G, K). Scale bars: 50 μ m.

MSA-P cases but much lower than that of GFAP-positive cells in MSA-C cases (Fig. 3E). Moreover, we confirmed strong positive correlations of mean marker-positive cell densities in the putamen of MSA-P cases (Fig. 3F).

To evaluate the precise ratio of GFAP and STING upregulation in astrocytes in the putamen, we conducted double immunohistochemical labeling in all cases. Fig. 4A shows a representative MSA-P case. The colocalization ratio of GFAP and STING was high in astrocytes of MSA-P, but not of MSA-C, cases (Fig. 4E). Moreover, double-labeling immunofluorescence staining and confocal imaging analysis showed the colocalization of GFAP and STING (Fig. 4G-J), GFAP, and TBK1 (Fig. 4K-N) in reactive astrocytes of putamen of MSA-P.

There was little overlap between STING-positive cells and CD68-positive cells in all cases (Fig. 4B, MSA-P case). The distribution of GCIs was also different from that of STING-positive cells in all cases (Fig. 4C, MSA-P case). The co-localization of GFAP and TBK1 (Fig. 4D, MSA-P case) was comparable to that of GFAP and STING (Fig. 4F).

4. Discussion

In the present study, we reported that the densities of STING- and TBK1-immunopositive astrocytes were increased in the putamen and S. nigra of autopsied MSA brains, and this increase was higher in MSA-P cases than in MSA-C cases. STING- and TBK1-positive astrocytes were morphologically compatible with gemistocytic astrocytes and immunopositive for GFAP. Quantitative analyses of double-stainings showed that the density of STING- and TBK1-positive astrocytes correlated with the degree of GFAP-positive astrocytes in MSA lesions.

STING is a pattern recognition receptor that initiates in the presence of released DNA the production of type I IFNs and cytokines. STING is activated by DNA sensors that recognize the released DNA from viruses, tumors, or injured cells. DNA sensors include cGAS [12], DDX41 [13], and so on. Upon activation, STING translocates from the endoplasmic reticulum (ER) membrane to an ER-Golgi intermediate compartment or the Golgi apparatus. During this process, activated STING interacts with TBK1 and produces interferon regulatory factor 3 (IRF3) dimer, which binds to interferon-stimulated responsive elements in the nucleus and leads to type I IFN production [12]. Some studies also reported that STING is involved in the production of NF- κ B-dependent inflammatory cytokines [12,18].

Although the STING pathway was originally described in immune cells as a response mechanism to bacterial and viral infections [14], recent studies have shown that STING is also activated by DNA from injured cells in cancer [17], traumatic brain injury [19], and autoimmune diseases [16]. The STING pathway also plays a pathogenic role in astrocytes [16,17] and microglia [20] in the CNS. In neurodegenerative diseases, damaged mitochondria can also activate the STING pathway. In a mouse model of Parkinson's disease, mitochondrial DNA released by impaired mitophagy activates the STING pathway [15]. Moreover, fragmented mitochondria released from microglia also activate neurotoxic astrocytes and propagate inflammatory neurodegeneration in AD, ALS, and Huntington's disease models [21]. Mitochondrial dysregulation has also been reported in iPSC-derived dopaminergic neurons of patients with MSA [22]. Based on these reports, released mitochondrial DNA may activate the astrocytic STING pathway in MSA-P.

α -synuclein was also reported to induce astrocyte activation [4]. Prusiner group has also shown that α -synuclein can accumulate and propagate in primary transgenic murine astrocytes expressing human variants of α -syn [23]. α -synuclein-induced astrogliosis could also be attributed to the STING pathway of astrocytes in MSA-P. Once STING is activated, a positive feedback loop by increasing STING expression via IFN stimulation amplifies this pathway [24]. Continuous activation of STING is assumed to contribute to chronic inflammation and neurodegeneration [24,25]. Moreover, our specific findings of MSA-P may be explained by the regional heterogeneity of astrocytes [26]. Daniels et al. showed that the reactivity to IFN β differs between cortical and

cerebellar astrocytes and affects the regulation of the blood-brain barrier permeability in these regions [27]. Actually, in the putamen in the MSA-P, iron deposition [28] and T cell infiltration [7] were reported to be correlated with strong astrogliosis. However, in the S. nigra of the present study, it was suggested that the ratio of STING-positive astrocytes to GFAP-positive astrocytes (Fig. 2B-F) was reflected by the degree of astrogliosis among MSA-P cases and that it may depend on the degree of GCI burden according to cases. We need to investigate how the activation of astrocytes in each region in MSA-P in the future.

The limitations of our study are associated with the postmortem samples that we could use. Our immunohistochemistry data was not enough to show the direct evidence of the activation of the STING pathway in the astrocytes. In the future, we intend to clarify this issue in other MSA disease models.

In conclusion, our findings suggest that further investigations of the STING pathway in astrocytes may provide important cues to elucidate specific pathomechanism in MSA-P, thereby identifying novel therapeutic strategies.

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CRediT authorship contribution statement

Yutaka Inoue: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft. **Takashi Ayaki:** Conceptualization, Validation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Writing - review & editing. **Tomoyuki Ishimoto:** Validation, Investigation, Methodology, Software. **Hodaka Yamakado:** Funding acquisition, Project administration, Resources, Writing - review & editing. **Takakuni Maki:** Funding acquisition, Resources, Supervision. **Shuichi Matsuzawa:** Funding acquisition, Resources, Supervision. **Nobukatsu Sawamoto:** Funding acquisition, Supervision. **Ryosuke Takahashi:** Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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