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Clinical characteristics of autoimmune GFAP astrocytopathy

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ABSTRACT

The clinical features of autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy remain to be elucidated. We describe here the clinical features of 14 patients with GFAP astrocytopathy confirmed by detection of GFAP-IgG in cerebrospinal fluid (CSF). The novel findings of this study are as follows. First, over half of the patients presented with movement disorders (tremor, myoclonus, and ataxia), autonomic dysfunction (mainly urinary dysfunction), and hyponatremia. Second, most patients showed transient elevation of adenosine deaminase activity levels in CSF. Finally, some patients showed bilateral hyperintensities in the posterior part of the thalamus on brain magnetic resonance imaging.

1. Introduction

Autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy is a spectrum of immunotherapy-responsive autoimmune inflammatory central nervous system (CNS) disorders that is distinct from infectious meningoencephalitis and idiopathic inflammatory CNS disorders, such as multiple sclerosis (MS), vasculitis, and sarcoidosis (Fang et al., 2016; Flanagan et al., 2017). Detection of GFAP-IgG in cerebrospinal fluid (CSF) by both tissue and cell-based testing (not by western blotting or enzyme-linked immunosorbent assays) is a biomarker of autoimmune GFAP astrocytopathy. The most clinically sensitive and specific diagnostic biomarker of this disorder is the detection of autoantibodies in CSF against GFAPα, which is the predominant intermediate filament protein in adult astrocytes (Flanagan et al., 2017). Several reports describe the clinical and immunological characteristics of autoimmune GFAP astrocytopathy (Fang et al., 2016; Flanagan et al., 2017; Long et al., 2018). This disorder manifests as a distinctive corticosteroid-responsive meningoencephalomyelitis or limited form thereof, with lymphocytic pleocytosis often accompanied by a hallmark brain linear perivascular radial gadolinium enhancement on magnetic resonance imaging (MRI) (Flanagan et al., 2017; Zekeridou et al., 2018). This disorder has been reported in several countries, including USA, China, Italy and UK (Fang et al., 2016; Iorio et al., 2018; Shan et al., 2018; Zarkali et al., 2018). However, patients who are only positive for serum GFAP-IgG have been described with non-inflammatory neurological diseases (Flanagan et al., 2017; Zekeridou et al., 2018). Moreover, utilizing one assay alone may yield nonspecific results (Kunchok et al., 2019). Thus, we investigated the clinical features and outcomes of patients with autoimmune GFAP astrocytopathy confirmed by detection of GFAP-IgG in CSF by both tissue-based and transfected cell-based immunofluorescence assays.

2. Subjects and methods

2.1. Subjects

We enrolled 225 patients with inflammatory CNS diseases and 35 patients with non-inflammatory neurological diseases, who were admitted to our hospital (Department of Neurology, Gifu University Graduate School of Medicine, Gifu, Japan) between October 2002 and October 2018. Among these 225 patients with inflammatory CNS diseases, 98 had autoimmune CNS diseases [53 autoimmune encephalitis, 19 multiple sclerosis, 15 neuromyelitis optica spectrum disorder, 5 anti-myelin oligodendrocyte glycoprotein (MOG) antibody-associated diseases, 6 neuropsychiatric systemic lupus erythematosus], 58 had infectious CNS diseases (30 viral infection, 12 bacterial infection, 9 neurosyphilis, 3 cryptococcus, 2 tuberculosis, 2 toxoplasmosis), and 69 had inflammatory CNS diseases of unknown etiology (27 meningitis, 8 encephalitis, 25 meningoencephalitis/meningoencephalomyelitis, 9 myelitis). Among the 35 patients with non-inflammatory neurological diseases, 22 had psychosomatic disorder, 9 had migraine, and 4 had epilepsy. A summary of the patients is presented in Table 1. CSF samples were collected from all 260 patients and stored at −30 °C until analysis. We investigated GFAP-IgG in CSF samples from all 260 patients. Initially, CSF from all patients was tested for autoantibodies against GFAPα using a cell based assay (CBA). We then performed immunofluorescence assays using frozen rat brain sections and CSF samples from patients determined to be GFAPα autoantibody-positive

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Table 1
Patients including 14 with CSF GFAP-IgG.

Clinical diagnosis	Number	Sex (female)	Age, years-old	Positive CSF GFAP-IgG
		Number (%)	Median [range]	Number
Inflammatory CNS diseases	225	104 (46)	45 [15–91]	14
Autoimmune CNS diseases	98	60 (62)	45 [30–61]	5
Autoimmune encephalitis	53	24 (45)	41 [16–82]	5
Multiple sclerosis	19	15 (79)	47 [26–64]	0
NMOSD	15	13 (87)	50 [16–79]	0
MOG Ab-associated disease	5	4 (80)	26 [18–37]	0
NPSLE	6	4 (67)	47 [24–69]	0
Infectious CNS diseases	58	17 (29)	54 [20–82]	1
Viral infection	30	10 (33)	62 [20–82]	0
Bacterial infection	12	3 (25)	63 [21–73]	0
Neurosyphilis	9	1 (11)	49 [37–77]	1
Cryptoccocus	3	1 (33)	57 [53–60]	0
Tuberculosis	2	2 (100)	44, 54	0
Toxoplasmosis	2	0 (0)	31, 39	0
Inflammatory CNS diseases of unknown etiology	69	27 (39)	41 [15–91]	8
Meningitis	27	11 (41)	35 [16–77]	0
Encephalitis	8	2 (25)	71 [17–81]	0
Meningoencephalitis/Meningoencephalomyelitis	25	9 (36)	39 [15–78]	8
Myelitis	9	5 (56)	54 [19–91]	0
Non-inflammatory neurological diseases	35	19 (46)	41 [15–82]	0
Psychosomatic disorder	22	10 (45)	39 [15–82]	0
Migraine	9	7 (78)	41 [16–62]	0
Epilepsy	4	2 (50)	45 [27–75]	0

CSF: cerebrospinal fluid, CNS: central nervous system, GFAP: glial fibrillary acidic protein, MOG Ab: myelin oligodendrocyte glycoprotein antibody, NMOSD: neuromyelitis optica spectrum disorder, NPSLE: neuropsychiatric systemic lupus erythematosus.

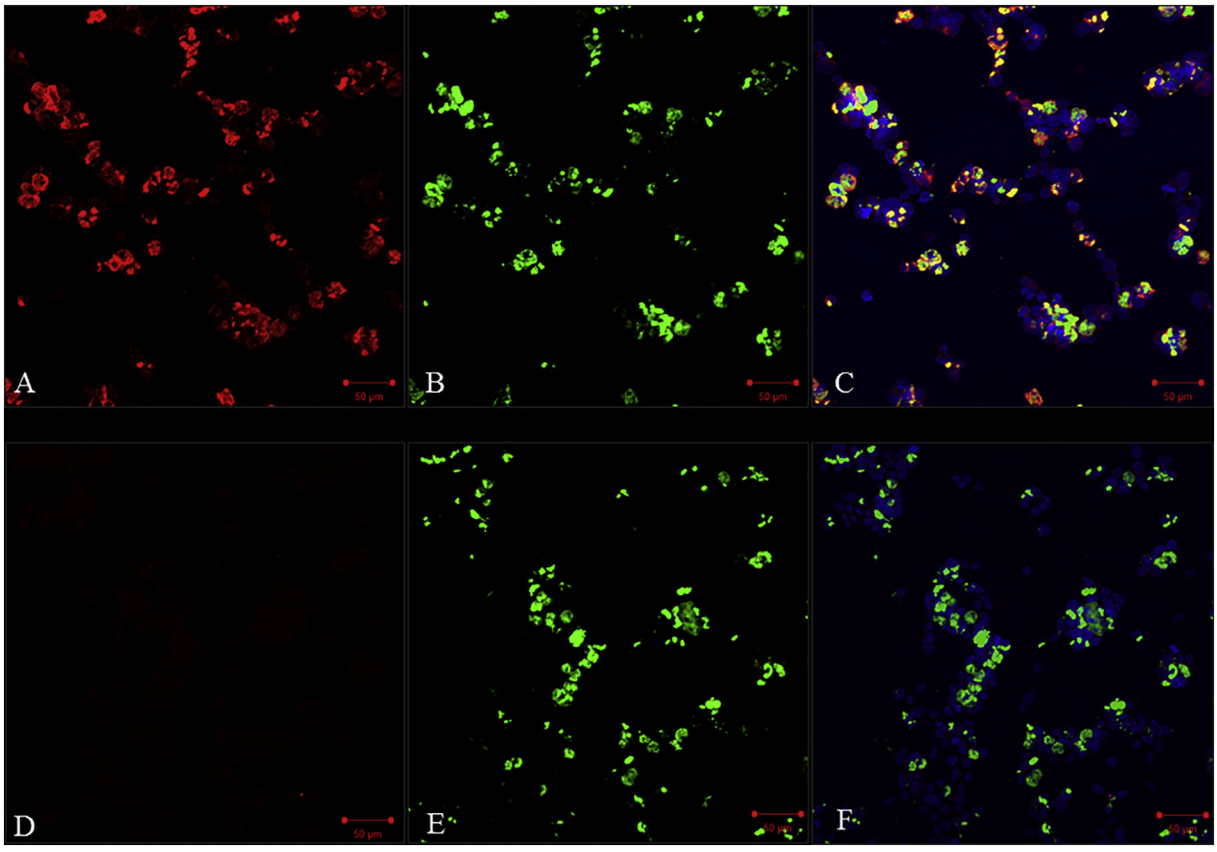

Fig. 1. Cell-based assay (CBA) of GFAPα-transfected HEK293 cells. HEK293 cells stably expressing green fluorescent protein (GFP)-tagged GFAPα (B, E). Glial fibrillary acidic protein (GFAP) IgG was detected in the cerebrospinal fluid (CSF) of patients with autoimmune GFAP astrocytopathy (A), but not in the CSF of patients with meningoencephalitis of unknown etiology (D). Colocalization of patient's IgG and GFAPα is yellow in merged images (C). DNA is stained with 4,6-diamidino-2-phenylindole (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by CBA. Finally, we investigated the clinical features and outcomes in patients confirmed to be CSF GFAP-IgG-positive by both CBA and tissue-based immunofluorescence assay. Routine CSF analysis of patients with meningitis and meningoencephalitis included total white cell counts (neutrophils or lymphocytes), protein level, glucose level, adenosine deaminase activity (ADA) level, gram stain and cultures. Informed consent was obtained from all patients for the secondary use of CSF samples. This study was approved by the institutional review board of the Gifu University Graduate School of Medicine, Gifu, Japan.

2.2. Cell-based assay

We screened for CSF GFAP-IgG using a CBA according to a previous report (Flanagan et al., 2017). The CBA employed human embryonic kidney 293 cells transfected with a plasmid from OriGene (Rockville, MD) that encodes a single *Homo sapiens* GFAP transcript variant 1 (RG204548; pCMV6-AC-GFAP-α-GFP). Transfected cells were fixed with 2% paraformaldehyde for 20 min, and permeabilized with 0.2% Triton-X-100/1% bovine serum albumin (BSA)/phosphate-buffered saline (PBS) for 10 min. The fixed cells were incubated with 1% BSA/PBS at 4 °C overnight to block nonspecific IgG binding. Cells were then exposed to diluted patient CSF (1:4) for 1 h, washed in PBS, then exposed to Cy3-conjugated donkey anti-human IgG (1:1500, Jackson ImmunoResearch, West Grove, PA) for 1 h.

2.3. Immunohistochemical assay

We performed immunohistochemical analysis using ready-to-use frozen rat brain sagittal sections (RF-201-SS, ZYAGEN, CA) and CSF samples from the patients determined to be GFAPα autoantibody-positive by CBA. Frozen sections were fixed in 4% paraformaldehyde at room temperature for 15 min, and permeabilized with 0.2% Triton-X-100/PBS for 10 min. After washing in PBS, normal goat serum (10%) was applied for 30 min to block nonspecific IgG binding. The sections were then incubated with diluted patient CSF (1:4) at 4 °C overnight. After washing in PBS, the sections were incubated with Alexa-Fluor 488 goat anti-human IgG (1:1500, Molecular Probes, Eugene, OR) for 1 h.

3. Results

3.1. Screening patients for autoimmune GFAP astrocytopathy

Among 260 patients tested, we detected autoantibodies against GFAPα in CSF from 14 patients by CBA (Fig. 1). CSF from all 14 patients showed immunoreactivity against astrocytes in pial, subpial and periventricular regions of the rat brain (Fig. 2). In some of these patients, the CSF also showed immunoreactivity against astrocytes in the cerebellum and cerebral white matter. The clinical diagnoses of these 14 patients with CSF GFAP-IgG were 5 patients with autoimmune encephalitis [4 patients with acute disseminated encephalomyelitis (ADEM) and 1 patient with anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis], 1 patient with neurosyphilis, and 8 patients with meningoencephalitis/meningoencephalomyelitis of unknown etiology (Table 1).

3.2. Clinical symptoms of patients with CSF GFAP-IgG

The demographics and clinical characteristics of the 14 patients with CSF GFAP-IgG are summarized in Tables 2 and 3. The median age was 44 years (range: 18–66 years) and six patients were females (43%). Past histories and complications on admission were diabetes mellitus (n = 4, 29%), hypertension (n = 2, 14%), prostate cancer (complete remission, n = 1, 7%), and acute pancreatitis (n = 1, 7%).

The initial symptoms were fever (n = 13, 93%), headache (n = 11, 79%), nausea (n = 4, 29%), anorexia (n = 3, 21%), fatigue (n = 2, 14%), and neck pain (n = 2, 14%). The median time from onset to hospitalization was 9.5 days (range: 4–22 days). The neurological findings during hospitalization were consciousness disturbance (n = 11, 79%), meningeal sign (n = 10, 71%), tremor and/or myoclonus (n = 9, 64%), hyperreflexia (n = 8, 57%), autonomic dysfunction (n = 8, 57%), limb and/or truncal ataxia (n = 6, 43%), psychosis (n = 5, 36%), respiratory failure (n = 4, 29%), recent memory disturbance (n = 3, 21%), convulsion (n = 2, 14%), and paraplegia (n = 2, 14%). The most common complication during hospitalization was hyponatremia (serum Na level < 135 mEq/L) (n = 8, 57%), followed by thromboembolism (n = 3, 21%). For tumor screening, we performed whole body computed tomography in all patients except one, who was pregnant. We also performed pelvic MRI in two patients and 18F-fluorodeoxyglucose-positron emission tomography (FDG-PET) in one patient. As a result, ovarian teratoma was found in two patients, one of which had anti-NMDAR encephalitis. We examined coexisting neural autoantibodies using a cell based assay. All 14 patients were negative for anti-leucine-rich glioma-inactivated protein 1 (LGI1), and anti-contactin associated protein 2 (Caspr2) antibodies. Five patients with autoimmune encephalitis and five patients with meningoencephalitis/meningoencephalomyelitis of unknown etiology were negative for anti-MOG antibodies. Five patients with autoimmune encephalitis were negative for anti-NMDAR antibodies except for one patient with NMDAR encephalitis. All five patients with autoimmune encephalitis were negative for anti-aquaporin 4 (AQP4) antibodies.

3.3. CSF findings in patients with CSF GFAP-IgG

CSF findings in the 14 patients with CSF GFAP-IgG are summarized in Table 4. On admission, all 14 patients showed lymphocyte-predominant pleocytosis (median: 148/μL, range: 25–378/μL), and elevated protein levels (median: 195 mg/dL, range: 71–286 mg/dL). In most patients, the CSF pleocytosis lasted several months (Fig. 3A). Most patients showed transiently increased CSF ADA levels in the first month from onset (Fig. 3B). The median level of the highest CSF ADA level of each patient was 13 IU/L. At last CSF examination, we confirmed the negative conversion of the anti-GFAPα antibody in five patients by CBA.

3.4. Neuroimaging and electroencephalogram findings in patients with CSF GFAP-IgG

Neuroimaging and electroencephalography findings of patients with CSF GFAP-IgG are summarized in Table 5. On brain MRI, nine out of 14 patients (64%) showed abnormal hyperintensity lesions on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images (Fig. 4 A–E). The abnormal hyperintensity MRI lesions were in the basal ganglia (n = 8), thalamus (n = 6), cerebral white matter (n = 5), brainstem (n = 5), internal capsule (n = 2), and hypothalamus (n = 2). On gadolinium-enhanced brain MRI, seven out of nine patients showed abnormal enhancement images. Four patients showed linear perivascular radial enhancement patterns in cerebral white matter (Fig. 4F). Two patients showed enhancement of the brainstem surface and cervical spinal cord. One patient showed enhancement of the left temporal lobe meninges. Two out of eight patients, who underwent spine MRI, showed intramedullary hyperintensity lesions on T2-weighted images. Six out of seven patients, who underwent enhanced spine MRI, showed abnormal enhancement images. Among these six patients, five patients showed enhancement of the medullary cone surface, and one patient showed intramedullary enhancement at C2–C5 vertebral levels. We performed brain 99mTc-hexamethylpropyleneamine oxime (HMPAO) single-photon emission computed tomography (SPECT) in four patients, and brain 123I-N-isopropyl-p-iodoamphetamine (IMP) SPECT in three patients. All seven patients had decreased cerebral blood flow in the frontal lobes. We performed electroencephalography in 10 patients, and all patients showed diffuse slow waves. No patients showed epileptic discharges.

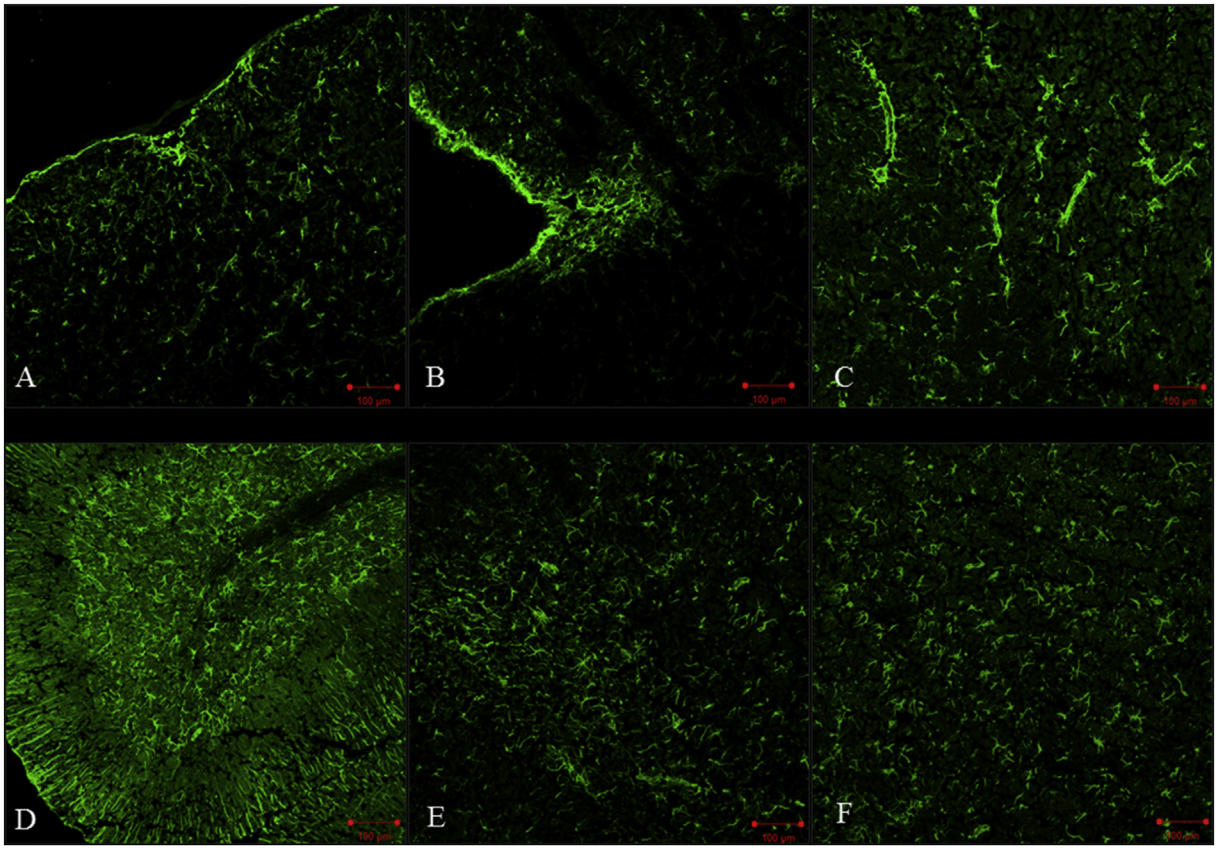

Fig. 2. Tissue-based immunofluorescence assay using frozen rat brain sagittal sections. Immunoreactivity of patients' cerebrospinal fluid (CSF)-IgG was observed in astrocytes of the pial, subpial (A), periventricular (B), and perivascular regions (C). Some patient's CSF-IgG also showed immunoreactivity against the Bergmann glia of the cerebellum (D) and cerebral white matter (E, F).

Table 2
Demographics of 14 patients with autoimmune GFAP astrocytopathy.

Demographic	Patients, Number (%)	Median [range]
Age at onset, years-old		44 [18–66]
Female	6 (43)	
Past history/Complications on admission		
Diabetes	4 (29)	
Hypertension	2 (14)	
Prostate cancer (complete remission)	1 (7)	
Acute pancreatitis	1 (7)	
Time from onset to hospitalization, day		9.5 [4–22]
Complications during hospitalization		
Hyponatremia	8 (57)	
Thromboembolism	3 (21)	
Paralytic ileus	2 (14)	
Pneumonia	2 (14)	
Tumor		
Ovarian teratoma	2 (14)	

GFAP: glial fibrillary acidic protein,

3.5. Treatments and outcomes of patients with CSF GFAP-IgG

The treatments and outcomes of patients with CSF GFAP-IgG are summarized in Table 6. All patients except one were treated with corticosteroid therapy. The median time from onset to start of corticosteroid therapy was 14 days (range 8–71 days). Eleven patients (79%) were treated with methylprednisolone at 1 g/day for 3 days several times (median: 2 times/patient, range 1–4 times/patient). Four patients (29%) were treated with intravenous dexamethasone therapy. After these steroid therapies, seven patients (50%) were treated with oral prednisolone (PSL). The median starting dose and duration of oral PSL therapy was 60 mg/day (range 20–60 mg/day), and 177 days (range 49–789 days), respectively. Two patients (14%) were treated with intravenous immunoglobulin therapy. Treatments other than immunotherapy administrated during hospitalization were, acyclovir; 14 patients (100%), antibiotics; 11 patients (79%), anti-tubercular agents; 5 patients (36%), anti-mycobacterial agents; 4 patients (29%), anticonvulsants; 4 patients (29%), and intravenous anesthetics; 3 patients (21%). Three patients (21%) needed mechanical ventilation. After treatments, the modified Rankin Scale (mRS) scores were decreased (median mRS on admission: 5, range 3–5; median mRS on discharge: 2, range 1–4; median mRS at last follow-up: 0.5, range 0–2). The median follow-up period was 13 months (range 1–60 months). The median hospitalization period was 48 days (range 21–184 days). The most common sequelae at last follow-up was autonomic dysfunction (3, 21%) including urinary dysfunction (3, 21%). In this study, no patients had relapsed up to the last follow-up period.

4. Discussion

In this study, among 225 patients with inflammatory CNS diseases, we identified 14 (6.2%) autoimmune GFAP astrocytopathy patients with CSF GFAP-IgG by both tissue-based and transfected cell-based immunofluorescence assays. The occurrence of autoimmune GFAP astrocytopathy was almost the same as that of anti-NMDAR encephalitis (n = 13) in our hospital. This result shows that autoimmune GFAP astrocytopathy is not a rare inflammatory CNS disease. The 14 patients were mostly diagnosed as meningoencephalitis/meningoencephalomyelitis of unknown etiology or ADEM. The patient with neurosyphilis was not infected with HIV and was positive for serum Treponema pallidum latex agglutination (TPLA), rapid plasma reagin (RPR), and CSF fluorescent treponemal antibody-absorbed (FTA-ABS) tests. She

Table 3
Clinical symptoms of 14 patients with autoimmune GFAP astrocytopathy.

No	Age, Gender	Clinical diagnosis	CSF cell counts (/μL)	Brain MRI		Initial symptoms	Consciousness disturbance	Meningeal sign	Tremor/ Myoclonus	Hyperreflexia	Autonomic dysfunction	Ataxia	Psychosis	Respiratory failure
				Thalamus	Linear									
1	23, F	NMDAR	102	−	NE	fever, headache	+	+	+	+	+	+	+	+
2	27, M	ADEM	34	+	−	fever, headache	+	+	−	+	+	−	−	+
3	18, M	ADEM	56	+	+	fever, headache	+	+	−	−	+	−	−	+
4	31, F	ADEM	378	+	+	fever, headache	+	+	+	+	+	−	−	−
5	31, M	ADEM	158	−	+	fever, headache, nausea, vomiting	+	+	−	−	+	+	−	+
6	66, M	ME	25	−	NE	general fatigue, dizziness, anorexia	+	−	−	−	−	+	+	−
7	52, F	ME	364	−	NE	fever, headache	+	+	+	−	−	−	+	−
8	47, M	ME	288	−	+	fever, headache, nausea	−	−	+	+	−	−	−	−
9	41, F	ME	136	+	NE	fever, headache, neck and low back pain	+	+	+	+	−	−	−	−
10	21, F	ME	207	+	NE	fever, headache, nausea	−	+	−	+	−	−	−	−
11	48, M	MEM	174	−	−	fever, headache neck pain	+	−	+	−	+	+	+	−
12	58, M	MEM	159	+	−	fever, headache anorexia, nausea	−	+	+	+	+	+	+	−
13	66, M	MEM	38	−	−	fever, anorexia	+	+	+	+	−	−	−	−
14	63, F	Neurosyphilis	232	−	−	fever, general fatigue	+	−	+	−	+	+	−	−
		Total, Number (%)		6 (43)	4 (44)	fever 13 (93) headache 11 (79)	11 (79)	10 (71)	9 (64)	8 (57)	8 (57)	6 (43)	5 (36)	4 (29)

ADEM: acute disseminated encephalomyelitis, CSF: cerebrospinal fluid, Linear: linear radial enhancement pattern, ME: meningoencephalitis of unknown etiology, MEM: meningoencephalomyelitis of unknown etiology, MRI: magnetic resonance imaging, NE: not examined, NMDAR: anti-*N*-methyl-D-aspartate receptor encephalitis, Thalamus: bilateral hyperintensities of the posterior part of the thalamus.

Table 4
CSF findings of 14 patients with autoimmune GFAP astrocytopathy.

	Median [range]
CSF examination on admission	
Cell count (/μL)	148 [25–378]
Mononuclear cell count (/μL)	138 [25–367]
Protein level (mg/dL)	195 [71–286]
CSF examination during hospitalization	
Highest level of adenosine deaminase activity (ADA) (IU/L)	13 [7–16.2]
Last CSF examination	
Period of last CSF examination from onset (day)	62 [17–202]
Cell count (/μL)	11 [2–69]
Protein level (mg/dL)	58 [30–115]

CSF: cerebrospinal fluid, GFAP: glial fibrillary acidic protein.

presented with headache and behavioral abnormalities followed by consciousness disturbance, dysarthria, tremor, truncal ataxia, and dysuria in the subacute clinical course. These clinical symptoms were similar to those of the other patients with autoimmune GFAP astrocytopathy. We consider that this patient is a parainfectious case of autoimmune GFAP astrocytopathy, triggered by syphilis infection. Anti-AQP4 and anti-MOG antibodies are sometimes detected in a subset of ADEM (Pohl et al., 2016). In this study, all four patients diagnosed as ADEM were negative for these two antibodies. We suspect that adult ADEM is often quite distinctly different than pediatric ADEM.

We also retrospectively revealed the characteristic clinical features of these 14 patients. The peak age of onset among our patients was in the 40s, and men and women were affected equally. The most common preceding symptoms were fever and headache, and symptoms of meningoencephalitis presented 1 or 2 weeks later. The most common neurological findings during hospitalization included consciousness disturbance and meningeal signs. These demographic and clinical characteristics among our patients resemble those of previous reports (Dubey et al., 2018; Flanagan et al., 2017). However, over half of the 14 autoimmune GFAP astrocytopathy patients presented with movement disorders (tremor, myoclonus and ataxia) and autonomic dysfunction (mainly urinary dysfunction), which were previously reported as rare neurological accompaniments of autoimmune GFAP astrocytopathy

Table 5
Neuroimaging and electroencephalogram findings of 14 patients with autoimmune GFAP astrocytopathy.

Examination	Patients, Number (%)
Brain MRI	14
Abnormal hyperintensity lesions on T2WI /FLAIR images	9 (64)
Basal ganglia	8 (57)
Thalamus	6 (43)
Cerebral white matter	5 (36)
Brainstem	5 (36)
Internal capsule	2 (14)
Hypothalamus	2 (14)
Gadolinium-enhanced brain MRI	9
Abnormal enhancement images	7 (78)
Linear radial enhancement pattern of cerebral white matter	4 (44)
Enhancement of brainstem surface and cervical spinal cord	2 (19)
Enhancement of left temporal lobe meninges	1 (11)
Spine MRI	8
Intramedullary hyperintensity lesions on T2WI	2 (25)
Gadolinium-enhanced spine MRI	7
Abnormal enhancement images	6 (86)
Enhancement of medullary cone surface	5 (71)
Intramedullary enhancement	1 (14)
SPECT images	7
Hypoperfusion SPECT images	7 (100)
Frontal lobe	7 (100)
Cerebellum	3 (43)
Parietal lobe	3 (43)
Posterior lobe	2 (29)
Temporal lobe	1 (14)
Brainstem	1 (14)
Electroencephalography	10
Diffuse slow waves	10 (100)

FLAIR: fluid-attenuated inversion recovery, GFAP: glial fibrillary acidic protein, MRI: magnetic resonance imaging, SPECT: single-photon emission computed tomography, T2WI: T2-weighted image.

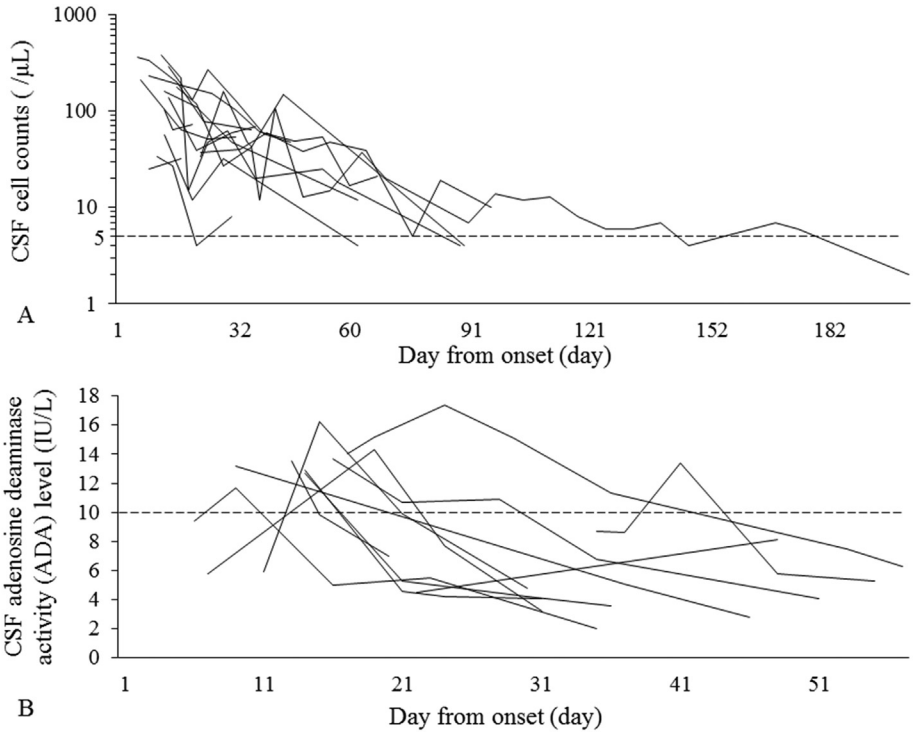

Fig. 3. Cerebrospinal fluid (CSF) findings of patients with autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy. Most patients showed CSF pleocytosis over several months (A), and showed transient increased levels of CSF adenosine deaminase activity (ADA) during the first month after onset (B). The cut off value of CSF cell counts (5 cells/μL) (A) and the general cut off value (10 IU/L) that is used for differentiation between tuberculous meningitis and non-tuberculous meningitis (B) are shown with dashed lines.

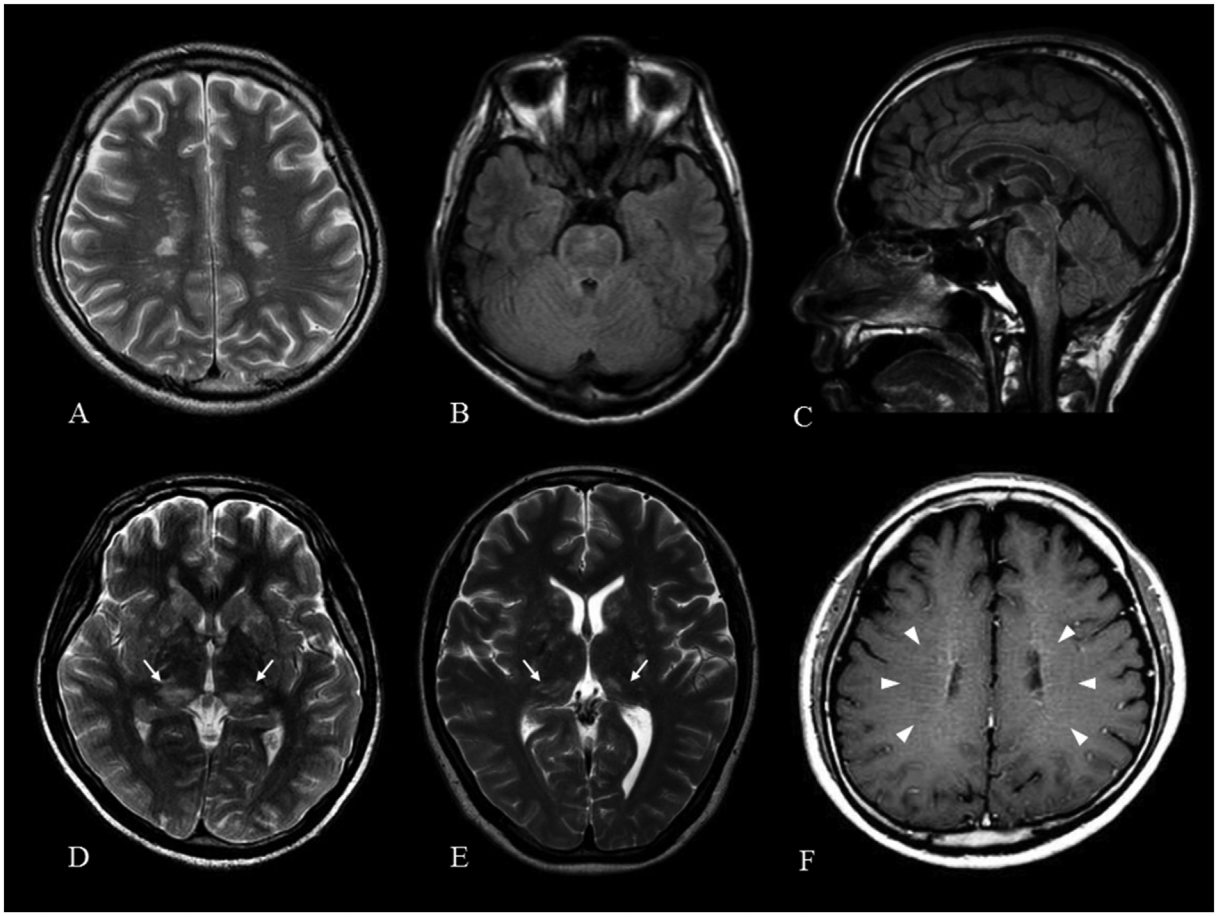

Fig. 4. Brain magnetic resonance imaging (MRI) of patients with autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy. The abnormal hyperintensity lesions on T2-weighted and fluid-attenuated inversion recovery images were observed in cerebral white matter (A), brainstem (B, C), and basal ganglia (D). The bilateral hyperintensities of the posterior part of the thalamus (arrows) were characteristic findings of autoimmune GFAP astrocytopathy (D, E). Gadolinium-enhanced brain MRI showed a linear perivascular radial enhancement pattern (arrowheads) (F).

(Kunchok et al., 2019). We suggest that patients with meningoencephalitis of unknown etiology presenting with these clinical symptoms should be considered for autoimmune GFAP astrocytopathy. The most common complication during hospitalization was hyponatremia. The syndrome of inappropriate antidiuretic hormone secretion (SIADH) may be a cause of hyponatremia. In this study, two patients had ovarian teratoma and one of them had a clinical course relatively typical of NMDAR encephalitis. This result is consistent with a previous report that described the only common oncological association as ovarian teratoma and that the most common neural IgG coexisting with CSF GFAP-IgG was an anti-NMDAR antibody (Kunchok et al., 2019; Yang et al., 2018).

Our patients with autoimmune GFAP astrocytopathy had characteristic CSF findings. In most patients, lymphocytic pleocytosis continued over several months. One patient showed lymphocytic pleocytosis for 6 months. It was hard to clarify the etiology of meningoencephalitis in most hospitalized patients. Moreover, most patients showed transiently increased CSF ADA levels during the first month from onset. The median of the highest CSF ADA level of each patient was 13 IU/L. A CSF ADA level of 10 IU/L is the standard cut off value for differentiation between tuberculous meningitis and non-tuberculous meningitis (Moghtaderi et al., 2010; Raviraj et al., 2017). In fact, five patients were initially suspected as tuberculosis meningitis and were administered anti-tubercular agents. However, all of them showed negative PCR and culture results for *Mycobacterium tuberculosis*. A previous report described a patient with autoimmune GFAP astrocytopathy who was initially diagnosed as tuberculosis meningitis (Iorio et al., 2018). We suggest that high CSF ADA levels are observed in patients with not only tuberculous meningitis but also with autoimmune GFAP astrocytopathy. The reason for the transient elevation of ADA levels during the early stage of this disease is uncertain. ADA plays an important role in growth and differentiation of lymphocytes and macrophages (Carson and Seegmillerm, 1976; Hovi et al., 1976). We suggest that elevated ADA levels might be associated with immunological pathology during the early stage of autoimmune GFAP astrocytopathy.

On brain MRI, two-thirds of patients have abnormalities on T2-weighted and FLAIR sequences. The most common abnormal hyperintensity lesions were in the basal ganglia, followed by the thalamus. We suggest that the bilateral hyperintensities of the posterior part of the thalamus are characteristic findings of autoimmune GFAP astrocytopathy. It has been reported that brain linear perivascular radial gadolinium enhancement patterns are a hallmark of autoimmune GFAP astrocytopathy (Fang et al., 2016; Flanagan et al., 2017). In this study, four out of nine patients who underwent the enhanced brain MRI showed this finding. All seven patients who underwent SPECT had decreased cerebral blood flow in the frontal lobes. This finding might be characteristic of autoimmune GFAP astrocytopathy; however, larger studies are needed for confirmation.

Corticosteroid-responsiveness is also a hallmark of autoimmune GFAP astrocytopathy (Flanagan et al., 2017; Kunchok et al., 2019). In this study, all patients except one were treated with corticosteroid therapy. Although some patients presented with severe disabilities, such as requirement for mechanical ventilation because of brainstem

Table 6
Therapy and outcome of 14 patients with autoimmune GFAP astrocytopathy.

	Patients, Number (%)	Median [range]
Therapy		
Steroid therapy	13 (93)	
Day from onset to start of steroid therapy		14 [8–71]
mPSL pulse	11 (79)	
Times of mPSL pulse, number/patient		2 [1–4]
Dexamethasone	4 (29)	
Oral prednisolone (PSL)	7 (50)	
Starting dose of oral PSL, mg/day		60 [20–60]
Duration of oral PSL, day		177 [49–789]
Intravenous immunoglobulin (IVIg)	2 (14)	
Others		
Acyclovir	14 (100)	
Antibiotics	11 (79)	
Anti-tubercular agents	5 (36)	
Anti-mycobacterial agents	4 (29)	
Anti-convulsants	4 (29)	
Intravenous anesthetics	3 (21)	
Mechanical ventilation	3 (21)	
Outcome		
Modified Rankin Scale		
On admission		5 [3–5]
On discharge		2 [1–4]
At last follow up		0.5 [0–2]
Hospitalization period, day		48 [21–184]
Long-term follow-up		
Follow up period, month		13 [1–60]
Sequelae at last follow up		
Autonomic dysfunctions	3 (21)	
Urinary dysfunction	3 (21)	
Orthostatic hypotension	1 (7)	
Anhidrosis	1 (7)	
Erectile dysfunction	1 (7)	
Ejaculatory disorder	1 (7)	
Dysesthesia of bilateral foot	1 (7)	
Relapse case	0 (0)	

GFAP: glial fibrillary acidic protein.
mPSL: methylprednisolone.

involvement, all patients had favorable outcomes (mRS ≤ 2) at the last follow-up. Urinary dysfunction, erectile dysfunction, ejaculatory disorder, and dysesthesia in bilateral feet were recognized in some patients as sequela, and might be caused by damage to the medullary cone, whose surface was enhanced on spinal MRI. Approximately 20–50% of patients with autoimmune GFAP astrocytopathy have relapsing courses (Flanagan et al., 2017; Kunchok et al., 2019; Long et al., 2018; Yang et al., 2017). In this study, half the patients required oral prednisolone therapy, but all patients discontinued it and had not relapsed at the last follow-up. We suggest that the sequelae and relapse can be avoided by early administration of immunotherapy before irreversible damage and by careful withdraw of prednisolone while observing a patient's symptoms and CSF findings. The main limitations of this study are that it is a single center retrospective study and that the number of autoimmune GFAP astrocytopathy patients studied was small. Additionally the follow up period for some of the patients was short, meaning that sequelae and relapse may not be fully evaluated. Data from a large number of patients with long-term followed up is necessary to develop standard treatment regimens for autoimmune GFAP astrocytopathy and to clarify outcomes.

In conclusion, autoimmune GFAP astrocytopathy is not a rare corticosteroid-responsive meningoencephalomyelitis in Japan. Its clinical characteristics are similar to those previously reported in patients with autoimmune GFAP astrocytopathy. However, we obtained novel findings of this disease as follows. First, movement disorders, including tremor, myoclonus, and ataxia, and autonomic dysfunction, including urinary dysfunction and hyponatremia, are key symptoms of suspected autoimmune GFAP astrocytopathy. Second, the lymphocytic pleocytosis lasts for several months and transient elevation of ADA levels is observed in many patients. Finally, both brain linear perivascular radial gadolinium-enhancement patterns and bilateral hyperintensities in the posterior part of the thalamus are characteristic findings and hallmarks of autoimmune GFAP astrocytopathy. Because the timing of corticosteroid therapy may affect a patient's prognosis, CSF GFAP-IgG should be examined in patients with the above clinical features.

Author contributions

AK designed the study, analyzed the data and drafted the manuscript. AT, NY, and YH collected the data. TS supervised this study. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that they have no competing interests.

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