Table 4
Summary of characteristics of patients according to age group.

Age	Female (%)	Asymptomatic (%)	Etiology 1st / 2nd / 3rd	%	Localized cavity (%)
<10	51.11	40.9	Chiari type I	40.6	36.9
			Chiari type II	34.8	
			Other	14.5	
10–19	66.07	23.9	Chiari type I	78.8	36.2
			Idiopathic	6.2	
			Other	5.3	
20–29	52.63	14.0	Chiari type I	47.4	47.1
			Idiopathic	22.8	
			Trauma	14.0	
30–39	46.34	20.5	Chiari type I	49.4	39.0
			Idiopathic	17.3	
			Trauma	14.8	
40–49	55.17	15.3	Chiari type I	55.9	27.8
			Idiopathic	18.6	
			Spinal cord tumor	10.2	
50–59	69.74	14.5	Chiari type I	42.1	40.6
			Idiopathic	23.7	
			Spinal cord tumor	10.5	
60–69	54.55	11.5	Chiari type I	28.2	42.9
			Idiopathic	24.4	
			Trauma	16.7	
>70	66.67	24.3	Idiopathic	37.8	40.0
			Chiari type I	27.0	
			Arachnoiditis	13.5	

and that of idiopathic syringomyelia was 15.8% according to the second survey.

The prevalence of syringomyelia in this survey is lower than that in previous studies that used different methods for estimation [5,6]. Estimation of prevalence in this survey was based on patients who were referred to a hospital for evaluation or treatment. Therefore, the data from patients whose syringomyelia was stable and who had discontinued their ambulatory care were not collected in this study. It is noteworthy that the early detection of syringomyelia by MRI can allow for early interventions, including surgery. Early diagnosis and intervention are more likely to lead to a positive outcome, and may therefore reduce the number of patients requiring ambulatory care. The lower number of patients diagnosed in the years preceding 2005 (Fig. 1-D) is consistent with our speculation. However, these results show the characteristics of ambulatory care among syringomyelia patients.

The etiology of syringomyelia can include Chiari malformation, trauma, arachnoiditis, and idiopathic origin, among other causes. In our study, Chiari malformations, including both types I and II, were the most common cause in both children and adults, and this finding is consistent with those of previous studies [7,11]. In particular, Chiari malformation is more frequent in children than in adults. These results may be associated with the widespread availability of MRI, which contributes to early diagnoses in cases of syringomyelia caused by Chiari malformation. Interestingly, idiopathic syringomyelia was the second most common cause according to our survey. Bogdanov et al. suggested that idiopathic syringomyelia is associated with a small posterior fossa with a narrow cerebrospinal fluid (CSF) space as well as with Chiari I malformation [12]. It is possible that some of the cases of idiopathic syringomyelia in our survey may be attributable to a small posterior

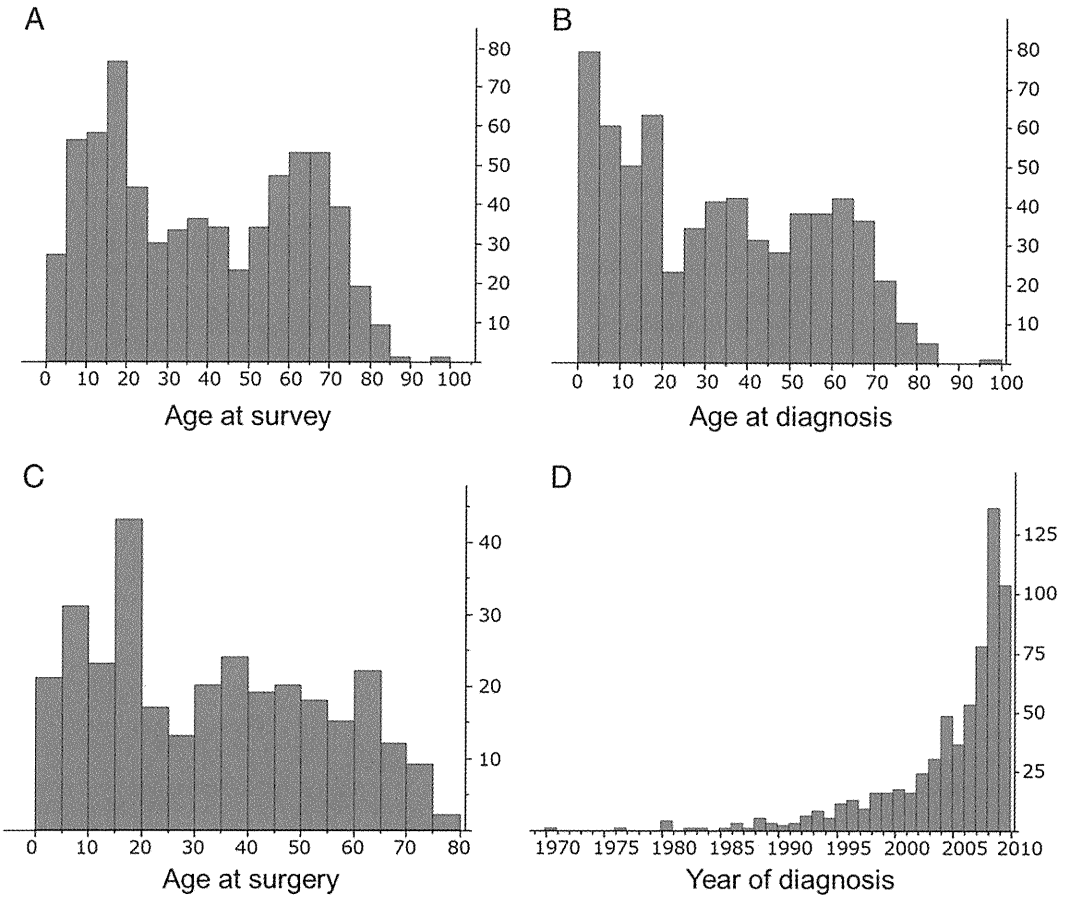

Fig. 1. (A) Histogram showing age distribution of patients at time of survey. (B) Histogram showing age distribution of patients at diagnosis. (C) Histogram showing age distribution at time of surgery. (D) Histogram showing the diagnosis by year.

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fossa. Holly et al. described slit-like syrinx cavities characterized by remnants of the central canal and an asymptomatic clinical course [13]. Therefore, idiopathic syringomyelia has several potential causes, including congenital remnants of the central canal and acquired dilations by a small posterior fossa. Hida et al. reported an association between syringomyelia with Chiari I malformation and birth injuries [14]. In this study, patients with problem at delivery accounted for 2.0% of symptomatic syringomyelia cases, but it had a higher unknown/missing proportion in the past history. Nakamura et al. discuss 2 types of idiopathic syringomyelia: localized and extended. Localized syringomyelia is associated with congenital enlargement of the central canal of the spinal cord and can be managed conservatively [15]. Actually, most of the patients with idiopathic cases in our study did not undergo surgical treatment. Idiopathic syringomyelia might be less progressive than syringomyelia with other causes.

Asymptomatic syringomyelia comprised 22.7% of all syringomyelia cases in our second survey. Prior to this survey, the proportion of asymptomatic syringomyelia cases was unknown. Cases of a few patients with asymptomatic syringomyelia caused by a brain tumor of the posterior fossa have been previously reported [16–18]. The infrequency of asymptomatic syringomyelia seems inconsistent with our survey results. There are 2 possible explanations for the relatively high proportion of asymptomatic syringomyelia in our survey. Firstly, the symptoms of patients who did not complain because of their age were underestimated. Secondly, the availability of MRI in Japan has resulted in an increase in the number of incidental diagnoses of asymptomatic syringomyelia including slit-like syrinx cavities.

Resolution of syringomyelia without surgical treatment was observed in 17 patients (3.2% of symptomatic patients) in our second survey. Spontaneous resolution of syringomyelia has recently been found to be more common than previously thought [19]. The mechanisms involved in the development and spontaneous resolution of syringomyelia are unclear despite multiple hypotheses [20]. The number of patients with spontaneous resolution may be underestimated because cases of asymptomatic syringomyelia patients who had not sought consultation were not evaluated in our survey.

Symptoms of syringomyelia include pain, sensory disturbance, and amyotrophy. Bogdanov et al. reported that 90% of patients had unilateral or bilateral sensory disturbances, while 79% of patients experienced weakness or wasting of the upper limbs [21].

Familial syringomyelia cases with autosomal dominant or recessive inheritance have been reported [22,23]. Chatel et al. suggested that the incidence of familial syringomyelia is approximately 2% [24]. However, a large-scale survey has not yet been conducted to determine the proportion of familial cases. In our study, familial syringomyelia comprised only 2 cases (0.6%) of patients with a reported family history. Although a potentially large number of patients who have been lost to follow-up affect the accuracy of the proportion of syringomyelia, familial syringomyelia cases are extremely rare.

This study has several limitations. Firstly, the prevalence of syringomyelia reported in this study was calculated using the estimated number of ambulatory patients. Cases of patients who did not receive ambulatory care in the past year were not evaluated. Therefore, the potential number of syringomyelia patients may be larger than that reported in this study. Secondly, this cross-sectional survey could not evaluate the entire clinical course of syringomyelia. The disease progression from asymptomatic to symptomatic is particularly unclear. The clinical course of idiopathic cases is also unclear. Further investigation is required to determine the most appropriate evaluations and treatments for these patients. Thirdly, the response rates in this study were 73% and 59% in the first and second stage surveys, respectively. Characteristics of patients whose cases were not reported in the second survey are unknown. The effect of this selection bias on our results is also unknown.

Finally, the definition of syringomyelia associated with spinal cord tumor has been changing, and peritumoral cysts have been differentiated from other distinct forms of syringomyelia. In this study, syringomyelia associated with spinal cord tumor was regarded as merely 1 type of syringomyelia.

Taken together, the findings of our survey can contribute to the development of healthcare services for syringomyelia patients. Knowledge of the characteristics of asymptomatic and symptomatic syringomyelia patients without surgical treatment can be useful for the optimization of those services. Further evaluations of the potential number of non-ambulatory syringomyelia patients should be performed to estimate the precise prevalence of syringomyelia.

In conclusion, we have investigated the epidemiology of syringomyelia in Japan. Asymptomatic and idiopathic syringomyelia cases are more common than was previously believed. The widespread availability of MRI scanners has potentially contributed to the early diagnosis of these cases.

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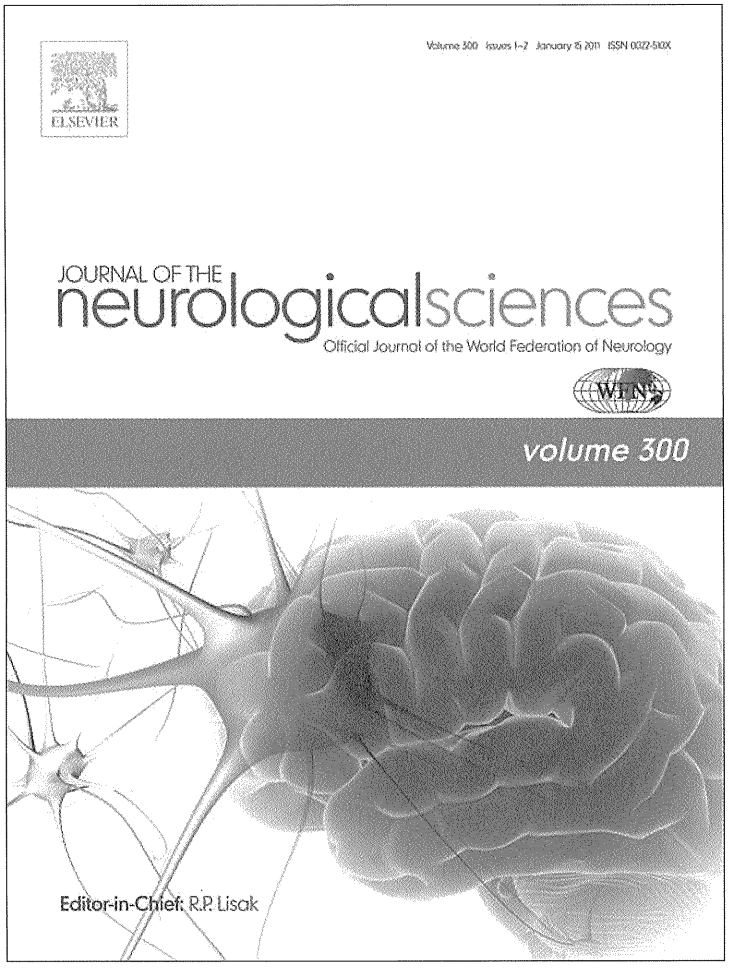

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A serological analysis of viral and bacterial infections associated with neuromyelitis optica

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ABSTRACT

To evaluate the role of infections in the development of neuromyelitis optica (NMO), 19 patients positive for anti-aquaporin-4 antibody were screened for 24 viral and bacterial infections. Serological evidence of recent viral infection was found in 7 of 15 patients screened during the acute phase of the neurologic illness, which was a significantly more frequent rate of infection than seen in the control group of 33 patients with neurodegenerative, metabolic, or vertebral diseases (47% *versus* 15%). Mumps virus and human herpes viruses were the frequent causal agents, although there was no statistical difference in frequency between the two groups. Most patients with identified recent infection had monophasic or recurrent myelitis without evidence of optic nerve involvement and small number of total clinical relapses. Disease history tended to be shorter in patients with identified recent infection than those without, and an expanded long spinal cord lesion in magnetic resonance imaging was rarely found in patients with identified recent infection, although statistical significance could not be shown. These findings indicate that, not single, but various viral infections, can be associated with the development of NMO during the early stages of the illness, although the exact pathogenesis of NMO has yet to be clarified.

1. Introduction

Neuromyelitis optica (NMO) is a disease entity that has recently been distinguished from multiple sclerosis (MS) based on clinical and immunological characteristics, especially the presence of serum anti-aquaporin-4 (AQP4) IgG antibody [1]. Although anti-AQP4 antibody appears to play the pathogenic role in the development of NMO, the exact pathogenesis of NMO remains unknown. Case analyses have demonstrated that in the Mayo Clinic, 25% of patients with monophasic or relapsing NMO had antecedent viral illness and that 15% of patients in Italy with relapsing NMO had a history of fever or infectious disease within four weeks before their clinical attack [2,3]. These findings strongly suggest that acute infections are related to the onset and the relapse of NMO, at least in a part of the patients.

Single case reports have suggested that human cytomegalovirus (CMV), varicella-zoster virus (VZV), Epstein–Barr virus (EBV), hepatitis A virus, human immunodeficiency virus (HIV), dengue virus, *Mycoplasma pneumoniae*, and *Mycobacterium tuberculosis* infections are related to the development of NMO and anti-AQP4 antibody-related myelitis [4–11]. However, there has been no comprehensive analysis of infections in NMO. We previously reported a case of NMO, in which chronic infection with human T lymphotropic virus type 1 (HTLV-1) was also evident in serum and cerebrospinal fluid analyses [12]. T cells infected by retroviruses, including HTLV-1, can induce polyclonal B cell activation [13], indicating the possibility that chronic HTLV-1 infection is a risk factor for the development of NMO. In this study, we serologically screened for various viral and bacterial infections in patients with NMO and related conditions to identify antecedent infectious agents, as well as chronic persistent infections, associated with risk for NMO development.

2. Patients and methods

2.1. Patients

Nineteen patients with various neurological deficits (median age, 53 [range, 16–71]; male/female, 2/17), all of whom were seen at the Yamaguchi University Hospital and judged positive for anti-AQP4 IgG antibody in a cell based assay [14], were included as patients with "NMO-related conditions." Their clinical diagnoses were as follows: NMO fulfilling proposed diagnostic criteria [15] ($N=10$), monophasic or recurrent myelitis ($N=7$), monophasic optic neuritis ($N=1$), and medial longitudinal fasciculus syndrome followed by acute myelitis

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($N=1$). Five (26%) of the 19 patients with NMO-related conditions had been treated with interferon β-1b ($N=3$) or oral low dose prednisolone ($N=2$) to prevent the recurrence of neurological deficit at the time of sampling, whereas the other 14 received neither immunosuppression nor immunomodulation therapies. Serum samples were taken within one month after the onset or recurrence of the disease in 15 of the 19 patients, all of the 15 patients did not receive the treatments for the acute attack (high dose steroid or plasmapheresis) before blood sampling. A control group of patients (median age, 59 [range, 17–77]; male/female, 20/13), who were receiving neither immunosuppression nor immunomodulation drugs, were those with neurodegenerative, metabolic, or vertebral diseases ($N=33$). Informed consent was provided by all participants for serological analyses.

2.2. Infectious serology

Serum antibodies or antigen for 24 viral and bacterial infections were screened using the following methods: virus-specific IgM antibodies (for herpes simplex virus [HSV], VZV, CMV, EBV [against viral capsid antigen], parvovirus B19, rubella virus, measles virus, and mumps virus) by commercial enzyme-linked immunosorbent assay (ELISA) kits (Denka Seiken, Tokyo, Japan); IgG/IgM/IgA antibodies specific for *Campylobacter jejuni* and *Haemophilus influenzae* by the ELISA systems established for determination of antecedent infection in Guillain–Barré syndrome and its clinical variants [16]; *M. pneumoniae* (Serodia-Mycon II test kit, Fujirebio, Tokyo, Japan) and HTLV-1 (SRL, Inc, Tokyo, Japan) by particle agglutination assays; adenovirus, influenzae viruses A and B, respiratory syncytial virus, and rotavirus by complement fixation assays (SRL, Inc, Tokyo, Japan); human parainfluenza viruses A/B/C by hemagglutination inhibition assays (SRL, Inc, Tokyo, Japan); *Treponema pallidam* by hemagglutination assay; and hepatitis B virus (surface antigen), hepatitis C virus, and HIV by chemi-luminescent immunoassays.

2.3. Data analysis

Differences in frequency of infections between the groups were analyzed using the Fisher exact test. Differences in medians were examined by the Mann–Whitney *U* test. All statistical analyses were performed using SPSS 12.0 J software (SPSS Inc., Chicago, IL). Differences were considered significant for 2-sided *P* values less than 0.05.

3. Results

Statistical analysis did not identify any agent which was significantly more common in the group of patients with NMO-related conditions compared to the control group (Table 1). However, virus-specific IgM antibodies, which indicate recent infection, were seen in 7 (47%) of 15 patients with NMO-related conditions in whom serum samples were taken within 1 month after the onset or recurrence of the disease, which was significantly more common than in the control group (5 [15%] of 33; $P=0.03$; odds ratio = 4.9; 95% confidence interval = 1.2–19.7). None of the 4 patients in whom sera were taken during the remission phase of the illness had virus-specific IgM antibodies ($P=0.54$; compared to the control group). The most frequent infectious agent in patients with anti-AQP4 antibody was mumps virus ($N=3$; 20%). In 1 of the 3 patients with serological evidence of mumps virus, mumps-specific IgM antibody was confirmed to have disappeared within 3 months after the neurological attack. Viruses identified in the 4 other patients positive for IgM antibodies were human herpes viruses (HSV, VZV, EBV, and CMV), although 2 of these patients were each concomitantly positive for two viruses (HSV and VZV, or HSV and EBV) and the other 2 each for VZV and CMV. No patients were positive for anti-HTLV-1 antibody, except for the case we have previously reported [12]. Antibody titer against influenza B virus was elevated (16 or more) in 2 (13%) of the 15 patients with acute NMO-related conditions, which was more frequent than in the control group (1 of 32 subjects; 3.1%), although the difference did not reach significance ($P=0.24$).

The clinical diagnoses of all 3 patients with mumps-specific IgM antibody (26-year-old female, 53-year-old female, and 59-year-old male) were recurrent myelitis without other neurological deficits, including optic neuritis, and these patients had low Expanded Disability Status Scores (0.0, 2.0, and 3.0, respectively). Similarly, 2 of the 4 patients with IgM antibodies against human herpes viruses (median

Table 1
Infectious serology in the patients with neuromyelitis optica (NMO)-related conditions.

Agents	Methods	NMO-related conditions		Controls[b] ($N=33$)	*P* value[c]
		Total ($N=19$)	Acute phase[a] ($N=15$)		
Acute infections					
Herpes simplex virus	ELISA (IgM)	2 (11%)	2 (13%)	1 (3.0%)	0.23
Varicella-zoster virus	ELISA (IgM)	2 (11%)	2 (13%)	0	0.09
Cytomegalovirus	ELISA (IgM)	1 (5.3%)	1 (6.7%)	1 (3.0%)	0.53
Epstein–Barr virus	ELISA (VCA-IgM)	1 (5.3%)	1 (6.7%)	1 (3.0%)	0.53
Parvovirus B19	ELISA (IgM)	0	0	NE	
Rubella virus	ELISA (IgM)	0	0	NE	
Measles virus	ELISA (IgM)	0	0	NE	
Mumps virus	ELISA (IgM)	3 (16%)	3 (20%)	2 (6.1%)	0.31
Campylobacter jejuni	ELISA (IgG/IgM/IgA)	0	0	NE	
Haemophilus influenzae	ELISA (IgG/IgM/IgA)	0	0	NE	
Mycoplasma pneumoniae	PA	1 (5.3%)	1/14 (7.1%)	2 (6.1%)	1.0
Chronic infections					
HTLV-1	PA	1 (5.3%)[d]	1 (6.7%)[d]	NE	
Hepatitis B	CLIA (HBsAg)	0/12	0/10	NE	
Hepatitis C	CLIA (Ab)	1/12 (8.3%)	1/10 (10%)	NE	
Human immunodeficiency virus	CLIA	0/12	0/10	NE	
Treponema pallidum	HA (TPHA)	1/12 (8.3%)	1/10 (10%)	NE	

HTLV-1 = human T lymphotropic virus type 1; NE = not examined; Ag = antigen; Ab = antibody;
ELISA = enzyme-linked immunosorbent assay; PA = particle agglutination;
CLIA = chemi-luminescent immunoassay; HA = hemagglutination
[a] Serum samples available within 1 month after the onset or the recurrence of the neurological disease.
[b] Patients with neurodegenerative, metabolic, or vertebral diseases.
[c] Two-tailed *P* value (acute NMO-related conditions *vs* controls).
[d] Previously reported case[12].

age, 52 years; all females) were diagnosed with myelitis (monophasic or recurrent) without optic neuritis, and only 1 received a clinical diagnosis of NMO. In contrast, 6 (75%) of 8 patients without virus-specific IgM antibodies received a clinical diagnosis of NMO, significantly more frequent than the 7 patients with virus-specific IgM antibodies ($P=0.04$; odds ratio = 2.9; 95% confidence interval = 1.3–255.7).

The number of total clinical attacks at time of blood sampling was significantly smaller in the 7 patients with NMO-related conditions who were positive for virus-specific IgM antibodies (median, 2; range, 1–4) than in those who were negative (median, 4; range, 1–8; $P=0.02$). Similarly, the median time from the onset of disease to serum sampling was 11 months (range, 16 days to 3 years) in the patients with the IgM antibodies, which was shorter than the median of 4 years (range, 14 days to 16 years) in those without the antibodies, although the difference did not reach significance ($P=0.07$). An expanded long spinal cord lesion (3 or more vertebral segments in length) seen on spinal magnetic resonance imaging (MRI) was somewhat less common in patients with virus-specific IgM antibodies (50%, 3 of 6 patients in whom MRI was performed) than in those without (75%, 6 of 8 patients) ($P=0.33$). Other neurological features, cerebrospinal fluid data (cell counts and protein level), and brain imaging did not differ between the groups of patients.

4. Discussion

This study is the first to comprehensively screen for infections in patients with NMO and its related conditions. Results revealed serological evidence of acute viral infections in about half of the patients with anti-AQP4 antibody detected during an acute phase of a neurological attack. The patients with acute infections were characterized by short disease history and acute myelitis without evidence of optic nerve involvement, which is an incomplete neurological presentation that does not satisfy the criteria for the diagnosis of NMO. By contrast, there were several patients in whom recent infection was not identified, and they commonly met the neurological and radiological criteria of NMO, and had long disease histories. These findings indicate that acute viral infections could be associated with the development of NMO during an early stage of the illness, whereas this seems not to be true in the late stages of the illness.

Various infectious agents, including EBV, have been suggested as triggers of the onset of MS, but there has not been enough evidence to draw conclusions [17]. The difficulty in collecting sufficient evidence may be due to infrequent antecedent infectious symptoms, complex pathogenesis including both genetic and environmental etiologies, and, in particular, the fact that MS is a heterogeneous disorder. In contrast, the high prevalence of serum anti-AQP4 IgG antibody indicates that NMO and its related conditions are relatively homogenous disorders, urging us to examine the possible contribution of specific infectious agents to the development of the disease. In the present study, immunosuppression/modulation therapies were given only in 5 (26%) of the 19 patients with NMO-related conditions before the serum sampling. Therefore, the close association of NMO-related conditions with acute virus infections does not appear to be due to the preceding therapies.

We identified the mumps virus, an enveloped RNA virus in the paramyxovirus family, as the most frequent agent of infections related to acute attacks of NMO-related illness. This study did not intend to investigate whether there was a history of antecedent infectious symptoms, and retrospective analysis of the patients' records found no patients who reported any antecedent symptoms, including parotid gland swelling. However, one third of patients who are infected by mumps virus are asymptomatic, and often in symptomatic patients, the symptoms are not specific, resembling upper respiratory tract infections [18]. In general, the elevation of virus-specific IgM antibody titers indicates acute primary viral infection or acute reactivation, and in this serological study we measured the IgM antibodies for screening mumps and some other virus infections. It is also known that several patients have persistent IgM antibodies specific for viruses including mumps virus and human herpes viruses, for a long time after infection, even after 3 years [19,20]. In this study, however, IgM anti-mumps antibody in 1 patient with NMO-related condition was confirmed to have disappeared 3 months after the neurological attack. Moreover, the commercially available ELISA system we used in this study had very low seropositive rate (0.3%) in 336 healthy persons, and was confirmed to show that anti-mumps IgM antibody had disappeared within 5 months after the acute infection in 14 patients with mumps-related parotitis [21]. We therefore believe that close association of NMO-related conditions with mumps virus was not due to limited methodology in this serological study.

Mumps virus infection can be accompanied by various neurological disorders, including encephalitis, sensorineural deafness, facial palsy and Guillain–Barré syndrome [18]. Interestingly, a case report of a 10-year-old boy developing mumps during acute myelitis also described a very long spinal cord lesion (from C3 to T12) on MRI, which is characteristic of NMO, although anti-AQP4 antibody was not mentioned [22]. Moreover, the optic chiasma was primarily perturbed in a case of acute optic neuritis following mumps virus infection [23]. Although this paper did not mention the presence of anti-AQP4 antibody, this clinical picture suggests that this patient also had an anti-AQP4 antibody-related disorder [24]. A population-based case-control study did not reveal the association of histories of mumps infection and measles–mumps–rubella vaccination with risk for MS [25], but these histories, as well as antecedent mumps infection, should be clarified in patients with anti-AQP4 antibody-related disorders.

A healthy patient positive for anti-AQP4 antibody for more than 10 years was reported to have suffered a clinical attack of acute myelitis following skin eruption suggestive of viral infection [26]. This case report raises the possibility that, in patients with NMO, viral infections play a role in increased blood–brain barrier permeability, which allows the autoantibody to cross the blood–brain barrier, although we have no data regarding leakage from blood to cerebrospinal fluid in the patients included in this study. In contrast, it remains unclear whether viral infection can trigger NMO-associated autoimmunity. In general, viruses can trigger autoimmunity through molecular mimicry and its adjuvant effects during the initiation of disease, and can promote autoimmune responses through bystander activation with or without antigen spreading [17]. Several cases have been reported in which mumps infection appeared to precede the onset of diabetes mellitus type 1, and it has been hypothesized that infection with mumps virus may induce autoimmunity via increased release of IL-1 and IL-6 and upregulated expression of HLA molecule in mumps virus-infected pancreatic beta cells [27]. Our study showed that the patients identified with acute viral infection had a short disease history of the illness, and therefore we might hypothesize that acute viral infection activates the immune system, causing initiation of autoimmunity, and thereby the development of NMO and its related disorders.

Conflict of interest statement

The authors report no conflicts of interest.

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ORIGINAL COMMUNICATION

GQ1b-seronegative Fisher syndrome: clinical features and new serological markers

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Abstract IgG anti-GQ1b antibodies are a powerful serological marker for the diagnosis of Fisher syndrome (FS), but little is known regarding serological markers in FS patients that do not have the autoantibodies. The authors analyzed IgG antibodies against gangliosides other than GQ1b, ganglioside complexes, and ganglioside-like lipo-oligosaccharide (LOS) of *Campylobacter jejuni* isolates from FS patients. We identified 24 (12%) patients with GQ1b-seronegative FS among 207 FS patients who had been referred to our laboratory for anti-ganglioside antibody testing. Patients with GQ1b-seronegative FS were male and had a history of antecedent gastrointestinal illness more frequently than FS patients with IgG anti-GQ1b antibodies. Other clinical features during the illness were not distinguishing for GQ1b-seronegative FS. Four (17%) of 24 patients with GQ1b-seronegative FS had IgG antibodies against single gangliosides such as GM1b, GD1a, or GT1a. Antibodies against GM1 and GT1a complex were detected in four GQ1b-seronegative FS patients, three of whom did not have antibodies against single gangliosides. Mass spectrometry analysis showed that *C. jejuni* isolates from FS patients had GD1c-, GalNAc-GM1b-, or GalNAc-GD1c-like LOS, and not GQ1b-like LOS, highlighting the utility of examining serum antibodies against these ganglioside mimics in GQ1b-seronegative FS patients. Seven (29%) had IgG antibodies against the LOS from *C. jejuni* strains expressing GD1c-, GalNAc-GM1b-, or GalNAc-GD1c-like LOS. These findings suggest that IgG antibodies against GM1b, GD1c, GalNAc-GM1b, and ganglioside complexes are serological markers for GQ1b-seronegative Fisher syndrome.

Keywords Fisher syndrome · Anti-GQ1b antibody · *Campylobacter jejuni* · Ganglioside complex · Lipo-oligosaccharide

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Introduction

Fisher syndrome (FS) is the most common clinical variant of Guillain–Barré syndrome (GBS) characterized by acute onset of ophthalmoplegia, ataxia, and areflexia. A landmark study identified IgG autoantibodies against GQ1b ganglioside as a serological marker in FS [1], and subsequent studies estimated the highly frequent detection of the antibodies between 83 and 100% of FS patients [2–4]. From a serological point of view, FS is much more uniform

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than the axonal subtype of GBS associated with IgG autoantibodies to GM1, GM1b, GD1a, or GalNAc-GD1a [5, 6]. It has been found that a mixture of two gangliosides (ganglioside complex) can generate new epitopes that differ from those of the constituents and may be targeted by serum autoantibodies from FS patients [7]. However, little effort has been made to identify novel autoantibodies in the minority of FS patients that are negative for anti-GQ1b antibodies.

Our prospective case–control study has shown that *Campylobacter jejuni* is the most frequently identified antecedent agent in FS [8]. A GQ1b-mimicking structure on the bacteria is hypothesized to be the key trigger for the generation of anti-GQ1b antibodies in *C. jejuni*-related FS. Unexpectedly, however, several *C. jejuni* isolates from FS patients did not express GQ1b-like lipo-oligosaccharide (LOS), and instead expressed GT1a-, GD3-, or GD1c-like LOS (Fig. 1) [8–11]. These findings led us to hypothesize that ganglioside-like LOS other than GQ1b-like LOS could trigger the production of unidentified pathogenic autoantibodies in GQ1b-seronegative patients and induce the development of FS.

In the present study, we retrospectively selected FS patients negative for IgG anti-GQ1b antibodies and investigated their clinical features. We analyzed IgG antibodies against other single gangliosides, ganglioside complexes, and ganglioside-like LOS of several *C. jejuni* isolates from FS patients in order to identify serological markers for anti-GQ1b antibody-negative FS patients.

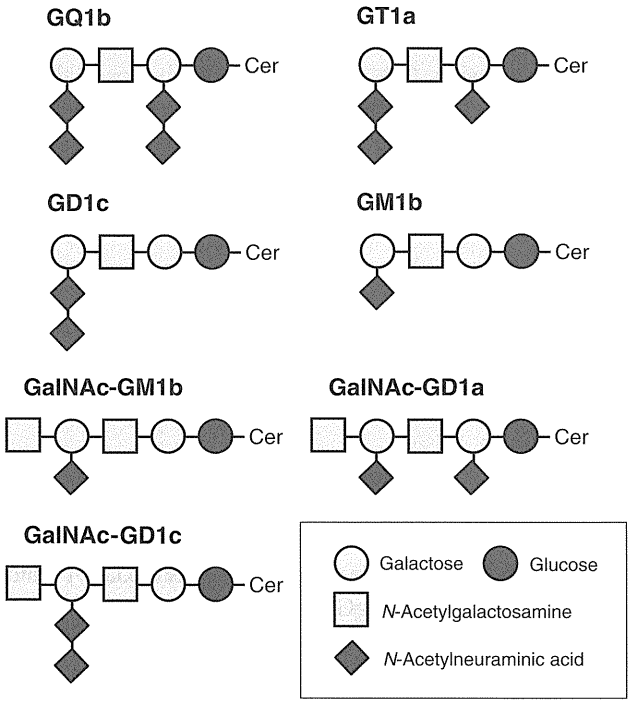

Fig. 1 Carbohydrate sequence of the gangliosides, *Cer* ceramide

Methods

Patients

We received requests to test serum anti-ganglioside antibodies from 207 patients presenting with FS from February 2000 to July 2002. All patients fulfilled the clinical criteria, which included (1) progressive, relatively symmetric ophthalmoplegia and ataxia for 4 weeks, (2) hyporeflexia or areflexia, (3) preserved limb strength (five or four on the Medical Research Council scale), and (4) features that rule out the other diagnoses such as vascular disease involving the brainstem, Wernicke encephalopathy, botulism, myasthenia gravis, brainstem tumor, pituitary apoplexy, acute disseminated encephalomyelitis, multiple sclerosis, neuro-Behçet disease, vasculitis, lymphoma, and Creutzfeldt–Jakob disease [4]. One of the authors (M.K.) reviewed the patients' medical records to ascertain diagnoses and neurological findings. Pretreatment serum samples were obtained during the acute phase of the illness. Sera from 40 healthy individuals were used as healthy controls (HC) and sera from 34 patients with neurodegenerative, metabolic, or vertebral diseases as disease controls (DC). Informed consent was provided by all participants for serological analyses. The study protocol was approved by the local Ethic Committee at Dokkyo Medical University.

Anti-ganglioside antibody testing and infectious serology

Serum IgG antibodies against isolated gangliosides (GM2, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, GT1b, or GQ1b; 10 pmol/well) were measured by ELISA as previously described [12]. Sera were considered positive if the optical density (OD) was 0.5 or more at a serum dilution of 1:500. IgG antibodies to GM1 and GT1a complex (GM1/GT1a) were similarly tested using a mixture of GM1 and GT1a (each 5 pmol/well) as the target antigen. Anti-GM1/GT1a antibodies were judged positive if the OD of anti-GM1/GT1a antibodies was 0.5 greater than the sum of ODs of GM1 and GT1a assayed individually. Antibodies against other combinations of gangliosides (GM1/GD1a, GM1/GQ1b, GD1a/GT1a, GD1a/GQ1b, or GT1a/GQ1b) were similarly analyzed. By these criteria, none of the sera from 40 HC and 34 DC were positive for anti-ganglioside complex antibodies. Antecedent *C. jejuni* and *Haemophilus influenzae* infections were serologically examined as described [8].

Mass spectrometry analysis of *C. jejuni* isolates

Four *C. jejuni* strains (GC033, GC068, GC149, and GC219) isolated from patients with FS with or without

overlapping GBS were used for LOS antibody analysis. Three patients were positive for IgG anti-GQ1b antibodies, and one (with strain GC149) was negative. These strains were used for anti-LOS antibody testing because they have defined ganglioside mimics in their LOS outer cores. All of these strains were included in our previous study [13], and the LOS outer core structures have been reported for two of them: GC149 [14–16] and GC033 [10, 17, 18]. The LOS outer core structures of GC068 and GC219 were determined in this work. Overnight growth of the strains on an agar plate was done as described [19], except that we used 60 μg/ml proteinase K, 200 μg/mL RNase A, and 100 μg/mL DNase I. The *O*-deacylated LOS sample was analyzed by capillary electrophoresis-electrospray ionization mass spectrometry (CE–ESI–MS), as described [20]. Classification of the LOS biosynthesis gene locus and *cst-II* genotype (Thr/Asn 51) were performed as described [13, 15].

Anti-*C. jejuni* LOS antibody testing

Serum IgG antibodies against *C. jejuni* LOS were examined by ELISA using crude LOS fractions [21]. Briefly, *C. jejuni* was grown at 37°C for 48 h on blood agar plates in a 5% oxygen, 10% carbon dioxide atmosphere. The bacterium then was suspended in sterile PBS and adjusted to an OD of 0.4 at 650 nm. A 1.5 ml aliquot of the suspension was centrifuged at 14,000 *g* for 1.5 min, and the pellets were resuspended in 300 μl of distilled water. This suspension was boiled for 10 min, cooled, 100 μg of proteinase K (Roche Diagnostics Corporation, Indianapolis, IN, USA) was added, and the suspension was incubated at 60°C for 60 min. Thereafter, 0.5 μl of this lysate was mixed with 50 μl methanol and then dried in a microtiter plate. Patient sera were diluted 1:500 with PBS containing 0.5% casein then added to the wells, after which the plate was incubated overnight at 4°C. After washing (0.05% Tween 20 in PBS), peroxidase-conjugated anti-human IgG (Dako, Glostrup, Denmark; 1:1,000) was added. Plates were kept at 20°C for 2 h prior to developing. Serum was considered positive when antibody OD was 1.0 or more.

Statistical analyses

Differences in frequency between the groups were analyzed using the Fisher exact test. Differences in medians were examined by the Mann–Whitney *U* test. Differences were considered significant for two-sided *P* values < 0.05. Statistical calculations were made with SPSS 19 software (IBM Japan Ltd, Tokyo, Japan).

Results

FS patients negative for anti-GQ1b IgG antibodies

Among the 207 FS patients, 24 (12%) were negative for IgG anti-GQ1b antibodies [GQ1b-seronegative FS; median age, 56 years (range, 6–74); male/female, 22/2] (Table 1). None of the 24 patients had IgM anti-GQ1b antibodies. Twenty-three (96%) patients reported antecedent infectious symptoms indicative of respiratory tract infection [$N = 15$ (63%)] or gastroenteritis [$N = 7$ (29%)]. Serological evidence of recent *C. jejuni* infection was found in three (13%) GQ1b-seronegative FS patients, and none had evidence of *H. influenzae* infection. The most frequent initial symptom was diplopia ($N = 13$ [54%]), followed by gait disturbance [$N = 8$ (33%)]. As in typical FS with anti-GQ1b antibodies, external ophthalmoparesis was abduction-predominate [9/20 (45%)], and frequently accompanied neurological deficits seen during the acute phase of illness were objective sensory disturbance [13/23 (54%)], mydriasis [6/14 (43%)], bulbar palsy [7/23 (29%)], and facial palsy [6/24 (25%)]. CSF albuminocytological dissociation was seen in 70% (14/20) of the GQ1b-seronegative FS patients.

Due to the difficulty of retroactively obtaining data from the patients included in this study, clinical features of the patients with GQ1b-seronegative FS were compared to those of GQ1b-positive FS patients included in our previous study [22]. Statistical analysis showed that patients with GQ1b-seronegative FS more frequently were male [$P = 0.002$, odds ratio (OR) 7.3, 95% confidence interval (CI) 2.0–27] and had a history of antecedent gastrointestinal symptoms ($P = 0.02$, OR 3.7, 95% CI 1.3–10.3) (Table 1). A history of antecedent upper respiratory infectious symptoms ($P = 0.02$, OR 0.26, 95% CI 0.10–0.71) and the onset of diplopia ($P = 0.046$, OR 0.38, 95% CI 0.15–0.96) were rarer in GQ1b-seronegative FS, but the frequencies of neurological features during the illness did not differ between the groups.

Ganglioside mimics of FS-related *C. jejuni* LOS

As mentioned above, GT1a-, GD3-, or GD1c-like LOS have been indentified in *C. jejuni* isolates from FS patients (Fig. 1) [8–11], whereas GQ1b-like LOS has not been identified. We used four FS-related *C. jejuni* strains with defined LOS outer core structures for the serological analyses described below. *C. jejuni* GC033 was reported to display a GD1c mimic [10]. *C. jejuni* GC149 was shown to express a mixture of ganglioside mimics through phase variation [16], and GC149 can display mimics of GD3, GT3, GQ3, GT1a, and Gal-GM1a in its LOS outer core

Table 1 Comparison of clinical features between Fisher syndrome patients with and without IgG anti-GQ1b antibodies

	IgG anti-GQ1b antibodies		
	Negative $N = 24$	Positive $N = 110$[a]	Two-sided P value
Age: median (range)	56 (6–74)	41 (2–78)	NS
Sex: male/female	22/2	66/44	0.002
Prior symptoms			
Any	23/24 (96%)	–	–
URTI	15/24 (63%)	95/110 (86%)	0.02
GI	7/24 (29%)	11/110 (10%)	0.02
Initial symptoms			
Diplopia	13/24 (54%)	83/110 (75%)	0.046
Gait disturbance	8/24 (33%)	36/110 (32%)	NS
Dysarthria	2/24 (8.3%)	4/110 (3.6%)	NS
Blepharoptosis	1/24 (4.2%)	3/110 (2.7%)	NS
Neurological features during the illness			
Abduction-predominance of EOP	9/20 (45%)	ND	–
IOP	6/14 (43%)	41/110 (37%)	NS
Nystagmus	4/10 (40%)	16/110 (16%)	NS
Facial palsy	6/24 (25%)	25/110 (23%)	NS
Bulbar palsy	7/24 (29%)	20/100 (20%)	NS
Sensory disturbance	13/23 (54%)	55/99 (56%)	NS
Autonomic disturbance	1/23 (4.3%)	ND	–
Campylobacter jejuni serology	3/24 (13%)	ND	–
Haemophilus influenzae serology	0/24	ND	–
Albuminocytologic dissociation in CSF	14/20 (70%)	62/94 (66%)	NS

NS not significant, *URTI* upper respiratory tract infectious symptoms, *GI* gastrointestinal infectious symptoms, *EOP* external ophthalmoparesis, *ND* not described, *IOP* internal ophthalmoparesis, *CSF* cerebrospinal fluid

[a] Reported previously [22]

(Table 2). Mass spectrometric analysis of *O*-deacylated samples was used to propose LOS outer core structures for strains GC068 and GC219 (Table 2). The mass species observed for strain GC068 are consistent with mixed GD1c-, GalNAc-GM1b-, and GalNAc-GD1c-like structures in the LOS outer core (Supplemental Table 1). The mass species observed for strain GC219 are consistent with an LOS outer core displaying a GalNAc-GM1b-like structure (Supplemental Table 1). LOS from these four FS-related *C. jejuni* strains (GC033, GC068, GC149, and GC219) were used as antigens for the following serological analyses of GQ1b-seronegative FS patients and control groups.

Serological analyses

IgG antibodies against single gangliosides other than GQ1b were detected in 4 (17%) of the 24 patients with GQ1b-seronegative FS (Table 3). Anti-GT1a and anti-GM1b antibodies were detected in two patients, each one of whom showed isolated elevation of the antibodies (Nos. 3 and 4 in Table 4). Antibodies against ganglioside complexes were positive in four (17%) of the GQ1b-seronegative FS patients, three of whom were negative for antibodies against all single gangliosides examined. Among anti-ganglioside complex antibodies, anti-GM1/GT1a antibodies were detected in all four patients positive for anti-ganglioside complex antibodies.

IgG antibodies against *C. jejuni* LOS from the four strains with defined structures were positive in seven (29%) of the 24 GQ1b-seronegative FS patients, and slightly above that of HC (5/40 [13%]; $P = 0.11$) (Table 3). Three of the seven patients with anti-LOS antibodies were negative for antibodies against all single gangliosides and ganglioside complexes. Patient IgG reacted with a variety of GalNAc-GM1b-like structures (six [25%] of 24 patients with GQ1b-seronegative FS versus 5/40 [13%] in HCs; $P = 0.30$), mixed GD1c- and GalNAc-GM1b-like structures (four [17%] versus two [5%]; $P = 0.19$), mixed GD3-, GT1a-, GT3-, GQ3-, Gal-GM1a-like structures (four [17%] versus zero [0%]; $P = 0.02$; OR, 17.8; 95% CI, 2.1–147), and GD1c-like structures (three [13%] versus one [2.5%]; $P = 0.14$). Four (12%) DC sera were scored

Table 2 Lipo-polysaccharide structures of *Campylobacter jejuni* isolates from patients with Fisher syndrome with or without overlapping Guillain–Barré syndrome

C. jejuni	Serogroup (serotype)	LOS biosynthesis class[a]	*cst-II* genotype	Ganglionside-mimic of LOS	Patient's diagnosis	IgG anti-ganglioside Ab titers[b] in patients	
						GQ1b	Others
GC033	D[c]	A	Asn51	GD1c	FS	32,000	GT1a (32,000)
GC219	HS:2	B	Asn51	GalNAc-GM1b	FS	8,000	GT1a (16,000)
GC068	HS:2	Unclassified	–	GD1c, GalNAc-GM1b, and GalNAc-GD1c	FS/GBS	32,000	GD1a/GT1a/GT1b (8,000)
Gc149	HS:1	R	Asn51	GD3, GT3, GT1a, GQ3, and Gal-GM1a	FS	(−)	(−)

LOS lipo-oligosaccharide, *Ab* antibody, *FS* Fisher syndrome; *GBS* Guillain–Barré syndrome; *NT* not tested

[a] Classified based on the organization of gene content in LOS biosynthesis locus

[b] Tested antigens were GM2, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, and GT1b gangliosides (cut-off < titer less than 500)

[c] HS:4, HS:13, HS:16, HS:43, HS:50

Table 3 Summary of serological findings

IgG antibodies against	Fisher syndrome IgG anti-GQ1b antibodies		DC $N = 34$	HC $N = 40$	Two-sided P value	
	Negative $N = 24$	Positive $N = 30$[d]			GQ1b-seronegative vs DC	GQ1b-seronegative vs HC
Isolated ganglioside (non-GQ1b)[a]	4 (17%)	29 (97%)	0	0	0.03[e]	0.02[h]
Ganglioside complex[b]	4 (17%)	15 (50%)	0	0	0.03[f]	0.02[i]
Campylobacter lipo-oligosaccharide[c]	7 (29%)	29 (97%)	4 (12%)	5 (13%)	NS	NS
Any	10 (42%)	30 (100%)	4 (12%)	5 (13%)	0.01[g]	0.01[j]

DC disease control, *HC* healthy control, *NS* not significant

[a] Tested antigens were GM2, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, and GT1b gangliosides

[b] Tested antigens were GM1/GD1a, GM1/GT1a, GM1/GQ1b, GD1a/GT1a, GD1a/GQ1b, and GT1a/GQ1b complexes

[c] Tested antigens were GD1c-, GalNAc-GM1b-, mixed GD1c/GalNAc-GM1b-, and mixed GD3/GT1a/GT3/GQ3-mimicking lipo-oligosaccharides of *Campylobacter jejuni* isolates (GC033, GC219, GC068, and GC149, respectively) from patients with Fisher syndrome with or without overlapping Guillain–Barré syndrome

[d] Selected at random from 183 patients with GQ1b-seropositive Fisher syndrome

[e] Odds ratio (OR) 15.1, 95% confidence interval (CI) 1.7–130.3

[f] OR 15.1, 95% CI 1.7–130.3

[g] OR 5.4, 95% CI 1.4–20.0

[h] OR 17.8, 95% CI 2.1–147.7

[i] OR 17.8, 95% CI 2.1–147.7

[j] OR 5.0, 95% CI 1.4–17.3

positive for antibodies against GC068 LOS [mixed-GD1c/GalNAc-GM1b/GalNAc-GD1c-mimics], and none of the DC sera for the other LOS.

Clinical features of FS patients negative for IgG antibodies to GQ1b, but positive for antibodies against other gangliosides and *C. jejuni* LOS

All seven GQ1b-seronegative but anti-ganglioside (single ganglioside or ganglioside complex) IgG-positive patients were male, although other clinical features, including antecedent infectious symptoms and neurological deficits, were unremarkable (Tables 4 and 5). Similar unremarkable clinical findings were common in the GQ1b-seronegative, *C. jejuni* ganglioside-like LOS-seropositive FS patients. These findings suggest that clinical features are not helpful in identifying GQ1b-seronegative FS patients with other anti-ganglioside antibodies. It is noteworthy that histories of antecedent gastrointestinal symptoms were available for only two of seven ganglioside-like

Table 4 Patients with GQ1b-seronegative Fisher syndrome who showed seropositive results for other antibodies

No	Age/ sex	Accident symptom	Infectious serology	IgG antibodies against		
				Isolated ganglioside	Ganglioside complex	Ganglioside-like LOS[c]
1	19/M	GI	(−)	GT1a, GM1b	(−)	GC033, GC068, and GC149
2	28/M	(−)	*C. jejuni*	GD1a, GalNAc-GD1a	(−)	GC033, GC219, GC068 and GC149
3	60/M	Fever	(−)	GT1a	GM1/GT1a, GM1/GQ1b	GC219, and GC149
4	63/M	Chill	(−)	GM1b	(−)	(−)
5	15/M	GI, URTI, fever	*C. jejuni*	(−)	GM1/GT1a, GM1/GQ1b	(−)
6	24/M	GI, URTI, fever	(−)	(−)	GM1/GT1a, GM1/GQ1b	GC219
7	54/M	URTI, fever	(−)	(−)	GM1/GT1a	(−)
8	22/M	URTI, fever	*C. jejuni*	(−)	(−)	GC033, GC219, and GC149
9	48/F	Fever, joint pain	(−)	(−)	(−)	GC219, GC068
10	28/M	URTI	(−)	(−)	(−)	GC068

GI gastrointestinal infection, *URTI* upper respiratory tract infection, *LOS* lipo-oligosaccharide

[a] Tested antigens were GM2, GM1, GM1b, GD1a, GalNAc-GD1a, GD1b, GT1a, and GT1b gangliosides

[b] Tested antigens were GM1/GD1a, GM1/GT1a, GM1/GQ1b, GD1a/GT1a, GD1a/GQ1b, and GT1a/GQ1b complexes

[c] Tested antigens were LOSs from *Campylobacter jejuni* isolates GC033 (GD1c-like), GC219(GalNAc-GM1b-like), GC068 (mixed GD1c/GalNAc-GM1b/GalNAc-GD1c-like), and GC149 (mixed GD3/GT1a/GT3/GQ3/Gal-GM1a-like) from patients with Fisher syndrome with or without overlapping Guillain–Barré syndrome

Table 5 Neurological features of the patients described in Table 4

No	Initial symptom	Ophthalmoparesis		Ptosis	FP	BP	Sensory disturbance	Ataxia	Others
		External	Internal						
1	Nasal voice	Only obduction disturbance	(+)	ND	(−)	(+)	Vibration↓	Truncal	–
2	Gait disturbance	Only obduction disturbance	ND	ND	(−)	(−)	(−)	Truncal	Good recover (without treatment)
3	Double vision	Total	(−)	(+)	(+)	(+)	Vibration↓	Unknown in detail	Neurological onset after lung cancer operation
4	Double vision	Obduction and upgaze disturbance	ND	ND	(−)	(−)	(−)	Limb, truncal	–
5	Double vision, Gait disturbance	Obduction-dominant	(+)	ND	(−)	(+)	(−)	Limb, truncal	Good recovery after IAT
6	Double vision	Only obduction disturbance	ND	ND	(−)	(−)	Distal dysesthesia	Unknown in detail	Relapse (first onset, 14 years old), good recovery after IVIg
7	Double vision	Unknown in detail	ND	ND	(−)	(−)	(−)	Truncal	–
8	Gait disturbance	Obduction-dominant	(−)	ND	(−)	(+)	Distal hypesthesia	Truncal	–
9	Ptosis, Gait disturbance	Unknown in detail	(+)	(+)	(+)	(−)	Distal hypesthesia	Truncal	Good recovery (without treatment)
10	Gait disturbance	Obduction-dominant	ND	ND	(−)	(−)	Distal paresthesia	Truncal	–

FP facial palsy, *BP* bulbar palsy, *ND* not described, *IAT* immunoadsorption therapy, *IVIg* intravenous immunoglobulin

LOS-reactive patients, and that serological evidence of recent *C. jejuni* infection was also shown in other two patients. This indicates that detecting anti-*C. jejuni* LOS antibodies was not due to cross-reaction with *C. jejuni* protein, which was used as antigen in serological assays for this infection, and that GD1c and GalNAc-GM1b-like

LOS antibodies were present irrespective of the type of antecedent infections.

Discussion

Disease pathology due to acetylcholine receptor antibodies has been clearly demonstrated for myasthenia gravis, although approximately 20% of myasthenia gravis patients with generalized disease lack the autoantibodies (so called seronegative myasthenia gravis), and some instead have antibodies reactive to a muscle specific kinase [23]. We have found a similar situation in FS: although most FS patients have anti-GQ1b autoantibodies, some FS patients have other autoantibodies, and clinical features are not useful for identifying GQ1b-seronegative FS patients. There are several individual case reports of GQ1b-seronegative FS, most of which describe a clinical picture atypical for FS, such as early infant onset (2 years old), complication of Burkitt's lymphoma, marked oculomotor nerve disturbance without abducens palsy predominance, and one-sided horizontal gaze palsy, irrespective of coexisting anti-ganglioside antibodies [24–27]. Unlike these reports, we did not find any obvious clinical differences between patients with and without anti-GQ1b antibodies, except for male dominance and antecedent gastrointestinal symptoms, and rarer onset of diplopia. This is the first study to extensively describe the clinical features of GQ1b-seronegative FS patients.

We found 24 GQ1b-seronegative FS patients among 207 FS cases and showed that some GQ1b-seronegative FS patients had IgG antibodies against single gangliosides such as GM1b (8.3%), GD1a (4.2%), GalNAc-GD1a (4.2%), or GT1a (8.3%). This is in agreement with our previous findings that some patients with GQ1b-seronegative FS have antibodies to GM1b (9/76 [12%]), GalNAc-GD1a (9/76 [12%]), or GD1b (9/76 [12%]) [4, 28]. A pioneering study found that anti-GT1a antibodies coexisted with anti-GQ1b antibodies in all FS patients, although both antibodies cross-reacted with each other, and a biochemical analysis detected GQ1b, but not GT1a, expression in human oculomotor nerves, but not GT1a. The authors speculated that anti-GQ1b antibodies are the primary effector in FS pathogenesis [2]. In contrast, our biochemical analysis detected GT1a as well as GQ1b in human oculomotor nerves [29]. The current study data, as well as our biochemical findings, raise the possibility that antibodies against gangliosides other than GQ1b play a pathogenic role in the development of FS, although negative result of anti-GQ1b antibodies might be partially due to insufficient sensitivity of the antibody assay used, as that of acetylcholine receptor antibodies in myasthenia gravis. Administration of anti-GQ1b monoclonal antibody can cause general weakness and respiratory failure in mice, but it remains unclear whether anti-GQ1b antibodies can induce FS-like deficits [30]. To investigate which anti-ganglioside antibodies play a pathogenic role of FS development, establishment of FS animal model by immunization with gangliosides or passive transfer of the antibodies is needed.

One recent study showed that three patients with GQ1b-seronegative FS had antibodies against ganglioside complexes [31]. Our larger study, including 24 GQ1b-seronegative FS patients, found that the frequency of GQ1b-seronegative FS patients with IgG reactive to heterologous ganglioside complexes was 17% (4 of 24 patients). Moreover, the GQ1b-seronegative FS patients with anti-ganglioside complex antibodies in our study did not show distinguishing clinical features, suggesting that clinical features are not helpful in identifying these patients among GQ1b-seronegative FS cases. In agreement with a previous report [31], our data showed that combinations of GT1a/GM1 and GQ1b/GM1, which contains a total of two sialic acids in the terminal residues, could be the alternative target antigens for serum IgG antibodies in patients with GQ1b-seronegative FS. GQ1b and GT1a are expressed diffusely in human cranial nerves [29], and therefore conspicuous ophthalmoplegia in FS appears not be explained only by the uneven expression of GQ1b and GT1a in cranial nerves. In order to clarify the molecular mechanisms underpinning FS pathogenesis, future studies should determine distribution of the alternative epitopes formed by ganglioside complexes containing two sialic acids in the terminal residues in the human nervous system to clarify the molecular mechanism in developing unique clinical picture of FS.

Structural analysis of the LOS of GC068 and GC219 confirmed previous studies that showed that FS-related *C. jejuni* isolates frequently displayed a GD1c-like LOS, and not the precise GQ1b-like LOS [8–11]. We also observed GalNAc-GM1b- and GalNAc-GD1c-like LOS, both of which had not been previously described in *C. jejuni*. GD1c, GQ1b, and GT1a have in common the structure (NeuAc α2–8 NeuAc α2–3 Gal β1–3 GalNAc) in the terminal residues (Fig. 1). All five patients with FS or FS/GBS overlap, from whom GD1c-like LOS-bearing *C. jejuni* was isolated (GC033 and GC068 in Table 2 and GC041, GC107, and GC125, unpublished data), had anti-GQ1b antibodies. It therefore can be assumed that GD1c-like LOS triggers the production of anti-GD1c antibodies that cross-react with GQ1b in some FS patients following *C. jejuni* enteritis. Mechanistically, IgG autoantibodies in FS patients are speculated to have higher affinity for GD1c than GQ1b, and our finding that some GQ1b-seronegative FS patients have antibodies against GD1c-like LOS supports this assertion. Anti-GD1c antibody testing should be performed using authentic ganglioside as the antigen to confirm our speculation.

Expression of GalNAc-GM1b-like LOS on FS-related *C. jejuni* strains suggests three possible mechanisms in the development of FS. First, GalNAc-GM1b-like LOS might be associated with production of antibodies against GM1b and GalNAc-GD1a. GalNAc-GM1b has a carbohydrate sequence (GalNAc β1-4 [NeuAc α2–3] Gal β1–3 GalNAc) in the terminal moiety in common with GalNAc-GD1a, and the inner moiety (NeuAc α2–3 Gal β1–3 GalNAc) is also common to GM1b (Fig. 1). In GBS and chronic neuropathies, anti-GalNAc-GM1b antibodies (IgG or IgM) can coexist with antibodies against GalNAc-GD1a or GM1b, and cross-react with each other [32–35]. Based on these findings, GalNAc-GM1b-like LOS may act as an immunogen for producing antibodies against GalNAc-GD1a and GM1b, which contribute to FS pathogenesis. This is supported by the detection of anti-GM1b and anti-GalNAc-GD1a autoantibodies in GQ1b-seronegative FS patients in this and another study [4], and the detection of anti-GalNAc-GD1a antibodies in a patient from whom *C. jejuni* bearing GalNAc-GM1b-like LOS (GC051, unpublished data) had been isolated. Second, anti-GalNAc-GM1b antibodies themselves might initiate the development of FS. GalNAc-GM1b is expressed in the brains of several animals, although it is unclear if this minor ganglioside is expressed in the human nervous system [32]. Some individuals may express GalNAc-GM1b as well as GQ1b in oculomotor nerves. Finally, GalNAc-GM1b-like LOS might be associated with the production of anti-GQ1b antibodies. Although non-reducing terminal of oligosaccharide of GalNAc-GM1b is different from that of GQ1b in looking at the second dimensional structure, the three-dimensional structure can be identical, as in the case of GM1b and GalNAc-GD1a [36]. This alternative explanation seems the most likely, as we found anti-GQ1b antibodies in all three patients with FS or FS/GBS overlap from whom *C. jejuni* bearing GalNAc-GM1b-like LOS [GC219 and GC068 in Table 2; GC051 (unpublished data)] was cultured. Thus, the presence of anti-GalNAc-GM1b antibodies should be examined using authentic ganglioside as an antigen.

In conclusion, this is the first study to examine the clinical and serological features of patients with GQ1b-seronegative FS. We showed that the clinical features of GQ1b-seronegative FS were similar to those of GQ1b-seropositive FS, except for extreme male predominance and a history of antecedent gastrointestinal illness. IgG antibodies against GM1b, GD1c, GalNAc-GM1b, and ganglioside complexes were found to be serological markers of GQ1b-seronegative FS, although a pathogenic role of these autoantibodies requires further study.

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Conflicts of interest None.

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