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Spinal cord ring enhancement in patients with neuromyelitis optica

Yokote H, Nose Y, Ishibashi S, Tanaka K, Takahashi T, Fujihara K, Yokota T, Mizusawa H. Spinal cord ring enhancement in patients with neuromyelitis optica.
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Objectives – Clinical and pathological significance of gadolinium (Gd)-enhancing pattern on magnetic resonance imaging (MRI), including ring enhancement (RE), is well documented in multiple sclerosis but not in neuromyelitis optica (NMO), especially in the spinal cord. The purpose of this study is to examine the prevalence of spinal cord RE in NMO and to determine the association between clinical characteristics and spinal cord RE. *Materials and methods* – We retrospectively examined Gd-enhanced spinal cord MRI scans, during the acute phase, in patients with anti-aquaporin 4-positive NMO, including NMO spectrum disorder. We then analysed their clinical features and MRI imaging characteristics of spinal cord lesions. *Results* – Of the 30 patients with NMO, we enrolled 12 patients with 16 Gd-enhanced spinal cord MRI scans in this study. Five scans revealed RE (31.2%). Male ratio, as well as myelin basic protein (MBP) levels, in the cerebrospinal fluid (CSF) of patients with RE was significantly higher than those of patients without RE ($P = 0.018$, $P = 0.026$, respectively). *Conclusions* – Spinal cord RE is common in patients with NMO. Higher MBP levels in the CSF of patients with RE can be associated with a higher degree of myelin damage.

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Key words: demyelination; magnetic resonance imaging; multiple sclerosis; myelin basic protein; neuromyelitis optica; ring enhancement; spinal cord

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Introduction

Neuromyelitis optica (NMO) is an inflammatory disease that predominantly affects the optic nerves and the spinal cord and is characterized by longitudinally extensive spinal cord lesions (1). Enhancement of T1-weighted magnetic resonance imaging (MRI) after administration of gadolinium (Gd) in NMO indicates increased blood–brain barrier (BBB) permeability with active inflammation. In multiple sclerosis (MS), lesions are classified based on nodular enhancement (NE) or ring enhancement (RE) patterns on MRI (2). Reports indicate that 23% of new enhancing lesions in the brains of MS patients exhibit RE in MRI (3). RE is also common in acute spinal cord lesions (4). One study suggests that RE lesions are associated with more severe clinical disease status than are lesions without RE (2). However, this association between RE and disease severity was not found in another study (5). In NMO, however, little is known about Gd-enhancing pattern except for the cloud-like enhancement in the brain that is specific to NMO (6). The purpose of this study was to find out the frequency of RE in the spinal cord during the acute phase of NMO and to characterize the clinical manifestation of NMO in patients with spinal cord RE compared to those in patients without RE.

Patients and methods

This retrospective case–control study was performed on all patients admitted to two hospitals, from April 2007 to November 2012, who were clinically diagnosed with acute phase of NMO including NMO spectrum disorder (7, 8). The inclusion criteria were as follows: (i) at least one clinical attack of transverse myelitis; (ii) positivity

for serum anti-aquaporin 4 (AQP4) antibody; and (iii) clinically acute phase with spinal cord gadolinium enhancement. We collected the demographic data, information on the Kurtzke Expanded Disability Status Scale (EDSS), past medical history and the results of diagnostic tests at the time of admission. Anti-AQP4 antibodies were measured by a previously described cell-based assay (9, 10). We excluded patients without AQP4 antibody from this study, because pathogenesis of NMO without anti-AQP4 antibody has not been well understood.

NMO patients underwent spinal cord MRI scans. We acquired MRI scans using 1.5 T or 3 T scanners including Signa HDxt (GE Healthcare, Milwaukee, WI, USA) and EXCELART (Toshiba Medical Systems, Tochigi, Japan), and MAGNETOM Verio (Siemens AG Medical Solutions, Erlandgen, Germany). With a few modifications depending on the type of MRI scanners used, the acquisition parameters were as follows: a T1-weighted sagittal fast spin echo (FSE-XL) sequence without gadolinium injection (repetition time [TR], 420 ms; echo time [TE], 8.9 ms; matrix size, 320 × 224; field of view [FOV], 240 mm; slice thickness, 2 mm; slice space, 0.4 mm); a T1-weighted sagittal/axial FSE-XL sequence with gadolinium injection (TR, 475/600 ms; TE, 8.8/8.5 ms; matrix size, 320 × 192/256 × 192; FOV, 240/160 mm; slice thickness, 2.0/4.0 mm; slice space, 0.4/1.0 mm); a fat-saturated T1-weighted sagittal/axial FSE-XL sequence with gadolinium injection (TR, 520/680 ms; TE, 8.8/8.2 ms; matrix size, 320 × 192/256 × 192; FOV, 240/160 mm; slice thickness, 2.0/4.0 mm; slice space, 0.4/1.0 mm); a fast spin echo (FRFSE-XL) sequence with T2-weighted sagittal/axial image (TR, 3900/4000 ms; TE, 102/102 ms; matrix size, 320 × 224/256 × 192; FOV, 240/160 mm; slice thickness, 2.0/4.0 mm; slice space, 0.4/1.0 mm). The time-interval between Gd administration and scan acquisition was approximately 4–10 min.

The pattern of gadolinium enhancement was classified as either 'presence of ring' or 'absence of ring', in the axial or sagittal dimension of the spinal cord (Fig. 1).

We compared demographic and clinical features of anti-AQP4 positive NMO patients including age, sex, prior use of disease modifying therapy including immunosuppressive therapy (DMT), EDSS, MRI findings and cerebrospinal fluid (CSF) findings between NMO patients with spinal cord RE and those without RE.

We performed statistical analysis using R version 2.13.1. We used Welch's *t*-test to compare serial data between different patient groups.

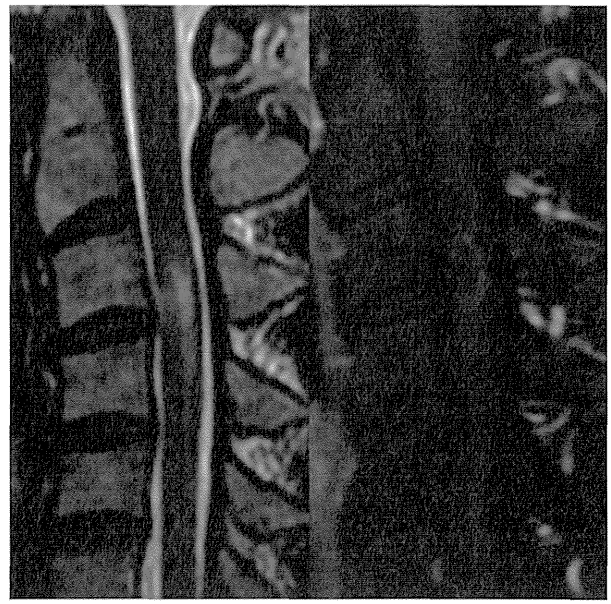

Figure 1. Ring enhancement (RE) on spinal cord magnetic resonance imaging (MRI). Sagittal T2-weighted image shows hyperintense lesion extending from C3 to C4 levels. RE on fat-saturated T1-weighted image after gadolinium administration is shown.

Categorical data were compared using Mann–Whitney *U*-test or Fisher's exact test.

Results

Among 30 patients with NMO, 12 NMO patients (nine female), with 16 Gd-enhanced spinal cord MRI scans, were enrolled in this study. Four patients had two scans at the different event times. Mean age at the time of imaging was 54.4 years (range, 26–80 years). The median initial EDSS score was 3.0 (range, 1.0–7.5) at the time of the imaging study. We prescribed immunomodulatory therapy for five patients (31.3%). Mean duration from symptom onset to MRI scan was 9.9 ± 8.4 days. Seven (43.8%) scans had cervical spinal cord lesions whereas 11 (68.8%) scans had thoracic spinal cord lesions. There were two (12.5%) scans that showed both cervical and thoracic spinal cord lesions. Mean lesion length was 5.4 vertebrae (range, 2–16). Five scans (31.2%) from five patients revealed RE pattern whereas 11 (68.8%) scans had non-RE pattern. The rate of male NMO patients was significantly higher in RE group compared with non-RE group. ($P < 0.018$, Table 1). There is no difference in Gd-enhancing pattern between the different scanners. Percentages of RE pattern were similar between 3 T and 1.5 T scanners, 50% (1 of 2) and 28.6% (4 of 14), respectively. RE lesions

Table 1 Patient characteristics and comparison between MRI scans with RE and without RE

	RE+ $n = 5$[b]	RE− $n = 11$[a,b]	P
Age (years)	51.2 ± 22.4	55.9 ± 7.6	0.67
Female *n*, (%)	2 (40.0)	11 (100)	0.018
DMT *n*, (%)	1 (20.0)	4 (36.4)	1.00
Initial EDSS	3.0	3.0	0.91
Residual EDSS	1.5	2.0	0.30
ΔEDSS	1.5	1.0	0.31
Lesion length (vertebrae)	6.4 ± 5.5	5.0 ± 2.8	0.61
Duration from onset to MRI (days)	17.8 ± 10.1	6.4 ± 4.6	0.063
Disease duration (years)	0.6 ± 1.3	6.2 ± 8.7	0.062
Age at disease onset (years)	50.8 ± 22.9	49.3 ± 14.6	0.900
Optic neuritis (%)	1 (20.0)	6 (54.5)	0.31
Brain lesions (%)	0 (0)	4 (40.0)	0.23
CSF			
Cell count (/3 μl)	23.3 ± 11.9	10.8 ± 14.1	0.14
Protein	65.0 ± 14.7	56.9 ± 17.6	0.41
MBP	442 ± 177	97.4 ± 75.8	0.026

RE, ring enhancement; DMT, disease modifying therapy; EDSS, Expanded Disability Scale Score; MBP, myelin basic protein; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.
[a]Brain MRI were available in 10 without RE.
[b]CSF data were available in 4 with RE and 9 without RE.

were centrally located in two patients with two MRI scans whereas peripherally located in three patients with three scans. Non-RE lesions were centrally located in one patient with one MRI scan, peripherally located in six patients with six scans, and diffusely located in three patients with four scans. Concurrent brain MRI showed non-specific white matter T2-high intensity lesions in four patients with six scans of 15 scans available (40.0%), but not any Gd-enhanced lesions and 'NMO-like' periependymal lesions of the third and forth ventricles, dorsal medulla lesions or tumefactive white matter lesions. There were no significant differences in the rate of brain involvement between the RE and non-RE groups ($P = 0.23$, Table 1).

Initial EDSS score was similar between NMO patients with RE and those without RE (Table 1). We also obtained EDSS during the residual stage after the treatment in the acute phase. Mean duration from initial EDSS to residual EDSS was 7.0 ± 5.0 months. When we defined the ΔEDSS as the change from basal to residual EDSS, median ΔEDSS was 1.5 in NMO patients with RE and 1.0 in NMO patients without RE ($P = 0.31$, Table 1).

Cerebrospinal fluid data were available for 10 patients with 13 MRI scans: three patients had two lumbar punctures at the different event times. Although cell count and total protein level in CSF did not differ between groups, myelin basic protein (MBP) levels in CSF in patients with RE were significantly higher than those in patients without RE ($P = 0.026$, Table 1).

Discussion

Here, we firstly described that, among the patients who were positive for serum AQP4 antibody, 31.2% of patients who had Gd-positive spinal cord lesions had RE pattern in the acute phase. Moreover, we observed spinal cord RE lesions in patients characterized as 'greater male ratio'. RE lesions were also associated with higher MBP levels in CSF than were non-RE lesions.

Gd-enhancing pattern on brain MRI in patients with MS has been well discussed, and a recent paper suggests that RE can be associated with timing of MRI scan (11).

Blood–brain barrier disruption in new MS or NMO lesions can be detected by MRI after intravenous injection of gadolinium, a contrast agent that shortens the longitudinal T1 relaxation time. BBB opening is usually associated with new lesions and consequently with inflammation originating in the area surrounding parenchymal microvessels (11). MS lesions have been classified as RE and NE pattern on Gd-enhanced MRI, and the clinical and pathological significance of enhancing pattern have been discussed (2, 5). Recent study using dynamic contrast-enhanced MRI for relapsing remitting MS described that distinct enhancement patterns were consequence of the timing of image acquisition after gadolinium administration (11). They observed that nodular lesions enhanced from centre to periphery could expand over the course of days and change their enhancement dynamics to a ring pattern, enhanced from periphery to centre. In this study, we did not investigate the dynamic change of Gd-enhancing pattern. The duration from symptom onset to MRI scan tended to be longer in NMO patients with RE compared to those without RE although they did not significantly differ suggesting that RE pattern might just represent the later stage of Gd-enhancing dynamics.

This study demonstrated that 31.2% of Gd-positive spinal cord lesions in NMO patients showed RE pattern. In acute phase of MS patients, Klawiter et al. (4) described that the prevalence of spinal cord RE was 20 patients (55.6%) of 36 patients with Gd-enhancing spinal cord lesion. These results indicate that spinal cord RE is common in NMO patients as well as MS patients.

We found that spinal cord RE lesions were more observed in male patients than in female

patients. Considering the significant predominance of female patients in the NMO group (7), this gender difference is a striking characteristic of NMO patients with spinal cord RE lesion. However, we cannot conclude that RE lesion is characteristic of male NMO patients from this small study and further larger studies are needed to confirm this result.

Significantly higher MBP levels in CSF from NMO patients with RE suggest that RE is associated with more severe myelin damage compared to NMO patients without RE. Myelin damage can be due to larger tissue destruction following severe inflammation, or primary demyelination. Although CSF cell count, as well as lesion length in the spinal cord, was not significantly different between NMO patients with RE and those without RE, it is possible that severe inflammation leads to secondary larger myelin damage. Recently, in patients with NMO, the levels of CSF glial fibrillary acidic protein (GFAP) were reported to be significantly higher than that in MS and other neurological disease. Moreover, CSF–GFAP levels were associated with EDSS or spinal lesion length in the acute phase, and with EDSS at 6-month follow-up, suggesting that astrocytic damage is a key feature of NMO (12). Therefore, evaluating CSF–GFAP levels can be helpful for confirming this idea. It might also be informative to evaluate axonal damage using biomarker including CSF tau or cystatin c (13).

On the other hand, significantly higher MBP levels in CSF from NMO patients with RE might suggest primary demyelination, based on the observation that type six lesions, defined as lesion similar to primary demyelination, were seen in four of seven patients with NMO (14). Misu et al. (15) recently reported that active lesion in NMO displays a wider spectrum of pathology than previously thought showing six different types (14). Lesions of type 1, 2 and 3 reflect typical NMO lesions presenting complement deposition, associated with granulocyte infiltration and astrocyte necrosis. This is followed by demyelination, leading to global tissue destruction accompanied, sometimes, by Wallerian degeneration. Type 4 and type 5 lesions are characterized by clasmatodendrosis of astrocytes in the absence of complement activation, defined by cytoplasmic swelling and vacuolation in astrocytes, beading and dissolution of their foot processes, and nuclear alteration. A type 6 lesion similar to primary demyelination in association with oligodendrocyte apoptosis and astrocytic clasmatodendrosis, but with the preservation of axons. This type 6 lesion resembles a subset of active MS lesions defined by primary demyelination with oligodendrocyte apoptosis (14, 16). Therefore, higher MBP levels in CSF from patients with RE in our study can be associated with higher degree of demyelination and might suggest distinct pathogenesis from NMO patients without RE.

Small sample size is one of the major limitations of this study. There were nearly but not significant differences in duration from onset to MRI as well as disease duration between RE+ and RE− group (Table 1, $P = 0.063$ and 0.062, respectively). This lack of statistical significance might be due to small sample size. Larger sample size is also desirable when discussing an association between spinal cord RE and sex ratio. The other limitations include the use of four different types of MRI scanners and non-standardized protocols. Further, more CSF markers including GFAP, tau and cystatin c need to be analysed for a better understanding of the pathogenesis of spinal cord RE in NMO patients.

In this study, we describe the RE pattern on the spinal cord of patients with NMO. RE pattern on the spinal cord is associated with higher CSF MBP levels than for non-RE pattern due either to larger tissue destruction or demyelination. We also consider that RE pattern on spinal cord might be associated with timing of MRI scan. Further studies are warranted to clarify the exact mechanisms of RE.

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Conflict of interest

The authors have no conflict of interest to declare.

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Original Research Paper

Latitude and *HLA-DRB1* alleles independently affect the emergence of cerebrospinal fluid IgG abnormality in multiple sclerosis

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Abstract

Background: It is unclear whether the prevalence of oligoclonal IgG bands (OCBs) in multiple sclerosis (MS) is different between northern and southern regions of Asia.

Objective: This study aimed to compare the prevalence of OCBs and positive cerebrospinal fluid (CSF) findings between northern and southern regions of Japan and to investigate the association of these CSF findings with *HLA-DRB1* alleles.

Methods: The study included 180 MS patients from Hokkaido (northern Japan) and 184 patients from Kyushu (southern Japan). The IgG index was defined as increased if it was >0.658. Presence of CSF OCBs and/or increased IgG index was defined as positive CSF findings.

Results: Positive CSF findings and OCB positivity were significantly higher in MS patients from Hokkaido than in those from Kyushu ($p < 0.0001$ for both). Logistic regression analysis revealed that after adjusting for covariates that can be related to abnormal CSF IgG production, the geographic region (Hokkaido) showed odds ratios (ORs) of 4.08 and 2.57, whereas the *HLA-DRB1*04:05* allele showed ORs of 0.36 and 0.30 for positive CSF findings and OCB positivity, respectively.

Conclusions: The results indicate that latitude and *HLA-DRB1* alleles independently affect the emergence of CSF IgG abnormalities in Japanese patients with MS.

Keywords: Multiple sclerosis, oligoclonal IgG band, IgG index, cerebrospinal fluid, human leukocyte antigen, MRI

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Introduction

The prevalence of multiple sclerosis (MS) in Asia, especially East and South Asia, is lower than that in Western countries.[1] In Japan, the prevalence of MS in the Tokachi province on the northernmost island of Japan was 16.2/100,000 in 2011, which was the highest in eastern Asia.[2] The prevalence and incidence of MS in Japan is increasing,[2,3] and a small but statistically significant north-south gradient exists with regard to the prevalence of MS.[4]

It is considered that increased synthesis of cerebrospinal fluid (CSF) immunoglobulin (Ig)Gs relative to that of serum Igs (an increased IgG index) and/or the presence of intrathecal oligoclonal IgG bands (OCBs) are typical in patients with MS.[5] In fact, it has been reported that the CSF IgG index is elevated in up to 90% of MS patients,[6] and a recent meta-analysis demonstrated that approximately 90% of MS patients in Western countries are OCB positive.[7] However, studies from Asian countries suggest that OCB positivity in MS patients is not as high as that observed in Western countries,[8–11] and the diagnostic sensitivity of OCBs may be lower in non-Caucasian patients.[12] Conversely, recent studies have suggested a significant relationship between latitude and OCB positivity or provided evidence of

Figure 1. Map of Japan. Samples were collected from multiple sclerosis (MS) patients from Hokkaido, a northern island, and Kyushu, a southern island. Sapporo is the main city of Hokkaido and is located at 43 degrees North; Fukuoka is the main city of Kyushu and is located at 33 degrees North.

intrathecally produced IgG in patients from Western countries.[7,12,13] However, it remains unclear whether this relationship exists in Asian populations.

The human leukocyte antigen (*HLA*) gene region has long been implicated as a major player in the susceptibility of developing MS, and the *HLA-DRB1*15* allele is strongly associated with MS.[14] In southern Japan, the frequency of the *HLA-DRB1*15* allele was also higher in patients with MS than in healthy controls (26.2% vs. 16.4%).[15] On the other hand, the *HLA-DRB1*15* allele has been reported to be associated with OCB-positive MS patients in Western countries and Australia.[16–18] Previous studies from Japan have also demonstrated that the *HLA-DRB1*15* allele is associated with OCB-positive MS and that the *HLA-DR*04* allele is associated with OCB-negative MS.[8,19]

In this study, we investigated whether the rates of OCB positivity and elevated IgG index are different in MS patients between the northern and southern main islands (which exhibit a difference in latitude of approximately 10 degrees) of Japan. Furthermore, we also investigated whether *HLA-DRB1*04:05* and **15:01* alleles affect the differences in OCB positivity and elevated IgG index in MS patients from these two islands of Japan.

Materials and methods

Patients

MS was defined using the 2010 revised McDonald criteria.[20] Patients with neuromyelitis optica (NMO)[21] or NMO spectrum disorders[22] were excluded from this study. In total, 180 MS patients were recruited from five institutes (Sapporo Neurology Clinic, Sapporo Medical University, Japanese Red Cross Asahikawa Hospital, Obihiro Kosei General Hospital, and Hokkaido Medical Center) in Hokkaido Island, northern Japan, and 184 MS patients were recruited from one institute (Kyushu University Hospital) in Kyushu Island, southern Japan (Figure 1). The clinical profiles of the patients are shown in Table 1. The female:male ratio was 136:44 and 126:58, the mean age at onset (± standard deviation, SD) was 30.1 ± 10.5 and 31.8 ± 13.0 years, and the mean disease duration was 11.7 ± 9.5 and 9.5 ± 8.4 years in patients from Hokkaido and Kyushu,

Table 1. Clinical profiles of MS patients from Hokkaido and Kyushu.

		Hokkaido (n = 180)	Kyushu (n = 184)	p value
Female (F) : Male (M)		136:44	126:58	n.s.
Age at onset (years)[a]		30.1 ± 10.5	31.8 ± 13.0	n.s.
Disease duration (years)[a]		11.7 ± 9.5	9.5 ± 8.4	**<0.05**
EDSS at blood sampling for DNA[a]		2.5 ± 2.4	3.3 ± 2.4	**<0.001**
MSSS[a]		3.2 ± 2.9	4.6 ± 2.9	**<0.0001**
Annualized relapse rate [a]		0.61 ± 0.54	0.58 ± 0.64	n.s.
Barkhof criteria [b]		139/180 (77.2%)	126/172 (73.3%)	n.s.
OCB		93/147 (63.3%)	75/181 (41.4%)	**<0.0001**
IgG index[a]		0.99 ± 0.50	0.71 ± 0.28	**<0.0001**
Increased IgG index[c]		114/153 (74.5%)	43/106 (40.6%)	**<0.0001**
*HLA-DRB1**				
04:05 (+)	Total	70/180 (38.9%)	71/179 (39.7%)	n.s.
	F	50/136 (36.8%)	48/122 (39.3%)	n.s.
	M	20 /44 (45.5%)	23/57 (40.4%)	
15:01 (+)	Total	60/180 (33.3%)	46/179 (25.7%)	n.s.
	F	45/136 (33.1%)	33/122 (27.1%)	n.s.
	M	15/44 (34.1%)	13/57 (22.8%)	
04:05 (+) and *15:01* (−)	Total	53/180 (29.4%)	62/179 (34.6%)	n.s.
	F	39/136 (28.4%)	40/122 (32.8%)	n.s.
	M	14/44 (31.8%)	22/57 (38.6%)	
04:05 (−) and *15:01* (+)	Total	43/180 (23.9%)	37/179 (20.7%)	n.s.
	F	34/136 (25.0%)	25/122 (20.5%)	n.s.
	M	9/44 (20.5%)	12/57 (21.1%)	

[a]Values represent mean ± SD.
[b]Brain MRI lesions that meet the Barkhof criteria.[20]
[c]Positive CSF findings: oligoclonal IgG band (OCB) positivity and/or elevated IgG index.
[d]Increased IgG index: upper normal limit = 0.658, according to our previous study.
EDSS: Expanded Disability Status Scale; MSSS: Multiple Sclerosis Severity Score; n.s.: not significant.

respectively. The Expanded Disability Status Scale (EDSS)[23] at blood sampling for DNA and the Multiple Sclerosis Severity Score (MSSS)[24] were significantly higher in MS patients from Kyushu than in those from Hokkaido (Table 1). The frequency of patients with Barkhof brain lesions[25] was not significantly different between Hokkaido and Kyushu (Table 1). The ethics committee of each institution approved this study, and informed consent was obtained from each participant.

CSF findings

OCBs were determined by isoelectric focusing because this is the most sensitive method for OCB determination.[9,26] OCBs were considered positive when two or more bands in the gamma region of CSF not appearing in the serum were detected by the electrophoresis pattern.[9] OCBs of patients in all institutes included in this study were analyzed by the Mitsubishi Chemical Medience Corporation (currently LSI Medience Corporation, Tokyo, Japan). CSF and serum samples were sent to one of the company's local institutes for laboratory processing, and analyzed for OCBs by an experienced person. The IgG index is calculated as (CSF IgG/serum IgG)/(CSF albumin/serum albumin). The IgG index was considered to be elevated if it was >0.658.[27] Presence of OCBs and/or an elevated IgG index was defined as a positive CSF finding.

HLA-DRB1 *genotyping*

The genotypes of *HLA-DRB1* alleles from the patients were determined by hybridization between the products of polymerase chain reaction amplification of the *HLA-DRB1* gene and sequence-specific oligonucleotide probes, as described previously.[28]

Table 2. Comparison of the prevalence of positive CSF findings and OCB positivity in MS patients from Hokkaido and Kyushu.

Region	Positive CSF findings[a]			
	Total MS patients	*p* value	MS patients with Barkhof brain lesions	*p* value
Hokkaido	133/180 (73.9%)	**<0.0001**	106/139 (76.3%)	**<0.0001**
Kyushu	80/184 (43.5%)		61/126 (48.4%)	
Region	OCB positivity			
	Total MS patients	*p* value	MS patients with Barkhof brain lesions	*p* value
Hokkaido	93/147 (63.3%)	**<0.0001**	74/113 (65.5%)	**<0.01**
Kyushu	75/181 (41.4%)		56/123 (45.5%)	

[a]Positive CSF findings: oligoclonal IgG band (OCB) positivity and/or elevated IgG index. CSF: cerebrospinal fluid; MS: multiple sclerosis.

Statistical analysis

Statistical analyses were performed using JMP 9.0.2 (SAS Institute Inc, Cary, NC, USA). Welch's *t* test was used for comparison of the clinical data of MS patients from Hokkaido and Kyushu. Comparisons between *HLA* alleles and positive CSF findings or OCB positivity were performed using Fisher's exact probability test. Logistic regression was used to analyze the association between OCB abnormality and the following factors: geographic region (Hokkaido or Kyushu), *HLA-DRB1* alleles, and sex. We calculated odds ratios (ORs) adjusted for these factors. Sex was used as a covariate because female sex is a risk factor for MS. The Mann-Whitney *U* test was used to compare MSSS between CSF-positive and -negative MS patients or between OCB-positive and -negative MS patients. Statistical significance was set at $p < 0.05$.

Results

OCB positivity and increased IgG index in MS patients from Hokkaido and Kyushu

We first compared the rates of positive CSF findings in MS patients from Hokkaido and Kyushu (Table 2). The rates of positive CSF findings and OCB positivity were significantly higher in patients from Hokkaido than in those from Kyushu (Table 2). In MS patients with Barkhof brain lesions, the rates of positive CSF findings and OCB positivity were slightly higher than the corresponding values for the entire cohort, and the differences in the rates between patients from Hokkaido and Kyushu were retained (Table 2).

Association of OCB positivity and increased IgG index with HLA-DRB1*04:05 *or* *15:01 *alleles in MS patients from Hokkaido and Kyushu*

It has been considered that *HLA-DRB1*04:05* and **15:01* alleles may affect OCB positivity or increased IgG index.[8,15] First, we investigated whether there were any differences in the frequencies of *HLA-DRB1*04:05* or **15:01* alleles between MS patients from Hokkaido and Kyushu and found that the frequencies of *HLA-DRB1*04:05* and **15:01* alleles were almost similar in MS patients from these two regions (Table 1). Subsequently, to investigate the association between each allele and CSF findings or OCB positivity, we compared the rates of positive CSF findings and OCB positivity between individuals carrying only one of these alleles in a combined cohort of patients from Hokkaido and Kyushu. The results indicated that the rates of positive CSF findings and OCB positivity were significantly higher in *HLA-DRB1*04:05*-negative and *HLA-DRB1*15:01*-positive MS patients than in *HLA-DRB1*04:05*-positive and *HLA-DRB1*15:01*-negative patients (Table 3).

Logistic regression analysis

Prior to the calculation of ORs by logistic regression analysis, we evaluated whether there were interactions between factors that may be associated with abnormal IgG production in the CSF (geographic region, *HLA-DRB1* alleles, and sex). One significant interaction between the *HLA-DRB1*15:01* allele and sex, which may induce positive CSF findings, was detected ($p = 0.0449$). Therefore, we added this interaction as a covariate in logistic regression analysis of positive CSF findings. After adjusting for *HLA-DRB1*04:05* and **15:01* alleles and sex, the geographic region (Hokkaido to Kyushu) was found to be significantly associated with positive CSF findings and OCB positivity (OR = 4.08, 95% confidence interval (CI) = 2.57–6.60, $p < 0.0001$ and OR = 2.57, 95% CI = 1.60–4.19, $p < 0.0001$, respectively). In addition, after adjusting for the geographic region, *HLA-DRB1*15:01* allele, and sex, we found that the *HLA-DRB1*04:05* allele was also significantly associated with positive CSF findings and

Table 3. Comparison of the prevalence of positive CSF findings and OCB positivity in MS patients from Hokkaido and Kyushu according to the carrier status of *HLA-DRB1*04:05* and **15:01* alleles.

Locus	Allele	CSF findings[a]		*p* value
		Positive	Negative	
*HLA-DRB1**	*04:05* (+) and *15:01* (−)	51/115 (44.4%)	64/115 (55.7%)	**<0.0001**
	04:05 (−) and *15:01* (+)	58/80 (72.5%)	22/80 (27.5%)	
Locus	Allele	OCB		*p* value
		Positive	Negative	
*HLA-DRB1**	*04:05* (+) and *15:01* (−)	33/108 (30.6%)	75/108 (69.4%)	**<0.0001**
	04:05 (−) and *15:01* (+)	51/72 (70.8%)	21/72 (29.2%)	

[a]Positive CSF findings: oligoclonal IgG band (OCB) positivity and/or elevated IgG index; negative CSF findings: neither presence of OCBs nor elevated IgG index. CSF: cerebrospinal fluid; MS: multiple sclerosis; HLA: human leukocyte antigen; Ig: immunoglobulin.

Table 4. Logistic regression analysis of the association between CSF abnormalities and possible factors (geographic region, *HLA-DRB1* alleles, and sex).

		Positive CSF findings[a]		
		Adjusted OR	95% CI	*p* value
Region (Hokkaido)		**4.08**	**2.57–6.60**	**<0.0001**
*HLA-DRB1**	*04:05* allele	**0.36**	**0.22–0.57**	**<0.0001**
	15:01 allele	0.92	0.52–1.62	n.s.
Sex (female)		1.50	0.86–2.63	n.s.
		OCB positivity		
		Adjusted OR	95% CI	*p* value
Region (Hokkaido)		**2.57**	**1.60–4.19**	**<0.0001**
*HLA-DRB1**	*04:05* allele	**0.30**	**0.18–0.49**	**<0.0001**
	15:01 allele	1.66	0.97–2.85	0.0650
Sex (female)		1.44	0.85–2.43	n.s.

[a]Positive CSF findings: oligoclonal IgG band (OCB) positivity and/or elevated IgG index; Negative CSF findings: neither presence of OCBs nor elevated IgG index. CSF: cerebrospinal fluid; HLA: human leukocyte antigen; Ig: immunoglobulin; 95% CI: 95% confidence interval; OR: odds ratio; n.s.: not significant.

OCB positivity (OR = 0.36, 95% CI = 0.22–0.57, $p < 0.0001$ and OR = 0.30, 95% CI = 0.18–0.49, $p < 0.0001$, respectively). Although the effect of the *HLA-DRB1*15:01* allele did not reach significance after adjustment for the other covariates, a significant trend was observed in estimating the risk for OCB positivity (OR = 1.66, 95% CI = 0.97–2.85, $p = 0.0650$). There was no definite correlation between sex and CSF abnormality (Table 4).

Association of OCB positivity and/or increased IgG index with age at onset in MS patients

Age at disease onset was similar between MS patients with positive and negative CSF findings or positive and negative OCBs in both Hokkaido and Kyushu (Table 5).

Association of OCB positivity and/or increased IgG index with MSSS in MS patients

MSSS was similar between MS patients with positive and negative CSF findings or positive and negative OCBs in both Hokkaido and Kyushu (Table 6).

Discussion

In the present study, we demonstrated that OCB positivity was significantly higher in MS patients from the northern main island, Hokkaido, than in patients from the southern main island, Kyushu. This finding suggests that OCB positivity in Japanese MS patients may be closely correlated with latitude, because there is a difference in latitude of approximately 10 degrees between the two regions. A similar relationship has