**Table 4**
Comparison of brain MRI findings between NMO and MS.

| | NMO patients ($n=38$) | MS patients ($n=110$) |
|---|---|---|
| Barkhof brain lesions[a] | 7/38 (18.4%)* | 71/110 (64.6%)* |
| ≥9 T2 brain lesions | 8/38 (21.1%)* | 76/110 (69.1%)* |
| ≥1 Gd-enhanced lesion | 3/36 (8.3%)* | 35/108 (32.4%)* |
| ≥1 juxtacortical lesion | 15/38 (39.5%)* | 81/110 (73.6%)* |
| ≥1 periventricular lesion | 11/38 (29.0%)* | 81/110 (73.6%)* |
| ≥1 infratentorial lesion | 13/38 (34.2%)* | 72/110 (65.5%)* |
| Paty brain lesions[b] | 17/38 (44.7%)* | 92/110 (83.6%)* |
| Ovoid lesions | 12/38 (31.6%)* | 87/108 (80.6%)* |
| Atypical brain lesions | 10/38 (26.3%) | 23/110 (20.9%) |
| Extensive brain lesions | 5/38 (13.2%)* | 2/110 (1.8%)* |
| Bil. diencephalic lesions | 0/38 (0.0%) | 6/110 (5.5%) |
| Cavity formation | 3/38 (7.9%) | 16/110 (14.6%) |
| Extension from the cervical cord into brainstem | 3/38 (7.9%)* | 0/110 (0.0%)* |

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis; NMO = neuromyelitis optica.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].
* $P<0.05$.

typical NMO patients than MS patients ($P^{corr}<0.001$). The frequencies of OBs and an elevated IgG index were higher in MS patients than in typical NMO patients and NMO patients with brain lesions, but the differences did not reach statistical significance (Table 2).

The demographic features of patients with and without anti-AQP4 antibody are shown in Table 3. Female to male ratio, annualized relapse rate and frequencies of optic neuritis, severe optic neuritis, and LESCLs during the entire course, were significantly higher in anti-AQP4 antibody-positive patients than in anti-AQP4 antibody-negative patients ($P=0.034$, $P=0.0070$, $P<0.0001$, $P=0.00018$, and $P=0.00015$, respectively).

### 3.2. Brain MRI findings

The frequencies of brain lesions fulfilling the Barkhof [16] or Paty [13] criteria were significantly higher in MS patients than in NMO patients (64.6% vs. 18.4%, $P<0.0001$, and 83.6% vs. 44.7%, $P<0.0001$, respectively). The frequency of ovoid lesions was similarly higher in MS patients than that in NMO patients (80.6% vs. 31.6%, $P<0.001$). Although the frequency of total atypical brain lesions was not different between the two groups, extensive brain lesions (13.2% vs. 1.8%, $P=0.012$) and lesions extending from the cervical cord into the brainstem (7.9% vs. 0.0%, $P=0.016$) were significantly more common in NMO patients than in MS patients (Table 4).

When brain MRI features were compared among typical NMO patients, NMO patients with brain lesions, and MS patients, atypical brain lesions were most frequently found in NMO patients with brain lesions (Table 5). The frequencies of atypical brain lesions and extensive brain lesions were significantly higher in the NMO patients with brain lesions (52.9% and 29.4%, respectively) than in MS patients (20.9%, $P^{corr}=0.038$, and 1.8%, $P^{corr}=0.0013$, respectively) and typical NMO patients (4.8%, $P^{corr}=0.0056$, and 0%, $P^{corr}=0.037$ respectively). By contrast, ovoid lesions were significantly more commonly found in MS patients (80.6%) and NMO patients with brain lesions (64.7%) than in typical NMO patients (4.8%, $P^{corr}<0.001$ and $P^{corr}<0.001$, respectively).

In comparisons between those with and without anti-AQP4 antibody, brain lesions fulfilling the Barkhof criteria were significantly less common in anti-AQP4 antibody-positive patients (33.3%) than in anti-AQP4 antibody-negative patients (57.0%) ($P=0.033$), while fulfilment of the Paty criteria during the entire clinical course was observed nearly as frequently in anti-AQP4 antibody-positive patients as in anti-AQP4 antibody-negative ones (74.1% vs. 73.5%) (Table 6). Among the items of Barkhof's criteria, the frequency of patients having ≥nine T2 hyperintense lesions was significantly higher among anti-AQP4 antibody-negative patients than among anti-AQP4 antibody-positive patients ($P=0.031$) while the frequencies of patients with ≥1 gadolinium-enhanced lesion, those with ≥1 juxtacortical lesion, those with ≥1 periventricular lesion and those with ≥1

**Table 5**
Comparison of brain MRI findings among patients with typical NMO, NMO with brain lesions and MS.

| | Typical NMO patients ($n=21$) | NMO with brain lesions ($n=17$) | MS patients ($n=110$) |
|---|---|---|---|
| Barkhof brain lesions[a] | 0/21 (0.0%)*,** | 7/17 (41.2%)** | 71/110 (64.6%)* |
| ≥9 T2 brain lesions | 0/21 (0.0%)*,** | 8/17 (47.1%)** | 76/110 (69.1%)* |
| ≥1 Gd-enhanced lesion | 1/19 (5.3%)* | 2/17 (11.8%) | 35/108 (32.4%)* |
| ≥1 juxtacortical lesion | 6/21 (28.6%)* | 9/17 (52.9%) | 81/110 (73.6%)* |
| ≥1 periventricular lesion | 0/21 (0.0%)*,** | 11/17 (64.7%)** | 81/110 (73.6%)* |
| ≥1 infratentorial lesion | 3/21 (14.3%)*,** | 10/17 (58.8%)** | 72/110 (65.5%)* |
| Paty brain lesions[b] | 0/21 (0.0%)*,** | 17/17 (100.0%)** | 92/110 (83.6%)* |
| Ovoid lesions | 1/21 (4.8%)*,** | 11/17 (64.7%)** | 87/108 (80.6%)* |
| Atypical brain lesions | 1/21 (4.8%)* | 9/17 (52.9%)*,** | 23/110 (20.9%)** |
| Extensive brain lesions | 0/21 (0.0%)* | 5/17 (29.4%)*,** | 2/110 (1.8%)** |
| Cavity formation | 0/21 (0.0%) | 3/17 (17.7%) | 16/110 (14.6%) |
| Bil. diencephalic lesions | 0/21 (0.0%) | 0/17 (0.0%) | 6/110 (5.5%) |
| Extension from the cervical cord into brainstem | 1/21 (4.8%) | 2/17 (11.8%) | 0/110 (0.0%) |

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis; NMO = neuromyelitis optica.
*, ** Corrected $P<0.05$.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].

**Table 6**
Comparison of brain MRI findings between anti-AQP4 antibody-positive and -negative patients with NMO and MS.

| | Anti-AQP4 antibody-positive patients ($n=27$) | Anti-AQP4 antibody-negative patients ($n=121$) |
|---|---|---|
| Barkhof brain lesions[a] | 9/27 (33.3%)* | 69/121 (57.0%)* |
| ≥9 T2 brain lesions | 10/27 (37.0%)* | 74/121 (61.2%)* |
| ≥1 Gd-enhanced lesion | 4/27 (14.8%) | 34/117 (29.1%) |
| ≥1 juxtacortical lesion | 13/27 (48.2%) | 83/121 (68.6%) |
| ≥1 periventricular lesion | 13/27 (48.2%) | 78/121 (64.5%) |
| ≥1 infratentorial lesion | 12/27 (44.4%) | 73/121 (60.3%) |
| Paty brain lesions[b] | 20/27 (74.1%) | 89/121 (73.5%) |
| Ovoid lesions | 13/27 (48.2%)* | 86/119 (72.3%)* |
| Atypical brain lesions | 10/27 (37.0%) | 23/121 (19.0%) |
| Extensive brain lesions | 5/27 (18.5%)* | 2/121 (1.7%)* |
| Bil. diencephalic lesions | 0/27 (0.0%) | 6/121 (5.0%) |
| Cavity formation | 4/27 (14.8%) | 15/121 (12.4%) |
| Extension from the cervical cord into brainstem | 2/27 (7.4%) | 1/121 (0.8%) |

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis; NMO = neuromyelitis optica.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].
* $P<0.05$.

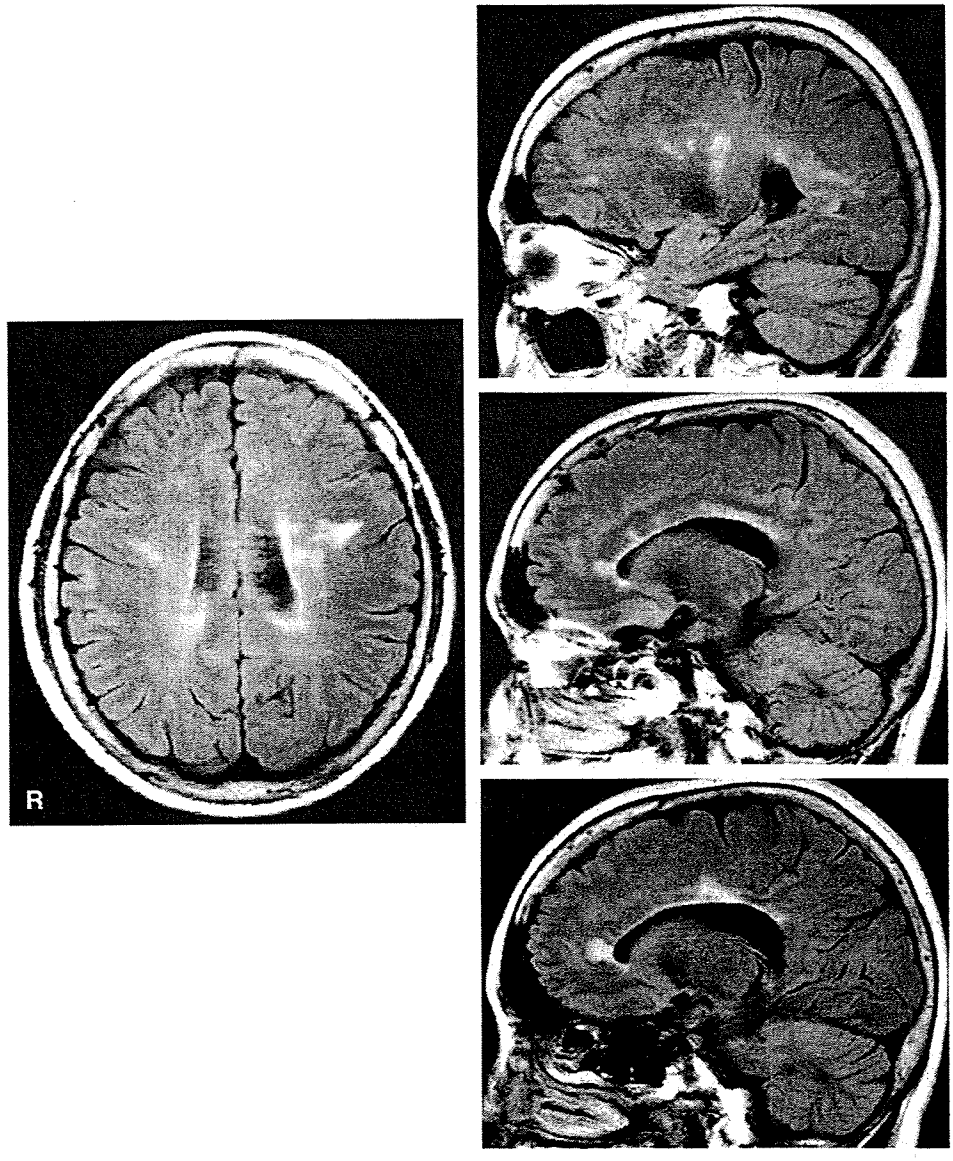



**Fig. 1.** Axial and sagittal fluid-attenuated inversion recovery (FLAIR) images in an MS patient with a high titre of anti-AQP4 antibody (1:4096). She had 15 years of illness and her EDSS score was 1 at the time of the MRI scan. Note the presence of ovoid periventricular lesions typical of MS.

**Table 7**
Comparison of demographic features between MS patients with and without CSF OB/high IgG index.

| | MS with OB/high IgG index ($n=54$) | MS without OB/high IgG index ($n=30$) |
|---|---|---|
| No. of female/male patients | 38/16 (2.4:1) | 18/12 (1.5:1) |
| Age at onset (years)[a] | 28.7 ± 10.4* | 35.9 ± 15.5* |
| Disease duration (years)[a] | 12.0 ± 10.1 | 10.9 ± 8.6 |
| Relapse rate[a] | 0.67 ± 0.42 | 0.71 ± 0.56 |
| EDSS score[a] | 3.8 ± 2.6 | 3.2 ± 2.5 |
| Frequency of symptoms: | | |
| Optic neuritis | 31/54 (57.4%) | 16/30 (53.3%) |
| Bilateral optic neuritis | 6/54 (11.1%) | 4/30 (13.3%) |
| Severe optic neuritis (FS ≥ 5) | 18/54 (33.3%) | 11/30 (36.7%) |
| Myelitis | 44/54 (81.5%) | 26/30 (86.7%) |
| Acute transverse myelitis | 8/54 (14.8%) | 8/30 (26.7%) |
| Secondary progression | 8/54 (14.8%) | 1/30 (3.3%) |
| CSF: | | |
| Marked pleocytosis (≥50/μl) | 2/52 (3.9%) | 2/30 (6.7%) |
| Neutrophilia (≥5/μl) | 3/48 (6.3%) | 1/30 (3.3%) |
| LESCLs during the entire course | 15/54 (27.8%) | 9/30 (30.0%) |

The upper normal range of the IgG index was derived from our previous study [24]. CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; OB = oligoclonal IgG bands.
[a] Means ± SD.
* $P<0.05$.

infratentorial lesion did not differ significantly between the two groups. About a half of patients with anti-AQP4 antibody had ovoid periventricular lesions (Fig. 1), but the frequency of ovoid lesions was less common in anti-AQP4 antibody-positive patients than in anti-AQP4 antibody-negative patients (48.2% vs. 72.3%, $P=0.022$). By contrast, atypical brain lesions were present more frequently in patients with anti-AQP4 antibody (37.0%) than in those without the antibody (19.0%) ($P=0.070$). Among these, extensive brain lesions were observed more commonly in anti-AQP4 antibody-positive patients (18.5%) than in antibody-negative patients (1.7%) ($P=0.0023$). However, the frequencies of other atypical lesions did not differ significantly between anti-AQP4 antibody-positive and -negative patients.

Finally, we compared clinical features between MS patients with and without CSF OB/high IgG index, and found that age at onset was significantly younger in MS patients with CSF OB/high IgG index than in MS patients without it ($P=0.036$) (Tables 7 and 8). Moreover, the frequencies of brain lesions fulfilling the Barkhof and Paty criteria and ovoid lesions were significantly higher in those with CSF OB/high IgG index than in those without it ($P=0.00040$, $P=0.014$, and $P=0.030$, respectively).

## 4. Discussion

By extensive analyses of bpboard1rain MRIs of Japanese patients with MS and NMO, we found that MS-like brain lesions were more common in anti-AQP4 antibody-negative patients than in those with the antibody, while extensive brain lesions were more frequently observed in the latter than in the former; however, about 30 to 50% of either NMO or anti-AQP4 antibody-positive patients had brain MRI lesions that were indistinguishable from those associated with MS. Surprisingly, anti-AQP4 antibody-positive patients had periventricular ovoid lesions more frequently than atypical brain lesions. Even in patients who met the revised NMO criteria [3], MS-like brain lesions, including periventricular ovoid lesions, were more frequently observed than atypical brain lesions in the present series. The presence of typical MS-like brain lesions, such as periventricular ovoid lesions, suggests that considerable overlap exists in MRI appearance between patients with NMO who have anti-AQP4 antibody and classical MS patients without anti-AQP4 antibody. The fact that we [11] and others [4,10] observed that around 10% of classical MS patients harbour NMO-IgG/anti-AQP4 antibody further supports such an overlap between the two conditions. In fact, among

**Table 8**
Comparison of brain MRI findings between MS patients with and without CSF OB/high IgG index.

| | MS with OB/high IgG index ($n=54$) | MS without OB/high IgG index ($n=30$) |
|---|---|---|
| Barkhof brain lesions[a] | 45/54 (83.3%)* | 13/30 (43.3%)* |
| ≥9 T2 brain lesions | 46/54 (85.2%)* | 16/30 (53.3%)* |
| ≥1 Gd-enhanced lesion | 19/53 (35.9%) | 8/30 (26.7%) |
| ≥1 juxtacortical lesion | 46/54 (85.2%)* | 19/30 (63.3%)* |
| ≥1 periventricular lesion | 46/54 (85.2%)* | 19/30 (63.3%)* |
| ≥1 infratentorial lesion | 40/54 (74.1%) | 17/30 (56.7%) |
| Paty brain lesions[b] | 51/54 (94.4%)* | 22/30 (73.3%)* |
| Ovoid lesions | 48/53 (90.6%)* | 21/30 (70.0%)* |
| Atypical brain lesions | 15/54 (27.8%) | 3/30 (10.0%) |
| Extensive brain lesions | 0/54 (0.0%) | 0/30 (0.0%) |
| Bil. diencephalic lesions | 5/54 (9.3%) | 0/30 (0.0%) |
| Cavity formation | 11/54 (20.4%) | 3/30 (10.0%) |
| Extension from the cervical cord into brainstem | 0/54 (0.0%) | 0/30 (0.0%) |

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].
* $P<0.05$.

patients with anti-AQP4 antibody, ovoid lesions were more commonly encountered than atypical brain lesions. It is therefore suggested that a common mechanism may in part be operative in these two conditions, especially in producing periventricular ovoid lesions, irrespective of the presence or absence of anti-AQP4 antibody. Ovoid periventricular lesions are said to be caused by T cells invading along the postcapillary high endothelial venules, which perpendicularly radiate from the lateral ventricular walls [18]. Thus, T cells might also play an important role in producing the brain lesions in the patients with anti-AQP4 antibody, but the target antigens could be different from those in patients without the antibody. We also previously reported cases showing seroconversion during the course of MS [11]. All of these findings support the notion that there are cases in whom anti-AQP4 antibody can be produced during the course of idiopathic demyelinating diseases, such as MS, and secondarily modify the clinical features, like anti-neurofascin antibody [19].

Among atypical brain lesions, only the extensive brain lesions seemed to be significantly more frequent in anti-AQP4 antibody-positive patients than in anti-AQP4 antibody-negative patients in our series. We previously reported that extensive brain lesions showed a vasogenic oedema pattern on diffusion-weighted MRI [11,20]. In AQP4 knock-out mice, cytotoxic oedema is ameliorated [21] while vasogenic oedema becomes worse [22]. Destruction of AQP4 on astrocyte foot processes by complement activation by anti-AQP4 antibody might well retard the resolution of vasogenic oedema, which tends to cause extensive oedematous brain lesions associated with inflammation in patients with anti-AQP4 antibody.

In the present study, NMO patients with brain lesions showed a significantly higher annualized relapse rate than typical NMO patients, suggesting a high disease activity in the former. Indeed, frequencies of severe optic neuritis, LESCLs and cavity formation were all higher in the former than in the latter, although this difference was not statistically significant. In addition, anti-AQP4 antibody positivity rate was highest in NMO patients with brain lesions among the three groups examined. Therefore, development of brain lesions in NMO patients may reflect high disease activity and the presence of anti-AQP4 antibody, and thus be regarded as a warning sign for a grave clinical course.

The positivity rate of CSF OB/high IgG index in our MS patients was lower than those reported for Caucasians with MS [23]. However, the positivity rate was similar to those previously reported in Asian patients with MS [24–26]. The disparities between Western and Asian MS patients may be related to differences in genetic backgrounds. Interestingly, MS patients with CSF OB/high IgG index showed not only a significantly younger age at onset but also higher frequency of brain lesions fulfilling the Barkhof criteria [16] than those without it. These findings suggest that MS with CSF OB/high IgG index has similar features to classical Western-type MS, even in Asians.

In summary, up to a half of anti-AQP4 antibody-positive patients could develop classical MS-like brain lesions, which is even more frequent than the development of so-called atypical brain lesions. Because the presence of anti-AQP4 antibody can modify treatment response, as shown previously [11], anti-AQP4 antibody should be tested for even in patients with classical MS-like features, especially in Asians.

## Acknowledgments

This work was supported in part by grants from the Research Committees of Neuroimmunological Diseases, the Ministry of Health, Labour and Welfare, Japan and from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

## References

[1] de Seze J. Neuromyelitis optica. Arch Neurol 2003;60:1336–8.
[2] Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). Neurology 1999;53:1107–14.
[3] Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. Neurology 2006;66:1485–9.
[4] Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 2004;364:2106–12.
[5] Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 2005;202:473–7.
[6] Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG. Brain MRI abnormalities in neuromyelitis optica. Arch Neurol 2006;63:390–6.
[7] Lucchinetti CF, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 2002;125:1450–61.
[8] Roemer SF, Parisi JE, Lennon VA, Benarroch EE, Lassmann H, Bruck W, et al. Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. Brain 2007;130:1194–205.
[9] Misu T, Fujihara K, Kakita A, Konno H, Nakamura M, Watanabe S, et al. Loss of aquaporin-4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. Brain 2007;130:1224–34.
[10] Nakashima I, Fujihara K, Miyazawa I, Misu T, Narikawa K, Nakamura M, et al. Clinical and MRI features of Japanese patients with multiple sclerosis positive for NMO-IgG. J Neurol Neurosurg Psychiatry 2006;77:1073–5.
[11] Matsuoka T, Matsushita T, Kawano Y, Osoegawa M, Ochi H, Ishizu T, et al. Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. Brain 2007;130:1206–23.
[12] Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–31.
[13] Paty DW, Oger JJ, Kastrukoff LF, Hashimoto SA, Hooge JP, Eisen AA, et al. MRI in the diagnosis of MS: a prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. Neurology 1988;38:180–5.
[14] Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005;58:840–6.
[15] Su JJ, Osoegawa M, Minohara M, Tanaka M, Ishizu T, Mihara F, et al. Upregulation of vascular growth factors in multiple sclerosis: correlation with MRI findings. J Neurol Sci 2006;243:21–30.
[16] Barkhof F, Filippi M, Miller DH, Scheltens P, Campi A, Polman CH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain 1997;120:2059–69.
[17] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–52.
[18] Fog T. The topography of plaques in multiple sclerosis with special reference to cerebral plaques. Acta Neurol Scand 1965;15:1–161.
[19] Mathey EK, Derfuss T, Storch MK, Williams KR, Hales K, Woolley DR, et al. Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 2007;204:2363–72.
[20] Matsushita T, Isobe N, Matsuoka T, Ishizu T, Kawano Y, Yoshiura T, et al. Extensive vasogenic edema of anti-aquaporin-4 antibody-related brain lesions. Mult Scler 2009;15:1113–7.
[21] Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat Med 2000;6:159–63.
[22] Papadopoulos MC, Manley GT, Krishna S, Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J 2004;18:1291–3.
[23] Compston A, Coles A. Multiple sclerosis. Lancet 2002;359:1221–31.
[24] Kira J, Kanai T, Nishimura Y, Yamasaki K, Matsushita S, Kawano Y, et al. Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders. Ann Neurol 1996;40:569–74.
[25] Nakashima I, Fujihara K, Itoyama Y. Oligoclonal IgG bands in Japanese multiple sclerosis patients. J Neuroimmunol 1999;101:205–6.
[26] Li B, Dong H, Zhang J, Song X, Guo L. Cerebrospinal fluid IgG profiles and oligoclonal bands in Chinese patients with multiple sclerosis. Acta Neurol Scand 2007;115:319–24.

Research Paper

Multiple Sclerosis

Multiple Sclerosis
16(2) 147–155
© The Author(s), 2010.
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/1352458509355067
msj.sagepub.com
SAGE

# Influence of *HLA-DRB1* alleles on the susceptibility and resistance to multiple sclerosis in Japanese patients with respect to anti-aquaporin 4 antibody status

**N Isobe[1], T Matsushita[1], R Yamasaki[1], SV Ramagopalan[3], Y Kawano[1], Y Nishimura[3], GC Ebers[3] and J Kira[1]**

**Abstract**

**Background:** Epistatic interactions between human leukocyte antigen (*HLA*)-*DRB1* alleles alter multiple sclerosis (MS) risk in Caucasians. Such interactions have never been studied in Asian MS patients.

**Objective:** To investigate the influence of *HLA-DRB1* alleles, including epistatic interactions at this locus, in Japanese MS patients with and without the anti-aquaporin 4 (AQP4) antibody.

**Methods:** The *HLA-DRB1* locus was genotyped in 108 MS patients and 127 healthy controls. MS patients were further classified into two groups according to anti-AQP4 antibody status (27 positive and 81 negative).

**Results:** *HLA-DRB1*09* (adjusted odds ratio (OR) = 0.243, 95% confidence interval (CI) 0.099–0.533) and *HLA-DRB1*01* (adjusted OR = 0.327, 95% CI 0.103–0.873) decreased the incidence of anti-AQP4 antibody-negative MS. By contrast, *HLA-DRB1*12* increased the risk of anti-AQP4 antibody-positive MS (adjusted OR = 3.691, 95% CI 1.233–10.565). Individuals with *HLA-DRB1*09/15* decreased the risk of anti-AQP4 antibody-negative MS (adjusted OR = 0.164, 95% CI 0.026–0.593), while those with *HLA-DRB1*12/15* increased the risk of anti-AQP4 antibody-positive MS (adjusted OR = 10.870, 95% CI 2.004–81.752).

**Conclusions:** The ability of *HLA-DRB1*09* to reduce the risk of anti-AQP4 antibody-negative MS may arise from an interaction with *HLA-DRB1*15*. By contrast, *HLA-DRB1*12* increases susceptibility to anti-AQP4 antibody-positive MS, possibly via an interaction with *HLA-DRB1*15*.

**Keywords**

aquaporin 4, autoantibody, epistatic interaction, *HLA-DRB1*, multiple sclerosis, neuromyelitis optica

Date received: 3rd June 2009; accepted: 21st October 2009

## Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS), whereas neuromyelitis optica (NMO) is an inflammatory disease selectively affecting the optic nerves and spinal cord. The nosological position of NMO has long been a matter of debate. The recent discovery of a specific immunoglobulin G (IgG) against NMO,[1] designated NMO-IgG, targeting aquaporin 4 (AQP4),[2] suggests that NMO is a distinct disease entity with a fundamentally different aetiology from MS. MS is rare in Asians; however, when it appears, the selective but severe involvement of the optic nerves and spinal cord is characteristic.[3] This form, termed opticospinal MS (OSMS), has similar features to the relapsing form of NMO in Western populations.[4] Based on the detection of the NMO-IgG/anti-AQP4 antibody in 30–60% of Japanese OSMS patients,[5–7] OSMS has been suggested to be the same disease entity as the relapsing form of NMO.

The present authors previously reported on the existence of anti-AQP4 antibody-positive and -negative OSMS patients in Japan[6,7] and the differences in the clinical features between the two, including the responses to disease-modifying therapy; the former group were not responsive to interferon beta-1b while the latter did respond. We also revealed that the human leukocyte antigen (*HLA*)-*DPB1*0501* allele is

[1]Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.
[2]Department of Immunogenetics, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan.
[3]Wellcome Trust Centre for Human Genetics and Department of Clinical Neurology, University of Oxford, Oxford, UK.

**Corresponding author:**
Professor J Kira, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
Email: kira@neuro.med.kyushu-u.ac.jp

associated only with anti-AQP4 antibody-positive OSMS, but not with anti-AQP4 antibody-negative OSMS or classical (conventional) MS (CMS).[8] These findings collectively suggest that anti-AQP4 antibody-positive OSMS patients are distinct from anti-AQP4 antibody-negative MS patients. However, it is still uncertain whether the anti-AQP4 antibody directly causes NMO or if it is simply a disease-modifying factor in MS patients.

MS, like all complex traits, is determined by multiple genetic and environmental factors, and its features vary depending on genetic background.[9] The largest genetic effect comes from the major histocompatibility complex (MHC) class II region. In Caucasians, the allele *HLA-DRB1*15* is associated with MS. However, it was not until recently that possible epistatic interactions among *HLA* alleles have attracted the attention of MS researchers; it has now been shown that the alleles *HLA-DRB1*10*, *HLA-DRB1*01* and *HLA-DRB1*08* interact specifically with the *HLA-DRB1*15* allele to alter MS risk in Caucasians.[10,11] All previous HLA studies in Asian MS patients, with the exception of our above-mentioned study,[8] were carried out before the discovery of NMO-IgG. As the NMO-IgG/anti-AQP4 antibody was found in up to 25% of MS patients in a consecutive series of Japanese cases,[6,7] it is critical to clarify the NMO-IgG/anti-AQP4 antibody status before any HLA study. No study of Japanese MS patients has investigated interactions at the *HLA-DRB1* locus. We report here the first analysis of *HLA-DRB1* allelic associations and epistatic interactions in Japanese MS patients with and without the anti-AQP4 antibody.

## Materials and methods

### Patients and controls

The patients who enrolled in the present study all fulfilled the criteria for clinically definite relapsing–remitting MS, as defined by Poser et al.[12] and were thoroughly examined at the MS clinic in the Department of Neurology at Kyushu University Hospital between 1987 and 2007. Informed consent for the collection of DNA was obtained from 108 MS patients and 127 unrelated healthy controls (HCs). Among 108 MS patients, 27 (25.0%) were positive for anti-AQP4 antibody, 21 of whom (77.8%) also met the revised NMO criteria,[13] while 81 (75%) were negative for anti-AQP4 antibody, 7 of whom (8.6%) also met the NMO criteria.

### Anti-AQP4 antibody assay

The level of anti-AQP4 antibody was measured, as described previously,[6,7] using green fluorescent protein-AQP4 fusion protein-transfected human embryonic kidney cells. Serum samples diluted 1:4 were assayed for the anti-AQP4 antibody. Each sample was assayed at least twice, with the examiners blind to the origin of the specimens. Samples that gave a positive result twice were deemed to be positive.

### HLA-DRB1 genotyping

The genotypes of the *HLA-DRB1* alleles were determined by hybridization between the products of polymerase chain reaction (PCR) amplification of the *HLA-DRB1* genes and sequence-specific oligonucleotide probes, as described previously.[8,14]

### Statistical analysis

Allele frequencies among groups were compared using the chi-squared test or Fisher's exact probability test. To clarify the associations among *HLA-DRB1* alleles, we conducted multiple logistic regression analyses. The candidate variables were all of the two-digit *HLA-DRB1* allelotypes. All variables that could significantly improve the model of association were selected in a stepwise manner. Allelic effects were added or removed if $P < 0.05$ in the stepwise model selection. All analyses were performed using JMP 6.0.3 (SAS Institute, Cary, USA), except for Fisher's exact probability test, which was performed using the R package (R version 2.5.1, The R Foundation for Statistical Computing, Vienna, Austria). In all tests, statistical significance was set at $P < 0.05$.

## Results

### The influence of HLA-DRB1 alleles on MS susceptibility and resistance

The frequency of each *HLA-DRB1* allele was compared between MS patients and HCs (Table 1). Monovariate analysis revealed that MS patients had the *HLA-DRB1*09* allele less frequently than HCs (9.3% vs 29.1%, $P = 0.0001$). There was no significant difference in the frequency of the *HLA-DRB1*15* allele. By multiple logistic regression, *HLA-DRB1*09* was negatively associated with MS (adjusted odds ratio (OR) = 0.228, 95% confidence interval (CI) 0.102–0.472) and *HLA-DRB1*01* was also shown to be negatively associated with MS (adjusted OR = 0.394, 95% CI 0.154–0.934).

### The influence of HLA-DRB1 alleles on anti-AQP4 antibody-negative MS susceptibility and resistance

Among anti-AQP4 antibody-negative MS patients, the *HLA-DRB1*09* frequency was lower (9.9% vs 29.1%, $P = 0.0010$) and the *HLA-DRB1*04* higher (60.5% vs 40.2%, $P = 0.0042$) than among HCs (Table 2).

**Table 1.** Allelic ORs for MS for alleles at the *HLA-DRB1* locus

| *DRB1*X* | MS (*n* = 108) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 8 (7.4) | 18 (14.2) | 0.0994 | 0.484 | 0.202–1.163 | **0.0406** | 0.394 | 0.154–0.934 |
| *03* (%) | 2 (1.9) | 2 (1.6) | 1 | 1.179 | 0.163–8.515 | – | | |
| *04* (%) | 56 (51.9) | 51 (40.2) | 0.0728 | 1.605 | 0.956–2.694 | – | | |
| *07* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *08* (%) | 26 (24.1) | 30 (23.6) | 0.9354 | 1.025 | 0.562–1.872 | – | | |
| *09* (%) | 10 (9.3) | 37 (29.1) | **0.0001** | 0.248 | 0.117–0.528 | **0.0001** | 0.228 | 0.102–0.472 |
| *10* (%) | 1 (0.9) | 1 (0.8) | 1 | 1.178 | 0.073–19.053 | – | | |
| *11* (%) | 3 (2.8) | 3 (2.4) | 1 | 1.181 | 0.233–5.975 | – | | |
| *12* (%) | 13 (12.0) | 11 (8.7) | 0.3944 | 1.443 | 0.618–3.368 | – | | |
| *13* (%) | 10 (9.3) | 17 (13.4) | 0.4126 | 0.660 | 0.289–1.510 | – | | |
| *14* (%) | 16 (14.8) | 14 (11.0) | 0.4356 | 1.404 | 0.651–3.027 | – | | |
| *15* (%) | 40 (37.0) | 50 (39.4) | 0.7139 | 0.906 | 0.534–1.537 | – | | |
| *16* (%) | 2 (1.9) | 2 (1.6) | 1 | 1.179 | 0.163–8.515 | – | | |

CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; OR, odds ratio.

**Table 2.** Allelic ORs for anti-AQP4 antibody-negative MS for alleles at the *HLA-DRB1* locus

| *DRB1*X* | Anti-AQP4 Ab (–) MS (*n* = 81) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 5 (6.2) | 18 (14.2) | 0.0728 | 0.398 | 0.142–1.120 | **0.0362** | 0.327 | 0.103–0.873 |
| *03* (%) | 1 (1.2) | 2 (1.6) | 1 | 0.781 | 0.070–8.758 | – | | |
| *04* (%) | 49 (60.5) | 51 (40.2) | **0.0042** | 2.282 | 1.291–4.033 | – | | |
| *07* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *08* (%) | 17 (21.0) | 30 (23.6) | 0.6578 | 0.859 | 0.438–1.684 | – | | |
| *09* (%) | 8 (9.9) | 37 (29.1) | **0.0010** | 0.267 | 0.117–0.608 | **0.0008** | 0.243 | 0.099–0.533 |
| *10* (%) | 1 (1.2) | 1 (0.8) | 1 | 1.575 | 0.097–25.539 | – | | |
| *11* (%) | 2 (2.5) | 3 (2.4) | 1 | 1.046 | 0.171–6.402 | – | | |
| *12* (%) | 6 (7.4) | 11 (8.7) | 0.7475 | 0.844 | 0.299–2.378 | – | | |
| *13* (%) | 7 (8.6) | 17 (13.4) | 0.2964 | 0.612 | 0.242–1.549 | – | | |
| *14* (%) | 10 (12.4) | 14 (11.0) | 0.7710 | 1.137 | 0.479–2.698 | – | | |
| *15* (%) | 31 (38.3) | 50 (39.4) | 0.8741 | 0.955 | 0.539–1.692 | – | | |
| *16* (%) | 1 (1.2) | 2 (1.6) | 1 | 0.781 | 0.070–8.758 | – | | |

Ab, antibody; AQP4, aquaporin 4; CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; OR, odds ratio.

By multiple logistic analysis, *HLA-DRB1*09* (adjusted OR = 0.243, 95% CI 0.099–0.533) and *HLA-DRB1*01* (adjusted OR = 0.327, 95% CI 0.103–0.873) alleles were shown to be associated with a decreased risk of anti-AQP4 antibody-negative MS.

### *The influence of HLA-DRB1 alleles on susceptibility and resistance to anti-AQP4 antibody-positive MS*

*HLA-DRB1*12* frequency was higher among anti-AQP4 antibody-positive MS patients than HCs (25.9% vs 8.7%, $P = 0.0112$), while *HLA-DRB1*09* was under-represented in the former group (7.4% vs 29.1%, $P = 0.0260$). Logistic regression indicated that only *HLA-DRB1*12* was associated with a significantly increased risk of anti-AQP4 antibody-positive MS (adjusted OR = 3.691, 95% CI 1.233–10.565) (Table 3).

### *Interaction of the HLA-DRB1*09 allele with other alleles*

To test whether *HLA-DRB1*09* interacted with other *HLA-DRB1* alleles, allele frequencies were compared between *HLA-DRB1*09*-carrying MS patients and HCs. *HLA-DRB1*09*-carrying anti-AQP4 antibody-negative

**Table 3.** Allelic ORs for anti-AQP4 antibody-positive MS for alleles at the *HLA-DRB1* locus

| *DRB1*X* | Anti-AQP4 Ab (+) MS ($n=27$) | HCs ($n=127$) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 3 (11.1) | 18 (14.2) | 1 | 0.757 | 0.206–2.777 | – | | |
| *03* (%) | 1 (3.7) | 2 (1.6) | 0.4415 | 2.404 | 0.210–27.506 | – | | |
| *04* (%) | 7 (25.9) | 51 (40.2) | 0.1943 | 0.522 | 0.206–1.323 | – | | |
| *07* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *08* (%) | 9 (33.3) | 30 (23.6) | 0.2920 | 1.617 | 0.658–3.972 | – | | |
| *09* (%) | 2 (7.4) | 37 (29.1) | **0.0260** | 0.195 | 0.044–0.864 | – | | |
| *10* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *11* (%) | 1 (3.7) | 3 (2.4) | 0.5414 | 1.590 | 0.159–15.893 | – | | |
| *12* (%) | 7 (25.9) | 11 (8.7) | **0.0112** | 3.691 | 1.279–10.651 | **0.0157** | 3.691 | 1.233–10.565 |
| *13* (%) | 3 (11.1) | 17 (13.4) | 1 | 0.809 | 0.220–2.981 | – | | |
| *14* (%) | 6 (22.2) | 14 (11.0) | 0.1160 | 2.306 | 0.796–6.681 | – | | |
| *15* (%) | 9 (33.3) | 50 (39.4) | 0.5579 | 0.770 | 0.321–1.849 | – | | |
| *16* (%) | 1 (3.7) | 2 (1.6) | 0.4415 | 2.404 | 0.210–27.506 | – | | |

Ab, antibody; AQP4, aquaporin 4; CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; OR, odds ratio.

**Table 4.** Genotypic ORs for MS for individuals carrying *HLA-DRB1*09*

| *DRB1*X /09* | MS ($n=108$) | HCs ($n=127$) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 0 (0.0) | 2 (1.6) | 0.5011 | 0 | | – | | |
| *04* (%) | 3 (2.8) | 8 (6.3) | 0.2327 | 0.425 | 0.110–1.644 | – | | |
| *08* (%) | 1 (0.9) | 2 (1.6) | 1 | 0.584 | 0.052–6.532 | – | | |
| *09* (%) | 1 (0.9) | 2 (1.6) | 1 | 0.584 | 0.052–6.532 | – | | |
| *12* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *13* (%) | 1 (0.9) | 2 (1.6) | 1 | 0.584 | 0.052–6.532 | – | | |
| *14* (%) | 1 (0.9) | 2 (1.6) | 1 | 0.584 | 0.052–6.532 | – | | |
| *15* (%) | 3 (2.8) | 17 (13.4) | **0.0041** | 0.185 | 0.053–0.649 | **0.0084** | 0.185 | 0.042–0.570 |
| *16* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| Total (%) | 10 (9.3) | 37 (29.1) | | | | | | |

CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; OR, odds ratio.

MS patients and HCs, and *HLA-DRB1*09*-carrying anti-AQP4 antibody-positive MS patients and HCs. Individuals with *HLA-DRB1*09/15* had a decreased risk of not only MS (adjusted OR = 0.185, 95% CI 0.042–0.570) (Table 4), but also anti-AQP4 antibody-negative MS (adjusted OR = 0.164, 95% CI 0.026–0.593) (Table 5). There were no significant interactions between *HLA-DRB1*09* and other alleles in anti-AQP4 antibody-positive MS patients (data not shown).

### *Interaction of the HLA-DRB1*12 allele with other alleles*

As *HLA-DRB1*12* increased the risk of anti-AQP4 antibody-positive MS significantly, interactions between this allele and other alleles were also assessed (Table 6). Individuals with an *HLA-DRB1*12/15* genotype had an increased risk of anti-AQP4 antibody-positive MS (adjusted OR = 10.870, 95% CI 2.004–81.752). No other significant risk factor was found.

### *The influence of HLA-DRB1 alleles on the susceptibility and resistance to MS with respect to the NMO criteria*

In a group of 28 NMO patients who met the NMO criteria,[13] the frequency of *HLA-DRB1*09* was significantly lower (0.0% vs 29.1%, $P = 0.0003$) and that of *HLA-DRB1*12* (25.0% vs 8.7%, $P = 0.0146$) was significantly higher compared with healthy controls by monovariate analysis; however, no variable remained significant in the stepwise multiple logistic analysis (Table 7).

**Table 5.** Genotypic ORs for anti-AQP4 antibody-negative MS for individuals carrying *HLA-DRB1*09*

| *DRB1*X/09* | Anti-AQP4 Ab (−) MS (*n* = 81) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 0 (0.0) | 2 (1.6) | 0.5222 | 0 | | – | | |
| *04* (%) | 3 (3.7) | 8 (6.3) | 0.5342 | 0.572 | 0.147–2.223 | – | | |
| *08* (%) | 1 (1.2) | 2 (1.6) | 1 | 0.781 | 0.070–8.758 | – | | |
| *09* (%) | 1 (1.2) | 2 (1.6) | 1 | 0.781 | 0.070–8.758 | – | | |
| *12* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *13* (%) | 1 (1.2) | 2 (1.6) | 1 | 0.781 | 0.070–8.758 | – | | |
| *14* (%) | 0 (0.0) | 2 (1.6) | 0.5222 | 0 | | – | | |
| *15* (%) | 2 (2.5) | 17 (13.4) | **0.0066** | 0.164 | 0.037–0.729 | **0.0176** | 0.164 | 0.026–0.593 |
| *16* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| total (%) | 8 (9.9) | 37 (29.1) | | | | | | |

Ab, antibody; AQP4, aquaporin 4; CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; OR, odds ratio.

**Table 6.** Genotypic ORs for anti-AQP4 antibody-positive MS for individuals carrying *HLA-DRB1*12*

| *DRB1*X/12* | Anti-AQP4 Ab (+) MS (*n* = 27) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 1 (3.7) | 2 (1.6) | 0.4415 | 2.404 | 0.210–27.506 | – | | |
| *04* (%) | 0 (0.0) | 4 (3.2) | 1 | 0 | | – | | |
| *08* (%) | 1 (3.7) | 0 (0.0) | 0.1753 | | | – | | |
| *09* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *13* (%) | 1 (3.7) | 0 (0.0) | 0.1753 | | | – | | |
| *14* (%) | 0 (0.0) | 2 (1.6) | 1 | 0 | | – | | |
| *15* (%) | 4 (14.8) | 2 (1.6) | **0.0090** | 10.870 | 1.880–62.842 | **0.0077** | 10.870 | 2.004–81.752 |
| total (%) | 7 (25.9) | 11 (8.7) | | | | | | |

Ab, antibody; AQP4, aquaporin 4; CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; OR, odds ratio.

In the non-NMO MS patient group, excluding patients who met the NMO criteria and those who had the anti-AQP4 antibody but did not fulfil the NMO criteria (NMO spectrum disorders), monovariate analysis revealed that the frequencies of *HLA-DRB1*01* (4.1% vs 14.2%, $P = 0.0299$) and *HLA-DRB1*09* (10.8% vs 29.1%, $P = 0.0026$) were lower and that the frequency of *HLA-DRB1*04* was higher (63.5% vs 40.2%, $P = 0.0014$) than that of the healthy controls. By stepwise multiple logistic analysis, *HLA-DRB1*01* (adjusted OR = 0.257, 95% CI 0.058–0.826) and *HLA-DRB1*09* (adjusted OR = 0.323, 95% CI 0.129–0.735) significantly reduced the risk of non-NMO MS relative to healthy controls, while *HLA-DRB1*04* significantly increased that risk (adjusted OR = 1.917, 95% CI 1.028–3.602) (Table 8).

According to the results of the multivariate analysis, we next conducted an analysis for the interaction of either *HLA-DRB1*01*, *HLA-DRB1*04*, and *HLA-DRB1*09* with other alleles. For *HLA-DRB1*01*, no allele had a significant interaction (data not shown). For *HLA-DRB1*04*, *HLA-DRB1*04/04* (adjusted OR = 5.488, 95% CI 2.153–15.288), *HLA-DRB1*04/14* (adjusted OR = 4.482, 95% CI 1.285–17.869), and *HLA-DRB1*04/15* (adjusted OR = 2.561, 95% CI 1.022–6.435) significantly increased the risk of non-NMO MS (Table 9). Finally, for *HLA-DRB1*09*, *HLA-DRB1*09/15* significantly decreased the risk of non-NMO MS (adjusted OR = 0.180, 95% CI 0.028–0.652) (Table 10).

## Discussion

This study is the first to investigate interactions among *HLA-DRB1* alleles in Asian MS patients according to anti-AQP4 antibody status. Owing to the rarity of MS in

**Table 7.** Allelic ORs for NMO for alleles at the *HLA-DRB1* locus

| *DRB1*X* | NMO[a] (*n* = 28) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 5 (17.9) | 18 (14.2) | 0.6196 | 1.316 | 0.443–3.908 | – | | |
| *03* (%) | 2 (7.1) | 2 (1.6) | 0.1496 | 4.808 | 0.647–35.702 | – | | |
| *04* (%) | 9 (32.1) | 51 (40.2) | 0.4306 | 0.706 | 0.296–1.683 | – | | |
| *07* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *08* (%) | 8 (28.6) | 30 (23.6) | 0.5816 | 1.293 | 0.517–3.234 | – | | |
| *09* (%) | 0 (0.0) | 37 (29.1) | **0.0003** | 0 | | – | | |
| *10* (%) | 1 (3.6) | 1 (0.8) | 0.3296 | 4.667 | 0.283–76.957 | – | | |
| *11* (%) | 2 (7.1) | 3 (2.4) | 0.2218 | 3.180 | 0.506–19.989 | – | | |
| *12* (%) | 7 (25.0) | 11 (8.7) | **0.0146** | 3.515 | 1.223–10.100 | – | | |
| *13* (%) | 3 (10.7) | 17 (13.4) | 1 | 0.777 | 0.211–2.855 | – | | |
| *14* (%) | 5 (17.9) | 14 (11.0) | 0.3183 | 1.755 | 0.575–5.352 | – | | |
| *15* (%) | 8 (28.6) | 50 (39.4) | 0.2851 | 0.616 | 0.252–1.506 | – | | |
| *16* (%) | 1 (3.6) | 2 (1.6) | 0.4523 | 2.315 | 0.203–26.458 | – | | |

[a]NMO means those who fulfil the NMO criteria[13] among MS patients.
CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; NMO, neuromyelitis optica; OR, odds ratio.

**Table 8.** Allelic ORs for non-NMO MS for alleles at the *HLA-DRB1* locus

| *DRB1*X* | non-NMO MS[a] (*n* = 74) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 3 (4.1) | 18 (14.2) | **0.0299** | 0.256 | 0.073–0.901 | **0.0389** | 0.257 | 0.058–0.826 |
| *03* (%) | 0 (0.0) | 2 (1.6) | 0.5324 | 0 | | – | | |
| *04* (%) | 47 (63.5) | 51 (40.2) | **0.0014** | 2.594 | 1.436–4.687 | **0.0414** | 1.917 | 1.028–3.602 |
| *07* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *08* (%) | 15 (20.3) | 30 (23.6) | 0.5824 | 0.822 | 0.409–1.654 | – | | |
| *09* (%) | 8 (10.8) | 37 (29.1) | **0.0026** | 0.295 | 0.129–0.675 | **0.0101** | 0.323 | 0.129–0.735 |
| *10* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *11* (%) | 0 (0.0) | 3 (2.4) | 0.2987 | 0 | | – | | |
| *12* (%) | 4 (5.4) | 11 (8.7) | 0.5792 | 0.603 | 0.185–1.965 | – | | |
| *13* (%) | 6 (8.1) | 17 (13.4) | 0.2569 | 0.571 | 0.215–1.519 | – | | |
| *14* (%) | 10 (13.5) | 14 (11.0) | 0.5995 | 1.261 | 0.530–3.003 | – | | |
| *15* (%) | 30 (40.5) | 50 (39.4) | 0.8701 | 1.050 | 0.585–1.885 | – | | |
| *16* (%) | 1 (1.4) | 2 (1.6) | 1 | 0.856 | 0.076–9.607 | – | | |

[a]Both patients who met the NMO criteria and those who had anti-AQP4 antibody but did not fulfil the NMO criteria (NMO spectrum disorder) were excluded.
AQP4, aquaporin 4; CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; NMO, neuromyelitis optica; OR, odds ratio.

the Japanese population, the number of cases studied was relatively low, which reduced the statistical power of the present study. Nonetheless, we did find a protective effect of *HLA-DRB1*09* in anti-AQP4 antibody-negative MS patients and a predisposing effect of *HLA-DRB1*12* in anti-AQP4 antibody-positive MS. Moreover, epistatic interactions among *HLA-DRB1* alleles were distinct depending on the presence or absence of the anti-AQP4 antibody: the *HLA-DRB1*09/15* genotype was highly under-represented in anti-AQP4 antibody-negative MS patients compared with controls, whereas the *HLA-DRB1*12/15* genotype was over-represented in anti-AQP4 antibody-positive MS patients.

A protective effect of *HLA-DRB1*01* in anti-AQP4 antibody-negative MS patients is in good accord with findings in Caucasians.[10,11,15] We and others had previously reported that the frequencies of HLA-DR9 antigen[16] and the *HLA-DRB1*09*[17] allele were significantly lower in MS patients compared with controls. In the present study, we have demonstrated that *HLA-DRB1*09* is a protective factor for anti-AQP4 antibody-negative MS. *HLA-DRB1*09* is one of the

**Table 9.** Genotypic ORs for non-NMO MS for individuals carrying *HLA-DRB1*04*

| *DRB1*X/04* | Non-NMO MS[a] (*n* = 74) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| 01 (%) | 1 (1.4) | 4 (3.2) | 0.6537 | 0.421 | 0.046–3.841 | — | | |
| 04 (%) | 15 (20.3) | 7 (5.5) | **0.0012** | 0.229 | 0.089–0.593 | **0.0006** | 5.488 | 2.153–15.288 |
| 06 (%) | 0 (0.0) | 1 (0.8) | 1 | – | | – | | |
| 07 (%) | 0 (0.0) | 1 (0.8) | 1 | – | | – | | |
| 08 (%) | 5 (6.8) | 9 (7.1) | 1 | 1.053 | 0.339–3.268 | – | | |
| 09 (%) | 3 (4.1) | 8 (6.3) | 0.7494 | 0.629 | 0.162–2.447 | – | | |
| 12 (%) | 1 (1.4) | 4 (3.2) | 0.6537 | 0.421 | 0.046–3.841 | – | | |
| 13 (%) | 4 (5.4) | 2 (1.6) | 0.1955 | 0.280 | 0.050–1.567 | – | | |
| 14 (%) | 7 (9.5) | 4 (3.2) | 0.1031 | 0.311 | 0.088–1.102 | **0.0217** | 4.482 | 1.285–17.869 |
| 15 (%) | 11 (14.9) | 11 (8.7) | 0.1743 | 0.543 | 0.223–1.323 | **0.0429** | 2.561 | 1.022–6.435 |
| total (%) | 47 (63.5) | 51 (40.2) | | | | | | |

[a]Both patients who met the NMO criteria and those who had anti-AQP4 antibody but did not fulfil the NMO criteria (NMO spectrum disorder) were excluded.
AQP4, aquaporin 4; CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; NMO, neuromyelitis optica; OR, odds ratio.

**Table 10.** Genotypic ORs for non-NMO MS for individuals carrying *HLA-DRB1*09*

| *DRB1*X/09* | Non-NMO MS[a] (*n* = 74) | HCs (*n* = 127) | Crude *P* | Crude OR | 95% CI | Adjusted *P* | Adjusted OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *01* (%) | 0 (0.0) | 2 (1.6) | 0.5324 | 0 | | – | | |
| *04* (%) | 3 (4.1) | 8 (6.3) | 0.7494 | 0.629 | 0.162–2.447 | – | | |
| *08* (%) | 1 (1.4) | 2 (1.6) | 1 | 0.856 | 0.076–9.607 | – | | |
| *09* (%) | 1 (1.4) | 2 (1.6) | 1 | 0.856 | 0.076–9.607 | – | | |
| *12* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| *13* (%) | 1 (1.4) | 2 (1.6) | 1 | 0.856 | 0.076–9.607 | – | | |
| *14* (%) | 0 (0.0) | 2 (1.6) | 0.5324 | 0 | | – | | |
| *15* (%) | 2 (2.7) | 17 (13.4) | **0.0121** | 0.180 | 0.040–0.802 | **0.0244** | 0.180 | 0.028–0.652 |
| *16* (%) | 0 (0.0) | 1 (0.8) | 1 | 0 | | – | | |
| total (%) | 8 (10.8) | 37 (29.1) | | | | | | |

[a]Both patients who met the NMO criteria and those who had anti-AQP4 antibody but did not fulfil the NMO criteria (NMO spectrum disorder) were excluded.
AQP4, aquaporin 4; CI, confidence interval; HCs, healthy controls; MS, multiple sclerosis; NMO, neuromyelitis optica; OR, odds ratio.

most common alleles in the Japanese population, but is quite rare in the Caucasian populations,[18] which may make the protective effect of *HLA-DRB1*09* in individuals of Northern European descent difficult to detect. As individuals with a *HLA-DRB1*09/15* genotype had a decreased risk of anti-AQP4 antibody-negative MS, the protective effect of *HLA-DRB1*09* may come from reducing the susceptibility effect of *HLA-DRB1*15*, which is clearly associated with Caucasian MS. The effect of *HLA-DRB1*09* on the risk-increasing effect of *HLA-DRB1*15* may explain the observation that the frequency of *HLA-DRB1*15* was not over-represented in anti-AQP4 antibody-negative MS patients. The risk-reducing effects of *HLA-DRB1*01* and *HLA-DRB1*09*, and of the *HLA-DRB1*09/15* genotype was also observed in non-NMO MS, further supporting the protective actions of these genes in MS. A number of mechanisms for the protection exerted by resistance alleles have been proposed, including the generation of antigen-specific suppressor thymus (T)-cells,[19] deletion of autoreactive T-cells,[20] and the alteration of the immune response through poor engagement of encephalitogenic peptides;[21] however, none of this has yet been proven. Alternatively, other genes in linkage dysequilibrium with the resistance alleles could be interacting in *cis* or *trans* to reduce MS risk.[11] Recently, *HLA-DRB1*09* was also shown to be negatively associated with ulcerative colitis in

Japanese patients.[22,23] Collectively, it is assumed that *HLA-DRB1*09*, or some gene(s) in linkage dysequilibrium with it, protects against certain autoimmune diseases, at least in the Japanese population.

In addition, *HLA-DRB1*09* also significantly decreased the risk of anti-AQP4 antibody-positive MS in monovariate analysis. We previously reported that *HLA-DPB1*0501* increases the risk of OSMS,[24] especially anti-AQP4 antibody-positive OSMS.[8] However, the effects of *HLA-DRB1*09* and *HLA-DRB1*12* observed in the present study are independent of *HLA-DPB1*0501* (data not shown) and *HLA-DRB1* and *-DPB1* alleles are not in tight linkage dysequilibrium in the Japanese population. It is thus suggested that although there is a great difference in terms of clinical and pathological features among patients who have anti-AQP4 antibodies and those who do not, there appears to be some genetic similarity between these groups with regards to the protection conferred by *HLA-DRB1*09*.

Although our results indicate some genetic overlap at the *HLA-DRB1* gene locus in terms of resistance to anti-AQP4 antibody-positive and -negative MS in Japanese patients, disease susceptibility alleles appear to be distinct between patients with different anti-AQP4 antibody status. Based on these results, we suggest that *HLA-DRB1*12* acts to increase the risk of anti-AQP4 antibody-positive MS, but has no effect on the risk of anti-AQP4 antibody-negative MS. *HLA-DRB1*12* appears to have similar effects in patients meeting the NMO criteria to those with anti-AQP4 antibody; however, the effects were significant only when looking at anti-AQP4 antibody-positive patients, suggesting that the effects are more anti-AQP4 antibody-related rather than NMO criteria-related. Interestingly, *HLA-DRB1*12* has been reported to increase the risk of allergic disorders, such as asthma,[25] urticaria,[26] and food allergy.[27] In allergic disorders, type 2 helper T (Th2) cells play a pivotal role. A contribution of Th2 cells is also suggested in both NMO and OSMS cases with anti-AQP4 antibody: eosinophil infiltration in the CNS lesions, heightened humoral immune responses and increases in the levels of Th2 cytokines in peripheral blood and CSF are observed.[7,28–30] Thus, *HLA-DRB1*12* may confer susceptibility to anti-AQP4 antibody-positive MS and NMO through Th2 cell-mediated mechanisms.

We found a significant association of *HLA-DRB1*04* with non-NMO MS, which only became evident after excluding two sets of patients; those who met the NMO criteria and those who had anti-AQP4 antibody but who did not fulfil the NMO criteria. *HLA-DRB1*04/04*, *HLA-DRB1*04/14*, and *HLA-DRB1*04/15* genotypes increased the risk of non-NMO MS and the risk effect was especially pronounced in patients carrying *HLA-DRB1*04* in both alleles. HLA-DR4 was previously shown to be associated with MS in Sardinia,[31,32] the Canaries,[33] and Turkey.[34] Indeed, even in a Japanese population, exclusion of patients with NMO and NMO spectrum disorders resulted in the same conclusion, indicating an association of *HLA-DRB1*04* with non-NMO MS. Thus, *HLA-DRB1*04* is considered to be a susceptibility gene for non-NMO MS, even in East Asians. *HLA-DRB1*15* may contribute to increase the risk of non-NMO MS via an interaction with *HLA-DRB1*04* in the Japanese patients.

Recently, Cree et al.[35] reported that among African Americans, no OSMS patients with the anti-AQP4 antibody carried the *HLA-DRB1*15* allele; however, there was no significant difference in the frequency of the allele between healthy controls and the OSMS patients grouped irrespective of anti-AQP4 antibody status. We also found no significant difference in the *HLA-DRB1*15* frequency between anti-AQP4 antibody-positive MS patients and the controls, yet it was a little lower in the former than in the latter. Although the possibility of a false positive cannot be discarded, because of the small number of anti-AQP4 antibody-positive MS patients, an interaction between *HLA-DRB1*12* and *HLA-DRB1*15* was shown to increase the risk of anti-AQP4 antibody-positive MS in Japanese patients. The genetic risk for the development of anti-AQP4 antibody autoimmunity may vary with ethnic background. In any case, the present findings are preliminary due to the small sample size. The influence of the *DRB1* allele on anti-AQP4 antibody-positive MS deserves further studies in a larger cohort.

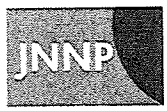
## Acknowledgments

We wish to thank Professor Jorge Oksenberg (Department of Neurology, University of California-San Francisco, USA) for valuable comments on the manuscript. This work was supported, in part, by grants from the Research Committees of Neuroimmunological Diseases, the Ministry of Health, Labor and Welfare, Japan, and from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

## References

1. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004; 364: 2106–2112.
2. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* 2005; 202: 473–477.
3. Kira J. Multiple sclerosis in the Japanese population. *Lancet Neurol* 2003; 2: 117–127.
4. Kira J, Kanai T, Nishimura Y, et al. Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders. *Ann Neurol* 1996; 40: 569–574.

5. Nakashima I, Fujihara K, Miyazawa I, et al. Clinical and MRI features of Japanese patients with multiple sclerosis positive for NMO-IgG. *J Neurol Neurosurg Psychiatry* 2006; 77: 1073–1075.
6. Matsuoka T, Matsushita T, Kawano Y, et al. Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. *Brain* 2007; 130: 1206–1223.
7. Matsushita T, Isobe N, Matsuoka T, et al. Aquaporin-4 autoimmune syndrome and anti-aquaporin-4 antibody-negative opticospinal multiple sclerosis in Japanese. *Mult Scler* 2009; 15: 837–847.
8. Matsushita T, Matsuoka T, Isobe N, et al. Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders. *Tissue Antigens* 2009; 73: 171–176.
9. Ebers GC. Environmental factors and multiple sclerosis. *Lancet Neurol* 2008; 7: 268–277.
10. Dyment DA, Herrera BM, Cader MZ, et al. Complex interactions among MHC haplotypes in multiple sclerosis: susceptibility and resistance. *Hum Mol Genet* 2005; 14: 2019–2026.
11. Ramagopalan SV, Morris AP, Dyment DA, et al. The inheritance of resistance alleles in multiple sclerosis. *PLoS Genet* 2007; 3: 1607–1613.
12. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983; 13: 227–231.
13. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006; 66: 1485–1489.
14. Kimura A, Sasazuki T. 11th International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In: Tsuji K, Aizawa M, Sasazuki T (eds), *HLA 1991: Proceedings of the 11th International Histocompatibility Workshop and Conference*. Vol. 1, Oxford: Oxford University Press, 1992, p. 397–419.
15. DeLuca GC, Ramagopalan SV, Herrera BM, et al. An extremes of outcome strategy provides evidence that multiple sclerosis severity is determined by alleles at the HLA-DRB1 locus. *Proc Natl Acad Sci USA* 2007; 104: 20896–20901.
16. Fukazawa T, Hamada T, Tashiro K, et al. HLA profiles of multiple sclerosis in Hokkaido, the northernmost island of Japan. *Acta Neurol Scand* 1992; 86: 517–520.
17. Matsuoka T, Matsushita T, Osoegawa M, et al. Association of the HLA-DRB1 alleles with characteristic MRI features of Asian multiple sclerosis. *Mult Scler* 2008; 14: 1181–1190.
18. Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T. Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In: Tsuji K, Aizawa M, Sasazuki T (eds), *HLA 1991: Proceedings of the 11th International Histocompatibility Workshop and Conference*. Vol. 1, Oxford: Oxford University Press, 1992, p. 1065–1220.
19. Nishimura Y, Sasazuki T. Suppressor T cells control the HLA-linked low responsiveness to streptococcal antigen in man. *Nature* 1983; 302: 67–69.
20. Gregersen JW, Kranc KR, Ke X, et al. Functional epistasis on a common MHC haplotype associated with multiple sclerosis. *Nature* 2006; 443: 574–577.
21. Barcellos LF, Sawcer S, Ramsay PP, et al. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. *Hum Mol Genet* 2006; 15: 2813–2824.
22. Mochida A, Kinouchi Y, Negoro K, et al. Butyrophilin-like 2 gene is associated with ulcerative colitis in the Japanese under strong linkage disequilibrium with *HLA-DRB1*1502*. *Tissue Antigens* 2007; 70: 128–135.
23. Matsumura Y, Kinouchi Y, Nomura E, et al. HLA-DRB1 alleles influence clinical phenotypes in Japanese patients with ulcerative colitis. *Tissue Antigens* 2008; 71: 447–452.
24. Yamasaki K, Horiuchi I, Minohara M, et al. HLA-DPB1*0501-associated opticospinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies. *Brain* 1999; 122: 1689–1696.
25. Movahedi M, Moin M, Gharagozlou M, et al. Association of HLA class II alleles with childhood asthma and Total IgE levels. *Iran J Allergy Asthma Immunol* 2008; 7: 215–220.
26. Chen J, Tan Z, Li J, Xiong P. Association of HLA-DRB1, DQB1 alleles with chronic urticaria. *J Huazhong Univ Sci Technol Med Sci* 2005; 25: 354–356.
27. Boehncke WH, Loeliger C, Kuehnl P, Kalbacher H, Böhm BO, Gall H. Identification of HLA-DR and -DQ alleles conferring susceptibility to pollen allergy and pollen associated food allergy. *Clin Exp Allergy* 1998; 28: 434–441.
28. Lucchinetti CF, Mandler RN, McGavern D, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* 2002; 125: 1450–1461.
29. Ishizu T, Osoegawa M, Mei FJ, et al. Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. *Brain* 2005; 128: 988–1002.
30. Tanaka M, Matsushita T, Tateishi T, et al. Distinct CSF cytokine/chemokine profiles in atopic myelitis and other causes of myelitis. *Neurology* 2008; 71: 974–981.
31. Marrosu MG, Muntoni F, Murru MR, et al. Sardinian multiple sclerosis is associated with HLA-DR4: a serologic and molecular analysis. *Neurology* 1988; 38: 1749–1753.
32. Marrosu MG, Murru MR, Costa G, Murru R, Muntoni F, Cucca F. DRB1-DQA1-DQB1 loci and multiple sclerosis predisposition in the Sardinian population. *Hum Mol Genet* 1998; 7: 1235–1237.
33. Coraddu F, Reyes-Yanez MP, Parra A, et al. HLA associations with multiple sclerosis in the Canary Islands. *J Neuroimmunol* 1998; 87: 130–135.
34. Saruhan-Direskeneli G, Esin S, Baykan-Kurt B, Örnek İ, Vaughan R, Eraksoy M. HLA-DR and -DQ associations with multiple sclerosis in Turkey. *Hum Immunol* 1997; 55: 59–65.
35. Cree BA, Reich DE, Khan O, et al. Modification of Multiple Sclerosis Phenotypes by African Ancestry at HLA. *Arch Neurol* 2009; 66: 226–233.



# Phenotypic spectrum of hereditary neuralgic amyotrophy caused by the SEPT9 R88W mutation

M Ueda, N Kawamura, T Tateishi, et al.

*J Neurol Neurosurg Psychiatry* 2010 81: 94-96
doi: 10.1136/jnnp.2008.168260

Short report

# Phenotypic spectrum of hereditary neuralgic amyotrophy caused by the *SEPT9* R88W mutation

M Ueda, N Kawamura, T Tateishi, N Sakae, K Motomura, Y Ohyagi, J-i Kira

Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Correspondence to: Professor J-i Kira, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; kira@neuro.med.kyushu-u.ac.jp

MU and NK contributed equally.

Received 18 November 2008
Revised 9 January 2009
Accepted 12 January 2009

## ABSTRACT

**Background:** Hereditary neuralgic amyotrophy (HNA), also known as hereditary brachial plexus neuropathy, has phenotypic and genetic heterogeneity. Mutations in the septin 9 (*SEPT9*) gene were recently identified in some HNA patients. The phenotypic spectrum of HNA caused by *SEPT9* mutations is not well known.

**Objective:** To characterise the phenotype of a large family of HNA patients with the *SEPT9* R88W mutation.

**Methods:** We report clinical, electrophysiological, neuroimaging and genetic findings of six HNA patients from a Japanese family.

**Results:** All 17 neuropathic episodes identified were selectively and asymmetrically distributed in the upper-limb nerves. Severe pain was an initial symptom in 16 episodes (94%). Motor weakness occurred in 15 (88%) and sensory signs in 10 (59%). A minor dysmorphism, hypotelorism, was seen in all. Nerve conduction studies revealed focal demyelination as well as prominent axonal degeneration changes. Needle electromyography revealed chronic neurogenic patterns only in the upper limbs. An MRI study showed a gadolinium-enhanced brachial plexus. The missense mutation c.262C>T; p.R88W was found in exon 2 of *SEPT9* in all patients.

**Conclusions:** The *SEPT9* R88W mutation in this family causes selective involvement of the brachial plexus and upper-limb nerves. Wider and more universal recognition of clinical hallmarks and genetic counselling are of diagnostic importance for HNA caused by the *SEPT9* mutation.

Hereditary neuralgic amyotrophy (HNA), also known as hereditary brachial plexus neuropathy, is a rare autosomal dominant disorder involving recurrent episodes of painful brachial plexus neuropathies.[1] Minor dysmorphism and triggers preceding the neuropathic episode were observed in some HNA patients.[2] HNA has phenotypic heterogeneity in terms of the distribution of neuropathic episodes (selective brachial plexus neuropathy or neuropathic involvement other than the upper-limb nerves) and disease course (classical relapsing-remitting type or chronic undulating type).[3 4]

Previous genetic linkage analysis mapped the HNA locus to chromosome 17q25.[5] Recently, three genetic mutations in septin 9 (*SEPT9*), a cytoskeletal filament forming protein, were identified in HNA families of European origin.[6] By contrast, linkage to chromosome 17q25 has been excluded in some HNA families, suggesting genetic heterogeneity in HNA.[7] Although the clinical features of HNA have been extensively described, some of the neurological features of patients carrying *SEPT9* mutations have been described only in two families.[8 9] Here we report detailed clinical, electrophysiological and neuroradiological findings in a large Japanese HNA family with the *SEPT9* R88W mutation, with the aim of characterising the phenotype of HNA caused by the *SEPT9* mutation.

## METHODS

Six HNA patients from a Japanese pedigree (fig 1A) underwent clinical interviews, neurological examinations, conventional nerve conduction study (NCS) and needle electromyography (EMG) in the remission stage after written informed consent was obtained. Activities of daily living were assessed using a modified Rankin scale.[10] Conduction block and temporal dispersion were defined according to the American Association of Electrodiagnostic Medicine guidelines.[11] An MRI scan of the brachial plexus was performed in one patient in the acute stage. We performed genetic tests for *SEPT9* mutations using peripheral blood samples obtained from six patients and one unaffected family member, as previously described.[6]

## RESULTS

### Clinical features

The clinical data are summarised in table 1. The mean age at disease onset was 18.3 years. Past medical history was not significant, except for diabetes mellitus in one (patient II-5). Motor signs were multifocally and asymmetrically documented only in the upper limbs (fig 1B). Sensory signs were less prominent than motor signs. The grades on the modified Rankin scale ranged between 0 (asymptomatic) and 2 (slight disability). The minor dysmorphisms we observed included hypotelorism in six patients (fig 1C), short stature in five patients, and skin creases and deformed auricles in two patients.

### Neuropathic episodes

All 17 neuropathic episodes we identified were selectively distributed only in the upper limbs. There were preceding triggers, including strenuous work, childbirth, a minor traffic accident and repeated muscular injections, in seven episodes (41%). The triggers were not always the same in each patient. Pain was an initial symptom in 94% of episodes. Motor weakness was observed in 88%; sensory symptoms were observed in 59%. One patient (II-5) received an intravenous immunoglobulin (IVIG) infusion, which resulted in mild improvement of muscle power, hypaesthesia and decreased compound muscle action potential amplitudes in the median nerve. The patients completely recovered from the symptoms following 41% of the neuropathic episodes. In fifteen

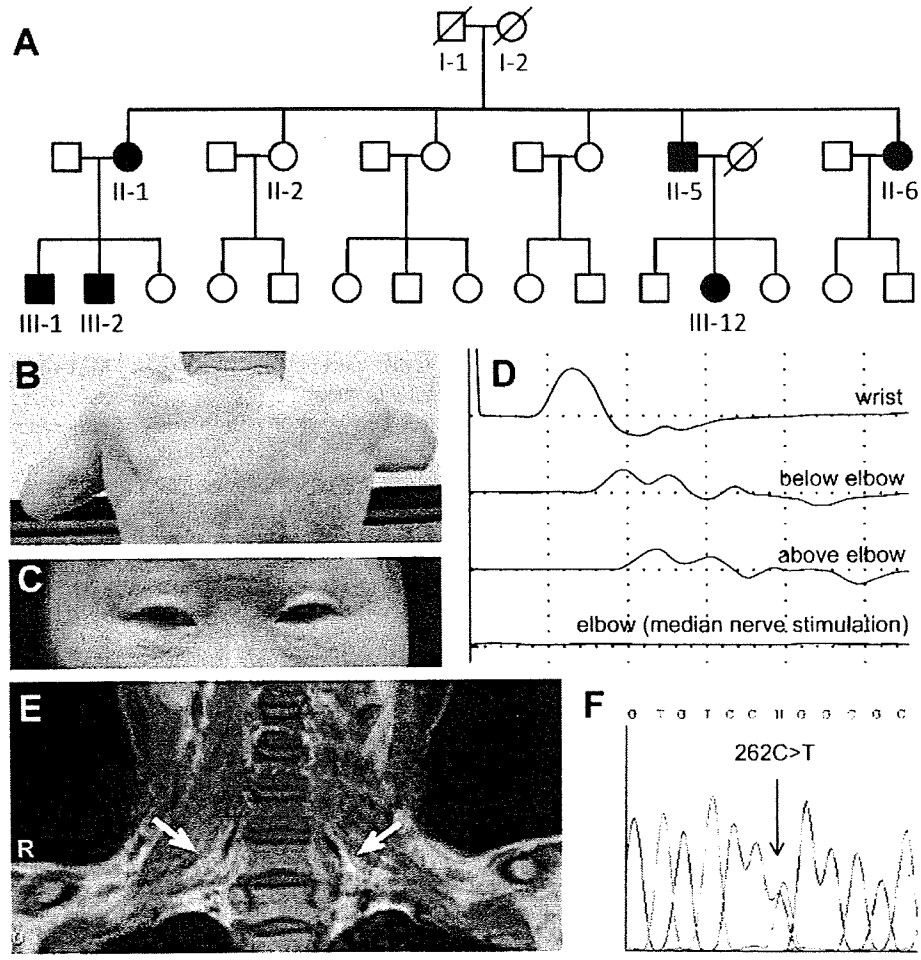



**Figure 1** Clinical findings in patients. (A) Pedigree of the present family. Squares, males; circles, females; filled, affected; diagonal line, deceased. (B) Asymmetrical atrophy and weakness of shoulder girdle muscles. (C) Hypotelorism (close-set eyes) seen in a patient. (D) An abnormal temporal dispersion in the left ulnar motor nerve between the wrist and below the elbow. Simultaneous median nerve stimulation at the elbow. This elicited no action potential in the digital minimal abductor muscle, indicating an absence of Martin–Gruber anastomosis. Scale = 5 mV/division, 5 ms/division. (E) T1-weighted MRI image showing the gadolinium-enhanced lower brachial plexus (arrows) of a patient. (F) A heterozygous *SEPT9* mutation; c.262C>T, in exon 2 of a patient.

episodes (88%), the patients presented with classical relapsing-remitting courses, while two episodes were chronically undulating.

### Electrophysiological findings

The NCS revealed axonal alterations in the upper limbs of all patients and in the lower limbs of a patient with diabetes (table 1). Demyelinating features, such as decreased conduction velocities and prolonged latencies, were documented in five patients. One patient (III-1) showed a partial conduction block at the median nerve between the wrist and elbow. Two patients showed temporal dispersions at the median nerves between the wrist and elbow (Patient II-1) and at the ulnar nerve (Patient II-5, fig 1D). EMG disclosed multifocally or diffusely distributed chronic neurogenic patterns only in the upper limbs of all patients.

**Table 1** Clinical and electrophysiological findings in patients

| Patient | II-1 | II-5 | II-6 | III-1 | III-2 | III-12 |
|---|---|---|---|---|---|---|
| Clinical findings | | | | | | |
| Age (years)/sex | 69/F | 60/M | 58/F | 43/M | 41/M | 32/F |
| Age at the first episode (years) | 28 | 12 | 22 | 18 | 11 | 19 |
| No of episodes | 2 | 3 | 2 | 4 | 4 | 2 |
| Motor weakness at UE (R/L) | −1/−2 | −1/−3 | N/N | −1/−2 | N/−2 | N/−1 |
| Sensory loss at UE (R/L) | N/−2 | −1/−1 | N/N | N/N | N/−2 | N/−1 |
| mRS | 2 | 2 | 0 | 2 | 1 | 1 |
| Nerve conduction study | | | | | | |
| Axonal degeneration changes at UE | + (S) | + (M, S) | + (S) | + (S) | + (M) | + (S) |
| Demyelinative changes at UE | + (M) | + (M) | + (M, S) | + (M) | + (M) | – |
| Focal demyelination at UE | TD | TD | – | CB | – | – |
| Abnormalities at LE | – | + (S) | – | | – | |

−1, −2 and −3 represent mild, moderate, and severe degree of signs.
CB, conduction block; LE, lower extremity; M, motor nerve; mRS, modified Rankin Scale (0, asymptomatic; 1, no significant disability despite symptoms; 2, slight disability; 3, moderate disability; 4, moderately severe disability; 5, severe disability); N, normal; R/L, right/left; S, sensory nerve; TD, temporal dispersion; UE, upper extremity.

### Neuroimaging findings

An MRI scan in one patient (II-6), taken during an acute attack, showed T2 hyperintensities and gadolinium enhancement in the brachial plexus (fig 1E).

### Genetic findings

In all of the HNA patients, sequencing of exon 2 of *SEPT9* revealed the point mutation c.262C>T; p.R88W (fig 1F), but this mutation was not found in the unaffected family member (II-2).

## DISCUSSION

We described a Japanese HNA family with a *SEPT9* R88W mutation, members of which have the following characteristics: (1) the upper-limb nerves, especially the brachial plexus, are selectively involved; (2) hypotelorism is highly diagnostic among minor dysmorphic features; (3) electrophysiological focal demyelination can occur in addition to axonal degeneration changes. The number of relapses and the age at the first episode were not correlated with the patient's recovery and disability. The diabetic state in one patient (II-5), who had the greatest disability, might be a factor aggravating HNA.

The selective brachial plexus neuropathy phenotype in this family is in contrast to the findings of a previous large HNA study showing a high frequency (56%) of clinical involvement of the nerves other than upper-limb nerves.[4] This discrepancy may result from the genetic heterogeneity of HNA. Although this is a family study, another reported HNA family carrying the *SEPT9* R88W mutation also showed selective involvement of the brachial plexus.[5] This *SEPT9* mutation is clearly related to a highly preferential involvement of the brachial plexus among HNA patients.

Focal demyelinations in our electrophysiological findings may be secondary to primary axonal alterations. However, such secondary focal demyelinations are typically transitory. An alternative explanation is that glial components, such as Schwann cells and myelin, are another target in HNA caused by *SEPT9* mutations. Of interest is a previous histological study that showed minor onion bulb formations in an HNA patient,[12] suggestive of Schwann cell alterations. SEPT9 is highly expressed in Schwann cells in peripheral nerves.[13] The pathological *SEPT9* mutation results in dysfunction of Rho/Rhotekin signalling,[13] which is essential for proper myelination[14] and determines T cell function.[15] The highly frequent painful onset, contrast-enhancement of the brachial plexus and some responsiveness to IVIG in our patients suggest an inflammatory contribution to brachial plexus attacks associated with the *SEPT9* mutation. Thus, it is possible that the inflammatory attacks by the genetically altered immune system on peripheral nerves, where Schwann cells abundantly produce mutated SEPT9 proteins, resulted in the focal demyelination.

The present study is the first report of a *SEPT9* mutation in a non-Caucasian population, suggesting a worldwide distribution of *SEPT9* mutations. Wider and more universal recognition of clinical hallmarks and genetic counselling are of diagnostic importance for this potentially inflammatory-mediated and therapeutically interventional disorder.[13]

**Funding:** Supported in part by grants from the Ministry of Health, Labour and Welfare, Japan (JK) and from the Ministry of Education, Culture, Sports, Science and Technology, Japan (NK).

**Competing interests:** None.

**Ethics approval:** Ethics approval was provided by the Ethics Committee of Kyushu University.

**Patient consent:** Obtained.

**Provenance and peer review:** Not commissioned; externally peer reviewed.

## REFERENCES

1. **Klein CJ,** Windebank AJ. Hereditary brachial plexus neuropathy. In: Dyck PJ, Thomas PK, eds. *Peripheral neuropathy*. 4th edn. Philadelphia: Elsevier, 2005:1753–67.
2. **Jeannet PY,** Watts GD, Bird TD, *et al*. Craniofacial and cutaneous findings expand the phenotype of hereditary neuralgic amyotrophy. *Neurology* 2001;**57**:1963–8.
3. **van Alfen N,** van Engelen BG, Reinders JW, *et al*. The natural history of hereditary neuralgic amyotrophy in the Dutch population: two distinct types? *Brain* 2000;**123**:718–23.
4. **van Alfen N,** van Engelen BG. The clinical spectrum of neuralgic amyotrophy in 246 cases. *Brain* 2006;**129**:438–50.
5. **Pellegrino JE,** Rebbeck TR, Brown MJ, *et al*. Mapping of hereditary neuralgic amyotrophy (familial brachial plexus neuropathy) to distal chromosome 17q. *Neurology* 1996;**46**:1128–32.
6. **Kuhlenbäumer G,** Hannibal MC, Nelis E, *et al*. Mutations in SEPT9 cause hereditary neuralgic amyotrophy. *Nat Genet* 2005;**37**:1044–6.
7. **Watts GD,** O'Briant KC, Borreson TE, *et al*. Evidence for genetic heterogeneity in hereditary neuralgic amyotrophy. *Neurology* 2001;**56**:675–8.
8. **Hoque R,** Schwendimann RN, Kelley RE, *et al*. Painful brachial plexopathies in SEPT9 mutations: adverse outcome related to comorbid states. *J Clin Neuromuscul Dis* 2008;**9**:379–84.
9. **Laccone F,** Hannibal M, Neesen J, *et al*. Dysmorphic syndrome of hereditary neuralgic amyotrophy associated with a SEPT9 gene mutation—a family study. *Clin Genet* 2008;**74**:279–83.
10. **van Swieten JC,** Koudstaal PJ, Visser MC, *et al*. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988;**19**:604–7.
11. **American Association of Electrodiagnostic Medicine,** Olney RK. Guidelines in electrodiagnostic medicine. Consensus criteria for the diagnosis of partial conduction block. *Muscle Nerve Suppl* 1999;**8**:S225–9.
12. **Klein CJ,** Dyck PJ, Friedenberg SM, *et al*. Inflammation and neuropathic attacks in hereditary brachial plexus neuropathy. *J Neurol Neurosurg Psychiatry* 2002;**73**:45–50.
13. **Sudo K,** Ito H, Iwamoto I, *et al*. SEPT9 sequence alternations causing hereditary neuralgic amyotrophy are associated with altered interactions with SEPT4/SEPT11 and resistance to Rho/Rhotekin-signaling. *Hum Mutat* 2007;**28**:1005–13.
14. **Stendel C,** Roos A, Deconinck T, *et al*. Peripheral nerve demyelination caused by a mutant Rho GTPase guanine nucleotide exchange factor, frabin/FGD4. *Am J Hum Genet* 2007;**81**:158–64.
15. **Waiczies S,** Bendix I, Prozorovski T, *et al*. Geranylgeranylation but not GTP loading determines rho migratory function in T cells. *J Immunol* 2007;**179**:6024–32.

# Targeted lipidomics reveals mPGES-1-PGE2 as a therapeutic target for multiple sclerosis

Yasuyuki Kihara[a], Takuya Matsushita[b], Yoshihiro Kita[a], Satoshi Uematsu[c], Shizuo Akira[c], Jun-ichi Kira[b], Satoshi Ishii[a,d,1], and Takao Shimizu[a,1]

[a]Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; [b]Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; [c]Immunology Frontier Research Center, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan; and [d]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Tokyo, Japan

Edited by K. Frank Austen, Brigham and Women's Hospital, Boston, MA, and approved October 29, 2009 (received for review June 25, 2009)

**The arachidonic acid (AA) cascade produces eicosanoids, such as prostaglandins (PGs), that regulate physiological and pathological functions. Although various nonsteroidal anti-inflammatory drugs have been developed, blocking upstream components (cyclooxygenase-1 and -2) of the AA cascade leads to severe side effects, including gastrointestinal ulcers and cardiovascular events, respectively, due to the complexity of the AA cascade. Here, using an AA cascade-targeted lipidomics approach, we report that microsomal PGE synthase 1 (mPGES-1) plays a key role in experimental autoimmune encephalomyelitis (EAE). Eicosanoids (mainly $PGD_2$) are produced constitutively in the spinal cord of naive mice. However, in EAE lesions, the $PGE_2$ pathway is favored and the $PGD_2$, $PGI_2$, and 5-lipoxygenase pathways are attenuated. Furthermore, mPGES-$1^{-/-}$ mice showed less severe symptoms of EAE and lower production of IL-17 and IFN-γ than mPGES-$1^{+/+}$ mice. Expression of $PGE_2$ receptors (EP1, EP2, and EP4) was elevated in EAE lesions and correlated with clinical symptoms. Immunohistochemistry on central nervous systems of EAE mice and multiple sclerosis (MS) patients revealed overt expression of mPGES-1 protein in microglia/macrophages. Thus, the mPGES-1-$PGE_2$-EPs axis of the AA cascade may exacerbate EAE pathology. Our findings have important implications for the design of therapies for MS.**

autoimmunity | demyelination | lipid mediator | mass spectrometry | $T_h17$

Multiple sclerosis (MS) is the most prevalent autoimmune disorder of the central nervous system (CNS), with neurological symptoms caused by inflammation and demyelination (1). Studies of experimental autoimmune encephalomyelitis (EAE), an animal model for MS, have shown that autoreactive T cells secreting IL-17 ($T_H17$ cells) and IFN-γ ($T_H1$ cells) are involved in EAE/MS pathogenesis (2–4).

In an effort to understand and treat this complex disease, lipids have emerged as one of the targets for developing drugs. Membrane lipids have been identified as autoantigens in MS and EAE pathologies (5). In addition to membrane components, lipids play roles in cell–cell interaction as autacoids. Arachidonic acid (AA) is released from membrane glycerophospholipids by the action of cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$) (6, 7). Released AA is further converted into prostaglandins (PGs), leukotrienes (LTs), lipoxins, and hydroxy-eicosatetraenoic acids (HETEs), collectively termed eicosanoids, by cyclooxygenases (COXs), lipoxygenases (LOs), and terminal enzymes (8). Involvement of eicosanoids and related lipid mediators has been reported in EAE, collagen-induced arthritis, and other immunological disorders (6, 7, 9). Previously, we and others clearly demonstrated the importance of cPLA$_2\alpha$ in EAE pathology by genetically and pharmacologically ablated mouse studies (10–12). Furthermore, cPLA$_2\alpha$ expression and activities were up-regulated in the spinal cords (SCs) of EAE mice (13). However, it remains totally elusive which eicosanoids downstream of cPLA$_2\alpha$ are involved in the EAE induction and exacerbation. Miyamoto et al. (14) demonstrated that COX-$2^{-/-}$ mice develop EAE with comparable severity to wild-type control mice, while indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), prevents EAE. However, all mice treated with effective doses of indomethacin died of gastrointestinal bleeding (14). On the other hand, 5-LO$^{-/-}$ or 12/15-LO$^{-/-}$ mice developed more severe EAE than control wild-type mice (15), while pharmacological studies showed different results (12). Previous sketchy studies to focus on individual eicosanoids in EAE or MS patients have obvious limitations, as >20 different eicosanoids are produced by the concerted actions of cPLA$_2\alpha$ and downstream enzymes (Fig. S1) (16–18). To overcome such complexity and some controversy, we evaluated the cascade in the SCs of naive and EAE mice by the nonbiased top-down approach (AA cascade-targeted lipidomics and transcriptomics) and confirmed the results from knockout studies.

## Results

**AA Cascade-Targeted Transcriptomics Imply That AA Cascade Plays Important Roles in EAE Pathology.** C57BL/6 mice were immunized with a myelin oligodendrocyte glycoprotein 35–55 ($MOG_{35-55}$) peptide to induce EAE and monitored daily (Fig. 1*A*). As described in refs. 13 and 19, the course of the disease is divided into induction, acute, and chronic phases in accordance with the clinical symptoms (Fig. 1*A*). We collected the SCs, spleen, and plasma of naive mice and EAE mice in these three phases.

To determine which enzymes and receptors in the AA cascade are involved in EAE pathology, AA cascade-targeted transcriptomics of the SCs was performed by quantitative RT-PCR (Fig. 1*B*). Components immediately downstream of cPLA$_2\alpha$ are COX-1/2, 5-LO/FLAP, and 12/15-LO, whose expression levels were highly up-regulated (Fig. 1*B*). Large part of terminal enzymes and receptors, such as microsomal PGE synthase 1 (mPGES-1), were substantially up-regulated in the SCs of EAE mice (Fig. 1*B*). However, expression levels of PGI synthase (PGIS) and lipocalin-type PGDS (L-PGDS) were down-regulated in the induction and acute phases of EAE, respectively, and then returned to the basal levels in the chronic phase (Fig. 1*B*). The $PGI_2$, $PGE_2$, and LT receptor (IP, EP1/2/4, BLT1, and CysLT1) gene expression was up-regulated in the acute phase of EAE (Fig. 1*B*). A correlation between the gene expression and the clinical score was observed for COX-1, H-PGDS, EP1/2/4, BLT1, CysLT1, etc. (Fig. S2). These results imply that AA cascade profoundly affects the pathogenesis of EAE.

**AA Cascade-Targeted Lipidomics Reveals That the $PGE_2$ Pathway Is Favored in EAE Lesions.** The lipidomics approaches disclosed the constitutive production of eicosanoids in the SCs of naive mice and

Author contributions: Y. Kihara, S.I., and T.S. designed research; Y. Kihara, T.M., and Y. Kita performed research; S.U. and S.A. contributed new reagents/analytic tools; Y. Kihara, T.M., and J.-i.K. analyzed data; and Y. Kihara, S.I., and T.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]To whom correspondence may be addressed. E-mail: tshimizu@m.u-tokyo.ac.jp or mame@m.u-tokyo.ac.jp.

This article contains supporting information online at www.pnas.org/cgi/content/full/0906891106/DCSupplemental.

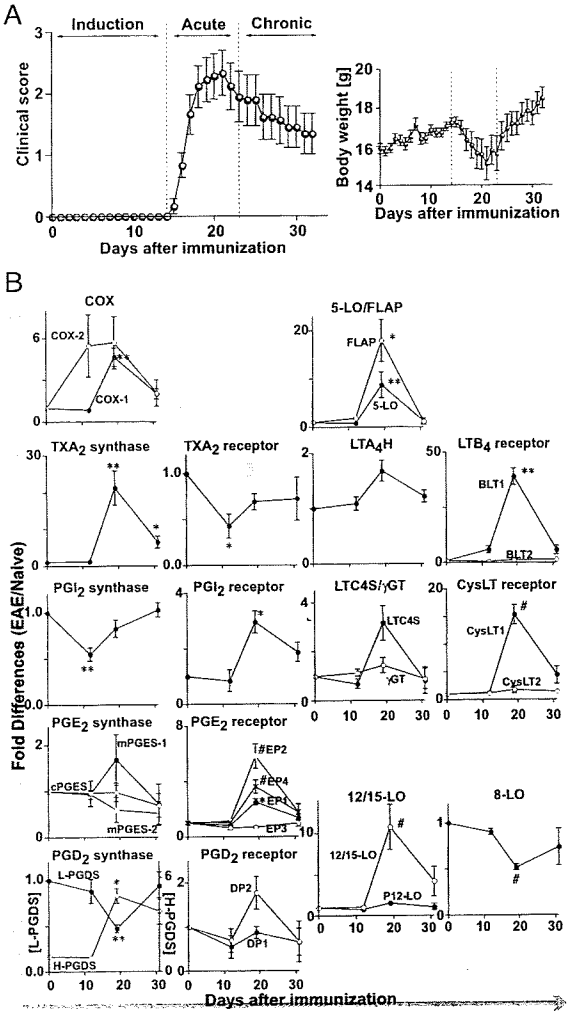



Fig. 1. AA cascade-targeted transcriptomics analysis. (*A*) C57BL/6 female mice were immunized with the $MOG_{35-55}$ peptide. Mice were monitored and weighed daily. Data are the mean clinical score and body weight ± SEM of eight animals. (*B*) Expression of AA cascade related transcripts was estimated using the comparative $C_T$ method with the SCs of naive mice and EAE mice in the induction, acute, and chronic phases ($n$ = 6, 5, 6, and 5 animals, respectively). The relative abundance of mRNA levels in EAE mice compared with naive mice is shown. Data represent means ± SEM. #, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$ compared with naive mice using the Kruskal-Wallis test with Dunn's post-hoc test.

the AA cascade dynamics during EAE (Fig. 2). Approximately 90% of the eicosanoids in the SCs of naive mice were derived from the COX pathway (Fig. S3). The rank order of eicosanoids in the SCs of naive mice was the following: $PGD_2$ and metabolites > $PGE_2$ and metabolites >6-keto-$PGF_{1\alpha}$ > $PGF_{2\alpha}$ and metabolites > cysteinyl LTs >11-HETE >12-HETE >15-HETE > thromboxane $B_2$ ($TXB_2$) > 5-HETE >8-HETE > $LTB_4$ (Fig. 2*A*). Cluster analysis demonstrated pathway-dependent fluctuations in the AA cascade, such as COX (clusters I and III) and LO (cluster II), during the course of EAE (Fig. 2*B*). Eicosanoids belonging to cluster I, such as $PGD_2$ and 6-keto-$PGF_{1\alpha}$, were markedly suppressed in the acute phase and returned to the basal levels in the chronic phase (Fig. 2 *A* and *B*). Suppression of these metabolites was probably caused by the fluctuation of L-PGDS and PGIS, respectively (Fig. 1*B*). 5-LO metabolites ($LTB_4$, $LTC_4$, $LTD_4$, and 5-HETE) belonging to cluster II considerably diminished during the disease course (Fig. 2 *A* and *B*), although the up-regulation of 5-LO pathway components were remarkable (Fig. 1*B*). In the cluster III, the levels of $PGE_2$ and 13, 14-dihydro-15-keto-$PGE_2$ (DHK-$PGE_2$, a major tissue metabolite of $PGE_2$) were increased, thereby making them the major eicosanoids in acute phase SCs instead of $PGD_2$ and its metabolites (Fig. 2*A*). In the chronic phase of EAE, the level of $PGE_2$ remained higher than in naive mice (Fig. 2*A*). Among PGESs, only mPGES-1 was up-regulated in the SCs of EAE mice (Fig. 1*B*), suggesting that this is the key enzyme for the $PGE_2$ accumulation in EAE lesions. Cluster IV consists of only platelet-activating factor (PAF), which is structurally distinct from the eicosanoids. We previously reported that PAF exacerbates inflammation in the chronic phase of EAE by the enhancement of phagocytosis in microglia/macrophages and subsequent production of TNF-α (19). Furthermore, there was a strong correlation between PAF levels and clinical scores of EAE (13, 19). However, in the current study, no eicosanoid was correlated with the clinical scores of EAE. Although we also measured eicosanoid levels in the spleen and plasma, there were only a few fluctuations in these samples (Fig. S4). These results suggest that the AA cascade was strictly regulated in the SCs of naive mice and this regulation was disrupted by EAE pathology.

Next, correlations between the pathways were analyzed to understand the selectivity of the downstream pathways in the AA cascade (Fig. 2*C*, Fig. S5, and Table S1), because the cluster analysis revealed the pathway-dependent fluctuations. The COX pathway (PGs, TXs, and 11-HETE) correlated with the 5-LO pathway (LTs and 5-HETE) in naive mice (Fig. 2*C*). Of note, AA metabolism was shifted into the COX pathway, rather than the 5-LO pathway, in the acute phase of EAE (Fig. 2*C*). Within the COX pathway, the $PGE_2$ pathway was correlated with both the $PGD_2$ and $PGI_2$ pathways in naive mice (Fig. S5 and Table S1). After the induction phase, the $PGE_2$ pathway was correlated with both the $PGD_2$ and $PGI_2$ pathways, while $PGE_2$ production was facilitated, as shown by the small slope (Fig. S5). Correlations between other pathways (Table S1), such as the $PGD_2$ and $PGI_2$ pathways (Fig. S5), were largely conserved throughout the disease course, as indicated by the stable slopes. Taken together, the COX-mPGES-1-$PGE_2$-EP axis may aggravate EAE pathology.

**mPGES-1 Exacerbates EAE Pathology Through $T_H1$ and $T_H17$ Cytokine Production.** To further elucidate the roles of $PGE_2$ pathway in EAE, we particularly focused on mPGES-1, a key enzyme responsible for $PGE_2$ production in inflammation (20–22). In EAE lesions, mPGES-1 was colocalized with F4/80, a marker for macrophages, but not with CD4 (Fig. 3*A*). $PGE_2$ production in SCs of EAE mice was completely blocked by disruption of the mPGES-1 gene (Fig. 3*B*), suggesting that $PGE_2$ production in EAE lesions depends on mPGES-1 expressed in macrophages/microglia. The clinical course of EAE in mPGES-$1^{-/-}$ mice was less severe than in mPGES-$1^{+/+}$ mice (Fig. 3*C*), consistent with the lower cumulative scores in mPGES-$1^{-/-}$ mice (Table S2). To understand the mechanisms underlying the attenuated symptoms of EAE in mPGES-$1^{-/-}$ mice, proliferation and cytokine production of T cells in response to the $MOG_{35-55}$ peptide were investigated (Fig. 3 *D*–*F*). When stimulated with the $MOG_{35-55}$ peptide, cells isolated from lymph nodes (LNs) of immunized mPGES-$1^{+/+}$ and mPGES-$1^{-/-}$ mice proliferated similarly (Fig. 3*D*). However, LN cells from mPGES-$1^{-/-}$ mice produced significantly lower levels of cytokines (TNF-α, IFN-γ, IL-6, and IL-17) than mPGES-$1^{+/+}$ mice (Fig. 3*E*). Flow cytometry of $MOG_{35-55}$-treated $CD4^+$ T cells also revealed that IFN-γ and IL-17 production in mPGES-$1^{-/-}$ mice was reduced (Fig. 3*F*). Taken together, $PGE_2$ derived from mPGES-1 appears to support $T_H1$ and $T_H17$ cytokine production in EAE lesions.