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].
* $P<0.05$.

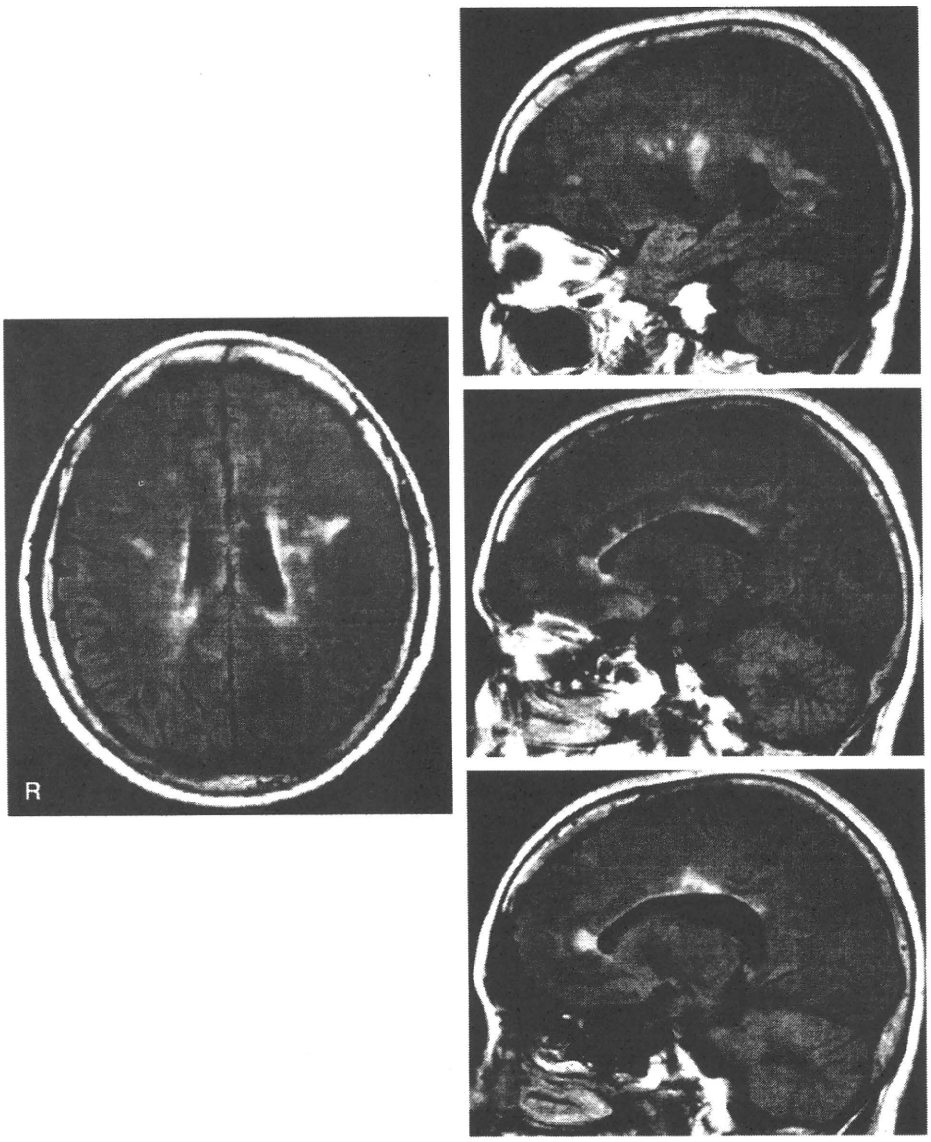


Fig. 1. Axial and sagittal fluid-attenuated inversion recovery (FLAIR) images in an MS patient with a high titre of anti-AQP4 antibody (1:4096). She had 15 years of illness and her EDSS score was 1 at the time of the MRI scan. Note the presence of ovoid periventricular lesions typical of MS.

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.

Clinical/Scientific Notes

Kazumasa Okada, MD, PhD
Takuya Matsushita, MD, PhD
Jun-ichi Kira, MD, PhD
Sadatoshi Tsuji, MD, PhD

B-CELL ACTIVATING FACTOR OF THE TNF FAMILY IS UPREGULATED IN NEUROMYELITIS OPTICA

Neuromyelitis optica (NMO) is characterized by optic neuritis and longitudinally extensive transverse myelitis, and serum NMO-IgG antibody against aquaporin-4 (AQP4) has been implicated in the pathogenesis.[1] It has been reported that NMO patients are often complicated by either serum non-organ-specific autoantibodies and autoimmune diseases such as systemic lupus erythematosus (SLE) or Sjögren syndrome (SS).[1] Efficacy of interferon (IFN)-β for the prevention of NMO relapses is not supported in contrast to that for multiple sclerosis (MS).[2] These findings suggest that humoral immunity plays a pivotal role in the pathogenesis of NMO.

B-cell activating factor of the tumor necrosis factor family (BAFF) is a key molecule involved in the differentiation and survival of B cells, and this molecule also promotes immunoglobulin production.[3] Previous studies have shown that serum level of BAFF is increased and correlates with disease activity and titers of pathogenic autoantibodies in SLE and SS.[3] BAFF is implicated in the establishment and maintenance of autoantibody-associated autoimmune diseases.

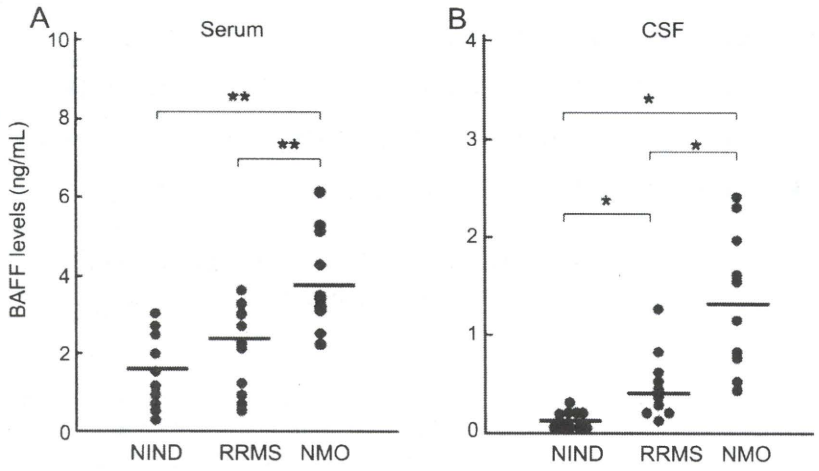
Since it has been speculated that more intense humoral immune responses are involved in the pathogenesis of NMO than MS, we investigated whether BAFF in serum and CSF is increased in NMO.

Methods. Serum and CSF were collected from patients with relapsing-remitting MS (RRMS) and patients with NMO during the acute relapse phase. Patients with NMO included 7 women and 3 men with an age range of 24 to 55 years (median 43), Expanded Disability Status Scale range of 4.0–8.5 (median 6.0), and disease duration range of 2.0–10.5 years (median 5.2) at the sampling of CSF and serum. Patients with RRMS included 5 women and 5 men with an age range of 19–55 years (median 37), EDSS range of 2.0–5.0 (median 3.0), and disease duration range of 2.2–6.3 years (median 4.2) at the sampling of CSF and serum. None of the patients with NMO or RRMS had any history of treatment with any immunosuppressants or IFN-β at the time of sampling. All patients with NMO demonstrated longitudinally extending transverse myelitis (LETM) and serum anti-AQP4 antibody which was assayed using AQP4-transfected HEK293 cells previously described.[4] Two patients with NMO and 2 patients with RRMS were SS-A antibody positive without clinical SS. As a control, we examined 5 women and 5 men with an age range of 28–56 years (median 43) who had various noninflammatory neurologic diseases, including 2 patients with amyotrophic lateral sclerosis, 3 patients with migraine, 3 patients with multiple system atrophy, and 2 patients with Alzheimer disease. Diagnosis of NMO and RRMS was based on the revised criteria for NMO and McDonald criteria for RRMS.[5,6] CSF and serum samples were stored at −80°C until assay. The internal review board of our institution approved the study. BAFF was measured by ELISA kit (Bender MedSystems, Vienna, Austria) according to the manufacturer's protocol. Mann-Whitney test was performed for statistical analysis and $p < 0.05$ was considered significant.

Results. The serum BAFF level was significantly elevated in patients with NMO (mean ± SD: 3.85 ± 1.18 ng/mL) than patients with RRMS (2.28 ± 1.0 ng/mL) and patients with noninflammatory neurologic diseases (NIND) (1.60 ± 0.97 ng/mL) (figure). The CSF BAFF level also was significantly higher in patients with NMO (1.34 ± 0.68 ng/mL) than patients with RRMS (0.49 ± 0.32 ng/mL) and patients with NIND (0.10 ± 0.09 ng/mL). Patients with RRMS show a higher level of CSF BAFF than patients with NIND. There was no significant difference in the serum BAFF level between patients with RRMS and patients with NIND.

Discussion. The finding that patients with NMO showed increased serum levels of BAFF, while patients with RRMS and patients with NIND did not, may support the pivotal roll of B cells and humoral immunity in the pathogenesis of NMO, because BAFF is a key molecule for the differentiation and survival of B cells and immunoglobulin production.[3] Upregulation of BAFF is thought to promote and maintain NMO–immunoglobulin G production in

Figure B-cell activating factor of the tumor necrosis factor family (BAFF) levels in serum (A) and CSF (B)



BAFF levels detected by ELISA in CSF and serum of neuromyelitis optica (NMO) patients (n = 10), relapsing-remitting MS (RRMS) patients (n = 10), and noninflammatory neurologic diseases (NIND) patients (n = 10). The line denotes the mean value. $^{*}p < 0.05$, $^{**}p < 0.01$.

the systemic immune system of patients with NMO. Moreover, the finding may be in line with previous reports of the excellent prevention of NMO relapses by rituximab, which depletes the CD20-positive B cell lineage, and the augmentation of NMO relapses by IFN-β, which induces BAFF and augments systemic autoimmune diseases such as SLE and SS.[2,3,7]

Increased levels of BAFF in CSF were found in both patients with RRMS and patients with NMO. Previous study has shown that BAFF expression is increased in MS lesions and activated astrocytes are a strong source of BAFF.[3] Upregulated BAFF in the CSF may reflect the local production in lesions of the CNS in both RRMS and NMO.

From the Department of Neurology (K.O., S.T.), School of Medicine, University of Occupational and Environmental Health, Kitakyushu; and Department of Neurology (T.M., J.-i.K.), Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Disclosure: Dr. Okada and Dr. Matsushita report no disclosures. Dr. Kira has received funding for travel and speaker honoraria from Bayer Schering Pharma; serves on the editorial boards of Multiple Sclerosis, the *Open Neurology Journal, and* Autoimmune Diseases, *on the editorial advisory board of* The Year in Neurology, *and as a journal editor of* Rinsho Shinkeigaku; *serves as a consultant for Biogen Idec; and receives research support from the Ministry of Health, Labor and Welfare, Japan. Dr. Tsuji reports no disclosures.*

Received July 28, 2009. Accepted in final form October 27, 2009.

Address correspondence and reprint requests to Dr. Kazumasa Okada, Department of Neurology, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan; gion@med.uoeh-u.ac.jp.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Kazumasa Okada.

1. Wingerchuk DM, Lennon VA, Lucchinetti CF, et al. The spectrum of neuromyelitis optica. Lancet Neurol 2007;6:805–815.
2. Krumbholz M, Faber H, Steinmeyer F, et al. Interferon-B increases BAFF levels in multiple sclerosis: implication for B cell autoimmunity. Brain 2008;131:1455–1463.
3. Mackay F, Silveira PA, Brink R. B cells and the BAFF/APRIL axis: fast-forward on autoimmunity and signaling. Curr Opin Immunol 2007;19:327–336.
4. 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.
5. Wingerchuk DM, Lennon VA, Pittock SJ, et al. Revised diagnostic criteria for neuromyelitis optica. Neurology 2006;66:1485–1489.
6. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald criteria." Ann Neurol 2005;58:840–846.
7. Jacob A, Weinshenker BG, Violich I, et al. Treatment of neuromyelitis optica with rituximab. Arch Neurol 2008;65:1443–1448.

Research Paper

Multiple Sclerosis

Multiple Sclerosis
16(2) 147–155

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.

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.[3 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

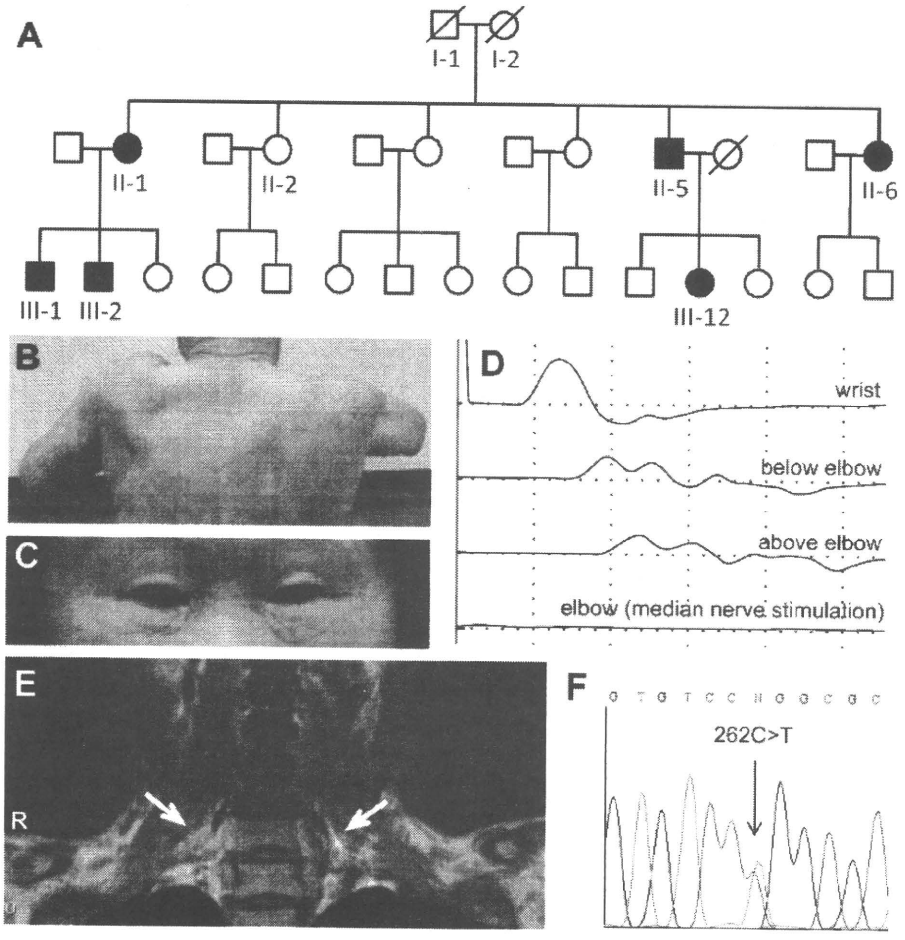


Figure 1 Clinical findings in patients. (A) Pedigree of the present family. Squares, males; circles, females; filled, affected; diagonal line, deceased. (B) Asymmetrical atrophy and weakness of shoulder girdle muscles. (C) Hypotelorism (close-set eyes) seen in a patient. (D) An abnormal temporal dispersion 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.[9] 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.[12]

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.

NeuroImage 51 (2010) 412–420

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Oscillatory gamma synchronization binds the primary and secondary somatosensory areas in humans

Koichi Hagiwara [a,b,1], Tsuyoshi Okamoto [c,1], Hiroshi Shigeto [a,b,*], Katsuya Ogata [b], Yuko Somehara [b], Takuya Matsushita [a], Jun-ichi Kira [a], Shozo Tobimatsu [b]

[a] Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[b] Department of Clinical Neurophysiology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[c] Digital Organ, Digital Medicine Initiative, Kyushu University, Fukuoka, Japan

ARTICLE INFO

Article history:
Received 1 September 2009
Revised 14 December 2009
Accepted 2 February 2010
Available online 10 February 2010

Keywords:
Primary somatosensory cortex (SI)
Secondary somatosensory cortex (SII)
Magnetoencephalography (MEG)
Functional connectivity
Induced gamma
Phase-locking value (PLV)

ABSTRACT

Induced gamma activity has a key role in the temporal binding of distributed cortico-cortical processing. To elucidate the neural synchronization in the early-stage somatosensory processing, we studied the functional connectivity between the primary and secondary somatosensory cortices (SI and SII) in healthy subjects using magnetoencephalography (MEG) with excellent spatiotemporal resolution. First, somatosensory-evoked magnetic fields were recorded to determine the locations of each cortical activity. Then we analyzed the phase-locking values (PLVs) of the induced gamma activity to assess neural synchrony within the somatosensory cortical network. We also assessed PLVs in patients with multiple sclerosis (MS) to validate our PLV analysis in evaluating the inter-areal functional connectivity, which can often be impaired in MS. The PLVs of the induced gamma activity were calculated for each pair of unaveraged MEG signals that represented the activities of the contralateral SI and bilateral SII areas. Analysis of PLVs between the SI and SII areas showed significantly increased PLVs for gamma-band activities, starting at an early post-stimulus stage in normal controls, whereas this increase in PLVs was apparently diminished in MS. The PLV analysis provided evidence for early-latency, gamma-band neuronal synchronization between the SI and SII areas in normal controls. Our study first demonstrates the gamma-band synchrony in the early-stage human somatosensory processing.

Introduction

In the somatosensory system, the primary and secondary somatosensory cortices (SI and SII) comprise the early stage of the hierarchical organization. Numerous neurophysiological studies have examined the functional differences between the two cortical areas in humans. Electrophysiological studies of evoked responses have often reported differences in activation timing between SI and SII (Pons et al., 1987; Mauguière et al., 1997; Inui et al., 2004), with the SI response occurring earlier than the SII responses. There are also studies with direct intracortical recording in epilepsy patients, which proved that SI and SII were activated in a sequential manner (Frot and Mauguière, 1999; Balzamo et al., 2004). The typical characteristics of SII include sensitivity towards higher order functions, such as sensorimotor integrations, attention, unitary body image, and integration of nociceptive and non-nociceptive information (for a review, see Lin and Forss, 2002). Anatomically, it has been shown that the neurons in SII have larger and more complex receptive fields than those in SI (for a review, see Iwamura, 1998). Therefore, SII is often considered to be a hierarchically higher cortical area than SI.

Less is known about the functional connectivity between SI and SII in humans. Apart from the activation sequence of the cortical areas, few studies have evaluated functional connectivity between SI and SII in humans. Recently, it has become evident that neuronal synchronization plays an important role in distributed cortico-cortical processing: induced gamma activity could be related to the temporal binding of spatially distributed information processing in the mammalian brain (Tallon-Baudry and Bertrand, 1999; Engel and Singer, 2001; Buzsáki and Draguhn, 2004; Knight, 2007). To date, however, whether the temporal binding mechanism with the gamma-band activity relates to the somatosensory processing between SI and SII has not been demonstrated. To clarify this issue, we applied magnetoencephalography (MEG) to evaluate neural synchrony in the gamma-band during the median nerve stimulation in healthy subjects. We analyzed phase-locking values (PLVs) of induced gamma activity between the somatosensory cortices. To validate our PLV analysis in evaluating functional connectivity between the distributed cortical areas, we also assessed PLVs in patients with

* Corresponding author. Department of Clinical Neurophysiology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Fax: +81 92 642 5352.
E-mail address: shigetou@neuro.med.kyushu-u.ac.jp (H. Shigeto).
[1] These authors contributed equally to this work.

1053-8119/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2010.02.001

clinically definite multiple sclerosis (MS). MS is known to cause multiple disconnections between distributed regions of the brain owing to demyelination and axonal loss in the central nervous system (Calabrese and Penner, 2007; Dineen et al., 2009; He et al., 2009). Here we provide evidence for early-stage, gamma-band neuronal synchronization between SI and SII for somatosensory information processing in humans.

Methods

Subjects

Twenty-three healthy volunteers (18 women and 5 men, mean age 37.3 ± 10.6 years) and 23 patients with clinically definite MS (18 women and 5 men, mean age 38.8 ± 8.1 years) according to the revised McDonald criteria (Polman et al., 2005) participated in this study. With respect to the MS patients, the mean duration of disease was 10.0 ± 7.3 years, and the mean Kurtzke Expanded Disability Status Scale Score (Kurtzke, 1983) was 2.7 ± 2.7. The clinical courses of the patients were relapsing–remitting in 17 patients, secondary progressive in four patients, and primary progressive in two patients. All the patients showed only mild disability and were ambulatory. On the basis of clinical histories obtained during routine follow-up, clinically evident sensory symptoms were documented in the right arm in 10 patients, and in the left arm, in nine patients. Somatosensory-evoked potentials were measured in 19 patients, and either delayed central conduction time or an absent N20 response was observed in four patients in response to right median nerve stimulation and seven patients in response to left median nerve stimulation. Nineteen patients showed typical brain lesions fulfilling the Barkhof magnetic resonance imaging (MRI) criteria (Barkhof et al., 1997): nine T2 hyperintense lesions present in nineteen patients, at least one infratentorial lesion present in seventeen patients, at least one juxtacortical lesion present in seventeen patients, at least three periventricular lesions present in twenty-two patients. In most patients, their clinical manifestations were usually caused by lesions in the optic nerves, brainstem, cerebellum, and spinal cord. No patients showed cognitive decline. None of the patients had anti-aquaporin-4 antibody, as confirmed by immunofluorescence technique (Matsuoka et al., 2007). Four patients were examined during a relapse period. This study was approved by the local ethics committees of our university, and written consent was obtained from all subjects.

Stimuli

Left and right median nerves were stimulated at the wrist in a separate recording with constant current pulses of 0.2-ms duration. The stimulus intensity was adjusted above the motor threshold to produce slight contraction of the abductor pollicis brevis muscle. At this intensity, large myelinated fibers but not small ones were stimulated. The stimuli were given pseudo-randomly, and the inter-stimulus interval ranged from 2.5 to 3.5 s (mean interval: 3 s) to avoid habituation of SII responses.

Data acquisition and processing

The MEG signals were acquired using a whole-head 306-channel sensor array (Vectorview, ELEKTA Neuromag, Helsinki) that comprises 102 identical triple-sensor elements. Each sensor element consists of two orthogonal planar-type gradiometers and one magnetometer. In this study, we analyzed MEG data recorded by the 204-channel planar-type gradiometers (gradiometers reduce external artifact signals, including geomagnetic signals and other environmental artifact signals). Prior to the recording, four head position indicator (HPI) coils were attached to the scalp, and a 3D digitizer was used to measure anatomical landmarks of the head with respect to the HPI coils. During the recording, subjects lay on a bed in a magnetically shielded room with their heads positioned inside the helmet-shaped sensor array. The precise location of the head with respect to the sensor array was determined using the HPI coils. The recording bandpass filter was 0.03–1500 Hz, and the sampling rate was 5 kHz. The subjects were instructed to keep their eyes open and not to sleep; their vigilance levels were monitored by spontaneous MEG signals over the parieto-occipital areas and a video camera positioned in a shielded room. During the stimulation, we only stored raw data for off-line analysis. A spatiotemporal signal space separation (tSSS) method was applied off-line to the recorded raw data. tSSS is a software method that removes artifact signals arising from outside the sensor helmet (Taulu and Simola, 2006), and thus, theoretically, only artifact-free raw data were stored for further analysis.

Data analysis

Analysis of somatosensory-evoked magnetic fields (SEFs)

First, we analyzed conventional SEFs to determine the sensors representing activities of SI and SII. Off-line averaging of SEFs was performed using the tSSS-reconstructed raw data in the following condition: the analysis epoch was 50 ms before and 250 ms after the stimulus, and 100–120 responses were averaged for each median nerve. The averaged responses were digitally bandpass filtered in the 0.3–150 Hz range with a notch filter of 60 Hz. The prestimulus period from −50 ms to −10 ms was used as a baseline. The peak latencies and amplitudes of SEF waveforms were determined by root-mean-square (RMS) waveforms reconstructed from the two orthogonal gradiometers to better identify the sensors showing the maximal responses (Kida et al., 2006). We analyzed evoked responses generated by three sources: the SI area in the hemisphere contralateral to the median nerve stimulation (cSI) and the bilateral secondary somatosensory areas (cSII and iSII, for contralateral and ipsilateral SII areas, respectively). For responses in cSI, three deflections (i.e., N20m, P35m, and P60m) were recorded. For each of the five deflections (N20m, P35m, P60m, cSII, and iSII), we searched for the sensor with the maximum amplitude, and peak latencies were determined at the time point showing the maximal amplitude. In the somatosensory paradigm used in this study, several cortical areas other than SI and SII could be activated such as the posterior parietal cortex and the mesial cortex of the paracentral lobule (Forss et al., 1994, 1996; Mauguière et al., 1997). Here, we focused on SI and SII responses to elucidate the functional interaction of these somatosensory cortices.

Equivalent current dipoles (ECDs) that explained the most dominant sources of each deflection were calculated by a least-squares fit using approximately 30 channels around the sensor with the maximum response. For analysis of the bilateral SII responses, the ECD of the P35m deflection (if P35m was not apparent, then P60m) was subtracted from the original waveforms using the signal space projection (SSP) method to remove the magnetic fields of cSI, because the isocontour fields of cSII could be hidden by those of cSI. The ECD analysis yielded the three-dimensional locations and strengths of the ECDs in a spherical conductor model. Because MRI was performed only in four subjects from the control group, the centers of the individual head coordinate system were standardized using the center of device coordinate ($x=0$ mm, $y=0$ mm, $z=40$ mm) in both groups. Only the ECDs with goodness-of-fit values exceeding 80% were accepted for statistical analyses.

The peak latencies and amplitudes of the RMS waveforms were examined by Mann–Whitney *U*-test. The correlation between the latencies of cSI and bilateral SII responses was assessed by calculating Pearson's correlation coefficient. A Chi-square test was used to compare the numbers of evoked deflections between normal subjects and MS

patients. A probability level of 0.05 or less was considered to represent a significant difference throughout the statistical examinations.

Phase-locking analysis of gamma-band cortical activity

To evaluate the neuronal synchronization between the SI and SII areas, we calculated the PLVs of the stimulus-related gamma-band activity (Lachaux et al., 1999). First, to analyze oscillatory gamma-band activity, a continuous wavelet transform was applied to the tSSS reconstructed raw data. A temporal-frequency response is given by the temporal convolution of an MEG signal with the wavelet centering at center frequency f_0 and time t:

$$\Phi_k^m(t,f_0) = \int_{-\infty}^{\infty} s_k^m(\tau)\Psi_{f_0}{}^*(\tau - t)d\tau$$

where $s_k^m(\tau)$ represents the signal of kth trial recorded by channel m. In this transformation, we used a complex Morlet wavelet:

$$\Psi_{f_0}(\tau) = \frac{1}{\sqrt{\omega_0}} exp\left(-\frac{\tau^2}{2\sigma^2}\right) exp(i\omega_0\tau)$$

where $\omega_0 = 2\pi f_0$, $\omega_0\sigma = 7$. The wavelet analysis yielded induced gamma-band activities. Epochs for the wavelet transformation ranged from −250 to 350 ms relative to the stimulus onset. Second, we selected a sensor with maximal RMS amplitude of N20m deflection as a sensor representing cSI activity, and sensors with maximal RMS amplitudes of cSII and iSII responses were determined as sensors

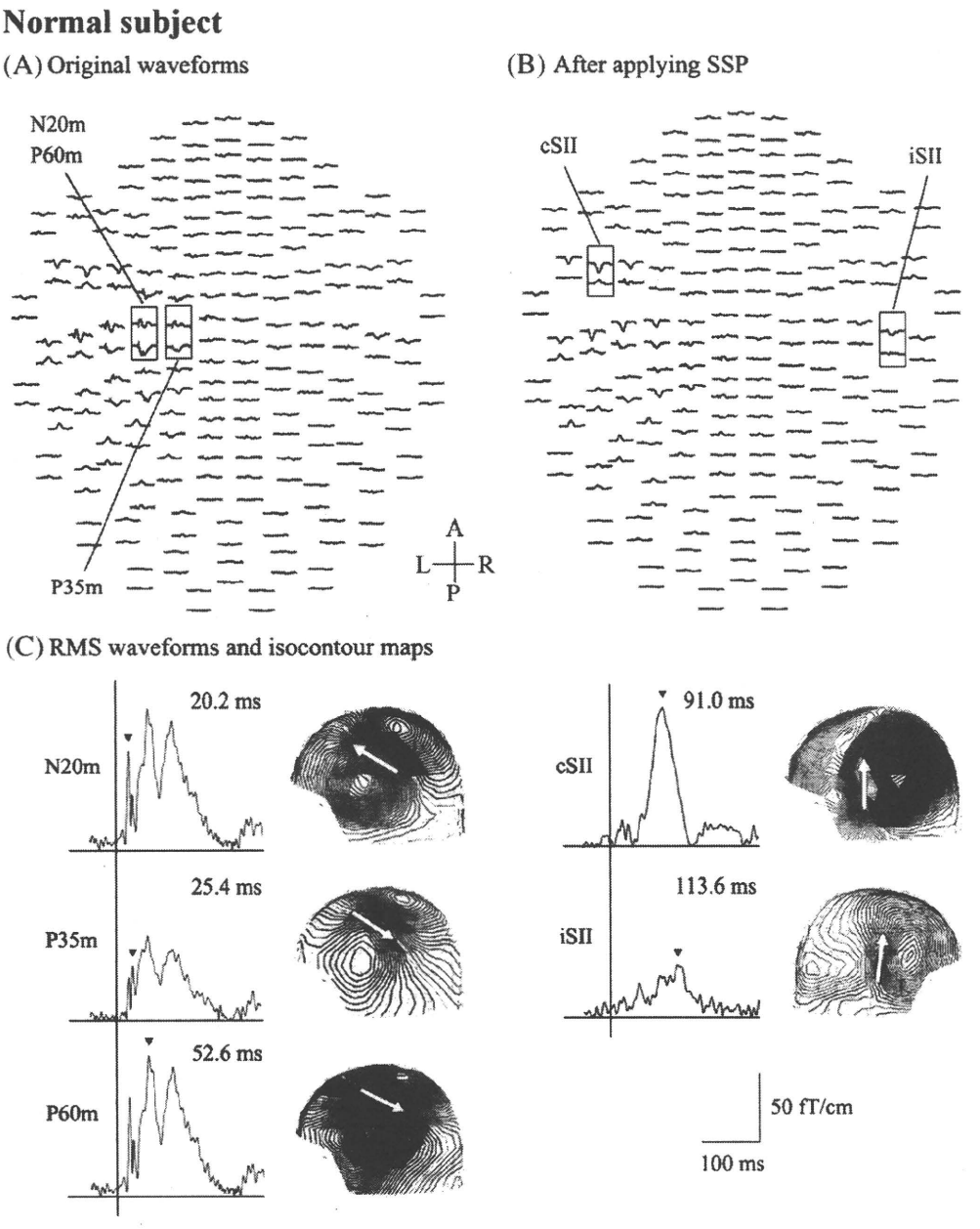


Fig. 1. Analysis of SEFs in a representative normal subject. (A) Original SEF waveforms are averaged from the tSSS reconstructed signals. (B) After subtracting the P35m response from the original waveforms by the SSP method, the sensors show only bilateral SII responses. (C) RMS waveforms at sensors with maximal peak amplitude of each deflection and corresponding field distributions (isocontour maps). The red lines indicate outgoing magnetic signals, while the blue lines ingoing magnetic signals. The arrows denote the current direction of the corresponding ECDs.