pitals of the South Japan MS Genetics Consortium (Coinvestigators). Samples from 265 patients and 158 unrelated healthy controls (HCs) were collected between 1987 and 2010. Among the 265 patients, 73 were positive for an anti-aquaporin-4 (AQP4) antibody while 192 were negative. Sixty-five patients positive for anti-AQP4 antibody and 13 patients negative for the antibody met the NMO criteria[5] and were regarded as patients with NMO. Additionally, the present patients, excluding 20 patients with primary progressive MS, were also classified into 107 conventional MS (CMS) and 138 opticospinal MS (OSMS) including spinal and brainstem-spinal MS.[7]

The SNP rs6897932 in the IL-7RA gene was genotyped by real-time PCR using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA). The genotype of each sample was defined automatically by allele-specific fluorescence, using an ABI Prism 7500 sequence detection system (Applied Biosystems). The test for Hardy-Weinberg equilibrium demonstrated that the HCs and cases of all the other subgroups were in allelic population equilibrium. The allele and genotype frequencies among the groups were compared using Fisher exact test. Uncorrected $p$ values were corrected by Bonferroni-Dunn correction to calculate corrected $p$ ($p_{corr}$) values.

***Standard protocol approvals, registrations, and patient consents.*** This study was approved by each institutional ethics committee. Informed consent was obtained from all participants.

**Results and discussion.** The frequencies of both the C allele and the CC genotype of SNP rs6897932 in the *IL-7RA* gene in patients with non-NMO MS were significantly higher than those of HCs (table). However, there was no significant difference in the frequency of either the C allele or the CC genotype between HCs and patients with NMO. The frequencies of both the C allele and the CC genotype were significantly higher in patients with CMS than in HCs (90.65% vs 79.75%, $p_{corr} = 0.0020$, odds ratio [OR] = 2.46, 95% confidence interval [CI] 1.44–4.21, and 81.31% vs 63.29%, $p_{corr} = 0.0048$, OR = 2.52, 95% CI 1.41–4.52, respectively), but not in patients with OSMS (87.68% vs 79.75%, and 75.30% vs 63.29%, respectively, $p_{corr} > 0.05$).

This study revealed a significant association of the SNP rs6897932 of *IL-7RA* gene with non-NMO MS in Japanese populations. In a case-control study conducted by the International Multiple Sclerosis Genetics Consortium among white populations, the C allele of rs6897932 was shown to be a susceptibility allele for MS, with an OR of 1.18 (1.11–1.26),[3] while a North European group reported similar findings; the OR for the C allele was 1.32 (1.11–1.54).[2] By contrast, our OR for the C allele in non-NMO MS was much higher than those in the abovementioned studies, although the number of participants in this study was small. We assume that this allele is a much stronger risk factor for non-NMO MS in Asians than in Caucasians. Moreover, since in the present series we found no significant difference in either allele or phenotypic frequency of *HLA-DRB1*1501* between non-NMO MS and HC (12.6% vs 8.9% and 23.5% vs 17.7%, respectively, $p_{corr} > 0.05$), we suggest that in Asians, the effect of rs6897932 in IL-7RA is greater than that of *HLA-DRB1*1501*, which is the strongest MS susceptibility gene allele in Caucasians. Finally, because of the rar-

Table Allele and genotype frequencies for *IL-7RA* SNP rs6897932 among patients with NMO, non-NMO MS, and healthy controls

| | HC (n = 158), n (%) | NMO (n = 78), n (%) | NMO vs HC, C/T[a] $p_{corr}$ | NMO vs HC, C/T[a] OR (95% CI) | Non-NMO MS (n = 187), n (%) | Non-NMO MS vs HC, C/T[a] $p_{corr}$ | Non-NMO MS vs HC, C/T[a] OR (95% CI) |
|---|---|---|---|---|---|---|---|
| **Allele frequencies** | | | | | | | |
| T allele | 64 (20.25) | 20 (12.82) | 0.1644 | 1.73 (1.00–2.97) | 40 (10.70) | 0.0018[b] | 2.12 (1.38–3.25) |
| C allele | 252 (79.75) | 136 (87.18) | | | 334 (89.30) | | |
| | | | NMO vs HC, CC/TT+TC[c] | | | Non-NMO MS vs HC, CC/TT+TC[c] | |
| **Genotype frequencies** | | | | | | | |
| TT | 6 (3.80) | 0 (0.00) | 0.3180 | 1.68 (0.92–3.07) | 0 (0.00) | 0.0056[b] | 2.13 (1.32–3.43) |
| TC | 52 (32.91) | 20 (25.64) | | | 40 (21.39) | | |
| CC | 100 (63.29) | 58 (74.36) | | | 147 (78.61) | | |

Abbreviations: CI = confidence interval; HC = healthy controls; *IL-7RA* = interleukin-7 receptor $\alpha$ gene; MS = multiple sclerosis; NMO = neuromyelitis optica; OR = odds ratio; $p_{corr}$ = corrected $p$ value; SNP = single nucleotide polymorphism.

[a] $\chi^2$ Test was used to see the effect of C allele vs T allele.

[b] Significant.

[c] Fisher exact test was used to assess the statistical significance of CC vs TT+TC (recessive model for C).

ity of NMO, its sample size was not large in the present study and larger cohort studies are required to confirm our findings.

*These authors contributed equally.

*From the Department of Neurology (L.F., N.I., S.Y., T.Y., T.M., K.M., H.D., J.K.), Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka; Department of Clinical Neuroscience and Therapeutics (K.O.), Hiroshima University Graduate School of Biomedical Sciences, Hiroshima; Department of Neurology (K.M.), Kinki University School of Medicine, Osaka; and Department of Geriatric Medicine, Medicine and Bioscience (Y.K.), Graduate School of Medicine, Ehime University, Matsuyama, Japan.*

*Study funding: Supported in part by a Japan-China Sasakawa Medical Fellowship, a grant (H20-Nanchi-Ippan-016) from the Research Committees of Neuroimmunological Diseases, Ministry of Health, Labour and Welfare, Japan, and a grant-in-aid (B; no. 22390178) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.*

*Disclosure: Dr. Fang receives research support from a Japan-China Sasakawa Medical Fellowship. Dr. Isobe, Dr. Yoshimura, and Dr. Yonekawa report no disclosures. Dr. Matsushita has received speaker honoraria from Bayer Schering Pharma and Mitsubishi Tanabe Pharma Corporation; and receives research support from Bayer Schering Pharma and the Ministry of Health, Labour and Welfare of Japan. Dr. Masaki, Dr. Doi, Dr. Ochi, Dr. Miyamoto, and Dr. Kawano report no disclosures. Dr. Kira has received funding for travel and/or speaker honoraria from Bayer Schering Pharma and Biogen Idec; serves as a consultant for Biogen Idec; serves on the editorial board of* Multiple Sclerosis, The Open Neurology Journal, *and* Journal of the Neurological Sciences; *serves as a consultant for Biogen Idec; and receives research support from the Ministry of Health, Labour and Welfare, Japan, the Japan Science and Technology Agency, and the Ministry of Education, Science, Sports and Culture, Japan.*

*Received November 1, 2010. Accepted in final form March 2, 2011.*

*Address correspondence and reprint requests to Dr. Jun-ichi 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*



## AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Le Fang, Dr. Noriko Isobe, and Dr. Satoshi Yoshimura.

## COINVESTIGATORS

Susumu Kusunoki, MD, PhD (Kinki University, Chairman), Saburo Sakoda, MD, PhD (Osaka University, Chairman), Tatsuo Kohriyama, MD, PhD (Hiroshima University, Site Investigator), Masayasu Matsumoto, MD, PhD (Hiroshima University, Chairman), Takeshi Kanda, MD, PhD (Yamaguchi University, Chairman), Tetsuro Miki, MD, PhD (Ehime University, Chairman), Kazumasa Okada, MD, PhD (University of Occupational and Environmental Health, Site Investigator), and Sadatoshi Tsuji, MD, PhD (University of Occupational and Environmental Health, Chairman).

## ACKNOWLEDGMENT

The authors received technical support from the Research Support Center, Graduate School of Medical Sciences, Kyushu University.

1. Gregory SG, Schmidt S, Seth P, et al. Interleukin 7 receptor a chain (IL7R) shows allelic and functional association with multiple sclerosis. Nat Genet 2007;39:1083–1091.
2. Lundmark F, Duvefelt K, Iacobaeus E, et al. Variation in interleukin 7 receptor α chain (IL7R) influences risk of multiple sclerosis. Nat Genet 2007;39:1108–1113.
3. International Multiple Sclerosis Genetics Consortium. Risk alleles for multiple sclerosis identified by a genome-wide study. N Engl J Med 2007;357:851–862.
4. Chen X, Fang L, Song S, et al. Thymic regulation of autoimmune disease by accelerated differentiation of Foxp3 + regulatory T cells through IL-7 signaling pathway. J Immunol 2009;183:6135–6144.
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. 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.

Genes and Immunity (2011) 12, 523–530

www.nature.com/gene

ORIGINAL ARTICLE

# SNP-based analysis of the HLA locus in Japanese multiple sclerosis patients

JP McElroy[1], N Isobe[2], PA Gourraud[1], SJ Caillier[1], T Matsushita[3], T Kohriyama[4], K Miyamoto[5], Y Nakatsuji[6], T Miki[7], SL Hauser[1], JR Oksenberg[1] and J Kira[2]

[1]*Department of Neurology, University of California, San Francisco (UCSF), San Francisco, CA, USA;* [2]*Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;* [3]*Department of Clinical Neuroimmunology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;* [4]*Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan;* [5]*Department of Neurology, Kinki University School of Medicine, Osaka, Japan;* [6]*Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan and* [7]*Department of Geriatric Medicine, Graduate School of Medicine, Ehime University, Ehime, Japan*

*Although several major histocompatibility complex (MHC)-wide single-nucleotide polymorphism (SNP) studies have been performed in populations of European descent, none have been performed in Asian populations. The objective of this study was to identify human leukocyte antigen (HLA) loci associated with multiple sclerosis (MS) in a Japanese population genotyped for 3534 MHC region SNPs. Using a logistic regression model, two SNPs (MHC Class III SNP rs422951 in the NOTCH4 gene and MHC Class II SNP rs3997849, susceptible alleles A and G, respectively) were independently associated with MS susceptibility (204 patients; 280 controls), two (MHC Class II SNP rs660895 and MHC Class I SNP rs2269704 in the NRM gene, susceptible alleles G and G, respectively) with aquaporin-4− (AQP4−) MS susceptibility (149 patients; 280 controls) and a single SNP (MHC Class II SNP rs1694112, susceptible allele G) was significant when contrasting AQP4+ against AQP4− patients. Haplotype analysis revealed a large susceptible association, likely DRB1*04 or a locus included in the DRB1*04 haplotype, with AQP4− MS, which excluded DRB1*15:01. This study is the largest study of the HLA's contribution to MS in Japanese individuals.*

Genes and Immunity (2011) **12,** 523–530; doi:10.1038/gene.2011.25; published online 9 June 2011

**Keywords:** *multiple sclerosis; HLA; Japanese; MHC; AQP4*

## Introduction

Multiple sclerosis (MS) is the prototypic disease of central nervous system (CNS) myelin and is considered to be autoimmune in origin. Although the exact cause of MS is unknown, there is an unequivocal, if partial, genetic contribution to its pathogenesis.[1,2] Although several loci with modest replicable effects on MS susceptibility have been identified (mostly in studies of populations of European descent), the only genetic region identified with a large, consistently replicated effect in all populations is the human leukocyte antigen (HLA) region on chromosome 6p21.3. The primary signal arises from the *HLA-DRB1* gene in the Class II segment of the locus, more specifically the **15:01* allele of *DRB1*, but complex hierarchical allelic effects, copy number and *cis–trans* regulatory interactions across the entire region, including a protective signal in the Class I region, have been reported as well.[3–9]

Correspondence: Dr JP McElroy, Department of Neurology, University of California, UCSF, 513 Parnassus Avenue, Room S-256, Box 0435, San Francisco, CA 94143, USA.
E-mail: Joseph.P.McElroy@gmail.com
Received 14 January 2011; revised and accepted 5 April 2011; published online 9 June 2011

MS is relatively rare in Asians, but clinical heterogeneity is worth noting. Some have a disease, termed conventional MS (CMS), which is indistinguishable from MS in western countries (disseminated lesions in the CNS), whereas others have a variant, termed opticospinal MS (OSMS), which involves predominantly the optic nerve and spinal cord.[10] The exact relationship between CMS and OSMS is uncertain; OSMS might represent a true variant of CMS or a phenocopy that is biologically unrelated to CMS. HLA data suggest that the two forms are immunogenetically distinct. In studies of the HLA in Japanese MS populations, CMS was associated with *HLA-DRB1*15:01*,[10] whereas OSMS was associated with the centromeric *HLA-DPB1* locus,[10–12] both Class II major histocompatibility complex (MHC) genes. Recently, autoantibodies against the cell membrane water channel aquaporin-4 (AQP4), a specific biomarker for neuromyelitis optica (NMO), were identified in a proportion of patients with OSMS, leading to a reclassification of this entity based on seropositivity to AQP4. Because the distinction between MS and NMO (an inflammatory disease affecting only the optic nerves and spinal cord) and their etiologies is not clear, especially in the Japanese population where there is a higher relative prevalence of OSMS,[13] a well-defined biomarker such as AQP4 seropositivity may be more useful for stratification

for genetic analyses. Indeed, in Japanese populations, *HLA-DRB1*12* was found to be a risk factor for anti-AQP4 antibody-positive patients, but not antibody-negative MS.[14] However, a comprehensive investigation of the entire HLA region in Japanese MS has yet to be performed.

The advent of large-scale single-nucleotide polymorphism (SNP)-based genotyping allowed broad analyses of the HLA region in MS. For example, several whole-genome association studies have been completed in individuals of European descent,[15–23] which included thousands of SNPs in the HLA region. Most of these studies validated the strong Class II *HLA-DRB1*15:01* association with disease susceptibility, as well as HLA Class I[20,24] and a *DRB1*03:01-DQB1*02:01* (ref. 23) associations. In addition, a few studies have focused exclusively on SNPs in the HLA region using customized arrays, confirming the presence of a disease locus in the Class I region.[6,9,24] To date, significant Class I associations have not been identified in Japanese MS. The unique patterns of linkage disequilibrium (LD) between different ethnic groups have represented a powerful means to delineate causative disease-associated variants in the HLA region.[25–27] The objective of this study is to identify HLA loci associated with MS in a Japanese data set using a high-density SNP array. In addition, because of the complexities of defining disease subclasses and the resulting sizes of those subclasses in this population, a biomarker (presence or absence of autoantibodies against AQP-4) was used instead to stratify the population for further refinement of genetic associations.

## Results

*Population parameters*

A total of 280 healthy controls (HC) and 204 individuals with MS with data for 3534 HLA region SNPs remained after all quality control (QC) steps (Table 1). In all, 46% of the HC and 75% of the individuals with MS were women (gender was included as a covariate for all analyses). A total of 55 of all patients were positive for anti-AQP4 antibody (AQP4+). Among 193 cases that fulfill the revised McDonald criteria,[28] 51 cases (26.4%) are positive for the anti-AQP4 antibody.

**Table 1** Clinical phenotypes

| | *Anti-AQP4Ab+* (n=*55*) | | *Anti-AQP4Ab–* (n=*149*) | |
|---|---|---|---|---|
| | *NMO*[a]+ (n=*38*) | *NMO–* (*n*=*17*) | *NMO+* (*n*=*8*) | *NMO–* (*n*=*141*) |
| McDonald criteria[b] (+) (*n*=193) | 35 | 16 | 8 | 134 |
| CMS (*n*=110) | 9 | 9 | 1 | 91 |
| OSMS (*n*=52) | 25 | 3 | 7 | 17 |
| Others[c] (*n*=31) | 1 | 4 | 0 | 26 |
| McDonald criteria (–) and criteria for CIS[d] (+) (*n*=11) | 3 | 1 | 0 | 7 |

Abbreviations: Ab, antibody; AQP4, aquaporin-4; CIS, clinically isolated syndrdome; CMS, conventional multiple sclerosis; NMO, neuromyelitis optica; OSMS, opticospinal multiple sclerosis.
[a]The revised criteria for NMO by Wingerchuk *et al.*[50]
[b]The revised McDonald criteria by Polman *et al.*[28]
[c]Those cases who fulfill the revised McDonald criteria, but did not meet the criteria for CMS or OSMS.
[d]CIS criteria by Dalton *et al.*[41]

Multidimensional scaling was used to determine the ethnic relationship between the Japanese MS cohort and 12 HapMap populations. By plotting the first by the second dimension, all patients and controls cluster with the HapMap Japanese and Chinese populations (Supplementary Figure 1), verifying that they are indeed East Asian. Plotting all informative dimensions ($n=7$) separately, patients and controls always cluster with the HapMap Japanese population, including dimension 7, which separates the Chinese from the Japanese populations (Figure 1), verifying that the experimental sample is non-Chinese East Asian (genotyping data for other East Asian populations was not available).

*Association analyses*

The two SNPs typically used to infer *DRB1*15* status (rs3135388 and rs9271366 (ref. 29)) were not useful for determining **15:01* carrier status and copy number in this data set. The SNP rs3135388 is fixed for the G allele in the current population, as well as the HapMap Japanese population, and rs9271366 captured in our data set both the **15:01* and **15:02* alleles. The association between rs9271366 and all MS and AQP4– MS was not significant (comparison-wise $P=0.33$ and 0.15, respectively). Across the (classical) HLA-typed individuals, 48% of the **15* alleles in the data set were **15:02*, indicating that the **15:02* alleles are likely diluting the risk effect of the **15:01* alleles for this particular SNP. Therefore, only *DRB1*15:01* presence or absence data, from *DRB1*15:01*-specific primers,[25] was used for the experimental sample and was moderately associated with MS ($P=0.014$) and AQP4– MS ($P=0.01$); however, this association was modest compared with the SNP associations (see below: trend test, $P=10^{-6}$–$10^{-7}$).

Two SNPs were associated with MS using the iterative model (Table 2). Rs422951 in the Class III region had the most significant association with MS ($P=2.9\times10^{-6}$; odds ratio (OR)=0.4; 32 296 360 bp) (Supplementary Figure 2A). This SNP results in a missense mutation in *NOTCH4*. After fitting rs422951 into the model, rs3997849 was the most significant SNP ($P=8.1\times10^{-5}$; OR=0.5; 32 790 379 bp). The SNP rs3997849 is in the HLA Class II region, closest to the *HLA-DQA2* gene (26 761 bp away). When fitting both SNPs as covariates in the model, no other SNPs were significant for MS at false discovery rate (FDR) $P<0.1$.

Two SNPs were significant for AQP4– patients vs controls using the iterative model (Table 2). SNP rs660895 was significant ($P=6.2\times10^{-7}$; OR=2.23; 32 685 357 bp) with no SNPs in the model (Supplementary Figure 2B). This SNP is in the Class II region and is closest to the *HLA-DRB1* and *HLA-DRB5* genes (19 817 bp away). After fitting rs660895 into the model, rs2269704 was the most significant SNP ($P=1.64\times10^{-4}$; OR=0.27; 30 764 931 bp). This SNP is in the Class I region and is in an intron of the *NRM29/NRM* gene. After fitting these two SNPs, no other SNPs were significant for AQP4– MS susceptibility at FDR $P<0.1$.

Only a single SNP, rs1694112, was significant ($P=1.34\times10^{-5}$; OR=3.30; 32 757 641 bp) when contrasting AQP4– vs AQP4+ patients (Supplementary Figure 2C). This SNP is in the Class II region and is closest to

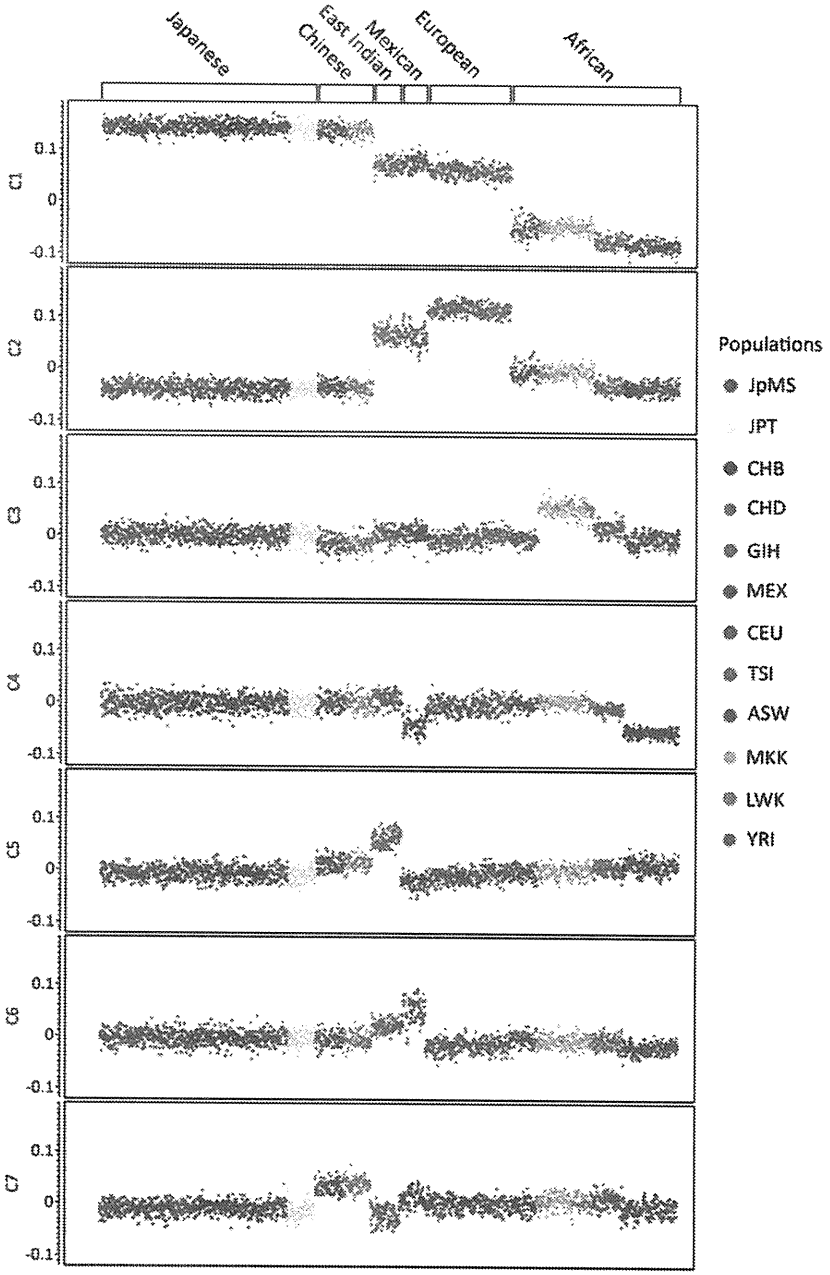


**Figure 1** Plot of each of the first seven multidimensional scaling dimensions individually. The Japanese MS (JpMS) sample always clusters with the HapMap Japanese population, including dimension 7, which separates the Chinese from the Japanese populations. The *y* axis is the value for each of the first seven dimensions. ASW, African ancestry in Southwest USA; CEU, CEPH (NW European); CHB, Han Chinese in Beijing, China; CHD, Chinese in Denver, Colorado; GIH, Gujarati Indians in Houston, Texas; JPT, Japanese in Tokyo, Japan; LWK, Luhya in Webuye, Kenya; MEX, Mexican ancestry in Los Angeles, California; MKK, Maasai in Kinyawa, Kenya; TSI, Tuscan in Italy; YRI, Yoruban in Ibadan, Nigeria.

the *HLA-DQB1* gene (15 197 bp away). No SNPs were significant (FDR $P>0.1$) when contrasting AQP4+ patients vs controls.

*Haplotype analyses*

Haplotype analyses were performed to further clarify the roles of the significant SNPs in tagging causative variation (Table 3). For MS, using a haplotype dosage model, three Class II–class III haplotypes (rs422951–rs3997849: G–G, G–A, A–A) were at least suggestively (comparison-wise $P<0.1$) associated in the resistant direction (OR $<1$), and the remaining haplotype (rs422951–rs3997849: A–G) was significantly associated in the susceptible direction. All three 'resistant' haplotypes are significantly associated with resistance when fit together in the multivariate model. For AQP4–demyelinating patients vs controls, two haplotypes (rs660895–rs2269704: A–G, A–A) were significantly associated in the resistant direction, whereas a single haplotype (rs660895–rs2269704: G–G) was associated

**Table 2** SNPs significantly (FDR $P = 0.1$) associated with MS in the Japanese sample

| *Comparison* | N *SNPs in model* | *SNP* | *Raw P-value* | *FDR P* | *Odds ratio* | *Susceptible allele* | *Position* | *Class* | *Closest gene* | *Distance to closest gene (bp)* |
|---|---|---|---|---|---|---|---|---|---|---|
| MS vs cont. | 0 | rs422951 | 2.94E−06 | 6.08E−03 | 0.4 | A | 32 296 360 | III | *NOTCH4* | 0 |
| MS vs cont. | 1 | rs3997849 | 8.07E−05 | 5.59E−02 | 0.5 | G | 32 790 379 | II | *HLA-DQA2* | 26 761 |
| AQP4− vs cont. | 0 | rs660895 | 6.23E−07 | 6.61E−04 | 2.23 | G | 32 685 357 | II | *HLA-DRB1, HLA-DRB5* | 19 817 |
| AQP4− vs cont. | 1 | rs2269704 | 1.64E−04 | 8.49E−02 | 0.27 | G | 30 764 931 | I | *NRM29,NRM* | 0 |
| AQP4+ vs AQP4− | 0 | rs1694112 | 1.34E−05 | 4.74E−02 | 3.3 | G | 32 757 641 | II | *HLA-DQB1* | 15 197 |

Abbreviations: AQP, aquaporin-4; FDR, false discovery rate; LD, linkage disequilibrium; MS, multiple sclerosis; SNP, single-nucleotide polymorphism.
The most significant SNP from each iteration was fit as a covariate for the remaining iterations (*N* SNPs in Model) to remove associations resulting from LD with that SNP. Gender was an additional covariate in all iterations. Minor allele is reference for odds ratios. MS vs Cont = all patients ($n = 204$) vs controls ($n = 280$); AQP4− vs controls = AQP4− patients ($n = 149$) vs controls ($n = 280$); AQP4+ vs AQP4− = AQP4+ patients ($n = 55$) vs AQP4− patients ($n = 149$).

**Table 3** Haplotype associations with MS susceptibility

| *Comparison* | *SNPs* | *Haplotype* | *OR* | P-*value* | *Frequency* |
|---|---|---|---|---|---|
| (a) *Individual haplotype dosage* | | | | | |
| MS vs controls | rs422951–rs3997849 | GG | 0.41 | 3.46E−06 | 0.18 |
| MS vs controls | rs422951–rs3997849 | AG | 2.45 | 5.17E−10 | 0.6 |
| MS vs controls | rs422951–rs3997849 | GA | 0.33 | 8.46E−02 | 0.01 |
| MS vs controls | rs422951–rs3997849 | AA | 0.62 | 5.18E−03 | 0.21 |
| AQP4− vs controls | rs660895–rs2269704 | GG | 2.32 | 9.04E−08 | 0.3 |
| AQP4− vs controls | rs660895–rs2269704 | AG | 0.71 | 2.12E−02 | 0.6 |
| AQP4− vs controls | rs660895–rs2269704 | GA | 0.43 | 2.76E−01 | 0.01 |
| AQP4− vs controls | rs660895–rs2269704 | AA | 0.22 | 1.93E−06 | 0.09 |

| *Comparison* | *SNPs* | *Haplotype* | *OR* | P-*value* |
|---|---|---|---|---|
| (b) *Multivariate model* | | | | |
| MS vs controls | rs422951–rs3997849 | GG | 0.33 | 4.01E−08 |
| MS vs controls | rs422951–rs3997849 | GA | 0.25 | 3.06E−02 |
| MS vs controls | rs422951–rs3997849 | AA | 0.49 | 4.49E−05 |
| AQP4− vs controls | rs660895–rs2269704 | AG | 0.5 | 1.22E−05 |
| AQP4− vs controls | rs660895–rs2269704 | AA | 0.13 | 1.54E−09 |

Abbreviations: AQP, aquaporin-4; MS, multiple sclerosis; OR, odds ratio; SNP, single-nucleotide polymorphism.
Haplotypes formed from the two SNPs significant for each for MS and AQP4− MS. (a) Association of dosage of each haplotype with MS susceptibility (MS vs controls) or AQP4− MS susceptibility (AQP4− vs controls). (b) Multivariate model with all three susceptible haplotypes for all MS and both significant susceptible haplotypes for AQP4− MS. Gender was fit as a covariate in all models.

with susceptibility. Both resistant haplotypes were highly significant when fit in the multivariate model.

*Haplotype tagging of* DRB1 *alleles*

As the *HLA-DRB1* gene, and specifically the **15:01* allele, has been shown in populations of European descent and in Japanese to have an association with MS susceptibility, the two-locus haplotypes associated with MS and the single SNP associated with AQP4− vs AQP4+ patients were scrutinized for their ability to tag *DRB1* alleles in the subset of individuals ($n = 218$) with *DRB1* data (Supplementary Table 1; Supplementary Figure 3). For the MS group, the most significantly associated and only susceptible haplotype was AG (rs422951–rs3997849). This haplotype captures 100% of the *DRB1*15:01* alleles, which may explain part of the association with MS, but also captures most of the **08:03*, **08:02* and **04* alleles. The association of this haplotype with MS could be due to any single allele or combinations of these *DRB1* alleles. Interestingly, the 53% of the A–A resistant haplotypes contain *DRB1*15:02* alleles, and all of the **15:02* alleles are captured by this haplotype. The GG haplotype, which is the most associated resistant haplotype, is fairly evenly split between three *DRB1* alleles: **01:01*, **13:02* and **09:01*. The G–A haplotype is at such a low frequency in the population (1.4%) that interpretations of *DRB1* allele tagging for this haplotype are likely not meaningful.

For the AQP4− vs HC comparisons, the most significantly associated and only susceptible haplotype was GG (rs660895–rs2269704). Interestingly, this haplotype excludes *DRB1*15:01*, but includes all of the **08:02* and **12:02* alleles and most of the **04* alleles. The most associated resistant haplotype (A–A) mostly contains

**13:02* alleles (sensitivity = 75%; positive predictive value = 75%). The A–G haplotype, which is mildly associated with resistance, captures all of the **15:01* and **15:02* alleles, and the G–A haplotype is too rare to make sound conclusions. Finally, the SNP associated with AQP4− vs AQP4+ does not capture any single *DRB1* allele well enough to draw *DRB1*-specific conclusions.

## Discussion

The HLA region has repeatedly shown a strong association with MS in studies of individuals of European descent.[2] The *HLA-DRB1*15:01* allele, as observed through classical HLA typing and **15:01* tagging SNP studies, is the likely source of the major HLA effect in individuals of European descent. In the Japanese population, which has a higher frequency of OSMS than populations of European descent, the **15:01* allele was found to be associated with CMS only.[10] This study investigated the association between 6040 HLA region SNPs with MS in 204 Japanese patients and 280 Japanese controls.

A total of 2506 SNPs were removed from the analysis owing to QC. The majority (65%) of these SNPs were removed because of minor allele frequency $<0.05$. This is not surprising considering that most known SNPs were discovered in non-Asian populations, and that there is less genetic variation in the Japanese population as compared with many of populations in which the SNPs were identified. The total number of SNPs passing QC and remaining in the analysis was 3534. The reduction in the total number of SNPs from QC decreased the coverage of the MHC region to an average of 707 SNPs per Mb.

Although the number of individuals were modest for an association study ($n = 280$, 204, 149 and 55 for controls, all patients, AQP4− and AQP4+ patients, respectively), this study is the largest of its kind in Japanese MS. For the main analysis with 204 patients, the power, calculated using the Power for Genetic Association Analyses program[30] (co-dominant model, disease prevalence = 0.001, disease allele frequency = 0.2, marker allele frequency = 0.2, effective degrees of freedom = 3429 (calculated using the EDF program included with Power for Genetic Association Analyses), $\alpha = 0.1$), was good to detect large effects (power = 0.97 for relative risk = 3), but not moderate effects (power = 0.4 for relative risk = 2). Power was considerably less for the other analyses with fewer patients, as expected (data not shown). Consequently, as in any study of the genetics of a complex disease, this study likely does not capture all biologically associated loci or loci with small effects if they are present. The results and discussion herein therefore pertain to those effects that the study had the power to identify.

Fitting the most significant SNP from an MHC-wide analysis as a covariate in subsequent MHC-wide analyses identified two SNPs that were significantly (FDR $P = 0.1$) associated with MS. It was decided *a priori* to use an FDR $P = 0.1$ to strike a balance between power and false negatives. At this significance level, 90% of all significant associations are expected to be true positives. The sequential method allows the determination of multiple SNPs that are associated independent of the LD they share with a more significant SNP. The most significantly associated SNP (rs422951) was in the Class III region and results in a missense mutation (Thr → Ala) in the *NOTCH4* gene. *NOTCH4* is involved in cell differentiation, proliferation and apoptosis, and has been implicated as a schizophrenia-associated locus.[31] Although possible associations have been identified for other autoimmune diseases,[9] a *NOTCH4* genetic association independent of *DRB1* has not been previously shown for MS. However, the NOTCH signaling pathway may be important in T-cell activation, oligodendrocyte differentiation, remyelination and has been suggested as a target for treatment of MS.[32] Therefore, the association of the *NOTCH4* missense mutation with MS may be of great importance to identifying treatments for Japanese MS patients. The secondarily MS-associated SNP (rs3997849) is closest to the *HLA-DQA2* gene in the Class II region. This gene encodes a protein involved in antigen presentation, and although previously studied in MS in a population of European descent,[33] the gene has not been previously shown to be associated with MS independent of *DRB1*.

Classification of patients into MS or NMO disease is complex in the Japanese population. Many patients fall into both OSMS and NMO groups, and several non-NMO patients are AQP4 seropositive (Table 1). Therefore, AQP4 seropositivity, rather than disease class, was used to stratify the data for subsequent analysis. Two SNPs were significantly associated with AQP4− MS. The SNP rs660895 was the primary associated SNP, and is closest to the *DRB1* gene. *DRB1* is the strongest and most replicated associated gene with MS in populations of European descent.[2] When fitting rs660895 in the model, rs2269704, which is located in an intron of the Class I *NRM29/NRM* gene, was the most significantly associated SNP. This gene is a nuclear envelope membrane protein, and no evidence of previous associations of this gene with MS could be identified. Finally, contrasting AQP4+ and AQP4−, MS patients identified a single significantly associated SNP (rs1694112), which is closest to the *HLA-DQB1* gene.

It should be noted that both SNPs significant for all MS, rs422951 and rs3997849, were both significant for AQP4− patients vs controls (FDR $P = 0.003$ and 0.06, respectively) in the same direction, and rs660895 and rs2269704 in all MS (FDR $P = 0.006$ and 0.01, respectively). Thus, it can be concluded that the differences in top associations between the two analyses, all demyelinating and AQP4− demyelinating, is likely due to random statistical fluctuations that occur when making a small modification to a data set (removing 55 AQP4+ individuals), rather than true differences being identified by subsetting the data.

Although it is interesting, and possibly informative, to discuss the genes closest to the identified significant SNPs, the extensive and intricate LD patterns in the MHC leads to the possibility that these SNPs may be tagging a causative mutation further away than the closest genes. As *DRB1* is the classical MS-associated gene in individuals of European descent and evidence of association has been reported in Japanese MS, haplotypes formed by the SNPs were scrutinized for the ability to tag *DRB1* alleles in 218 Japanese individuals for whom four-digit *DRB1* data were available. For the analysis of

all MS, the single susceptible haplotype contained all of the *DRB1*15:01* alleles. However, because this haplotype also tagged several other *DRB1* alleles well, it is not possible to determine which of the *DRB1* alleles are responsible for the association. The most highly associated resistant haplotype (G–G) mostly contained **01:01, *13:02* and **09:01* alleles. *DRB1*01* and **09* alleles have previously been shown to confer resistance to MS and AQP4– MS,[14] and it is likely the same association identified in this data set.

For the AQP4– haplotype analyses, G–G and A–G haplotypes made up 90% of the total haplotypes. The most strongly associated haplotype was G–G ($P = 9.04 \times 10^{-8}$; OR = 2.32). Seventy-five percent of this haplotype contains **04* alleles. The association of this haplotype is particularly striking when considering that it excludes **15:01*, and considering that the **15:01* effect is included in the non-GG haplotypes in the analysis (**15:01* is found exclusively with the A–G haplotype). This haplotype is tagging a non-**15:01* allele, which has a large effect, which could be larger than the **15:01* effect, on AQP4– MS susceptibility in the Japanese population. The very modest associations between **15:01* presence/absence and general MS and AQP4– MS also support the observation that the **15:01* haplotype may have a reduced role in Japanese MS as compared with MS in western populations. Isobe *et al.*[14] also identified a **04* association with AQP4– MS, and it is therefore this allele group that is the likely source of the susceptible association or part of a susceptible haplotype.

Although an association of *DRB1*15:01* with conventional Japanese MS has been reported previously,[10,34,35] a lack of a *DRB1*15* association, except when stratifying by **09* or **12*, has also been reported in Japanese MS.[14] The results of the present study clarify the **15* association. In this study, with a larger sample, presence/absence of **15:01* was found to be modestly associated with MS and AQP4– MS. However, the SNP that tags both **15:01* and **15:02* (rs9271366) was not associated with either disease classification. This result indicates that the non-associated **15:02* alleles are diluting the modest association of **15:01* when only two-digit genotypes are available. If **15:01* is indeed the causative allele in western populations, this finding is surprising considering that **15:02* proteins are expected to present the same antigens as proteins derived from **15:01*.[36] However, **15:02* was also identified as MS non-associated in two very small previously published studies,[37,38] and it has been shown that the two alleles may have differing effects with regard to aplastic anemia.[36] The difference in effect of the two alleles may be due to the single amino-acid difference between proteins from the two alleles, LD with another associated mutation or differences in expression between the two alleles. Because of the large association of **15:01* with MS in individuals of European descent and moderate association in other ethnicities, determining the mechanism for the difference in association between **15:01* and **15:02* may greatly increase our understanding of the molecular causes of MS.

Through conditional analysis, Lincoln *et al.*[39] found that the Class II haplotype block and *DRB1* accounted for most of the MHC-associated MS susceptibility in two populations of European descent, and that Class III associations could be explained by LD with Class II genes. Other studies have suggested, through haplotype analysis, that *DRB1*15:01* interacts with other genes in the Class II region to cause susceptibility, or that *DRB1*15:01* is part of a susceptible haplotype, but itself is not the causative genetic factor for the strongest genetic association with MS in individuals of European descent. This study also finds that in the Japanese MS population, the *DRB1*15:01* allele is not part of the major MS susceptible haplotype in AQP4– patients.

In conclusion, the objective of this study was to elucidate the effects of the HLA in Japanese MS. This study is the largest study of the HLA's contribution to MS in Japanese individuals. Haplotype analysis revealed a large susceptible association, likely *DRB1*04* or a locus in LD with *DRB1*04* alleles, with AQP4– MS, which excluded *DRB1*15:01* and other loci sharing a haplotype with *DRB1*15:01*. Several resistant haplotypes were identified, but it is difficult to say whether these haplotypes truly harbor resistant alleles or whether they only appear resistant when opposed to the susceptible haplotypes. Finally, although a very modest association of *DRB1*15:01* with MS was observed, *DRB1*15:02* was not associated. Because of the similarities of the proteins from these two alleles, differing only at a single amino acid, further studies to understand the nature of this difference, whether it be functional or haplotypic, could greatly increase our understanding of the molecular mechanisms leading to MS.

## Materials and methods

*Subjects*

All samples from Japanese cases were collected in the Neurology Departments of the University Hospitals of the South Japan MS Genetics Consortium, which comprises the following six universities, all located in southwestern Japan: Kyushu University, Yamaguchi University, Ehime University, Hiroshima University, Kinki University and Osaka University. The final data set consisted of DNAs from 280 control (HC) and 204 patients with MS (193 cases who fulfilled the revised McDonald criteria[28] and 11 cases who at least met the criteria of clinically isolated syndrome[41] and were suggestive of MS), genotyped on a custom Infinium iSelect HD Custom Genotyping BeadChip (Illumina Inc., San Diego, CA, USA) for 6040 MHC region SNPs. SNPs were selected by previously described methods,[27,42,43] and included an additional 4431 non-chromosome six SNPs genotyped for assessment of population stratification. Anti-AQP4 antibody was measured in all patients using green uorescent proteinAQP4 fusion protein-transfected human embryonic kidney cells as described previously.[14] All participants gave written informed consent. This study was approved by the UCSF institutional review board, and the institutional ethical committees at each university of the South Japan MS Genetics Consortium.

*DRB1* genotypes were available for 218 Japanese individuals. The *HLA-DRB1* alleles of the subjects were determined by hybridization of sequence-specific oligonucleotide probes in specific amplicons, as described elsewhere.[44] In addition, 264 controls and 203 patients were typed for *DRB1*15:01* presence/absence using validated gene-specific TaqMan assays as described by Caillier *et al.*[25]

*Quality control*

*SNPs.* SNPs were removed from the data set for missing genotypes greater than 5%, violation of HardyWeinberg equilibrium ($P<0.001$), or having a minor allele frequency less than 5%. SNPs were also removed if they were significantly ($P<0.001$) differently missing between all patients and controls (PLINK—test-missing) or if they were non-randomly missing ($P<0.001$) with respect to their expected genotypes derived from nearby SNPs in LD (PLINK—test-mishap). After all QC, 3534 HLA region SNPs remained. All genomic positions reported correspond to NCBI SNP build 129.[45]

*Samples.* All individuals with 10% or more missing genotypes were removed from analysis. To verify ethnicity, multidimensional scaling was used to cluster the experimental sample with data from 12 HapMap populations. Data for 705 non-chromosome six SNPs were common between all data sets and used for the analysis. Plots were generated for the first component onto the second component, and for each informative component (components 1–7) separately.

To identify population stratification, principal components were calculated for each individual using the 3668 non-chromosome six SNPs remaining after QC. Based on the scree plot, the first three components were considered informative (Supplementary Figure 4). Visual examination of a three-dimensional plot of the first three components identified three obvious outliers (Supplementary Figure 5). $T^2$ statistic analysis (Supplementary Figure 6) identified six outliers (including the three identified by visual examination), and data for all six samples were removed from the study.

*Statistical analyses*

*Association analyses.* Logistic regression (PLINK—logistic) was used to determine the association between HLA SNPs and MS, AQP4+ MS and AQP4− MS. An additive genetic model was assumed and gender included as a covariate for all analyses. Following the methods of McElroy *et al.*,[27] for each trait multiple rounds of analyses were performed, with each successive round including the most significant SNPs from the previous rounds, until no SNPs were significant at an FDR[46] of 0.1. This method facilitates the identification of multiple associated SNPs that are not redundantly associated with the trait through LD.

*Haplotype analyses.* Haplotypes were estimated for the significant SNPs (PLINK—hap). Haplotype effects were determined by weighted logistic regression of disease status onto haplotype dosage, with gender as a covariate to identify specific haplotypes that may be tagging trait-associated loci. Four-digit *HLA-DRB1* genotypes were available for a subset of individuals ($n=218$). To investigate how haplotypes of significant SNPs relate to *HLA-DRB1* allelic polymorphism, haplotype frequencies were estimated by maximum-likelihood implemented in an Expectation Maximization algorithm.[47] Positive predictive values and sensitivities of the SNPs to predict *HLA-DRB1* alleles at the haplotype level were computed. All analyses were completed using PLINK (version 1.07),[48] JMP Genomics 4.1 (SAS Institute Inc., Cary, NC, USA) and R (version 2.9),[49] unless otherwise noted.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

We thank the MS patients and controls who participated in this study. We thank RR Lincoln, R Guerrero, H Mousavi and A Santaniello for sample and database management. This work was supported by grants from the National Institute of Health U19AI067152, RO1NS46297 and National Multiple Sclerosis Society RG3060C8. JPM is a post-doctoral fellow supported by the National MS Society. NI is a post-doctoral fellow supported by The Association for Preventive Medicine of Japan. The members of the South Japan Multiple Sclerosis Genetic Consortium are J Kira, N Isobe, T Matsushita and S Yoshimura (Kyushu University), S Kusunoki and K Miyamoto (Kinki University), S Sakoda and Y Nakatsuji (Osaka University), T Kohriyama, K Ochi and M Matsumoto (Hiroshima University), T Kanda (Yamaguchi University) and T Miki and Y Kawano (Ehime University).

## References

1 Ebers GC, Yee IM, Sadovnick AD, Duquette P. Conjugal multiple sclerosis: population-based prevalence and recurrence risks in offspring. Canadian Collaborative Study Group. *Ann Neurol* 2000; **48**: 927–931.
2 McElroy JP, Oksenberg JR. Multiple sclerosis genetics. *Curr Top Microbiol Immunol* 2008; **318**: 45–72.
3 Barcellos LF, Oksenberg JR, Begovich AB, Martin ER, Schmidt S, Vittinghoff E *et al.* HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course. *Am J Hum Genet* 2003; **72**: 710–716.
4 Barcellos LF, Sawcer S, Ramsay PP, Baranzini SE, Thomson G, Briggs F *et al.* Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. *Hum Mol Genet* 2006; **15**: 2813–2824.
5 Brassat D, Salemi G, Barcellos LF, McNeill G, Proia P, Hauser SL *et al.* The HLA locus and multiple sclerosis in Sicily. *Neurology* 2005; **64**: 361–363.
6 Cree BA, Rioux JD, McCauley JL, Gourraud PA, Goyette P, McElroy J *et al.* A major histocompatibility Class I locus contributes to multiple sclerosis susceptibility independently from HLA-DRB1*15:01. *PLoS One* 2010; **5**: e11296.
7 Dyment DA, Herrera BM, Cader MZ, Willer CJ, Lincoln MR, Sadovnick AD *et al.* Complex interactions among MHC haplotypes in multiple sclerosis: susceptibility and resistance. *Hum Mol Genet* 2005; **14**: 2019–2026.
8 Link J, Lorentzen AR, Kockum I, Duvefelt K, Lie BA, Celius EG *et al.* Two HLA class I genes independently associated with multiple sclerosis. *J Neuroimmunol* 2010; **226**: 172–176.
9 Rioux JD, Goyette P, Vyse TJ, Hammarstrom L, Fernando MM, Green T *et al.* Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. *Proc Natl Acad Sci USA* 2009; **106**: 18680–18685.
10 Kira J. Multiple sclerosis in the Japanese population. *Lancet Neurol* 2003; **2**: 117–127.
11 Fukazawa T, Kikuchi S, Miyagishi R, Miyazaki Y, Yabe I, Hamada T *et al.* HLA-dPB1*0501 is not uniquely associated with opticospinal multiple sclerosis in Japanese patients. Important role of DPB1*0301. *Mult Scler* 2006; **12**: 19–23.
12 Matsushita T, Matsuoka T, Isobe N, Kawano Y, Minohara M, Shi 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.

13 Kira J. Neuromyelitis optica and opticospinal multiple sclerosis: mechanisms and pathogenesis. *Pathophysiology* 2011; **18**: 69–79.
14 Isobe N, Matsushita T, Yamasaki R, Ramagopalan SV, Kawano Y, Nishimura Y *et al.* Influence of HLA-DRB1 alleles on the susceptibility and resistance to multiple sclerosis in Japanese patients with respect to anti-aquaporin 4 antibody status. *Mult Scler* 2010; **16**: 147–155.
15 ANZgene. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nat Genet* 2009; **41**: 824–828.
16 Aulchenko YS, Hoppenbrouwers IA, Ramagopalan SV, Broer L, Jafari N, Hillert J *et al.* Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. *Nat Genet* 2008; **40**: 1402–1403.
17 Baranzini SE. The genetics of autoimmune diseases: a networked perspective. *Curr Opin Immunol* 2009; **21**: 596–605.
18 Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A *et al.* Association scan of 14 500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007; **39**: 1329–1337.
19 Comabella M, Craig DW, Camina-Tato M, Morcillo C, Lopez C, Navarro A *et al.* Identification of a novel risk locus for multiple sclerosis at 13q31.3 by a pooled genome-wide scan of 500 000 single nucleotide polymorphisms. *PLoS One* 2008; **3**: e3490.
20 De Jager PL, Jia X, Wang J, de Bakker PI, Ottoboni L, Aggarwal NT *et al.* Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nat Genet* 2009; **41**: 776–782.
21 IMSGC. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med* 2007; **357**: 851–862.
22 Jakkula E, Leppa V, Sulonen AM, Varilo T, Kallio S, Kemppinen A *et al.* Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. *Am J Hum Genet* 2010; **86**: 285–291.
23 Sanna S, Pitzalis M, Zoledziewska M, Zara I, Sidore C, Murru R *et al.* Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. *Nat Genet* 2010; **42**: 495–497.
24 Baranzini SE, Wang J, Gibson RA, Galwey N, Naegelin Y, Barkhof F *et al.* Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum Mol Genet* 2009; **18**: 767–778.
25 Caillier SJ, Briggs F, Cree BA, Baranzini SE, Fernandez-Vina M, Ramsay PP *et al.* Uncoupling the roles of HLA-DRB1 and HLA-DRB5 genes in multiple sclerosis. *J Immunol* 2008; **181**: 5473–5480.
26 Oksenberg JR, Barcellos LF, Cree BA, Baranzini SE, Bugawan TL, Khan O *et al.* Mapping multiple sclerosis susceptibility to the HLA-DR locus in African Americans. *Am J Hum Genet* 2004; **74**: 160–167.
27 McElroy JP, Cree BA, Caillier SJ, Gregersen PK, Herbert J, Khan OA *et al.* Refining the association of MHC with multiple sclerosis in African Americans. *Hum Mol Genet* 2010; **19**: 3080–3088.
28 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**: 840–846.
29 de Bakker PI, McVean G, Sabeti PC, Miretti MM, Green T, Marchini J *et al.* A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* 2006; **38**: 1166–1172.
30 Menashe I, Rosenberg PS, Chen BE. PGA: power calculator for case–control genetic association analyses. *BMC Genet* 2008; **9**: 36.
31 Glessner JT, Hakonarson H. Common variants in polygenic schizophrenia. *Genome Biol* 2009; **10**: 236.
32 Jurynczyk M, Selmaj K. Notch: a new player in MS mechanisms. *J Neuroimmunol* 2010; **218**: 3–11.
33 Duvefelt K, Anderson M, Fogdell-Hahn A, Hillert J. A NOTCH4 association with multiple sclerosis is secondary to HLA-DR*1501. *Tissue Antigens* 2004; **63**: 13–20.
34 Fukazawa T, Kikuchi S, Miyagishi R, Niino M, Yabe I, Hamada T *et al.* CTLA-4 gene polymorphism is not associated with conventional multiple sclerosis in Japanese. *J Neuroimmunol* 2005; **159**: 225–229.
35 Miyagishi R, Niino M, Fukazawa T, Yabe I, Kikuchi S, Tashiro K. C–C chemokine receptor 2 gene polymorphism in Japanese patients with multiple sclerosis. *J Neuroimmunol* 2003; **145**: 135–138.
36 Sugimori C, Yamazaki H, Feng X, Mochizuki K, Kondo Y, Takami A *et al.* Roles of DRB1 *1501 and DRB1 *1502 in the pathogenesis of aplastic anemia. *Exp Hematol* 2007; **35**: 13–20.
37 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**: 569–574.
38 Ma JJ, Nishimura M, Mine H, Saji H, Ohta M, Saida K *et al.* HLA-DRB1 and tumor necrosis factor gene polymorphisms in Japanese patients with multiple sclerosis. *J Neuroimmunol* 1998; **92**: 109–112.
39 Lincoln MR, Montpetit A, Cader MZ, Saarela J, Dyment DA, Tiislar M *et al.* A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. *Nat Genet* 2005; **37**: 1108–1112.
40 Lincoln MR, Ramagopalan SV, Chao MJ, Herrera BM, Deluca GC, Orton SM *et al.* Epistasis among HLA-DRB1, HLA-DQA1, and HLA-DQB1 loci determines multiple sclerosis susceptibility. *Proc Natl Acad Sci USA* 2009; **106**: 7542–7547.
41 Dalton CM, Brex PA, Miszkiel KA, Hickman SJ, MacManus DG, Plant GT *et al.* Application of the new McDonald criteria to patients with clinically isolated syndromes suggestive of multiple sclerosis. *Ann Neurol* 2002; **52**: 47–53.
42 de Bakker PI, Burtt NP, Graham RR, Guiducci C, Yelensky R, Drake JA *et al.* Transferability of tag SNPs in genetic association studies in multiple populations. *Nat Genet* 2006; **38**: 1298–1303.
43 Leslie S, Donnelly P, McVean G. A statistical method for predicting classical HLA alleles from SNP data. *Am J Hum Genet* 2008; **82**: 48–56.
44 Tsuji K, Aizawa M, Sasazuki T (eds). 11th International Histocompatibility Workshop Reference Protocol for the HLA DNA Typing Technique. *HLA 1991: Proceedings of the 11th International Histocompatibility Workshop and Conference.* Oxford University Press, 1992.
45 NCBI. *Database of Single Nucleotide Polymorphisms (dbSNP): dbSNP Build ID.* National Center for Biotechnology Information, National Library of Medicine: Bethesda, MD. p 129.
46 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc Ser B* 1995; **57**: 289–300.
47 Gourraud PA, Genin E, Cambon-Thomsen A. Handling missing values in population data: consequences for maximum likelihood estimation of haplotype frequencies. *Eur J Hum Genet* 2004; **12**: 805–812.
48 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559–575.
49 R Development Core Team. *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing: Vienna, Austria, 2009.
50 Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006; **66**: 1485–1489.

Supplementary Information accompanies the paper on Genes and Immunity website (http://www.nature.com/gene)

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.

*Neurology Asia 2011; 16(2) : 139 – 142*

# T cell reactivities to myelin protein-derived peptides in neuromyelitis optica patients with anti-aquaporin-4 antibody

[1]Tomomi Yonekawa *MD*, [1,2]Takuya Matsushita *MD PhD*, [1]Motozumi Minohara *MD PhD*, [1]Noriko Isobe *MD PhD*, [1]Katsuhisa Masaki *MD PhD*, [1]Satoshi Yoshimura *MD*, [3]Yasuharu Nishimura *MD PhD*, [1]Jun-ichi Kira *MD PhD*

*[1]Department of Neurology, Neurological Institute, and [2]Department of Clinical Neuroimmunology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, and [3]Division of Immunogenetics, Department of Neuroscience and Immunology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan*

### *Abstract*

We previously reported the establishment of major myelin protein-derived T cell lines from 11 patients with multiple sclerosis. In the present study, we determined anti-aquaporin-4 (AQP4) antibody status in these patients and classified them into five patients with anti-AQP4 antibody who met the criteria for neuromyelitis optica (NMO) or NMO spectrum disorders, and six patients without anti-AQP4 antibody who fulfilled the revised McDonald criteria for multiple sclerosis. T cell lines reactive to myelin oligodendrocyte glycoprotein, proteolipid protein and myelin basic protein were detected in 5/5, 3/5 and 3/5 of the anti-AQP4 antibody-positive patients, respectively, and in 5/6, 4/6 and 4/6 of the anti-AQP4 antibody-negative ones, respectively. T cell lines from most of these patients showed inter- or intra-molecular epitope spreading, irrespective of anti-AQP4 antibody status. These findings suggest that T cells are stimulated in vivo against major myelin proteins in anti-AQP4 antibody-positive patients with NMO/NMO spectrum disorders.

## INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that is generally considered to be mediated by myelin-autoreactive T cells. By contrast, neuromyelitis optica (NMO) is characterized by severe and selective involvement of the optic nerves and spinal cord. Recently, a specific IgG against NMO, designated NMO-IgG, targeting aquaporin-4 (AQP4), was described.[1,2] Because of the high specificity of NMO-IgG/anti-AQP4 antibody and the selective loss of AQP4 from acute lesions in autopsied NMO spinal cord specimens[3], NMO has been claimed to be a distinct disease entity with a fundamentally different causal mechanism from MS. The demyelination in NMO is proposed to be secondarily produced following damage to the astrocyte foot process, where AQP4 is localized.[3]

In Asians, selective and severe involvement of the optic nerves and spinal cord is characteristic, and there are two distinct subtypes of MS: the opticospinal form of MS (OSMS), which has similar features to the relapsing-remitting form of NMO in Western populations[4], and the conventional form of MS, which is associated with disseminated lesions in the CNS[4], similar to classical MS in Western populations. Because a fraction of OSMS patients also have anti-AQP4 antibodies[1,5], OSMS is suggested to be the same disease as NMO. Although both NMO and OSMS are claimed to be primary astrogliopathies, it remains unknown how anti-AQP4 antibody present in the peripheral blood enters into the CNS across the blood brain barrier (BBB) to initiate parenchymatous inflammation, and how astrocyte foot process damage produces extensive demyelination.

We previously reported the establishment of T cell lines (TCLs) reactive to major myelin proteins, such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), by stimulating peripheral blood T cells from MS patients with a myelin peptide mixture, before the discovery of NMO-IgG.[6] Therefore, in the present study, we aimed to

*Address correspondence to:* Jun-ichi Kira MD PhD, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel: +81-92-642-5340. Fax: +81-92-642-5352, E-mail: kira@neuro.med.kyushu-u.ac.jp