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Risk *HLA-DRB1* alleles differentially influence brain and lesion volumes in Japanese patients with multiple sclerosis

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ABSTRACT

Background: The effects of distinct *HLA* alleles on the brain and lesion volumes remain to be established, particularly in non-Caucasian populations. Two distinct susceptibility alleles, *DRB1*15:01* and *DRB1*04:05*, are prevalent in the Japanese population; we therefore aimed to clarify the effects of *HLA-DRB1* alleles on brain and lesion volumes in multiple sclerosis (MS).

Methods: A total of 66 patients with MS (50 relapsing remitting, 16 progressive) underwent brain MRI volumetry measuring fluid-attenuated inversion recovery (FLAIR) and T1 lesion volumes, and normalized whole-brain (NWBV), white matter (NWMV), gray matter (NGMV), cortical gray matter (NCGMV), deep gray matter (NDGMV) and thalamus (NTV) volumes, and *HLA-DRB1* genotyping.

Results: Carriers of *HLA-DRB1*15:01*(+)**04:05*(−) and *HLA-DRB1*15:01*(−)**04:05*(+) comprised 25.8% and 31.8% of patients, respectively. *HLA-DRB1*15:01* carriers showed negative correlations between disease duration and NWBV ($r_s = -0.484$, $p = .036$), NWMV ($r_s = -0.593$, $p = .008$), and NTV ($r_s = -0.572$, $p = .011$), and positive correlations between disease duration and FLAIR ($r_s = 0.539$, $p = .017$) and T1 lesion volumes ($r_s = 0.545$, $p = .016$). By contrast, no significant correlation of any MRI parameters with disease duration was found in *HLA-DRB1*04:05* carriers. *HLA-DRB1*15:01* carriers had a significantly faster reduction in NWBV and NWMV by disease duration and smaller NDGMV than *DRB1*15:01* non-carriers, whereas *HLA-DRB1*04:05* carriers had a significantly slower increase in FLAIR and T1 lesion volumes than *HLA-DRB1*04:05* non-carriers.

Conclusions: Our study suggests that distinct *HLA-DRB1* alleles could differentially influence brain and lesion volumes over the disease course of MS.

1. Introduction

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system. Clinical features of MS differ by race. For example, Japanese patients with MS have a milder disease course than patients in the United Kingdom [1] and less frequently encounter cerebellar hemispheric lesions than Caucasian patients with MS [2]. Likewise, African American patients with MS show a more severe course and higher lesion volumes than patients of European descent in the United States [3–5].

MS is affected by genetic and environmental factors [6], which could explain the differences in clinical features between races. The class II sub-region of the *human leukocyte antigen* (*HLA*) has the strongest genetic influence on MS susceptibility. In Caucasian patients, *HLA-DRB1*15:01* is an established risk allele for MS [7], while in Japanese patients, *DRB1*15:01* and *DRB1*04:05* are the most prevalent *HLA* risk alleles for MS [8,9]. The prevalence of *HLA* alleles varies according to race. Nearly half of European patients with MS carry *DRB1*15:01*, while *DRB1*04:05* is rare [10]. By contrast, in Japanese patients with MS, phenotype frequencies of *DRB1*04:05* and *DRB1*15:01* have been reported to be 21.1%–44.8% and 23.3%–28.8%, respectively [8,9,11].

Brain MRI volumetric studies have revealed the decline of whole and regional brain volumes over the disease course in Caucasian patients with MS [12–15]. However, only a few studies have analyzed the

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Table 1
Baseline characteristics of participants.

	All patients with MS (n = 66)
Female rate, n (%)	46 (69.7)
Subtype, n (%)	PMS 16 (24.2) RRMS 50 (75.8)
Age at time of MRI scan, years[a]	47.0 (38.0–53.0)
Age at first symptom, years[a]	29.0 (22.8–40.3)
Disease duration, years[a]	13.0 (8.0–20.5)
EDSS scores[a]	2.5 (1.0–5.6)
Patients with DMDs or immunosuppressive agents, n (%)	54 (81.8)
OCBs, n (%)	29/59 (45.8)
Increased IgG index, n (%)	16/41 (39.0)
*HLA-DRB1*15:01*(+)**04:05*(−), n (%)	17 (25.8)
*HLA-DRB1*15:01*(−)**04:05*(+), n (%)	21 (31.8)
*HLA-DRB1*15:01*(+)**04:05*(+), n (%)	2 (3.0)
*HLA-DRB1*15:01*(−)**04:05*(−), n (%)	26 (39.4)

DMDs: Disease-modifying drugs; EDSS: Expanded Disability Status Scale of Kurtzke; MS: multiple sclerosis; PMS: progressive MS; RRMS: relapsing-remitting MS; OCBs: oligoclonal IgG bands.
[a] Values are expressed as the median (interquartile range).

Table 2
Baseline brain MRI findings of participants.

	All patients with MS (n = 66)
FLAIR lesion volume (ml)	11.8 (7.2–19.8)
Periventricular volume (ml)	10.7 (6.0–18.7)
Juxtacortical volume (ml)	0.12 (0.04–0.25)
Infratentorial volume (ml)	0.00 (0.00–0.03)
Deep white matter volume (ml)	0.54 (0.33–0.91)
T1 lesion volume (ml)	6.4 (2.9–10.4)
NWBV (ml)	1450 (1390–1510)
NGMV (ml)	882 (851–927)
NCGMV (ml)	838 (802–879)
NDGMV (ml)	45.0 (41.8–47.7)
NTV (ml)	15.0 (13.2–16.4)
NWMV (ml)	556 (525–593)

Values are expressed as the median (interquartile range).
NCGMV: normalized cortical gray matter volume; NDGMV: normalized deep gray matter volume; NGMV: normalized gray matter volume; NTV: normalized thalamus volume; NWBV: normalized whole brain volume; NWMV: normalized white matter volume.

differences in whole and regional brain volume according to *HLA* alleles. *HLA-DRB1*15:01* was reported to be positively associated with brain white matter lesion volumes, and negatively associated with normalized brain parenchymal volumes in Caucasian patients with MS [16]. In addition, *HLA-DRB1*15:01* was associated with increased contrast enhancing-lesion number and volumes, and had a trend toward increased T2 lesion volumes [17]. However, other studies have reported that the heterogeneity of *HLA-DRB1* does not influence clinical manifestations of MS [18–21]. Thus, the contribution of *DRB1* genes to clinical manifestations, including MRI findings, remains to be established.

In Japanese patients with MS, we previously reported that *HLA-DRB1*04:05* was associated with fewer brain lesions that fulfilled the Barkhof criteria and fewer intracortical lesions, in addition to a milder clinical course, younger age at disease onset, and fewer cerebrospinal fluid (CSF) IgG abnormalities [22,23]. Two distinct susceptibility alleles, *DRB1*15:01* and *DRB1*04:05*, are relatively prevalent in Japanese patients [8,9,11], which makes it easier to make comparisons of the effects of different *HLA* alleles on clinical manifestations. Therefore, in this study, we investigated the influence of *HLA-DRB1* alleles on brain and lesion volumes in Japanese patients with MS.

Table 3
Comparison of the clinical features in patients with MS according to the presence or absence of *HLA-DRB1*15:01* and/or **04:05*.

	15:01 (+) *04:05* (−) (n = 17)	*15:01* (−) *04:05* (+) (n = 21)	*15:01* (+) *04:05* (+) (n = 2)	*15:01* (−) *04:05* (−) (n = 26)	p value				
					15:01 (+) *04:05* (−) vs. *15:01* (−) *04:05* (−)	*15:01* (−) *04:05* (+) vs. *15:01* (−) *04:05* (−)	*15:01* (+) *04:05* (+) vs. *15:01* (−) *04:05* (−)	*15:01* (+) *04:05* (−) vs. *15:01* (−) *04:05* (+)
Female rate, n (%)	14 (82.4)	15 (71.4)	1 (50.0)	16 (61.5)	0.185	0.547	NA[b]	0.476
Subtype, n (%)	PMS 4 (23.5) RRMS 13 (76.5)	PMS 4 (19.0) RRMS 17 (81.0)	PMS 1 (50.0) RRMS 1 (50.0)	PMS 7 (26.9) RRMS 19 (73.1)	1.000	0.731	NA[b]	1.000
Age at time of MRI scan, years[a]	47.0 (40.0–50.5)	48.0 (37.0–54.5)	46.0 (39.0–53.0)	46.5 (37.3–53.8)	0.901	0.889	NA[b]	0.713
Age at first symptom, years[a]	25.0 (23.0–37.5)	29.0 (22.0–43.5)	42.5 (36.0–49.0)	27.0 (20.5–34.5)	0.980	0.386	NA[b]	0.445
Disease duration, years[a]	18.0 (7.5–23.0)	11.0 (7.5–11.0)	3.0	13.0 (10.0–20.8)	1.000	0.234	NA[b]	0.411
EDSS scores[a]	3.0 (2.0–4.5)	2.0 (1.0–4.5)	1.5 (1.0–2.0)	3.0 (1.4–6.5)	0.871	0.327	NA[b]	0.147
Patients with DMDs or immunosuppressive agents, n (%)	14 (82.4)	18 (85.7)	2 (100.0)	20 (76.9)	1.000	0.711	NA[b]	1.000
OCBs, n (%)	8/14 (57.1)	8/20 (40.0)	1/2 (50.0)	10/23 (43.5)	0.508	1.000	NA[b]	0.487
Increased IgG index, n (%)	8/13 (61.5)	5/16 (31.3)	2/2 (100.0)	7/23 (30.4)	0.090	1.000	NA[b]	0.144

DMDs: Disease-modifying drugs; EDSS: Expanded Disability Status Scale of Kurtzke; MS: multiple sclerosis; PMS: progressive MS; RRMS: relapsing-remitting MS; OCBs: oligoclonal IgG bands.
[a] Values are expressed as the median (interquartile range).
[b] As there were only 2 patients with *HLA-DRB1*15:01* (+) **04:05* (+), these data were not included in the statistical analyses (NA).

Table 4
Comparison of the brain MRI parameters in patients with MS according to the presence or absence of *HLA-DRB1*15:01* and/or **04:05*.

	15:01 (+) *04:05* (−) (n = 17)	*15:01* (−) *04:05* (+) (n = 21)	*15:01* (+) *04:05* (+) (n = 2)	*15:01* (−) *04:05* (−) (n = 26)	p value			
					15:01 (+) *04:05* (−) vs. *15:01* (−) *04:05* (−)	*15:01* (−) *04:05* (+) vs. *15:01* (−) *04:05* (−)	*15:01* (+) *04:05* (+) vs. *15:01* (−) *04:05* (−)	*15:01* (+) *04:05* (−) vs. *15:01* (−) *04:05* (+)
FLAIR lesion volume (ml)	11.9 (5.5–19.7)	11.6 (7.4–21.5)	12.2 (3.5–20.8)	11.6 (7.3–15.3)	0.951	0.797	NA[a]	0.849
Periventricular volume (ml)	11.4 (4.8–18.5)	8.8 (6.7–20.2)	11.8 (3.1–20.6)	10.7 (5.5–14.6)	0.823	0.814	NA[a]	0.942
Juxtacortical volume (ml)	0.13 (0.01–0.26)	0.10 (0.05–0.33)	0.00 (0.00–0.00)	0.15 (0.04–0.26)	0.756	0.983	NA[a]	0.670
Infratentorial volume (ml)	0.00 (0.00–0.06)	0.01 (0.00–0.02)	0.00 (0.00–0.00)	0.00 (0.00–0.03)	0.618	0.637	NA[a]	0.826
Deep white matter volume (ml)	0.57 (0.32–0.89)	0.52 (0.31–0.81)	0.33 (0.19–0.46)	0.62 (0.38–1.29)	0.412	0.586	NA[a]	0.814
T1 lesion volume (ml)	6.5 (2.1–13.1)	6.0 (3.2–12.5)	6.9 (0.9–12.8)	6.4 (3.0–9.2)	1.000	0.983	NA[a]	0.965
NWBV (ml)	1440 (1380–1510)	1450 (1400–1510)	1470 (1450–1480)	1430 (1340–1510)	0.766	0.441	NA[a]	0.618
NGMV (ml)	883 (834–930)	893 (864–931)	897 (894–890)	876 (836–917)	0.728	0.369	NA[a]	0.528
NCGMV (ml)	839 (793–884)	842 (826–884)	853 (851–854)	830 (790–874)	0.655	0.257	NA[a]	0.628
NDGMV (ml)	42.6 (40.3–46.1)	45.9 (41.8–49.8)	43.9 (42.3–45.5)	44.6 (41.8–48.1)	0.224	0.563	NA[a]	0.138
NTV (ml)	10.9 (9.4–12.0)	10.6 (9.8–11.6)	11.3 (11.1–11.6)	11.1 (9.1–12.2)	0.862	0.789	NA[a]	0.758
NWMV (ml)	557 (511–593)	553 (527–584)	571 (555–586)	564 (510–594)	0.882	0.932	NA[a]	0.895

Values are expressed as the median (interquartile range).
NCGMV: normalized cortical gray matter volume; NDGMV: normalized deep gray matter volume; NGMV: normalized gray matter volume; NTV: normalized thalamus volume; NWBV: normalized whole brain volume; NWMV: normalized white matter volume.
[a] As there were only 2 patients with *HLA-DRB1*15:01* (+) **04:05* (+), these data were not included in the statistical analyses (NA).

2. Material and methods

2.1. Participants

We enrolled a total of 76 patients with MS who were diagnosed according to the 2010 McDonald criteria for MS [24] and who provided written informed consent to receive both *HLA-DRB1* genotyping and volumetric brain MRI measurements between November 1, 2017 and December 31, 2018. All enrolled patients also fulfilled the newly published 2017 McDonald criteria for an MS diagnosis [25]. MRI data from 4 patients and genetic information from 8 patients were missing, leaving 66 patients with MS whose volumetric brain MRI measurement and *HLA-DRB1* genotyping were available, and who were subjected to further analyses. Anti-aquaporin 4 antibody was negative in all 66 patients; anti-myelin oligodendrocyte glycoprotein (MOG) antibodies were only examined in two patients, both of whom were negative. All participants were 20 years of age or older. Patients were thoroughly examined and regularly followed-up at Kyushu University Hospital, Fukuoka, Japan. This study protocol was approved by the Kyushu University Ethics Committee.

2.2. Clinical measures

Clinical information at the time of the MRI scan was collected from medical records, and included sex, subtype (relapsing remitting MS [RRMS] or progressive MS [PMS], age at the time of MRI, age at the first symptom, disease duration, Expanded Disability Status Scale (EDSS) of Kurtzke scores [26], oligoclonal IgG bands (OCBs; the presence of two or more bands in CSF was interpreted as positive), IgG index (an IgG index higher than 0.658 was considered as an increase according to a previous study [27]), and exposure to disease-modifying drugs (DMDs; including interferon-β-1a, interferon-β-1b, glatiramer acetate, fingolimod, dimethyl fumarate, and natalizumab) and immunosuppressive drugs (such as prednisolone, tacrolimus, and azathioprine). PMS was defined by progressive disability from the onset or following a relapsing course, independent of relapses, which included secondary-progressive and primary-progressive MS. RRMS is MS with a relapsing disease course that fulfils the McDonald criteria and has no evidence of disease progression [28]. OCBs were examined by either isoelectric focusing or agarose gel electrophoresis according to the availability of these study methods at the time of lumbar puncture. As a result, isoelectric focusing was used in 30 patients (50.8%), agarose gel electrophoresis in 15 patients (25.4%), and the method was unspecified in the remaining 14 patients (23.7%). However, the frequencies of the OCBs study methods, i.e., isoelectric focusing, agarose gel electrophoresis, and unspecified, were not significantly different between the *HLA-DRB1*15:01*(+)**04:05*(−), *DRB1*15:01*(−)**04:05*(+), *DRB1*15:01*(+)**04:05*(+), and *DRB1*15:01*(−)**04:05*(−) carriers (p = .201; Supplementary Table 1).

2.3. Image acquisition

All MRI studies were performed using a 3T system (Achieva, Philips, Best, the Netherlands). The typical imaging parameters were as follows: 3D-turbo-fluid-attenuated inversion recovery (FLAIR) imaging using repetition time (TR)/echo time (TE)/inversion time = 6000/230/2200 ms, field-of view (FOV) = 240 × 226 mm, matrix = 256 × 241, slice thickness = 1.4 mm, and acquisition time = 8 min 12 s. 3D sagittal T1-weighted gradient-echo imaging using TR/TE = 7.8/3.6 ms, FOV = 240 × 240 mm, matrix = 240 × 240, slice thickness = 1 mm, and acquisition time = 5 min 22 s. MRI scans were taken during a clinically stable period excluding the period within 4 weeks following the onset of relapses.

2.4. MRI analysis

All MRI parameters, namely, FLAIR lesion volume, T1 lesion

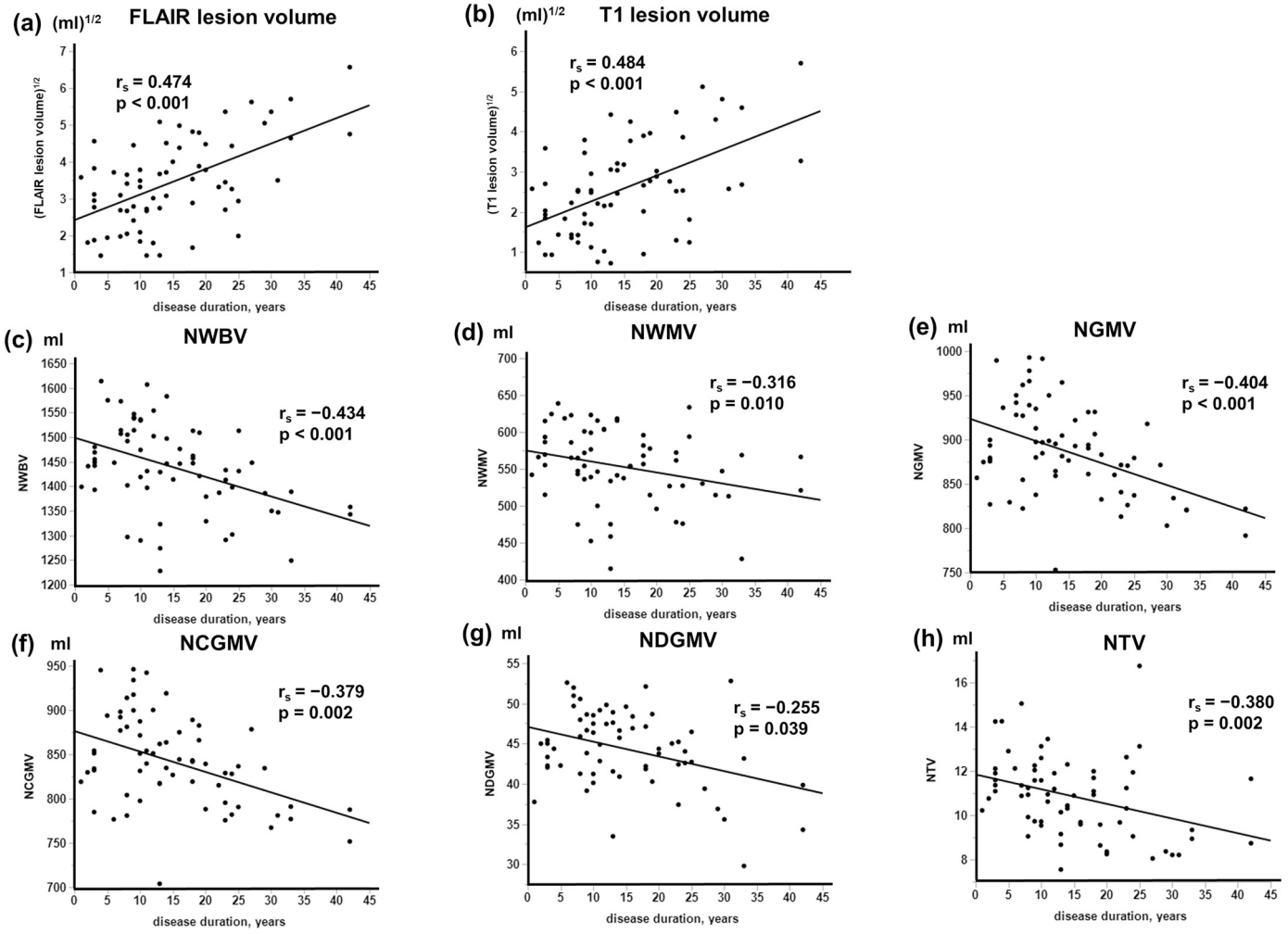

Fig. 1. Correlations of disease duration with brain lesions and volume in all patients with MS.
Scatter plots show the relationship between disease duration and brain lesion volumes (FLAIR (a) and T1 lesion volumes (b)) and brain volumes (normalized whole-brain volume [NWBV] (c); normalized white matter volume [NWMV] (d); normalized gray matter volume [NGMV] (e); normalized cortical gray matter volume [NCGMV] (f); normalized deep gray matter volume [NDGMV] (g); normalized thalamus volume [NTV] (h)). Lines represent the linear regression of the data. FLAIR (a) and T1 lesion volumes (b) are represented as the square root transformed to a normal distribution. r_s = Spearman's rank correlation coefficient.

volume, normalized whole-brain volume (NWBV), normalized gray matter volume (NGMV), normalized cortical gray matter volume (NCGMV), normalized deep gray matter volume (NDGVM), normalized thalamus volume (NTV), and normalized white matter volume (NWMV), were measured using *icobrain ms*, a scanner-independent software developed by *icometrix* (Leuven, Belgium).

2.5. HLA-DRB1 genotyping

The genotypes of the participants' *HLA-DRB1* alleles were determined by hybridization between polymerase chain reaction amplification products of the *HLA-DRB1* gene and sequence-specific oligonucleotide probes, as described previously [29].

2.6. Statistical analyses

All analyses were performed using statistical software (JMP 14.1.0; SAS Institute Inc., Cary, NC, USA). Fisher's exact probability test was used to compare categorical variables (sex, subtypes, presence of OCBs, and DMDs use), and the Mann–Whitney *U* test was used to compare continuous variables (age at the time of MRI, age at the first symptom, disease duration, EDSS scores, IgG index, and MRI parameters) between patients with and without *HLA-DRB1*15:01* or *04:05*. Correlations between MRI parameters and EDSS scores or disease duration were evaluated using Spearman's rank correlation. To identify the contributing clinical factors to MRI parameters, multivariate linear regression analyses were also performed, and included sex, age at first symptom, presence of OCBs, DMDs use, EDSS score and disease duration and EDSS scores as independent variables. When *HLA*-based differences in MRI parameters were assessed by multivariate linear regression analyses, *HLA-DRB1* alleles and interaction terms between disease duration and *HLA-DRB1 alleles* were additionally included in the models to test intercept and slope homogeneity between patients with and without each of the *HLA-DRB1* alleles. When the objective variables were not normally distributed, they were appropriately transformed. Statistical significance was set at $p < .05$.

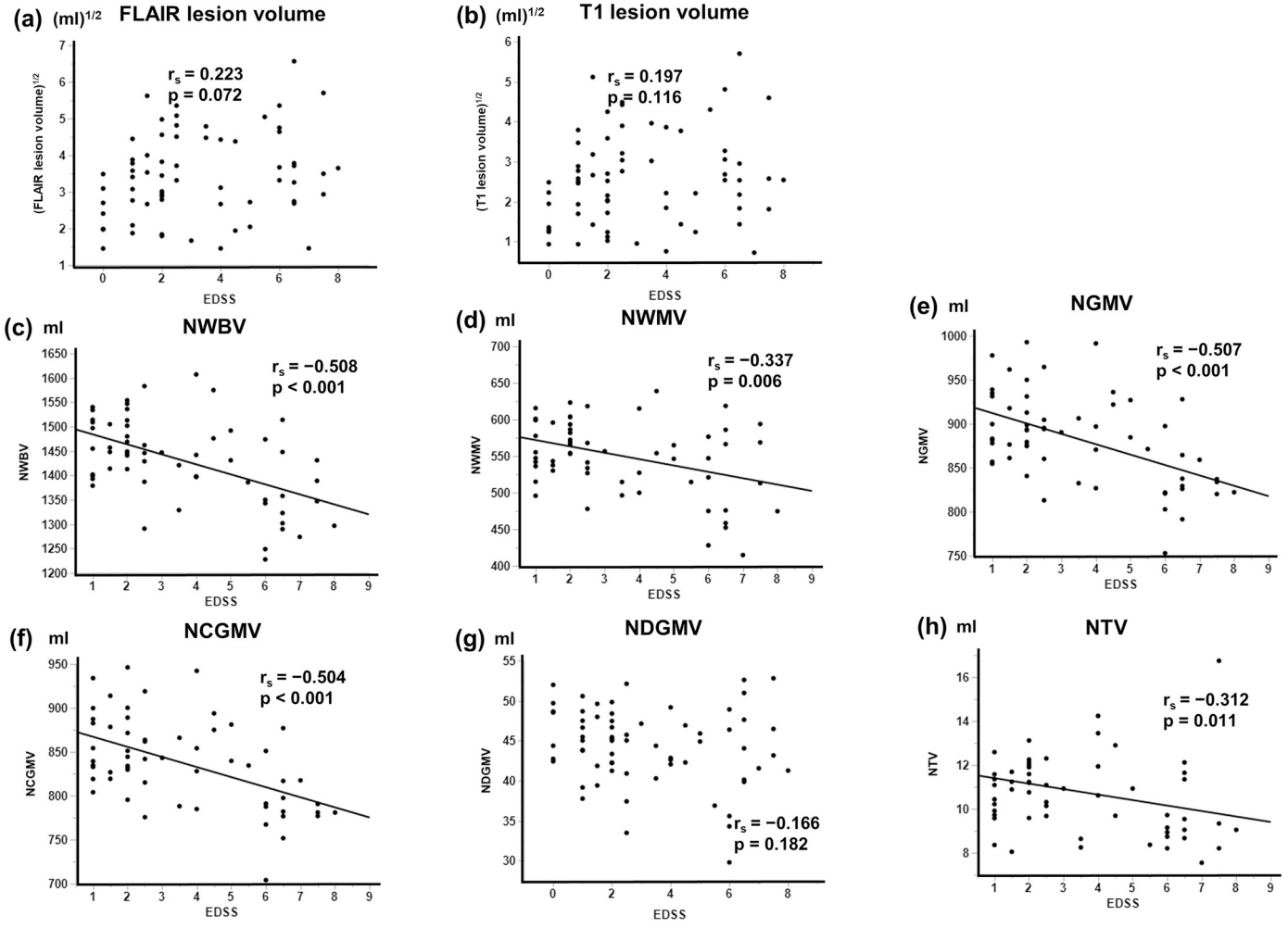

Fig. 2. Correlations between EDSS scores and brain lesions and volume in all patients with MS.
Scatter plots show the relationship between EDSS scores and brain lesion volumes (FLAIR (a) and T1 lesion volumes (b)) and brain volumes (normalized whole-brain volume [NWBV] (c); normalized white matter volume [NWMV] (d); normalized gray matter volume [NGMV] (e); normalized cortical gray matter volume [NCGMV] (f); normalized deep gray matter volume [NDGMV] (g); normalized thalamus volume [NTV] (h)). Lines represent the linear regression of the data. FLAIR (a) and T1 lesion volumes (b) are represented as the square root transformed to a normal distribution. r_s = Spearman's rank correlation coefficient.

3. Results

3.1. Clinical demographics and MRI measurements

Clinical demographics are shown in Table 1. Of the 66 patients, 46 were female and 20 male, and 50 patients had RRMS and 16 had PMS. The median age at time of MRI scans was 47.0 years, and the median disease duration was 13.0 years. The frequency of patients receiving DMDs or immunosuppressive therapy was 81.8%. The frequencies of patients treated with interferon-β, fingolimod, dimethyl fumarate, and glatiramer acetate were 27.8%, 48.1%, 20.4%, and 1.9%, respectively. No patients were being treated with natalizumab. The phenotypic frequencies of the *HLA-DRB1*15:01* was 28.8% and *HLA-DRB1*04:05* was 34.8%, which is comparable to previous reports [8,9,11] (Supplementary Table 2). The other major alleles were *DRB1*15:02* (n = 15, 22.7%), *DRB1*08:03* (n = 12, 18.2%), and *DRB1*09:01* (n = 7, 10.6%). MRI measures are shown in Table 2. The median FLAIR lesion volume, NWBV, NGMV, and NWMV were 11.8, 1450, 882, and 556 ml, respectively.

3.2. Comparison of clinical characteristics and MRI parameters according to HLA-DRB1*15:01 and DRB1*04:05 status

There were no significant differences in clinical and MRI findings, including treatment status, between *HLA-DRB1*15:01*(+)**04:05*(−), *HLA-DRB1*15:01*(−)**04:05*(+), *DRB1*15:01*(+)**04:05*(+), and *HLA-DRB1*15:01*(−)**04:05*(−) carriers (Tables 3 and 4). Frequencies in OCBs or increased IgG index between MS patients with HLA-*DRB1*15:01*(+)**04:05*(−) (n = 17) and those with *DRB1*15:01*(−)**04:05*(+) (n = 21), excluding both alleles-positive patients, were not significantly different (OCBs, 57.1% vs. 40.0%, p = .487; and increased IgG index, 61.5% vs. 31.3%, p = .144, respectively; Table 3).

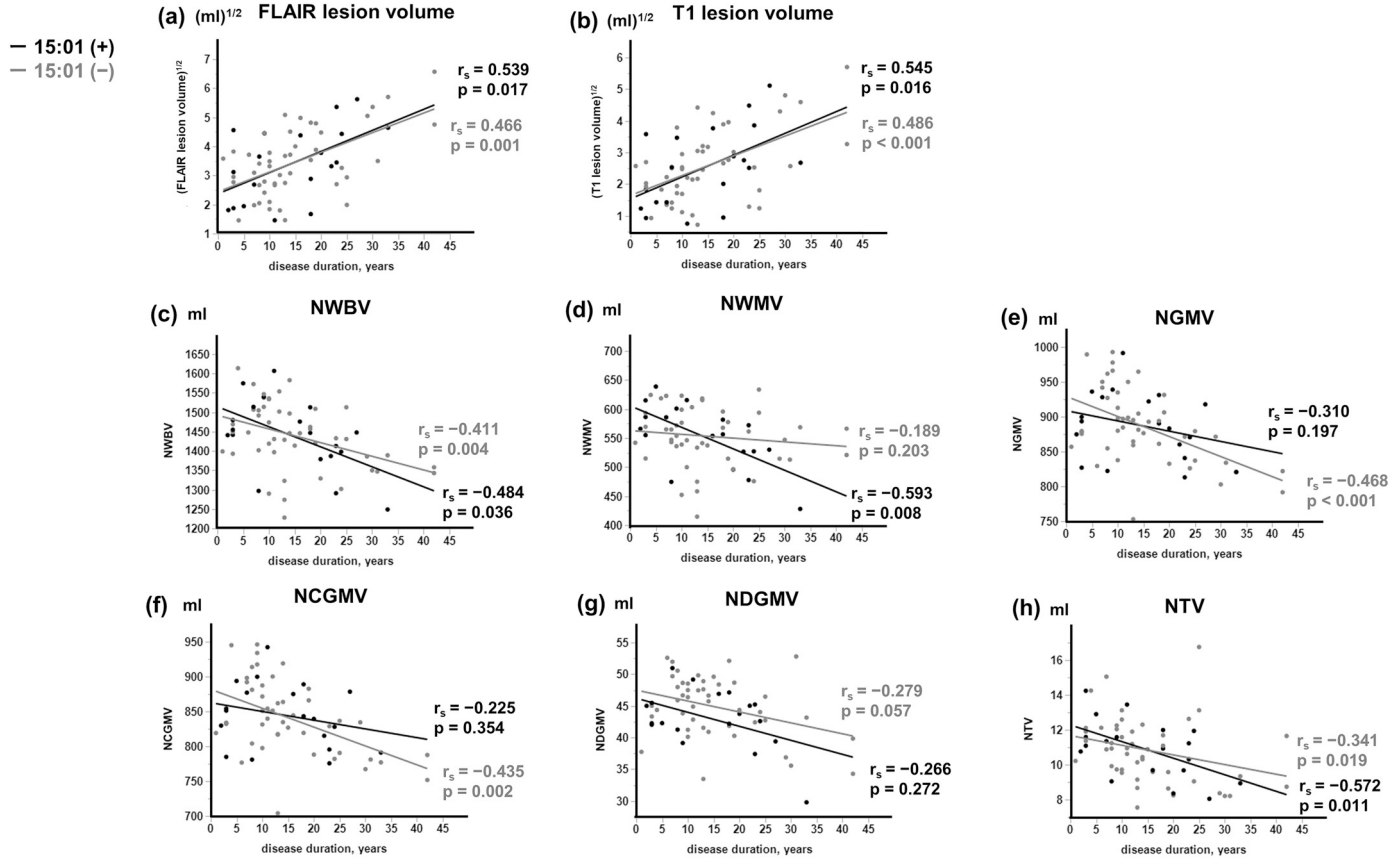

Fig. 3. Relationship between disease duration and MRI parameters in MS patients with and without *HLA-DRB1*15:01*.
Scatter plots show the relationship between disease duration and brain lesion volumes (FLAIR lesion volume (a) and T1 lesion volume (b)) and brain volumes (normalized whole brain volume [NWBV] (c); normalized white matter volume [NWMV] (d); normalized gray matter volume [NGMV] (e); normalized cortical gray matter volume [NCGMV] (f); normalized deep gray matter volume [NDGMV] (g); normalized thalamus volume [NTV] (h)) in *HLA-DRB1*15:01*-positive (black dots) and *HLA-DRB1*15:01*-negative (gray dots) patients. Lines represent the linear regression of the data in *HLA-DRB1*15:01*-positive (black line) and *HLA-DRB1*15:01*-negative (gray line) patients. FLAIR (a) and T1 lesion volumes (b) are represented as the square root transformed to a normal distribution. r_s = Spearman's rank correlation coefficient.

3.3. Correlation of brain and lesion volumes with disease duration and severity by univariate analysis

There were negative correlations between disease duration and NWBV ($r_s = -0.434$, $p < .001$), NWMV ($r_s = -0.316$, $p < .001$), NGMV ($r_s = -0.404$, $p < .001$), NCGMV ($r_s = -0.379$, $p = .002$), NDGMV ($r_s = -0.255$, $p = .039$), and NTV ($r_s = -0.380$, $p = .002$), and positive correlations between disease duration and FLAIR ($r_s = 0.474$, $p < .001$) and T1 lesion volumes ($r_s = 0.484$, $p < .001$) (Fig. 1). In addition, there were negative correlations between EDSS scores and NWBV ($r_s = -0.508$, $p < .001$), NWMV ($r_s = -0.337$, $p = .006$), NGMV ($r_s = -0.507$, $p < .001$), NCGMV ($r_s = -0.504$, $p < .001$), and NTV ($r_s = -0.312$, $p = .011$) (Fig. 2). FLAIR and T1 lesion volumes were not significantly associated with EDSS scores.

3.4. Correlation of brain and lesion volumes with disease duration and severity by multivariate analysis

We then investigated contributing factors to MRI parameters in all patients with MS using a multivariate analysis (Table 5). Disease duration was positively associated with lesion volume, and negatively associated with NWBV, NWMV, NGMV, NCGMV, and NTV. Age at first symptom was negatively associated with NWBV, NGMV, NCGMV, and NTV. Compared with male participants, female participants had greater NWBV, NGMV, and NCGMV. EDSS scores were negatively associated with NWBV.

3.5. Correlation of brain and lesion volumes with disease duration and severity according to HLA alleles by univariate analysis

Next, we assessed the correlation between MRI parameters and disease duration in patients with MS risk *HLA* alleles. There were negative correlations between disease duration and NWBV ($r_s = -0.484$, $p = .036$), NWMV ($r_s = -0.593$, $p = .008$), and NTV ($r_s = -0.572$, $p = .011$), and positive correlations between disease duration and FLAIR ($r_s = 0.539$, $p = .017$) and T1 lesion volumes ($r_s = 0.545$, $p = .016$) in the patients with *HLA-DRB1*15:01* (Fig. 3). By contrast, there were no significant correlations of any MRI parameters with disease duration in *HLA-DRB1*04:05* carriers (Fig. 4). No MRI parameters were significantly associated with EDSS scores in patients with *HLA-DRB1*15:01*. There was a significant negative correlation of NWBV with EDSS scores in those with *HLA-DRB1*04:05* ($r_s = -0.488$, $p = .018$).

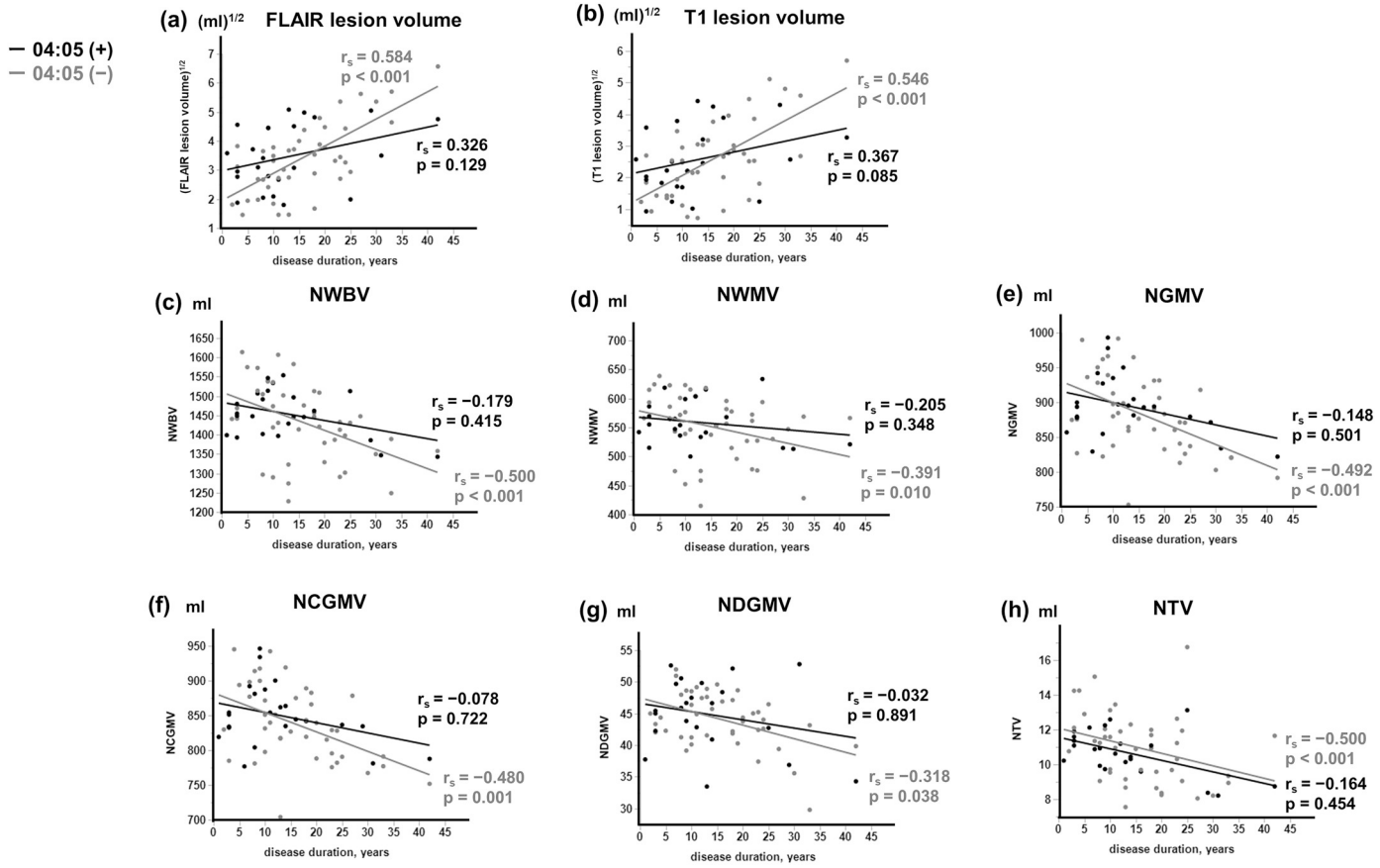

Fig. 4. Relationship between disease duration and MRI parameters in MS patients with and without *HLA-DRB1*04:05*.
Scatter plots show the relationship between disease duration and brain lesion volumes (FLAIR (a) and T1 lesion volumes (b)) and brain volumes (normalized whole brain volume [NWBV] (c); normalized white matter volume [NWMV] (d); normalized gray matter volume [NGMV] (e); normalized cortical gray matter volume [NCGMV] (f); normalized deep gray matter volume [NDGMV] (g); normalized thalamus volume [NTV] (h)) in *HLA-DRB1*04:05*-positive (black dots) and *HLA-DRB1*04:05*-negative (gray dots) patients. Lines represent the linear regression of the data in *HLA-DRB1*04:05*-positive (black line) and *HLA-DRB1*04:05*-negative (gray line) patients. FLAIR (a) and T1 lesion volumes (b) are represented as the square root transformed to a normal distribution. r_s = Spearman's rank correlation coefficient.

3.6. Correlation of brain and lesion volumes with disease duration and severity according to HLA alleles by multivariate analysis

We compared trends in the association of brain or lesion volume with disease duration between patients with and without each *HLA* allele. We found that patients with *HLA-DRB1*15:01* had a significantly faster reduction in NWBV and NWMV than those without *HLA-DRB1*15:01* (p = .010 and p = .003, respectively) (Table 6). Moreover, patients with *HLA-DRB1*15:01* had a significantly smaller NDGMV than those without (p = .044). *HLA-DRB1*04:05* carriers had a significantly slower increase in FLAIR lesion volume and T1 lesion volume than *HLA-DRB1*04:05* non-carriers (p = .035 and p = .046, respectively) (Table 7). To exclude the influence of the other allele, we performed the same multivariate analyses in patients without *HLA-DRB1*04:05* to assess the effect of *DRB1*15:01*, and in patients without *HLA-DRB1*15:01* to assess the effect of *DRB1*04:05*. These analyses confirmed the differences in volumetric reduction rates of NWBV and NWMV as well as accumulation rates of lesion volumes between *HLA-DRB1*15:01* carriers and *DRB1*15:01* non-carriers (Supplementary Table 3), and between *HLA-DRB1*04:05* carriers and *DRB1*04:05* non-carriers (Supplementary Table 4). The results were essentially same as those shown in Tables 6 and 7.

Finally, we directly compared MRI volumetric findings between MS patients with *HLA-DRB1*15:01*(+)**04:05*(−) and those with *HLA-DRB1*15:01*(−)**04:05*(+). As a result, *HLA-DRB1*15:01*(+)**04:05*(−) carriers had a significantly faster reduction in NWMV than *HLA-DRB1*15:01*(−)**04:05*(+) carriers (p = .016; Table 8). NWBV also tended to reduce faster in *HLA-DRB1*15:01*(+)**04:05*(−) carriers than in *HLA-DRB1*15:01*(−)**04:05*(+) carriers (p = .066). However, there were no significant between-group differences in FLAIR and T1 lesion volumes.

4. Discussion

In the present study, whole-brain (NWBV) and fractionated brain region volumes (NWMV, NGMV, NCGMV, NDGMV, and NTV) showed weak to moderate negative correlations with disease duration, and both FLAIR and T1 lesion volumes had moderate positive correlations with disease duration in Japanese patients with MS. When patients were separately analyzed according to the susceptibility *HLA-DRB1* alleles, these associations became stronger in *HLA-DRB1*15:01*(+) patients, but disappeared in *HLA-DRB1*04:05*(+) patients. Thus, the reduction of whole-brain and fractionated brain region volumes, as well as

Table 5
Multivariate analysis of factors contributing to MRI parameters in all patients with MS.

FLAIR lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.016	−0.311 to 0.278	0.912
Age at first symptoms	0.019	−0.011 to 0.049	0.204
OCBs	0.054	−0.254 to 0.362	0.725
DMD use	0.044	−0.349 to 0.436	0.824
EDSS scores	−0.067	−0.200 to 0.067	0.321
Disease duration	0.089	0.048 to 0.131	< 0.001

T1 lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	0.016	−0.268 to 0.299	0.912
Age at first symptoms	0.021	−0.008 to 0.049	0.156
OCBs	0.052	−0.245 to 0.348	0.727
DMD use	0.067	−0.311 to 0.445	0.723
EDSS scores	−0.097	−0.226 to 0.031	0.133
Disease duration	0.088	0.048 to 0.128	< 0.001

NWBV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	21.909	3.677 to 40.141	0.020
Age at first symptoms	−4.057	−5.902 to −2.211	< 0.001
OCBs	−2.549	−21.626 to 16.528	0.790
DMD use	−23.012	−47.327 to 1.303	0.063
EDSS scores	−9.420	−17.666 to −1.174	0.026
Disease duration	−5.949	−8.515 to −3.382	< 0.001

NWMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	4.138	−9.763 to 18.038	0.553
Age at first symptoms	−1.355	−2.762 to 0.052	0.059
OCBs	−2.245	−16.790 to 12.299	0.758
DMD use	−15.364	−33.903 to 3.174	0.102
EDSS scores	−5.664	−11.951 to 0.623	0.076
Disease duration	−2.286	−4.243 to −0.329	0.023

NGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	17.772	8.270 to 27.273	< 0.001
Age at first symptoms	−2.702	−3.664 to −1.740	< 0.001
OCBs	−0.303	−10.245 to 9.638	0.951
DMD use	−7.648	−20.319 to 5.024	0.231
EDSS scores	−3.756	−8.053 to 0.541	0.085
Disease duration	−3.663	−5.001 to −2.325	< 0.001

NCGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	18.346	9.200 to 27.492	< 0.001
Age at first symptoms	−2.767	−3.693 to −1.841	< 0.001
OCBs	−0.251	−9.821 to 9.319	0.958
DMD use	−8.565	−20.763 to 3.632	0.165
EDSS scores	−3.647	−7.783 to 0.490	0.083
Disease duration	−3.561	−4.849 to −2.274	< 0.001

NDGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.573	−1.930 to 0.785	0.401
Age at first symptoms	0.065	−0.072 to 0.202	0.347
OCBs	−0.052	−1.473 to 1.368	0.941
DMD use	0.917	−0.893 to 2.728	0.314
EDSS scores	−0.109	−0.724 to 0.505	0.722
Disease duration	−0.102	−0.293 to 0.089	0.290

NTV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.215	−0.685 to 0.256	0.364
Age at first symptoms	−0.064	−0.112 to −0.016	0.010
OCBs	−0.155	−0.647 to 0.337	0.530
DMD use	−0.458	−1.085 to 0.170	0.149
EDSS scores	−0.025	−0.238 to 0.187	0.812
Disease duration	−0.112	−0.179 to −0.046	0.001

The multivariate linear regression model to identify clinical factors contributing to MRI parameters included sex, age at first symptoms, OCBs, disease-modifying therapy, EDSS scores and disease duration.
DMD: Disease-modifying drug; EDSS: Expanded Disability Status Scale of Kurtzke; NCGMV: normalized cortical gray matter volume; NDGMV: normalized deep gray matter volume; NGMV: normalized gray matter volume; NTV: normalized thalamus volume; NWBV: normalized whole brain volume; NWMV: normalized white matter volume; OCBs: oligoclonal IgG bands.

increase of FLAIR and T1 lesion volumes over the disease course, are likely to be driven by patients with *HLA-DRB*15:01*. Absence of such correlations between disease duration and whole and regional brain volumes and between disease duration and lesion accumulation is characteristic of MS patients with *HLA-DRB1*04:05*. As a result, in Japanese patients with MS, *HLA-DRB1*15:01* carriers appear to show faster reduction of NWBV and NWMV than *HLA-DRB1*15:01* non-carriers. The faster reduction of NWBV in *HLA-DRB1*15:01* carriers was mostly attributable to the faster reduction of NWMV because NWMV occupies the large part of NWBV. By contrast, *HLA-DRB1*04:05* carriers showed slower accumulation of MRI lesions than *HLA-DRB1*04:05* non-carriers. These findings support the hypothesis that distinct *HLA-DRB1* alleles differentially influence brain and lesion volumes over the disease course.

Progressive decline of whole and regional brain volume over the disease course is true for both Caucasians and Japanese patients [13,14,30]. In a recent comparative MRI study [30], we investigated brain volume differences between Caucasian and Japanese patients with MS who enrolled in the fingolimod clinical trials [31,32]. In both races, NWBV, NCGMV, NDGMV, and NTV were significantly negatively correlated with disease duration, while NWMV was negatively correlated with disease duration in Caucasian patients only [30]. In the present study, NWMV was also significantly negatively associated with disease duration in Japanese patients with MS. This discrepancy could be explained by the fact that the present patients with MS were recruited from a single institution and showed a wide range of disease duration, while the patients from the clinical trial series mostly had short disease durations. The present study revealed that NWMV reduction over the disease course was significantly different according to the presence or absence of *HLA-DRB1*15:01*, whereby there was a faster reduction of NWMV in *DRB1*15:01* carriers than non-carriers. Accordingly, if large numbers of *DRB1*15:01* non-carriers (mainly *DRB1*04:05* carriers in Japanese patients with MS) are enrolled in a patient series, it may obscure the negative association between NWMV and disease duration.

In Caucasian patients with MS, a high-risk *HLA* genotype or higher *HLA* genetic burden has been associated with a faster reduction of whole-brain and gray matter volumes [33] and subcortical gray matter volume [34], which is mainly driven by the *HLA-DRB1*15:01* haplotype. Our findings of an association of *HLA-DRB1*15:01* with a faster reduction of whole-brain and regional brain volumes over the disease course in Japanese patients with MS is in line with these findings. We also found that patients with *HLA-DRB1*15:01* had significantly smaller NDGMV than those without, which is also in line with previous results [34]. In addition, Caucasian patients with MS with *HLA-DRB1*15:01* have been reported to show increased white matter lesion volumes [16] and enhanced lesion numbers and volumes compared with those without *HLA-DRB1*15:01* [17]. The relatively strong correlation between FLAIR and T1 lesion volumes and disease duration in the *HLA-DRB1*15:01* carriers is likely to reflect facilitated inflammatory lesion

Table 6
Multivariate analysis of factors contributing to MRI parameters in MS patients with *HLA-DRB1*15:01* and those without *HLA-DRB1*15:01*.

FLAIR lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.021	−0.321 to 0.279	0.887
Age at first symptoms	0.019	−0.011 to 0.049	0.211
OCBs	0.054	−0.262 to 0.370	0.732
DMD use	0.012	−0.410 to 0.433	0.956
EDSS scores	−0.061	−0.200 to 0.077	0.378
Disease duration	0.091	0.048 to 0.134	< 0.001
*HLA-DRB1*15:01*	0.060	−0.247 to 0.367	0.696
The interaction terms between disease duration and *HLA-DRB1*15:01*	0.007	−0.027 to 0.041	0.676

T1 lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	0.013	−0.277 to 0.303	0.929
Age at first symptoms	0.021	−0.009 to 0.050	0.163
OCBs	0.052	−0.253 to 0.357	0.732
DMD use	0.048	−0.359 to 0.455	0.814
EDSS scores	−0.094	−0.228 to 0.040	0.164
Disease duration	0.089	0.048 to 0.131	< 0.001
*HLA-DRB1*15:01*	0.032	−0.264 to 0.328	0.828
The interaction terms between disease duration and *HLA-DRB1*15:01*	0.004	−0.028 to 0.037	0.790

NWBV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	23.031	5.704 to 40.359	0.010
Age at first symptoms	−4.046	−5.796 to −2.295	< 0.001
OCBs	−3.861	−22.109 to 14.388	0.673
DMD use	−12.177	−36.513 to 12.159	0.320
EDSS scores	−11.575	−19.596 to −3.554	0.006
Disease duration	−6.664	−9.155 to −4.172	< 0.001
*HLA-DRB1*15:01*	−9.293	−27.01 to 8.426	0.297
The interaction terms between disease duration and *HLA-DRB1*15:01*	−2.625	−4.583 to −0.667	0.010

NWMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	4.844	−8.158 to 17.846	0.458
Age at first symptoms	−1.335	−2.649 to −0.022	0.047
OCBs	−3.876	−17.569 to 9.817	0.572
DMD use	−6.310	−24.571 to 11.950	0.491
EDSS scores	−7.651	−13.670 to −1.632	0.014
Disease duration	−2.906	−4.776 to −1.036	0.003
*HLA-DRB1*15:01*	−3.365	−16.662 to 9.931	0.613
The interaction terms between disease duration and *HLA-DRB1*15:01*	−2.284	−3.753 to −0.815	0.003

NGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	18.187	8.635 to 27.739	< 0.001
Age at first symptoms	−2.710	−3.675 to −1.745	< 0.001
OCBs	0.016	−10.044 to 10.076	0.998
DMD use	−5.866	−19.282 to 7.550	0.384
EDSS scores	−3.924	−8.346 to 0.498	0.081
Disease duration	−3.758	−5.131 to −2.384	< 0.001
*HLA-DRB1*15:01*	−5.928	−15.697 to 3.840	0.229
The interaction terms between disease duration and *HLA-DRB1*15:01*	−0.341	−1.420 to 0.739	0.529

NCGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	18.663	9.407 to 27.918	< 0.001
Age at first symptoms	−2.774	−3.709 to −1.839	< 0.001
OCBs	−0.004	−9.752 to 9.743	0.999
DMD use	−7.216	−20.214 to 5.783	0.270
EDSS scores	−3.772	−8.057 to 0.512	0.083
Disease duration	−3.633	−4.964 to −2.302	< 0.001
*HLA-DRB1*15:01*	−4.535	−14.000 to 4.930	0.341
The interaction terms between disease duration and *HLA-DRB1*15:01*	−0.257	−1.303 to 0.789	0.623

NDGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.474	−1.796 to 0.848	0.474
Age at first symptoms	0.063	−0.071 to 0.197	0.348

(*continued on next page*)

Table 6 (*continued*)

NDGMV (ml)	*Estimate*	*95% CI*	*p value*
OCBs	0.020	−1.373 to 1.412	0.977
DMD use	1.349	−0.508 to 3.206	0.151
EDSS scores	−0.152	−0.764 to 0.460	0.620
Disease duration	−0.125	−0.315 to 0.065	0.193
*HLA-DRB1*15:01*	−1.393	−2.745 to −0.041	0.044
The interaction terms between disease duration and *HLA-DRB1*15:01*	−0.083	−0.233 to 0.066	0.267

NTV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.210	−0.682 to 0.262	0.376
Age at first symptoms	−0.063	−0.111 to −0.016	0.010
OCBs	−0.192	−0.689 to 0.306	0.443
DMD use	−0.326	−0.990 to 0.337	0.328
EDSS scores	−0.059	−0.277 to 0.160	0.592
Disease duration	−0.122	−0.190 to −0.054	0.001
*HLA-DRB1*15:01*	0.058	−0.425 to 0.541	0.810
The interaction terms between disease duration and *HLA-DRB1*15:01*	−0.035	−0.089 to 0.018	0.190

The multivariate linear regression model to identify clinical and genetic factors contributing to MRI parameters included sex, age at first symptoms, OCBs, disease-modifying therapy, EDSS scores, disease duration, *HLA-DRB1*15:01*, and interaction terms between disease duration and *HLA-DRB1*15:01*.
DMD: Disease-modifying drug; EDSS: Expanded Disability Status Scale of Kurtzke; NCGMV: normalized cortical gray matter volume; NDGMV: normalized deep gray matter volume; NGMV: normalized gray matter volume; NTV: normalized thalamus volume; NWBV: normalized whole brain volume; NWMV: normalized white matter volume; OCBs: oligoclonal IgG bands.

accumulation in this *HLA* haplotype. Heightened intrathecal inflammatory and immune responses in *HLA-DRB1*15:01* carriers, as shown by enhanced inflammatory infiltrates in the autopsied brain [35] and high frequency of CSF IgG abnormalities [23], could contribute to the faster brain volume reduction and lesion accumulation among races in *HLA-DRB1*15:01* carriers than in *HLA-DRB1*15:01* non-carriers.

In MS patients with *HLA-DRB1*04:05*, we found a slower accumulation of FLAIR and T1 lesions over the disease course compared with those without *DRB1*04:05*, while we found no correlation between whole and regional brain volumes and disease duration. Although these findings could be caused by a lower detection power owing to a small sample size, the clear difference between *DRB1*15:01* carriers and *DRB1*04:05* carriers suggests some distinct disease mechanisms to be operative in these allele carriers.

A milder disease course and less intrathecal immune response, as indicated by fewer CSF IgG abnormalities, have repeatedly been reported in *DRB1*04:05* carriers [8,23,36]. However, the differences in the frequencies of CSF IgG abnormalities between exclusive *DRB1*04:05* and *DRB1*15:01* carriers did not reach statistical significance in the present study, most likely because of a small sample size. An autopsy study on MS brains has also reported that *DRB1*15:01* carriers have enhanced inflammatory infiltrates [35]. It is thus conceivable that *DRB1*04:05* carriers may have milder activation of the autoimmune system, including B cell responses, than *DRB1*15:01* carriers. These results indicate that activated inflammatory mechanisms or the degree of inflammation are different between MS patients with *DRB1*04:05* and those with *DRB1*15:01*. It is possible that the difference of autoantigens presented by each *HLA* molecule or the affinity of autoantigens to each *HLA* molecule may contribute to the difference in the brain volume via modulation of inflammatory reactions in the central nervous system tissue.

We found negative correlations of NWBV with EDSS scores in the patients with MS, which is consistent with previous studies in Caucasian patients [12,13,15]. Meanwhile, only a few studies have analyzed the correlation between brain volume and EDSS scores according to risk *HLA* alleles. Although Okuda et al. [16] reported that *HLA-DRB1*15:01* was associated with increased T2 lesion volumes and decreased NWBV, there was no association between *HLA-DRB1*15:01* and EDSS scores. Other studies have also reported that the presence or absence of *HLA-DRB1*15:01* was unrelated to EDSS scores [17,20,37]. In our study, neither *HLA-DRB1*15:01* nor *–DRB1*04:05* were significantly associated with EDSS scores. Furthermore, in *HLA-DRB1*15:01* carriers, there were no significant associations between brain MRI volumetric parameters and EDSS scores, despite the fact that whole-brain and all the fractionated brain region volumes showed a significant negative correlation with disease duration. To identify the MRI surrogate markers for disability, other parameters, such as cortical lesions, should be taken into account, especially considering that cortical lesions are not correlated with gray and white matter atrophy, but are correlated with both cognitive and physical impairment [22,38], and that *HLA-DRB1*15* augments the extent of inflammation in cortical lesions [35]. Interestingly, we also found that NWBV, NGMV, and NCGMV were associated with sex and age at the first symptom, and NTV was associated with age at the first symptom. These findings are in line with reports that older age at onset and being male are risk factors for a poor MS prognosis [39–41].

Our study has several limitations. First, the sample size was relatively small because of the rarity of MS in Japan [42]. This may have weakened the detection power of the present study. Nevertheless, we found significant differences in the volumetric findings by *HLA-DRB1* alleles, which is suggestive of the relatively strong effects of the two susceptibility alleles, *DRB1*15:01* and *04:05*. However, the effects of other minor susceptibility and resistance alleles in Japanese patients, including recently identified *HLA* class I alleles such as *HLA-B*39:01* and *HLA-B*15:01* [8,9,11], and the influence of genetic interactions on volumetric findings should be investigated in further large-scale studies. Second, we did not have MRI data of age and sex-matched healthy controls to compare with those of patients with MS. In particular, the lack of data of brain volume in Japanese healthy persons with determined *HLA* alleles means that we could not accurately evaluate the effects of the disease and the *HLA* on whole and regional brain volumes. Third, this was a cross-sectional study. Therefore, we could not estimate

Table 7
Multivariate analysis of factors contributing to MRI parameters in MS patients with *HLA-DRB1*04:05*(+) and those without *HLA-DRB1*04:05*(+).

FLAIR lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	0.063	−0.233 to 0.360	0.670
Age at first symptoms	0.014	−0.015 to 0.044	0.331
OCBs	0.076	−0.228 to 0.381	0.617
DMD use	−0.060	−0.456 to 0.335	0.760
EDSS scores	−0.042	−0.176 to 0.092	0.531
Disease duration	0.079	0.038 to 0.120	< 0.001
*HLA-DRB1*04:05*	0.044	−0.235 to 0.324	0.751
The interaction terms between disease duration and *HLA-DRB1*04:05*	−0.031	−0.061 to −0.002	0.035

T1 lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	0.090	−0.198 to 0.377	0.533
Age at first symptoms	0.017	−0.012 to 0.046	0.242
OCBs	0.065	−0.230 to 0.361	0.658
DMD use	−0.022	−0.405 to 0.361	0.909
EDSS scores	−0.079	−0.209 to 0.051	0.229
Disease duration	0.079	0.039 to 0.119	< 0.001
*HLA-DRB1*04:05*	0.005	−0.266 to 0.276	0.968
The interaction terms between disease duration and *HLA-DRB1*04:05*	−0.029	−0.057 to −0.001	0.046

NWBV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	20.195	1.217 to 39.173	0.038
Age at first symptoms	−4.139	−6.030 to −2.249	< 0.001
OCBs	−1.009	−20.495 to 18.477	0.918
DMD use	−22.990	−48.269 to 2.289	0.074
EDSS scores	−8.722	−17.306 to −0.138	0.047
Disease duration	−5.798	−8.447 to −3.148	< 0.001
*HLA-DRB1*04:05*	9.658	−8.236 to 27.551	0.284
The interaction terms between disease duration and *HLA-DRB1*04:05*	0.528	−1.333 to 2.388	0.572

NWMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	3.037	−11.546 to 17.620	0.678
Age at first symptoms	−1.378	−2.831 to 0.074	0.063
OCBs	−1.582	−16.555 to 13.391	0.833
DMD use	−14.992	−34.416 to 4.432	0.127
EDSS scores	−5.414	−12.009 to 1.182	0.106
Disease duration	−2.178	−4.214 to −0.142	0.037
*HLA-DRB1*04:05*	4.486	−9.263 to 18.235	0.515
The interaction terms between disease duration and *HLA-DRB1*04:05*	0.363	−1.066 to 1.792	0.612

NGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	17.158	7.255 to 27.061	0.001
Age at first symptoms	−2.761	−3.747 to −1.774	< 0.001
OCBs	0.573	−9.595 to 10.741	0.910
DMD use	−7.998	−21.189 to 5.193	0.229
EDSS scores	−3.309	−7.788 to 1.170	0.144
Disease duration	−3.620	−5.002 to −2.237	< 0.001
*HLA-DRB1*04:05*	5.172	−4.165 to 14.509	0.271
The interaction terms between disease duration and *HLA-DRB1*04:05*	0.165	−0.806 to 1.135	0.735

NCGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	17.874	8.335 to 27.412	< 0.001
Age at first symptoms	−2.829	−3.779 to −1.879	< 0.001
OCBs	0.609	−9.185 to 10.403	0.901
DMD use	−9.039	−21.745 to 3.666	0.159
EDSS scores	−3.190	−7.504 to 1.124	0.144
Disease duration	−3.534	−4.866 to −2.203	< 0.001
*HLA-DRB1*04:05*	4.958	−4.035 to 13.952	0.273
The interaction terms between disease duration and *HLA-DRB1*04:05*	0.113	−0.822 to 1.048	0.810

NDGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.714	−2.139 to 0.710	0.319
Age at first symptoms	0.068	−0.074 to 0.210	0.339
OCBs	−0.036	−1.499 to 1.427	0.961

(*continued on next page*)

Table 7 (*continued*)

NDGMV (ml)	*Estimate*	*95% CI*	*p value*
DMD use	1.041	−0.857 to 2.939	0.276
EDSS scores	−0.119	−0.763 to 0.525	0.712
Disease duration	−0.086	−0.285 to 0.113	0.391
*HLA-DRB1*04:05*	0.214	−1.129 to 1.558	0.750
The interaction terms between disease duration and *HLA-DRB1*04:05*	0.052	−0.088 to 0.191	0.460
NTV (ml)	***Estimate***	***95% CI***	***p value***
Sex (Female)	−0.206	−0.701 to 0.289	0.408
Age at first symptoms	−0.062	−0.111 to −0.013	0.015
OCBs	−0.179	−0.687 to 0.329	0.483
DMD use	−0.440	−1.100 to 0.219	0.186
EDSS scores	−0.039	−0.263 to 0.185	0.730
Disease duration	−0.113	−0.182 to −0.043	0.002
*HLA-DRB1*04:05*	−0.134	−0.601 to 0.333	0.567
The interaction terms between disease duration and *HLA-DRB1*04:05*	−0.001	−0.050 to 0.047	0.952

The multivariate linear regression model to identify clinical and genetic factors contributing to MRI parameters included sex, age at first symptoms, OCBs, disease-modifying therapy, EDSS scores, disease duration, *HLA-DRB1*04:05*, and interaction terms between disease duration and *HLA-DRB1*04:05*.
DMD: Disease-modifying drug; EDSS: Expanded Disability Status Scale of Kurtzke; NCGMV: normalized cortical gray matter volume; NDGMV: normalized deep gray matter volume; NGMV: normalized gray matter volume; NTV: normalized thalamus volume; NWBV: normalized whole brain volume; NWMV: normalized white matter volume; OCBs: oligoclonal IgG bands.

the exact annual rate of brain atrophy or annual development of brain lesion volume. Longitudinal prospective studies with appropriate controls are needed to evaluate these factors in the Japanese MS population. Fourth, we did not measure anti-MOG antibodies in most of the present patients with MS. However, given that anti-MOG antibodies are rarely found in adult patients with MS presenting with typical MRI features [43,44], we believe that this does not severely distort the present findings. Fifth, OCBs were not measured in all cases by isoelectric focusing, which has a higher sensitivity than agarose gel electrophoresis (77% vs. 23% in Japanese patients with MS) [45]. However, the availability of the OCBs study methods, i.e., isoelectric focusing, agarose gel electrophoresis, and unspecified, was not significantly different between *HLA* genotypes. In addition, when CSF IgG abnormalities were excluded from the multivariate analyses, the main contribution of *HLA* to MRI parameters did not change (data not shown). Therefore, we believe that this limitation did not severely affect the results. Finally, we did not measure spinal cord volume in the present study, partly because of the difficulty of performing spinal cord MRI at the same time as brain MRI at our institution. Given our recent findings that the upper cervical cord area is negatively associated with EDSS scores in Japanese patients with MS [46], it would be interesting to compare spinal cord atrophy between *HLA* alleles and analyze the correlations between spinal cord atrophy and brain MRI parameters in future work.

5. Conclusions

In *HLA-DRB1*15:01* carriers, we found negative correlations of NWBV, NWMV, and NTV and positive correlations of FLAIR and T1 lesion volumes with disease duration. In *HLA-DRB1*04:05* carriers, there were no significant correlations of any MRI parameters with disease duration. As a result, *HLA-DRB1*15:01* carriers showed a significantly faster reduction in NWBV and NWMV by disease duration and a smaller NDGMV than *HLA-DRB1*15:01* non-carriers, while *HLA-DRB1*04:05* carriers showed a significantly slower increase in FLAIR and T1 lesion volumes than *HLA-DRB1*04:05* non-carriers. These findings suggest that *HLA-DRB1* alleles can distinctly influence brain and lesion volumes over the disease course in MS.

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Ethical approval

All the procedures conducted with the participants of this study were carried out according to the Declaration of Helsinki. This study protocol was approved by the Kyushu University Ethics Committee.

Informed consent

All participants provided written informed consent.

Author contributions

All authors contributed to the study conception and design. Data collection and analyses were performed by Shoko Fukumoto, Yuri Nakamura, and Mitsuru Watanabe. Yuri Nakamura, Mitsuru Watanabe, Takuya Matsushita, Noriko Isobe, Ayako Sakoda, Akio Hiwatashi, Ryo Yamasaki, Akira Tsujino, and Jun-ichi Kira provided critical discussion of the results. The results were interpreted by Shoko Fukumoto, Yuri Nakamura, Mitsuru Watanabe, and Jun-ichi Kira. The first and revised drafts of the manuscript were written by Shoko Fukumoto, Mitsuru Watanabe, and Jun-ichi Kira. All authors read and approved the final manuscript.

Table 8
Multivariate analysis of factors contributing to MRI parameters in MS patients with *HLA-DRB1*15:01*(+)**04:05*(−) and those with *HLA-DRB1*15:01*(−)**04:05*(+).

FLAIR lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	0.204	−0.322 to 0.730	0.432
Age at first symptoms	0.018	−0.033 to 0.069	0.483
OCBs	−0.217	−0.765 to 0.331	0.423
DMD use	−0.100	−0.825 to 0.626	0.780
EDSS scores	−0.058	−0.286 to 0.169	0.602
Disease duration	0.063	−0.001 to 0.127	0.054
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	0.047	−0.376 to 0.470	0.821
The interaction terms between disease duration and *HLA*	0.030	−0.020 to 0.081	0.230

T1 lesion volume (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	0.282	−0.231 to 0.796	0.268
Age at first symptoms	0.016	−0.034 to 0.065	0.524
OCBs	−0.276	−0.811 to 0.259	0.298
DMD use	0.014	−0.694 to 0.721	0.969
EDSS scores	−0.157	−0.379 to 0.064	0.156
Disease duration	0.056	−0.006 to 0.119	0.074
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	0.090	−0.323 to 0.503	0.657
The interaction terms between disease duration and *HLA*	0.018	−0.032 to 0.067	0.464

NWBV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	9.335	−20.759 to 39.428	0.529
Age at first symptoms	−3.098	−6.009 to −0.186	0.038
OCBs	15.726	−15.626 to 47.078	0.312
DMD use	4.990	−36.504 to 46.483	0.806
EDSS scores	−7.321	−20.321 to 5.679	0.257
Disease duration	−5.455	−9.116 to −1.795	0.005
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	−14.243	−38.440 to 9.954	0.237
The interaction terms between disease duration and *HLA*	−2.701	−5.592 to 0.191	0.066

NWMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−11.862	−31.960 to 8.236	0.236
Age at first symptoms	−0.048	−1.993 to 1.896	0.960
OCBs	6.598	−14.341 to 27.537	0.522
DMD use	6.214	−21.498 to 33.926	0.648
EDSS scores	−6.062	−14.744 to 2.621	0.163
Disease duration	−1.967	−4.412 to 0.478	0.110
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	−3.186	−19.347 to 12.974	0.688
The interaction terms between disease duration and *HLA*	−2.418	−4.349 to −0.487	0.016

NGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	21.197	5.055 to 37.338	0.012
Age at first symptoms	−3.050	−4.611 to −1.488	0.001
OCBs	9.128	−7.688 to 25.945	0.274
DMD use	−1.224	−23.481 to 21.032	0.911
EDSS scores	−1.259	−8.232 to 5.714	0.713
Disease duration	−3.488	−5.452 to −1.525	0.001
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	−11.057	−24.036 to 1.922	0.092
The interaction terms between disease duration and *HLA*	−0.283	−1.834 to 1.268	0.711

NCGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	22.130	6.584 to 37.676	0.007
Age at first symptoms	−3.113	−4.617 to −1.609	< 0.001
OCBs	8.730	−7.466 to 24.926	0.278
DMD use	−3.356	−24.791 to 18.079	0.750
EDSS scores	−1.310	−8.026 to 5.405	0.691
Disease duration	−3.354	−5.245 to −1.463	0.001
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	−9.829	−22.329 to 2.671	0.118
The interaction terms between disease duration and *HLA*	−0.166	−1.660 to 1.328	0.821

NDGMV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.932	−3.727 to 1.863	0.498
Age at first symptoms	0.063	−0.207 to 0.334	0.634
OCBs	0.400	−2.512 to 3.312	0.780

(*continued on next page*)

Table 8 (*continued*)

NDGMV (ml)	*Estimate*	*95% CI*	*p value*
DMD use	2.130	−1.723 to 5.984	0.266
EDSS scores	0.051	−1.156 to 1.259	0.931
Disease duration	−0.134	−0.474 to 0.206	0.425
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	−1.229	−3.476 to 1.019	0.271
The interaction terms between disease duration and *HLA*	−0.117	−0.385 to 0.152	0.380

NTV (ml)	*Estimate*	*95% CI*	*p value*
Sex (Female)	−0.542	−1.175 to 0.091	0.090
Age at first symptoms	−0.045	−0.106 to 0.016	0.144
OCBs	−0.024	−0.684 to 0.635	0.940
DMD use	−0.270	−1.143 to 0.603	0.530
EDSS scores	−0.016	−0.290 to 0.257	0.905
Disease duration	−0.116	−0.193 to −0.039	0.005
HLA [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)]	0.168	−0.341 to 0.677	0.504
The interaction terms between disease duration and *HLA*	−0.029	−0.090 to 0.031	0.328

The multivariate linear regression model to identify clinical and genetic factors contributing to MRI parameters included sex, age at first symptoms, OCBs, disease-modifying therapy, EDSS scores, disease duration, *HLA* [*DRB1*15:01*(+)**04:05*(−) vs. *DRB1*15:01*(−)**04:05*(+)], and interaction terms between disease duration and *HLA*.

DMD: Disease-modifying drug; EDSS: Expanded Disability Status Scale of Kurtzke; NCGMV: normalized cortical gray matter volume; NDGMV: normalized deep gray matter volume; NGMV: normalized gray matter volume; NTV: normalized thalamus volume; NWBV: normalized whole brain volume; NWMV: normalized white matter volume; OCBs: oligoclonal IgG bands.

Declaration of Competing Interest

Dr. Fukumoto, Dr. Hiwatashi, and Dr. Yamasaki declare no conflicts of interest. Dr. Nakamura received grant support from Mitsubishi Tanabe Pharma, Bayer Yakuhin, Ltd., and Japan Blood Products Organization, and received speaker honoraria from Novartis Pharma. Dr. Watanabe received speaker honoraria and consultant fees from Novartis Pharma. Dr. Matsushita received speaker honoraria payments from Mitsubishi Tanabe Pharma, Takeda Pharmaceutical Company, and Biogen Japan. Dr. Isobe received grant support from Mitsubishi Tanabe Pharma, Osoegawa Neurology Clinic, Bayer Yakuhin, Ltd., and Japan Blood Products Organization. Dr. Kira received consultant fees, speaking fees and/or honoraria from Novartis Pharma, Mitsubishi Tanabe Pharma, Boehringer Ingelheim, Teijin Pharma, Takeda Pharmaceutical Company, Otsuka Pharmaceutical, Astellas Pharma, Pfizer Japan, Sumitomo Dainippon Pharma, and Eisai.

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Appendix A. Supplementary data

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