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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

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 = .007) 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. *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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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.

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.

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	15:01(+)04:05(-)	15:01 (-) 04:05 (+)	15:01(+)04:05(+)	15:01 (-) 04:05 (-)	p value			
	(/1 = U)	(TZ = U)	(7 = 11)	(07 = u)	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 (+)
2 emale 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)	PMS 4 (19.0)	PMS 1 (50.0)	PMS 7 (26.9)	1.000	0.731	NA ^b	1.000
	RRMS 13 (76.5)	RRMS 17 (81.0)	RRMS 1 (50.0)	RRMS 19 (73.1)				
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
² atients with DMDs or immunosuppressive	14 (82.4)	18 (85.7)	2 (100.0)	20 (76.9)	1.000	0.711	NA ^b	1.000
agents, n (%)								
DCBs, 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
ncreased 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
MDe: Disease-modifizing drugs: FDSC: F	Vrnanded Disability Sta	this Scale of Kurtzker	MS: multinle sclerosis	. DMS: progressive MS	s. RRMS. relansing.re	mitting MS: OCBs: oli	oorlonal IoG hands	

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

Table 3

2

As there were only 2 patients with HLA-DRB1*15:01 (+), *04:05 (+), these data were not included in the statistical analyses (NA). Values are expressed as the median (interquartile range)

Ā

	15:01 (+) 04:05 (-)	15:01 (-) 04:05 (+)	15:01 (+) 04:05 (+)	15:01 (-) 04:05 (-)	<i>p</i> value			
		(17 - 11)	(7 - 11)	(oz – m)	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

As there were only 2 patients with HLA-DRB1 *15:01 (+) *04:05 (+), these data were not included in the statistical analyses (NA). NWMV: normalized white matter volume.

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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

Table 4



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 wholebrain 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 noncarriers (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

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
A co ot first sumstance	0.010	0.011 to 0.2/0	0.012
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
Disease duration	0.005	0.040 10 0.131	< 0.001
T1 logion volume (ml)	Ectimate	05% CI	n yalua
11 lesion volume (mi)	Estimate	95% 01	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
FDSS scores	-0.097	-0.226 to 0.031	0 1 3 3
Disease duration	0.007	0.220 10 0.031	0.135
Disease duration	0.088	0.048 10 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
FDSS scores	-9/20	-17666 to -1174	0.005
ED35 scores	- 9.420	-17.000 to -1.174	0.020
Disease duration	- 5.949	-8.515 to -3.382	< 0.001
NUAD 017 (1)	Fedding and a	05% 01	
	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 upo	15 264	22,002 to 2174	0.102
DMD use	-15.504	- 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	-3664 to -1740	< 0.001
Age at hist symptoms	- 2.702	- 3.004 10 - 1.740	< 0.001
OCBS	-0.303	-10.245 10 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
NCOMU (1)	R. du	05% 01	
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
FDSS scores	-3647	-7.783 to 0.490	0.083
Disease duration	2.561	4 940 to 0.190	0.000 < 0.001
Disease duration	- 5.501	-4.849 10 -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	-0109	-0.724 to 0.505	0 722
Disassa duration	0.100	0.202 to 0.000	0.722
Disease uniation	-0.102	- 0.293 10 0.089	0.290
NTV (ml)	Estimate	95% CI	p value
	0.01-	0.007	1
Sex (Female)	-0.215	-0.685 to 0.256	0.364
Age at first symptoms	-0.064	-0.112 to -0.016	0.010
0			

Table 5 (con	ntinued)
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NTV (ml)	Estimate	95% CI	p value
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* haplo-type. 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

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	0.007
Age at mist symptoms	0.054	-0.262 to 0.370	0.211
DMD use	0.012	-0.202 to 0.370	0.752
EDSS scores	-0.061	-0.200 to 0.077	0.330
ED33 scores	0.001	-0.200 to 0.077	0.378
	0.051	-0.247 ± 0.267	0.606
The interaction terms between disease duration and HIA DPR1*15:01	0.000	-0.27 to 0.307	0.090
The interaction terms between disease duration and <i>ILA-DADI</i> 13.01	0.007	-0.027 10 0.041	0.070
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
			0.04 -
Sex (Female)	23.031	5.704 to 40.359	0.010
Age at first symptoms	- 4.046	-5.796 to -2.295	< 0.001
DVD8	- 3.861	- 22.109 to 14.388	0.673
DMD use	-12.1//	- 36.513 to 12.159	0.320
EDSS scores	-11.5/5	- 19.596 to - 3.554	0.006
Disease duration	- 6.664	-9.155 to -4.1/2	< 0.001
HLA-DKB1^15:01	-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	-3675 to -1745	< 0.001
OCBs	0.016	-10.044 to 10.076	0.001
DMD 11se	-5.866	-19282 to 7550	0.384
FDSS scores	- 3 924	-8346 to 0.498	0.081
Disease duration	- 3 758	-5131 to -2384	< 0.001
HLA-DRB1*15:01	-5.928	-15.697 to 3.840	0 229
The interaction terms between disease duration and <i>HLA-DRB1*15:01</i>	-0.341	-1.420 to 0.739	0.529
NCGMV (ml)	Fstimate	95% CI	n value
	Estimate	2070 GI	p vulue
Sex (Female)	18.663	9.407 to 27.918	< 0.001
Age at first symptoms	-2.774	-3.709 to -1.839	< 0.001
	-0.004	-9.752 to 9.743	0.999
	- /.210	- 20.214 to 5.783	0.270
ED35 Stores	-3.//2	- 8.05/ 10 0.512	0.083
	- 3.033	-4.904 to $-2.302-14 000 to 4 920$	0.241
The interaction terms between discass duration and ULA DDD1*15.01	- 4.335	- 14.000 to 4.930	0.341
The interaction terms between disease duration and <i>nLA-DKB1^15:01</i>	-0.237	- 1.303 10 0./89	0.023
NDGMV (ml)	Estimate	95% CI	p value
NDGMV (mi) Sex (Female)	<i>Estimate</i>	95% CI	p value 0.474
NDGMV (mi) Sex (Female) Age at first symptoms	Estimate - 0.474 0.063	95% CI - 1.796 to 0.848 - 0.071 to 0.197	p value 0.474 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
Sex (Female) Age at first symptoms	-0.210 -0.063	-0.682 to 0.262 -0.111 to -0.016	0.376 0.010
Sex (Female) Age at first symptoms OCBs	-0.210 -0.063 -0.192	-0.682 to 0.262 -0.111 to -0.016 -0.689 to 0.306	0.376 0.010 0.443
Sex (Female) Age at first symptoms OCBs DMD use	-0.210 -0.063 -0.192 -0.326	-0.682 to 0.262 -0.111 to -0.016 -0.689 to 0.306 -0.990 to 0.337	0.376 0.010 0.443 0.328
Sex (Female) Age at first symptoms OCBs DMD use EDSS scores	-0.210 -0.063 -0.192 -0.326 -0.059	-0.682 to 0.262 -0.111 to -0.016 -0.689 to 0.306 -0.990 to 0.337 -0.277 to 0.160	0.376 0.010 0.443 0.328 0.592
Sex (Female) Age at first symptoms OCBs DMD use EDSS scores Disease duration	$\begin{array}{c} -0.210\\ -0.063\\ -0.192\\ -0.326\\ -0.059\\ -0.122\end{array}$	$\begin{array}{r} -0.682 \ {\rm to} \ 0.262 \\ -0.111 \ {\rm to} \ -0.016 \\ -0.689 \ {\rm to} \ 0.306 \\ -0.990 \ {\rm to} \ 0.337 \\ -0.277 \ {\rm to} \ 0.160 \\ -0.190 \ {\rm to} \ -0.054 \end{array}$	0.376 0.010 0.443 0.328 0.592 0.001
Sex (Female) Age at first symptoms OCBs DMD use EDSS scores Disease duration <i>HLA-DRB1*15:01</i>	$\begin{array}{c} -0.210\\ -0.063\\ -0.192\\ -0.326\\ -0.059\\ -0.122\\ 0.058\end{array}$	$\begin{array}{r} - 0.682 \ {\rm to} \ 0.262 \\ - 0.111 \ {\rm to} \ - 0.016 \\ - 0.689 \ {\rm to} \ 0.306 \\ - 0.990 \ {\rm to} \ 0.337 \\ - 0.277 \ {\rm to} \ 0.160 \\ - 0.190 \ {\rm to} \ - 0.054 \\ - 0.425 \ {\rm to} \ 0.541 \end{array}$	0.376 0.010 0.443 0.328 0.592 0.001 0.810

The multivariate linear regression model to identify clinical and genetic factors contributing to MRI parameters included sex, age at first symptoms, OCBs, diseasemodifying 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

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
0 (D	0.000	0.000 +- 0.000	0.670
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
HI 4-DRB1*04-05	0.044	= 0.235 to 0.324	0.751
The interaction terms between disease duration and HIA DRP1*04:05	0.021	0.235 to 0.324	0.025
The interaction terms between disease duration and <i>TLA-DAD1</i> 07.05	-0.031	-0.001 10 -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 1156	-0.022	-0.405 to 0.361	0.909
EDSS scores	-0.079	-0.209 to 0.051	0.220
ED35 scores	-0.079		0.229 < 0.001
Disease duration	0.079	0.039 10 0.119	< 0.001
HLA-DRB1*04:05	0.005	-0.266 to 0.276	0.968
The interaction terms between disease duration and <i>HLA-DRB1*04:05</i>	-0.029	-0.057 to -0.001	0.046
NWRV (ml)	Fstimate	95% CI	n value
	Estimate	7570 GI	p vuille
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	-8722	-17306 to -0.138	0.047
Disease duration	- 5 798	-8.447 to -3.148	< 0.01
	- 3.798	- 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
	0.000	1000 0 10/2	0.012
NGMV (ml)	Estimate	95% CI	p value
Sex (Female)	17 158	7 255 to 27 061	0.001
Age at first symptoms	-2.761	-3747 to -1774	< 0.001
OCPo	0.572	$0.505 \pm 0.10.741$	0.001
	7.009	21 100 to E 102	0.910
DMD use	- 7.998	-21.189 to 5.195	0.229
Eliss scores	- 3.309	-/./88 t0 1.1/U	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	n value
room (m)	Lounau	2070 GI	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
HI A_DRR1*04.05	4 958	-4 035 to 13 952	0.272
The interpolity terms between diverse low-time and that DDD1404.05	0 110		0.2/3
The interaction terms between disease duration and HLA-DKB1*04:05	0.113	- 0.822 10 1.048	0.810
NDGMV (ml)	Estimate	95% CI	p value
	0.514	0.100 - 0.710	0.010
Sex (remale)	-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, diseasemodifying 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.

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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 (Eemale)	- 11 862	-31,960 to 8,236	0.226
Age at first symptoms	- 0.048	-1.902 to 1.806	0.230
OCBe	6 598	-14341 to 27537	0.500
DMD use	6 214	-21498 to 33.926	0.522
EDSS scores	-6.062	-14744 to 2621	0.040
Disease duration	-1 967	-4.412 to 0.478	0.100
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 <i>HLA</i>	-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)	- 0.022	- 2 727 to 1 969	0 400
Age at first symptoms	- 0.932	-3.727 to 1.803	0.498
	0.003	-0.207 to 0.334	0.034
ورتي 1	0.400	-2.512 10 3.512	0.780

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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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References

- L. Piccolo, G. Kumar, I. Nakashima, et al., Multiple sclerosis in Japan appears to be a milder disease compared to the UK, J. Neurol. 262 (2015) 831–836, https://doi. org/10.1007/s00415-015-7637-3.
- [2] I. Nakashima, K. Fujihara, M. Okita, et al., Clinical and MRI study of brain stem and cerebellar involvement in Japanese patients with multiple sclerosis, J. Neurol. Neurosurg. Psychiatry 67 (1999) 153–157.
- [3] B. Weinstock-Guttmann, L.D. Jacobs, C.M. Brownscheidle, et al., Multiple sclerosis characteristics in African American patients in the New York state multiple sclerosis consortium, Mult. Scler. J. 9 (2003) 293–298.
- [4] B.A. Cree, O. Khan, D. Bourdette, et al., Clinical characteristics of African Americans vs Caucasian Americans with multiple sclerosis, Neurology 63 (2004) 2039–2045.
- [5] B. Weinstock-Guttman, M. Ramanathan, K. Hashmi, et al., Increased tissue damage and lesion volumes in African Americans with multiple sclerosis, Neurology. 74

(2010) 538-544, https://doi.org/10.1212/WNL.0b013e3181cff6fb.

- [6] G. Ebers, Environmental factors and multiple sclerosis, Lancet Neurol. 7 (2008) 268–277, https://doi.org/10.1016/S1474-4422(08)70042-5.
- [7] S. Sawcer, G. Hellenthal, M. Pirinen, et al., Genetic risk and a primary role for cellmediated immune mechanisms in multiple sclerosis, Nature. 476 (2011) 214–219, https://doi.org/10.1038/nature10251.
- [8] S. Yoshimura, N. Isobe, T. Yonekawa, et al., Genetic and infectious profiles of Japanese multiple sclerosis patients, PLoS One 7 (2012) e48592, https://doi.org/ 10.1371/journal.pone.0048592.
- [9] K. Ogawa, T. Okuno, K. Hosomichi, et al., Next-generation sequencing identifies contribution of both class I and II HLA genes on susceptibility of multiple sclerosis in Japanese, J. Neuroinflammation 16 (2019) 162, https://doi.org/10.1186/ s12974-019-1551-z.
- [10] F.B.S. Briggs, J.C. Yu, M.F. Davis, et al., Multiple sclerosis risk factors contribute to onset heterogeneity, Mult. Scler. Relat. Disord. 28 (2019) 11–16, https://doi.org/ 10.1016/j.msard.2018.12.007.
- [11] Y. Nakamura, T. Matsushita, S. Sato, et al., Latitude and HLA-DRB1*04:05 independently influence disease severity in Japanese multiple sclerosis: a cross-sectional study, J. Neuroinflammation 13 (2016) 239, https://doi.org/10.1186/ s12974-016-0695-3.
- [12] A. Eshaghi, F. Prados, W.J. Brownlee, et al., Deep gray matter volume loss drives disability worsening in multiple sclerosis, Ann. Neurol. 83 (2018) 210–222, https:// doi.org/10.1002/ana.25145.
- [13] N. De Stefano, M.L. Stromillo, A. Giorgio, et al., Establishing pathological cut-offs of brain atrophy rates in multiple sclerosis, J. Neurol. Neurosurg. Psychiatry 87 (2016) 93–99, https://doi.org/10.1136/jnnp-2014-309903.
- [14] S. Datta, T.D. Staewen, S.S. Cofield, et al., Regional gray matter atrophy in relapsing remitting multiple sclerosis: baseline analysis of multi-center data, Mult. Scler. Relat. Disord. 4 (2015) 124–136, https://doi.org/10.1016/j.msard.2015.01.004.
- [15] M.D. Steenwijk, J.J. Geurts, M. Daams, et al., Cortical atrophy patterns in multiple sclerosis are non-random and clinically relevant, Brain. 139 (2016) 115–126, https://doi.org/10.1093/brain/awv337.
- [16] D.T. Okuda, R. Srinivasan, J.R. Oksenberg, et al., Genotype-phenotype correlations in multiple sclerosis: HLA genes influence disease severity inferred by 1HMR spectroscopy and MRI measures, Brain. 132 (2009) 250–259, https://doi.org/10. 1093/brain/awn301.
- [17] D. Horakova, R. Zivadinov, B. Weinstock-Guttman, et al., *HLA DRB1*1501* is only modestly associated with lesion burden at the first demyelinating event, J. Neuroimmunol. 236 (2011) 76–80, https://doi.org/10.1016/j.jneuroim.2011.04. 011.
- [18] A. Van der Walt, J. Stankovich, M. Bahlo, et al., Heterogeneity at the HLA-DRB1 allelic variation locus does not influence multiple sclerosis disease severity, brain atrophy or cognition, Mult. Scler. J. 17 (2011) 344–352, https://doi.org/10.1177/ 1352458510389101.
- [19] T. Kalincik, C.R. Guttmann, J. Krasecsky, et al., Multiple sclerosis susceptibility loci do not alter clinical and MRI outcomes in clinically isolated syndrome, Genes Immun. 14 (2013) 244–248, https://doi.org/10.1038/gene.2013.17.
- [20] Ö. Yaldizli, V. Sethi, M. Pardini, et al., HLA-DRB*1501 associations with magnetic resonance imaging measures of grey matter pathology in multiple sclerosis, Mult. Scler. Relat. Disord. 7 (2016) 47–52, https://doi.org/10.1016/j.msard.2016.03. 003.
- [21] Ö. Yaldizli, V. Sethi, M. Pardini, et al., Response to the commentary of Yates RL and

DeLuca GC on the study: HLA-DRB1*1501 associations with magnetic resonance imaging measures of grey matter pathology in multiple sclerosis, Mult. Scler. Relat. Disord. 19 (2018) 168–170, https://doi.org/10.1016/j.msard.2016.08.006.

- [22] K. Shinoda, T. Matsushita, Y. Nakamura, et al., *HLA-DRB1**04:05 allele is associated with intracortical lesions on three-dimensional double inversion recovery images in Japanese patients with multiple sclerosis, Mult. Scler. J. 24 (2018) 710–720, https://doi.org/10.1177/1352458517707067.
- [23] S. Yoshimura, N. Isobe, T. Matsushita, et al., Genetic and infectious profiles influence cerebrospinal fluid IgG abnormality in Japanese multiple sclerosis patients, PLoS One 9 (2014) e95367, https://doi.org/10.1371/journal.pone.0095367.
- [24] C.H. Polman, S.C. Reingold, B. Banwell, et al., Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria, Ann. Neurol. 69 (2011) 292–302, https://doi.org/10.1002/ana.22366.
- [25] A.J. Thompson, B.L. Banwell, F. Barkhof, et al., Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria, Lancet Neurol. 17 (2018) 162–173, https://doi. org/10.1016/S1474-4422(17)30470-2.
- [26] J.F. Kurtzke, Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS), Neurology 33 (1983) 1444–1452, https://doi.org/10. 1212/wnl.33.11.1444.
- [27] J. Kira, T. Kanai, Y. Nishimura, et al., Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders, Ann. Neurol. 40 (1996) 569–574.
- [28] F.D. Lublin, S.C. Reingold, J.A. Cohen, et al., Defining the clinical course of multiple sclerosis: the 2013 revisions, Neurology. 83 (2014) 278–286, https://doi.org/10. 1212/WNL.00000000000560.
- [29] T. Matsushita, T. Matsuoka, N. Isobe, et al., Association of the HLA-DPB1*0501 allele with anti-apuaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders, Tissue Antigens 73 (2009) 171–176, https://doi.org/10.1111/j.1399-0039.2008.01172.x.
- [30] Y. Nakamura, L. Gaetano, T. Matsushita, et al., A comparison of brain magnetic resonance imaging lesions in multiple sclerosis by race with reference to disability progression, J. Neuroinflammation 15 (2018) 255, https://doi.org/10.1186/ s12974-018-1295-1.
- [31] L. Kappos, J. Antel, G. Comi, et al., Oral fingolimod (FTY720) for relapsing multiple sclerosis, N. Engl. J. Med. 355 (2006) 1124–1140.
- [32] T. Saida, S. Kikuchi, Y. Itoyama, et al., A randomized, controlled trial of fingolimod (FTY720) in Japanese patients with multiple sclerosis, Mult. Scler. 18 (2012) 1269–1277, https://doi.org/10.1177/1352458511435984.
- [33] L. Lorefice, G. Fenu, C. Sardu, et al., Multiple sclerosis and HLA genotypes: a possible influence on brain atrophy, Mult. Scler. J. 25 (2019) 23–30, https://doi.org/ 10.1177/1352458517739989.
- [34] N. Isobe, A. Keshavan, P.A. Gourraud, et al., Association of HLA genetic risk burden

with disease phenotypes in multiple sclerosis, JAMA Neurol. 73 (2016) 795–802, https://doi.org/10.1001/jamaneurol.2016.0980.

- [35] R.L. Yates, M.M. Esiri, J. Palace, et al., The influence of *HLA-DRB1*15* on motor cortical pathology in multiple sclerosis, Neuropathol. Appl. Neurobiol. 41 (2015) 371–384, https://doi.org/10.1111/nan.12165.
- [36] M. Niino, S. Sato, T. Fukazawa, et al., Latitude and HLA-DRB1 alleles independently affect the emergence of cerebrospinal fluid IgG abnormality in multiple sclerosis, Mult. Scler. 21 (2015) 1111–1120, https://doi.org/10.1177/1352458514560924.
- [37] R. Balnytė, D. Rastenytė, I. Ulozienė, et al., The significance of HLA DRB1*1501 and oligoclonal bands in multiple sclerosis: clinical features and disability, Med. (Kaunas) 47 (2011) 368–373.
- [38] F. Matsushita, H. Kida, K.I. Tabei, et al., Clinical significance of cortical lesions in patients with multiple sclerosis: a neuropsychological and neuroimaging study, Brain Behav. 8 (2018) e00934, https://doi.org/10.1002/brb3.934.
- [39] C.C. Vasconcelos, J.C. Aurenção, L.C. Thuler, et al., Prognostic factors associated with long-term disability and secondary progression in patients with multiple sclerosis, Mult. Scler. Relat. Disord. 8 (2016) 27–34, https://doi.org/10.1016/j. msard.2016.03.011.
- [40] C. Confavreux, S. Vukusic, P. Adeleine, Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process, Brain. 236 (2003) 770–782, https://doi.org/10.1093/brain/awg081.
- [41] S. Baghizadeh, M.A. Sahraian, N. Beladimoghadam, Clinical and demographic factors affecting disease severity in patients with multiple sclerosis, Iran J. Neurol. 12 (2013) 1–8.
- [42] E. Leray, T. Moreau, A. Fromont, et al., Epidemiology of multiple sclerosis, Rev. Neurol. (Paris) 172 (2016) 3–13, https://doi.org/10.1016/j.neurol.2015.10.006.
- [43] M. Jurynczyk, R. Geraldes, F. Probert, et al., Distinct brain imaging characteristics of autoantibody-mediated CNS conditions and multiple sclerosis, Brain. 140 (2017) 617–627, https://doi.org/10.1093/brain/aww350.
- [44] Á. Cobo-Calvo, H. d'Indy, A. Ruiz, et al., Frequency of myelin oligodendrocyte glycoprotein antibody in multiple sclerosis: A multicenter cross-sectional study, Neurol. Neuroimmunol. Neuroinflamm. 7 (2019), https://doi.org/10.1212/NXI. 000000000000649 pii: e649.
- [45] I. Nakashima, K. Fujihara, S. Sato, Oligoclonal IgG bands in Japanese patients with multiple sclerosis. A comparative study between isoelectric focusing with IgG immunofixation and high-resolution agarose gel electrophoresis, J. Neuroimmunol. 159 (2005) 133–136, https://doi.org/10.1016/j.jneuroim.2004.09.011.
- [46] Y. Nakamura, Z. Liu, S. Fukumoto, et al., Spinal cord involvement by atrophy and associations with disability are different between multiple sclerosis and neuromyelitis optica spectrum disorder, Eur. J. Neurol. 27 (2020) 92–99, https://doi.org/ 10.1111/ene.14038.