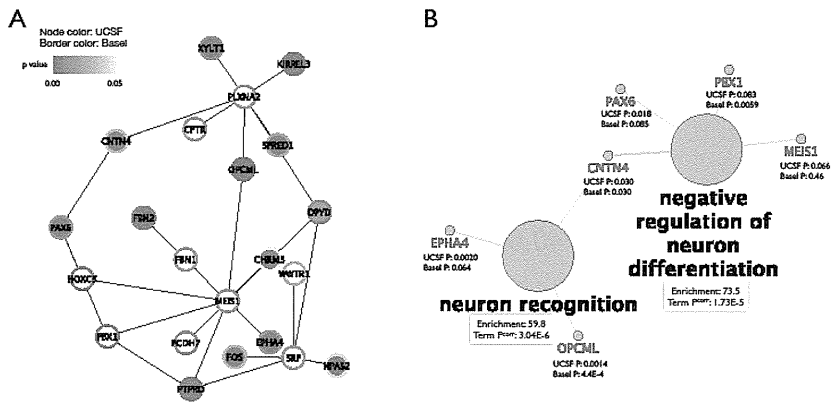

Figure 3. (**A**) A representative subnetwork enriched with genes with significant p values in intersection nets (inferior parietal cortex). Node and the border color represents p value in UCSF and Basel data set respectively. Non-significant genes are displayed as white circle or circle with gray border. (**B**) Detected categories enriched with genes which are included in a subnetwork shown in (A).

Table 1. Demographic features

	UCSF		Basel	P value	
	Case (557)	Control (75)	Case (218)	Case vs. control in UCSF	UCSF case vs. Basel case
Female (%)	68.8	65.3	71.9	0.6	0.55
Age (mean±SD)	43.1±9.8	40.6±10.4	43.7±10.9	0.15	0.5
Onset age (mean±SD)	34.6±9.4	N/A	32.2±9.6	N/A	0.0019
Disease duration	8.5±8.8	N/A	11.7±8.9	N/A	2.80E-08
Disease course (CIS/RR/SP/PP/PR)	90/398/49/18/2	N/A	9/148/38/10/8	N/A	6.40E-06
EDSS (mean±SD)	1.9±1.6	N/A	3.0±1.7	N/A	<2.2E-16

Table 2. Comparing adjusted thickness between cases and controls

Region		Left		Right	
		Estimate	P	Estimate	P
Frontal lobe	Frontal Pole	3.05E-03	9.34E-01	-1.57E-02	6.59E-01
	Medial Orbital Frontal	3.94E-02	6.90E-02	3.91E-02	6.05E-02
	Lateral Orbital Frontal	-3.28E-02	6.65E-02	-2.14E-02	2.28E-01
	Pars Orbitalis	-1.66E-02	4.91E-01	1.65E-02	5.03E-01
	Pars Triangularis	-1.66E-02	4.91E-01	1.65E-02	5.03E-01
	Pars Opercularis	-1.82E-02	2.31E-01	-8.49E-03	6.05E-01
	Superior Frontal*	-4.50E-02	1.27E-03	-3.00E-02	3.90E-02
	Rostral Middle Frontal	-9.69E-03	4.38E-01	-3.18E-02	1.70E-02
	Caudal Middle Frontal	-3.43E-02	1.87E-02	-1.26E-02	4.35E-01
	Paracentral*	-4.31E-02	1.09E-02	-6.22E-02	1.73E-04
	Precentral	-3.77E-02	6.88E-03	-1.36E-02	3.19E-01
Parietal lobe	Precuneus*	-6.86E-02	7.78E-06	-5.94E-02	1.90E-04
	Postcentral	-1.49E-02	2.91E-01	-1.25E-02	3.77E-01
	Superior Parietal*	-4.56E-02	9.39E-04	-4.39E-02	2.51E-03
	Supramarginal*	-5.16E-02	3.80E-04	-1.97E-02	1.68E-01
	Inferior Parietal*	-7.20E-02	6.97E-06	-4.47E-02	6.44E-03
Temporal lobe	Transverse Temporal	3.42E-02	1.42E-01	6.50E-02	1.00E-02
	Superior Temporal	2.82E-04	9.87E-01	-2.18E-02	2.07E-01
	Banks of STS*	-9.03E-02	1.49E-06	-8.14E-02	3.03E-05
	Middle Temporal	-1.58E-02	3.70E-01	-2.22E-02	1.75E-01
	Inferior Temporal	-2.96E-02	1.00E-01	-2.66E-02	1.44E-01
	Fusiform*	-6.59E-02	2.00E-04	-4.56E-02	1.38E-02
	Temporal Pole	9.87E-02	8.56E-02	1.50E-01	7.78E-03

Occipital lobe	Cuneus	-1.80E-02	2.80E-01	-1.05E-02	5.23E-01
	Pericalcarine	3.06E-03	8.53E-01	-9.25E-03	5.87E-01
	Lateral Occipital	-2.68E-02	8.61E-02	-3.37E-02	2.75E-02
	Lingual	-4.20E-02	6.81E-03	-2.41E-02	1.01E-01
Limbic system	Rostral Anterior Cingulate	-3.64E-03	9.15E-01	7.13E-02	3.08E-02
	Caudal Anterior Cingulate	5.36E-02	1.44E-01	4.69E-02	1.32E-01
	Posteriorcingulate	-1.66E-02	3.85E-01	-4.07E-02	4.48E-02
	Isthmus Cingulate*	-1.93E-01	2.11E-12	-1.47E-01	1.96E-08
	Parahippocampal	2.03E-02	5.79E-01	5.01E-02	1.18E-01
	Entorhinal	-1.33E-01	6.69E-03	-8.48E-03	8.74E-01
Insula	Insula	-3.17E-02	1.19E-01	-4.64E-02	2.17E-02

Estimate describes the difference of adjusted mean thickness between cases and controls.

* Regions of interest. P value corrected by Bonferroni method less than 0.05 in one hemisphere and nominal p value less than 0.05 in the contralateral hemisphere.

Table 3. Summary of enriched network modules in each ROI

	Enriched networks		Size of union net		Intersection nodes	Enrichment	Pemutation P value	Enriched networks in intersection
	UCSF	Basel	UCSF	Basel				
Banks sts	30	26	1809	1847	701	3.31	3.76E-01	2
Fusiform	25	28	1632	1829	642	3.35	3.20E-01	2
Inferior parietal	27	25	1834	1758	714	3.71	3.84E-02	3
Isthmus cingulate	27	32	1701	2012	732	3.53	1.36E-01	3
Paracentral	32	36	1963	2087	784	2.91	9.34E-01	2
Precuneus	25	32	1592	1994	663	3.24	5.26E-01	3
Superior frontal	30	31	1867	1934	733	3.17	6.33E-01	4
Superior parietal	26	23	1659	1707	610	3.29	4.24E-01	3
Supramarginal	26	30	1818	1916	709	3.14	6.70E-01	3

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Decreased serum vitamin D levels in Japanese patients with multiple sclerosis

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ABSTRACT

Data regarding vitamin D in multiple sclerosis (MS) in Asia are limited. We investigated whether Japanese MS patients show decreased serum 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], and vitamin D-binding protein (DBP) during winter. Mean serum 25(OH)D and 1,25$(OH)_2$D levels were significantly lower in MS patients than in controls. There were no significant differences in serum 25(OH)D, 1,25$(OH)_2$D, and DBP levels between patients or between controls from northern Japan (Hokkaido) and southern Japan (Kyushu). Serum vitamin D levels were low in Japanese MS patients but did not differ in patients from northern and southern Japan.

1. Introduction

Low serum vitamin D is associated with risk of multiple sclerosis (MS) and poor prognosis (Munger et al., 2006; van der Mei et al., 2007; Gelfand et al., 2011; Amezcua et al., 2012; Salzer et al., 2012), possibly because of changes in neuroimmune function and neural repair capacity. Several immune cell types express vitamin D receptors (VDRs) and vitamin D metabolites are important modulators of immune system development and function (Niino, 2010 for review). Vitamin D receptors are also expressed by neurons and glial cells in the central nervous system (CNS), where vitamin D promotes neurite outgrowth, maturation and differentiation, and release of neurotrophins, suggesting that vitamin D may prevent neurodegeneration and promote CNS repair (Smolders et al., 2011).

The two major metabolites of vitamin D, 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the latter derived from 25(OH)D by 1α-hydroxylase-mediated hydroxylation, are potent immunomodulators that function mainly through VDRs. There are substantial differences in the half-lives of 1,25$(OH)_2$D (10–20 h) and 25(OH)D (15 days) (Jones, 2008); therefore, the more stable 25(OH)D is commonly used as a measure of individual vitamin D status. Vitamin D-binding protein (DBP) is the major plasma carrier of vitamin D and its metabolites. Binding to DBP acts to regulate the bioavailability of 1,25$(OH)_2$D as it buffers the levels of free metabolites and thus affords a degree of protection against short-term seasonal or diet-induced fluctuations (White and Cooke, 2000). Furthermore, DBP may have immune functions independent of vitamin D transport (Chun, 2012).

The geographic epidemiology of MS suggests that vitamin D is a modifiable risk factor (Simpson et al., 2011). In addition, serum vitamin D levels differ by ethnicity and so may be a critical intermediary in the ethnic variation in MS incidence. There have been several studies on the association between vitamin D and MS in patients from Europe and North America (Soilu-Hänninen et al., 2005; Munger et al., 2006; Kragt et al., 2009; Amezcua et al., 2012; Gelfand et al., 2011) but few in Asian patient populations (Niino et al., 2013a; Pandit et al., 2013). Serum vitamin D in Asians appears lower than in Caucasians (Niino et al., 2013b), suggesting a distinct relationship among vitamin D intake, seasonal synthesis, metabolism, and MS risk.

The aims of this study were to evaluate serum levels of 1,25$(OH)_2$D (an active form of vitamin D), 25(OH)D (the stable transportable metabolite) and DBP during winter in Japanese MS patients. We also compared winter serum vitamin D levels between patient populations living in the northern or southern region of Japan because it was

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Table 1
Clinical profiles of healthy controls and patients with multiple sclerosis in Hokkaido and Kyushu regions.

	Healthy controls	Patients		
		RRMS	SPMS	Total
Number (female/male)	40 (20/20)	40 (20/20)	30 (14/16)	70 (34/36)
Age at blood puncture[a] (range)	45.5 ± 9.3 (29–60)	44.3 ± 9.3 (28–69)	47.8 ± 12.6 (22–74)	45.8 ± 10.9 (22–74)
Age at onset[a] (range)		32.1 ± 10.3 (18–60)	29.0 ± 12.3 (10–58)	30.7 ± 11.2 (10–60)
EDSS[a] (range)		2.1 ± 1.5 (1.0–7.0)	5.6 ± 1.7 (2.5–8.5)	3.6 ± 2.3 (1.0–8.5)
MSSS[a] (range)		2.25 ± 2.16 (0.26–9.08)	5.64 ± 2.59 (1.45–9.49)	3.70 ± 2.88 (0.26–9.49)
Number of patients treated with DMDs				
IFNβ-1a im		7	5	12
IFNβ-1b sc		13	7	20
Fingolimod		12	11	23
Number of patients receiving vitamin D intake				
Alfacalcidol (0.5 μg/day)	0	0	2	2

RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary-progressive multiple sclerosis; EDSS, Expanded Disability Status Scale; MSSS, Multiple Sclerosis Severity Score; DMDs, disease modifying drugs, IFN, interferon; sc, subcutaneous injection; im, intramuscular injection.
[a] Data are mean ± standard deviation (SD).

reported that there is a significant difference in solar radiation between north and south Japan, particularly during winter (Miyauchi et al., 2013). Furthermore, as a study from the Netherlands reported that low serum 25(OH)D was associated with higher Expanded Disability Status Scale (EDSS) scores (Smolders et al., 2008), we examined the association of vitamin D with disability level and disease progression in the same patient population.

2. Materials and methods

2.1. Patients and controls

In Hokkaido, patients with MS were recruited from Hokkaido Medical Center and Sapporo Neurology Clinic (Sapporo, Japan), and healthy control subjects were recruited from hospital staff from Hokkaido Medical Center. All subjects in Hokkaido resided in or near Sapporo City. In Kyushu, patients with MS were recruited from Kyushu University Hospital (Fukuoka, Japan), and healthy control subjects were recruited from Kyushu University Hospital staff. All subjects in Kyushu resided in or near Fukuoka City. Sapporo, the prefectural city of Hokkaido Prefecture, and Fukuoka the prefectural city of Fukuoka Prefecture, are located at 43° and 33° north, respectively. Subject recruitment followed institutional review board approval, and informed consent was obtained from all subjects. All patients were diagnosed with MS using the 2010 revised McDonald criteria (Polman et al., 2011), and relapsing–remitting MS (RRMS) and secondary-progressive MS (SPMS) forms were distinguished by the definition of Lublin and Reingold (1996). Blood samples from RRMS patients were collected at remitting stage at least 2 months after steroid treatment. Patients with neuromyelitis optica (NMO) (Wingerchuk et al., 2006) or NMO spectrum disorders (Wingerchuk et al., 2007) were excluded. Clinical profiles of all MS patients and healthy controls are shown in Table 1. In both Hokkaido and Kyushu regions, 20 RRMS patients and fifteen SPMS patients were included. Both patient groups and the two subgroups (RRMS and SPMS) had nearly equal or equal sex ratios (Table 2). There were no significant differences in age at blood collection among RRMS patients, SPMS patients, and healthy controls within and between regions (Table 2). Disease severity was evaluated

Table 2
Clinical profiles of healthy controls and patients with multiple sclerosis.

	Healthy controls	Patients		
		RRMS	SPMS	Total
Hokkaido				
Number (female/male)	20 (10/10)	20 (10/10)	15 (8/7)	35 (18/17)
Age at blood puncture[a] (range)	45.4 ± 9.2 (29–56)	43.3 ± 7.5 (28–61)	46.4 ± 12.2 (22–62)	44.6 ± 9.7 (22–62)
Age at onset[a] (range)		30.7 ± 9.1 (18–50)	27.5 ± 11.6 (10–48)	29.3 ± 10.2 (10–50)
EDSS[a] (range)		2.2 ± 1.6 (1.0–7.0)	6.0 ± 1.7 (2.5–8.5)	3.8 ± 2.5 (1.0–8.5)
MSSS[a] (range)		2.24 ± 2.12 (0.26–8.63)	6.34 ± 2.50 (2.03–9.48)	4.00 ± 3.06 (0.26–9.48)
Number of patients treated with DMDs				
IFNβ-1a im		4	3	7
IFNβ-1b sc		7	4	11
Fingolimod		5	5	10
Number of patients receiving vitamin D intake				
Alfacalcidol (0.5 μg/day)	0	0	1	1
Kyushu				
Number (female/male)	20 (10/10)	20 (10/10)	15 (6/9)	35 (16/19)
Age at blood puncture[a] (range)	45.6 ± 9.7 (29–60)	45.3 ± 11.0 (28–69)	49.3 ± 13.3 (22–74)	47.0 ± 12.0 (22–74)
Age at onset[a] (range)		33.4 ± 11.5 (19–0)	30.5 ± 13.2 (11–58)	32.1 ± 12.1 (11–60)
EDSS[a] (range)		2.1 ± 1.4 (1.0–6.0)	5.1 ± 1.7 (3.0–8.5)	3.4 ± 2.1 (1.0–8.5)
MSSS[a] (range)		2.25 ± 2.25 (0.26–9.08)	4.93 ± 2.56 (1.45–9.49)	3.40 ± 2.71 (0.26–9.49)
Number of patients treated with DMDs				
IFNβ-1a im		3	2	5
IFNβ-1b sc		6	3	9
Fingolimod		7	6	13
Number of patients receiving vitamin D intake				
Alfacalcidol (0.5 μg/day)	0	0	1	1

RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary-progressive multiple sclerosis; EDSS, Expanded Disability Status Scale; MSSS, Multiple Sclerosis Severity Score; DMDs, disease modifying drugs, IFN, interferon; sc, subcutaneous injection; im, intramuscular injection.
[a] Data are mean ± standard deviation (SD).

using the EDSS (Kurtzke, 1983) and progression of disability was assessed using the Multiple Sclerosis Severity Score (MSSS) (Roxburgh et al., 2005).

2.2. Measurement of 1,25(OH)$_2$D, 25(OH)D, and DBP

Serum samples for 1,25(OH)$_2$D, 25(OH)D, and DBP measurements were obtained from one blood sample per individual. Serum samples from patients and healthy individuals were frozen at −80 °C until required for analysis. Levels of 1,25(OH)$_2$D were measured using a standard radioimmunoassay (RIA) protocol (Immunodiagnostic Systems Limited, Boldon, UK) at the laboratory of SRL, Inc. (Tokyo, Japan), and serum 25(OH)D levels were measured using a 125I RIA Kit (DiaSorin Catalog No. 68100E, Stillwater, Minnesota, USA) at the laboratory of SRL, Inc. (Tokyo, Japan). Levels of DBP by enzyme linked immunosorbent assays (ELISAs) (Immundiagnostik, Bensheim, Germany). Serum samples were collected between January 1 and February 28, 2014.

2.3. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Group means were compared among healthy control subjects, RRMS patients, and SPMS patients by analysis of variance (ANOVA) followed by Tukey's multi-comparison test using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Means of the total healthy control group and patient group (RRMS plus SPMS patients) were compared by the Mann–Whitney *U* test. Similarly, interregional group means were compared by Mann–Whitney *U* test. Spearman rank correlation was used to assess the correlation between serum vitamin D status and disease severity. A $p < 0.05$ was considered significant.

3. Results

3.1. Serum levels of 1,25(OH)$_2$D, 25(OH)D, and DBP in healthy controls and patients with MS

Serum levels of 1,25(OH)$_2$D, 25(OH)D, and DBP were compared between healthy controls and MS patients, and the MS groups were divided into the RRMS group and SPMS group. Serum 1,25(OH)$_2$D was significantly lower in MS patients (60.2 ± 16.8 pg/mL) than in healthy controls (71.2 ± 21.8 pg/mL). In addition, on comparing the healthy controls, the RRMS group, and the SPMS group, we found that the SPMS patient subgroup had the lowest 1,25(OH)$_2$D level (58.8 ± 17.9 pg/mL) (Fig. 1A and D). Similarly, serum 25(OH)D in the total MS patient group (17.1 ± 6.8 ng/mL) was significantly lower than healthy

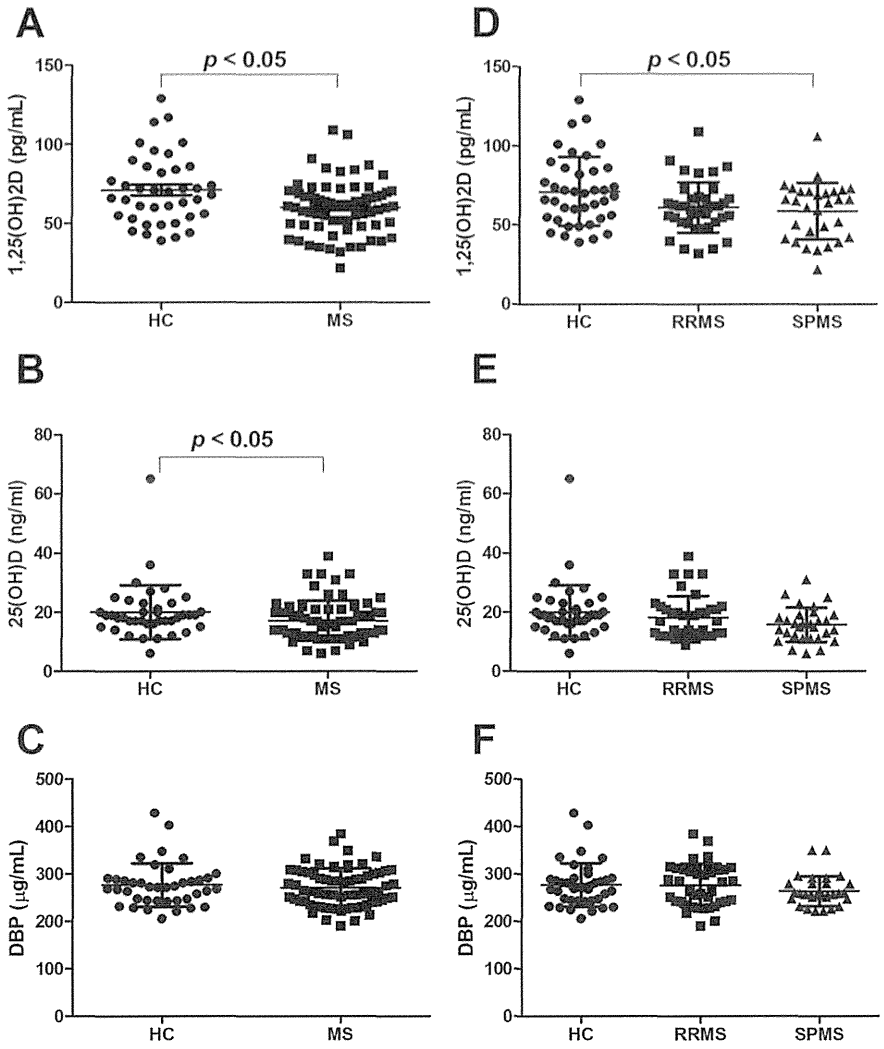

Fig. 1. Serum levels of 1,25(OH)$_2$D, 25(OH)D, and DBP in healthy controls and MS patients. Serum levels of 1,25(OH)$_2$D and 25(OH)D were significantly lower in MS patients than healthy controls ($p < 0.05$ and $p < 0.01$, respectively; Mann–Whitney *U* test) (A, B). Serum levels of 1,25(OH)$_2$D were significantly lower in SPMS patients than healthy controls ($p < 0.05$; ANOVA followed by Tukey's multicomparison test) (D). Serum levels of 25(OH)D in SPMS patients were lower than those of healthy controls and RRMS patients, although this was not statistically significant ($p = 0.0762$; ANOVA followed by Tukey's multicomparison test) (E). Serum levels of DBP did not differ between healthy controls and MS patients (C, F). HC; healthy controls, MS; patients with MS (relapsing–remitting MS and secondary-progressive MS), RRMS; relapsing–remitting MS, SPMS; secondary-progressive MS, DBP; vitamin D-binding protein.

controls (20.0 ± 9.2 ng/mL). In addition, on comparing the healthy controls, the RRMS group, and the SPMS group, the SPMS patient subgroup had the lowest 25(OH)D level (15.7 ± 5.8 ng/mL) although this difference was not statistically significant (Fig. 1B and E). In contrast, levels of DBP in healthy controls (276.7 ± 46.0 μg/mL) and MS patients (271.2 ± 40.8 μg/mL) did not significantly differ (Fig. 1C and F).

3.2. Serum levels of 1,25(OH)$_2$D, 25(OH)D, and DBP in subjects from northern and southern Japan (Hokkaido and Kyushu)

Serum levels of 1,25(OH)$_2$D, 25(OH)D, and DBP were compared between Hokkaido in northern Japan and Kyushu in southern Japan to investigate whether the difference in latitude affects serum vitamin D levels in either patient or control groups. In healthy controls, levels of 1,25(OH)$_2$D did not significantly differ between Hokkaido and Kyushu (73.0 ± 18.8 pg/mL vs. 69.5 ± 24.8 pg/mL). In MS patients, 1,25(OH)$_2$D did not significantly differ between Hokkaido and Kyushu (64.1 ± 17.8 pg/mL vs. 55.8 ± 14.3 pg/mL). Similarly, levels of 25(OH)D did not differ between regions in healthy controls (Hokkaido: 20.4 ± 11.7 ng/mL vs. Kyushu: 19.6 ± 6.2 ng/mL) or MS patients (Hokkaido: 17.7 ± 6.7 ng/mL vs. Kyushu: 16.5 ± 6.9 ng/mL). Serum DBP did not differ between regions for either controls (Hokkaido: 260.9 ± 32.1 μg/mL vs. Kyushu: 292.6 ± 52.8 μg/mL) or MS patients (Hokkaido: 265.5 ± 36.3 μg/mL vs. Kyushu: 278.1 ± 44.5 μg/mL).

3.3. Serum levels of 1,25(OH)$_2$D, 25(OH)D, and DBP in subjects in between MS patients treated with IFNβ and without IFNβ

Thirty-two MS patients had received IFNβ and 38 patients had not when blood samples were collected. There were no significant differences in EDSS scores between MS patients treated with IFNβ and those not treated (data not shown). As this treatment may alter vitamin D status (Stewart et al., 2012), 1,25(OH)$_2$D, 25(OH)D, and DBP levels were compared between these treatment groups. Patients treated with IFNβ had significantly higher serum levels of 1,25(OH)$_2$D compared with those not treated with IFNβ (Fig. 2A). In contrast, neither 25(OH)D nor DBP differed between treatment groups (Fig. 2B and C).

3.4. Correlations between serum vitamin D status (1,25(OH)$_2$D, 25(OH)D, DBP) and disease severity scores (EDSS and MSSS) for MS patients

There were no significant associations between serum status for 1,25(OH)$_2$D, 25(OH)D, or DBP and EDSS or MSSS in MS patients (Fig. 3).

4. Discussion

The greatest effect of vitamin D status on MS risk reported to date was an inverse association between serum 25(OH)D and MS risk among Caucasians, suggesting that higher circulating levels of 25(OH)D may reduce MS risk (Munger et al., 2006). Furthermore, several studies with primarily Caucasian populations found that patients with MS had significantly lower 25(OH)D levels than controls (Soilu-Hänninen et al., 2005; Kragt et al., 2009). Individuals with darker skin have a higher risk of vitamin D deficiency because melanin competes with 7-dehydrocholesterol for the absorption of UV light, which is required for vitamin D synthesis (Armas et al., 2007). Serum 25(OH)D levels are substantially lower in Africans than Caucasians (Munger et al., 2006). In a comparative study from Southern California, serum 25(OH)D levels were significantly lower among Hispanics with MS than Caucasians with MS (Amezcua et al., 2012). Healthy vitamin D status appears to differ among ethnicities; therefore, the association of vitamin D level with MS risk may also differ among ethnicities. However, a study of African-Americans also demonstrated lower serum 25(OH)D levels in patients with MS compared with controls (Gelfand et al., 2011). In addition, serum levels of 25(OH)D were significantly lower in MS patients than healthy controls in Iran (Shaygannejad et al., 2010) and India (Pandit et al., 2013). In the current study, serum 25(OH)D levels and 1,25(OH)$_2$D levels were significantly lower in MS patients than in controls. Thus, the correlation between vitamin D status and MS risk appears to hold across ethnicities.

There is a 10° difference in latitude between Sapporo in the north and Fukuoka in the south, and a significant difference in solar radiation has also been reported in Japan between north and south, particularly in winter (Miyauchi et al., 2013). However, our study found no differences in 25(OH)D and 1,25(OH)$_2$D levels in controls and in MS patients between Hokkaido and Kyushu. The reasons for this are unclear, but may stem from differences in dietary intake. In fact, it is speculated that dietary consumption of vitamin D in Scandinavia may contribute to the effect of latitude on MS prevalence (Simpson et al., 2011). In Japan, much fish is consumed, which may affect vitamin D levels. In addition, many MS patients may avoid the outdoors in winter, even in the south, which could normalize vitamin D levels between regions. Further studies are needed to determine why there were no latitudinal differences in serum 25(OH)D and 1,25(OH)$_2$D levels in Japan. While we found no regional difference in serum vitamin D status, a small but

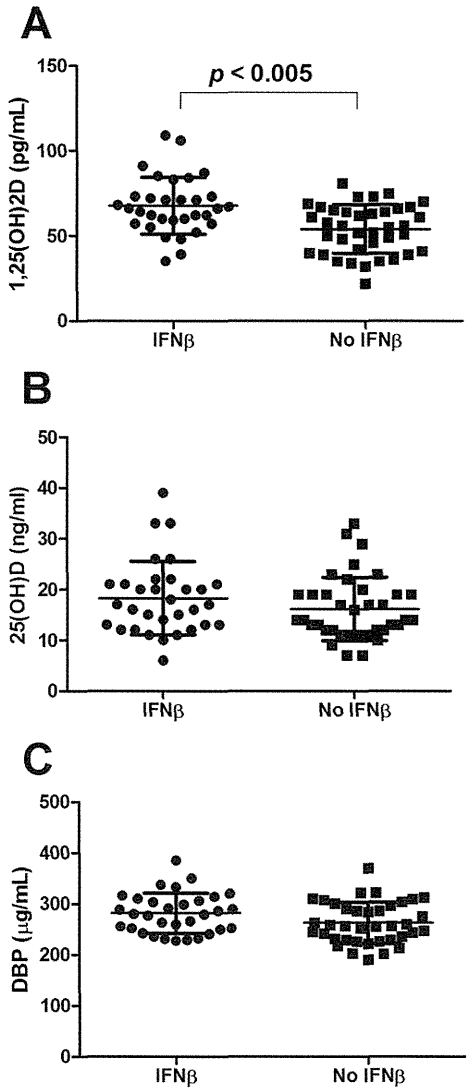

Fig. 2. Comparison of serum 1,25(OH)$_2$D, 25(OH)D, and DBP levels between MS patients treated with interferon β and untreated patients. MS patients treated with IFNβ had significantly higher 1,25(OH)2D levels than untreated MS patients (A). No significant differences in 25(OH)D and DBP were found between MS patients with and without IFNβ treatment (B, C). IFNβ; MS patients treated with IFNβ, No IFNβ; MS patients who were not treated with IFNβ.

statistically significant north–south gradient of MS prevalence has been documented in Japan (Kira, 2003), which is consistent with regional differences in sun exposure. However, it was recently proposed that sun exposure and vitamin D status may have independent effects on the risk of CNS demyelination (Lucas et al., 2011). Both in vivo and in vitro studies indicate that ultraviolet radiation (UVR) can suppress the immune system independent of vitamin D. Continuous treatment with UVR dramatically suppressed clinical signs of experimental autoimmune encephalomyelitis (EAE), a ubiquitous animal model of MS, independent of vitamin D production (Becklund et al., 2010; Breuer et al., 2014).

In a previous study of a Japanese population, SPMS patients had significantly lower levels of 25(OH)D than controls in summer (Niino et al., 2013a). In the current study, SPMS patients also showed a tendency to present lower 25(OH)D levels than controls in winter. Possible explanations include a propensity for these patients to stay indoors regardless of season, lowering serum 25(OH)D. Alternatively, inherently lower serum 25(OH)D may increase the risk of SPMS. Vitamin D has strong immune-modulating effects, including the ability to decrease IL-17 production (Daniel et al., 2008), a key cytokine in MS pathogenesis. Furthermore, an in vitro study showed that low doses of vitamin D protected mesencephalic dopaminergic neurons against combined L-buthionine sulfoximine and 1-methyl-4-phenylpyridine toxicity (Shinpo et al., 2000). Thus, vitamin D may directly or indirectly suppress neurodegeneration in the CNS. Further studies are required to address why SPMS patients had lower vitamin D levels than other MS groups.

This study presents some limitations. First, it included a relatively small sample size. MS prevalence in Japan is lower than that in western countries, and it was limited to samples collected through two winter months. There is a possibility that our data may not represent all MS groups in Japan because of a relatively small sample size. Furthermore, healthy control subjects were recruited from hospital staff and not from the general population. Hospital staff may be healthier than the general population, possibly biasing control data from what would be expected from the general population.

A recent study demonstrated that MS patients treated with IFNβ had significantly higher serum 25(OH)D levels than untreated subjects and suggested that IFNβ therapy enhances production of vitamin D from sun exposure (Stewart et al., 2012). In our study, serum $1,25(OH)_2D$ levels

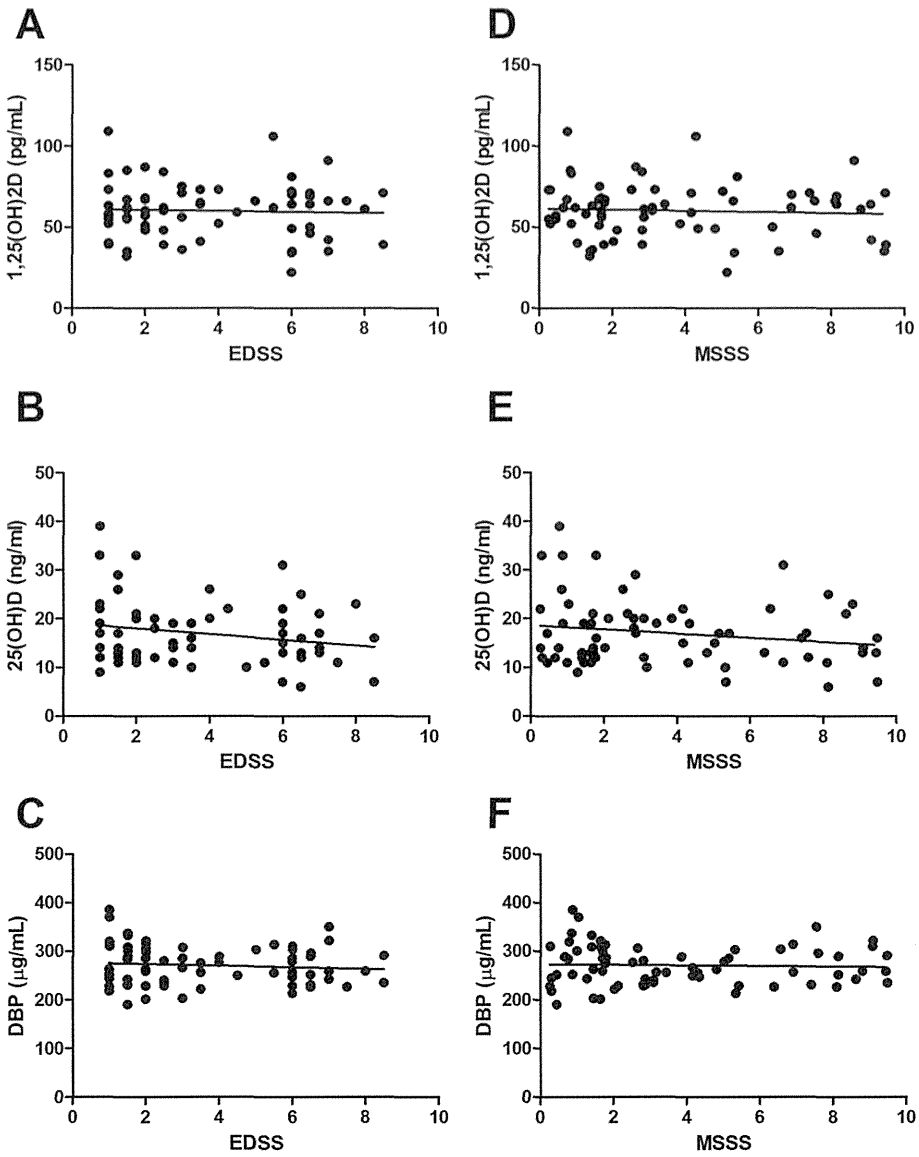

Fig. 3. Correlation between serum vitamin D status and disease severity. EDSS or MSSS and serum levels of $1,25(OH)_2D$, 25(OH)D, or DBP did not correlate significantly. EDSS; Expanded Disability Status Scale, MSSS; Multiple Sclerosis Severity Score.

were also elevated in MS patients treated with IFNβ, although there were no significant differences in serum 25(OH)D levels. Our data suggested that IFNβ may increase vitamin D levels regardless of ethnicity in patients with MS.

5. Conclusions

Serum vitamin D levels were lower in Japanese MS patients compared to age- and sex-matched healthy controls, and supplementation of vitamin D may be a simple but effective treatment option for MS.

Disclosures

None of the authors have a financial interest in the publication of the contents of this article or have a relationship with any company with such financial interests.

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