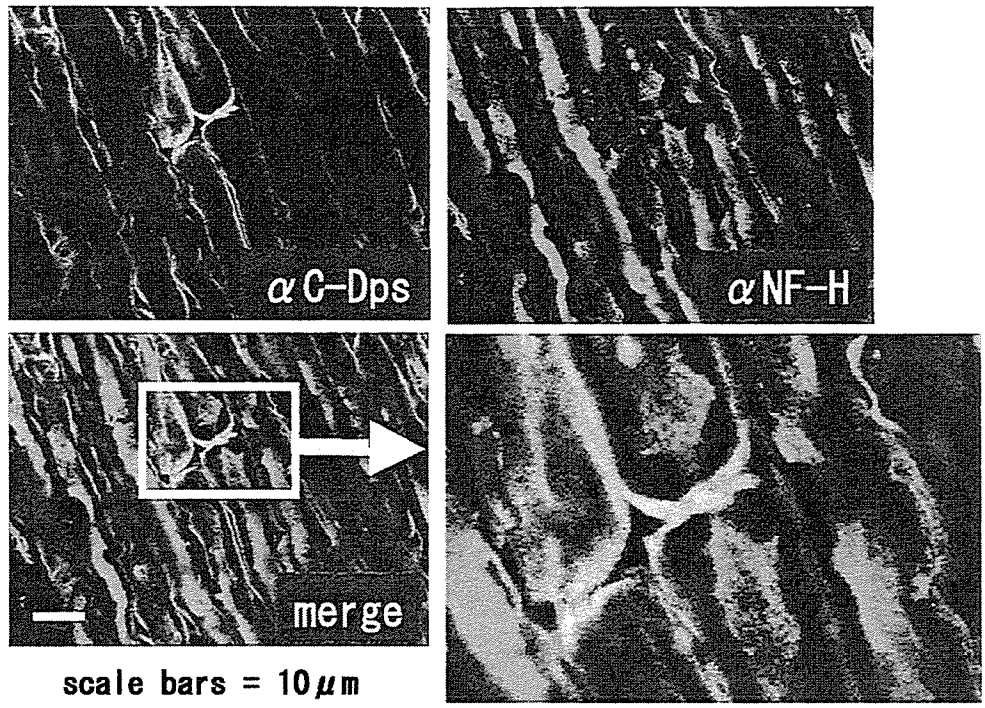

図3　C-Dps蛋白の末梢神経への沈着

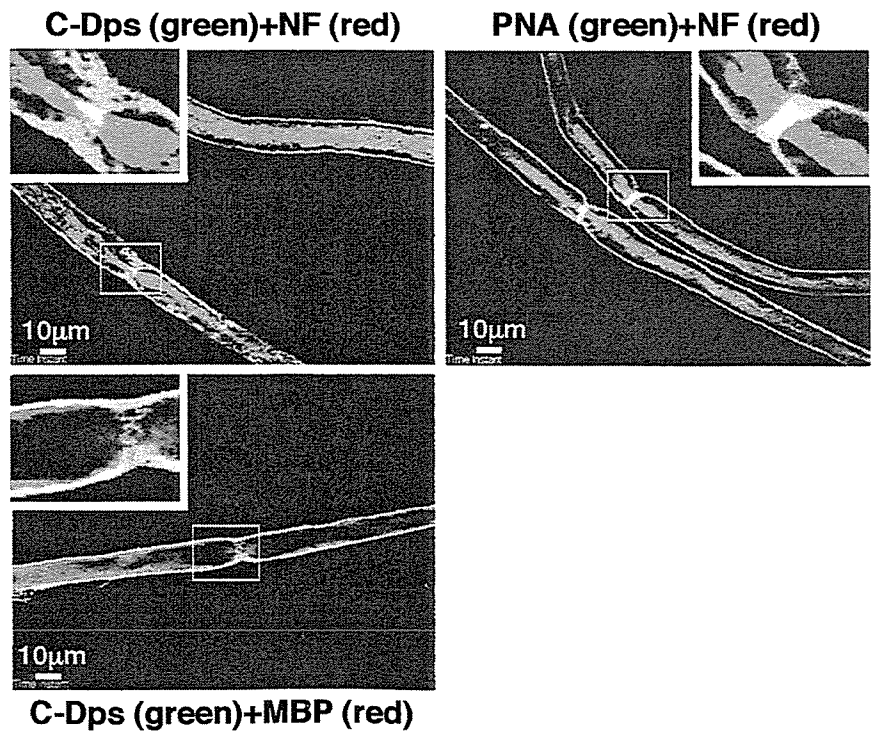

図4　C-Dps蛋白の末梢神経への沈着
（解きほぐし標本）

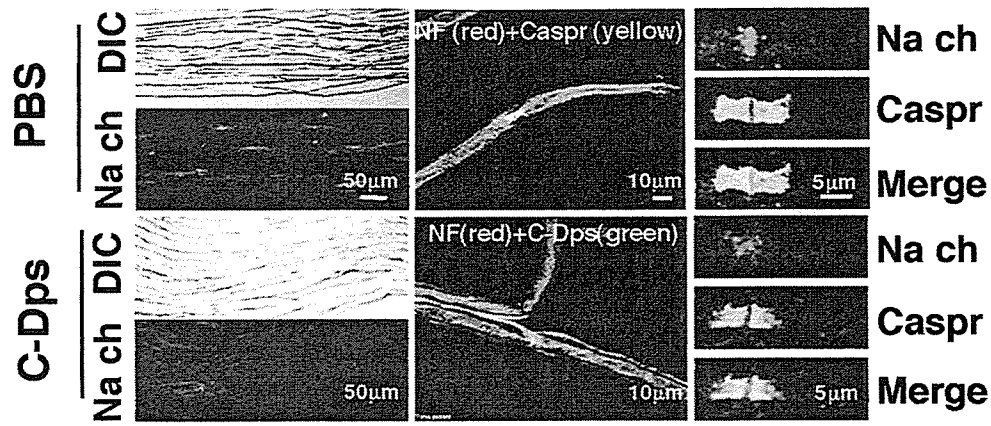

図5　C-Dps蛋白によるNaチャンネル異常

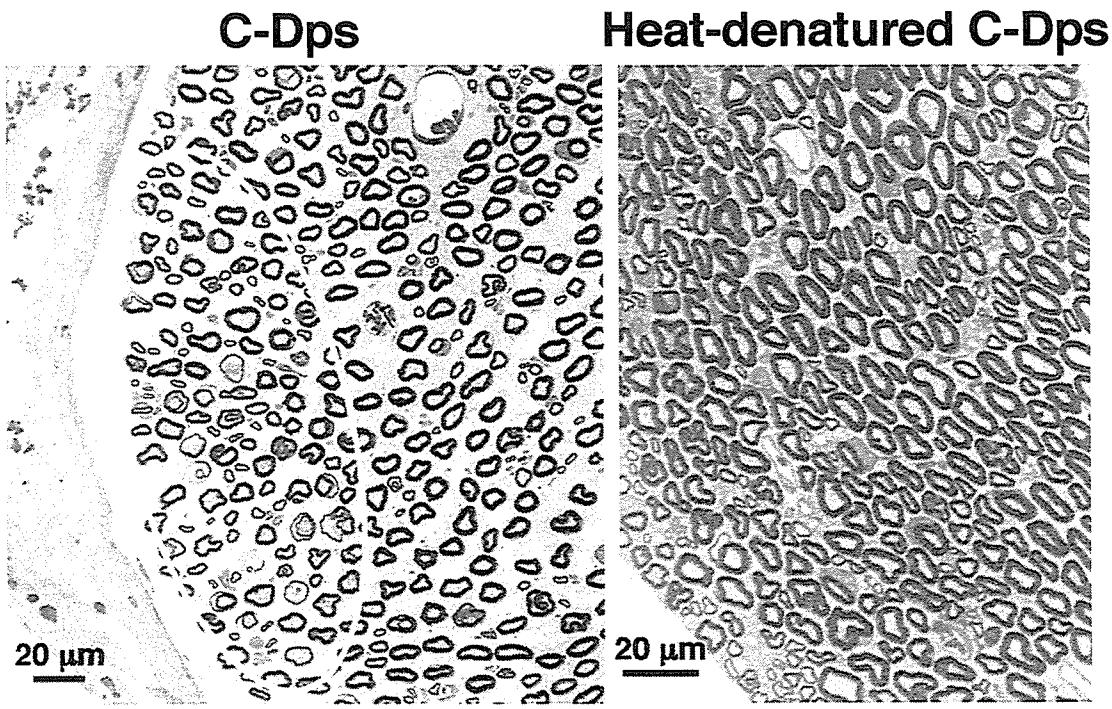

図6　C-Dps蛋白による末梢神経傷害

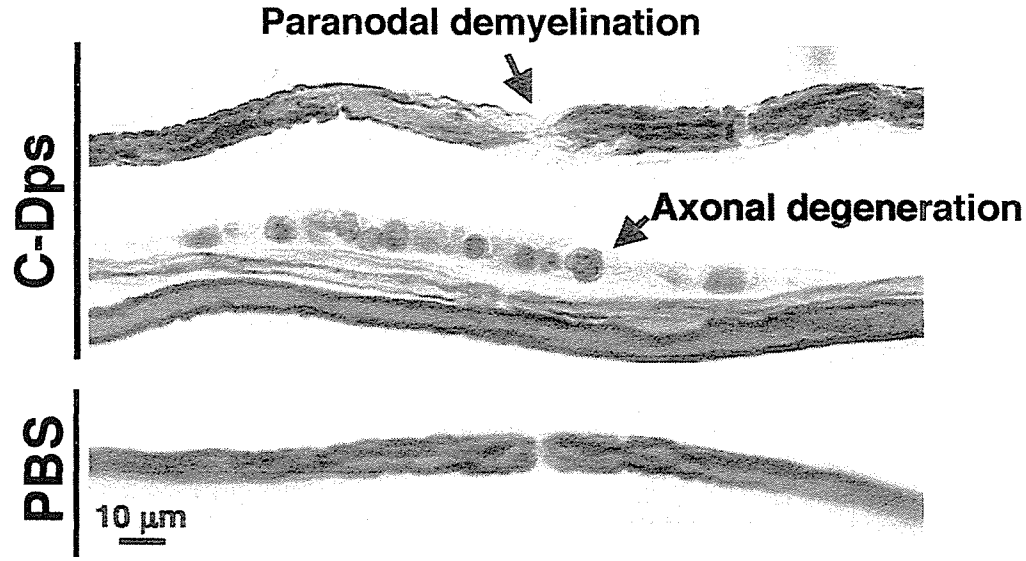

図7　C-Dps蛋白による末梢神経傷害
（解きほぐし標本）

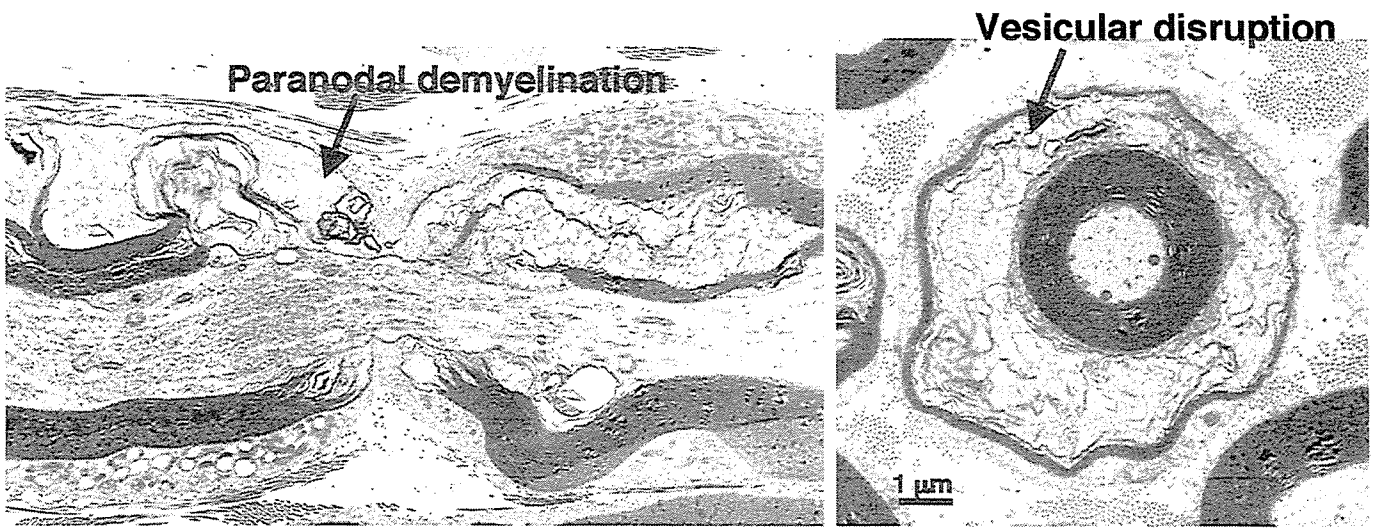

図8　C-Dps蛋白による末梢神経傷害（電顕）

研究成果の刊行に関する一覧表

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研究成果の刊行物

原　　著

Anterior Horn Cell Involvement in Myelitis with Atopic Diathesis (Atopic Myelitis)

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Abstract To clarify the involvement of anterior horn cells in myelitis with atopic diathesis (atopic myelitis), 20 patients with atopic myelitis were subjected to neurological evaluation, concentric needle electromyography (EMG), spinal cord magnetic resonance imaging (MRI) and motor and somatosensory evoked potentials. Apparent muscle atrophy was present only 1 of 20 patients (5 %) and the rests clinically showed no lower motor neuron sign. On needle EMG, 12 patients (60 %) showed varying degrees of lower motor neuron involvement. On-going denervation potentials, such as fasciculation potentials, fibrillation potentials and positive sharp waves, were seen in 5 patients and chronic neurogenic patterns, such as giant and polyphasic motor unit potentials with reduced recruitment patterns, in 12 patients. In 4 patients, the segments of lower motor neuron involvement on needle EMG were beyond those of the spinal cord lesions shown by MRI. In 2 patients showing on-going denervation potentials, such immunotherapies as plasma exchange and intravenous immunoglobulins, were applied and effective clinically as well as electrophysiologically. Therefore, varying degrees of subclinical anterior horn cell involvement seems to be common in atopic myelitis and reversible by immunotherapy.

Key words : anterior horn cell ; myelitis ; atopy ; mite ; electromyography

Introduction

We reported occurrences of myelitis accompanied with atopic disorders in Japanese for the first time and proposed to name it atopic myelitis (AM)[1)2)3)]. Following our reports, several similar cases have also been reported in the Japanese literatures[4)5)6)] and the recent nationwide survey in Japan disclosed the presence of many patients with myelitis and atopic diathesis[7)]. Thus, atopic diathesis appears to be one of risk factors for developing spinal cord inflammation.

On the other hand, an acute poliomyelitis-like illness following asthma attacks is well known as asthmatic amyotrophy (Hopkins syndrome)[8)]. Although the previously reported cases were confined to children, we reported occurrences of Hopkins syndrome in adults[9)]. We also reported an association between airway allergy and juvenile muscular atrophy of distal upper extremity (JMADUE) (Hirayama's disease)[10)], in which anterior horn cells in the lower cervical cord were preferentially involved. In addition, we recently found a significant association of lower motor neuron disease (LMND) with asthma by a prospective study on the past and present history of common allergic disorders in patients with neurologic diseases[11)]. These observations suggest a link

between allergy and anterior horn cell damage. In agreement with our clinical observations, mediators of allergic inflammation, such as cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2), have recently been reported to be increased in the spinal cord[12] as well as in the cerebrospinal fluid (CSF) of amyotrophic lateral sclerosis (ALS) patients[13].

The results of these studies prompted us to examine needle electromyography (EMG) in a series of such patients with AM, in order to classify lower motor neuron involvement in this condition. We found that subclinical anterior horn cell involvement was common in this condition, which further strengthens the link between allergic tendency and anterior horn cell damage.

Materials and methods

Materials

The materials consisted of 20 patients with AM. AM was defined as myelitis of unknown cause with either[1] hyperIgEaemia and allergen-specific IgE positivity or[2] coexistent atopic diseases[1)2)3)]. The demographic characteristics of these patients are summarized in Table 1. Eight had atopic dermatitis (AD), 7 had allergic rhinitis (AR), 2 had bronchial asthma (BA) and 13 had others (food allergy, metal allergy, allergic conjunctivitis, drug allergy and urticaria) and five had hyperIgEaemia and allergen-specific IgE (atopic diathesis) without any atopic disorders. All AM patients had abnormalities indicating the spinal cord lesions by spinal cord MRI and / or evoked potentials such as motor evoked potentials (MEPs), somatosensory evoked potentials (SEPs). AM patients were 13 men and 7 women, whose age at examination was 37.6±12.3 years (mean±SD), and age at onset was 35.9±12.4 years (mean±SD). Duration of the disease at needle electromyography (EMG) evaluation was 26.5±38.3 months (mean±SD). All patients had allergen-specific IgE (either mite antigen or cedar pollen-specific IgE) and all but one patient showed hyperIgEaemia at the time of evaluation. Severity of the disease was graded by Kurtzkes Expanded Disability Status Scale (EDSS)[14], and the

Table 1 Clinicolaboratory findings of patients with myelitis and atopic diathesis

No.	Age	Sex	Coexistent atopic dis.				Serum IgE	Allergen-specific IgE			Clinically determined	MRI	MEP	SEP	Age at	Disease duration	EDSS
			AD	AR	BA	others	(U/ml)	*D.pteronyssinus*	*D.farnae*	ceder pollen	lesions				onset	to EMG (months)	score
1	42	M	−	+	−	+	314	+	+	+	C	C5-6	A	N	40	48	3.5
2	38	M	−	−	−	−	782	+	+	+	C	C1-3	N	A	37	7	2.5
3	42	M	+	−	−	−	1726	+	+	+	C	C3-7	N	A	42	2	2
4	36	M	−	−	−	+	1517	+	+	+	C	C6	A	A	34	24	2.5
5	27	M	+	+	−	+	712	+	+	+	C	C1-7	N	N	27	60	3
6	36	M	−	−	−	+	256	+	−	−	C	C6	N	N	36	1	3.5
7	20	M	+	−	+	+	1170	+	+	−	C	C3-5	N	A	20	1	3
8	61	M	+	−	−	+	530	−	−	+	C-Th	C2-4	A	A	54	120	7
9	47	M	−	−	−	+	292	+	+	−	Th	N	A	N	47	14	6
10	42	F	−	−	−	+	3361	+	+	+	C	C1-7	ND	ND	41	6	2
11	58	F	−	−	−	−	314	+	+	+	C	C3	A	A	57	5	4.5
12	53	F	−	+	−	+	1810	+	+	+	Th	C3-7	A	A	53	2	6.5
13	38	F	+	−	−	+	4400	+	+	+	C	N	A	N	38	1	2.5
14	16	M	−	−	−	−	1810	+	+	+	C-Th	N	N	A	12	48	2.5
15	50	M	+	+	+	+	1206	+	+	−	C	N	N	A	49	10	3
16	31	M	+	+	−	+	124	+	+	+	C	N	N	A	30	17	3.5
17	33	M	−	−	−	−	493	+	+	+	C	C2-3	A	A	22	132	3.5
18	34	F	+	+	−	−	1020	+	+	+	Th	Th4-11	A	A	32	24	2.5
19	22	F	−	−	−	−	257	+	+	−	C	C3-4	ND	A	21	6	2
20	25	F	−	+	−	+	287	+	+	+	C-Th	C6	N	A	25	1	3

atopic dis.=atopic diseases, AD=atopic dermatitis, AR=allergic rhinnitis, BA=bronchial asthma, *D. pteronyssinus*=*Dermatophagoides pteronyssinus*, *D. farinae* = *Dermatophagoides farinae*. M=male, F=female, C=Cervical cord, Th=Thoracic cord, A=abnormal, N=normal, ND=not determined. HyperIgEaemia was defined as serum IgE level higher than 250 U / ml. Cut off value of allergen-specific IgE was 0.34 IU / ml. The EDSS scores before treatment are shown.

mean EDSS score before treatment was 3.4±1.5 (mean±SD).

Electrophysiological studies

Concentric needle EMG was performed on at least one upper and one lower limb muscles (biceps, triceps, first dorsal interossei (FDI), vastus lateralis (VL), gastrocnemius, tibialis anterior (TA) at rest, at weak voluntary contraction and at maximum voluntary contraction in all patients. We checked 6 findings such as fasciculation potentials, fibrillation potentials, positive sharp waves (PSW), high amplitude (≧ 5000μV) motor unit potentials (MUPs), polyphasic (≧3 phases) MUPs. SEPs and MEPs in upper and lower limbs were recorded as descrived previously[15)16)17)]. In brief, the peak latencies of N9 (Erb's point), N13 (C7 cervical spine) and N20 (C3 or C4) were measured for the median SEPs. For the tibial SEPs, the peak latencies of N20 (Th12 thoracic spine) and P37 (Cz') were measured. The upper limb (UL) MEPs were recorded from the abductor pollicis brevis muscle by stimulating the hand motor area, cervical roots and Erb's point with a figure 8-shaped magnetic coil. The lower limb (LL) MEPs were recorded from the abductor hallucis muscle by stimulating leg motor area and lumbar roots. EPs were classified as abnormal if the latencies and the central conduction time exceeded more than 3 SDs above the means of the controls or if any component was absent.

Immunotherapy

Two patients (cases 5 and 12 in Table 1) showing on-going denervation potentials on needle EMG were subjected to plasma exchange (PE) and / or intravenous immunoglobulin (IVIG). Each patient received three PEs using Spectra (COBE) at three-to eight-day intervals. The entire procedure was carried out in a closed circuit. Briefly, the patient's blood was obtained from a forearme vein and delivered to a single-stage channel where centrifugation separated blood into plasma and blood cells. Blood cells were returned to the patient's vein together with the replacement solution, which consisted of 2.3% albumin, Na 119 mEq / L, K 3.6 mEq / L, Ca 2.5 mEq / L and lactate 25.2 mEq / L. Approximately 2.0 L (40 ml / kg) of plasma was replaced in each procedure. In case 5, immunoglobulin (Glovenin-I, Nihonseiyaku) was infused into the patient in dosages of 0.4 gm / kg / day in one infusion over several hours for 5 days.

Results

Clinical findings

Only 1 of 20 (5 %) patients showed definite muscle atrophy (left biceps brachii muscle in case 7), and none had fasciculation.

EMG findings

On needle EMG, 12 of 20 patients (60 %) had definite neurogenic patterns (Table 2). On-going denervation potentials were observed in 5 patients (25 %), such as fasciculation potentials in 1 (case 5), fibrillation potentials in 5 (cases 2, 5, 8, 10 and 12) and, PSW in 3 (cases 2, 5 and 8) (Table 2). Chronic neurogenic patterns with reduced recruitment were found in 12 patients (60 %), such as giant MUPs in 9 patients (cases 1, 2, 4, 5 and 8-12) and polyphasic MUPs in 9 patients (cases 1, 3, 5-10 and 12). In case 13, equivocal neurogenic patterns (giant MUPs without reduced recruitment) were observed. Among 11 patients who had both MRI lesions and neurogenic patterns, 4 patients (36 %) had lower motor neuron involvement on needle EMG beyond the segments of the spinal cord lesions demonstrated by MRI (right vastus lateralis in case 2, right tibialis anterior in case 5,

Table 2 Needle EMG findings in patients with myelitis and atopic diathesis

	Fasciculation potential	Fibrillation potential	PSW	Giant MUP	Polyphasic MUP	Recruitment pattern
1	–	–	–	Bil. Triceps, FDI	Rt. triceps	reduced
2	–	Rt. FDI	Rt. FDI	Rt. vastus lateralis	–	reduced
3	–	–	–	–	Rt. ext. carpi ulnaris	reduced
4	–	–	–	Rt. abductor pollicis brevis	–	reduced
5	Rt. FDI, Rt. tibialis anterior	Rt. biceps	Rt. biceps	Rt. biceps	Rt. Biceps, Rt. tibialis anterior	reduced
	–	–	–	–	Bil. abductor pollicis brevis	
6	–	–	–	–	Rt. abductor pollicis brevis	reduced
7	–	Rt. FDI	Rt. FDI	Rt. FDI, Rt. gastrocnemius	Lt. Biceps, Lt. triceps	reduced
8	–	–	–	Lt. tibialis anterior, Lt. gastrocnemius	Rt. gastrocnemius	reduced
9	–	Rt. flex. pollicis brevis	–	Rt. flex. pollicis brevis	Lt. gastrocnemius	reduced
10	–	–	–	Rt. FDI, Lt. rectus femoris	Rt. flex. pollicis brevis	reduced
11	–	Lt. abductor digiti minimi	–	Bil. FDI, Lt. triceps	–	reduced
12	–	–	–	Rt. FDI	Lt. triceps	reduced
13	–	–	–	–	–	normal
14	–	–	–	–	–	normal
15	–	–	–	–	–	normal
16	–	–	–	–	–	normal
17	–	–	–	–	–	normal
18	–	–	–	–	–	normal
19	–	–	–	–	–	normal
20	–	–	–	–	–	normal

Bil.=bilateral, Rt.=right, Lt.=Left, PSW=Positive sharp wave, MUP=motor unit potential, FDI=First dorsal interossei, flex.=flexor, ext.=extensor.

right gastrocnemius in case 8 and left rectus femoris in case 11). In addition, these 4 patients had no compressive lesions on lumbar MRI.

Effects of immunotherapy

Muscle weakness as well as on-going denervation potentials were improved by immunotherapy such as PE or PE plus IVIG in both patients tried (cases 5 and 12). In case 5, muscle strength improved as follows: deltoid 4 (right) / 5 (left) to 5 / 5, finger flexor 3+/ 5 to 4 / 5, finger extensor 3+/ 5 to 4 / 5, iliopsoas 2+/ 3+ to 3+/ 4+, tibialis anterior 0 / 3 to 1-/ 4 and gastrocnemius 0 / 3 to 1-/ 4 on the Medical Research Council (MRC) scale. Fasciculation potentials in right first dorsal interossei and right tibialis anterior and fibrillation potentials and positive sharp waves in right biceps were disappeared on needle EMG performed 7 days after immunotherapy (three PEs followed by IVIG). MRI lesions were unchanged before and after the treatment in this case. In case 12, muscle strength improved as follows: deltoid 5 (right) / 4+ (left) to 5 / 5, triceps 4 / 4 to 5 / 5, wrist extensor 4+ / 4 to 5 / 5, wrist flexor 4+ / 4 to 5 / 5, iliopsoas 1 / 1 to 5 / 5, hamstrings 1 / 1 to 5 / 5, quadriceps 3 / 3 to 5 / 5, tibialis anterior 3 / 4 to 5 / 5, gastrocnemius 3 / 4 to 5 / 5, toe extensor 3 / 4 to 5 / 5, toe flexor 3 / 4 to 5 / 4+ on the MRC scale. Fibrillation potentials in left abductor digiti minimi muscle was disappeared on needle EMG performed on 9 days after three PEs. MRI was not studied after the treatment in case 12. Chronic neurogenic patterns were unchanged in both cases.

Discussion

The present study disclosed the frequent involvement of lower motor neurons in patients with AM. Among 13 patients showing the lower motor neuron involvement on needle EMG, only 1 had the definite muscle atrophy and none showed fascicula-

tion, suggesting that the lower motor neuron involvement is subclinical in most cases. Frequent lower motor neuron involvement in the relatively early course of the disease is consistent to the pathological observation that axons as well as myelin were lost in the biopsied spinal cord lesions[7)18)]. These findings are thus supposed to discriminate this condition from multiple sclerosis (MS).

It is important that one-third of the patients had subclinical involvement of the lower motor neurons in the spinal cord segments other than the overt spinal cord lesions shown by MRI. It thus suggests the scattered involvement of the spinal cord by the disease process, yet the preferential site of involvement is the cervical cord.

Hopkins syndrome, JMADUE (Hirayama' s disease) and sporadic LMND have been shown to be more or less associated with airway allergy, such as bronchial asthma, allergic rhinitis and pollinosis[8)9)10)11)]. The results of the present study add AM to the list of lower motor neuron involvement associated with allergy, yet the degree of lower motor neuron involvement is mild and mostly subclinical. These observations support the notion that allergic tendency is one of the risk factors for lower motor neuron damage.

On the other hand, the neuropathology of the biopsied spinal cord lesions in atopic myelitis revealed it to be eosinophilic inflammation[7)18)]. Such neuropathological findings are in good agreement with the typical atopic disorders, such as bronchial asthma, allergic rhinitis and atopic dermatitis, where eosinophil infiltration is one of the cardinal features[19)20)]. Activated eosinophil products, such as eosinophil cationic proteins (ECP), have been shown to be deposited in the spinal cord tissues[7)]. ECP causes membrane damage through the formation of transmembrane channel pore[21)]. Such eosinophil products are well known to be neurotoxic[22)23)], yet the mechanism of neurotoxicity is ill-defined. Therefore, neurotoxic eosinophil products may in part contribute to the anterior horn cell damage in this condition.

We recently reported that PE is also beneficial for the lower motor neuron damage in JMADUE associated with airway allergy[24)]. Effectiveness of immunotherapy in lower motor neuron involvement of AM and JMADUE further supports the notion that immunological process contributes to the lower motor neuron damage in these conditions. PE may be beneficial for the motor neuron damage by removing mediators of allergic inflammation, anti-neuronal autoantibodies and Th2 cytokines enhancing their production.

Conclusion

In the present study, we found that subclinical lower motor neuron involvement is common in AM. Although the precise mechanism remains to be elucidated, it is likely to be immune-mediated and reversible by immunotherapy, and therefore such immunotherapies as PE and IVIG may thus be worth trying in the lower motor neuron involvement associated with atopic diathesis.

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Platelet-activating factor acetylhydrolase gene polymorphism and its activity in Japanese patients with multiple sclerosis

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Abstract

We evaluated the association of the plasma platelet-activating factor acetylhydrolase (PAF-AH) gene polymorphism ($G^{994} \rightarrow T$) and PAF-AH activity with susceptibility and severity of multiple sclerosis (MS) in Japanese. DNA was collected from 216 patients with clinically definite MS (65 opticospinal MS (OS-MS) and 151 conventional MS (C-MS)) and from 213 healthy controls. The missense mutation $G^{994} \rightarrow T$ that disrupts the PAF-AH activity was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). No statistically significant difference in the frequency of genotypes and alleles of the plasma PAF-AH polymorphism was observed among OS-MS patients, C-MS patients and healthy controls. However, the missense mutation tended to be associated with the severity of OS-MS, especially in females (GT/TT genotypes; 51.7% in female rapidly progressive OS-MS vs. 26.6% in female controls, $p = 0.0870$). Moreover, PAF-AH activities were significantly lower in MS than in controls, irrespective of clinical subtypes, among those carrying the identical polymorphism in terms of nucleotide position 994 of the PAF-AH gene. These findings suggest that the PAF-AH gene missense mutation has no relation to either susceptibility or severity of C-MS, yet its activity is down-regulated, and that the mutation has no relation with susceptibility of OS-MS, yet it may confer the severity of female OS-MS.

Keywords: Polymorphism; Platelet-activating factor acetylhydrolase; Multiple sclerosis; Opticospinal MS; Conventional MS

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of unknown etiology influenced by genetic background (Bell and Lathrop, 1996; Chataway et al., 1998). In Asian patients with MS, severe and selective involvement of the optic nerves and spinal cord is characteristic. We previously reported the existence of two subtypes of MS in Japanese; namely opticospinal MS (OS-MS), in which the clinically estimated main lesions are confined to the optic nerves and spinal cord, and conventional MS (C-MS) showing disseminated lesions in the central nervous system (CNS), including the cerebrum, cerebellum or brainstem (Kira et al., 1996; Yamasaki et al., 1999). OS-MS has distinct features, such as a marked female preponderance, higher age at onset, higher Kurtzke's expanded disability status scale (EDSS) scores (Kurtzke, 1983) resulting from severe visual impairment and marked spinal cord dysfunction, and a lower number of brain lesions on MRI compared with C-MS. Severe inflammatory destruction is suggested in OS-MS because of the higher cell counts and amounts of protein in the cerebrospinal fluid (CSF), as well as long swollen lesions extending over several vertebral segments on spinal cord MRI (Kira, 2003). Pathological studies have also revealed severe inflammation and vascular changes in OS-MS lesions (Ikuta et al., 1982; Tabira and Tateishi, 1982). Gene polymorphism in HLA-DR (DRB1*1501) (Kira et al., 1996; Ma et al., 1998), Vitamin D receptor

Abbreviations: OS-MS, opticospinal multiple sclerosis; C-MS, conventional multiple sclerosis; EDSS, Kurtzke's expanded disability status scale; PAF-AH, platelet-activating factor acetylhydrolase.

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(Fukazawa et al., 1999; Niino et al., 2000) and osteopontin (Niino et al., 2003) were reported to be associated with susceptibility of C-MS while estrogen receptor polymorphism was associated with the severity of C-MS in Japanese (Kikuchi et al., 2002); however all of these results except for HLA-DRB1*1501 have not been independently confirmed by other groups and therefore still require further confirmatory studies. On the other hand, in OS-MS only the HLA-DPB1*0501 allele has been shown to be a disease-susceptibility gene (Ito et al., 1998; Yamasaki et al., 1999; Fukazawa et al., 2000), and no genetic background explaining the severe inflammation and vascular changes has ever been demonstrated.

There is a report showing an elevation in platelet-activating factor (PAF) in the CSF and plasma of relapsing–remitting MS patients, and the authors claimed that PAF is responsible for the early breakdown of the blood–brain barrier (BBB) in MS (Callea et al., 1999). The notion is supported by the observation that PAF receptors are upregulated in MS lesions (Lock et al., 2002). PAF is a biologically active phospholipid implicated as a mediator of physiological processes, such as the signaling and activation of proinflammatory cells and alteration of vascular permeability. In addition, PAF is also a very potent chemotactic stimulus for inflammatory cells such as eosinophils (Wardlaw et al., 1986) and polymorphonuclear neutrophils (O'Flaherty et al., 1981).

PAF is inactivated by plasma PAF acetylhydrolase (PAF-AH), which has its gene located in chromosome 6p12-21.1 and comprises 12 exons spanning at least 45 kilobase of DNA (Stafforini et al., 1996). In Japanese, a single point mutation (G → T transversion) at nucleotide position 994 in exon 9 results in a Val → Phe substitution at amino acid residue 279 of the mature protein, and is responsible for the loss of catalytic activity, has been shown to occur in 4% of the population, whereas no such mutation has ever been reported in Caucasians (Miwa et al., 1988). In previous reports, this mutation has been held up as a genetic risk factor for Japanese patients with vascular or autoimmune inflammatory diseases, such as stroke (Hiramoto et al., 1997), nonfamilial dilated cardiomyopathy (Ichihara et al., 1998), coronary artery disease (Yamada et al., 1998), and asthma (Stafforini et al., 1999). Moreover, the plasma PAF-AH gene mutation has been reported to influence the degree of proteinuria and the extent of mesangial proliferation in childhood IgA nephropathy (Tanaka et al., 1999).

Whether or not this missense mutation in the PAF-AH gene confers OS-MS susceptibility and severity in Japanese is of interest since the OS-MS is characterized by severe inflammation and vascular disruptions (Ikuta et al., 1982; Tabira and Tateishi, 1982; Kira, 2003). Moreover, OS-MS is preferentially seen in Japanese, who are reported to have higher frequency of the PAF-AH gene missense mutation, and is rare in Caucasians, in whom such mutation has not been reported. The present study was thus designed to ascertain the associations between the PAF-AH gene mutation and PAF-AH activities in Japanese MS patients by clinical subtypes.

2. Patients and methods

2.1. Patients

A total of 216 patients (56 men and 160 women) with clinically definite MS, according to the recommended diagnostic criteria (McDonald et al., 2001), were recruited from the Department of Neurology, Kyushu University Hospital, and the Department of Neurology, Hokkaido University Hospital, and Hokuyukai Neurological Hospital (Table 1). Hematological and biochemical studies and serologic tests for syphilis were performed in all patients. None of the patients were seropositive for human T-cell leukemia virus type 1. Age at examination was 41.5 ± 13.0 years (mean ± S.D.) and at disease onset 30.1 ± 12.0 years (mean ± S.D.). After at least a 1-year observation period, 169 were diagnosed as relapsing–remitting type MS and 47 as secondary progressive type MS, in which the onset of progressive disease was defined as continual worsening of symptoms and signs for a period at least 6 months, with or without superimposed relapses (Lublin and Reingold, 1996; Confavreux et al., 2000). Primary progressive MS was not included in the present study. MS severity was evaluated by Kurtzke's Expanded Disability Status Scale (EDSS) scores (Kurtzke, 1983). EDSS scores were 3.8 ± 2.6 (mean ± S.D.) at the time of examination. In the present study, MS patients were classified into two subtypes before genotyping of the plasma PAF-AH according to the clinical criteria described previously (Kira et al., 1996; Yamasaki et al., 1999). The patients, whose clinically estimated lesions were confined to the optic nerve and

Table 1
Clinical profiles of MS patients

	MS (n=216)	OS-MS (n=65)	C-MS (n=151)
Male:Female*	56:160	9:56	47:104
Age at disease onset[a,**]	30.1 ± 12.0	38.1 ± 13.9	26.7 ± 9.2
Age at examination[a,**]	41.5 ± 13.0	49.3 ± 13.4	38.2 ± 11.3
Disease duration[a]	11.4 ± 8.7	11.2 ± 8.6	11.5 ± 8.8
EDSS score[b,**]	3.8 ± 2.6	4.8 ± 2.3	3.5 ± 2.7
Rapidly progressive patients (%)**	29.3	52.3	29.8
Clinical course**			
R-R	169	62	107
S-P	47	3	44

OS-MS, opticospinal form multiple sclerosis; C-MS, conventional form multiple sclerosis; R-R, relapsing–remitting course; S-P, secondary progressive course; EDSS, Kurtzke's Expanded Disability Status Scale.
[a] Mean ± S.D. (years).
[b] Mean ± S.D.
*p<0.05 (OS-MS vs. C-MS).
**p<0.001 (OS-MS vs. C-MS).

spinal cord, were classified as having OS-MS and had no clinical evidence of disease in either the cerebrum or the cerebellum. The rest of the MS patients, who showed involvement of multiple sites in the CNS including the cerebrum, cerebellum, or brainstem, were classified as having C-MS. In each MS group, those who had a grave disability (EDSS score 5 and more than 5) within 10 years were classified as rapidly progressive type. Sixty-three of 216 (29.2%, 34 OS-MS and 29 C-MS) were considered to have a rapidly progressive disease. Moreover, in each MS group, those who had not a grave disability (EDSS score less than 5) beyond 10 years were classified as non-rapidly progressive type; 41 of 216 (19.0%, 12 OS-MS and 29 C-MS) were considered to have a non-rapidly progressive disease. The rests (112 patients; 19 OS-MS patients and 93 C-MS patients) were undetermined at the time of examination, since the observation period was less than 10 years and the EDSS scores were less than 5. The control group was composed of 89 unrelated healthy men and 124 unrelated healthy women (mean age $\pm$ S.D. $= 34.7 \pm 10.1$ years). Subjects' consent was obtained in accord with the declaration of Helsinki, and the Ethical Committee of the Institution in which the work was performed gave its approval.

2.2. Genotyping of the plasma PAF-AH

Total blood genomic DNA was extracted from leukocytes with a QIAamp DNA Blood Midi Kit (QIAGEN, Tokyo, Japan) following the manufacturer's instructions. The genotype of the plasma PAF-AH was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the method of Stafforini et al. (Stafforini et al., 1996) without knowledge of the samples' clinical diagnosis. The sense primer (5′-CTATAAATTTA-TATCATGCTT-3′) and antisense primer (5′-TTTAC-TATTCTCTTGCTTTAC-3′) were used. Reactions were performed in a total volume of 50 μl containing 0.5 μg genomic DNA, 20 pmol of each primer, 0.2 mmol/l each of dATP, dGTP, dCTP, and dTTP, 1 U Taq DNA polymerase (Takara, Otsu, Japan), 50 pmol/l KCl, 1.5 mmol/l $MgCl_2$, and 10 nmol/l Tris hydrochloride (pH 8.3). The thermocycling procedure consisted of an initial denaturation at 94 °C for 5 min; five cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min; 30 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. The genotype of the plasma PAF-AH was confirmed by digestion of the PCR products with the restriction endonuclease *Mae II* (Stafforini et al., 1996).

PCR products were analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. Since the $G^{994} \rightarrow T$ transversion produces a new restriction site for *Mae II*, genotypes were classified as GG (normal), GT (heterozygous) and TT (homozygous deficient).

2.3. Measurement of PAF-AH activity

PAF-AH activity was measured using a spectrophotometric method with an H-7170 automatic analyzer (HITACHI) as described previously (Kosaka et al., 2000, 2001). Briefly, a 3-μl volume of sample and 240 μl of Reagent 1 (200 mmol/l NaCl, 5 mmol/l EDTA, 10 mmol/l sodium 1-non-anesulfonate, 10 mmol/l CHAPS, and 200 mmol/l HEPES, pH 7.6) were mixed, and preincubated at 37 °C for 5 min. The reaction was then started by adding 80 μl of Reagent 2 (19 mmol/l citric acid monohydrate, 9.5 mmol/l sodium 1-non-anesulfonate, and 4.8 mmol/l L-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, pH 4.5). Absorption was measured at 2 and 5 min after the addition of the substrate solution (Reagent 2). The activities were calculated using the differences between the absorbances of the above measuring points and the extinction coefficient of 4-nitrophenol. The sera from 24 OS-MS patients, 28 C-MS patients and 82 healthy controls and the plasma from eight OS-MS patients, five C-MS patients and 43 healthy controls were measured for their PAF-AH activities. Samples from OS-MS and C-MS patients were analyzed at a clinical remission phase in all patients and, in addition, in 13 patients with OS-MS and in nine patients with C-MS PAF-AH activities were analyzed both at the time of clinical relapse (within 2 weeks after the onset of acute exacerbation) and the time of clinical remission. Here we defined MS patients in remission as those who had been clinically stable for at least 1 month without any immunosuppressive medication, such as corticosteroids, interferon beta and azathioprine; and MS patients in relapse as those who developed new symptoms or a significant aggravation of pre-existing symptoms lasting for more than 2 days.

2.4. Statistical analysis

Plasma PAF-AH genotypes and allele frequencies were compared between OS-MS, C-MS and healthy controls, using Chi-square tests. As the calculated p values were not corrected for multiple comparisons, p values were multiplied by a factor of 10 (the number of comparisons) to calculate the corrected p values. The association between plasma PAF-AH activities and serum PAF-AH activities was examined using Pearson's correlation coefficient. Serum PAF-AH activities in the three genotypes were compared using the Kruskal–Wallis H test followed by multiple comparisons. Once differences among clinical groups were identified, we compared the results using one-way analysis of variance followed by Bonferroni's correction to determine statistical differences after multiple comparisons. In addition, for serum PAF activities, other comparisons were conducted using the Kruskal–Wallis H test followed by multiple comparisons. Values of $p < 0.05$ were considered statistically signifi-

Table 2
Genotype and allele frequencies for the PAF-AH gene mutation ($G^{994} \rightarrow T$) in MS patients and healthy controls

PAF-AH	MS			OS-MS			C-MS			Healthy controls		
	Total ($n=216$)	Male ($n=56$)	Female ($n=160$)	Total ($n=65$)	Male ($n=9$)	Female ($n=56$)	Total ($n=151$)	Male ($n=47$)	Female ($n=104$)	Total ($n=213$)	Male ($n=89$)	Female ($n=124$)
Genotype frequency												
GG	159 (73.6)	41 (73.2)	118 (73.7)	44 (67.7)	8 (88.9)	36 (64.3)	115 (76.2)	33 (70.2)	82 (78.8)	155 (72.8)	64 (71.9)	91 (73.4)
GT	54 (25.0)	14 (25.0)	40 (25.0)	20 (30.8)	1 (11.1)	19 (33.9)	34 (22.5)	13 (27.7)	21 (20.2)	53 (24.9)	21 (23.6)	32 (25.8)
TT	3 (1.4)	1 (1.8)	2 (1.3)	1 (1.5)	0 (0.0)	1 (1.8)	2 (1.3)	1 (2.1)	1 (1.0)	5 (2.3)	4 (4.5)	1 (0.8)
Allele frequency												
Allele G	372 (86.1)	96 (85.7)	276 (86.2)	108 (83.1)	17 (94.4)	91 (81.2)	264 (87.4)	79 (84.0)	185 (88.9)	363 (85.2)	149 (83.7)	214 (86.3)
Allele T	60 (13.9)	16 (14.3)	44 (13.8)	22 (16.9)	1 (5.6)	21 (18.8)	38 (12.6)	15 (16.0)	23 (11.1)	63 (14.8)	29 (16.3)	34 (13.7)

OS-MS, opticospinal form multiple sclerosis; C-MS, conventional form multiple sclerosis. The table indicates frequency of genotypes and alleles (percentage).

cant. Statistical analyses were performed with StatView/Mac software.

3. Results

3.1. Plasma PAF-AH genotype and allele frequencies in MS

The proportions of plasma PAF-AH genotypes (GG, GT, and TT) and alleles (G allele, T allele) in OS-MS patients, C-MS patients and healthy controls are shown in Table 2. In control subjects, the genotype frequencies are similar to those found in other Japanese studies (Hiramoto et al., 1997; Ichihara et al., 1998; Yamada et al., 1998; Stafforini et al., 1999; Tanaka et al., 1999). No statistically significant difference in the frequency of genotypes and alleles of the plasma PAF-AH polymorphism was observed among OS-MS patients, C-MS patients and healthy controls.

3.2. Plasma PAF-AH genotype and allele frequency changes by severity in each MS subgroup

In the rapidly progressive OS-MS subgroup, the frequency of the GT/TT genotypes was higher than in healthy

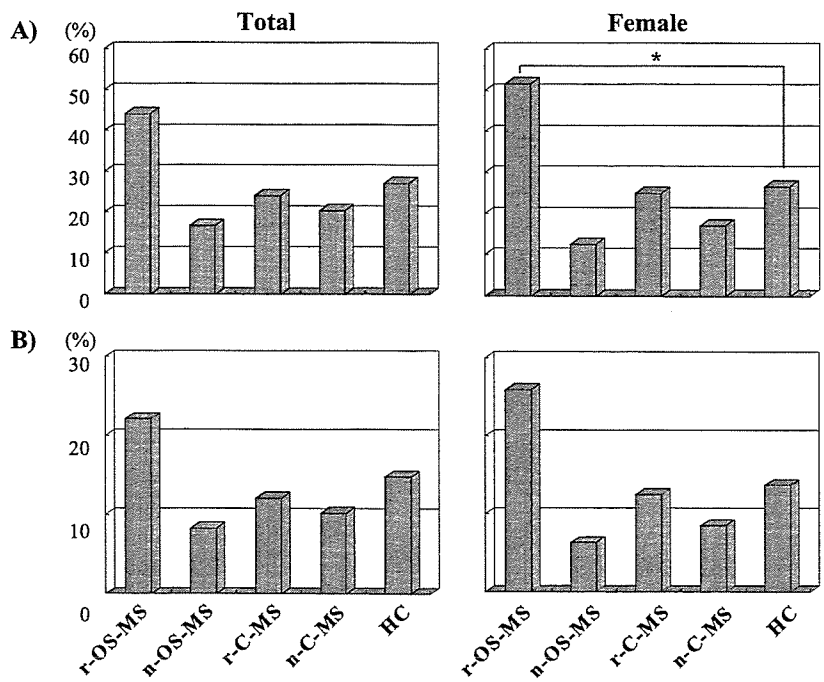

Fig. 1. Comparison of frequencies of GT/TT genotypes (A) and T allele (B) among rapidly progressive OS-MS, non-rapidly progressive OS-MS, rapidly progressive C-MS, non-rapidly progressive C-MS and healthy controls in total (left) and female (right) subjects. The asterisks (*) indicates that the frequency of GT/TT genotypes in female rapidly progressive OS-MS tended to be higher than in female healthy controls (15 of 29 female rapidly progressive OS-MS (51.7%) vs. 33 of 124 female healthy control (26.6%), $p=0.087$). No statistically significant difference in the frequency of genotypes and alleles of the plasma PAF-AH polymorphism was observed in other comparisons. r-OS-MS; rapidly progressive opticospinal multiple sclerosis ($n=34$); n-OS-MS, non-rapidly progressive opticospinal multiple sclerosis ($n=12$); r-C-MS, rapidly progressive conventional multiple sclerosis ($n=29$); n-C-MS, non-rapidly progressive conventional multiple sclerosis ($n=54$); HC, healthy controls ($n=213$).

controls (44.1% vs. 27.2%), but did not reach statistical significance. The frequency of GT/TT genotypes in female patients with rapidly progressive OS-MS tended to be higher than those in female healthy controls (51.7% vs. 26.6%, $p=0.087$) (Fig. 1). In female OS-MS patients, although the frequency of GT/TT genotype was much higher in the rapidly progressive type than in the non-rapidly progressive type (51.7% vs. 12.5%), it did not reach a statistical significance due to the small sample size. As for C-MS, no statistically significant difference in the frequency of either genotypes or alleles of the plasma PAF-AH polymorphism was observed.

3.3. Plasma and serum PAF-AH activities in each MS subgroup and healthy controls

As the number of sera stored in −80 °C and available for the present study was much larger than that of deep-frozen plasma, we first measured PAF-AH activities in both plasma and serum and evaluated the correlation between the two. Plasma PAF-AH activities significantly correlated with serum PAF-AH activities in MS patients and healthy controls ($r=0.9949$, $p<0.0001$), and therefore the serum PAF-AH activities were used for the statistical comparisons that followed. When we assorted serum PAF-AH activities in the MS subgroups and healthy controls into three groups according to genotype, we found that in each group, enzyme activities were significantly higher in subjects with the GG genotype (523.2 ± 133.7 IU/l) than those with GT or TT genotype (GT; 233.0 ± 52.8 IU/l, TT; 40.8 ± 4.2 IU/l, respectively), and also significantly higher in individuals with the GT genotype than in those with the TT genotype ($p<0.0001$) (Fig. 2). In addition, when we assayed PAF-AH activities at the time of a clinical relapse and at the time of a clinical remission in 22 MS patients, no statistically significant difference was observed ($p>0.1$). In subjects with the GG genotype and those with the GT genotype, PAF-AH activities in MS patients were significantly lower than those in healthy controls (GG genotype; 493.7 ± 146.0 vs. 546.9 ± 131.0 IU/l, $p=0.0223$, GT genotype; 204.7 ± 47.1 vs. 258.0 ± 45.2 IU/l, $p=0.0044$). In OS-MS subjects with the GG genotype, PAF-AH activities in rapidly progressive patients were lower than those in other patients (473.4 ± 81.1 IU/l in 5 vs. 505.9 ± 131.3 IU/l in 10). Moreover, in OS-MS subjects with the GT genotype, PAF-AH activities in rapidly progressive patients were lower than those in other patients (213.0 ± 45.7 IU/l in 6 vs. 241.0 ± 23.3 IU/l in 2). However, these comparisons did not reach a statistical significance due to the small sample size.

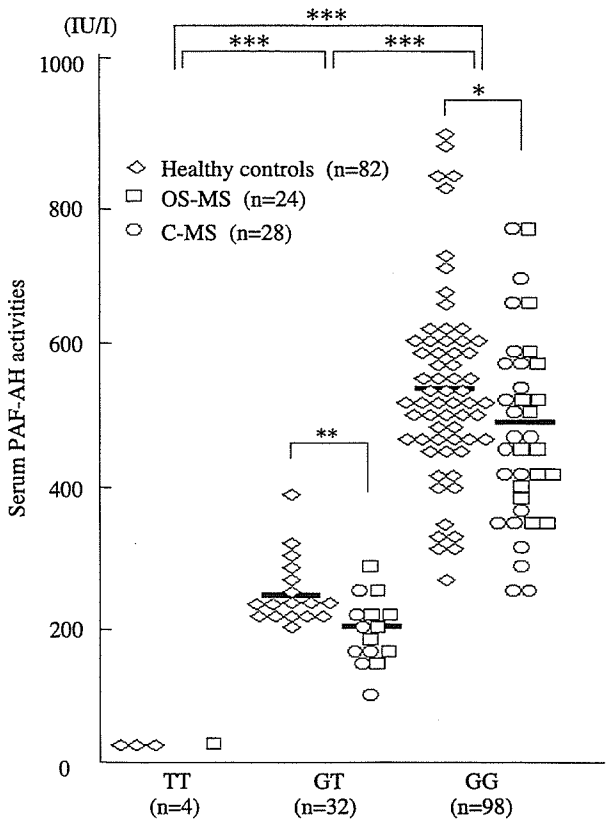

Fig. 2. Serum PAF-AH activities in healthy controls, and OS-MS and C-MS patients. Serum PAF-AH activities are significantly higher in subjects with the GG genotype (523.2 ± 133.7 IU/l) than those with GT or TT genotypes (233.0 ± 52.8 and 40.8 ± 4.2 IU/l, respectively), and also in individuals with GT genotype compared to those with TT ($p<0.0001$). In subjects with GG and GT genotypes, PAF-AH activities in MS patients are significantly lower than in healthy controls (GG genotype, 493.7 ± 46.0 vs. 546.9 ± 131.0 IU/l, $p=0.0223$; GT genotype, 204.7 ± 47.1 vs. 258.0 ± 45.2 IU/l, $p=0.0044$). The asterisks, *, ** and ***, indicate $p<0.05$, $p<0.01$ and $p<0.0001$, respectively. Each bar indicates the mean value of each group.

4. Discussion

In the present study, no significant association of the inactivating mutation of the PAF-AH gene with MS or with either subtype of MS was found, although the PAF-AH activities in MS were significantly lower than in healthy controls, even among populations carrying identical polymorphisms in terms of nucleotide position 994 of the PAF-AH gene. It is therefore suggested that the PAF-AH gene inactivating polymorphism plays no role in MS susceptibility.

In C-MS, the frequency of the PAF-AH gene missense mutation did not differ either by gender or by disease severity; while in OS-MS it tended to be higher in female patients with the rapidly progressive type than in female controls and female patients with the non-rapidly progressive type; however, the difference did not reach statistical significance due to the small sample size. It might suggest that the missense mutation is a possible genetic severity factor for OS-MS in female. Such a difference in the association of the missense mutation with diseases by gender has also been reported in cardiac infarction; in which