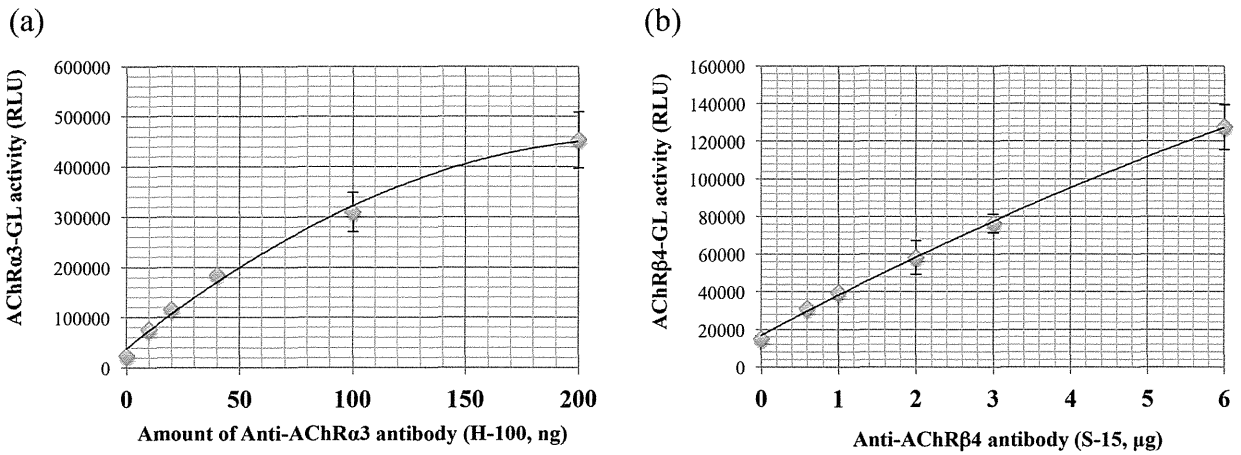

Fig 3. Confirmation of the LIPS assay system for the gAChRα3 or β4 with ready-made antibodies. The anti-gAChRα3 antibody (H-100) and the anti gAChRβ4 antibody (S-15) bound the gAChRα3-GL and the gAChRβ4 reporters, respectively in a dose-dependent manner (a and b). The X-axis indicates the amount of ready-made gAChRα3 or β4 antibody used. The Y-axis indicates the gAChRα3 or β4-GL activity. The line with closed diamonds shows the results that were obtained in this experiment.

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gAChR subunit-specific antibodies. As shown In Fig. 3, there is a dose-dependent response was observed between the amount of ready-made gAChR subunit-specific antibody and the gAChR subunit-specific luciferase reporter activity. These data demonstrate that each gAChR subunit-specific antibody properly bounds gAChRα3-GL or gAChRβ4-GL.

Detection of the autoantibodies to the gAChR in patients with AAG

Both the anti-gAChRα3 and anti-gAChRβ4 antibodies were determined by the LIPS assay to be present in 0.0% (0 of 73) of the HC. In contrast, 48% (24 of 50) of the sera from patients with AAG were positive for autoantibodies ($p < 0.001$, Fig. 4A). The anti-gAChRα3 antibodies were detected in 23 samples and the anti-gAChRβ4 antibodies were detected in seven samples (14.0%), as shown in Fig. 4B ($p < 0.001$). Both of the antibodies were detected in six samples. The mean anti-gAChRα3 antibody levels in the HC and the DC were 0.305 A.I. and 0.336 A.I., respectively. These levels were significantly lower than the mean level in the AAG samples with a mean level of 1.210 A.I. ($p < 0.001$, Fig. 4A). Similarly, the mean anti-gAChRβ4 antibody level in HC was 0.367 A.I. and DC was 0.302 A.I., respectively. Those were significantly lower than the AAG samples with a mean level of 0.618 A.I. ($p < 0.001$, Fig. 4B). In the DC group, we detected anti-gAChRα3 antibodies in the serum of the patient with the suspected case of amyloid neuropathy.

Clinical profile of the anti-gAChR antibody-positive and -negative patients with AAG

The clinical characteristics of the patients are presented in Table 1. The age at the onset and the duration of the autonomic symptoms were 48.8 ± 20.1 (mean ± standard deviation) years and 3.7 ± 6.9 years, respectively. The patterns of mode were divided into subacute and gradual groups, according to the duration to the peak of autonomic symptoms. Half of the patients with AAG had subacute onsets and half had chronic progressive presentations. Gastrointestinal tract symptoms were the most frequently observed (92.0%). Table 1 compares the clinical features between patients who were positive and those who were negative for the anti-gAChR

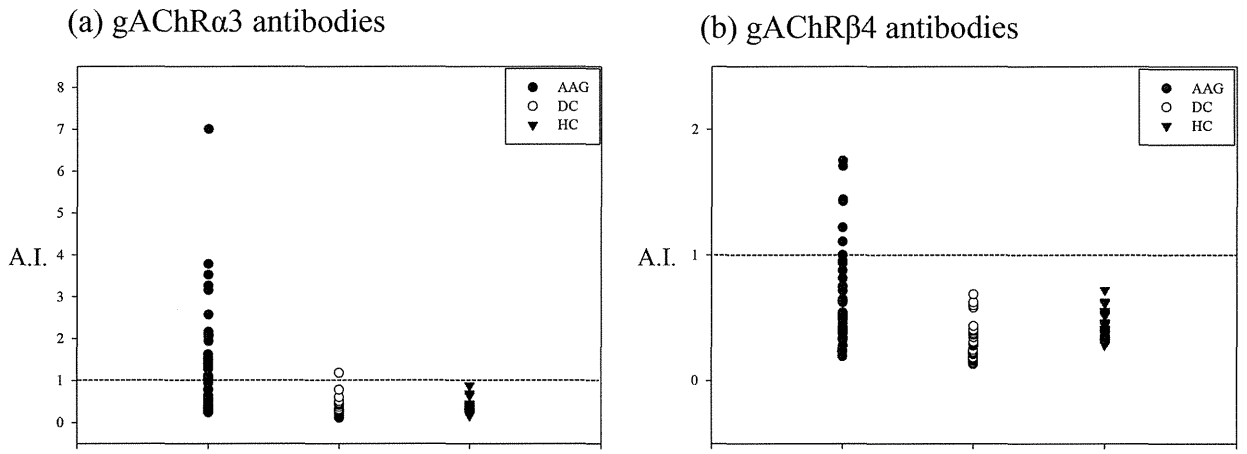

Fig 4. LIPS for gAChR in the sera from patients with autoimmune autonomic ganglionopathy (AAG) and controls. We tested the sera from patients with AAG, disease controls (DC), and healthy controls (HC). a) Anti-gAChRα3 antibodies were detected in 23 samples. The mean anti-gAChRα3 antibody level in the HC was 0.305 antibody index (A.I.), which was significantly lower than in the AAG samples with a mean level of 1.210 A.I. ($p < 0.001$). b) Anti-gAChRβ4 antibodies were also detected in seven samples, as shown in Fig. 4B ($p = 0.005$). The mean anti-gAChRβ4 antibody level in the HC was 0.367 A.I., which was significantly lower than the measn level of 0.618 A.I. in the AAG samples ($p < 0.001$).

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antibodies. Gradual onset was more common in the anti-gAChR antibody-positive patients than in the antibody-negative patients (62.5% *vs.* 38.5%). No significant differences in the clinical findings were noted, except for a higher frequency of other autoimmune disease complications in the patients who were positive for the anti-gAChR antibody compared to the antibody-negative patients (37.5% *vs.* 8.0%, $p = 0.012$).

Clinical characteristics and autonomic symptoms of the patients with AAG who were anti-gAChR antibody-positive (Table 2)

Table 2 summarizes the clinical characteristics and autonomic symptoms of the patients with AAG. An antecedent event was reported in four patients shortly before the initiation of autonomic symptoms. In our study, major autonomic symptoms, including orthostatic, sicca, sudomotor, papillary, gastrointestinal, and urinary symptoms were analyzed. Orthostatic hypotension for orthostatic intolerance and gastrointestinal tract symptoms were observed in 20 (83.3%) patients and 22 (91.7%) patients, respectively. Gastrointestinal tract symptoms were composed of various digestive system problems, such as constipation (n = 14), early satiety (n = 4), vomiting (n = 4), abdominal pain (n = 4), anorexia (n = 4), diarrhea (n = 4), ileus (n = 3), alternate stool abnormality (n = 3), taste impairment (n = 1), and achalasia (n = 3). Pupillary dysfunction was observed in 11 patients, including two patients who had Adie's tonic pupil. The initial symptoms of seropositive AAG/acute pandysautonomia (APD) in 15 patients (62.5%) were orthostatic hypotension, involving lightheadedness, orthostatic intolerance, or syncope. The autonomic manifestations of the anti-gAChR antibody-positive patients were widespread, and they affected both sympathetic and parasympathetic functions. However, 3 patients (Patient 6, 16, and 17) had only one symptom (disturbance of the digestive system, bladder dysfunction, and orthostatic hypotension, respectively). Patient 6 had a history of recurrent ileus and severe abdominal pain. She previously had an operation to remove a sigmoid volvulus 1 year before the onset of repeated ileus and abdominal pain. As for the other symptoms, attacks of coughing were observed in four patients (16.7%), and six patients (Patients 1, 5, 11, 12, 14, and 15) had a subjective numbness or superficial sensory disturbance in the

Table 2. Clinical and autonomic characteristics at baseline of anti-gAChR Ab positive AAG patients.

Patient	Age	Sex	Onset age	Duration	Onset [a]	AE [b]	OH, OI	Sicca	Coughing episodes	HI, AH	Pupil abnormality [c]	GI [d]	Bladder dysfunction	Sexual dysfunction	Other clinical features	Complication: endocrine disorder, autoimmune disease	Complication: tumor
1	75	M	59	16	Gradual	–	+	+	–	+	+	+	+	+	Numbness	–	–
2[53]	60	M	60	0	Subacute	+	+	+	–	+	+	+	+	–	–	Still disease susp.	–
3	39	F	39	0	Subacute	–	+	+	–	–	–	+	–		–	ANA positive	–
4	26	F	21	5	Gradual	–	–	+	–	+	+	+	+		–	Amenorrhea	Ovarian tumor
5	68	M	53	15	Gradual	–	+	–	–	–	–	+	+	+	Numbness	–	–
6	37	F	35	2	Gradual	–	–	–	–	–	–	+	–	–	–	–	–
7	45	M	45	0	Subacute	+	+	+	–	+	+	+	+	+	–	–	–
8	60	M	60	0	Subacute	–	+	+	–	+	+	+	+	+	Mental symptom	–	–
9	79	F	77	2	Gradual	–	+	–	–	–	+	+	+		Dementia	PBC, Hashimoto dis.	–
10	78	M	78	0	Subacute	–	+	–	–	+	–	+	–	–	–	–	–
11	67	M	59	8	Gradual	–	+	+	–	–	–	+	–	–	Numbness	–	–
12	49	M	12	37	Gradual	–	+	+	+	+	+	+	+	+	Sensory disturbance	–	–
13	73	F	66	7	Gradual	–	+	+	–	+	–	+	+		–	–	Mediastinal tumor
14	16	M	16	0	Subacute	–	+	–	+	+	+	+	–	–	Numbness, character change	SIADH	–
15	56	M	55	1	Gradual	–	+	–	+	+	–	+		+	Numbness	PMR, panhypopituitarism	–
16	54	F	35	19	Gradual	–	–	–	–	–	–	–	+		–	–	–
17	84	F	84	0	Gradual	–	+	–	+	–	–	+	+		–	–	Paranasal cancer
18	37	F	37	0	Subacute	–	+	+	–	+	+	+	+		–	SLE, SS	–
19	38	F	39	0	Subacute	–	+	–	–	–	–	–	–		–	Graves' dis.	Ovarian tumor
20	68	F	66	2	Gradual	–	+	+	–	+	–	+	+		–	RA, SS	–
21	46	F	39	7	Gradual	+	+	–	–	+	–	+	+		–	RA, fibromyalgia	–
22	49	F	47	2	Gradual	–	–	+	–	–	–	+	–		–	PBC	–
23	6	F	6	0	Subacute	+	+	+	–	+	+	+	+		–	–	–
24	36	M	35	0.5	Gradual	–	+	+	–	+	+	+	+	+	–	–	–

Initial symptoms were expressed in bold.

AE = antecedent event; OH = orthostatic hypotension; OI = orthostatic intolerance; HI = heat intolerance; AH = anhidrosis; GI = gastrointestinal tract symptoms; α3 Ab = ganglionic acetylcholine receptor α3 antibody; β4 Ab = ganglionic acetylcholine receptor β4 antibody; A.I. = Antibody Index; AE = antecedent event; OI = orthostatic intolerance; OH = orthostatic hypotension; HI = heat intolerance; AH = anhidrosis; GI = gastrointestinal tract symptoms; Dept = department

a. Subacute = peak of autonomic failure within 3 months; gradual = gradual onset of chronic autoimmune autonomic ganglionopathy with the peak of autonomic failure after 3 months.

b. Patient 2 = fever up; 7 = epididymitis; 21 = influenza virus type A infection; 23 = fever up and cough.

c. Patient 4 and 7 = Adie's tonic pupil. The other cases had the abnormality of papillary reflex to light bilaterally or unilaterally.

d. Patient 1 = constipation; 2 = constipation; 3 = early satiety and vomiting; 4 = constipation; 5 = constipation; 6 = constipation, ileus, and sigmoid volvulus suspected; 7 = early satiety, vomiting, alternate stool abnormality, abdominal pain, and taste impairment; 8 = constipation; 9 = constipation; 10 = constipation; 11 = anorexia and diarrhea; 12 = diarrhea and achalasia; 13 = constipation, early satiety, vomiting, and ileus; 14 = abdominal pain, anorexia, and diarrhea; 15 = diarrhea; 17 = constipation, anorexia, and achalasia; 18 = alternate stool abnormality, abdominal pain, and anorexia; 20 = constipation; 21 = constipation; 22 = early satiety, ileus, alternate stool abnormality, and abdominal pain; 23 = constipation, vomiting, and achalasia; 24 = constipation.

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extremities or trunk. Psychiatric symptoms were observed in three patients (Patient 8, 9, and 14). Patient 8 demonstrated infantilization after the onset of autonomic symptoms and had frequent syncope with emotional strain. Patient 9 showed memory disturbances and apathy in daily living, such as domestic duties. Patient 14 also demonstrated character changes, such as the tendency to act in a childish manner. Three patients with the following endocrine disorders: amenorrhea (Patient 4), syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (Patient 14), and panhypopituitarism (Patient 15). Nine patients presented the other following autoimmune diseases: rheumatoid arthritis (n = 2), primary biliary cirrhosis (n = 2), secondary Sjögren's syndrome (n = 2), Still disease (n = 1), Hashimoto disease (n = 1), polymyalgia rheumatica (n = 1), systemic lupus erythematosus (n = 1), Graves' disease (n = 1), and fibromyalgia (n = 1).

Autonomic function studies of the anti-gAChR antibody-positive patients with AAG

Table 3 shows the results of the autonomic function studies of the anti-gAChR antibody-positive patients. Some information was missing because some of the hospitals did not have adequate equipment for the autonomic function tests. Of these 24 patients, abnormalities the orthostatic changes in systolic blood pressure and the $CV_{R\text{-}R}$ were observed at high rates (86.4% and 75.0%, respectively). Cardiac MIBG uptake (H/M ratio), which was measured by ^{123}I-MIBG myocardial scintigraphy in 11 anti-gAChR antibody-positive patients, was decreased in nine patients (81.8%). Urodynamic studies were performed in five of 24 patients. Residual urine of more than 50 mL was noted in four patients (80.0%). Sudomotor and cutaneous vasomotor functions were assessed by thermoregulatory sweat testing in three patients and a reduced ability to sweat was reported in two patients. Plasma norepinephrine values were measured in seven of 24 patients. Six had reduced supine norepinephrine below 100 $pg{\cdot}mL^{-1}$ (85.7%). Pupillary responses to 1% phenylephrine, 5% tyramine, and 5% cocaine were examined in two patients (Patients 2 and 14) and 0.1 to 0.125% pilocarpine in two patients (Patients 4 and 7) in a totally dark room. In the former test, remarkable mydriasis was observed as a pupillary response to the local instillation of 1% phenylephrine. In the latter test, the affected Adie's pupil reacted with miosis to low dose pilocarpine. We confirmed albuminocytologic dissociation in seven of 12 patients (58.3%) in a cerebrospinal fluid analysis.

Illustrative cases

Patient 1: A 75-year-old man who had been affected by AAG for at least 15 years and who had severe orthostatic hypotension (supine, 102/64 mmHg; sitting, 60/34 mmHg). He had recurrent syncope (8–10 times a day), dry mouth, heat intolerance, severe constipation, and great difficulty in passing urine (he required intermittent self-catheterization). An examination confirmed anisocoria and numbness in the all of his extremities. The serum levels of the anti-gAChR autoantibodies were 1.626 A.I. (α3) and 1.000 A.I. (β4). ^{123}I-MIBG myocardial scintigraphy revealed a decreased H/M ratio (early, 1.554; delay, 1.320, Fig. 5A). The normal values for the H/M range were from 1.9 to 2.8 with a mean of approximately 2.2. Based on the basis of the diagnosis of AAG, he was treated with two doses of intravenous methylprednisolone (IVMP), which was followed by intravenous immunoglobulin (IVIg), in order to reach a sustained improvement of the autonomic symptoms. He maintained the improvements in the orthostatic hypotension and experienced complete recovery of gastrointestinal function with IVMP and IVIg, which was followed by oral prednisolone. The levels of the anti-gAChR autoantibodies in Patient 1 converted to normal values after treatment (α3, 0.727 A.I.; β4, 0.473 A.

Table 3. Autonomic function tests at baseline of anti-gAChR Ab positive AAG patients.

Patient	Age	Sex	Anti-gAChRα3 Ab (A.I., LIPS)	Anti-gAChRβ4 Ab (A.I., LIPS)	Anti-gAChR Ab (nmol/L, RIP)	OH in HUTT	Decreased CV R-R	Decreased H/M ratio	Residual urine in urodynamic study	Abnormality of TST	Reduction of resting plasma NE	Abnormality of pupillary response	Albuminocytologic dissociation in CSF
1	75	M	1.626	1.000	0.060	+	+	+					–
2 [42)]	60	M	2.163	0.389	1.220	+	+		+		+	+	
3	39	F	1.000	0.638			–				–		
4	26	F	3.520	0.376		+	+			+	+	+	
5	68	M	2.046	1.219		+	–	+	+				+
6	37	F	1.352	0.636									
7	45	M	7.005	1.443	42.160	+	+		+		+	+	–
8	60	M	1.008	0.494		+	+			+	+		
9	79	F	1.027	0.935		+	+	+					–
10	78	M	1.419	0.737		+	+	+					–
11	67	M	3.265	1.107		+	+	+					+
12	49	M	0.937	1.428		+							
13	73	F	1.103	0.877		+	+	+					+
14	16	M	1.488	0.713	negative	+	+	+	+		–	+	+
15	56	M	3.781	1.708		+	+						+
16	54	F	2.084	0.525									
17	84	F	2.573	1.752		+	+	+					
18	37	F	1.054	0.523		+	–	+			+		+
19	38	F	2.101	0.488		+	+				+		–
20	68	F	1.542	0.952		+	+						
21	46	F	1.113	0.478		–	–	–		–			
22	49	F	3.154	0.336	0.100	–		–	–				
23	6	F	1.275	0.456		+	–						+
24	36	M	1.936	0.323		+	+						

Anti-gAChRα3 Ab = ganglionic acetylcholine receptor α3 antibody; Anti-gAChRβ4 Ab = ganglionic acetylcholine receptor β4 antibody; A.I. = Antibody Index; OH = orthostatic hypotension; HUTT = head-up tilt test; CV R-R = CV = coefficient of variation R-R interval; H/M ratio = heart-to-mediastinum ratio in ^{123}I-MIBG myocardial scintigraphy; TST = thermoregulatory sweat test; NE = norepinephrine; SSR = sympathetic skin response; QSART = quantitative sudomotor axon reflex test; CSF = cerebrospinal fluid

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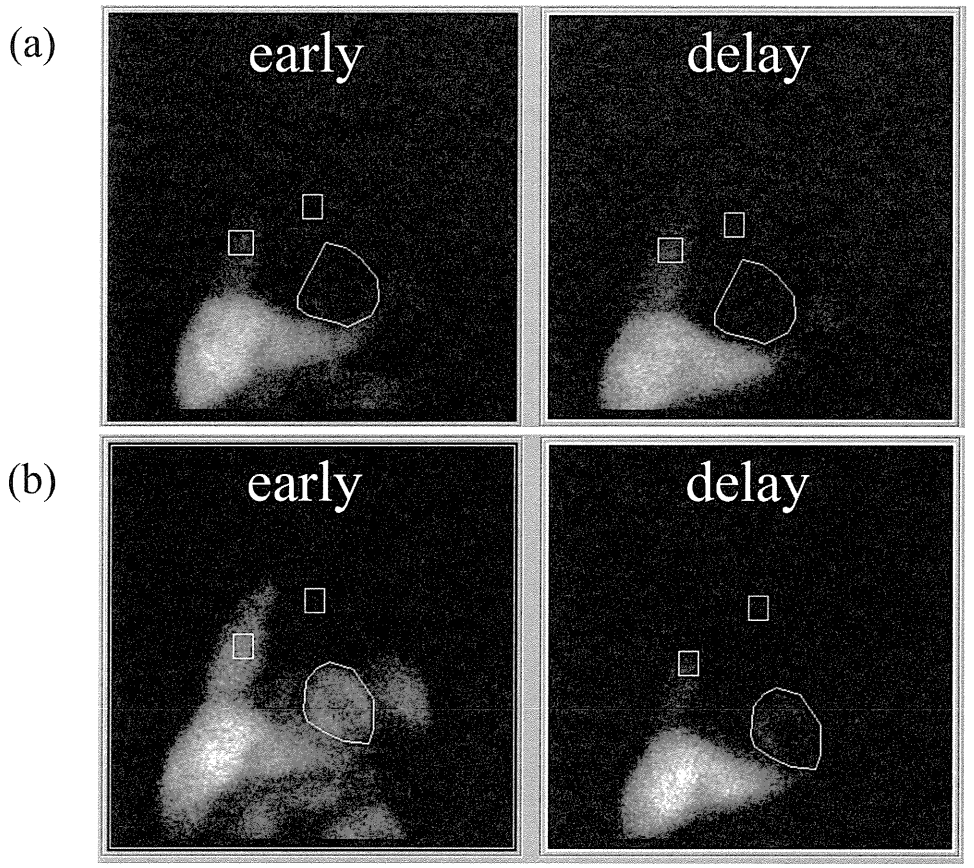

Fig 5. [^{123}I] meta-iodobenzylguanidine (MIBG) cardiac imaging. Early and Delayed. a)^{123}I-MIBG myocardial scintigraphy revealed that the heart/mediastinum (H/M) ratio was decreased at the baseline (early, 1.55; delay, 1.32). A reduced HM ratio indicates peripheral noradrenergic depletion. b) Combined immunomodulatory therapies resulted in a remarkable improvement in the H/M ratio (early, 2.38; delay, 2.23).

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I.), and treatment resulted in a remarkably improvement of H/M ratio (early, 2.380; delay, 2.231, Fig. 5B).

Patient 4: A 25-year-old woman who had been affected by AAG for at least 5 years and who had severe constipation. She developed orthostatic intolerance, dry mouth and eye (left), photophobia, and reduced sweating on the right side of the face. An examination revealed Adie's tonic pupil (left), loss of deep tendon reflexes in the four extremities, preserved sensations, and normal muscle strength. We confirmed the orthostatic hypotension (supine, 108/58 mmHg; standing, 75/32 mmHg). Autonomic function tests confirmed generalized autonomic failure. She had developed several autonomic symptoms since the first episode of amenorrhea at the age of 20. The level of the gAChRα3 antibody was 3.520 A.I. She was treated with plasma exchange (PE) and showed improvements in orthostatic intolerance, deep tendon reflexes, and Adie's tonic pupil in response to PE that was followed by oral prednisolone. Surprisingly, menstruation started again after a series of immunotherapy. The level s of the anti-gAChRα3 antibody remained positive in this patient, but with a substantially reduced (1.151 A.I.).

Comparison of the RIP and the LIPS assay for the detection of the autoantibodies to the gAChR

Eight samples from patients who were previously measured for gAChR antibodies by the RIP were also evaluated by the LIPS assay. Seventy-five percent (6/8) of the samples demonstrated perfect agreement on both assays. Anti-gAChRα3 antibodies were detected in Patient 14 by the LIPS assay, but this sample was seronegative in the RIP (Table 3). Another sample demonstrated an opposite pattern. We further evaluated the correlation between the level of the anti-gAChRα3 antibody that was measured by LIPS and the level of the anti-gAChR antibody that was measured by the RIP in four seropositive samples. Some of the samples tracked each other well between the two assays.

Discussion

Since the gAChR was identified as an autoantigen that is associated with the pathogenesis of AAG, it has been a challenge to search for gAChR-specific autoantibodies. RIA and cell-based assays (CBA) have been available for the detection of autoantibodies to the gAChR in the sera of patients with AAG [1,5]. In this study, we established the LIPS assay for the detection of gAChR antibodies and examined AAG cases with subunit-specific antibodies for gAChR in Japan for the first time.

For the the detection of subunit-specific antibodies to the ion-channels, Ching et al. have reported that the use of the LIPS method to detects the subunit-specific autoantibody of the AChR in patients with myasthenia gravis [21]. Myasthenia gravis (MG) is an autoimmune channelopathy that is caused by autoantibodies to the apparatus of the neuromuscular junctions (NMJs). In about 80% of the patient sera, autoantibodies to the muscle-type of the nicotinic AChR are detected [22]. It has been known that the AChR is a pentamer that is composed of four subunits (α1, β1, γ or δ, and ε). Ching et al have demonstrated that in LIPS using the α1 AChR subunit that is fused to *Renilla* luciferase in LIPS was partly useful for the detection of subunit-specific antibodies of the AChR. In the present study, gAChR autoantibodies were detected by LIPS in about 48% of the patients with AAG, which indicated that our data on the frequency of the gAChR autoantibodies matched the results of the previous study that used the RIP. Furthermore, the levels of the gAChR antibody that were estimated by LIPS (the current group) and the RIP (reported by studies from the Steven Vernino Laboratory and Mayo Medical Laboratories [Patient 1, 2, 7, 14, and 22, as shown in Table 3]) showed a similar tendency. Together, these results demonstrated that the LIPS exhibits a similar performance to the RIP, at least in the detection of autoantibodies to gAChR. Needless to say there is still a slight possibility that a pentamer-specific antibody exists in the AAG patient serum, because these results matched incompletely. In addition, we have identified a subunit-specific antibody for the gAChR, the anti-AChRβ4 antibody for the first time. Another subunit specific antibody for gAChR, anti-AChRα3 antibody, causes several autonomic dysfunctions in an experimental AAG model [6,7,23]. However, it is unclear whether the anti-AChRβ4 antibody is involved in the pathogenesis of AAG. Further studies are necessary to prove this, and we are planning to test the autoantibodies for other subunits of the nicotinic AChR (e.g., α5, β2).

AAG has two patterns of onset. In the present study, a gradual mode of onset was more common in the seropositive group than in the group that was seronegative for anti-gAChR antibodies, although Sandroni et al. have reported that subacute onset was more common in the seropositive group [24]. There was no difference between the demographic features of the seropositive patients in gradual onset and subacute AAG in seropositive patients (S2 Table. Demographic features of patients with subacute AAG/APD and gradual AAG/APD) and no relationship between antibody status and the temporal profile. Subacute AAG was often

preceded by an illness that was presumed to be a viral or bacterial infection. Patients with chronic AAG with mild or restricted autonomic failure usually present with low antibody levels whereas high levels of antibodies are associated with severe acute/subacute AAG subtypes. However, the highest A.I., which was found in Patient 7, was associated with chronic AAG and severe autonomic dysfunction. These findings suggested that it is necessary to compile information about patients with AAG more precisely. The main findings of the clinical and autonomic characteristics were that patients with AAG were associated with a high rate of orthostatic hypotension for orthostatic intolerance and upper and lower gastrointestinal tract symptoms in both the seropositive and seronegative group. There was no significant difference in the occurrence of autonomic symptoms between the seropositive and seronegative group. It remains possible that unknown autoantibodies and other causative agents exist in the seronegative group. Clinically, it is difficult to distinguish the chronic form of seronegative AAG from other degenerative disorders of the autonomic nervous system (e.g., pure autonomic failure). Of note, three patients had achalasia in the seropositive group. The pathogenesis of achalasia remains unclear, but anti-gAChR antibodies might contribute to it. All of the anti-gAChR antibody-positive patients, except for Patients 6, 16, and 19, presented pandysautonomia. Patient 6 had been diagnosed with a chronic intestinal pseudo obstruction (CIPO) before being testing with the autoantibodies. Our data were consistent with prior reports that showed that patients with limited autonomic symptoms demonstrated a relatively lower A.I. Sandroni et al. have previously reviewed other autonomic neuropathies that are associated with the gAChR antibody. Occasional positivity to gAChR antibodies has been found in postural orthostatic tachycardia, CIPO, chronic idiopathic anhidrosis, and distal small fiber neuropathy [25]. All of these were listed as possible clinical phenotypes of anti-gAChR autoimmunity, although they show low antibody levels. The results of our study indicated a similar tendency and suggested a possible correlation between antibody levels and the phenotype of the dysautonomia. In the present study, however, we were not able to perform a correlational analysis between the level of A.I. levels and the clinical severity of the autonomic symptoms because we did not perform a quantitative assessment for autonomic dysfunction. Moreover, several research groups have reported that patients with other neuroimmunological disorders (myasthenia gravis, Lambert-Eaton myasthenic syndrome, Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy) have autoantibodies to gAChR and autonomic symptoms [26–28]. We need to verify the pathogenicity of these autoantibodies and the correlation between antibody levels and disease severity.

The Patients with AAG in Japan were younger and more male predominant than those in Western countries [8]. In the present study, Japanese patients with AAG also had a lower age at onset. The seronegative and seropositive groups did not differ significantly in age and gender, although the seropositive group showed a female predominance. Of the 29 Japanese patients previously reported, 10 (34.5%) had coughing episodes and 12 (41.4%) had psychiatric symptoms. The present study found several anti-AChR antibody-positive patients with coughs (Patients 12, 14, 15, and 17) and psychiatric symptoms (Patients 8, 9, and 14), but these patients had a low frequency when compared with the findings of previous reports. Baker et al. reported encephalopathy co-occurring with classic subacute AAG [29]. They revealed the presence of antibodies that were directed against both the central α4 and α7 nAChRs in the serum of the patient. That study is the first case report of patients with AAG presenting with antibodies directed against both peripheral and central nAChRs. Watson et al. found antibodies that specifically blocked the function of α7 nAChRs in patients with Rasmussen encephalitis [30]. Therefore, it may be necessary to establish a LIPS assay for the detection of autoantibodies to these nAChRs. Six AAG anti-gAChR antibody-positive patients (Patients 1, 5, 11, 12, 14, and 15) complained of subjective numbness as an extra autonomic symptom, and the

numbness might have been caused by extensive disturbance of the sympathetic and parasympathetic nervous system. However, this is an important symptom that may distinguish AAG from other disorders such as acute autonomic sensory neuropathy, Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy. A nerve conduction study could be performed to assess this, and a nerve biopsy may be necessary in the patient with AAG.

The seropositive group had a significant overrepresentation of autoimmune diseases. Autonomic dysfunctions have been reported in association with Sjögren's syndrome [31,32], systemic sclerosis [33], systemic lupus erythematosus [34], rheumatoid arthritis [35,36], and mixed connective-tissue disease [37]. Although AAG and the other autoimmune diseases can coexist due to the same background of autoimmunity, few reports have referred to anti-gAChR antibodies in these autoimmune diseases. In a previous report, the coexistence of AAG and Sjögren's syndrome coexistence was found in two patients [38,39]. The frequency of positive anti-gAChR antibody status in large populations or among patients with autonomic neuropathy in Sjögren's syndrome has not been determined. We believe it is important to clarify the clinical and immunological characteristics of the coexistence of AAG and the other autoimmune diseases. Additionally, attention should be paid to the endocrine disorders that are complications of AAG, because there were five patients (Patients 4, 9, 14, 15, and 19) with endocrine disorders in the study. Three of the five patients who were seropositive for the anti-gAChR antibodies presented with amenorrhea, SIADH, and panhypopituitarism. Several Japanese neurologists have already reported cases of acute pandysautonomia or acute autonomic and sensory neuropathy (AASN) with amenorrhea and/or SIADH [40–42]. They have suggested that patients with autonomic neuropathy might have both peripheral and central nervous system manifestations. The roles of nAChRs are associated with cholinergic neurotransmission, the modulation of dopamine function, the influence of inflammation, and the activity of the hypothalamic-pituitary-adrenal axis [43,44]. We presumed that nAChRs are involved in a variety of neurological systems that are implicated in the pathophysiology of central nervous system involvement including endocrine disorders and psychiatric symptoms.

Ganglionic AChR antibodies have the potential to impair autonomic ganglionic synaptic transmission [9,45,46]. Because both sympathetic and parasympathetic ganglia utilize nicotinic cholinergic synapses, antibodies that interfere with ganglionic transmission could cause pandysautonomia. Our clinical and serological observations suggest that pandysautonomia may be mediated by autoantibodies that interfere with autonomic ganglionic transmission. The patients who were identified in the present study had a failure of both the sympathetic (orthostatic hypotension and anhidrosis) and parasympathetic functions (gut dysmotility, sicca complex, and pupil abnormalities). The autonomic function tests that were performed in this study were incomplete and inadequate, but most of the results (H/M ratio in ^{123}I-MIBG myocardial scintigraphy and pupillary response to local instillation) demonstrated postganglionic parasympathetic involvement. Kimpinski et al. characterized the unique sudomotor dysfunction in AAG as widespread, predominantly postganglionic, and a result of lesions at both the ganglia and distal axon [47]. Manganelli et al. have also demonstrated in a sudomotor function study and skin biopsy findings, that there is postganglionic autonomic damage in patients with AAG [48]. They attributed this damage to prolonged and severe impaired synaptic transmission. These reports coincide with our deduction that the anatomic pattern of autonomic dysfunction was predominantly postganglionic.

Another important observation was that the impairment of the autonomic function may be partially reversible in AAG. Patients 1 and 4 (the illustrative cases) improved in response to immunotherapy (plasmapheresis, IVMP, IVIg, and immunosuppressant drugs) with symptomatic therapy. We interpreted this improvement to be related to the correlation with a decrease in the levels of anti-gAChR antibodies in each case. Some patients with seropositive AAG

responded to treatment with IVMP, plasmapheresis, or IVIg, and most of these required combined or subsequent treatments to maintain the improvement [49–53]. The more severely affected patients who did not respond to IVMP or PP monotherapy benefited from combined therapy with other first line therapy and immunosuppressant agents, such as prednisolone, azathioprine, and rituximab [54]. They also required prolonged immunotherapy for sustained clinical improvement. These results suggest that antibody production may be ongoing, and not self-limited. Combined treatment with any of the immunosuppressant agents reduced antibody production to levels that were adequate for clinical benefit in our patients. In Patient 4, menstruation restarted after a series of immunotherapy, suggesting an autoimmune mechanism in this process. Thus, immunotherapy can also be efficacious for treatment of the endocrinological abnormalities of patients with AAG.

Some limitations existed in this study. The important limitations of this study were the retrospective design and the small number of subjects. A prospective, multi-center, and clinical observation is necessary to confirm the relationships between the antibody levels, symptoms, and results of the autonomic functions tests. Additional experiments and investigations are necessary to clarify the role of AChR β4 antibodies in the pathogenesis of AAG. In addition, we need to compare the sensitivity and specificity of LIPS with the RIP for the measurement of autoantibodies to gAChR in spiraling numbers.

Our results demonstrated the usefulness of the LIPS assay as a new tool for detecting autoantibodies against gAChR in the patients with AAG. Furthermore, follow-up studies with a greater number of patients with AAG are required to investigate the relationship between the levels of gAChR autoantibodies and clinical features.

Supporting Information

S1 Table. Detailed clinical characteristics of OND patients. The disease control (DC) consisted of 34 subjects with other neurological diseases (OND: mean age, 56.3 ± 20.4 years old, 19 males and 15 females).
(DOCX)

S2 Table. Demographic features of patients with subacute AAG/APD and gradual AAG-/APD. There was no difference between the demographic features of the seropositive patients in gradual onset and subacute AAG in seropositive patients and no relationship between antibody status and the temporal profile.
(DOCX)

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Author Contributions

Conceived and designed the experiments: SN OH. Performed the experiments: OH AM YM. Analyzed the data: SN OH HM. Contributed reagents/materials/analysis tools: SN M. Koga TK KM TS HK M. Kunimoto MH KK AM WS. Wrote the paper: SN OH HM.

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免疫性神経疾患

—基礎・臨床研究の最新知見—

V. 免疫性筋疾患

多発筋炎・皮膚筋炎・封入体筋炎・壊死性筋症

封入体筋炎

村田顕也

V 免疫性筋疾患

多発筋炎・皮膚筋炎・封入体筋炎・壊死性筋症

封入体筋炎

Sporadic inclusion body myositis

村田顕也

Key words：封入体筋炎，炎症性筋疾患，rimmed-vacuole，筋変性

はじめに

封入体筋炎(sporadic inclusion body myositis: sIBM)は，中高年以降に好発する後天性の筋疾患で[1]，多発筋炎(PM)，皮膚筋炎(DM)，免疫介在性壊死性筋炎とともに特発性炎症性筋疾患に分類されている．これまで，本邦ではまれな疾患と考えられていたが，近年の有病率は100万人あたり9.83であり，欧米水準にまで達した[2]．現在国内の患者数は1,000-1,500人前後と推定され，平成27年1月1日からの指定難病に含まれた．sIBMの病理像は，通常の炎症性変化に加えて，異常タンパク沈着による筋変性変化を伴う．また，PM，DMと異なり，各種免疫療法への反応性が低いことから，本症が1次的な自己免疫性筋疾患であるのか，2次的な炎症・自己免疫機序を有する筋疾患であるのかについては議論がある．

1 臨床症状

sIBM 53例の検討では，大腿四頭筋や手指屈曲の筋力低下が特徴的で，垂れ足や嚥下障害を伴うこともある[3]．筋力低下は，経過とともに手指伸筋群・上肢近位部・傍脊柱筋に及び，dropped-headやcamptocormiaを呈することもある．大腿部や前腕尺側の筋萎縮の有無の検討が重要である．

自覚的には，大腿四頭筋の障害により階段昇降や椅子からの立ち上がりが困難となり転倒回数が増加する．深指屈筋の筋力低下により，鍵が回しにくい，ネクタイが結びにくい，ペットボトルのふたが開けにくい，ボタンがかけにくいといった指先の巧緻障害を訴える．上肢の筋力低下は，左右非対称であり，通常は非利き手側の障害が高度である．sIBMでは，手外筋である深指屈筋と長母指屈筋が障害され，骨間筋・虫様筋・長母指外転筋などの手内筋は保たれる．握力検査では深指屈筋や長母指屈筋の筋力低下を，虫様筋を用いてMP関節で補うため，異常を見いだせないことも多い．

嚥下障害の病変の主座は，輪状咽頭筋の開大障害である．経過中に約40％の症例で出現するが，本人の自覚が乏しく体重減少や誤嚥性肺炎で気づくことが多い．嚥下造影検査で確認されるcricopharyngeal barは，食道入口部の開大障害により狭窄している部分(sIBMでは輪状咽頭筋部の筋は萎縮し結合織が増生している)を造影剤が押し広げながら流れる際に出現する見かけ上の隆起様病変で，器質的変化ではない．このbar(**図1**)は，検査に使用する造影剤の性状(例えば，水が多いもの，ゼリー状のもの)により，その形態が変わり，顔咽頭筋型筋ジストロフィーでも確認され，本症に特異的な所見で

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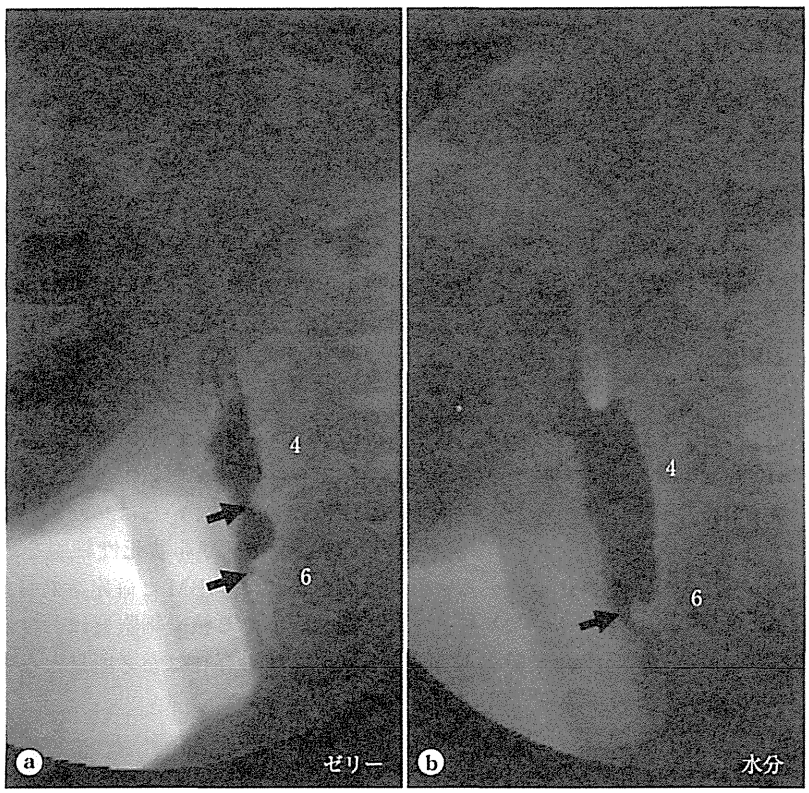

図1 Cricopharyngeal bar

a. ゼリー嚥下時，b. 水分嚥下時．

嚥下造影にてC5-6にて隆起病変を認める．この隆起病変は，ゼリー(a)と水分が多い嚥下剤(b)の使用により形態が異なるため，器質性変化でないと推測される．

はないが慢性の嚥下障害を示唆し[4]，病像把握に有益である．通常感覚障害は出現しないが，30％の症例では，電気生理学的検査や臨床所見にて末梢性感覚神経障害を呈する[5]．15％の症例で，進行性エリテマトーデス・Sjögren症候群・全身性強皮症・サルコイドーシスといった自己免疫疾患を併発する[6]．PM，DMと異なり心筋炎や肺疾患を併発することは少なく，悪性腫瘍の合併も少ない．血清CK値は正常か正常値の10倍以内の値を呈する．sIBMの約20％の症例で，抗核抗体陽性となるが，筋炎特異抗体は通常陰性である．

針筋電図検査では，自発電位と刺入時電位が亢進し，多相性の低振幅運動単位電位と早期漸増を認める．一般に，筋線維直径が大きいほど，神経筋接合部から筋線維の方向に沿って流れる活動電位の伝播速度(神経筋伝導速度)が早くなる．また，再生筋線維では，神経筋伝導速度は低下する．つまり，筋線維に大小不同がみられると，各筋線維の同期性が不良となり，神経筋伝導速度の時間的なばらつきも大きくなり多相性を呈する．このような多相性で長持続の運動単位は，慢性進行性の炎症性筋疾患(PM，DM)でも高頻度に認められるが，sIBMでは，さらに高振幅の多相性電位が目立ち，なかにはfasciculation potentialを呈するため神経原性変化と判断され，sIBMを筋萎縮性側索硬化症(ALS)と誤診することも多々あり注意を要する[7]．

2 病像の自然経過

本邦では厚生労働科学研究費補助金難治性疾

患克服研究事業「封入体筋炎(IBM)の臨床病理学的調査および診断基準の精度向上に関する研究班」(研究代表者 東北大学 青木正志教授 平成22-23年度)が，臨床的・病理学的にsIBMと確定された121例の臨床特徴を検討している．それによると，男女比は1.23：1で男性にやや多く，初発年齢は64.4±8.6歳(40-81歳)，初発症状は74％が大腿四頭筋の脱力であった．嚥下障害は23％にみられ生命予後を左右する要因であった．CK値は511.2±361.8(30-2,401) IU/Lであった．初発症状出現後，診断確定までに52.7±47.6(4-288)カ月を要していた．またHTLV-1やHCV抗体陽性患者が20％，家族歴があるものが5例いた．

3 画像診断

MRIは生検部位の決定や治療介入時の効果判定にも重要である．T1強調画像(T1WI)，T2強調画像(T2WI)で高信号を呈し，脂肪抑制T2画像(FST2)で等信号になる部位が脂肪組織に相当し，T1WI低～等信号，T2WIで高信号を呈する部位が，FST2でも高信号を呈していれば，炎症性(浮腫性)病変と判断する．ただし，脂肪組織の混在が多いとT1，T2高信号部位が，FST2でも抑制されず高信号を呈することもあり，炎症性変化部位との鑑別が困難な場合がある．sIBMでは前腕部は深指屈筋と浅指屈筋の障害が高度であるが同一の筋群でも障害の程度が様々である．各指の深指屈筋の筋力低下の程度と脂肪混在の程度とは一致する(図2)．一方，下肢は大腿四頭筋の病変が高度で，経過ともに内転筋群，大腿屈筋群へと病変部位が進展する(図3)．

4 診断基準

sIBMの研究は，Chouら[8]が，慢性の多発筋炎のうち核内や細胞質内にmyxovirus様の封入体を有した症例の検討から始まったことから，筋細胞内の縁取り空胞(rimmed-vacuole: RV)や管状フィラメントの存在といった病理所見が重要な要素であった[9,10]．しかし，2011年のENMCの診断基準では，臨床症状(①罹病期間が12カ月を超える．②発症年齢が45歳を超える．③膝の伸展力が股関節屈曲力より弱く，かつ手指屈筋力が肩外転力より弱い，④血清CK値が正常の15倍を超えない)さえ満たせば，①間質内への細胞浸潤，②主要組織適合抗原(MHC) Class Iの発現の亢進，③RV，④タンパク(アミロイドやp62，SMI 31など)，もしくは15-18 nmのフィラメントの沈着といったsIBMに特徴的な病理的特徴のうち1つ以上の条件を満たせばclinically defined IBMとして，診断可能となった[11]．本邦でも診断基準が策定され，IBMに特徴的な臨床徴候を有していれば，筋内鞘への単核球浸潤に加え，RVや非壊死性細胞を取り囲む細胞浸潤があれば，IBMと確定診断可能となった．電顕で観察する核や細胞質におけるフィラメント状封入体の存在は参考所見となった(表1)[12]．

5 筋病理

sIBM患者の筋生検所見は，炎症と筋変性の要素が混在する．筋線維は円形で大小不同を呈し，筋細胞壊死・再生像と細胞浸潤を認める．炎症細胞の主体はCD8陽性のT細胞で，これらがendomysiumに浸潤し，MHC Class Iを発現している非壊死筋線維を取り囲んでいる．このような多発筋炎の病理像に加え，sIBMではRVが確認される．RVは，ゴモリトリクローム変法にて赤く染まる顆粒で縁取られた筋細胞内の空胞である．通常，RVを有する筋線維への炎症細胞浸潤は認められない(図4)．RVは，通常の多発筋炎でも認めることがあるが，少数である．筋細胞内にユビキチンやアミロイドβタンパク，リン酸化タウタンパクが凝集している．アミロイドβの凝集体は，RVの中や近傍で確認されることが多く，コンゴレッドでも陽性である．また，赤色ぼろ線維(ragged red fiber: RRF)やcytochrome C oxidase(CCO)染色にて染色性が低下した筋線維がみられ，ミトコンドリア異常が想定される．診断のためには，当初は，電子顕微鏡を用いて核や細胞質内における15-18

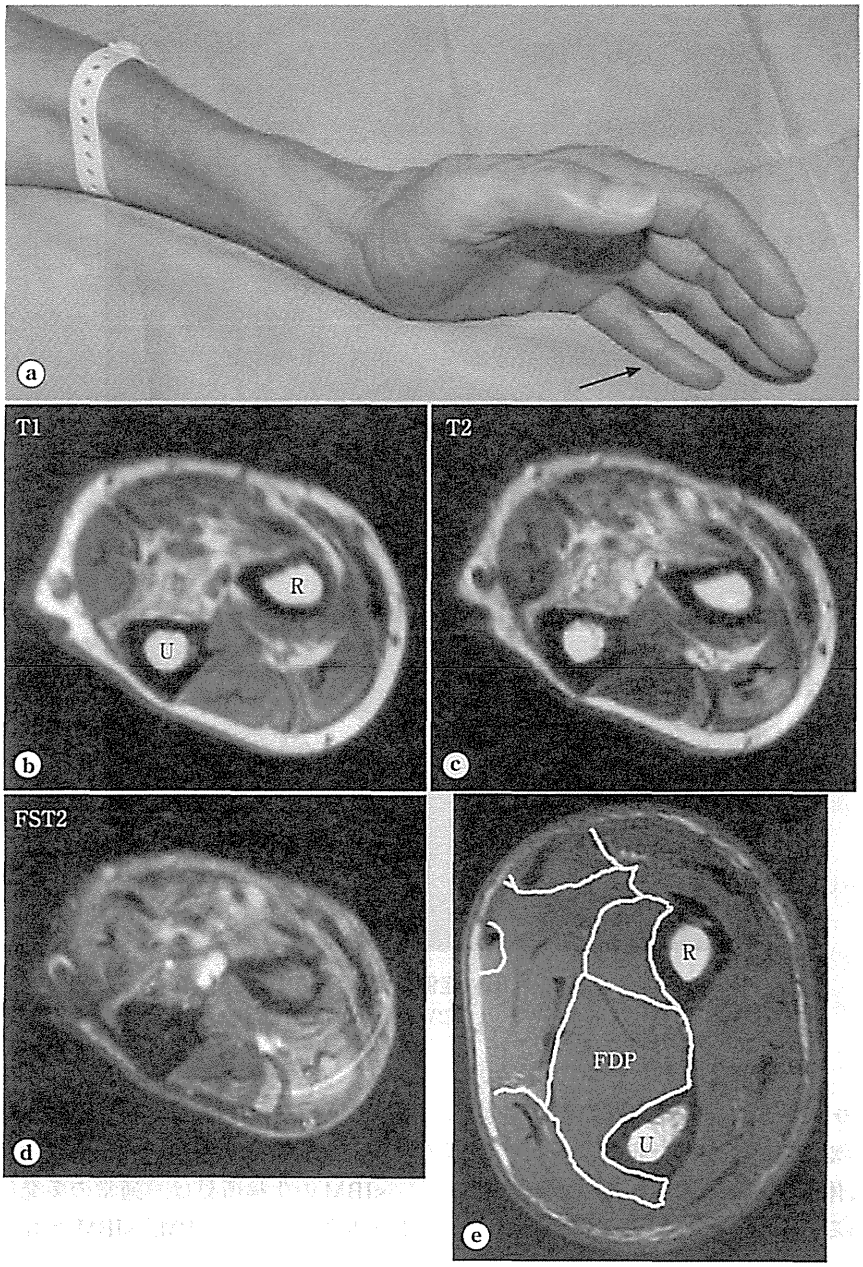

図2　sIBM症例の左深指屈筋の脂肪性変化

a. 左前腕部外形. b–e. 左前腕部MRI(b・e: T1強調像, c: T2強調像, d: 脂肪抑制画像). b–d. sIBM症例. e. 正常対照.

R: 橈骨, U: 尺骨, FDP: 深指屈筋.

sIBMでは, 深指屈筋の障害のため, 2–5指のDIP関節での指の屈曲が困難となる(a, 矢印). FDPは, 橈骨(R)と尺骨(U)の間の全骨間膜に接して存在する(e). sIBMではFDPが萎縮し, 脂肪混在が著明となる.

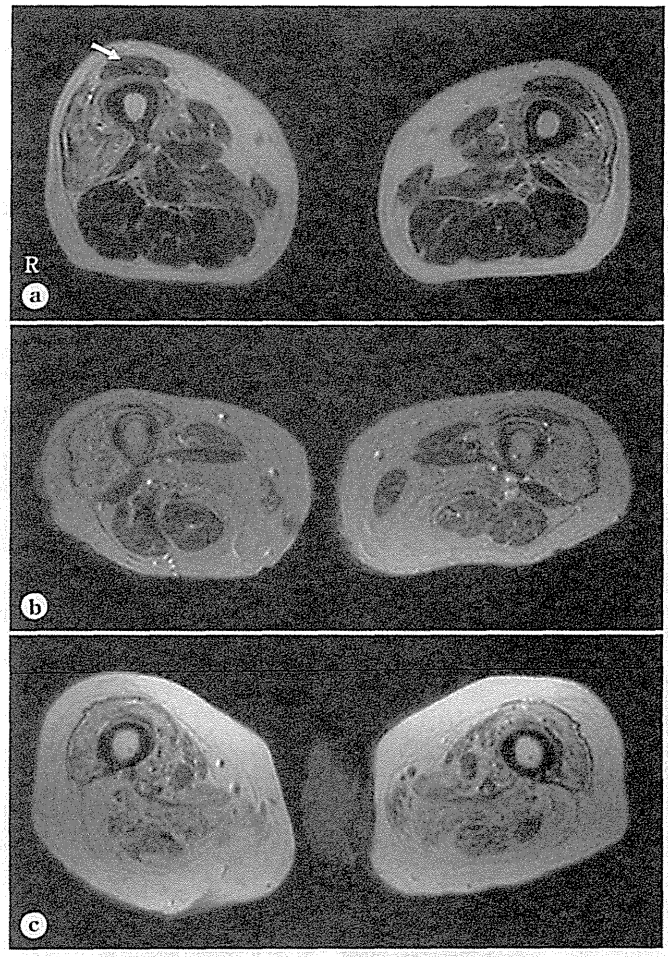

図 3　sIBM 症例の大腿部 MRI
a. T1W2. b. T2W2. c. 脂肪抑制.

nm のフィラメント状封入体の存在を明らかにするのが必要であったが，近年では，ユビキチン陽性封入体やアミロイド β の沈着が重要となってきている.

6　発症機序

sIBM の明確な発症機序は不明で，ウイルス感染，有毒タンパクの集積，自己免疫，筋核変性，小胞体ストレス，オートファジーやプロテアソーム機能低下など様々な説が提唱されている.

7　炎症の関与

sIBM の生検所見は，通常の多発筋炎の病理像と類似している．PM，sIBM とも MHC Class I を発現した非壊死線維を，CD8 陽性のリンパ球が取り囲んでいる．CD8 は，パーフォリンを放出し，筋細胞膜を破壊し，筋細胞内に浸潤する．CD8 陽性細胞は，グランザイムを放出し，筋細胞を壊死させる．パーフォリンを介したこのシステムは，抗原特異性が高いとされている[13]．MHC は，T 細胞に抗原提示し，T 細胞の活性化を誘発するが，その際に costimulatory molecule が関与する．筋細胞膜には B7 ファミ

表1 暫定版：封入体筋炎診断基準(厚生労働科学研究費補助金 難治性疾患克服研究事業封入体筋炎(IBM)の臨床病理学的調査および診断基準の精度向上に関する研究班(H22-難治-一般-117))

◎診断に有用な特徴

A. 臨床的特徴

a. 他の部位に比して大腿四頭筋または手指屈筋(特に深指屈筋)が侵される進行性の筋力低下および筋萎縮

b. 筋力低下は数カ月以上の経過で緩徐に進行する

＊多くは発症後5年前後で日常生活に支障をきたす．数週間で歩行不能などの急性の経過はとらない

c. 発症年齢は40歳以上

d. 安静時の血清CK値は2,000 IU/Lを越えない

(以下は参考所見)

・嚥下障害がみられる

・針筋電図では早期動員，PSW/Fibrillation/CRDの存在

B. 筋生検所見

筋内鞘への単核球浸潤を伴っており，かつ以下の所見を認める

a. 縁取り空胞を伴う筋線維

b. 非壊死線維への単核球の侵入や単核球による包囲

(以下は参考所見)

・筋線維の壊死・再生

・免疫染色が可能なら非壊死線維への単核細胞浸潤は主にCD8陽性T細胞

・形態学的に正常な筋線維におけるMHC class I発現

・筋線維内のユビキチン陽性封入体とアミロイド沈着

・COX染色陰性の筋線維：年齢に比して高頻度

・(電子顕微鏡にて)核や細胞質における16-20 nmのフィラメント状封入体の存在

◎合併しうる病態

HIV，HTLV-I，C型肝炎ウイルス感染症

◎除外すべき疾患

・縁取り空胞を伴う筋疾患*(眼咽頭型筋ジストロフィー・縁取り空胞を伴う遠位型ミオパチー・多発筋炎を含む)

・他の炎症性筋疾患(多発筋炎・皮膚筋炎)

・筋萎縮性側索硬化症などの運動ニューロン病

*Myofibrillar myopathy(FHL1，Desmin，Filamin-C，Myotilin，BAG3，ZASP，Plectin変異例)やBecker型筋ジストロフィーも縁取り空胞が出現しうるので鑑別として念頭に入れる．特に家族性の場合は検討を要する

◎診断カテゴリー：診断には筋生検の施行が必須である

Definite AのA a-dおよびBのa，bの全てを満たすもの

Probable AのA a-dおよびBのa，bのうち，いずれか5項目を満たすもの

Possible AのA a-dのみ満たすもの(筋生検でBのa，bのいずれもみられないもの)

◎文献

・Griggs RC，Askanas V，DiMauro S，et al: Ann Neurol 1995; 38: 705-713.

・Needham M，Mastaglia FL: Lancet Neurol 2007; 6: 620-631.

リーのCD80，CD86が発現し，Tリンパ球には，そのリガンドとして，CD28，CTLA-4が発現している．CD28とB7ファミリーが結合すると正のシグナルとなり，リンパ球増生が進み，CTLA-4と結合すると負のシグナルとなり，リンパ球の増生が抑制される[14]．MHC ClassⅠやB7ファミリーは，インターフェロンγやTNF(tumor necrosis factor)αなどのサイトカインやケモカインで誘導されるが[15]，これらのサイトカインやケモカインはIBM患者の生検筋で発現が増強している[16]．

sIBMでは，endomysiumに浸潤するTリンパ球のT細胞レセプターのタイプはある程度限られている．また，HIV(human immunodefi-