Full-length research article

Prevalence of anti-NT5C1A antibodies in Japanese patients with autoimmune rheumatic diseases in comparison with other patient cohorts

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Abstract

Background: Sporadic inclusion body myositis (sIBM) is usually classified as an idiopathic inflammatory myopathies. Although the diagnosis of sIBM is sometimes challenging, recent studies have shown that the autoantibodies against cytosolic 5'-nucleotidase 1A (NT5C1A) are the possible diagnostic biomarker for sIBM. Few reports have shown the frequencies of anti-NT5C1A antibodies in systemic autoimmune rheumatic diseases (SARDs) using large cohorts of SARDs.

Methods: Serum samples obtained from 314 patients including dermatomyositis (DM) (n=144), systemic lupus erythematosus (SLE) (n=50), systemic sclerosis (SSc) (n=50), Sjögren's syndrome (SS) (n=50), polymyositis (PM) (n=10) and mixed connective tissue disease (n=10), and healthy controls (n=42) in addition to 10 patients with typical sIBM were analysed for the presence of autoantibodies using full-length recombinant NT5C1A ELISA.

Results: Japanese patients with DM (11%), PM (10%), SLE (6%), SSc (8%) or SS (4%) had anti-NT5C1A antibodies at lower frequencies than patients with sIBM. Interestingly, 4 of 17 DM/PM patients with anti-NT5C1A antibodies were found to have no other myositis-specific/associated autoantibodies.

Conclusions: There is a wide heterogeneity of anti-NT5C1A antibody immunoreactivity. Some populations of SARDs are positive for anti-NT5C1A are also positive for anti-NT5C1A. However, the anti-NT5C1A frequencies in the patients with SARDs are low also in Japanese.

Key words: anti-NT5C1A antibody, dermatomyositis, ethnicity, inclusion body myositis, systemic autoimmune rheumatic disease

1. Introduction

The idiopathic inflammatory myopathies (IIMs) are a group of systemic autoimmune rheumatic diseases (SARDs) that include polymyositis (PM) and dermatomyositis (DM) [1]. Several myositis-specific autoantibodies (MSAs), which have been regarded as mutually exclusive, are associated with PM/DM. Although sporadic inclusion body myositis (sIBM) is usually classified as an IIM, sIBM is distinguished from other IIMs by its histological changes and resistance to immunosuppressive therapies [2].

In 2011, autoantibodies against a 43kDa protein that were specific for sIBM were reported, and in 2013, the target of these antibodies was identified as cytosolic 5'-nucleotidase 1A (NT5C1A) (2). Recent studies have reported that anti-NT5C1A antibodies were detected in sera of 33-76% patients with sIBM and that their overall specificity ranged between 87% and 100% (reviewed in [3]). However, very few reports have shown the frequencies of the antibodies in SARDs using large cohorts of more than 100 patients with SARDs [3-5]. There have been also no reports on the frequencies of the antibodies in SARDs from Asian countries. We aimed to establish a quantitative assay for anti-NT5C1A antibodies and to clarify the positivity of those antibodies in Japanese patients with various SARDs as well as in healthy controls (HCs) in this study.

2. Methods

2.1. Patients and Sera

We screened 144 patients with DM (62 with classic, 48 with clinically amyopathic, 22 with cancer-associated, 12 with juvenile), 50 with systemic lupus erythematosus (SLE), 50 with systemic sclerosis (SSc), 50 with Sjögren's syndrome (SS), 10 with PM, 10 with mixed connective tissue disease and 10 with sIBM, in addition to 42 individuals as HCs. For each SARD, we used the same established diagnostic criteria that we used in our previous study [6]. Ten patients with typical sIBM fulfilled the two criteria for clinically defined sIBM [7, 8]. All serum samples were obtained at Department of Dermatology of Nagoya University Hospital except for samples from sIBM patients which were obtained at Department of Neurology of the same hospital. This study was approved by the ethics committee of Nagoya University Graduate School of Medicine. We obtained written informed consent from all the Japanese patients and HCs.

2.2. ELISA and Immunoprecipitation using recombinant protein

The full-length cDNA clone of NT5C1A (Open Biosystems, Waltham, USA) and the SP6 Quick Coupled Transcription/Translation System (Promega, Madison, USA) were used to produce biotinylated recombinant proteins. Antigen-capture ELISA was performed according to our published protocols [6]. Autoantibodies against MDA5, Mi-2, NXP-2, TIF1γ, Ku70/80, SAE1/2, Jo-1, PL-7, PL-12, EJ, KS, and PM-Scl75/100 were measured for patients with DM/PM by in-house ELISA. Anti-MLH1 and -PMS1 antibodies, which are

myositis-associated autoantibodies, were also measured by the ELISA published in our previous study [9]. Autoantibodies against Ro52 were measured by a commercial ELISA (Abnova, Taipei, Taiwan).

Immunoprecipitation was performed using biotinylated recombinant proteins as previously described [10] except for washing buffer. In this study, in order to increase stringency, the washing buffer contained 0.5M NaCl, 0.5% NP-40 and 0.5% Triton X-100.

2.3. Statistical analysis

Fisher's exact probability test and Bonferroni's correction were used for comparison of frequencies. Values were evaluated by Mann–Whitney U test. $P < 0.05$ was considered significant.

3. Results

3.1. Detection of anti-NT5C1A antibodies by ELISA and immunoprecipitation

In the cohort of HC and sIBM, 1 HC sample showed much higher reactivity than the other 41 HC samples while 8 of the 10 sIBM samples were highly reactive in ELISA. Immunoprecipitation with biotinylated NT5C1A protein was used to confirm these reactivities. The 8 ELISA-reactive sIBM samples immunoprecipitated recombinants (Figure 1), as did as the 1 ELISA-reactive HC sample (data not shown), even using the stringent washing buffer. The ELISA units were calculated by a standard curve created with a serially diluted sIBM

serum showing the highest values. The cutoff value was determined as the mean of the units obtained from 41 non-reactive sera from the HCs + 5 standard deviations (SDs) (0.79 unit) (Figure 2).

3.2. Screening of anti-NT5C1A antibodies in patients with various SARDs

Next, we examined 314 serum samples from patients with various SARDs. The anti-NT5C1A frequencies in the disease groups are shown in Tables 1 and 2. In total, 26 (8.3%) patients were positive for anti-NT5C1A in ELISA. The sera from these 26 patients were also positive in immunoprecipitation assays using the stringent washing buffer (data not shown). The anti-NT5C1A frequencies in the patients with SARDs were not statistically different from those in previous studies, except for SS (Table 2). The anti-NT5C1A frequency in the Japanese patients with SS (2/50, 4%) was significantly lower than that in the Caucasian patients with SS (10/44, 23% or 8/22, 36%) [4, 5] although it was not significantly different from that in the American patients (0/20, 0%) by one study [3]. The anti-NT5C1A frequency in the total patients with SS of the 3 previous studies [3-5] (18/86, 21%) was significantly higher than that in the Japanese patients with SS (2/50, 4%) ($P<0.007$). There was no statistical difference in ELISA units for anti-NT5C1A-positive patients between the sIBM group and the non-sIBM group by Mann-Whitney U tests (Figure 2).

Of the 17 anti-NT5C1A-positive DM/PM patients, 3 patients had anti-MDA5 antibodies, and 3 had anti-TIF1γ. Anti-Jo-1, -Mi-2, -Ku, -NXP2, -MLH1 antibodies were

independently found in the other patients. The frequencies of these concomitant antibodies were not significantly different between the anti-NT5C1A-positive and -negative DM/PM patients (data not shown). Four (24%) of these 17 DM/PM patients were found to have no concomitant autoantibodies. Also in the previous study, 4 (29%) of the 14 anti-NT5C1A-positive DM patients had no MSAs [3]. Kramp *et al.* showed that anti-NT5C1A-positive sIBM patients had a higher frequency of anti-Ro52 (32%) [3]; however, none of our patients with sIBM and only 3 of the 27 anti-NT5C1A-positive non-sIBM patients had anti-Ro52. Although only a few cases with anti-NT5C1A-positive DM/PM were performed with muscle biopsy, no pathological changes similar to sIBM were found.

Performance characteristics (sensitivity / specificity / odds ratio [95% confidence interval]) for sIBM vs. other forms of IIM were (80.0% / 89.0% / 32.24 [6.32-164.43]) and for sIBM vs. other controls were (80.0% / 94.4% / 67.11 [12.39-363.41]).

4. Discussion

This is the first study of anti-NT5C1A antibodies using sera from various SARDs patients in addition to sIBM patients from an Asian country. Although our sIBM cohort is small, our ELISA to detect anti-NT5C1A antibody showed very high sensitivity and rather low specificity. These results may be due to the sampling bias of their all being typical cases

or due to differences in experimental methods. Since none of our patients with sIBM was investigated by electron microscopy, we selected serum samples from clinically typical patients with sIBM. However, very recently, Lilleker *et al.* reported no relationship between a diagnostic classification of 'possible' IBM versus 'definite' or 'pathologically/clinically defined' IBM and anti-NT5C1A antibody status [11]. Future studies on the anti-NT5C1A frequency in Japanese patients with sIBM using large cohorts should be interesting.

Our ELISA used biotinylated recombinant protein produced by in vitro transcription and translation using rabbit reticulocyte lysate. Direct comparison for the detection of autoantibodies in our previous study [10] showed the similar specificities and sensitivities in immunoprecipitation assays using between cultured cell extract and our recombinant protein. In this study, the results obtained by our ELISA, in which the setting cut-off value was relatively high (more than mean + 5 SDs), showed a good agreement with the results by immunoprecipitation with the high stringent washing buffer.

Two previous studies showed the significantly higher prevalence of anti-NT5C1A antibodies in SS patients than that in our SS patients (Table 1). Future studies are necessary to confirm whether these differences might be due to sample sizes, ethnic differences or using different diagnostic criteria of SS [12]. The significance of anti-NT5C1A autoantibodies in patients with non-IBM autoimmune diseases is unclear and will require further investigation. An increased incidence of SSc, SLE and SS with sIBM is supported by the literature [13-18].

This might highlight underlying immune mechanisms that are common to these diseases.

In this study, a total of 26 patients with various SARDs had anti-NT5C1A antibodies. The antibody-positive SLE and SS patients were concomitantly positive for anti-ds DNA and anti-SS-A (Ro60), respectively, but also the antibody-positive SSc patients had also anti-Topoisomerase I or anti-centromere antibody (data not shown). “Monospecific”, which means no concomitance of disease-specific/associated autoantibody, anti-NT5C1A-positive patients were found only in patients with DM. Future studies are needed whether anti-NT5C1A antibodies are useful not only for the diagnosis of sIBM but also for the diagnosis of DM.

In summary, the present study clarified the following: there is a wide heterogeneity of anti-NT5C1A antibody immunoreactivity. Some populations of SARDs are positive for anti-NT5C1A antibodies and HCs are also positive for anti-NT5C1A antibodies. However, the anti-NT5C1 frequencies in patients with SARDs are low also in Japanese. Future collaborative studies for evaluating our sera with other assays promise to be interesting.

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Table I. Patient's groups and anti-NT5C1A-antibody frequencies

Clinical group	Age range*	Age, mean* ± S.D.	Sex M : F	Total n	anti-NT5C1A n (%)	95% confidence interval (%, %)
Sporadic inclusion body myositis	55 - 78	67 ± 6	8 : 2	10	8 (80.0)	(49.0, 94.3)
Total dermatomyositis	3 - 85	53 ± 18	34 : 110	144	16 (11.1)	(7.0, 17.3)
Classic DM	3 - 84	54 ± 16	14 : 48	62	9 (14.5)	(7.8, 25.3)
Clinically amyopathic DM	48 - 80	53 ± 13	7 : 41	48	5 (7.1)	(4.5, 22.2)
Cancer-associated DM	16 - 80	49 ± 17	6 : 16	22	0	(0, 14.9)
Juvenile DM	3 - 32	18 ± 9	7 : 5	12	2 (16.7)	(4.7, 44.8)
Polymyositis	17 - 81	59 ± 20	2 : 8	10	1 (10.0)	(1.8, 40.4)
Systemic lupus erythematosus	10 - 67	38 ± 14	6 : 44	50	3 (6.0)	(2.1, 16.2)
Systemic sclerosis	26 - 83	57 ± 14	2 : 48	50	4 (8.0)	(3.2, 18.8)
Sjögren's syndrome	24 - 75	49 ± 16	6 : 44	50	2 (4.0)	(1.1, 13.5)
Mixed connective tissue disease	24 - 60	42 ± 25	0 : 10	10	0	(0, 27.8)
Healthy control	22 - 72	47 ± 35	16 : 26	42	1 (2.4)	(0.4, 12.3)

* Age at the time of sera collection. In juvenile DM, 2 patients were originally seen at other hospitals far from ours. Their intervals between disease onset and serum sampling were 26 and 28 years.

M : F = male : female; DM: dermatomyositis; N.T.: not tested.

Table II. Anti-NT5C1A-antibody frequencies in systemic autoimmune rheumatic diseases in different cohorts.

Ref.	Japan (this study)		California, USA (3)		Maryland, USA (4)		Europe (multicenter) (5)	
Method	ELISA using recombinant full-length protein*		ELISA using recombinant full-length protein**		Western blot with recombinant protein***		ELISA using 3 synthetic peptides of 23 amino acids	
	α-NT.(+) (n) / examined (n)	Frequency (%)	α-NT.(+) (n) / examined (n)	Frequency (%)	α-NT.(+) (n) / examined (n)	Frequency (%)	α-NT.(+) (n) / examined (n)	Frequency (%)
DM	16 / 144	11	0 / 4	0	24 / 159	15	8 / 185	4
PM	1 /10	10	0 / 7	0	2 / 42	5		
SLE	3 / 50	6	2 / 33	6	13 / 96	14	9 / 44	20
SSc	4 / 50	8	2 / 20	10	N.T.	-	1 / 44	2
SS	2 / 50	4[a]	0 / 20	0	10 / 44	23	8 / 22	36
RA	N.T.	-	1 / 20	5	N.T.	-	1 / 44	2
Others	MCTD 0 /10	0	N.T.	-	N.T.	-	OL 0 / 12	0
Total	26 / 314	8	5 / 104	5	49 / 341	14	27 / 351	8
IBM	8 / 10	80	11 / 31	35	71 / 117	61	88 / 238	37
HC	1 / 42	2	1 / 52	2	2 / 42	5	unknown/35	unknown

* biotinylated *in vitro* transcription and translation product. ** produced in *Escherichia coli*. *** expressed in HEK293 cells.

[a] $P<0.05$ vs. study (4) and $P<0.005$ vs. study (5).

α-NT.: anti-NT5C1A antibody; DM: dermatomyositis; MCTD: mixed connective tissue disease; N.T.: not tested; OL: polymyositis-scleroderma overlap; PM: polymyositis; SLE: systemic lupus erythematosus; SS: Sjögren's syndrome; SSc: systemic sclerosis

Figure 1. Detection of anti-NT5C1A antibodies in immunoprecipitation analysis

Immunoprecipitation of recombinant NT5C1A by sporadic inclusion body myositis patient or control sera. Lanes 1-10: sera from the different patients with sporadic inclusion body myositis. Lane HC: healthy control serum. In.: The input was the biotinylated protein used for the immunoprecipitation assay. (+): positive; (-): negative.

Figure 2. Detection of anti-NT5C1A antibodies in ELISA

ELISA units of anti-NT5C1A antibodies in serum samples from patients with sporadic inclusion body myositis, from patients with various systemic autoimmune rheumatic diseases and from healthy controls. The broken line indicates the cutoff value: 0.79 unit. sIBM: sporadic inclusion body myositis; DM: dermatomyositis; HC: healthy control; MCTD: mixed connective tissue disease; PM: polymyositis; SLE: systemic lupus erythematosus; SS: Sjögren's syndrome; SSc: systemic sclerosis.

Figure 1

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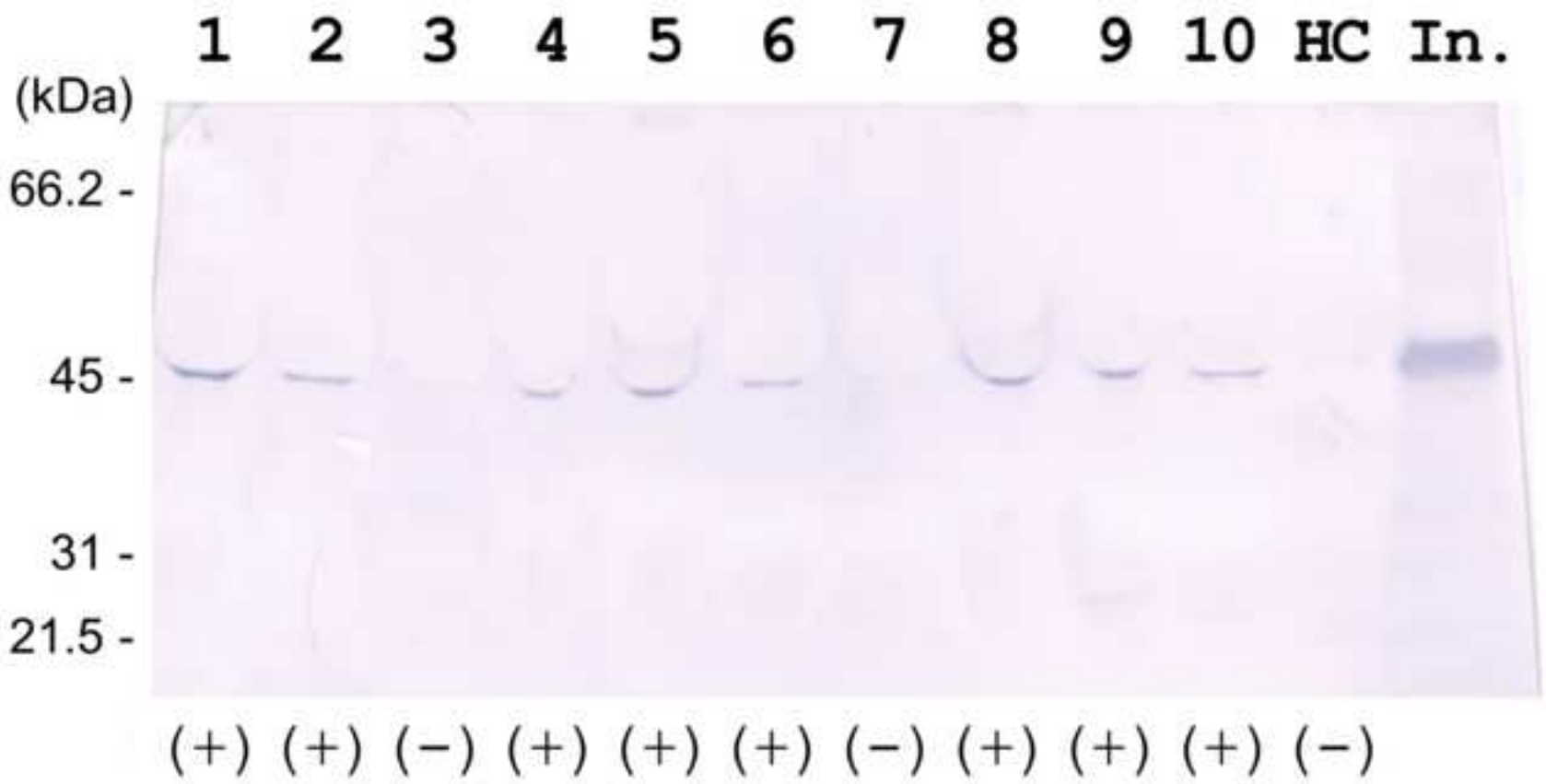

Figure 2

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