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BRIEF REPORT

Distinct profiles of myositis-specific autoantibodies in Chinese and Japanese patients with polymyositis/dermatomyositis

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Abstract The study aims to comprehensively assess the profiles of myositis-specific autoantibodies (MSAs) in Chinese patients with polymyositis (PM)/dermatomyositis (DM) and compare them with a Japanese cohort. One hundred forty-five Chinese patients (68 classic DM, 25 clinically amyopathic DM [CADM], and 52 PM) and 165 Japanese patients (56 classic DM, 52 CADM, and 57 PM) were recruited. MSAs were measured with immunoprecipitation, enzyme-linked immunosorbent assay, or immunoprecipitation–immunoblotting. MSA frequencies were compared. The overall frequency of anti-melanoma differentiation-associated gene 5 (MDA5) antibodies was significantly higher in the Chinese patients than in the Japanese cohort (36.6 % [53/145] versus 15.8 % [26/165], respectively, $P<0.001$), whereas the frequencies of anti-signal recognition particle (SRP) antibodies (1.4 % [2/145] versus 7.9 % [13/165], respectively, $P=0.008$) and anti-aminoacyl-transfer RNA synthetase (anti-ARS) antibodies (27.6 % [40/145] versus 40 % [66/165], respectively, $P=0.02$,) were significantly lower. The significantly lower frequency of anti-ARS antibodies and significantly higher frequency of anti-MDA5 antibodies in the Chinese patients were observed in the classic DM subset (14.7 % [10/68] versus 46.4 % [26/56], respectively, $P<0.001$, and 45.6 % [31/68] versus 5.4 % [3/56], respectively, $P<0.001$) and CADM subset (8.0 % [2/25] versus 28.8 % [15/52], respectively, $P=0.04$, and 88.0 % [22/25] versus 44.2 % [23/52], respectively, $P=0.0002$), but not in the PM subset. The first detailed profile of MSAs in Chinese patients with PM/DM was established. The differences in MSA frequencies in the Chinese cohort and Japanese cohort suggest underlying genetic and/or environmental differences between these two populations.

Key Messages

- *A significantly higher frequency of anti-melanoma differentiation-associated gene 5 (MDA5) antibodies was observed in Chinese patients with polymyositis/dermatomyositis (PM/DM) than in Japanese patients.*
- *Our findings suggest that distinct genetic and/or local environmental factors affect Chinese and Japanese patients with PM/DM, who have been considered a "homogeneous" population in previous studies.*

Keywords Autoantibodies<Tissues or models · Myositis<Rheumatic diseases

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Polymyositis/dermatomyositis (PM/DM) is a systemic idiopathic inflammatory myositis. Patients with PM/DM usually present with skin rash and muscle weakness at the onset of the disease, but lung involvement and underlying malignancy are the most important causes of morbidity and mortality [1]. In the past decade, a new subset of DM patients, presenting with hallmark skin manifestations but with no clinically significant muscle involvement, was described as displaying "clinically amyopathic dermatomyositis" (CADM) by Sonthiemer [2].

Accordingly, PM/DM comprises at least three important subsets: PM, classic DM, and CADM.

Myositis-specific autoantibodies (MSAs) are a group of autoantibodies specifically expressed in patients with PM/DM. To date, over 10 MSAs have been identified in different ethnicities [3]. Recent studies have shown that these MSAs are mutually exclusive and are associated with distinct and unique clinical features, treatment outcomes, and prognoses. We previously reported an antibody directed against melanoma differentiation-associated gene 5 (MDA5) specifically expressed in patients with CADM with rapidly progressive interstitial lung disease (RP-ILD) [4]. Reports mainly from eastern Asia (Japan, China, and Korea) have suggested that the anti-MDA5 antibody is associated with high mortality and poor prognoses. Our recent meta-analysis revealed that the pooled sensitivity and specificity of anti-MDA5 antibodies in identifying RP-ILD in DM are 77 and 86 %, respectively [5].

Several studies have demonstrated differences in the MSA profiles of different ethnicities or different geographic areas. However, to our knowledge, the MSA profiles of Chinese and Japanese patients with PM/DM have not been compared, although they have often been designated as "Asian" and regarded as a relatively homogeneous population in previous studies [3]. Because no commercial assays or convenient methods are available, as far as we know, MSAs have not been comprehensively analyzed in Chinese patients with PM/DM. In this study, we comprehensively investigated the profiles of MSAs in Chinese patients with PM/DM from a single referral medical center. When compared with those of a Japanese cohort, we found distinct distributions in the MSA profiles of Chinese patients.

Materials and methods

Subjects In total, 145 Chinese patients (52 PM, 68 classic DM, and 25 CADM) and 165 Japanese patients with (57 PM, 56 classic DM, and 52 CADM) PM/DM were recruited consecutively (after informed consent was given) between March 2010 and December 2012 from the rheumatology clinics of the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China, and the Keio University Hospital, Tokyo, Japan, respectively. The clinical settings of these hospitals were identical in that they are both referral medical centers located in a metropolitan center. Informed consent was obtained from each subject before blood sampling. The study protocol was approved by the Ethics Review Committees of the Affiliated Drum Tower Hospital and the Affiliated Hospital of Keio University Medical School. The diagnoses were based on the criteria proposed by Bohan and Peter for probable or definite PM and classic DM. The diagnosis of CADM was made according to the criteria suggested by Sontheimer [2] and Sato and Kuwana [6]. Muscle biopsy was performed in 19 patients. The ethnicity of all Chinese patients was Han Chinese. The presence of ILD was determined with high-resolution computed tomography [7]. RP-ILD was defined as worsening radiological interstitial changes with progressive dyspnea and hypoxemia within 1 month of the onset of respiratory symptoms [4]. The baseline demographic data, clinical data, and laboratory data at presentation were taken from hospital records.

Antibody detection Anti-MDA5 antibody was detected with an enzyme-linked immunosorbent assay (ELISA), as described previously [4]. Anti-aminoacyl-transfer RNA synthetase (anti-ARS), anti-signal recognition particle (SRP), and anti-Ku antibodies were determined with immunoprecipitation (IPP), as described previously [8]. Briefly, 2 mg of protein A–Sepharose beads were coated with 10 μl of patient serum, washed, and incubated with an extract of 6×10^6 unlabeled HeLa cells. The immunoprecipitates were recovered with phenol extraction, separated with 7–8 M urea 10 % polyacrylamide gel electrophoresis (PAGE), and visualized with silver stain. Immunoprecipitation–immunoblotting assays were used to detect anti-transcriptional intermediary factor 1γ (anti-TIF1γ), anti-Mi2, anti-small ubiquitin-like modifier activating enzyme (anti-SAE), and anti-nuclear matrix protein NXP2 (anti-NXP2) antibodies [9], with some modifications. The following antibodies were used for immunoblotting: mouse polyclonal anti-NXP2 antibody, mouse monoclonal anti-Mi-2 antibody (clone ab54603), and rabbit polyclonal anti-TIF1γ antibody (Abcam, Cambridge, UK), and rabbit polyclonal anti-SAE2 antibody and rabbit polyclonal anti-Ku80 antibody (Bethyl Laboratories, Montgomery, TX).

Statistical analyses The χ^2 or Fisher's exact test was used to compare categorical data. Student's *t* test was used to compare continuous data. *P* values <0.05 were considered to indicate statistical significance. All analyses were performed using the SPSS software (ver. 16.0) or Prism (GraphPad Software, San Diego, CA).

Results

In total, 145 Chinese patients and 165 Japanese patients with PM/DM were enrolled in this study. As shown in Table 1, there were no differences between the Chinese and Japanese cohorts in their age at diagnosis, distribution of sexes, prevalence of muscle weakness, arthritis, Raynaud's phenomenon, skin ulceration, ILD, or RP-ILD. Compared with the Japanese patients, more Chinese patients presented with fever at disease onset (14.5 vs 24.8 %, respectively, $P=0.02$). We also observed a significantly higher frequency of malignancy in the Japanese cohort than in the Chinese cohort (14.5 vs 5.5 %,

Table 1 Comparison of the clinical features of patients with PM/DM in Chinese and Japanese cohorts

Features	Chinese cohort (n=145)	Japanese cohort (n=165)	P
Age at diagnosis, years	49.3	51.2	NS
Sex, male/female	55/90	47/118	NS
Muscle weakness, no. (%)	110 (75.9)	110 (66.7)	NS
Arthritis, no. (%)	29 (20.0)	44 (26.7)	NS
Fever, no. (%)	36 (24.8)	24 (14.5)	0.02
Raynaud's phenomenon, no. (%)	15 (10.3)	28 (17.0)	NS
Skin ulceration, no. (%)	14 (9.7)	14 (8.5)	NS
ILD, no. (%)	103 (71.0)	105 (63.6)	NS
Rapidly progressive ILD, no. (%)	27 (18.6)	20 (12.1)	NS
Malignancy, no. (%)	8 (5.5)	24 (14.5)	0.009
Diagnosis			
Classic DM, no. (%)	68 (46.9)	56 (33.9)	0.02
CADM, no. (%)	25 (17.2)	52 (31.5)	0.004
PM, no. (%)	52 (35.9)	57 (34.5)	NS

PM/DM polymyositis/dermatomyositis, *ILD* interstitial lung disease, *CADM* clinically amyopathic myositis

respectively, P=0.009). In the DM subset (including classic DM and CADM), RP-ILD was more frequent in the Chinese patients than in the Japanese patients (29 vs 16.7 %, respectively, P=0.04). Compared with the Japanese patients, more Chinese patients were diagnosed with classic DM (33.9 vs 46.9 %, respectively, P=0.02), but fewer patients with CADM (31.5 vs 17.2 %, respectively, P=0.004).

We next compared the MSA distributions in these two populations. As shown in Table 2, seven MSAs (not anti-ARS antibodies) were detected in the total population of PM/DM patients at various frequencies: anti-MDA5, anti-NXP2, anti-TIFγ, anti-SRP, anti-Mi-2, anti-Ku, and anti-SAE antibodies. Although the overall frequencies of most of MSAs were similar in the Chinese cohort and Japanese cohort with PM/DM, a significantly higher frequency of anti-MDA5 antibody (36.6 vs 15.8 %, respectively, P<0.001) and significantly lower frequencies of anti-ARS (27.6 vs 40.0 %, respectively, P=0.02) and anti-SRP antibodies (1.4 vs 7.9 %, respectively, P=0.008) were found in the Chinese patients (Table 2). When we compared the prevalence of the MSAs in different PM/DM subsets, a significantly lower frequency of anti-ARS antibodies and a significantly higher frequency of anti-MDA5 antibodies were observed in the Chinese patients in both the classic DM subset (14.7 vs 46.4 %, P<0.001 and 45.6 vs 5.4 %, P<0.001, respectively) and the CADM subset (8.0 vs 28.8 %, P=0.04, and 88.0 vs 44.2 %, P=0.0002, respectively), but these differences were not observed in the PM subset. Anti-SRP antibodies were also more frequent in Japanese patients with PM than in Chinese patients with PM (21.1 vs 3.8 %, respectively, P=0.009). Consistent with our previous observation [5], anti-MDA5 antibodies were detected exclusively in DM patients (including classic DM and CADM) in both the Chinese and Japanese cohorts, but were absent in PM patients.

Discussion

In this study, we comprehensively analyzed, for the first time, the MSA profiles in a Chinese PM/DM cohort from a single center. All known MSAs were detected in the Chinese patients. Although the frequencies of most of them were similar to those found in Japanese patients with PM/DM, several distinct features of their distributions were identified. The most noteworthy finding of this study is the strikingly high frequency of anti-MDA5 antibodies (57 %, 53/93) in Chinese patients with DM (including classic DM and CADM). This frequency is considerably higher than that in a Mediterranean population, estimated to be about 12 % in DM patients [10]. Consistent with our previous findings, anti-MDA5 antibodies were present in the Chinese patients with DM, regardless of their grouping into classic DM or CADM, whereas most of the anti-MDA5-positive patients in the Japanese cohort had CADM. The well-known relationship between anti-MDA5 antibodies and RP-ILD may account for the high prevalence of RP-ILD observed in Chinese DM patients [5, 10]. Given the poor prognosis of patients with DM and RP-ILD, these observations suggest that the closely monitoring and novel therapeutic strategies should be addressed in Chinese patients with anti-MDA5, regardless of the DM subtypes.

To date, the exact mechanisms that influence the expression of anti-MDA5 antibodies remain unknown, but both

Table 2 Distribution of myositis-specific antibodies in patients with PM/DM in Chinese and Japanese cohorts

Myositis-specific antibodies	All PM/DM			Classic DM			CADM			PM		
	Chinese (n=145)	Japanese (n=165)	P	Chinese (n=68)	Japanese (n=56)	P	Chinese (n=25)	Japanese (n=52)	P	Chinese (n=52)	Japanese (n=57)	P
Anti-ARS, no. (%)	40 (27.6)	66 (40.0)	0.02	10 (14.7)	26 (46.4)	<0.001	2 (8.0)	15 (28.8)	0.04	28 (53.8)	25 (43.9)	NS
Anti-MDA5, no. (%)	53 (36.6)	26 (15.8)	<0.001	31 (45.6)	3 (5.4)	<0.001	22 (88.0)	23 (44.2)	0.0002	0	0	NS
Anti-NXP2, no. (%)	7 (4.8)	6 (3.6)	NS	6 (8.8)	5 (8.9)	NS	0	1 (1.9)	NS	1 (1.9)	0	NS
Anti-TIF1γ, no. (%)	8 (5.5)	14 (8.5)	NS	8 (11.8)	12 (21.4)	NS	0	2 (3.8)	NS	0	0	NS
Anti-SRP, no. (%)	2 (1.4)	13 (7.9)	0.008	0	1 (1.8)	NS	0	0	NS	2 (3.8)	12 (21.1)	0.009
Anti-Mi-2, no. (%)	6 (4.1)	4 (2.4)	NS	5 (7.4)	4 (7.1)	NS	1 (4.0)	0	NS	0	0	NS
Anti-Ku, no. (%)	1 (0.7)	1 (0.6)	NS	0	0	NS	0	0	NS	1 (1.9)	1 (1.8)	NS
Anti-SAE, no. (%)	1 (0.7)	1 (0.6)	NS	1 (1.5)	1 (1.8)	NS	0	0	NS	0	0	NS

Anti-ARS anti-aminoacyl-tRNA synthetase, *anti-MDA5* anti-melanoma differentiation-associated gene 5, *anti-NXP2*=anti-nuclear matrix protein NXP-2, *anti-TIF1γ* anti-transcriptional intermediary factor 1γ, *anti-SRP* anti-signal recognition particle, *anti-SAE* anti-small ubiquitin-like modifier activating enzyme, *NS* not significant

genetic and environmental factors may be involved. The relationships between MSAs and human leukocyte antigen (HLA) polymorphisms have been well recognized in the previous studies [11]. A strong association between anti-ARS antibodies and alleles of the 8.1 common ancestral haplotype has been confirmed in several idiopathic inflammatory myopathy studies in Caucasians [11, 12]. In contrast, anti-ARS antibodies appear to be associated with DRB1*0405 in Japanese patients [13]. In a small-sample study, Gono et al. reported an association between anti-MDA5 antibodies and DRB1*0101/*0405 in Japanese patients [14]. The combined allele frequencies of DRB1*0101 and DRB1*0405 are remarkably different between Japanese and Chinese (17.1 vs 7.8 %, respectively) [15, 16], suggesting the distinct genetic susceptibility to anti-MDA5 antibody in these two populations. In addition to genetic factors, some environmental factors may also affect MSA expression. Chinoy et al. demonstrated an interaction between DRB1*03 and smoking in the development of anti-Jo-1 antibodies [17]. A study in central Japan showed that from 1994 to 2010, the relative prevalence of CADM and anti-MDA-5-antibody-positive patients increased significantly, and the presence of anti-MDA-5 antibodies was inversely associated with the population of the patients' city of residence [18]. In the present study, both the Chinese and Japanese cohorts were recruited from local referral medical centers. The differences in MSA prevalence between the Chinese and Japanese cohorts may be attributable to genetic and/or local environmental factors, both of which should be investigated in the future.

We also observed a significantly lower frequency of malignancy in the Chinese patients than in the Japanese patients. Because the number of Chinese patients was relatively small in the present study, the accurate frequencies and clinical significance of anti-TIF1γ and anti-NXP2 antibodies in Chinese and Japanese patients with PM/DM require further investigation in larger populations.

Our study also had several limitations, including the relatively small sample sizes, inadequate screening for underlying malignancy, and the relatively short follow-up period in some patients. Furthermore, all patients were recruited from a single local referral center from each country, so may not be representative of their whole populations.

In summary, the first detailed profile of MSAs in Chinese patients with PM/DM was established. A significantly higher frequency of anti-MDA5 antibodies was observed in Chinese patients with PM/DM than in Japanese patients. Our findings suggest that distinct genetic and/or local environmental factors affect Chinese and Japanese patients with PM/DM, who have been considered a "homogeneous" population in previous studies.

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Competing interests MK holds a patent for an anti-MDA5-antibody-measuring kit.

Author contributions Z.C., W.H., Y.W., and Z.G. collected the samples and clinical data. Z.C. and M.K. performed the analyses and drafted the manuscript. M.K. and L.S. oversaw the whole project and helped to prepare the manuscript. All authors read and approved the manuscript.

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