

Coexistence of anti-HMGCR and anti-MDA5 identified by an unlabeled immunoprecipitation assay in a chinese patient cohort with myositis

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Abstract

Myositis-specific autoantibodies are important diagnostic and prognostic markers. The aim of our study is to detect anti-3-hydroxy 3-methylglutaryl coenzyme A reductase (anti-HMGCR) antibody using novel unlabeled immunoprecipitation (IP) assay and immunoblotting in Chinese patients with myositis and to clarify the features of anti-HMGCR-positive patients. In the present study, we established novel unlabeled IP assay and immunoblotting of HMGCR C-terminus for anti-HMGCR detection. The presence of anti-HMGCR was screened in 181 Chinese patients with myositis. The sera from 12 of 181 patients were positive for anti-HMGCR. The prevalence of anti-HMGCR autoantibody in our cohorts is about 6.6%. Unexpected, coexistence of anti-HMGCR and anti-melanoma differentiation-associated protein (anti-MDA5) were identified in 4 patients with characteristic rash and interstitial lung disease (ILD), but without myasthenia and elevated serum creatine kinase (CK) levels. Other anti-HMGCR positive patients without anti-MDA5 presented with severe proximal muscle weakness. Mean serum CK levels and lactate dehydrogenase (LDH) were significantly higher in anti-HMGCR-positive patients than in antibody-negative patients ($P < .05$). Muscle biopsies available from 6 anti-HMGCR-positive patients were characterized with prominent myofiber necrosis and regeneration, little or none of inflammatory cell infiltrates. None of anti-HMGCR positive patients in our cohort was exposed to statins. Our data suggested that anti-HMGCR were found to coexist frequently with anti-MDA5 identified by the established unlabeled IP assay and statin exposure is rare in Chinese myositis patients with anti-HMGCR.

Abbreviations: CK = creatine kinase, DM = dermatomyositis, HMGCR = 3-hydroxy 3-methylglutaryl coenzyme A reductase, IIMs = idiopathic inflammatory myopathies, ILD = interstitial lung disease, IP = immunoprecipitation, LDH = lactate dehydrogenase, MDA5 = melanoma differentiation-associated protein, MSAs = myositis-specific autoantibodies, MAAs = myositis-associated autoantibodies, PM = polymyositis.

Keywords: HMGCR, myositis, myositis-specific antibody, unlabelled protein immunoprecipitation

1. Introduction

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of diseases, collectively termed myositis, characterized by muscle weakness, fatigue, inflammation, and multisystem

involvement.^[1,2] Based on their characteristic histopathologic features, IIMs are composed of dermatomyositis (DM), polymyositis (PM), and inclusion body myositis (IBM). A fourth subtype with predominant necrosis and regeneration, but no or sparse inflammatory cells, is immune-mediated necrotizing myopathy.^[3]

Autoantibodies in myositis are grouped into myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs).^[4,5] Over recent years, an increasing number of MSAs including anti-synthetase autoantibodies (ASA),^[6,7] Anti-Mi-2,^[8] anti-small ubiquitin-like modifier activating enzyme (anti-SAE),^[9] anti-transcriptional intermediary factor 1 (anti-TIF1 γ),^[10] anti-nuclear matrix protein 2 (anti-NXP-2),^[11] anti-melanoma differentiation-associated protein 5 (anti-MDA5)^[12], anti-3-hydroxy 3-methylglutaryl coenzyme A reductase (HMGCR),^[13] anti-signal recognition particle (anti-SRP),^[14] and anti-cytoplasmic 5' nucleotidase 1A (anti-cN1A)^[15] have been identified and associated with distinct clinical phenotypes, making MSAs as important diagnostic and prognostic markers.^[5] Anti-SRP and anti-HMGCR are regarded as representative autoantibodies for immune-mediated necrotizing myopathy.

HMGCR is the pharmacologic target of statins, and statins reduce cholesterol levels by specifically inhibiting 3-hydroxy 3-methylglutaryl coenzyme A reductase.^[16] Christopher-Stine L et al identified a novel autoantibody targeting the relative molecular mass of 200 kDa and 100 kDa proteins in 16 cases of

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immune-mediated necrotizing myopathy.^[13] HMGCR was then identified as the 200 kDa and 100 kDa autoantigen by immunoprecipitating ³⁵S-methionine-labeled in vitro-translated HMGCR protein and this autoantibody only recognized the C-terminus of HMGCR spanning amino acids (aa) 340–888, but not N terminus (1–377aa).^[17] The prevalence of anti-HMGCR in 750 patients with suspected myopathy is about 6% and 92% of anti-HMGCR positive patients age 50 or older had statins-exposed history. Anti-HMGCR antibodies become a new marker to facilitate diagnosis and direct therapy for immune-mediated necrotizing myopathy. Werner JL and his colleagues found that anti-HMGCR antibodies were useful for monitoring the disease activity index.^[18] Therefore, Anti-HMGCR antibodies could assist diagnosis of myositis and might be defined as classification criteria in the future.^[19]

In the present report, we established an unlabeled immunoprecipitation (IP) assay and immunoblotting assay of HEK293 cells lysate overexpressing HMGCR C-terminus to screen anti-HMGCR antibody in sera from 181 adult myositis patients and confirmed the presence of anti-HMGCR antibody by *immunoblotting*. We also sought to clarify the features of anti-HMGCR-positive patients.

2. Materials and methods

2.1. Patients and sera

In the present study, we enrolled 181 adult Chinese patients with a Bohan and Peter diagnosis of DM/PM,^[20,21] who visited the Department of Rheumatology at the Xiangya Hospital of Central South University between July 2012 and August 2016. The following data were obtained from medical records: sex, age, lung function, muscle strength, chest computed tomography (CT) images, creatine kinase (CK) levels, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), Complement 3 (C3), and Complement 4 (C4), lactate dehydrogenase (LDH). Patients were classified as having cancer-associated DM if the internal malignancy was diagnosed within 3 years (before or after) of the DM diagnosis based on previous studies.^[24] For cancer, we routinely screen tumor markers including CEA, AFP, CA125, CA19–9, CA15–3 and perform screening of high incidence sites of solid tumors such as nasopharynx, thyroid, mammary gland, lung, and abdomen by computed tomography or positron emission tomography if needed. Aliquots of serum were frozen at –80°C until use. Serum samples from 30 health controls were also obtained. The study is in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board at the Xiangya Hospital of Central South University (approval number: 201703567). All study participants provided written informed consent before participation in the study.

2.2. Serological data

Anti-nuclear antibodies (ANA) were detected in all patients with myositis by indirect immunofluorescence using HEp-2 cells with a starting dilution of 1:80. Antibodies against nRNP/Sm, Sm, SSA, Ro-52, SSB, Scl-70, Jo-1, CENPB, nucleosomes, histones, and ribosomal P-protein were detected using a commercial line blot (EUROIMMUNE, Lübeck, Germany). The commercial myositis profile EUROLINE (DL1530–1601G, DL1530–1601–3G, DL1530–1601–4G) is not available in our clinical laboratory.

2.3. Unlabeled protein IP assay of HEK293 lysate overexpressing Myc-tagged HMGCR C-terminal fragment

Because anti-HMGCR autoantibody only recognized the C-terminus of HMGCR spanning amino acids (aa) 340–888, but not N terminus (1–377aa), we constructed pCMV-Myc-HMGCR plasmid, which contains Myc-tagged C-terminal fragment of HMGCR protein (GenBank: Q14149, 340–888aa). Human HEK293 cells (ATCC CRL-1573) were grown in DMEM supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD), 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA) in a humidified atmosphere of 5% CO₂ at 37°C. Transient transfections were carried out using Megatran 1.0 (TT200003, OriGene, MD) and pCMV-Myc-HMGCR plasmid. Cells were incubated for 48 hours after transfection and were freshly lysed in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) for western blot or protein IP assay. Unlabeled IP assay was performed following the described protocols with minor modifications (2012, 384: 128–134). Briefly, 20 µL of patient serum was incubated with 50 µL of a 50% slurry of pure Proteome Protein G magnetic beads (LSKMAGG10, Merk Milipore, Germany) suspended in 5% BSA in PBS. Incubation was carried out overnight at 4°C with mixing on a rotary mixer. Beads were washed 3 times with 0.1% Tween-20 in PBS. Beads were then incubated with 150 to 200 µg whole lysate from HEK293 cells overexpressing Myc-tagged HMGCR C-terminus for 2 hours at room temperature under mild spinning conditions. After washing 5 times with 0.1% Tween-20 in PBS, immunoprecipitated antigens were solubilized in 1 × SDS loading buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 100 mM dithiothreitol, and 10% glycerol), separated by 10% SDS-PAGE, and transferred to PVDF membrane. Then the membrane was detected with anti-Myc antibodies (SC-40, Santa Cruz Biotechnology, CA, 1:5000). The secondary antibody was HRP-conjugated anti-Mouse IgG (1:5000). After washing the antibody, the reaction was revealed by chemiluminescence using ECL Plus (Bio-Rad, Berkeley, CA).

2.4. Immunoblotting of HEK293 lysates overexpressing Myc-tagged HMGCR C-terminal fragment using anti-HMGCR-positive serum

Human HEK293 cells overexpressing Myc-tagged HMGCR C-terminal fragment were lysed in RIPA buffer, separated by 10% SDS-PAGE, and transferred to PVDF membrane. We tested the membrane via immunoblotting with an anti-HMGCR-positive serum as a reference serum or patient sera diluted 1:1000. The secondary antibody was HRP-conjugated Rabbit Anti-Human IgG H&L (1:5000; ab6759, Abcam).

2.5. Evaluation of pulmonary function and interstitial lung disease (ILD) diagnosis

ILD was diagnosed based on the respiratory symptoms such as dyspnea and the presence of typical features including ground-glass opacities, reticulation, or honeycombing on high-resolution computed tomography (HRCT) chest scan, performed by an experienced radiologist.^[22] When available, forced vital capacity and lung carbon monoxide transfer factor were used to evaluate the pulmonary function.

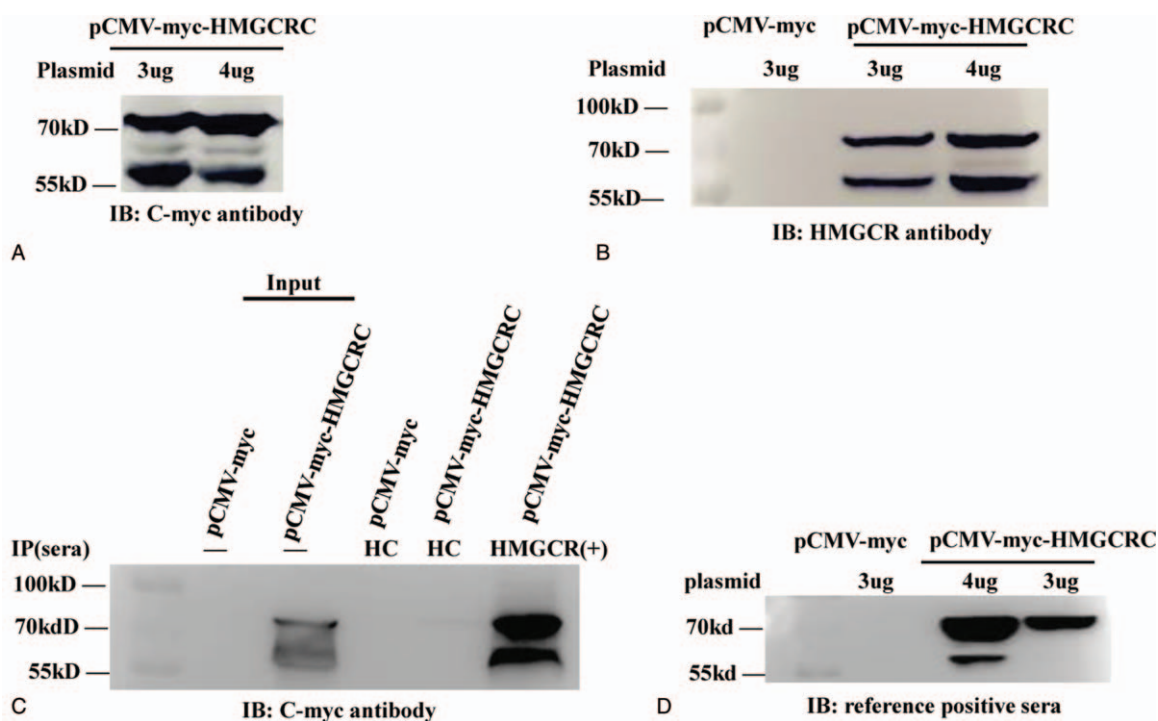


Figure 1. Establishment of unlabeled protein immunoprecipitation assay. HEK293 lysate overexpressing Myc-tagged HMGCRC C-terminus was used for detecting anti-HMGCRC autoantibody. (a and b) Overexpression of HMGCRC C-terminus in HEK293 cells confirmed by immunoblot using c-Myc antibody or HMGCRC antibody; (c) IP assay of HEK293 lysate overexpressing HMGCRC C-terminus using reference positive serum or healthy control serum; (d) Reactivities of reference HMGCRC-positive serum with HMGCRC C-terminal fragment encoded by pCMV-Myc-HMGCRC plasmid. HMGCRC=3-hydroxy 3-methylglutaryl coenzyme A reductase, IP=immunoprecipitation.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS Statistics program (Version 19.0) (SPSS, Chicago, IL). The Chi-square test or Fisher exact test was used for categorical variable. Continuous variables are compared using the Mann-Whitney test. Significance levels were set at P less than 0.05.

3. Results

3.1. Establishment of unlabeled protein IP assay for anti-HMGCRC detection

We established an unlabeled protein IP assay to detect anti-HMGCRC autoantibody in IIM patients because the commercial HMGCRC ELISA kit is not yet available in our clinical laboratory. The plasmid pCMV-myc-HMGCRC, containing C-terminus of HMGCRC protein (340–888aa) with Myc tag, was transiently transfected into HEK293 cells and the overexpression of HMGCRC C-terminus was detected at around 55 kDa and 70 kDa by c-Myc antibody (Fig. 1A). The specificity of HMGCRC C-terminus overexpression was confirmed using a commercial HMGCRC antibody (Fig. 1B). Because the full length of HMGCRC presented as 200 kDa and 100 kDa in HeLa cells, we affirmed that the relative molecular mass of 55 kDa and 70 kDa proteins, targeted by both c-Myc antibody and HMGCRC antibody, were C-terminus of HMGCRC protein. As shown in Figure 1C, the reference serum positive for anti-HMGCRC autoantibodies specifically immunoprecipitated HMGCRC C-terminus when it is overexpressed in HEK293 cells, but the health control serum did not. To exclude the possibility that autoantibodies against

any component of a protein complex interacting with HMGCRC C-terminus could theoretically immunoprecipitate the Myc-tagged HMGCRC and thus appear as anti-HMGCRC-positive in the IP assay, we also performed the immunoblot of HMGCRC C-terminus using reference HMGCRC-positive serum as the primary antibody. Reference positive sera showed strong reactivity with C-terminus of HMGCRC protein in the immunoblot (Fig. 1D).

3.2. Determine the prevalence of anti-HMGCRC autoantibody in Chinese myositis cohort by unlabeled IP assay and immunoblot

We performed the established IP assay to determine the presence of anti-HMGCRC autoantibody in the sera of 181 adult patients with myositis. In total, the anti-HMGCRC autoantibodies were detected in 13 sera (Fig. 2A and Table 1), while 30 normal control sera were negative (Fig. 2A, representative sera shown). Although IM48, IM84, IM90, D74, and D96 immunoprecipitated a little of HMGCRC C-terminus in the first screen, we did not detect the immunoprecipitated band anymore after repeat.

Then we confirmed the presence of anti-HMGCRC autoantibodies in all anti-HMGCRC positive sera and suspected positive sera (IM48, IM84, IM90, D74, and D96) by the immunoblotting of C-terminus using patient serum as primary antibody (Fig. 2B). Most anti-HMGCRC positive sera were strongly reactive with the C-terminus of HMGCRC except for IM46 serum (Fig. 2B). No reactivity of IM46 serum to C-terminus of HMGCRC in the immunoblot indicated that the serum from IM46 patient carried an unknown autoantibody against a component of protein complex interacting with HMGCRC C-terminus. As expected,

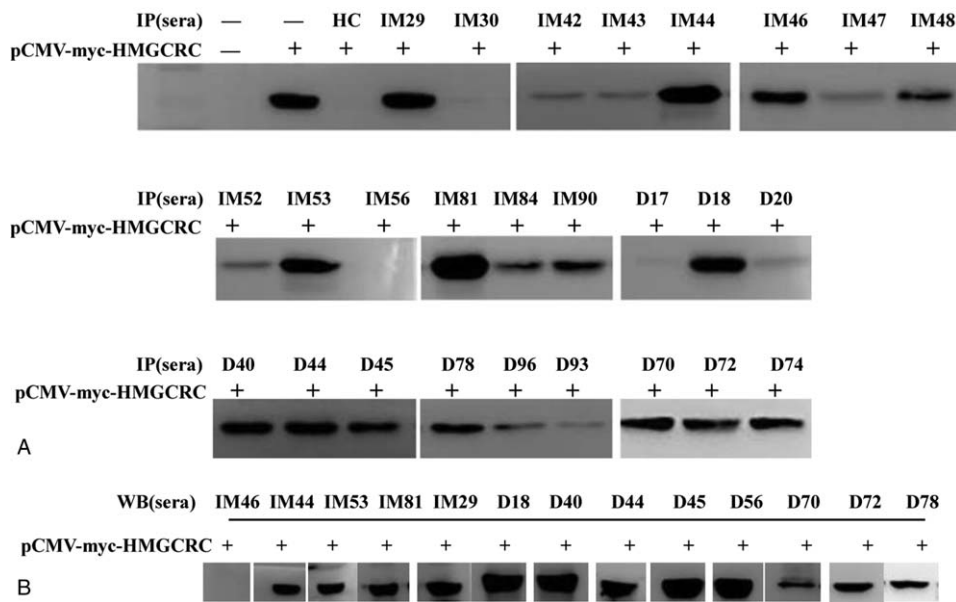


Figure 2. Detection of anti-HMGCR antibodies in myositis patients using unlabeled IP assay and immunoblot. (a) IP assay using HEK293 lysates overexpressing Myc-tagged HMGCR C-terminus. Sera from 181 myositis patients and 30 normal controls were assayed by IP. Representative anti-HMGCR-positive, anti-HMGCR-negative sera and health control sera are shown; (b) Immunoblot of Myc-tagged HMGCR C-terminus using all anti-HMGCR positive sera. HMGCR=3-hydroxy 3-methylglutaryl coenzyme A reductase, IP=immunoprecipitation.

IM48, IM84, IM90, D74, and D96 did not show any reactivity with the truncated HMGCR fragment in the immunoblot (data not shown). These data indicate that the developed IP assay combined with the immunoblot was a useful method for the detection of anti-HMGCR autoantibody in myositis.

3.3. Clinical and laboratory features of anti-HMGCR antibody-positive patients.

The prevalence of anti-HMGCR autoantibody in our cohorts with myositis is about 6.6%. Among MSAs/MAAs, we could detect the prevalence of Jo-1 (18.2%), SSA (16.6%), Ro-52 (50.8%), and SSB (2.2%) because the commercial myositis

profile EUROLINE (DL1530–1601G, DL1530–1601–3G, DL1530–1601–4G) is not available in our clinical laboratory. The clinical and laboratory features of patients carrying anti-HMGCR autoantibodies are summarized in Table 1. The anti-HMGCR antibody was found in 3.1% (4/128) of DM patients and 15.1% (8/53) of PM patients. Of the anti-HMGCR antibody-positive patients, 8 (66.7%) patients presented with proximal muscular weakness, 5 (42%) patients suffered myalgia, 3 (25%) of these patients had dysphagia, 4 (33%) had arthralgia, 5 (42%) experienced dyspnea, and 5 (42%) had ILD. It is interesting, 4 (33%) patients (D44, D56, D70, D78) were not only positive for anti-HMGCR antibody but also for anti-MDA5 antibody detected by in-house IP assay and ELISA

Table 1
Clinical and laboratory features of anti-HMGCR antibody-positive patients in our cohort.

Patient ID	Age at onset	Gender	Diagnosis	Muscle weakness	Myalgia	Dysphagia	Arthralgia	Dyspnea	ILD	ANA	Other MSA or MAA
IM029	47	F	PM	Y	N	N	N	N	N	negative	
IM044	55	M	PM	Y	Y	Y	N	N	N	negative	
IM053	42	M	PM	Y	N	N	N	N	N	negative	
IM081	62	F	PM	Y	N	Y	Y	Y	N	1:80, cytoplasmic pattern	SSA; SSB
D18	16	F	PM	Y	N	N	N	N	N	negative	
D40	44	F	PM	Y	Y	N	N	N	N	1:320, cytoplasmic pattern	SSA
D44	32	M	DM	N	N	N	Y	Y	Y	negative	MDA5
D45	58	M	PM	Y	Y	N	N	N	N	negative	
D56	39	M	DM	N	N	N	N	Y	Y	negative	MDA5
D70	43	F	DM	N	Y	Y	N	Y	Y	negative	MDA5
D72	57	F	PM	Y	N	N	Y	N	Y	1:80, cytoplasmic pattern	JO-1
D78	24	M	DM	N	Y	N	Y	Y	Y	1:80, cytoplasmic pattern	SSA; SSB; MDA5

ANA=antinuclear antibodies, ILD=interstitial lung disease, MAA=myositis-associated autoantibodies, MSA=myositis-specific autoantibodies, N=No, Y=yes.

Table 2**Demographic, clinical and laboratory features in anti-HMGCR positive and anti-HMGCR negative patients.**

	HMGCR (+) (n=12)	HMGCR (-) (n=169)	P
Demographic data			
Female	6 (50.0%)	113 (66.9%)	.19
Mean age, years (\pm SD)	43.1 (\pm 13.9)	50.1 (\pm 12.4)	.11
PM/DM	8 (66.7%)/4 (33.3%)	47 (27.8%)/122 (72.2%)	.008
Clinical and laboratory data			
Muscle weakness	8 (66.7%)	157 (92.9%)	.23
Heliotrope rash	2 (16.7%)	64 (37.9%)	.12
Gottron's papules or sign	4 (33.3%)	94 (55.6%)	.12
Myalgia	5 (41.7%)	69 (40.8%)	.49
Dysphagia	3 (25.0%)	50 (29.6%)	.51
Arthralgia	4 (33.3%)	91 (53.8%)	.14
Dyspnea	5 (41.7%)	59 (34.9%)	.43
Malignancy	0 (0%)	16 (9.5%)	0.11
elevation of CK, U/L	6617.2 (\pm 8407.2)	1858.3 (\pm 4630.1)	.012
LDH, U/L	901.6 (\pm 654.5)	485.6 (\pm 303.8)	.029
ANA ⁺	4 (33.3%)	107 (63.3%)	.041

ANA = antinuclear antibodies, CK = creatine kinase, LDH = lactate dehydrogenase, PM/DM = polymyositis/dermatomyositis, SD = standard deviation.

(Supplementary methods and figure S1, <http://links.lww.com/MD/C640>). In our cohort, the prevalence of anti-MDA5 is about 24.3%. Those patients with both anti-HMGCR and anti-MDA5 had typical DM skin rash (heliotrope rash or Gottron's sign) and ILD, but not myasthenia and markedly elevated CK levels. Anti-Jo-1 antibody was also detected in 1 anti-HMGCR positive patient complicated with myasthenia and ILD (D72). Most of these patients with ILD showed the features of cryptogenic organizing pneumonia on high-resolution computed tomography (HRCT) chest scan. Two patients positive for both anti-HMGCR and anti-MDA5 (D44, D56) did not respond to intensive immunosuppressive therapy and 1 died within 2 months, the other died within 16 months after skin rash onset. Ten out of 12 anti-HMGCR-positive patients are alive until now and respond well to corticosteroids, without or with other immunosuppressive agents. Antibodies against SSA or SSB were found in 3 or 2 patients positive for anti-HMGCR autoantibodies, respectively. Indirect immunofluorescence (IIF) using HEp-2 cells showed that 4 patients with anti-HMGCR antibodies displayed similar granular cytoplasmic patterns. Of note, none of anti-HMGCR positive patients had prior statin exposure including nature statins treatment. None of 30 health controls received statins. Among the anti-HMGCR negative patients with myositis, 8 patients had history of statin exposure.

Muscle biopsies were available from 6 anti-HMGCR antibody-positive patients. Five muscle biopsies showed a similar distinctive pathology pattern characterized by prominent and diffuse distribution of necrotic and regenerative myofibers with little or no evidence of endomysial or perivascular inflammatory cell infiltration that could be diagnosed as immune-mediated necrotizing myopathy (IMNM), according to European Neuromuscular Centre criteria (ENMC).^[23] One biopsy from the subject with anti-HMGCR and anti-MDA5 (D78) showed no dominant myofiber degeneration and necrosis, but a few enlarged capillaries.

3.4. Comparison of clinical and laboratory features between antibody-positive and antibody-negative patients

A comparison of the clinical features of the 12 anti-HMGCR-positive adult patients and 169 anti-HMGCR-negative adult

patients is shown in Table 2. Female and male ratio is consistent among the positive group and negative group. The average age of anti-HMGCR antibody-positive patients is not significantly different compared to those in the antibody-negative group. The majority (8/12) of anti-HMGCR patients has PM. Although the incidence of dyspnea (5/12, 41.7%) in anti-HMGCR positive group was higher than in the negative group (35%), the difference was not statistically significant. The levels of CK and LDH in anti-HMGCR antibody-positive patients were predominantly higher than those in antibody-negative patients ($P < .05$). This data is consistent with the higher muscle enzyme in immune-mediated necrotizing myopathy with anti-SRP and anti-HMGCR.^[26] The higher muscle enzyme could be attributed to the predominant necrosis of myofibers in immune-mediated necrotizing myopathy.

4. Discussion

This report describes the identification of anti-HMGCR autoantibodies in a Chinese patient cohort with myositis. A novel protein IP technique and immunoblotting were established to detect anti-HMGCR autoantibodies in the sera of patients with myositis. Among the published literature on anti-HMGCR autoantibodies, anti-HMGCR antibodies have been detected by IP of ^[35S] methionine-labeled cell extracts (13), enzyme-linked immunoassay (ELISA),^[24-26] and immunoblot. Recently, Drouot L also explored an addressable laser bead immunoassay (ALBIA) to detect and quantify anti-HMGCR antibodies.^[27] The radio-labeled IP assay is considered as the gold standard technique for determining the presence of defined autoantibodies and is more sensitive and specific than other laboratory tests. However, radiolabeled IP could not distinguish among components complexed to the primary antigen or autoantigens with similar molecular weights,^[28,29] and radioisotopes are used only in equipped laboratories and under rigorous control. HMGCR commercial ELISA (EUROIMMUNE, Lübeck, Germany) or in-house ELISA have been widely used.^[24-26] The advantage of HMGCR ELISA is to deal with large numbers of samples more quickly and efficiently than other immunologic techniques. However, ELISA process is labor intensive. The commercial Myositis Euroline 7 (IgG) line immunoassay including HMGCR

antigen (EUROIMMUNE, Lübeck, Germany) was not yet available in our clinical laboratory. Our unlabeled IP technique and immunoblotting assay can, therefore, be considered a valuable alternative to radiolabeled IP and a useful approach for the identification of anti-HMGCR antibodies in patient sera.

The present study shows that the prevalence of anti-HMGCR autoantibody in our cohorts with myositis is about 6.6%, which is consistent with previous study.^[17,24] Of note, none of anti-HMGCR positive patients in our group was exposed to statins including nature statins treatment. Another recent report in Chinese patients with myositis also demonstrated that only 3 (15%) out of 20 anti-HMGCR-positive patients with myositis had prior statin exposure, and 2 patients were exposed to statins in 5 anti-HMGCR-positive cases aged 50 or older.^[24] This percentage is much lower than other studies reported, ranging from 40% to 72.7%.^[17,18,30,27] Indeed, 40% to 70% of adult myositis with anti-HMGCR have no history of statin exposure, suggesting the presence of alternative disease triggers. It is well known that a history of statin exposure is not mandatory to develop anti-HMGCR positive immune-mediated necrotic myositis, being of some relevance only in patients older than 50. Mammen et al showed that the mean age of anti-HMGCR antibody-positive patients without statin exposure (aged 37 ± 17) was lower than that of statin-exposed anti-HMGCR-positive patients (aged 59 ± 9) (17). In fact, our group of anti-HMGCR-positive patients is young on average (aged 43 ± 14) and only 4 positive patients are older than 50 years. Recently, 4 patients with anti-HMGCR were identified in a large UK cohort of juvenile myositis, while statins are not typically prescribed to juvenile myositis.^[31] The significant difference related to statins exposure history ascribes to different ethnic backgrounds, age at onset and alternative disease triggers.

Although MSAs are highly selective and usually mutually exclusive, our data found for the first time that 4 patients carried both anti-HMGCR and anti-MDA5 antibodies, and 1 patient with anti-HMGCR antibody had Jo-1-positive anti-synthetase syndrome. The frequent coexistence of anti-HMGCR and anti-MDA5 antibodies in the present study may be attributed to our IP technique using truncated tagged-antigen overexpressed in HEK293 cells, which possesses the advantage of increasing assay sensitivity and specificity and recognizing the conformational epitopes and linear epitopes. However, the detection of myositis-specific autoantibody coexistence is not rare. For example, Ge Y et al observed that anti-Jo-1 antibodies were identified in 3 of anti-HMGCR antibody-positive patients.^[24] Mammen et al also reported that 1 patient with anti-HMGCR antibody had Jo-1 antibodies with ILD.^[17] Coexistence of anti-HMGCR and anti-SRP antibody was previously reported in 1 patient with myositis.^[26] These data suggested that anti-HMGCR antibody coexisted frequently with anti-MDA5 or anti-Jo-1 in patients with myositis.

Anti-HMGCR antibody is regarded as a representative autoantibody for immune-mediated necrotizing myopathy and helps differentiate immune-mediated necrotizing myopathy from muscular dystrophy without muscle biopsy. It might be defined as classification criteria in the future and the patients proven to have anti-HMGCR antibodies are encouraged to avoid muscle biopsy.^[18,26] Anti-HMGCR and anti-SRP autoantibodies should be evaluated early for patients with myositis especially when serum CK levels more than 1000 IU/L. Our data and other study demonstrated that the patients with anti-HMGCR responded well to corticosteroids, without or with other immunosuppressive agents such as Methotrexate, Azathioprine, Tacrolimus.

There are several major disadvantages of present study. The assays used to detect anti-HMGCR antibodies is a labor-consuming technique and is not suitable to quantify the titer of anti-HMGCR autoantibodies. Although the immunoblot of HMGCR C-terminus using patient sera is feasible, the fine mapping of linear epitopes is required to develop an immunoblot with high sensitivity. Additionally, the number of individuals in our myositis cohort and the number of anti-HMGCR-positive patients were relatively low. Furthermore, myositis disease activity assessment such as MMT8, MYOACT, MITAX is not available in our cohort, making it difficult to associate the disease activity and response to treatment with anti-HMGCR.

5. Conclusion

A novel IP assay and immunoblot were developed to detect patients with myositis carrying anti-HMGCR. Anti-MDA5 and anti-Jo-1 were found to coexist frequently with anti-HMGCR. Statin exposure is rare in Chinese myositis patients with anti-HMGCR.

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Author contributions

Li Huang, Li Wang, Yang Yang and Huan Chen performed the experiments; Yanjuan Liu, Ke Liu and Meidong Liu performed the analyses; Yizhi Xiao, Xiaoxia Zuo and Yisha Li were involved in the clinical studies; Hui Luo, Xianzhong Xiao and Huali Zhang designed the studies; and all authors were involved in writing and correcting the manuscript.

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