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Original article

Autoantibodies against four-and-a-half-LIM domain 1 (FHL1) in inflammatory myopathies: results from an Australian single-centre cohort

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Abstract

Objectives. To determine the prevalence and associations of autoantibodies targeting a muscle-specific autoantigen, four-and-a-half-LIM-domain 1 (FHL1), in South Australian patients with histologically-confirmed idiopathic inflammatory myopathies (IIM) and in patients with SSc.

Material and methods. Sera from patients with IIM ($n = 267$) from the South Australian Myositis Database (SAMD), SSc ($n = 174$) from the Australian Scleroderma Cohort Study (ASCS) and healthy controls (HC, $n = 100$) were analysed for anti-FHL1 autoantibodies by Enzyme-Linked ImmunoSorbent Assay (ELISA).

Results. Autoantibodies to FHL1 were more frequent in patients with IIM (37/267, 13.8%) compared with SSc (12/174, 7%) ($P < 0.02$) and HC (2/100, 2%) ($P < 0.001$). The most common IIM subtypes among FHL1$^+$ IIM patients were (32%) and IBM (2/37, 32%). No statistically significant differences in muscular or extra-muscular manifestations of IIM were found when comparing patients who were anti-FHL1$^+$ with their anti-FHL1$^-$ counterparts. In 29/37 (78%) anti-FHL1$^+$ patients, no myositis-specific autoantibodies (MSA) were present. In FHL1$^+$ muscle biopsies, there was less frequent infiltration by CD45$^+$ cells ($P = 0.04$). There was a trend for HLA alleles DRB1*07 and DRB1*15 to be more frequent in anti-FHL1$^+$ compared with anti-FHL1$^-$ patients (9/25 *vs* 19/113, $P = 0.09$ and 8/25 *vs* 15/114, $P = 0.09$, respectively).

Conclusions. We report a substantial prevalence (13.8%) of anti-FHL1 autoantibodies in a large cohort of patients with histologically confirmed IIM; 75% of these cases did not have a detectable myositis-specific autoantibody. Anti-FHL1 autoantibodies were also detected in a subgroup of patients with SSc (7%), indicating that anti-FHL1 autoantibodies may not be myositis-specific. The trend towards an HLA-DR association might indicate a specific immune response to the FHL1 protein.

Key words: PM, DM, IBM, myositis-specific autoantibodies, myositis-associated autoantibodies, SSc, anti-FHL1 autoantibodies

BASIC SCIENCE

Rheumatology key messages

- A prevalence of 14% of anti-FHL1 autoantibodies in idiopathic inflammatory myopathies was detected in a South Australian cohort.
- The majority of IIM patients with anti-FHL1 autoantibodies did not have myositis-specific antibodies.
- Anti-FHL1 autoantibodies were found in a lower frequency in patients with systemic sclerosis (7%).

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Introduction

The idiopathic inflammatory myopathies (IIM) are a group of chronic systemic autoimmune disorders that comprise a broad spectrum of clinical phenotypes with a common feature of skeletal muscle inflammation associated with progressive muscle weakness, leading potentially to chronic dysfunction and disability [1–3].

Distinct phenotypes are identified based on clinical features, histopathological findings in muscle tissue, and autoantibody profiles [4–12]. Currently, the umbrella-term IIM includes the disease subsets PM, DM, JDM, clinically amyopathic dermatomyositis (CADM), sporadic inclusion body myositis (IBM), immune-mediated necrotizing myopathy (IMNM) and antisynthetase syndrome (ASyS) [4–12].

Myositis-associated (MAA) and myositis-specific (MSA) autoantibodies are detected in up to 60–70% of IIM patients, are associated with distinct clinical phenotypes and may predict disease course [13, 14]. These autoantibodies are directed against ubiquitously expressed nuclear and cytoplasmic components involved in protein translocation, anti-viral response and gene transcription; however, none of these antibodies specifically target muscle-specific antigens.

Four-and-a-half-LIM-domain 1 (FHL1) is a muscle-specific antigen found in skeletal and heart muscle. Its structure comprises four and a half highly conserved LIM domains, which are cysteine-rich and further characterized by tandem zinc finger protein-interaction motifs [15, 16]. FHL1 is involved in differentiation, intracellular protein–protein interactions mainly with cytoskeletal proteins, maintenance of structural elements (e.g. sarcomere), and is a regulator of transcription factors (e.g. NFATc1) [17–19]. Mutations in the *FHL1* gene (position Xq26.3) were previously described in connection with X-linked myopathies, including reducing body myopathy (RBM), X-linked myopathy with postural muscle atrophy (XMPMA), scapuloperoneal myopathy, and Emery–Dreifuss muscular dystrophy [16, 20, 21].

A single study demonstrated the presence of autoantibodies targeting FHL1 in 25% of Caucasian patients with IIM, and the autoantibodies were associated with a propensity to severe skeletal muscle involvement, muscle fiber damage, muscle atrophy, vasculitis and dysphagia [15]. The presence of anti-FHL1 antibodies has not to date been reported in other myositis populations. Therefore, this study aimed to determine the prevalence and associations of anti-FHL1 autoantibodies in another population of adult patients with IIM and in patients with another systemic autoimmune disease, SSc as a comparator group.

Patients and methods

Patient population and disease activity assessment

The study comprised 267 adult patients diagnosed with IIM between 1992 and 2016. The diagnosis of myositis and its subgroups was based on the histopathological findings in muscle biopsies. The criteria used by the Anatomical Pathology Laboratory at South Australia (SA) Pathology are applied according to European Neuromuscular Centre (ENMC) and validated criteria [10, 22–24]. As a comparator group, 174 patients with SSc who fulfilled the 2013 ACR/EULAR Classification Criteria for SSc were included. In addition, samples from 100 healthy controls (HC) were provided by the Department of Human Immunology, SA Pathology.

IIM patients were identified from the South Australian Myositis Database (SAMD), a register of adult patients with a histological diagnosis of IIM. Recruitment to the SAMD is based on histological criteria that have previously been described for PM, DM and IBM [25]. Cases were recorded as IMNM when there was dominant myofiber necrosis and a paucity of muscle inflammation. The SAMD also includes patients with myositis not-otherwise-specified (MNOS) who have skeletal muscle inflammation though insufficient features to satisfy a diagnosis of PM, DM, IBM or IMNM (Supplementary Table S1, available at *Rheumatology* online). The diagnosis of ASyS was based on the presence of anti-Jo1 autoantibodies, plus one of the following features: interstitial lung disease, myositis, arthritis, Raynaud's phenomenon, fever, or mechanic's hands [12]. Serum samples were collected from these patients following the diagnosis of IIM (median 116 days post-biopsy, IQR 25–478 days) and stored at –80°C.

For IIM patients managed at the Royal Adelaide Hospital Rheumatology Unit, disease activity was evaluated prospectively at their usual clinic visits (every three months) following the diagnosis of IIM, using the disease activity core set measures proposed by the International Myositis Assessment Collaborative Studies group (IMACS): the muscle and extra-muscular components of the Myositis Disease Activity Assessment Tool by visual analogue scale (MYOACT-muscle, MYOACT-extra-muscular), physician global activity using visual analogue scales (VAS), creatine kinase (CK), manual muscle testing-8 (MMT8) and HAQ [26, 27].

Clinical and serological data concerning the SSc patients were obtained from the Australian Scleroderma Cohort Study database and included cutaneous subtypes, organ involvement, muscle strength, CK and autoantibodies as previously described [28].

Ethics

This study was approved by the Human Research Ethics Committee (RAH Protocol Number 051012f, HREC/15/RAH/98 and RAH Protocol Number 070405) and the Central Adelaide Local Health Network (CALHN) Governance (SSA/15/RAH/109). Patients and controls included in the study provided written informed consent for the use of their serum for research purposes.

Autoantibody detection and HLA typing

MSA and MAA were identified from serum samples by line immunoblot assay (Myositis Profile Euroline Blot,

Euroimmun profile 3, Lübeck, Germany, for antibodies against Mi-2, Ku, PM-Scl100, PM-Scl 75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52), indirect immunofluorescence of antinuclear antibodies (ANA) and by a commercial Enzyme-Linked ImmunoSorbent Assay (ELISA) for antibodies against extractable nuclear antigens (Ro, La, RNP, Sm) and anti-Jo-1. Anti-HMGCR was tested at baseline using a commercial ELISA (Pathwest, Perth, Australia) [29].

HLA typing for Class I and II alleles was performed by serology and DNA-based technology (allele-specific), respectively, by the Australian Red Cross Blood Service.

Muscle biopsy evaluation

Muscle samples were obtained for diagnostic purpose via open biopsy under general anesthetic or via needle biopsy under light sedation with a University College Hospital (UCH) needle and snap-frozen in liquid nitrogen. Biopsies underwent routine stains for inflammatory cells and were distinguished from non-inflammatory muscle conditions by routine use of histochemical, immunohistochemical and ultrastructural examination undertaken on all biopsies to exclude dystrophies, dysferlinopathy and other non-inflammatory muscle conditions. Diagnosis of subsets of IIM was according to established criteria and biopsies were regularly subjected to peer review [23, 24].

Anti-FHL1 antibody ELISA

Anti-FHL1 autoantibodies were detected in stored serum samples by indirect ELISA with minor modifications of the previously described methodology [15, 30]. Briefly, recombinant, full-length human His-tagged FHL1-protein was diluted in carbonate buffer, pH 9.6, and coated (0.25 μg/well) on a 384-well high-binding plate (Corning, Sigma-Arldrich, Burlington, MA, USA) for 4 h at room temperature. Plates were thereafter incubated overnight at 4°C with reducing buffer, pH 6.8 (100 mM Tris-HCl, 50 mM NaCl, 10 mM dithiothreitol); washed with PBS 0.05%-Tween (PBST) and blocked with PBST 0.1%BSA (PBST) (BSA, lyophilized powder, Sigma-Aldrich). Patient sera (diluted 1:500 in PBST 0.1%BSA) was added after washing the plates and incubated overnight at 4°C. Polyclonal rabbit anti-human IgG-alkaline phosphatase was added as the secondary antibody and incubated for 2 h (1:2000 in PBST, Dako, Agilent, Santa Clara, CA, USA).

Plates were developed using substrate phosphatase tablets (5 mg/tablet, Sigma-Aldrich) diluted in substrate solution (1 M diethanolamine pH 9.8, 0.5 mM $MgCl \times 6H2O$, 0.02% NaN_3) and the optical density (OD) was measured at an absorbance of 405 nm using SpectraMax Plus 384 microplate reader. The OD values were transformed to Arbitrary Units (AU) by interpolation of a sigmoidal 4PL $\log_{10}(x)$ standard curve consisting of 0.05–10 AU, where 1 AU = 1:500 dilution of a standard serum sample that was used in all ELISA plates. A cut-off of 1.06 AU units was considered positive. Characteristics of the diagnostic test are presented in Supplementary Fig. S1, available at *Rheumatology* online. All samples were tested in duplicates and the analysis was performed in samples with an intra-assay coefficient of variability (CV) <10% and inter-assay CV < 15% [31].

Statistical analysis

Descriptions of continuous variables are expressed as mean or median and standard deviation (SD) or interquartile range (IQR). Categorical variables are presented as frequencies and percentages. Differences between groups were analysed using the Student's *t test* or Mann–Whitney *U* test, depending on the normality of data for continuous variables, and Chi-squared or Fisher's-exact test for categorical variables. For allelic frequencies of the HLA class II, the values were corrected by the Bonferroni method according to the number of alleles analysed. The Kruskal–Wallis test with Dunn's correction was used for comparing the AU values of the different groups. GraphPad Prism 9.0 (GraphPad Software) and IBM SPSS Statistic V.27 were used for data management and statistical analyses. Two-tailed *P*-values <0.05 were considered statistically significant.

Results

Clinical and serologic characteristics of IIM, SSc, and HC

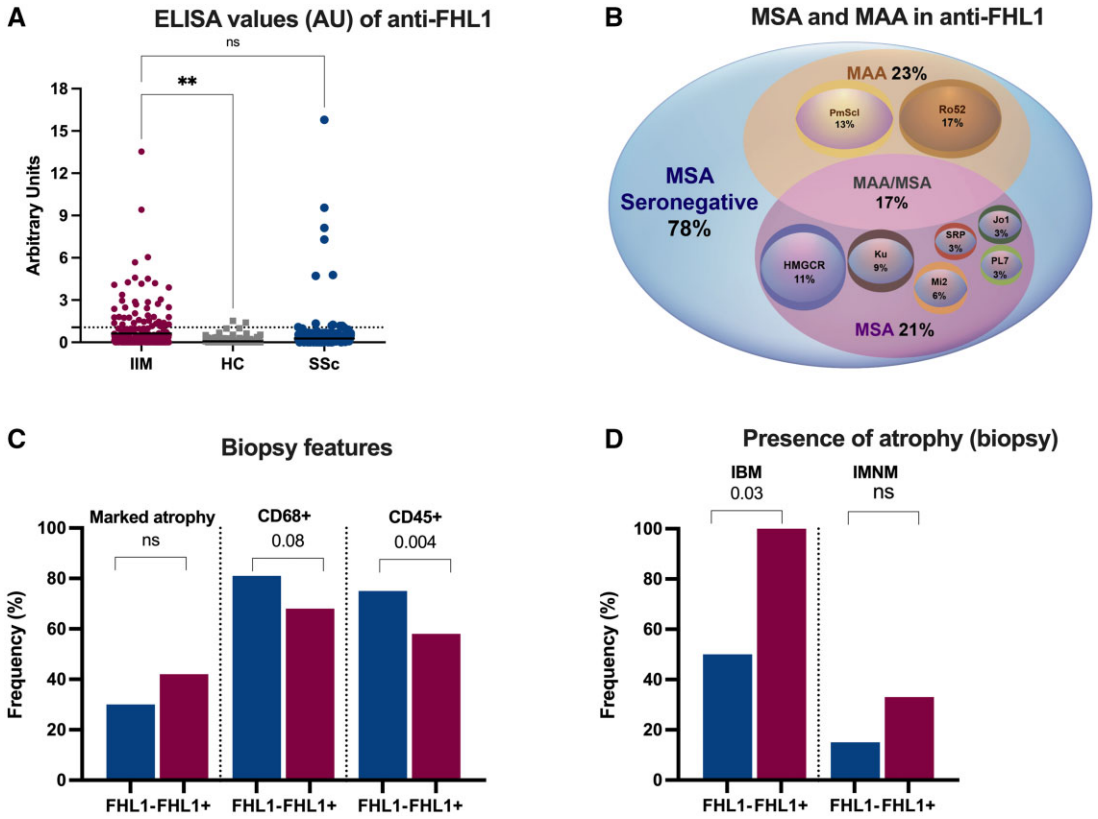
The IIM patient cohort ($n = 267$) comprised 159 (60%) females with a mean age (S.D.) at diagnosis of 54 (13) years. Disease subsets included DM ($n = 33$), PM ($n = 100$), IBM ($n = 71$), IMNM ($n = 47$) and MNOS ($n = 16$). ASyS ($n = 16$) included two patients with DM and 14 patients with the histological diagnosis of PM. Ethnicity was recorded in 234/267 IIM patients; 223/234 were Caucasian, two Indian, seven Asian, and two Indigenous Australian patients. No significant difference in demographic profile was identified between IIM patient subgroups. The SSc group comprised 144/174 (83%) females with a mean age (S.D.) of 60 (12) years; with a higher frequency of the limited subtype ($n = 142$) compared with the diffuse subtype ($n = 32$). No significant difference in demographic profile was identified between SSc patient subgroups. The HC group included 41/100 (41%) females with a mean age (S.D.) of 38 (15.4) years.

Anti-FHL1$^+$ autoantibodies were detected in 37/267 (14%) patients with IIM, in 12/174 (7%) patients with SSc and 2/100 (2%) of HC. Patients with IIM and SSc had significantly higher levels of anti-FHL1 autoantibodies compared with HC (mean AU 0.62 and 0.60, respectively, compared with 0.16 $P < 0.001$) (Fig. 1A).

Clinical and serologic characteristics of anti-FHL1+ IIM

The clinical characteristics and autoantibody profiles of IIM patients with and without anti-FHL1 autoantibodies

FIG. 1 Characteristics of IIM subgroups



(**A**) Sera from patients with IIM (DM, PM, IBM, IMNM and MNOS; $n = 267$), SSc (n = 174) and HC (n = 100) were analysed by ELISA using recombinant His-tagged FHL1. A cut-off value of 1.06 AU was calculated using a receiver operating characteristic (ROC) curve based on the HC. (**B**) Frequency of IIM patients presenting MSA and MAA in anti-FHL1$^+$ group. (**C**) Representative histological findings in the muscle biopsy regarding the frequency of marked atrophy (grade 4) and the infiltration by CD68$^+$ and CD45$^+$ cells. (**D**) Comparison of the frequency of atrophy in the muscle biopsy by IIM subgroup in IBM and IMNM in anti-FHL1$^+$ and anti-FHL1$^-$ groups. Statistical analysis for (**A**) was performed using Kruskal–Wallis test with Dunn's correction for multiple comparisons. Statistical analysis for (**C–D**) was performed using a 2-tailed Mann–Whitney *U* test. Each data point in (**A**) represents one individual and horizontal bars indicate the mean values. Asterisk indicates a significant difference, **P*-values < 0.05, ***P*-values < 0.01.HC: healthy controls; IIM: idiopathic inflammatory myopathy; IMNM: immune mediated necrotizing myopathy; MAA: myositis-associated autoantibodies; MNOS: myositis not-otherwise-specified; MSA: myositis-specific autoantibodies.

are presented in Table 1. Anti-FHL1 autoantibodies were more likely to be present in females (25/37, 68%), and within the subgroups of PM (15/37, 41%) and IBM (11/37, 29%), although this was not statistically different from the anti-FHL1$^-$ group.

In the anti-FHL1$^+$ group, 29/37 (78%) patients did not have any MSA, 27/35 (77%) patients were negative to all MAA and 23/35 (65%) patients were negative for both MSA and MAA, suggesting this antibody can be mutually exclusive of other MAA/MSA (Fig. 1B). Nevertheless, other MSA such as TIF1g, NXP2, MDA5 and cN1A were not evaluated in this cohort and were not considered as part of the analysis in this study. Median baseline CK and median peak CK levels (IU/l) were similar in the FHL1$^+$ group *vs* the FHL1$^-$ group [777 (IQR 300–1722) *vs* 743 (IQR 265–3150) and 561 (IQR 204–1868) *vs* 1096 (IQR 433–4094)].

As there is a genetic predisposition of HLA class II alleles to the formation of MSA, with HLA DRB1*03 and DRB1*04 shown to strongly predispose to MSA formation in a South Australian population [32], we next determined whether HLA Class II alleles predisposed to anti-FHL1 formation. Indeed, we found that HLA alleles DRB1*07 and DRB1*15 were more frequent in anti-FHL1$^+$ patients compared with anti-FHL1$^-$ IIM patients (Table 1, 9/25 *vs* 19/113, $P = 0.03$ and 8/25 *vs* 15/114, $P = 0.04$); however, these differences were not significant after correcting for multiple comparisons ($P = 0.09$; Supplementary Table S2, available at *Rheumatology* online).

Table 1 Characteristics of patients with idiopathic inflammatory myopathies (IIM) included in the FHL1 ELISA

	Anti-FHL1$^+$ $n = 37$ (14%)	Anti-FHL1$^-$ $n = 230$ (86%)	*P*-value
Female *n* (%)	25 (67.6%)	134 (58.5%)	0.29
Age, mean (S.D.), years	61 (12.9)	61 (12)	0.58
Disease duration (months) median (IQR)	22.5 (5–86)	12 (6–34)	0.22
Race (*n*, %)			0.069
Caucasian	30 (88%)	193 (97%)	
Indian	1 (3%)	1 (0.5%)	
Asian	3 (9%)	4 (2%)	
Indigenous	0 (0%)	2 (1%)	
IIM subtype			
DM	4 (11%)	29 (13%)	1.00
PM	15 (41%)	85 (37%)	0.67
IBM	11 (29%)	59 (26%)	0.38
IMNM	7 (19%)	41 (18%)	0.81
MNOS	0 (0%)	16 (7%)	0.13
[a]ASyS	1/16 (6.3%)	15/16 (94%)	0.48
Antibody profile			
MAA (*n*, %)			
Ro	2/35 (5.7%)	20/172 (11.6%)	0.38
La	0/35 (0%)	10/171 (5.8%)	0.21
Ro52	6/22 (27%)	40/85 (47%)	0.09
U1RNP	0/35 (0%)	3/171 (1.8%)	1.00
Scl-70	0/35	1/170 (0.5%)	1.00
PmScl	3/22 (13.6%)	8/64 (12.5%)	1.00
#MAA present			0.61
Absent	27/35 (77%)	128/181 (71%)	
One MAA	6/35 (17%)	28/181 (16%)	
Two MAA	1/35 (3%)	15/181 (8%)	
Three MAA	1/35 (3%)	10/181 (6)	
MSA (*n*, %)			
Ku	3/21 (14%)	2/61 (3.3%)	0.10
Mi2	2/21 (9.5%)	8/64 (12.5%)	1.00
PI7	1/20 (5%)	5/62 (8.1%)	1.00
PL12	0/20 (0%)	2/62 (3.2%)	1.00
Jo1	1/35 (3%)	8/171 (5%)	1.00
SRP	1/3 (33%)	3/40 (7.5%)	0.25
EJ	0/3	0/38	
OJ	0/3	1/38 (2.6%)	1.00
MDA5	0	1/38 (2.6%)	
HMGCR	4/35 (11.4%)	21/178 (11.8%)	1.0
#MSA present			0.03
Absent	29/37 (78%)	93/147 (63%)	
One MSA	5/37 (13%)	47/147 (32%)	
Two MSA	2/37 (5%)	7/147 (5%)	
Three MSA	1/37 (3%)	0/147 0	
Laboratory evaluation			
Baseline CK (median, IQR)	12/37 (777, 300–1722)	110/230 (743, 265–3150)	0.55
Peak CK (median, IQR)	13/37 (561, 204–1868)	112/230 (1096, 433–4094)	0.11
Clinical manifestations (*n*, %)			
Shawl sign	3/30 (10%)	14/190 (7.4%)	0.71
Heliotrope rash	2/30 (6.7%)	10/189 (5%)	0.67
Gottron papules	1/30 (3.3%)	16/190 (8.4%)	0.47
Raynaud	5/30 (16.7%)	31/181 (17%)	0.95
Sicca	1/30 (3%)	18/181 (10%)	0.48
Objective weakness	27/33 (82%)	161/191 (84%)	0.72
Upper limb weakness	23/32 (72%)	116/188 (62%)	0.27
Lower limb weakness	26/32 (81%)	156/190 (82%)	0.90
Proximal weakness	27/33 (82%)	158/190 (83%)	0.85
Distal weakness	8/33 (24%)	42/189 (22%)	0.79
Myalgia	10/31 (32%)	91/183 (50%)	0.07
Dysphagia	3/15 (20%)	32/113 (28%)	0.75

(continued)

Table 1 Continued

	Anti-FHL1$^+$ $n = 37$ (14%)	Anti-FHL1$^-$ $n = 230$ (86%)	*P*-value
Histological features (*n*, %)			
Vessel inflammation	2/25 (8%)	7/187 (4%)	0.28
Fibrous CT expansion	5/23 (22%)	43/146 (30%)	0.44
Presence of atrophy			
IBM	10/10 (100%)	30/59 (50%)	0.03
PM	8/8 (100%)	33/40 (82.5%)	0.25
DM	2/2 (100%)	16/16 (100%)	
IMNM	4/6 (67%)	30/33 (91%)	0.16
Necrosis	17/30 (57%)	140/205 (68%)	0.20
CD68$^+$	21/31 (68%)	168/207 (81%)	0.08
CD45$^+$	18/31 (58%)	156/207 (75%)	0.04

Results are expressed as *n* (%) if not otherwise specified. [a]ASyS group consisted of PM: $n = 14$ and DM $n = 2$ patients. ASyS:anti-synthtetase syndrome; CK: creatine kinase; IIM: idiopathic inflammatory myopathy; IMNM: immune mediated necrotizing myopathy; MAA: myositis associated autoantibodies; MNOS: myositis not-otherwise-specified; MSA: myositis specific autoantibodies.

There was no significant difference in muscular or extra-muscular manifestations of IIM in patients with or without anti-FHL1 antibodies, though there was a trend towards a reduced frequency of myalgia in anti-FHL1$^+$ patients ($P = 0.07$). Comparing IIM muscle biopsies with and without anti-FHL1 antibodies, there were no differences in histological scores of muscle atrophy though in FHL1$^+$ biopsies, there was less frequent infiltration by cells positive for CD45 ($P = 0.04$) and CD68 ($P = 0.08$) (Fig. 1C).

Anti-FHL1 autoantibodies in IBM

Since anti-FHL1 autoantibodies were first associated with dysphagia, distal muscle weakness, clinical atrophy and fiber necrosis [15], we performed a sub-analysis to identify the presence of these characteristics within FHL1$^+$ IBM patients. We identified 11 anti-FHL1$^+$ (eight female) and 59 anti-FHL1$^-$ (33 female) IBM patients. In anti-FHL1$^+$ IBM patients, there was a higher prevalence of atrophy in the muscle biopsy (100 *vs* 50%, $P = 0.03$) (Fig. 1D). The anti-FHL1$^+$ IBM patients (compared with anti-FHL1$^-$ IBM patients) also had a trend towards lower MMT8 scores ($P = 0.10$), higher median CK levels at baseline [1734U/l (225–904) *vs* 401U/l (147–805)], and higher peak CK levels [1090 (187–4597) *vs* 525 (225–904)], though these findings did not reach statistical significance. Both groups had similar median disease duration at the time of the serum collection (14 months, $P = 0.97$).

Anti-FHL1 autoantibodies in IMNM

In the group of IMNM, we detected seven anti-FHL1$^+$ (six female) and 41 anti-FHL1$^-$ (16 female) patients. Most of the patients in the anti-FHL1$^+$ IMNM group were female (86% *vs* 14%, $P = 0.03$), had a trend towards a higher degree of histological muscle atrophy (33% *vs* 15%, $P = 0.2$), less CD68+ (67% *vs* 83%, $P = 0.5$) and CD45+ (17% *vs* 53%, $P = 0.1$) infiltration, and less myalgia (33% *vs* 60%, $P = 0.3$), although this was not significantly different. There was a trend towards lower peak CK levels [1080U/l (993–1689) *vs* 3888U/l (1386–9965), $P = 0.13$] when compared with their anti-FHL1$^-$ IMNM counterparts.

Concurrence of anti-HMGCR

Antibodies to 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) were present in 4/35 (11.4%) of anti-FHL1$^+$ IIM patients, consistent with the 9.2% prevalence of anti-HMGCR we have previously reported in our IIM cohort [29]. Patients with dual antibodies targeting FHL1 and HMGCR showed less frequent infiltration of CD45$^+$ cells (25% *vs* 81%, $P = 0.05$) when compared with patients with only anti-HMGCR antibodies ($n = 21$).

Presence of anti-FHL1 autoantibodies in IIM patients seronegative for other MSA/MAA

There was no identifiable MSA in 122/184 (66%) or MAA in 155/216 (72%) of tested IIM patients, and 118/158 (75%) patients were seronegative for both MSA and MAA. This subgroup of monospecific anti-FHL1$^+$ enables an investigation into the clinical features associated with this antibody.

Similar to the general IIM cohort, patients with monospecific anti-FHL1$^+$ were most frequently diagnosed as PM (11/29, 30%) and IBM (9/29, 31%), followed by seronegative IMNM (5/29, 17%) and DM (4/29, 14%). These patients showed a trend towards more vessel inflammation in the muscle biopsy (10% *vs* 1%, $P = 0.07$), less myalgia (33% *vs* 54%, $P = 0.07$), lower CK median levels (459 *vs* 823, $P = 0.06$) and lower cellularity in the biopsy characterized by less CD68$^+$ (68% *vs* 85%, $P = 0.07$) and CD45$^+$ cells (64% *vs* 79%, $P = 0.10$) when compared with the anti-FHL1$^-$ counterpart

(Supplementary Table S3, available at *Rheumatology* online).

Anti-FHL1 autoantibodies in seronegative PM, IBM, IMNM and DM

In MSA seronegative PM, IBM, IMNM or DM, anti-FHL1 autoantibody levels were significantly higher in IBM and PM compared with DM. The levels of anti-FHL1 autoantibodies were not statistically different between the PM/IBM and IMNM groups (Supplementary Fig. S2, available at *Rheumatology* online).

When further characterizing patients with anti-FHL1$^+$ PM, vessel inflammation was present in 2/10 (20%) muscle biopsies compared with none in the anti-FHL1$^-$ and myalgia in 2/10 (20%) patients with anti-FHL1 autoantibodies, which was less frequent compared with the anti-FHL1$^-$ PM group.

Furthermore, the anti-FHL1$^+$ IBM group had a higher frequency of objective weakness (100% *vs* 87%, $P = 0.9$), upper limb weakness (86% *vs* 57%, $P = 0.2$), lower and proximal limb weakness (100% *vs* 84%, $P = 0.5$), distal weakness (63% *vs* 38%, $P = 0.2$), and marked atrophy in biopsies (88% *vs* 52%, $P = 0.06$) when compared with the anti-FHL1$^-$ IBM counterpart, although the differences were not statistically significant.

Anti-FHL1 autoantibodies in SSc

There were no differences regarding sex, age at diagnosis and disease duration between anti-FHL1$^+$ and anti-FHL1$^-$ SSc patients ($P = 0.69$, $P = 0.37$ and $P = 0.22$, respectively). However, a higher frequency of anti-FHL1 autoantibodies was observed in patients with limited *vs* diffuse SSc (91.7% *vs* 8.3% $P = 0.01$) (Table 2).

Given that FHL1 is a muscle-specific protein, and with the knowledge that patients with SSc may have symptoms and signs of inflammatory myopathies [33], we sought to determine whether anti-FHL1$^+$ SSc patients had a higher frequency of myopathy compared with anti-FHL1$^-$ patients. However, anti-FHL1$^+$ SSc patients did not have more frequent elevation of CK levels, or more severe weakness, and did not have more frequent clinical diagnosis of myositis or atrophy in the muscle biopsy compared with anti-FHL1$^-$ patients (Table 2).

Discussion

This is the first study to determine the prevalence of anti-FHL1 autoantibodies in a well-characterized cohort of patients with histologically confirmed IIM. We identified anti-FHL1 antibodies at a prevalence of 14%, which is somewhat lower than the originally reported 25% prevalence in a European cohort [15]. Importantly, we observed that in the majority (75%) of IIM patients with anti-FHL1 antibodies, they occurred in patients negative for known MSAs. Also, because of the histological criteria used to define the IIM subgroups, it has been possible to analyse a group of myositis now considered relatively rare such as PM. In addition, we found that anti-FHL1 autoantibodies occur with relatively low frequency in patients with SSc, more often in the limited than in the diffuse subtype.

The observation that anti-FHL1 autoantibodies were mainly detected in patients seronegative for already known MSA and MAA may indicate the diagnostic utility of this autoantibody in patients with suspected myositis; although confirmation in a larger cohort of patients with myositis and other autoimmune diseases as well as healthy individuals needs to be undertaken. Additionally, this is the second autoantibody to be associated with the subgroup PM, in addition to the rare anti-eukaryotic initiation factor 3 (eIF3) autoantibody [34].

The identification of anti-FHL1 autoantibodies mainly in the PM and IBM group is also of relevance regarding a potential role for these autoantibodies in the pathogenesis of these diseases. Both PM and IBM are characterized by cytotoxic CD8$^+$ T-cell infiltration with high levels of granzyme B and perforin expressed in the muscle tissue [35]. In this scenario, because the FHL1 protein is a muscle-specific antigen expressed in skeletal muscle, it can be hypothesized that exposure of FHL1 neo-epitopes could take place after tissue damage, with possible cytokine release and CD8$^+$ T-cell recruitment to the muscle, where FHL1 could be cleaved. The production of autoantibodies generated against potential neoepitopes may contribute to the initiation and propagation of inflammation.

The anti-FHL1$^+$ patients mainly presented with muscle symptoms and marked muscle fiber atrophy. But unlike the previous report, other findings reported initially to be associated with the presence of anti-FHL1 autoantibodies such as dysphagia, distal weakness and some histopathological features could not be replicated in this cohort [15]. In the subgroup of anti-FHL1$^+$ IBM, there was a trend towards higher baseline and peak CK levels and the presence of marked myofiber atrophy compared with the anti-FHL1$^-$ IBM patients. Although the low numbers of patients precluded demonstration of statistical significance, these results suggest the presence of more aggressive disease in the anti-FHL1$^+$ PM and IBM, which were identified as seronegative for MSAs. These findings suggest that the presence of this anti-FHL1 autoantibody could serve as a biomarker for severe disease in this subgroup of patients with IIM, especially in the seronegative group so far not linked to any MSA. Whether anti-FHL1 is involved in PM/IBM pathogenesis, or whether the antibody reflects an epiphenomenon or is indeed a secondary phenomenon to lymphocytic invasion of myofibres remains to be determined.

The finding of dual autoantibodies against FHL1 and HMGCR and the observations associated with this autoantibody in our patient population warrant confirmation in larger patient cohorts. Of note, there has been one report of a child with FHL1 gene mutation-associated reducing body myopathy who also had anti-HMGCR autoantibodies and muscle biopsy showing features of both reducing body myopathy and necrotizing myopathy [36].

Furthermore, our data indicate that alleles HLA-DRB1 *07 and *15 could be of relevance for the presence of

Table 2 Characteristics of patients with SSc included in the study

	Anti-FHL1$^+$ $n = 12$ (7%)	Anti-FHL1$^-$ $n = 162$ (93%)	*P*-value
Female *n* (%)	11 (91%)	133 (82%)	0.69
Age, mean (S.D.), years	63 (11)	60 (12)	0.37
Disease duration (months) median (IQR)	123 (26–201)	124 (76–226)	0.22
SSc subtype			
Diffuse	1 (8.3%)	31 (19%)	0.01
Limited	11 (91.7%)	131 (80%)	
Clinical manifestation (*n*, %)			
Reflux	8 (67%)	74 (46%)	0.16
Esophageal stricture	1 (8%)	11 (7%)	0.58
Esophageal dismotility	0 (0%)	23 (14%)	0.37
Bowel dismotility	0 (0%)	6 (4%)	1.00
Pseudo obstrution	0 (0%)	4 (3%)	0.82
Barret esophagous	1 (6%)	4 (3%)	0.30
GAVE	2 (17%)	11 (7%)	0.22
Malabsorption	0 (0%)	3/120 (2.3%)	0.90
Rectal prolapse	1/10 (10%)	3/137 (2%)	0.32
Dysphagia	7 (63%)	93/159 (59%)	0.19
Pulmonary arterial hypertension	1(8%)	21 (13%)	1.00
ILD	6 (50%)	63 (38%)	0.54
Muscle strength (0–5)			
Grade 5 (normal)	10 (83%)	101 (63%)	
Grade 4	1 (8%)	52 (33%)	
Grade 3	0 0	6 (4%)	
Grade 2	1 (8%)	0 (%)	
Grade 0 (paralysis)	0 (0%)	1 (0.6%)	
CK >150	3 (25%)	63 (39%)	0.53
Highest CK serum (median, IQR)	141 (108–179)	123 (84–178)	0.63
Clinical muscle atrophy	4 (33%)	38 (24%)	0.68
Myositis (biopsy)	2 (12.5%)	19 (12%)	0.95
Antibodies (*n*, %)			
ANA centromere	6 (50%)	72 (44%)	0.70
Anti-Scl-70	1 (8%)	22 (14%)	1.00
RNA-polymerase	1 (8%)	15 (9%)	1.00
Rheumatoid factor	2 (16%)	57 (35%)	0.34
Ro	0 (0%)	12 (8%)	1.00
Anti-PM/Scl	0 (0%)	2 (1%)	1.00

Results are expressed as *n* (%) if not otherwise specified. CK: creatine kinase; GAVE: gastric antral vascular ectasia; ILD: interstitial lung disease.

this autoantibody. This association differs from the original report from a Scandinavian cohort [15], where the HLA DRB1*03/13 was found in 21% of the patients. However, the small sample size of the anti-FHL1 patients in the current cohort limited the analysis of some alleles and our findings need to be validated in a larger cohort.

We found that 7% of patients with SSc also had anti-FHL1 autoantibodies, which is a similar frequency compared with the 6% previously reported [15]. It was noteworthy that patients in the SSc cohort, mainly with limited SSc, had similar titers of anti-FHL1 autoantibodies compared with patients with IIM, though an association with muscle disease was not demonstrable herein. This observation argues against the anti-FHL1 autoantibodies as being myositis-specific, although they do target a muscle-specific protein.

Though our study has advanced the understanding of anti-FHL1 autoantibodies in inflammatory muscle disease, some limitations need to be recognized. Firstly, while we studied a large cohort of IIM patients, some analyses were underpowered due to the low number of patients within individual IIM subgroups, hence some of these findings are based on non-significant trends in the data. Furthermore, it was not possible to identify significant statistical differences in the presence of muscular and extra-muscular manifestations in the IIM group comparing patients with anti-FHL1 autoantibodies with those without. Therefore, the presence of anti-FHL1 antibodies in DM, ASyS and IMNM could be underrepresented and will require investigation in larger cohorts. In addition, our IIM cohort was selected based on presence of defined histopathological features, which may explain the high frequency of PM and seronegative

cases, and low frequency of some IIM subgroups like the ASyS. Secondly, the retrospective nature of this analysis meant the clinical data was incomplete and some MSA could not be tested in the cohort such as TIF1g, NXP2, MDA5 and cN1A. Thirdly, the cross-sectional nature of our study precluded an investigation of whether anti-FHL1 autoantibodies predict the development of severe muscle atrophy and/or dysphagia or whether these autoantibodies are a bystander of chronic muscle inflammation, atrophy or disease activity. It is possible that other factors such as pharmacological treatment, time of diagnosis or exercise could influence the presence of anti-FHL1 autoantibodies. To analyse this effect, large-scale analyses of longitudinally collected patient sera measuring anti-FHL1 antibody titers and their response to treatment, exercise and expression of other MSA and MAA are required.

Conclusions

In conclusion, the present study provides basis for the anti-FHL1 antibody to be a novel autoantibody associated with IIM, preferentially associated with PM and IBM and associated with characteristic myopathology. It was not possible to identify a significant difference between muscular and extra-muscular clinical manifestations when comparing the IIM anti-FHL1^{+} and anti-FHL1^{-} autoantibody group. Future studies are needed to confirm that alleles HLA-DRB1*07 and *15 predispose to the formation of anti-FHL1 autoantibodies and to identify antigenic FHL1 epitopes, which will bring new insights into the mechanisms leading to anti-FHL1^{+} myopathies.

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Data availability statement

Patient-level data, the statistical analysis and the dataset specifications are available from the South Australian Myositis Database (SAMD) and from the Australian Scleroderma Cohort Study (ACSC) for researchers who meet the criteria for access to confidential data. The local ethics committees of the SAMD and the ACSC will maintain the ethical restrictions of the data. The Data Protection Officer of the Central Adelaide Local Health Network (CALHN) Governance will maintain the legal restrictions and appropriate codes of conduct. Patient-level data will be anonymised and study documents will be redacted to protect the privacy of the patients. Permission is required prior to access.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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