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-positive 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 positive with their anti-FHL1 negative counterparts. In 29/37 (78%) anti-FHL1 patients, no myositis-specific autoantibodies (MSA) were present. In FHL1-positive 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 negative 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

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%).
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 07/0405) 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,
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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.) 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 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 inter-quartile 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.

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Clinical and serologic characteristics of anti-FHL1+ IIM

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

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

| IIM Characteristics | Anti-FHL1+ | Anti-FHL1- |
|---------------------|------------|------------|
| Gender              | 159 females | 108 females |
| Age (years)         | 54 (±13)    | 54 (±13)    |
| Disease Subsets     | DM (33%)   | PM (100%)   |
|                     | IBM (71%)  | IMNM (47%)  |
|                     | MNOS (16%) | ASyS (16%)  |
| Ethnicity           | Caucasian (223) | Asian (2)  |
|                     | Indigenous (2) | Asian (2)  |
| Autoantibodies      | Anti-FHL1+ (37) | Anti-FHL1- (230) |
|                     | Anti-RNP | Anti-RNP |
|                     | Anti-Sm | Anti-Sm |
|                     | Anti-Jo-1 | Anti-Jo-1 |
|                     | Anti-HMGCR | Anti-HMGCR |

Table of characteristics and autoantibody profiles of IIM patients with and without anti-FHL1 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).

(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, ‘\(*\)’-values < 0.05, ‘**’-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.
Table 1 Characteristics of patients with idiopathic inflammatory myopathies (IIM) included in the FHL1 ELISA

| Characteristic                      | 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 (6–96)             | 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    |
| aASyS                               | 1/16 (6.3%)              | 15/16 (94%)               | 0.48    |
| Antibody profile                    |                          |                           |         |
| MAA (n, %)                          |                          |                           |         |
| Ro                                  | 2/35 (6.7%)              | 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    |
| Pl7                                 | 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    |
| MDAS                                | 0                        | 1/38 (2.6%)               |         |
| HMGCR                               | 4/35 (11.4%)             | 21/178 (11.8%)            | 1.00    |
| #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)
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 \( (100 \text{ vs } 50\%, \ P = 0.03) \) (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 \([1734 \text{ U/l (225–904)}]\) vs \([33 \text{ U/l (147–805)}]\), and higher peak CK levels \([1090 \text{ U/l (187–4597)}]\) vs \([33 \text{ U/l (82.5%)}]\), although this was not significantly different. There was a trend towards lower peak CK levels \([1080 \text{ U/l (993–1689)}]\) vs \([3888 \text{ U/l (1386–9965)}]\), \( P = 0.13 \) 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 \((25\% \text{ vs } 81\%, \ P = 0.05)\) 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 \((10\% \text{ vs } 1\%, \ P = 0.07)\), less myalgia \((33\% \text{ vs } 54\%, \ P = 0.07)\), lower CK median levels \((459 \text{ vs } 823, \ P = 0.06)\) and lower cellularity in the biopsy characterized by less CD68+ \((68\% \text{ vs } 85\%, \ P = 0.07)\) and CD45+ cells \((64\% \text{ vs } 79\%, \ P = 0.10)\) when compared with the anti-FHL1+ counterpart.
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 83% 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
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

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### Table 2 Characteristics of patients with SSc included in the study

| Anti-FHL1 | Anti-FHL1 | P-value |
|-----------|-----------|---------|
| Anti-FHL1+ | n = 12 | 7% | Anti-FHL1+ | n = 162 | 93% |
| Female | 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 obstruction | 0 (0%) | 4 (3%) | 0.82 |
| Barret esophagus | 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 | 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.
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, MDAS 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 data-set 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.

References

1. Lundberg IE, de Visser M, Werth VP. Classification of myositis. Nat Rev Rheumatol 2018;14:269–78.
2. Meyer A, Lannes B, Goetz J et al. Inflammatory myopathies: a new landscape. Joint Bone Spine 2018; 85:23–33.
3. Selva-O’Callaghan A, Pinal-Fernandez I, Trallero-Araguas E et al. Classification and management of adult inflammatory myopathies. Lancet Neurol 2018;17: 816–28.
4. Medsger TA Jr, Dawson WN Jr, Masi AT. The epidemiology of polymyositis. Am J Med 1970;48: 715–23.
5. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344–7.
6. Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292:403–7.
7. Love LA, Leff RL, Fraser DD et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine 1991;70:360–74.
8. Tanimoto K, Nakano K, Kano S et al. Classification criteria for polymyositis and dermatomyositis. J Rheumatol 1995;22:668–74.
9. Targoff IN, Miller FW, Medsger TA Jr, Oddis CV. Classification criteria for the idiopathic inflammatory myopathies. Curr Opin Rheumatol 1997;9:527–35.
10 Hoogendijk JE, Amato AA, Lecky BR et al. 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis. 10-12 October 2003, Naarden, The Netherlands. Neuromusc Disord 2004;14:337–45.

11 Troyanov Y, Targoff IN, Tremblay JL et al. Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. Medicine 2005;84:231–49.

12 Connors GR, Christopher-Stine L, Oddis CV, Danoff SK. 2005;84:231–49.

13 McHugh NJ, Tansley SL. Autoantibodies in myositis. Nat Rev Rheumatol 2011;4:290–302.

14 Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 2016;280:8–23.

15 Albrecht I, Wick C, Hallgren A et al. Development of autoantibodies against muscle-specific FHL1 in severe inflammatory myopathies. J Clin Invest 2015;125:4612–24.

16 Wilding BR, McGrath MJ, Bonne G, Mitchell CA. FHL1 mutants that cause clinically distinct human myopathies form protein aggregates and impair myoblast differentiation. J Cell Sci 2014;127:2269–81.

17 Cowling BS, McGrath MJ, Nguyen MA et al. Identification of FHL1 as a regulator of skeletal muscle mass: implications for human myopathy. J Cell Biol 2008;183:1033–48.

18 Bertrand AT, Bonnemann CG, Bonne G; FHL1 myopathy consortium. 199th ENMC international workshop: FHL1 related myopathies, June 7-9, 2013, Naarden, The Netherlands. Neuromusc Disord 2014;24:453–62.

19 Schessl J, Zou Y, McGrath MJ et al. Proteomic identification of FHL1 as the protein mutated in human reducing body myopathy. J Clin Invest 2008;118:904–12.

20 Gueneau L, Bertrand AT, Jais JP et al. Mutations of the FHL1 gene cause Emery-Dreifuss muscular dystrophy. Am J Hum Genet 2009;85:339–53.

21 Silva AMS, Camelo CG, Matsui-Junior C et al. Child neurology: a case of FHL1-related disease presenting as inflammatory myopathy. Neurology 2021;96:e1383–6.

22 Chahin N, Engel AG. Correlation of muscle biopsy, clinical course, and outcome in PM and sporadic IBM. Neurology 2008;70:418–24.

23 Dalakas MC. Pathophysiology of inflammatory and autoimmune myopathies. Presse Med 2011;40:e237–47.

24 Mastaglia FL, Phillips BA. Idiopathic inflammatory myopathies: epidemiology, classification, and diagnostic criteria. Rheum Dis Clin North Am 2002;28:723–41.

25 Limaye V, Luke C, Tucker G et al. The incidence and associations of malignancy in a large cohort of patients with biopsy-determined idiopathic inflammatory myositis. Rheumatol Int 2013;33:965–71.

26 Isenberg DA, Allen E, Farewell V et al. International consensus outcome measures for patients with idiopathic inflammatory myopathies. Development and initial validation of myositis activity and damage indices in patients with adult onset disease. Rheumatology 2004;43:49–54.

27 Miller FW, Rider LG, Chung YL et al. Proposed preliminary core set measures for disease outcome assessment in adult and juvenile idiopathic inflammatory myopathies. Rheumatology 2001;40:1262–73.

28 Proudmman SM, Huq M, Stevens W et al. What have multicentre registries across the world taught us about the disease features of systemic sclerosis? J Sclerod Related Disord 2017;2:169–82.

29 Limaye V, Bundell C, Hollingsworth P et al. Clinical and genetic associations of autoantibodies to 3-hydroxy-3-methyl-glutaryl-coenzyme a reductase in patients with immune-mediated myositis and necrotizing myopathy. Muscle Nerve 2015;52:196–203.

30 Salomonsson S, Dorner T, Theander E et al. A serologic marker for fetal risk of congenital heart block. Arthritis Rheum 2002;46:1233–41.

31 Liu X. Classification accuracy and cut point selection. Stat Med 2012;31:2676–86.

32 Limaye VS, Lester S, Bardy P et al. A three-way interplay of DR4, autoantibodies and synovitis in biopsy-proven idiopathic myositis. Rheumatol Int 2012;32:611–9.

33 Maundrell A, Proudman S, Limaye V. Prevalence of other connective tissue diseases in idiopathic inflammatory myopathies. Rheumatol Int 2019;39:1777–81.

34 Betteridge Z, Chinoy H, Vencovsky J et al. Identification of a novel autoantigen eukaryotic initiation factor 3 associated with polymyositis. Rheumatology 2020;59:1026–30.

35 Cherin P, Herson S, Creyon MC et al. Mechanisms of lysis by activated cytotoxic cells expressing perforin and granzyme-B genes and the protein TIA-1 in muscle biopsies of myositis. J Rheumatol 1996;23:1135–42.

36 Tanboon J, Sanmaneechai O, Charuvanij S et al. Concurrent positive anti-3-hydroxy-3-methylglutaryl-coenzyme a reductase antibody with reducing body myopathy: possible double trouble. Neuromusc Disord 2019;29:543–8.