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Anti-HMGCR myopathy may resemble limb-girdle muscular dystrophy

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Abstract

Objective
To determine the prevalence and clinical features of anti-HMGCR myopathy among patients with presumed limb-girdle muscular dystrophy (LGMD) in whom genetic testing has failed to elucidate causative mutations.

Methods
Patients with presumed LGMD and unrevealing genetic testing were selected based on a few clinico-pathologic features and tested for anti-HMGCR autoantibodies (n = 11). These clinico-pathologic features are peak creatine kinase (CK) greater than 1,000 IU/L and at least 3 of the following features: (1) limb-girdle pattern of weakness, (2) selective involvement of posterior thigh on clinical examination or muscle imaging, (3) dystrophic changes on muscle biopsy, and (4) no family history of muscular dystrophy.

Results
Six patients tested positive for anti-HMGCR autoantibodies. In 4, there was a presymptomatic phase, lasting as long as 10 years, characterized by elevated CK levels without weakness. Muscle biopsies revealed variable degrees of a dystrophic pathology without prominent inflammation. In an independent cohort of patients with anti-HMGCR myopathy, 17 of 51 (~33%) patients were initially presumed to have a form of LGMD based on clinico-pathologic features but were ultimately found to have anti-HMGCR myopathy. Most of these patients responded favorably to immunomodulatory therapies, evidenced by reduction of CK levels and improved strength.

Conclusions
Anti-HMGCR myopathy can resemble LGMD. Diagnosis of patients with a LGMD-like presentation of anti-HMGCR myopathy is critical because these patients may respond favorably to immunotherapy, especially those with shorter disease duration.

Glossary

ALBIA = addressable laser bead immunoassay; **CK** = creatine kinase; **FSHD** = facioscapulohumeral dystrophy; **IVIg** = intravenous immunoglobulin; **LGMD** = limb-girdle muscular dystrophy; **MRC** = Medical Research Council; **NGS** = next-generation sequencing; **PAS** = periodic acid Schiff; **STIR** = short tau inversion recovery; **WES** = whole exome sequencing; **WGS** = whole genome sequencing.

Limb-girdle muscular dystrophies (LGMDs) encompass a heterogeneous group of hereditary, degenerative myopathies that pose a major diagnostic challenge. Current genomics approaches do not identify a definitive genetic abnormality in 40%–60% of these patients (reviewed here[1]). Although most patients presenting with chronic, slowly progressive myopathies will be presumed to have a hereditary myopathy, and in most cases an LGMD, autoimmune myopathies can also present with a similar chronic disease course (reviewed here[2]).

Autoimmune anti-HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase) myopathy typically has an acute or subacute disease course in older adults with a history of statin exposure[3] and is defined by the presence of serum anti-HMGCR autoantibodies.[4] Yet, young adults and children without statin exposure may also develop anti-HMGCR myopathy, some of whom are anecdotally reported to present with a chronic, LGMD-like phenotype.[5–11] Thus, we hypothesized that some patients with presumed LGMD, specifically those in whom genetic testing has failed to elucidate causative mutations (i.e., "unrevealing genetic testing"), may actually have anti-HMGCR myopathy. We use the term "anti-HMGCR myopathy" to refer to a myopathy associated with anti-HMGCR autoantibodies.[12]

Using a few clinico-pathologic criteria, followed by autoantibody testing, we screened our cohort of patients with clinically suspected LGMD and unrevealing genetic testing and identified 6 patients (1 previously reported[6]) with anti-HMGCR myopathy. Furthermore, in a separate cohort, 17 patients with anti-HMGCR myopathy (~33%) were identified who were initially presumed to have LGMD based on a chronic disease course and clinico-pathologic features. A favorable treatment response could be documented for most patients. In this study, we expand the clinical spectrum of anti-HMGCR myopathy to include a chronic phenotype closely resembling LGMD, with important diagnostic repercussions given the treatment implications.

Methods

Standard protocol approvals, registrations, and patient consents

The National Institutes of Health (NIH) patients were evaluated under research protocols approved by the Institutional Review Boards of National Institute of Neurological Disorders and Stroke (NINDS) (protocol 12-N-0095) or the Undiagnosed Diseases Program, National Human Genome Research Institute (NHGRI) (protocol 15-HG-0130)

between January 2014 and December 2016. Written informed consent and/or assent (for minor patients) was obtained from each participant in the study.

Clinico-pathologic criteria for patient selection

Patients with presumed hereditary myopathy with unrevealing genetic testing (n = 128) were referred to the NIH for additional genetic and diagnostic evaluation. All patients had next-generation sequencing–based LGMD panel testing through commercial laboratories before referral to the NIH. The patients were included for anti-HMGCR autoantibody testing if they had elevated creatine kinase (CK) (peak level > 1,000 U/L) and met at least 3 of the following criteria: (1) limb-girdle pattern of weakness, (2) relatively more prominent involvement of the posterior thigh compartment compared with anterior thigh on manual muscle testing or imaging, (3) chronic myopathic changes as well as myofiber degeneration and regeneration on muscle biopsy, and (4) no family history of muscular dystrophy. These features were purposefully chosen to be compatible with both LGMD and anti-HMGCR myopathy.[6,9,13,14] Most patients had variable CK levels; thus, we included them in the study if they had at least 1 documented CK value greater than 1,000 U/L.

Patient evaluation and testing

Patients underwent clinical evaluation (history and neuromuscular examination), muscle MRI, muscle ultrasound, blood laboratory testing (CK and human leukocyte antigen [HLA] subtype testing), genetic testing, pulmonary function testing, and echocardiogram. DNA, blood samples, and tissue were obtained based on standard procedures. Testing for autoantibodies against HMGCR were performed using Clinical Laboratory Improvement Amendments-certified laboratories (RDL, CA or ARUP, UT). All positive results were confirmed using an immunoprecipitation assay as previously described.[4] The patients reported their overall symptoms and muscle strength using a general clinic questionnaire at each visit. Manual muscle testing using the Medical Research Council (MRC) grade was performed at each visit by at least 2 different neuromuscular neurologists. Discrepant ratings were consolidated at each visit at the bedside. Pediatric patients (P1 and P2) also underwent timed tests (e.g., getting up from supine to the standing position). Some patients also underwent serial handheld dynamometry (P3 and P4) at follow-up visits.

The patients who tested positive for anti-HMGCR autoantibodies (n = 6/11) were treated with immunosuppressive therapies and prospectively followed. The patients and the clinicians were not blinded to the treatments.

Muscle MRI and ultrasound

Muscle MRI was performed using conventional T1-weighted spin echo and short tau inversion recovery (STIR) of the lower extremities on a 3.0T Achieva Phillips, 3.0T Verio Siemens, or 1.5T Aera Siemens system. Noncontrast images were obtained from the pelvis, thighs, and lower legs in the axial plain. Slices were 5–10 mm thick. The gap between slices was 8–10 mm thick. Muscle ultrasound was performed using an upgraded Siemens S2000 with a 15 MHz linear probe.

Molecular genetic testing

Of the anti-HMGCR–positive patients (n = 6), 3 patients (P2 and her mother, P4 and his parents, P5 and his parents and unaffected sibling) had research-based whole exome sequencing (WES) testing at the NIH Intramural Sequencing Center using the SeqCap EZ Exome + UTR Library Kit (Roche, Nimblegen) and Illumina HiSeq2500 sequencing instruments. One patient (P6) underwent clinical WES (Baylor University) and whole genome sequencing (WGS) (HudsonAlpha, Huntsville, AL). All anti-HMGCR negative patients (n = 5) underwent WES at the NIH. Filtering of variants was carried out for 4 different segregation scenarios (de novo, recessive homozygous, recessive compound heterozygous, and isolated singleton proband) using a customized SQL script with the following parameters: minimum allele frequency less than 0.5% in the Exome Aggregate Consortium, National Heart, Lung, and Blood Institute (NHLBI) Exome Variant Server databases, and in the laboratory's aggregate exome variant database with 587 exomes; Combined Annotation Dependent Depletion score greater than 20; and coverage greater than 10 reads.

Validation cohort patients

The patients in the validation cohort were evaluated at the National Referral Center for rare neuromuscular diseases at the Pitié-Salpêtrière University Hospital (Paris, France) between 2000 and 2017. All patients with anti-HMGCR myopathy who were initially clinically suspected of having LGMD were identified. These patients had been evaluated as part of routine clinical visits in the outpatient clinic or during inpatient hospitalization at the Institute of Myology or internal medicine ward. Medical records were reviewed to assess clinical features, serum CK levels, muscle biopsy findings, electrodiagnostic studies, muscle MRI, and response to immunosuppressive therapies. Molecular and/or genetic testing for LGMD or other common hereditary myopathies was reviewed. Anti-HMGCR antibodies were identified using ELISA (Inova Diagnostics, Inc, San Diego, CA) or addressable laser bead immunoassay (ALBIA) (Rouen, France).[15] All of these patients also had a dot blot myositis profile (including anti-Jo1, -PL7, -PL12, -Pm/Scl, -Scl70, -Ku, -SRP, and -Mi-2 autoantibodies) using line immunoassays (Euroimmun—Germany or D-Tek—Belgium).

Data availability

Additional de-identified clinical data pertaining to this study are available on request from the authors.

Results

Identification and characterization of patients

Eleven patients met the clinico-pathologic entry criteria as described in the Methods section, 6 of whom (1 previously reported[6]) had positive anti-HMGCR autoantibodies, with titers 2–10 times the upper limit of normal. None of the 6 patients with anti-HMGCR myopathy tested positive for anti-SRP autoantibodies or other myositis-specific autoantibodies when tested. The summary of relevant clinical findings in the patients with anti-HMGCR autoantibodies is provided in table 1. In contrast to classic cases of anti-HMGCR myopathy, the disease course was indolent in these patients. Elevated CK, aspartate transaminase, or alanine transaminase (presumably originating from muscle), often preceded the onset of overt muscle weakness, in some cases by more than 10 years. None of the patients took statin drugs, but 1 patient (P6) took mushroom supplements (a natural source of statins) before developing muscle weakness.

A predominantly proximal pattern of muscle weakness was notable in all patients with near-complete sparing of distal muscles (e.g., anterior tibial group). Lower extremity weakness preceded upper extremity weakness and was more severe. Moderate to severe scapular winging was noted in 5 of the 6 patients, without selective involvement of scapular fixators. At the time of presentation, P1-P4 remained independently ambulatory. P5 relied on the assistance of a cane for outdoor ambulation, whereas P6 relied on a motorized wheelchair. Neck flexion, arm abduction (deltoid), elbow flexion, and to a lesser degree elbow extension were affected in the upper extremities. In the lower extremities, hip flexion, hip adduction, hip extension, hip abduction, knee flexion, and to a lesser degree knee extension were most commonly affected. Extraocular and facial muscles were spared.

Muscle imaging

Muscle MRI of the lower extremities showed a common pattern of involvement. Patients with shorter duration of disease showed minimal changes in T1 signal in the thighs or lower legs, whereas those with longer duration of disease showed T1 hyperintensity in paraspinal muscles, glutei, hamstrings, and adductors with variable involvement and atrophy of the quadriceps muscles. The gracilis muscle was relatively preserved (figure 1, A). In the lower leg, the pattern was more variable, with the medial gastrocnemius showing increased T1 signal in a heterogeneous and patchy distribution (3 of 6 patients). The tibialis anterior was relatively preserved (figure 1, B). In addition, thigh and lower leg muscles had hyperintense STIR signal with a heterogeneous and patchy distribution (figure 1, C). STIR signal hyperintensity was not limited to areas of T1 hyperintensity, suggestive of ongoing disease activity.

Muscle ultrasound showed an overall patchy and granular pattern of increased echogenicity in the patients with a shorter disease duration and more diffusely echodense muscles in those with longer disease duration. In the upper extremities, ultrasound

Table 1 Clinical characteristics of NIH patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	M	F	F	M	M	F
Age (y)	13.5	12	30	36	41	48
First sign, age (y)	Decline in running speed, 10	Could not jump or run, 8[a]	Elevated CK, 19	Difficulty rising from the floor, 20[b]	Elevated LFTs, 21	Dysphagia and elevated CK, 25
Disease duration (y)	3.5	4[a]	11	16[b]	20	23
CK (IU/L)	7,000–9,000	13,000–23,000	3,000–10,000	350–1,200	5,000	3,000–11,000
EMG/NCS	ND	Irritable myopathy	Irritable myopathy	Irritable myopathy	Irritable myopathy	Irritable myopathy
Genetic and molecular testing	Extended neuromuscular panel	LGMD panel and WES	LGMD panel and GAA dried blood test	LGMD panel; FSHD; DM2; and WES	LGMD panel; WES; and GAA dried blood test	LGMD panel; MFM panel; WES; and WGS
HLA-DRB1	07:01; 15:01	11:01; 11:02	11:01; 11:03	11:01; 11:04	01 or 15 (Amb)	07:01; 13:01
Echocardiogram	Normal	Normal	Normal	Normal	Normal	Normal
PFT	FVC 3.08 L (81%)	FVC 2.00 L (71%)	FVC 2.49 L (78%)	FVC 4.64 L (77%)	FVC 3.69 L (74%)	FVC 2.80 L (84%)
Other comments	Linear morphea scleroderma	Exotropia noted in early childhood	Dural sinus thrombosis while on oral contraceptive pills	Liver biopsy showed mild fatty liver disease; obstructive sleep apnea on BiPAP	Liver biopsy with normal results	Took mushroom supplements in her 20s; had bilateral calf herniation at 13 y

Abbreviations: Amb = ambiguous; CK = creatine kinase; DM2 = myotonic dystrophy type 2; FSHD = facioscapulohumeral muscular dystrophy; FVC = forced vital capacity; GAA = glucosidase alpha acid; LFT = liver function test; LGMD = limb-girdle muscular dystrophy; MFM = myofibrillar myopathy; ND = not done; PFT = pulmonary function test; WES = whole exome sequencing; WGS = whole genome sequencing.
[a] The patient was never able to ride a bike independently (normally achieved by age 5 years or earlier) or play with monkey bars in the playground.
[b] The patient was never able to perform a push-up or pull-up and was a slow runner in his teens.

highlighted selective involvement of certain muscle groups (e.g., biceps more than triceps) (figure 2).

Muscle histology

Severe myofiber atrophy, fiber size variability, and increased internalized nuclei were nearly universal findings (figure 3). Most biopsies included other chronic myopathic features such as splitting myofibers and increased endomysial fibrosis. Myofiber degeneration/necrosis and regeneration was a variable feature. Some biopsies showed many degenerating/necrotic myofibers, some actively undergoing myophagocytosis (P2), whereas others had only a single degenerating fiber (P4). A few biopsies showed a single or 2 small foci of chronic perivascular inflammation composed of macrophages and CD3-positive T cells. None of the biopsies showed prominent primary inflammation—nonnecrotic myofibers surrounded and/or invaded by chronic inflammatory cells. MHC-1 was increased in degenerating fibers and rare nonnecrotic fibers, but it appeared normal or only minimally increased in other areas. None of the patients had diagnostic changes in known LGMD proteins based on immunostaining.

A few additional nonspecific histologic findings were noted: muscle biopsy of P6 had inclusions in some muscle fibers that stained positive with periodic acid Schiff (PAS) stain, as well as with desmin, myotilin, and αβ-crystalllin immunostains. These PAS-positive inclusions were α-amylase sensitive and did not stain with adenosine triphosphatase or oxidative stains, suggesting that they do not contain polyglucosan bodies, myosin, or intermyofibrillar material. A single subsarcolemmal red-rimmed vacuole was noted in the muscle biopsy of P4.

Genetic testing

All anti-HMGCR–positive patients (n = 6) had undergone commercial genetic testing for common LGMDs using next-generation sequencing (NGS) panels before referral to the NIH (table 1). Because of the presence of asymmetric findings and scapular winging, 2 patients were tested for facioscapulohumeral dystrophy (FSHD). Other patients lacked typical features of FSHD and were not directly tested. Because of the preponderance of internalized nuclei in P4, myotonic dystrophy type 2 was also considered and ruled out by direct testing. Four patients also underwent WES, 3 at the NIH (P2, P4, and P5) and one through a commercial laboratory (P6). Because of the presence of myofibrillar inclusions in P6's muscle biopsy and a suspicion for an underlying myofibrillar myopathy, she also underwent WGS. None of the patients had pathogenically relevant variants (excluding benign variants) that matched familial segregation studies or mode of inheritance of the disease associated with the gene in question.

Figure 1 Muscle MRI
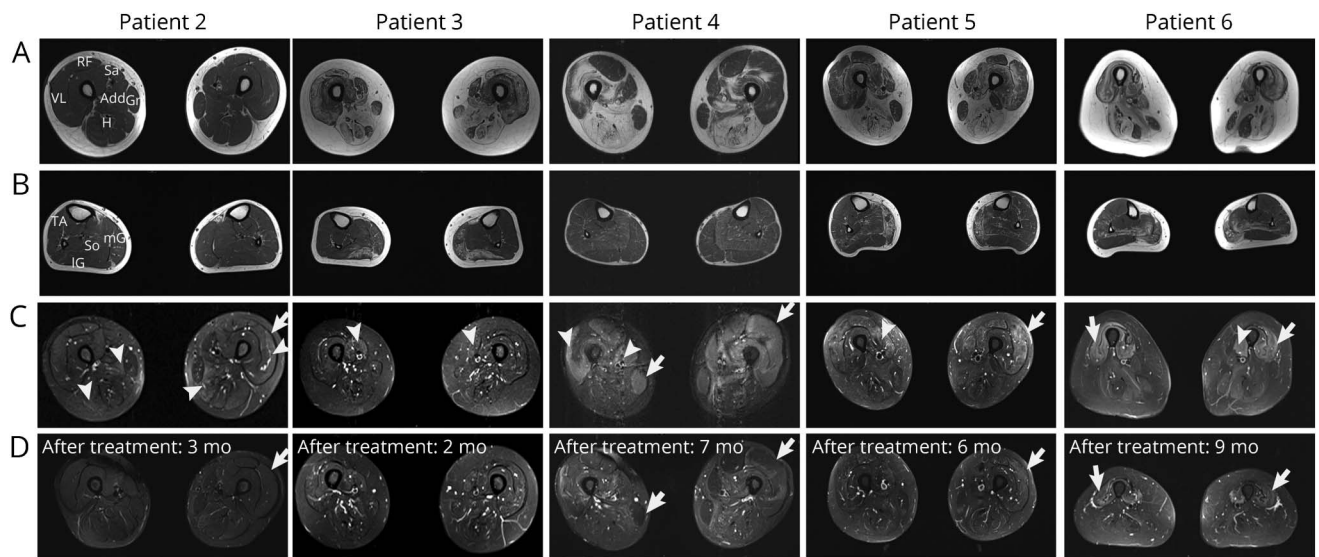
Muscle MRI showing increased T1 hyperintensity in the hamstrings, adductors, and variably in quadriceps muscles. Gracillis muscle appears relatively preserved (panel A). Medial gastrocnemius is variably involved in the lower leg (panel B). STIR signal is increased predominantly in the hamstrings, quadriceps, and adductor muscles (C, arrowheads). After treatment, STIR signal is decreased in most patients, especially those evaluated later after initiation of treatment (D, arrows). VL = vastus lateralis; RF = rectus femoris; Sa = sartorius; Add = adductor magnus; Gr = gracilis; H = hamstrings; TA = tibialis anterior; So = soleus; mG = medial gastrocnemius; lG = lateral gastrocnemius.

Most (∼70%) adult patients with anti-HMGCR myopathy possess the HLA-DRB1*11:01 allele,[16,17] whereas HLA DRB1*07:01 is recently reported in several pediatric patients with anti-HMGCR myopathy.[7] We tested these 6 patients for the presence of either allele. All but 1 patient had the HLA-DRB1*07:01 or 11:01 allele (table 1).

Follow-up after treatment

After confirmation of anti-HMGCR myopathy, all patients (n = 6) were treated with IV immunoglobulins (IVIg) and seen in follow-up at the NIH. Steroids (methylprednisolone, 750 mg every 3 weeks) were added to P6's regimen after 4 months of IVIg therapy. The patients were re-examined, CK was remeasured, and a muscle MRI was repeated. Most patients reported improved function and improved muscle strength. Manual muscle testing using MRC grading or handheld dynamometry showed improvement in muscle strength, at least in select muscle groups (table 2). P2 also showed significant improvement in timed rise from the floor, a commonly used outcome measure in pediatric muscular dystrophy.[18] All patients showed a marked reduction in CK levels. Most patients also had a marked reduction in muscle MRI STIR signal intensity (figure 1, D). The pediatric patients (n = 2; mean age 12 years), who had a shorter duration of symptoms before initiation of therapy, demonstrated the most dramatic improvements in strength and function to normal or near-normal levels (table 2).

Figure 2 Muscle ultrasound
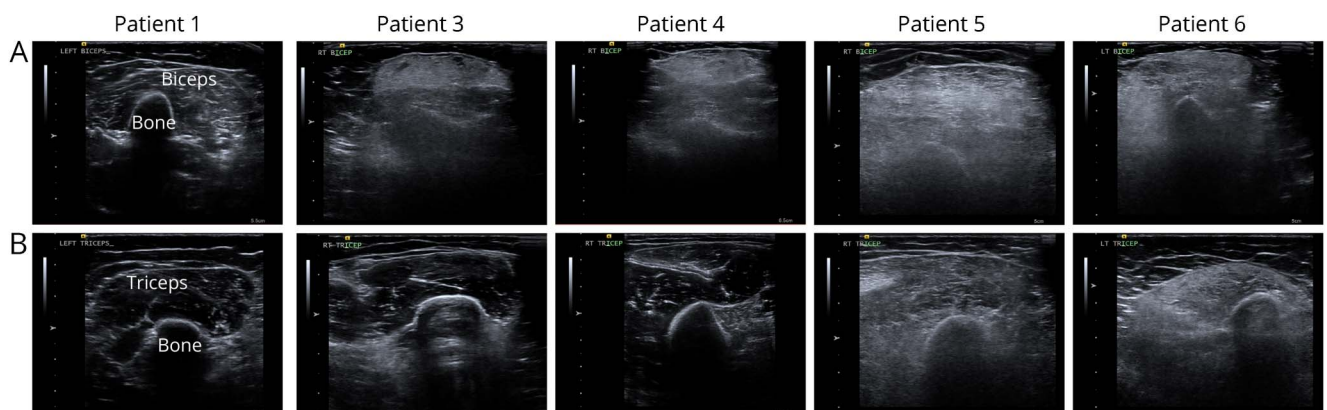
Muscle ultrasound showing selective involvement and increased echogenicity in the biceps muscle (row A) when compared with the triceps muscle (row B).

Figure 3 Muscle biopsy showing myofiber atrophy and degeneration
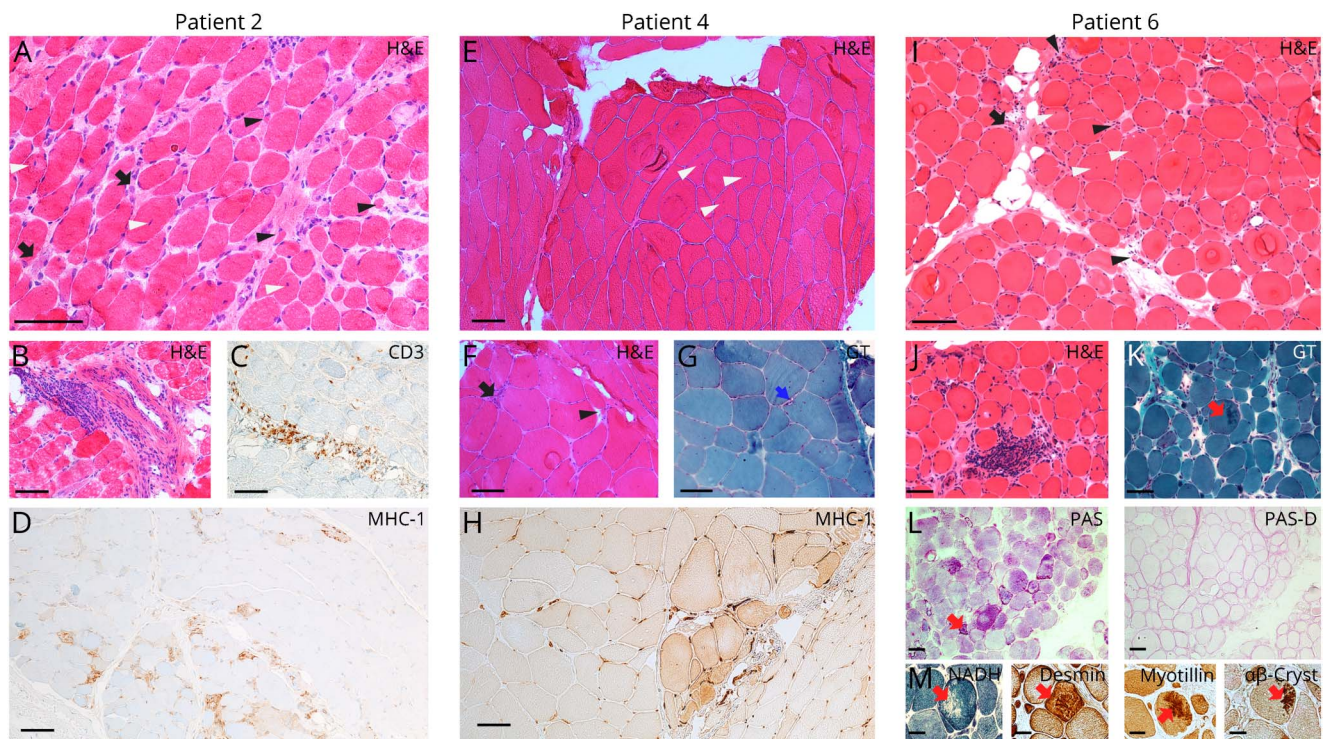
Muscle biopsy showing profound myofiber atrophy (black arrowhead, A, F, and I), myofiber degeneration (black arrows, A, F, and I), internalized nuclei (white arrowhead, A, E, and I), and increased endomysial fibrosis. Rare foci of chronic perivascular inflammation were noted in a few patients (B and J), consisting of macrophages, and CD3-positive T cells (C). Whorled fibers, a chronic myopathic change, were noted in patient 4 (E). A single red-rimmed vacuole was noted in patient 4 (blue arrow, G). Major histocompatibility complex-1 (MHC-1) immunostain was increased in degenerating fibers and nonspecifically in limited areas of the muscle biopsy (D and H). Patient 6 muscle biopsy had several myofibers with inclusions (red arrows K, L, and M) that stained positive with desmin, myotilin, αβ-crystallin, and periodic acid Schiff (PAS) but not after treatment with diastase (PAS-D). The inclusions did not stain positive on NADH stain. Scale bar = 100 μm (A–I, L); Scale bar = 50 μm (J, K, M).

After 1 year of follow-up, all patients remain on immunosuppression, and most continue to report improvement or stabilization of weakness; none report worsening weakness. P6 is able to ambulate with a walker for the first time in 2 years. P2 reports regaining the ability to run. P1 attempted to lengthen the treatment interval of IVIg to every 5 weeks and developed elevated CK levels (~1,100 U/L). A few months later, he noted a decline in athletic performance. Two months after returning to

Table 2 Response to treatment in patients with limb-girdle phenotype of anti-HMGCR myopathy

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Treatment	IVIg ~1.5 g/kg monthly	IVIg ~2g/kg monthly	IVIg ~2g/kg monthly	IVIg ~2g/kg monthly	IVIg ~2g/kg monthly	IVIg ~1.5g/kg + 750 mg methylprednisolone every 3 wk
Interval before re-evaluation	30 mo	18 mo	18 mo	7 mo	6 mo	9 mo
Reported functional improvement	Running speed	Walking speed	Walking speed	Rising from the floor	Walking speed and raising arms above the head	Rolling over in the bed, gait stability, walk with a walker, and raising arms
Manual muscle testing	Improved to normal	Improved	Improved	Stable/improved in select muscles only	Stable/improved in select muscles only	Stable/improved in select muscles only
CK before/after treatment (IU/L)	3,789/509	13,270/6,205	3,876/971	351/166	4,993/450	3,222/296
MRI STIR	Normalized	Normalized	Unchanged	Improved	Improved	Improved

Abbreviations: AA = arm abduction; CK = creatine kinase; EF = elbow flexion; HA = hip abduction; HE = hip extension; HF = hip flexion; IVIg = intravenous immunoglobulin; KE = knee extension; STIR = short tau inversion recovery; WE = wrist extension; WF = wrist flexion.

his previous IVIg schedule (every 4 weeks), his CK has decreased (~800 U/L), and his athletic performance has improved.

Validation cohort

We subsequently investigated another patient cohort in a large neuromuscular referral center in France. We asked the reverse question: how prevalent is a chronic, LGMD-like presentation among patients with already-established anti-HMGCR myopathy? Among 51 anti-HMGCR myopathy cases examined, 17 patients (~33%) were initially suspected to have LGMD based on clinical findings and muscle biopsy criteria, all without genetic diagnostic confirmation. All 17 patients underwent specific molecular or genetic testing for LGMD (including dystrophin [DMD], dysferlin [DYSF], sarcoglycans [SGCA, SGCB, SGCG, and SGCD], calpain-3 [CAPN3], caveolin-3 [CAV3], anoctamin 5 [ANO5], fukutin-related protein [FKRP]), or other hereditary myopathies such as acid maltase deficiency or type 2 myotonic dystrophy. These 17 patients had a clinical presentation and course of disease evolution similar to the initial cohort (table 3). Most patients had a prolonged disease course that initially presented with asymptomatic or oligosymptomatic hyper-CKemia, exercise intolerance, or myalgia (table e-1, links.lww.com/NXI/A90). A third of the patients had scapular winging. In those who had individual muscle group strength data available, the majority (~62%) had weakness of knee flexion greater than extension; however, 3 (23%) had knee extension greater then flexion weakness, whereas there was no difference in 2 (15%) patients. All patients displayed markedly elevated CK levels (mean: 6,580 U/L). Muscle histologic findings included myofiber degeneration and regeneration in

all patients and chronic myopathic changes in most patients. Most patients were treated with various regimens and immunosuppressive agents, although a few patients declined treatment, typically those with only mild weakness or asymptomatic hyperCKemia. Only a minority of these patients had exposure to statins (~24%). Overall, most patients treated with immunosuppressant therapies demonstrated improved muscle strength and functional capacity, especially those with relatively shorter disease duration (table e-2, links.lww.com/NXI/A91). Some patients with prolonged disease duration and evidence of severe muscle damage documented on muscle MRI did not show clear improvements in muscle strength testing. However, a decline in CK levels was seen in all treated patients. At the last follow-up, most of these patients (10 of 13; 77%) were still receiving IVIg either as monotherapy or in combination with another immunosuppressant.

Discussion

We have characterized in detail a less recognized yet clinically important phenotype of anti-HMGCR myopathy that mimics LGMD. In our cohort of patients with clinically suspected LGMD and unrevealing genetic testing, we preselected patients using 5 simple clinico-pathologic features and found a relatively high proportion (n = 6/11; ~55%) of patients with anti-HMGCR autoantibodies. These patients had a favorable response to immunosuppressive therapy. Because we preselected patients based on clinical features before autoantibody testing, we may have missed additional patients with anti-HMGCR myopathy with unexpected presentations and underestimated the overall prevalence in this cohort.

The overall clinico-pathologic presentation of these patients was indistinguishable from other patients with LGMD, including those with genetic confirmation. Illustrating this point, in the patients who tested negative for anti-HMGCR autoantibodies (n = 5/11), we subsequently found a patient with a second CAPN3 deletion mutation that was missed on the original NGS panel testing and another patient with GMPPB mutations, a gene that was not included in the NGS panel.

Analyzing an independent patient cohort with established anti-HMGCR myopathy, we found an ~33% prevalence for a LGMD-like presentation in these patients. The clinical features, disease course, and histopathologic findings of these patients were similar to the initial cohort (table 3). Thus, these patients independently corroborate the chronic, LGMD-like phenotype in a large subset of patients with anti-HMGCR myopathy. The preselection clinical criteria in the initial cohort were also found in most validation cohort patients; however, the pattern of weakness in the posterior thigh was not universally present. Thus, in future studies, less restrictive patient selection criteria may help identify additional patients.

A few criteria have been proposed to aid in establishing pathogenicity of an autoantibody (reviewed here[19]). Detecting

Table 3 Clinical characteristics and response to treatment in the validation cohort

Age (y); mean	17–66; 40
Disease duration (y); mean	1–26; 11
Female	N = 14/17 (82%)
Limb-girdle weakness	N = 16/17 (94%)
Scapular winging	N = 6/17 (35%)
Knee flexion weakness > knee extension weakness	N = 8/13 (62%)
Peak CK (U/L); mean	1,200–17,000; 6,580
Myopathic EMG	N = 17/17 (100%)
Muscle biopsy findings of chronic myopathy	N = 15/17 (88%)
Statin drug exposure	N = 4/17 (24%)
Improved strength after immunotherapy	N = 11/13 (85%)
CK after treatment (U/L); mean[a]	30–600; 201
IVIg at the last follow-up	N = 10/13 (77%)

Abbreviations: CK = creatine kinase; EMG = electromyography; IVIg = intravenous immunoglobulin.
[a] Checked in 13 patients.

anti-HMGCR autoantibodies falls short of providing definitive proof for an autoimmune disease in these patients, but our inference establishing this connection is supported by other data.

Anti-HMGCR autoantibodies are not found in healthy individuals or those with self-limited statin intolerance/myopathy.[20] Most importantly, genetically proven muscular dystrophy patients do not test positive for anti-HMGCR antibodies.[21] To exclude the possibility of ELISA false positives (estimated at ∼0.7%),[20] we confirmed the presence of anti-HMGCR autoantibodies in all the NIH LGMD-like patients using the gold standard immunoprecipitation assay. Similarly, the French anti-HMGCR cohort was tested using either a validated ELISA or ALBIA test.

CK levels are closely associated with disease activity in anti-HMGCR myopathy,[10,22] and all treated patients in our study showed unequivocal improvement in CK levels (tables 2 and 3). This was the case even in those patients never treated with steroids because steroids can result in nonspecific CK reduction irrespective of disease etiology. Notably, the very few patients in the validation cohort who did not pursue treatment had persistently elevated CK levels. Most treated patients also had functional gains or improvement on manual muscle strength testing and/or MRI STIR signal intensity (table 2 and figure 1, D). These improvements were most notable in those with shorter duration of disease who received immunotherapy within 4 years of symptom onset. The stabilization and/or improvements documented in these patients would be counter to the natural history of nearly all known LGMDs, especially in patients with greater than 1 year of follow-up. In parallel, the partial relapse of P1 after lengthening of IVIg interval and his subsequent improvement after return to the previous dosing interval also suggest a treatment response directly attributable to immunotherapy.

The frequency of 2 immunogenetic alleles, HLA-DRB1*11:01 and 07:01, is increased in adult and pediatric patients with subacute onset anti-HMGCR myopathy, respectively.[7,16,17] In the NIH cohort that was systematically tested for these HLA subtypes, all but 1 patient had either the HLA-DRB1*11:01 or 07:01 allele, which is much higher than what would be expected in the general population, providing further support for anti-HMGCR myopathy with a distinct phenotype in these patients.

It is conceptually possible that anti-HMGCR myopathy can co-occur with a genetic muscular dystrophy or even be triggered by it. The NIH anti-HMGCR–positive patients underwent extensive genetic testing by NGS panels (n = 6), WES (n = 4) and/or WGS (n = 1). The Salpêtrière validation cohort patients had less extensive but more directed molecular and genetic testing. Although these genetic tests were unrevealing, we cannot exclude this possibility in our cohort with absolute certainty, in part because of the limitations of current sequencing technologies; however, our findings make this possibility doubtful.

Our findings on muscle imaging suggest that without treatment, fatty replacement of muscle increases as the disease progresses, limiting the opportunity of a clinical response to treatment in later stages. There are very few reports about treatment of patients with unexplained hyperCKemia and anti-HMGCR myopathy[23]; however, it can be hypothesized that initiating immunotherapy during this stage may have the potential of preventing the clinical manifestations of the disease and require less aggressive immunosuppressive regimens. In our experience, regardless of disease duration, immunotherapy still provided a tangible benefit and, at the very least, seems to have prevented a further decline in motor function in patients with LGMD-like anti-HMGCR myopathy. Nonetheless, given the small number of patients and the observational nature of our study, treatment decisions should be individualized until more conclusive, prospective, and controlled studies are conducted. Long-term follow-up of patients is necessary to evaluate the possibility and timing of weaning or discontinuing immunosuppression in this patient population.

We propose testing for anti-HMGCR autoantibodies as part of the evaluation of all patients with suspected LGMD with unrevealing genetic testing or those with asymptomatic hyperCKemia without a family history. If the diagnosis of anti-HMGCR myopathy is serologically confirmed, treatment with immunosuppressant therapies should be considered.

Author contributions

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