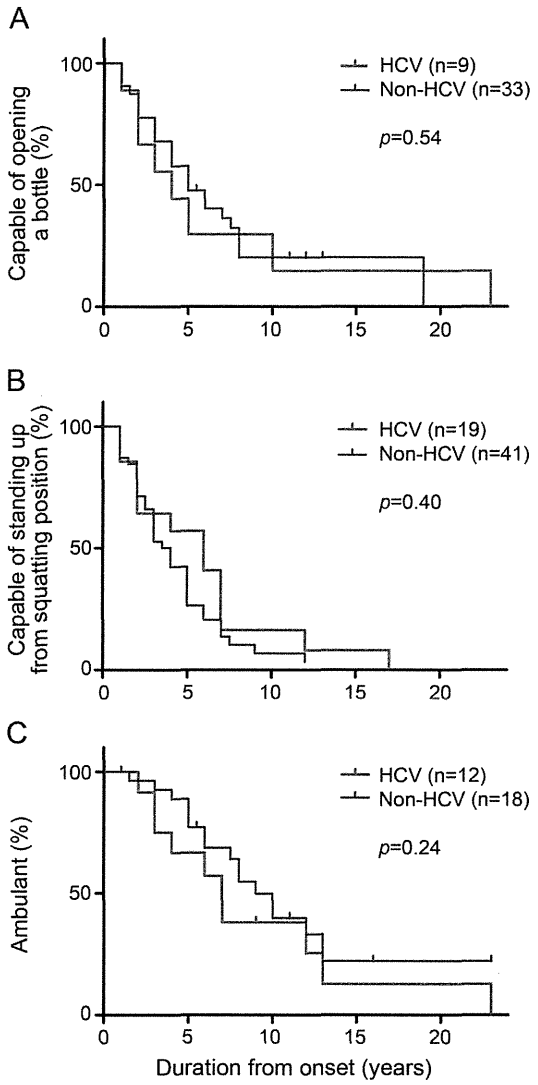
Figure 3 Development of symptoms



Kaplan-Meier curves to compare the durations from onset to each following status of patients with inclusion body myositis with hepatitis C virus (HCV) with that of the patients without HCV infection (non-HCV): inability to open a plastic bottle (A), inability to stand up from squatting position (B), and loss of ambulation (C). Medians of the periods were 4 vs 5 years, 6 vs 4 years, and 7 vs 9 years, respectively.

(1/44) of the patients with PM (OR 0.80, 95% CI 0.071–9.1). Two patients with IBM had both anti-HCV and anti-HTLV-1 Ab, and one had both anti-HCV Ab and HBV antigens.

Clinical and pathologic features of patients with IBM with HCV infection. Sex ratio, age at onset, serum creatine kinase level, and frequency of concurrent autoimmune diseases and detection of autoantibodies (antinuclear antibodies, anti-SS-A antibodies, and anti-SS-B antibodies) showed no significant difference between the IBM groups with and without anti-HCV Ab (table 1). There were no significant differences in the speed of development of the disease (figure 3).

No significant differences were observed in terms of the frequency of fibers with RVs and the myofiber staining pattern on immunohistochemistry for MHC class 1 and 2 (table 1).

Individual data of each subject are described in appendix e-2.

HCV infection in patients with IBM. Information about the development of muscle symptom after treatment for HCV was available in 29 patients with IBM with anti-HCV Ab. Among them, 15 patients had received treatment for HCV prior to the onset of muscle symptoms, and the most common drug was interferon (IFN)–α (table e-1). Of particular interest, patient 9 developed the symptom of IBM during the first course of IFN-α treatment, and it was aggravated during the second course. Similarly, patient 32 manifested the initial muscle symptoms during treatment with IFN-α with ribavirin. None of the 26 patients whose relevant data were available showed improvement in muscle weakness after treatment for HCV infection.

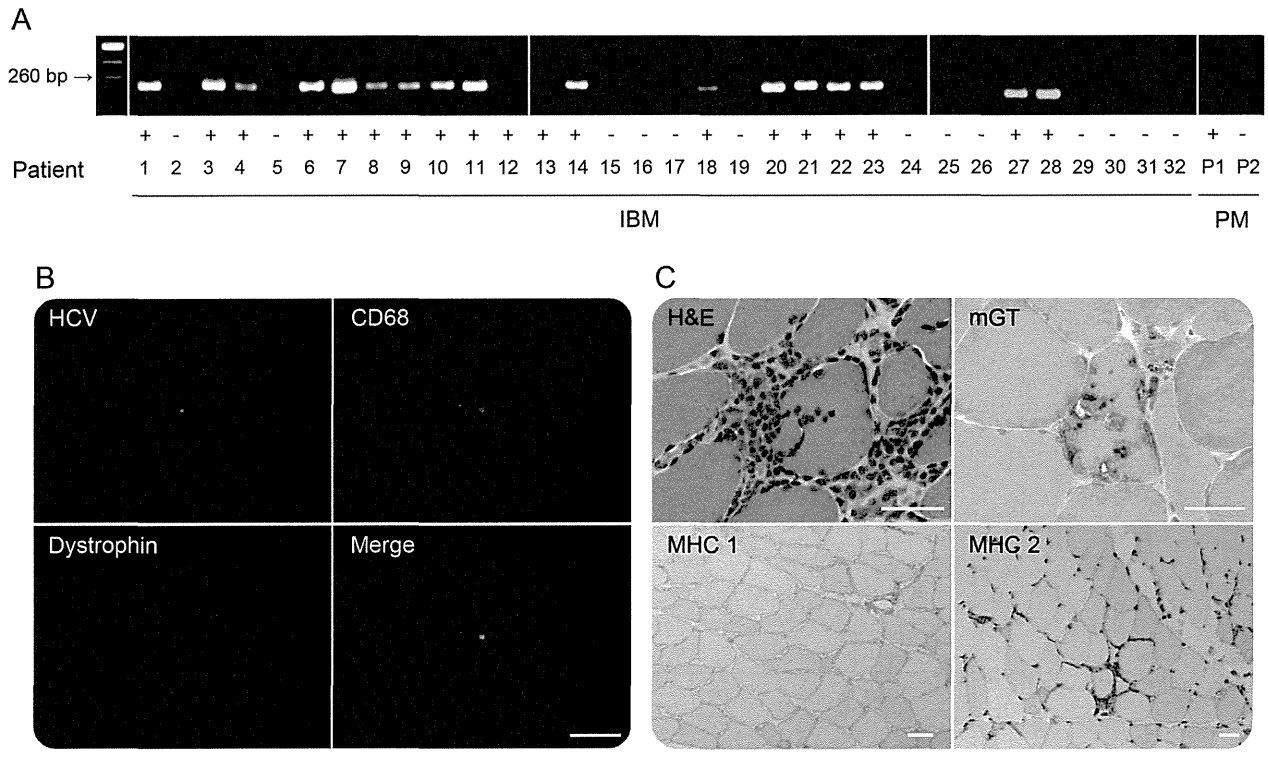
The data for genotype/serotype of the virus in serum were available in 11 patients: 7 had type 1 and 4 type 2 (table e-1).

RT-PCR for HCV-RNA in muscle tissues. HCV-RNA in the muscle specimens was detected in 19 (59%) of 32 patients with IBM with anti-HCV Ab (figure 4A). Meanwhile, HCV-RNA in muscle was found in 20 (95%) of 21 patients without IBM with anti-HCV Ab ($p = 0.0040$). In one of the 2 patients with PM with anti-HCV Ab, HCV-RNA was detected.

Immunohistochemistry for HCV peptide. To confirm the localization of HCV in muscles, we performed immunohistochemistry against HCV peptides. HCV peptides were detected in endomysium, mostly being localized within macrophages (figure 4B), but never within muscle fibers or T cells (not shown).

DISCUSSION Our study demonstrated significantly higher prevalence of HCV infection in the IBM cohort (28%) compared to the age-matched PM cohort (4.5%). The prevalence of HCV infection in the general Japanese population in their 60s in 2000 was estimated to be 3.4%,[15] indicating that the prevalence among patients with PM is virtually the same as that of the general population, while that among patients with IBM is one digit higher. These facts demonstrate a clear association between IBM and HCV infection. Nevertheless, clinical progression and extent of pathologic findings were essentially identical between HCV-positive and -negative patients with IBM, suggesting that HCV infection is most likely not a key determinant factor to develop IBM but rather serves as one of the triggers. This notion is in fact compatible with the fact that anti-HCV Ab were

Figure 4 HCV in muscle tissues



(A) RT-PCR for hepatitis C virus (HCV)–RNA in frozen muscle samples from patients with inclusion body myositis (IBM) and patients with polymyositis (PM) with anti-HCV antibodies (Ab). The 260-bp bands correspond to HCV-RNA. (B) Representative pictures of immunohistochemistry for HCV peptides (patient 14). HCV peptides are localized within a CD68-positive macrophage. Scale bar denotes 20 μm. (C) Muscle pathology of IBM shows endomysial mononuclear cellular infiltration on hematoxylin & eosin stain (H&E), rimmed vacuoles on modified Gomori trichrome stain (mGT), and major histocompatibility complex (MHC) 1 and 2 expression in muscle fibers. Scale bars denote 20 μm in (B) and 50 μm in (C).

negative in 72% of patients with IBM in our cohort. Interestingly, several articles reported the development of IBM in patients with retroviral infections such as HTLV-1 and HIV,[16–20] raising a possibility that some kinds of virus infection can be a trigger for IBM, although further studies are necessary to be more conclusive.

HCV infection is associated with not only hepatic diseases but also extrahepatic, often autoimmune, diseases, including mixed cryoglobulinemia, Sjögren syndrome, autoimmune thyroid diseases, lymphoproliferative disorders, diabetes mellitus, renal diseases, porphyria cutanea tarda, and oral lichen planus.[21] In mixed cryoglobulinemia and B-cell non-Hodgkin lymphoma, capture of HCV viral particle by V_H1-69+ B1 cells and marginal-zone B cells and following B-cell clonal expansion is considered a main cause.[22] Although underlying pathomechanism is not well-established in the other conditions, HCV infection may potentially elicit immunogenicity in humans.[23]

Interestingly, HCV-RNA was much less often detected in muscles from patients with IBM with HCV infection compared to patients without IBM with HCV infection, although this should be interpreted carefully due to possible contamination of infected blood. One possible interpretation would be that the pathogen may have been cleared when autoimmune disease with insidious onset with considerable subclinical period, such as IBM, becomes clinically evident, although the virus plays some pathomechanistic roles such as bystander activation.[24] Alternatively, HCV infection may not be directly involved in the pathogenesis of IBM.

Focusing on the localization of HCV in muscle tissues, viral peptides were seen within endomysial macrophages, not within muscle fibers, as in the case of IBM with HTLV-1/HIV infection.[16] In IBM with HTLV-1/HIV infection, viral-positive endomysial macrophages are considered to possibly facilitate the autoimmune process by secreting proinflammatory cytokines.[17]

Notably, 2 patients developed IBM during treatment for HCV infection (the only common drug was IFN-α). In addition, one case report described a patient with IBM with a similar clinical course.[8] Type I IFNs have potential to stimulate autoreactive memory B cells, leading to their differentiation into autoreactive plasmablasts and subsequent increased production of autoantibodies.[25] Therefore, type I

IFNs are considered to be associated with amplification of autoimmunity in autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis.[25] Interestingly, during the last decade, several studies have identified B-cell signatures in IBM pathogenesis, including the discovery of an autoantibody.[26,27] These facts may suggest that the use of IFN-α increased the risk for development or aggravation of IBM. However, the evidence is anecdotal and we cannot conclude an association between IFN-α therapy and IBM. Further studies are necessary to elucidate this issue.

The proportion of genotype/serotype of HCV in our IBM cohort would be equivalent to that of the general Japanese population: genotypes 1 and 2 are 70% and 30%, respectively.[28] Implication of a specific type of the virus with IBM pathogenesis seems less likely, although the available data were limited.

While there was no significant difference between the groups of IBM and PM, the prevalence of HTLV-1 infection in each group is higher than in the general population, although the prevalence in the PM group was obtained from a limited number of subjects (the prevalence in general Japanese people aged 60–64 years in 2006–2007 was estimated to be 1.5% in men and 1.7% in women).[29] Our results may be supportive of the theory that HTLV-1 infection is associated with both IBM and PM.[17,30]

While there has been controversy about diagnostic criteria for IBM,[1,31] the 2011 ENMC IBM Research Diagnostic Criteria were established under consensus of experts highly experienced in dealing with IBM.[10] They require all the positive pathologic findings to categorize a patient with clinicopathologically defined IBM.[10] However, analysis for aberrant protein accumulation and electromicroscopic observation would not be performed frequently in clinical practice except for specialized institutes. Notably in this study, all 138 patients with endomysial inflammatory infiltrate, which we judged as positive only when mononuclear cells surrounded and occasionally invaded non-necrotic fibers (figure 4C), and RV showed cytoplasmic accumulation of aberrant protein (p62 was detected in all). Furthermore, all 116 patients whose relevant clinical information was provided fulfilled clinical features for clinicopathologically defined IBM in the criteria except for one patient who was slightly younger than the age limit, suggesting that the presence of the strictly judged endomysial inflammatory cell infiltration and RV may be enough to make a diagnosis of IBM.

We are aware of a few limitations to this study. First, there may be a selection bias. We could not receive replies of the questionnaire survey from the physicians who saw 17 of 138 patients with IBM pathology. Physicians who have encountered a patient with IBM with HCV infection may have tended to reply more willingly, resulting in overestimation of the prevalence of HCV infection, although even if all of the no-reply cases were IBM without HCV infection, the prevalence (24.4% [32/131]) would be significantly higher than the control (OR 6.8, 95% CI 1.6–30). Second, in order to make a genuine IBM cohort, we applied the ENMC criteria strictly. While the criteria possess a high specificity, sensitivity is not high.[31] Thus, we cannot exclude a possibility that we may have missed patients with IBM lacking typical clinicopathologic features, which would affect the prevalence calculation. Finally, this study was performed only in a single cohort in one country while the prevalence of HCV infection and the prevailing genotype differ among different countries or regions. Therefore, there remains a possibility that this phenomenon may be unique to the Japanese population. Validation should be performed in several independent cohorts from different countries.

We have demonstrated that HCV infection is associated with IBM in a large Japanese cohort. Our results raise a possibility of a pathomechanistic link between the 2 conditions although further studies are necessary.

AUTHOR CONTRIBUTIONS

A.U. was involved in the conceptualization and design of the study, data analysis and interpretation, literature review, and drafting the manuscript. S.N., Y.K.H., R.S.T., and T.Y. were involved in technical or material support, data interpretation, and manuscript revision for intellectual content. I. Nonaka was involved in the supervision of pathologic analysis and manuscript revision for intellectual content. I. Nishino was involved in the supervision of all aspects including study design and data analysis and interpretation, and manuscript revision for intellectual content.

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DISCLOSURE

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Clinical/Scientific Notes

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PLASMA IP-10 LEVEL DISTINGUISHES INFLAMMATORY MYOPATHY

Discrimination of idiopathic inflammatory myopathy (IIM) from other muscle diseases is at times difficult in clinical practice even after muscle biopsy.[1]

Plasma or serum cytokine levels have been reported to be changed in various muscle diseases,[2–6] and thus we hypothesized that circulating cytokines could be a biomarker to distinguish IIM from other muscle diseases. We measured a variety of cytokines in plasma of patients with diverse muscle diseases and found that IP-10 (CXCL10) could be a potential biomarker.

Classification of evidence. This study provides Class III evidence that plasma IP-10 levels distinguish IIM from hereditary muscle diseases (HMD) (sensitivity 91%, specificity 90%).

Methods. We studied 100 patients with IIM (mean age 54.1 ± 23.2 years, 42 male and 58 female), including polymyositis (n = 19), dermatomyositis (n = 19), antisynthetase syndrome (n = 8), immune-mediated necrotizing myopathy (n = 17), and inclusion body myositis (IBM, n = 37). As non-IIM controls, we enrolled 50 patients with HMD (mean age 32.8 ± 20.2 years, 24 men and 26 women), including Duchenne muscular dystrophy/Becker muscular dystrophy (BMD, n = 6), limb-girdle muscular dystrophies (LGMD, n = 7: type 1B, n = 2; 2A, n = 1; 2B, n = 3; and 2L, n = 1), facioscapulohumeral muscular dystrophy (FSHD, n = 25), and *GNE* myopathy (n = 12). While patients with HMD did not receive any immunosuppressive therapy, we did not have full information in the patients with IIM. Diagnosis of IIM was made based upon findings on muscle pathology and autoantibody analyses and diagnosis of HMD was confirmed genetically or immunohistochemically. We chose subjects in a consecutive manner within each disease group.

We measured plasma levels of 27 cytokines (table e-1 on the *Neurology*® Web site at Neurology.org) with Bio-Plex Pro Human Cytokine Group I Panel 27-Plex in Bio-Plex 200 system (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Means of duplicate measurements were used for analyses. Statistical comparisons were performed by Kruskal-Wallis test followed by Dunn multiple comparison post test. A p value of less than 0.01 was considered statistically significant. We performed Spearman rank correlation test to determine correlation between cytokine levels and age and receiver operating characteristic (ROC) analysis to assess the differential diagnostic potential of cytokine levels. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA).

Standard protocol approvals, registrations, and patient consents. All the clinical information and materials used in the present study were obtained for diagnostic purposes and permitted for scientific use with written informed consent. All experiments in the study were approved by the ethical committee of the National Center of Neurology and Psychiatry.

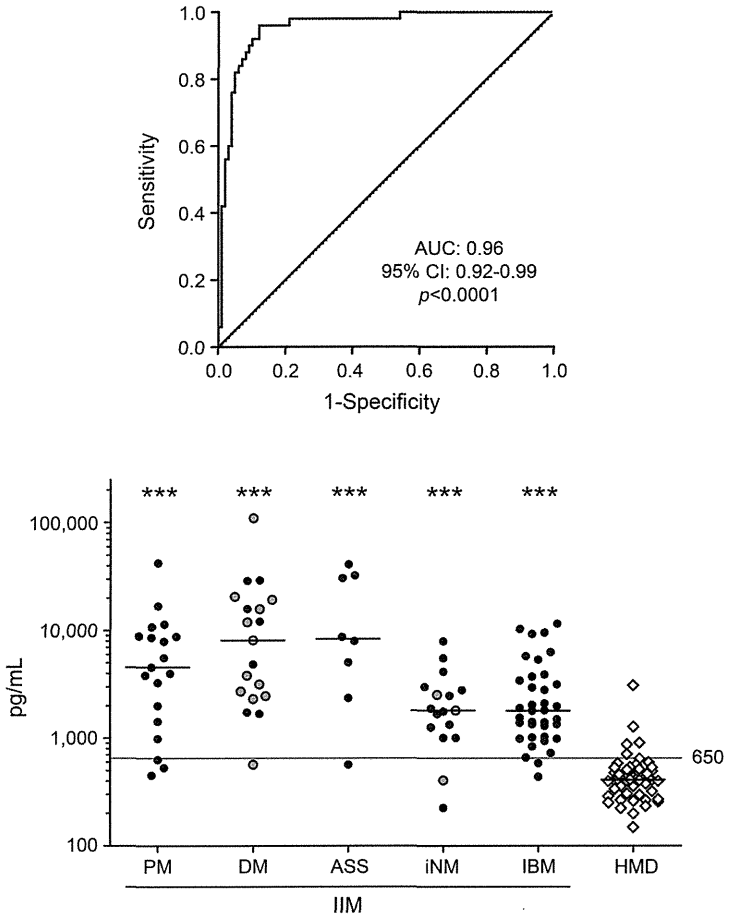
Results. IP-10 and eotaxin showed statistically significant differences between every IIM and HMD group (table e-1). No significant differences were seen among subtypes of IIM. There was no significant correlation between the level of IP-10 or eotaxin and patient age (table e-2).

ROC analysis revealed that IP-10 had a larger area under the curve than eotaxin (figure 1, figure e-1). Optimal accuracy of IP-10 as a differential diagnostic marker occurred at levels of 600–650 pg/mL, showing 91%. The cutoff level of 650 pg/mL gave 91% sensitivity and 90% specificity (figure 1). Even when HMD was limited to LGMD, FSHD, and BMD, which are more likely to be confused with IIM, the specificity was 91% (30/33). The sensitivity and specificity with optimal accuracy of each subset are described in table e-3.

Discussion. We demonstrated that plasma IP-10 level distinguishes IIM from HMD with high sensitivity and specificity. However, this study has a few limitations: (1) non-inflammatory acquired muscle diseases, such as toxic myopathy and endocrine myopathy, are not included; (2) it does not find a biomarker to differentiate IBM, which does not typically respond to immunosuppressive therapies, from other IIM; and (3) it lacks sufficient information about confounders

Supplemental data at Neurology.org

Figure 1 Receiver operating characteristic curve for IP-10 as a differential diagnostic test of idiopathic inflammatory myopathies and hereditary muscle diseases, and the plasma level in each patient



Each point represents the mean of duplicate measurements in semilog graph. Bars indicate median values. ***$p < 0.001$ when comparing IP-10 levels of each disease group to those of the hereditary muscle diseases (HMD) group. Gray circles indicate juvenile cases in the dermatomyositis group and cases with antimitochondrial antibodies in the immune-mediated necrotizing myopathy (iNM) group. Black circles in the iNM group indicate cases with anti-signal recognition particle antibodies. ASS = antisynthetase syndrome; AUC = area under the curve; CI = confidence interval; DM = dermatomyositis; IBM = inclusion body myositis; IIM = idiopathic inflammatory myopathy; PM = polymyositis.

such as severity of the diseases, treatments, and concurrent inflammatory diseases. Validation of the IP-10 level as a biomarker should be performed prospectively in larger and independent cohorts with different quantification kits.

From the Translational Medical Center (A.U., S.N., S.M., I.N.) and the National Institute of Neuroscience (A.U., S.N., W.S., H.N., S.M., T.Y., I.N.), National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan.

Author contributions: A.U. was involved in the conceptualization and design of the study, data analysis and interpretation, literature review, and drafting the manuscript. S.N., W.S., H.N., and S.M. were involved in technical or material support, data interpretation, and manuscript revision for intellectual content. T.Y. was involved in the supervision of data analysis and interpretation and manuscript revision for intellectual content. I.N. was involved in the supervision of all aspects, including study design and data analysis and interpretation, and manuscript revision for intellectual content.

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RESEARCH PAPER

Necklace cytoplasmic bodies in hereditary myopathy with early respiratory failure

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ABSTRACT

Background In hereditary myopathy with early respiratory failure (HMERF), cytoplasmic bodies (CBs) are often localised in subsarcolemmal regions, with necklace-like alignment (necklace CBs), in muscle fibres although their sensitivity and specificity are unknown.

Objective To elucidate the diagnostic value of the necklace CBs in the pathological diagnosis of HMERF among myofibrillar myopathies (MFMs).

Methods We sequenced the exon 343 of *TTN* gene (based on ENST00000589042), which encodes the fibronectin-3 (FN3) 119 domain of the A-band and is a mutational hot spot for HMERF, in genomic DNA from 187 patients from 175 unrelated families who were pathologically diagnosed as MFM. We assessed the sensitivity and specificity of the necklace CBs for HMERF by re-evaluating the muscle pathology of our patients with MFM.

Results *TTN* mutations were identified in 17 patients from 14 families, whose phenotypes were consistent with HMERF. Among them, 14 patients had necklace CBs. In contrast, none of other patients with MFM had necklace CBs except for one patient with reducing body myopathy. The sensitivity and specificity were 82% and 99%, respectively. Positive predictive value was 93% in the MFM cohort.

Conclusions The necklace CB is a useful diagnostic marker for HMERF. When muscle pathology shows necklace CBs, sequencing the FN3 119 domain of A-band in *TTN* should be considered.

INTRODUCTION

Hereditary myopathy with early respiratory failure (HMERF; OMIM 603689) is an adult-onset progressive myopathy characterised by early presentation of respiratory insufficiency usually during ambulant stage.[1–3] Pathologically, HMERF shares features of myofibrillar myopathy (MFM) besides the key finding of cytoplasmic bodies (CBs).[2 3] Its causative gene *TTN*, which encodes a gigantic protein, titin,[2 3] is known to be causative also for tibial muscular dystrophy, limb girdle muscular dystrophy type 2J, early-onset myopathy with fatal cardiomyopathy and dilated or hypertrophic cardiomyopathy.[4–10] Interestingly, all patients with HMERF so far identified carry a mutation in exon 343 (based on ENST00000589042) encoding the fibronectin-3 (FN3) 119 domain in the A-band region of titin.[2 3 11–17]

CBs are abnormal protein aggregates visualised usually as red-colored objects on modified Gomori trichrome stain and can be observed in a wide range of myopathic conditions. Nevertheless, they are often conspicuous in MFM and considered as one of the representative pathological findings in MFM.[18] In muscle specimens of HMERF, CBs are often located in the subsarcolemmal region,[12 13 14 19] with a necklace-like alignment, which here we call 'necklace CBs'. However, the utility of necklace CBs in the pathological diagnosis of HMERF is unknown. We therefore tested the sensitivity and specificity of necklace CBs in the diagnosis of HMERF.

METHODS

Patients

National Center of Neurology and Psychiatry (NCNP) functions as a referral centre for muscle pathology and muscle biopsy samples are sent from all over Japan. From 1991 to 2013, 187 patients from unrelated 175 Japanese families have been pathologically diagnosed as MFM at NCNP. In this cohort, mutations were found in known MFM-related genes: *DES*: 8 families (4.6%), *VCP*: 8 families (4.6%), *FLNC*: 6 families (3.4%), *DNAJB6*: 6 families (3.4%), *ZASP*: 5 families (2.9%), *FHL1*: 5 families (2.9%), *MYOT*: 4 families (2.3%) and *BAG3*: 1 family (0.6%). No mutation was identified in *CRYAB*. Clinical information at the time of muscle biopsy was available in all patients.

Genetic analysis

Genomic DNA was isolated from peripheral lymphocytes or frozen muscle as previously described.[20] Exon 343 of *TTN* was directly sequenced using ABI PRISM 3130 automated sequencer (PE Applied Biosystems). Sequence variants were assessed using publically available databases including 1000 Genomes Project database (http://www.1000genomes.org/), NHLBI Exome Sequencing Project 5400 database (http://evs.gs.washington.edu/EVS/), dbSNP135 (http://www.ncbi.nlm.nih.gov/SNP/) and Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB/); and

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Table 1 Clinical features of the patients with *TTN* variants

Patient Number	Age (years)/ sex	Relationship	Mutation (protein level)	Family history (years)	Initial manifestations (years)	Age at gait disturbance/ ambulant	Foot drop (years)	Respiratory disturbance (years)	Artificial ventilation (years)	Cardiac involve-ment	Dysphagia (years)	Other features
A-1	49/M	Father	p.C31712R		Tripping (46)	46/yes	Yes (NA)	Yes (48)	Yes (49)	RHF	No	No
A-2	26/M	Son			Tripping (20)	22/yes	Yes (22)	Yes*	No	No	No	No
B-1	45/M	Older brother	p.C31712R	Father: Foot drop; sudden death (42) Oldest brother: gait disturbance (32); sudden death (40)	Foot drop (31)	31/yes	Yes (31)	Yes (44)	Yes (45)	No	No	No
B-2	37/M	Younger brother			Foot drop (27)	27/yes	Yes (27)	Yes (34)	Yes (37)	No	No	No
C	34/F		p.C31712R		Fatiguability (26)	29/yes	Yes (31)	Yes (26)	Yes (31)	–	No	Difficulty in opening mouth
D	38/M		p.C31712R		Difficulty in lifting thigh (20)	20/no (32)	Yes (20 s)	Yes (29)	Yes (29)	STC	Yes (28) Artificial nutrition (35)	
E	40/M		p.C31712R	Mother: sudden death (38) Older sister: proximal muscle weakness; respiratory failure (35)	Fatiguability; respiratory failure (31)	31/yes	Yes (NA)	Yes (31)	Yes (37)	RHF, PH	–	
F	52/M		p.C31712R		Foot drop (47)	47/yes	Yes (47)	Yes (50)	Yes (52)	–	Yes (47)	
G-1	58/M	Father	p.C31712R	Grandfather of G-2: died of respiratory failure (45)	Tripping (57)	57/yes	Yes (58)	Yes*	–	–	–	
G-2	29/M	Son			Tripping (20)	20/yes	Yes (20 s)	Yes (29)	Yes (29)	RHF	–	
H	68/F		p.C31712R	Son: distal myopathy; sudden death	Difficulty in standing on right toe (56)	56/yes	No	Yes (68)	Yes (68)	RHF, Af	–	Head drop; forward bent posture
I	43/M		p.C31712R		Fatiguability; weight loss (39)	42/yes	No	Yes (39)	Yes (42)	–	Yes (42)	Myalgia muscle cramp
J	42/F		p.C31712Y		Difficulty lifting thighs (36)	38/Yes	No	Yes*	–	–	–	
K	38/M		p.G31791D		Gait disturbance (28)	28/Yes	Yes (30)	Yes*	Yes (38)	–	Yes (36)	Head drop
L	44/F		p.G31791R		Fatiguability; loss of appetite (40)	41/yes	No	Yes (40)	Yes (44)	STC	–	
M	40/M		p.G31791V	Mother and younger sister: lower leg muscle weakness	Gait disturbance (24)	24/yes	Yes (27)	Yes*	–	2° AVB (type 1)	–	
N	46/M		p.R31783_V31785del		Foot drop; difficulty in opening a bottle (41)	41/yes	Yes (41)	Yes*	–	–	–	Myalgia

*Asymptomatic but found by laboratory tests.
2° AVB (type 1), Mobitz type 1 second degree atrioventricular block; Af, atrial fibrillation; F, female; M, male; NA, not available; PH, pulmonary hypertension; RHF, right-sided heart failure; STC, sinus tachycardia.

softwares to predict functional effects of mutations such as PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/). The description of mutations of *TTN* conforms to Ensemble sequence ENST00000589042.

In this study a diagnosis of HMERF was made based on the presence of a mutation in exon 343 of *TTN*, although the possibility that mutations in other parts of the gene cause HMERF cannot be totally excluded.

Re-evaluation of muscle pathology

Muscle pathology was re-evaluated focusing on necklace CBs on modified Gomori trichrome. In the present study, we tentatively defined the presence of necklace CBs as at least two muscle fibres containing CBs exclusively localised in the subsarcolemmal area, covering more than 50% of circumference of each muscle fibre in three non-serial sections (each section was at least 250 μm apart and included at least 300 muscle fibres). CBs were evaluated in all MFM cases, and subsequently the sensitivity and specificity of necklace CBs for the diagnosis of HMERF were calculated. The positive predictive value (PPV) and 95% CIs were calculated with GraphPad Prism V.5.0 (Graph Pad Software, California, USA).

Electron microscopic observation

Biopsied muscle specimens were fixed in 2.5% glutaraldehyde and post-fixed with 2% osmium tetroxide. Semithin sections stained with toluidine blue were examined by light microscopy. Ultrastructural analysis was carried out on longitudinal and transverse ultrathin sections of muscles after staining with uranyl acetate and lead citrate, using Tecnai spirit transmission electron microscope (FEI, Hillsboro, Oregon, USA).

Re-evaluation of clinical data

The clinical information in our cohort together with previous reports[2 3 11–14] showed that respiratory insufficiency before being wheelchair users and selective involvement of semitendinosus muscles on muscle imaging were frequently observed, raising a possibility that these findings might be clues for the diagnosis of HMERF. We therefore reviewed clinical data of our patients with MFM in order to calculate sensitivities, specificities and PPVs of them at the time of muscle biopsy.

Ethics

All of the clinical information and materials used in this study were obtained for diagnostic purpose and permitted for scientific use with written informed consent. All experiments in this study were approved by the Ethical Committee of National Center of Neurology and Psychiatry.

RESULTS

Mutation analysis

Six different heterozygous mutations in exon 343 of *TTN* were identified in 17 patients from 14 families (table 1). Among them, two mutations, g.284701T>C (c.95134T>C; p.C31712R) and g.284939G>A (c.95372G>A; p.G31791D) were previously reported.[2 3 11 12 14] The former mutation was reported to be the most common in other populations.[11 12] This was also the case in our cohort and the mutation was shared by nine families, while all others were found in single families. The latter mutation was previously reported in a European-American family.[14] Among four novel mutations that we identified, three were missense: g.284702G>A (c.95135G>A; p.C31712Y), g.284938G>C (c.95371G>C; p.G31791R) and g.284939G>T (c.95372G>T; p.G31791V); and one was non-frameshift deletion: g.284913_284921delGAGGGCAGT (c.95346_95354del; p.R31783_V31785del). None of the variants was listed in the

Table 2 Laboratory findings at the time of muscle biopsy

Patient	CK IU/L (normal value)	Respiratory function (%VC, sitting position)	Selective involvement of muscles on imaging test: Semitendinosus muscle	Selective involvement of muscles on imaging test: Anterior compartment of lower legs	Muscle pathology: CB	Muscle pathology: Necklaces of CBs	Muscle pathology: RV
A-1 (49 years)	Normal (value: NA)	Abnormal (value: NA.)	NA	NA	+	+	+
A-2 (26 years)	65 (20–190)	77% (68%, lying)	+	+	+	+	+
B-1 (39 years)	425 (51–197)	84% → 67% (45 years)	+	+	+	+	–
B-2 (32 years)	659 (–200)	82% → 63% (37 years)	+	+	+	+	+
C (31 years)	488 (45–170)	32%	NA (+, 34 years)	NA (+, 34 years)	+	+	–
D (31 years)	375 (51–197)	VC: 0.97 L	+	–*	+	+	–
E (40 years)	234 (62–287)	ABG: $PaCO_2$ 86 mm Hg, PaO_2 56 mm Hg (RA)	+	+	+	+	–
F (50 years)	61 (NA)	41%	+	+	+	–	–
G-1 (58 years)	146 (50–170)	59%	+	+	+	–	–
G-2 (29 years)	142 (50–170)	31%	+	+	+	+	+
H (68 years)	140 (50–170)	ABG: PaCO2 60 mm Hg	+	+	+	+	–
I (43 years)	179 (62–287)	40%	+	+	+	–	–
J (42 years)	364 (45–163)	64%	+	+	+	+	+
K (34 years)	645 (51–197)	67% (55%, lying)	+	+	+	+	+
L (44 years)	139 (43–165)	36%	+	+	+	+	–
M (40 years)	190 (62–287)	61%	–*	+	+	+	+
N (46 years)	799 (62–287)	67%	+	+	+	+	+

In the column of Patient, the same alphabet indicates that they belong to the same family.
*Diffuse muscle involvement.
ABG, arterial blood gas; CB, cytoplasmic body; NA, not available; RV, rimmed vacuole; VC, vital capacity.

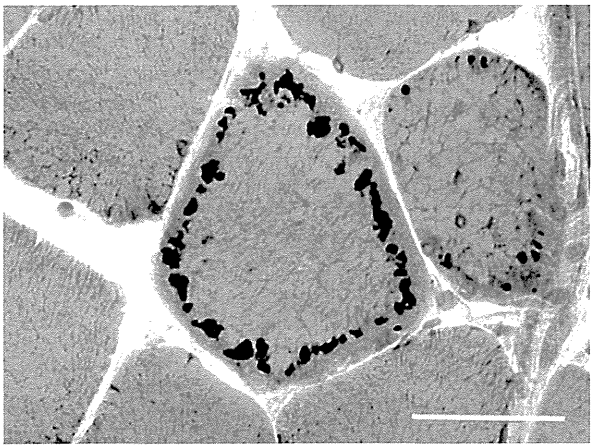

Figure 1 Necklace cytoplasmic bodies. Cytoplasmic bodies are located in line in subsarcolemmal region, often covering the total circumference of a muscle fibre. Modified Gomori trichrome stain. Bar: 50 μm.

Table 3 Cross tabulation of necklace cytoplasmic bodies and hereditary myopathy with early respiratory failure

	Patients with myofibrillar myopathies		
Necklace CBs	HMERF	Non-HMERF	Row total
+	14	1	15
% within column	82.4%	0.6%	
% within row	93.3%	6.7%	
–	3	169	172
% within column	17.6%	99.4%	
% within row	1.7%	98.3%	
Column total	17	170	187

CB, cytoplasmic body; HMERF, hereditary myopathy with early respiratory failure.

genomic variation databases. The mutated amino acids were highly conserved among species (UCSC Genome Browser). PolyPhen-2 predicted p.C31712Y, p.G31791R and p.G31791V mutations as probably damaging with scores 0.996, 1.000 and 1.000, respectively. Likewise, Mutation Taster predicted the p.C31712Y, p.G31791R, p.G31791V and p.V31782_A31784del mutations to be disease-causing with a probability of 1.000, 1.000, 1.000 and 0.998, respectively. No segregation analysis was possible in any family.

Clinical information of 17 participants with *TTN* mutations are summarised in tables 1 and 2 and online supplementary table. All patients show clinical signs consistent with HMERF previously reported.[1–3 11–14] The median age of onset was 31 years (range 20–57 years). Four patients developed dysphagia, and one of them required tube feeding.

Sensitivity and specificity of the necklace of CBs

Among 17 genetically-confirmed patients with HMERF, necklace CBs were found in 14 patients, comprising 0.1–0.8% of the muscle fibres (figure 1). In contrast, none of the 170 patients who had MFM other than HMERF had necklace CBs except for only one patient who had reducing body myopathy, which had been confirmed by the presence of reducing bodies in muscle fibres on menadione-linked α-glycerophosphate dehydrogenase (MAG) stain without substrate and a mutation in the second LIM domain of *FHL1* (g.60438G>A; c.377G>A; p.C126Y, based on ENST00000543669; online supplementary figure S1). Based on these results, the sensitivity and specificity of the necklace CBs in HMERF were calculated as 82% (14/ 17, 95% CI 57% to 96%) and 99% (169/170, 95% CI 97% to 100%), respectively (table 3). Since the prevalence of HMERF in the MFM cohort was 9.1% (17/ 187), the PPV was calculated as 93% (95% CI 68% to 100%) based on Bayes' theorem.

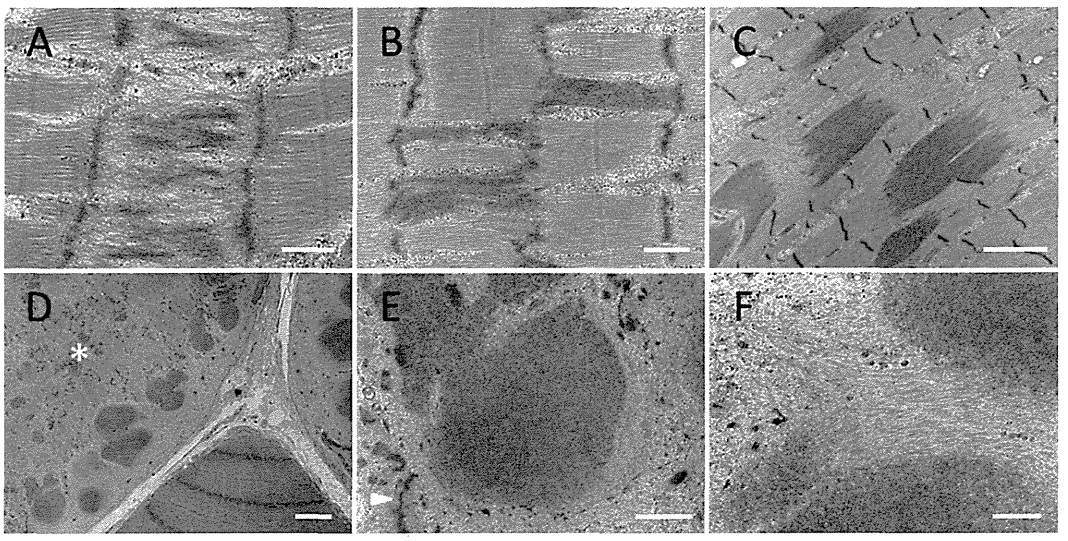

Figure 2 Electron microscope images. (A and B) Sarcomeric disarrangement limited to one sarcomere is observed. (C) Multiple electron-dense inclusions are present in association with Z lines. (D) A fibre containing necklace cytoplasmic bodies (CBs) shows marked myofibrillar disorganisation (asterisk), especially around the CBs. (E) CBs showing a necklace alignment with lower electron density as compared to that of Z line. △: a remnant of Z line. (F) Thin filamentous structure around the CBs. Lattice-like structure is not seen in the CBs. Bar: 0.5 μm (A, B and F), 1 μm (E), 2 μm (C), and 5 μm (D).

Muscle specimens of the three patients with HMERF had CBs, which are usually located in the subsarcolemmal regions, but did not show the definite necklace-like alignment pattern (online supplementary figure S2). Those three patients shared the same mutation, g.284701T>C (p.C31712R), albeit other nine patients harbouring the same mutation had definite necklace CBs.

Fibres with rimmed vacuoles were occasionally seen, but the sensitivity, specificity and PPV for HMERF were lower than those of the necklace CBs: 47% (8/ 17, 95% CI 23% to 72%), 61% (104/ 170, 95% CI 53% to 69%) and 11% (95% CI 20% to 48%), respectively.

Ultrastructural features

EM samples were available from four patients with HMERF. Sarcomeric disarrangement limited to one sarcomere was observed in all patients (figure 2A, B). In some areas, multiple electron-dense inclusions associated with Z line were surrounded by disorganised myofibrils (figure 2C). Fibres with necklace CBs were included only in one sample. These fibres showed marked myofibrillar disorganisation (figure 2D, asterisk), especially in the vicinity of the CBs (figure 2D, E). The CBs had a lower electron density as compared with that of the Z line and contained dense and mildly filamentous components, without lattice-like structure (figure 2E, F). Small number of CBs were partly surrounded by thin filaments (figure 2F).

Sensitivity and specificity of respiratory dysfunction and muscle imaging data

Using the data at the time of muscle biopsy about the respiratory function of 102 participants in the MFM cohort, the sensitivity, specificity and PPV of the respiratory insufficiency before being wheelchair users (below 80% of vital capacity or over 45 mm Hg of $PaCO_2$) were calculated as 88% (14/16, 95% CI 62% to 99%), 94% (81/86, 95% CI 87% to 98%) and 74% (95% CI 49% to 91%), respectively (table 2 and 4). In five non-HMERF participants presenting respiratory insufficiency during the ambulant stage, one had a mutation in *VCP*, but no causative genes were identified in four participants as far as we have screened.

As for the muscle imaging at the time of muscle biopsy, the sensitivity of selective muscle involvement of semitendinosus muscles as shown in figure 3 was 93% (14/ 15; table 4). The specificity could not be calculated due to the limited number of images available in non-HMERF participants.

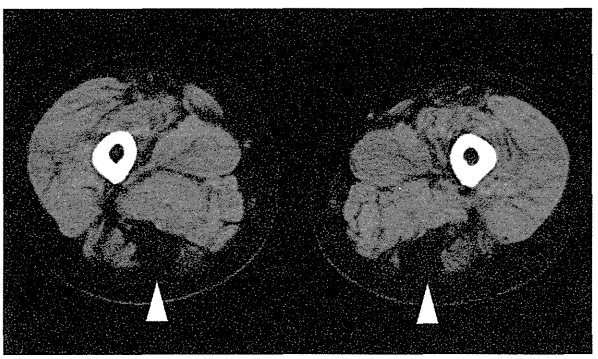

Figure 3 Muscle image. Representative image showing preferential involvement of semitendinosus muscles. CT images of skeletal muscles in proximal legs of the patient K. △: semitendinosus muscles.

DISCUSSION

We have demonstrated that necklace CBs had high specificity for a diagnosis of HMERF (99%), with sensitivity 82% and PPV 93% in our MFM cohort. In this study, all 15 patients with necklace CBs had HMERF except for only one who had reducing body myopathy due to a mutation in *FHL1*, without mutation in exon 343 of *TTN*. Although we judged this case as having necklace CBs retrospectively, CBs in this case are smaller in size and scattered in the subsarcolemmal region in muscle fibres, giving an appearance different from typical necklace CBs in HMERF (online supplementary figure S1), suggesting that the specificity of necklace CBs could be substantially 100%. In addition, muscle pathology of this patient showed typical reducing bodies in scattered fibres on MAG stain without substrate, and thus differential diagnosis between reducing body myopathy and HMERF was not a problem in practise.

Interestingly, even in three patients with HMERF without typical necklace CBs, CBs are aligned in the subsarcolemmal regions in muscle fibres, albeit they do not encompass more than half of the myofibre circumference (online supplementary figure S2), suggesting that this unique subsarcolemmal alignment pattern is likely to be related to the dysfunction of titin due to exon 343 mutations although the detailed mechanism is still unknown.

On electron microscopy (EM), CBs in the patients differ slightly from typical CBs seen in other various diseases. While typical CBs consists of a core with electron density similar to Z line,[21] the CBs in HMERF show lower density. Furthermore, thin filaments, which compose a halo of typical CBs, are rarely seen around the CBs. Interestingly, the sarcomeric disarrangement appears to progress in order of figure 2A, C, suggesting a possibility that this sarcomeric disarrangement may ultimately produce the CBs.

Several groups reported that CBs in HMREF are reactive to antibodies to myofibrillar proteins including myotilin, αB-crystallin, actin (phalloidin), filamin C, dystrophin, and γ-sarcoglycan, but not for titin on immunohistochemical analysis.[11 12 14 19] Although similar findings were obtained in our observation (data not shown), the pathophysiological significance of CBs in HMERF has still not been elucidated.

We found four novel variants in exon 343 of *TTN* including three heterozygous missense mutations [g.284702G>A (p.C31712Y), g.284938G>C (p.G31791R) and g.284939G>T (p.G31791V)], and a heterozygous deletion variant [g.284913_284921del (p.R31783_V31785del)]. These variants were not described in any of the publically available databases. Mutated amino acids are highly conserved among species. Also

Table 4 Sensitivity, specificity, and positive predictive value of laboratory findings for HMERF

	Respiratory disturbance in the ambulant stage	Selective affected semitendinosus muscles on muscle imaging	Necklace CBs on muscle pathology
Sensitivity	88% (14/16)	93% (14/15)	82% (14/17)
Specificity	94% (81/86)	NA	99% (169/170)
PPV	74%	NA	93%

At the time of muscle biopsy.
Respiratory disturbance: <80% of %VC or >45 mm Hg of $PaCO_2$. PPVs were calculated by the HMERF prevalence of 9.1% in the MFM cohort.
CB, cytoplasmic body; HMERF, hereditary myopathy with early respiratory failure; MFM, myofibrillar myopathy; NA, not available, PPV, positive predictive value; VC, vital capacity.

Table 5 Mutations in the FN3 119 domain of A-band in *TTN* in HMERF

DNA change	Amino acid change	Origin	Family	Reference
g.284693C>G	p.P31709R (p.P30068R)	French		3
g.284701T>C	p.C31712R (p.C30071R)	Swedish, British, Finnish, Italian, Spanish, Argentinian (European ancestry), East Indian, Japanese	A, B, C, D, E, F, G, H, I	2, 3, 11, 12, 14
g.284702G>A	p.C31712Y (p.C30071Y)	Japanese	J	*
g.284752T>C	p.W31729R (p.W30088R)	British		12
g.284754G>C	p.W31729C (p.W30088C)	German		12
g.284753G>T	p.W31729L (p.W30088L)	Japanese		13
g.284762C>T	p.P31732L (p.P30091 L)	Italian, French, British, Portuguese, Swedish		11, 12, 15, 16, 17
g.284913_284921del	p.R31783_V31785del (p.R30142_V30144del)	Japanese	N	*
g.284925C>G	p.N31786K (p.N30145 K)	British		11
g.284939G>A	p.G31791D (p.G30150D)	American (European ancestry), Japanese	K	14
g.284938G>C	p.G31791R (p.G30150R)	Japanese	L	*
g.284939G>T	p.G31791V (p.G30150V)	Japanese	M	*

*Possible novel mutation. Titin reference: ENST00000589042 and ENST00000591111, a former transcript, inside the brackets.
HMERF, hereditary myopathy with early respiratory failure.

the variants were predicted to be pathogenic by the plural prediction software programs. Furthermore, in single-base substitutions, other types of substitutions of the same amino acids (p.C31712R and p.G31791D) have already been reported in other families with HMERF (table 5).[2 3 11 12 14] Thus, although segregation analysis was not possible, the variants are highly likely to be pathogenic.

Previous reports suggested that HMERF might not be extremely rare in Caucasian populations.[11 12] In UK, in patients with HMERF with p.C31712R (p.C30071R, based on ENST00000591111), mutation in exon 343 of *TTN* was identified in 5.5% of the MFM cohort.[11] Patients have also been identified in Asian populations including Japanese and Indian, suggesting that patients with HMERF are likely to be distributed worldwide.[13 14 22] Here, we confirmed the presence of patients with HMERF in Japan. Furthermore, among all the 175 MFM families in our Japanese cohort, 14 families (8%) had HMERF with mutations in the exon 343 of *TTN*, which renders *TTN* the most frequent causative gene for MFM in our cohort although there still remains a possibility that there may be an undisclosed major causative gene as causative mutations have not been identified in more than 60% of the MFM families.

Clinical features of participants with HMERF described in this study coincide with those in previous reports for most parts: affected individuals usually present with predominant distal leg muscle weakness followed by chronic respiratory failure.[1–3 11–14] Interestingly, dysphagia was seen in 4 of the 17, which was rarely described in the literature.[13] Dysphagia seems to be mostly mild, but was severe in one patient, who required tube feeding.

Skeletal muscle imaging has been reported to show preferential involvement of semitendinosus, obturator, sartorius, gracilis, iliopsoas muscles and anterior compartment of lower legs, suggesting such imaging findings are useful for the diagnosis of HMERF.[3 11 12 19] Particularly, selective involvement of semitendinosus muscles is commonly observed. Our study showed a sensitivity of 93%, which is compatible with 95–100% reported by previous studies.[3 12] Unfortunately, skeletal muscle imaging was not available in many of our patients with MFM other than HMERF, and thus it was impossible to calculate the specificity and PPV of the selective involvement of semitendinosus muscles. However, it may not be so specific since such finding was observed also in other MFMs caused by mutations in *DES*, *CRYAB* and *MYOT*.[23 24]

In conclusion, the necklace CB is a useful pathological marker in the diagnosis of HMERF. When muscle pathology shows necklace CBs, sequencing the FN3 119 domain of the A-band in *TTN* should be considered.

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Contributors AU was involved in the conceptualisation and design of the study, data analysis and interpretation, literature review and drafting the manuscript. YKH was involved in the conceptualisation and design of the study, data analysis and interpretation, and manuscript revision for intellectual content. YO, MM-Y, MK, MM, MK, KO, TM, SS, YT, TK, TK, YI, NK, SY, RY, and JK were involved in the collection of clinical data. SM and SN were involved in the data interpretation (molecular data) and manuscript revision for intellectual content. IkN was involved in the supervision of pathological analysis and interpretation and manuscript revision for intellectual content. IcN was involved in the supervision of all aspects, including study design, data analysis and interpretation, and manuscript preparation.

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Competing interests None.

Ethics approval Ethical Committee of National Center of Neurology and Psychiatry.

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Necklace cytoplasmic bodies in hereditary myopathy with early respiratory failure

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Inflammatory myopathy with anti-signal recognition particle antibodies: case series of 100 patients

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Abstract

Background: Anti-signal recognition particle (SRP) antibodies are used as serological markers of necrotizing myopathy, which is characterized by many necrotic and regenerative muscle fibers without or with minimal inflammatory cell infiltration. The clinical spectrum associated with anti-SRP antibodies seems to be broad.

Objective: To describe the clinical characteristics, autoantibodies status, and neurological outcome associated with anti-SRP antibody.

Methods: We studied clinical and laboratory findings of 100 patients with inflammatory myopathy and anti-SRP antibodies. Anti-SRP antibodies in serum were detected by the presence of 7S RNA using RNA immunoprecipitation. In addition, enzyme-linked immunosorbent assays (ELISAs) using a 54-kD protein of SRP (SRP54) and 3-hydroxyl-3-methylglutatyl-coenzyme A reductase (HMGCR) were also conducted.

Results: The mean onset age of the 61 female and 39 male patients was 51 years (range 4–82 years); duration ≥ 12 months before diagnosis was seen in 23 cases. All patients presented limbs weakness; 63 had severe weakness, 70 neck weakness, 41 dysphagia, and 66 muscle atrophy. Extramuscular symptoms and associated disorders were infrequent. Creatine kinase levels were mostly more than 1000 IU/L. Histological diagnosis showed 84 patients had necrotizing myopathy, and apparent cell infiltration was observed in 16 patients. Anti-SRP54 antibodies were undetectable in 18 serum samples with autoantibodies to 7S RNA. Anti-HMGCR antibodies were positive in 3 patients without the statin treatment, however, were negative in 5 patients with statin-exposure at disease onset. All but 3 patients were treated by corticosteroids and 62 (77 %) of these 81 patients required additional immunotherapy. After 2-years treatment, 22 (27 %) of these 81 patients had poor neurological outcomes with modified Rankin scale scores of 3–5. Multivariate analysis revealed that pediatric disease onset was associated with the poor outcomes.

Conclusion: Anti-SRP antibodies are associated with different clinical courses and histological presentations.

Keywords: Signal recognition particle, Autoantibodies, Necrotizing myopathy, RNA immunoprecipitation, ELISA, Outcome

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Background

Signal recognition particle (SRP), which is a ubiquitous cytoplasmic RNA protein consisting of 7S RNA and 6 proteins with molecular weights of 9, 14, 19, 54, 68 and 72 kD, mediates the translocation of newly synthesized protein across the endoplasmic reticulum. Anti-SRP antibodies were first discovered in the serum of patients with clinical polymyositis by the presence of 7S RNA detected by RNA immunoprecipitation [1–3]. RNA immunoprecipitation is a powerful method for the detection of various autoantibodies, including those against aminoacyl transfer RNA synthetase (ARS). There is another method for detecting anti-SRP antibodies: an immunoassay using a 54-kD subunit protein of SRP (SRP54) as the antigen [4]. Immunoassays using SRP54 such as enzyme-linked immunosorbent assays (ELISAs) are easily performed and have the advantage of allowing the screening of many serum samples. However, comparisons of the RNA immunoprecipitation method and the SRP54 immunoassay method have not been conducted.

Based on an accumulation of clinical observations, it was reported that anti-SRP antibodies are associated with the severe and refractory myositis and that they can be regarded as myositis-specific antibodies [1]. Histological diagnoses have confirmed a tight association between anti-SRP antibodies and immune-mediated necrotizing myopathy [5–8]. Anti-SRP antibodies are now used as serological markers of necrotizing myopathy, which is characterized by many necrotic and regenerative muscle fibers without or with minimal inflammatory cell infiltration. Since there is a lack of information regarding the inflammatory processes in muscle histology, the detection of anti-SRP antibodies merely suggests an immune-mediated mechanism. The clinical spectrum associated with anti-SRP antibodies seems to be broad [9–12]. We hypothesized that anti-SRP antibodies could define a distinct subset of inflammatory myopathies, and the purpose of present study is to report the clinical characteristics, autoantibody status, and neurological outcome of 100 patients with inflammatory myopathy with anti-SRP antibody.

Methods

Patients

From 1997 to 2012, we followed 17 patients with inflammatory myopathy with anti-SRP antibody at Keio University Hospital. Between January 2008 and September 2012, we identified another 83 patients with anti-SRP antibodies who were referred from all over Japan to Keio University Hospital or the National Center of Neurology and Psychiatry. Anti-SRP antibodies were detected by RNA immunoprecipitation. The diagnosis of inflammatory myopathy was based on the histological diagnosis with clinical, electrophysiological, and radiological findings. Clinical information was retrospectively obtained by the authors or provided by referring physicians. This study was approved by the Institutional Review Boards at Keio University and the National Center of Neurology and Psychiatry.

Histology

Necrotizing myopathy was diagnosed based on the observation of many necrotic fibers as the predominant abnormal histological feature without or with minimal inflammatory cell infiltration [7, 8]. Sporadic inclusion body myositis was diagnosed by the identification of rimmed vacuoles with non-necrotic fibers invaded by mononuclear cells or increased major histocompatibility complex (MHC) class I expression. Polymyositis was diagnosed based on endomysial inflammation cell infiltrate surrounding or invading non-necrotic muscle fibers accompanied by ubiquitous MHC class I expression. Dermatomyositis was diagnosed by the identification of the presence of perifascicular atrophy [13, 14]. Other inflammatory myopathies were regarded as non-specific myositis including perimysial or endomysial inflammatory cell infiltration.

RNA immunoprecipitation

Ten-μl of serum was mixed with 2 mg of protein A-Sepharose CL-4B (Pharmacia Biotech AB) in 500 μl of immunoprecipitation buffer and incubated for 2 h. After washing 3 times with immunoprecipitation buffer, antigen-bound Sepharose beads were mixed with 100 μl of HeLa cell extract (6 × 10 cell equivalents per sample) for 2 h, and then 30 μl of 3 M sodium acetate, 30 μl of 10 % sodium dodecyl sulfate, and 300 μl of phenol:chloroform:isoamyl alcohol (50:50:1, containing 0.1 % 8-hydroxyquinoline) were added to extract the bound RNA. After ethanol precipitation, the RNA was resolved by using a 7 M urea-8 % polyacrylamide gel, and the gel was silver-stained (Bio-Rad). Immunoprecipitated RNA located in the 7S RNA lesion was regarded as anti-SRP antibody (Fig. 1).

ELISA

First, 96-well polyvinyl plates (Sumitomo Bakelite) were coated with recombinant SRP54 protein (Diarect) at 1 μg/ml diluted in phosphate buffered saline. The remaining blocking sites were blocked with 3 % bovine serum albumin. The wells were incubated with serum samples diluted 1:200 and subsequently with peroxidase-conjugated anti-human IgG (Jackson Immuno Research) diluted 1:100,000. The antibody binding was visualized by incubation with tetramethylbenzidine (1 mg/ml) in phosphate-citrate buffer. The reaction was stopped by 1 M sulfuric acid. The optical density at 450 nm (OD450) was read with an automatic plate reader (Biorad). Samples were tested in duplicate. In addition,

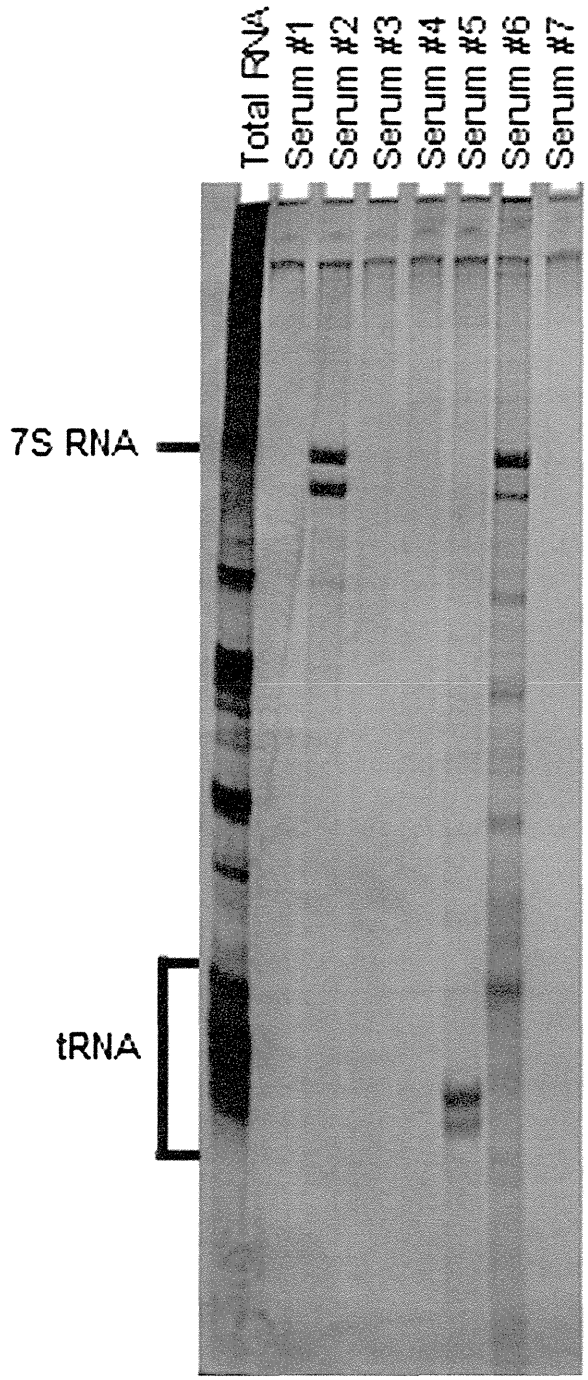

Fig. 1 RNA immunoprecipitation assay. Urea and 8 % PAGE of phenol-extracted immunoprecipitation from HeLa cell extracts were developed with silver staining. Total RNA indicates 7S RNA and transfer RNA regions. The serum samples #2 and #6 contained anti-signal recognition particle (SRP) antibodies. In contrast, one of autoantibodies against aminoacyl transfer RNA synthetase, anti-PL-7 antibody (anti-threonyl-tRNA synthetase) was found in serum #5

autoantibodies to 3-hydroxyl-3-methylglutatyl-coenzyme A reductase (HMGCR) were measured using an ELISA based on the original method with some modifications [15].

We also used sera from 46 myositis patients without autoantibodies, 46 patients with myasthenia gravis, 40 patients with Duchenne muscular dystrophy, and 40 normal healthy volunteers as controls.

Statistical analyses

Statistical analyses were performed using JMP version 9 statistical software (SAS Institute Inc.). Values of $p < 0.05$ were considered significant.

Results

Demographic features

The clinical features of the total 100 patients with anti-SRP antibodies are summarized in Table 1. There were 61 female, 39 males. The mean age of disease onset was 51.3 ± 19.3 years (range 4–82 years). The patients' ages at disease onset were distributed widely, and peaked in the 60s (Fig. 2). Pediatric disease onset at age ≤ 16 years was found in 8 patients.

Initial presentation

Antecedent infection was found in 7 patients. Statins were administered in 5 patients at disease onset. Inflammatory myopathy developed in 4 patients during the follow-up of another rheumatic disease. The durations of disease progression varied. The duration from the disease onset to the first examination was within 12 months in 77 patients, and ≥ 12 months in the other 23 patients.

With regard to the initial symptoms, weakness in the legs, such as difficulty in working or climbing stairs, was observed in 67 patients. Weakness in the arms was the initial symptom in 19 patients. The 7 patients with bulbar symptoms all suffered from difficulties in swallowing, but not in speaking. Among trunk muscles, neck weakness and muscle atrophy in the scapular muscles were reported in 5 and 2 patients, respectively.

Clinical features

All patients had limb weakness. The distribution of weakness was proximal-dominant and symmetrical, and affected legs more than arms. Severe limbs weakness with the grade ≤ 3/5 assessed by manual muscle strength (Medical Research Council scale grade) was observed in 63 patients. Laterality of limb weakness was seen in 16 patients.

Seventy patients experienced neck weakness. Among them, dropped head was observed in 7 patients. Dysphagia was observed in 41 patients, and nasogastric tubes were necessary in 5 patients. Facial and cardiac muscle involvement was infrequent. The vital capacity percentage in the respiratory function test was decreased in 12 patients, 2 of whom needed mechanical ventilation. Neurological examinations revealed muscle atrophy in 66 patients: among them, scapular winging was seen in

Table 1 Characteristics of the 100 patients with inflammatory myopathy with anti-SRP antibody

Findings	(n)
Females/males	61/39
Age at diseases onset	
Mean ± SD	51.3 ± 19.3
Age ≤ 15 years	8
Antecedent infection	7
Statin-exposure	5
Duration from the disease onset to the first examination	
≤12 months	77
>12 months	23
Initial symptoms	
Arms	19
Legs	67
Bulbar	7
Trunk	7
Muscle weakness	
Legs predominantly than arms	69
Severe limbs weakness	63
Laterality	16
Distal muscle dominant	3
Neck weakness	70
Dysphagia	41
Facial muscle involvement	10
Cardiac muscle involvement	2
Decreased capacity of respiratory function	12
Muscle atrophy	66
Scapular winging	10
Decreased deep tendon reflex	51
Myalgia	34
Extramuscular symptoms	
Fever	8
Skin rash	6
Arthritis	4
Raynaud phenomenon	7
Interstitial lung disease	13
Associated disorder	
Cancer	5
Rheumatic disease	9
Blood examination	
Creatine kinase (IU/L, mean ± SD)	6161 ± 4725
Elevated C-reactive protein	17
Antinuclear antibody positivity	5
Electromyography	
Spontaneous activity	41/86 (48 %)
Low-amplitude, short-duration motor unit potentials	79/86 (92 %)
Muscle images	
Atrophy on CT or MRI	46/79 (58 %)
Increased signals on T2 or STIR images	49/58 (84 %)
Histological diagnosis	
Necrotizing myopathy	84
Sporadic inclusion body myositis	0
Polymyositis	1
Dermatomyositis	1
Non-specific myositis	14
Autoantibodies	
7S RNA of SRP	100
54-kD protein of SRP	82
3-hydroxyl-3-methylglutatyl-coenzyme A reductase	3
Aminoacyl transfer RNA synthetase	0

SRP, signal recognition particle; STIR, short T1 inversion recovery

ten patients, which led physicians to make an initial diagnosis of facioscapulohumeral muscular dystrophy. Deep tendon reflexes were decreased or absent in 51 patients. Thirty-four patients reported Myalgia, especially in the early stage of disease.

With regard to extramuscular manifestations, the frequencies of fever, skin rash, arthritis, and Raynaud phenomenon were generally low. Chest CT revealed interstitial lung disease in 13 patients, all of whom had non-specific interstitial fibrosis, and their respiratory symptoms were generally mild. Malignancy (including lung, breast, stomach, ovarian and renal cancers) was discovered in 5 patients. Concomitant rheumatic diseases

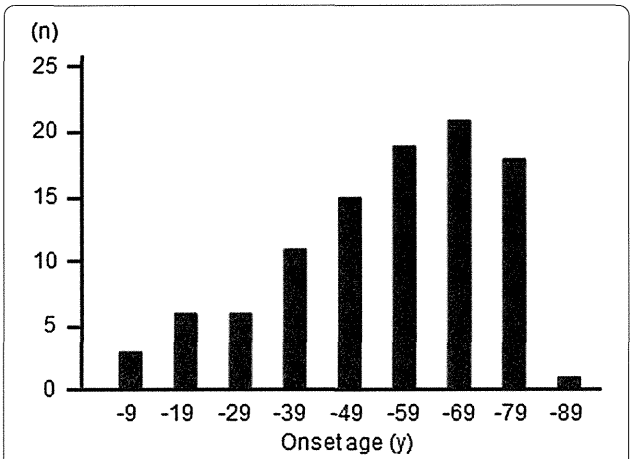

Fig. 2 Distribution of onset ages in the 100 patients with inflammatory myopathy with anti- SRP antibody

included Sjögren syndrome in 5 patients, systemic lupus erythematosus in 2, and rheumatic arthritis in 2 patients.

Laboratory, electrophysiological, and radiological tests

With regard to the routine laboratory findings, serum creatine kinase levels were markedly elevated to more than 1000 IU/L in all but 2 patients (mean 6161 IU/L, range 735–21,544 IU/L). In addition, the median on peaked creatine kinase level was 4998 IU/L (25–75 % interquartile range 3354–7464 IU/L). Elevation in C-reactive protein (≥1 mg/dL) was seen in 17 patients. Positivity of antinuclear antibody (≥1:160) was detected in only 5 patients.

We assessed the findings of electromyography from 86 patients. Needle electromyography revealed positive sharp waves and/or fibrillation potentials in 41 (48 %) of the 86 patients. Patients with chronic progression tended to lack the spontaneous activity. In contrast, myopathic motor unit potentials such as short-duration and low-amplitude motor units with early recruitment were detected in 92 % of the 86 patients.

Information obtained by muscle CT or MRI was available for 38 and 58 patients, respectively. The distribution of inflammation and muscle atrophy was detectable in muscle CT or MRI in the thighs (Fig. 3). Muscle atrophy on CT or MRI was seen in 46 (58 %) of 79 patients. High-intensity signal in T2 or short TI inversion recovery (STIR) images were observed in 49 (84 %) of 58 patients.

Histological diagnosis

With regard to the histological diagnoses, 84 patients had necrotizing myopathy, in which necrotic fibers > 1 % of total muscle fibers accompanied by regeneration fibers were observed. In contrast, there was apparent inflammatory cell infiltration in 16 patients. Inflammatory cell infiltration was regarded as positive when it was seen in the endomysial or perimysial regions, but not in the vicinity of necrotic fibers. Areas of invaded cells were in the endomysial region ($n = 5$), perimysial region ($n = 7$), or both ($n = 4$). The histological diagnoses of sporadic inclusion body myositis, polymyositis and dermatomyositis were made in 0, 1, and 1 patient, respectively. The other 14 patients had non-specific myositis.

Detection of autoantibodies

All 100 serum samples were positive for anti-SRP antibodies using RNA immunoprecipitation. RNA immunoprecipitation revealed additional autoantibodies including anti-Ro/SS-A or anti-La/SS-B in 11, anti-U1RNP in 4, anti-Ku in 1, anti-Th/To in 1, and anti-ribosome in 1 serum sample. It was noted that no serum contained anti-ARS antibodies.

In addition to the gold standard detection method, anti-SRP antibodies were also evaluated by an ELISA using recombinant SRP54 protein as an antigen. The anti-SRP54 antibody index was calculated as the OD450 of the samples divided by the OD450 of the referential serum. A total of 272 serum samples were examined (Fig. 4). When the cut-off value was set as the mean + 5 × the SD of the healthy control sera (anti-SRP54 antibody index: 0.37), positivity for the anti-SRP54 antibody was detected in 82 serum samples with anti-SRP antibodies detected by RNA immunoprecipitation. The disease control samples (including those with myositis, myasthenia gravis and Duchenne muscular dystrophy) were all negative for anti-SRP54 antibody. Importantly, 18 serum samples had autoantibody against 7S RNA of SRP, but not against SRP54 protein.

We evaluated the serial changes in anti-SRP54 antibody index in ten serum samples. The anti-SRP54 antibody index decreased from pre-treatment to post-treatment (Fig. 4b). Since the longitudinal follow-up was not enough, we could not determine the association between the serial change of anti-SRP54 antibody index and creatine kinase or muscle weakness. In contrast, we evaluated the peaked

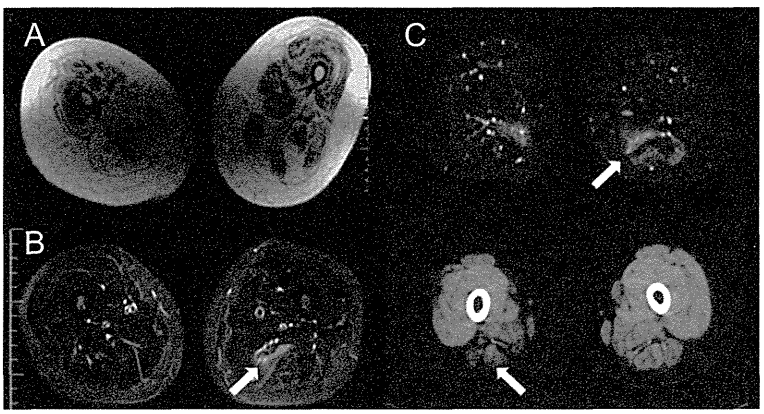

Fig. 3 Muscle images of thighs in patients with anti-SRP antibodies. **(a)** Muscle atrophy on MRI T1 images. **(b)** High signal intensity on MRI STIR images. **(c)** Increased signals on MRI STIR images (upper) and muscle atrophy in CT (lower)

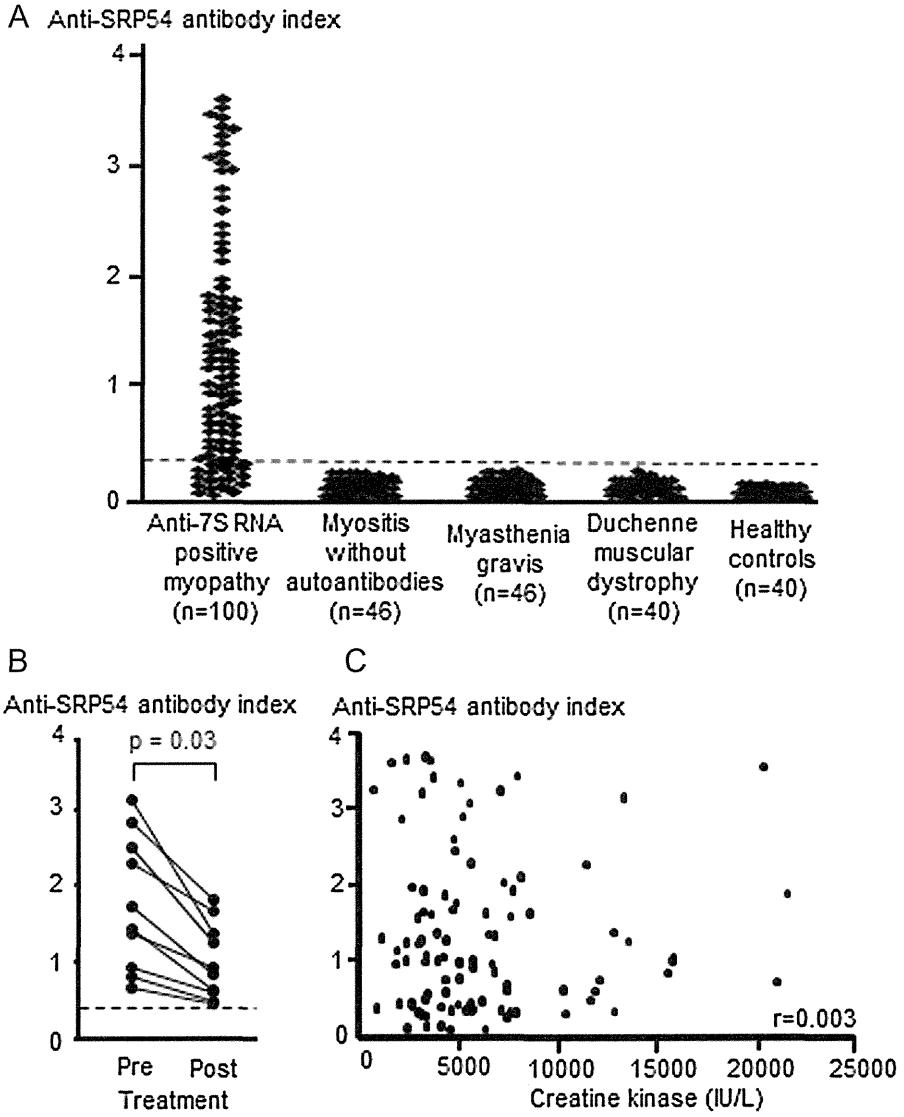

Fig. 4 SRP54 enzyme-linked immunosorbent assay (ELISA). **(a)** Antibodies reactive with recombinant SRP54 protein by ELISA in sera from patients with anti-7S RNA positive myopathy, myositis without autoantibodies, myasthenia gravis, muscular dystrophy, and healthy controls. The cut-off level for positivity of anti-SRP54 antibody index is indicated by the broken line. **(b)** Serial changes of anti-SRP54 antibody index between pre- and post-treatment in 10 patients with anti-SRP antibodies. **(c)** Correlation of anti-SRP54 antibody index determined by ELISA and serum creatine kinase in 100 patients with anti-SRP antibodies

serum creatine kinase values related with anti-SRP54 antibody index using same blood sample. There was no correlation between creatine kinase and anti-SRP54 antibody index in the 100 serum samples (r = 0.003, Fig. 4c).

To exclude the possibility of statin-induced myopathy, we also measured anti-HMGCR antibodies in the 100 serum samples. Anti-HMGCR antibodies were negative in the 5 patients with statin-exposure. Although anti-HMGCR antibodies were detected in 3 patients without the statin treatment, the titers of anti-HMGCR antibodies were much lower than those of the patients with statin-induced myopathy [15].

Treatment

We further evaluated the clinical course of the 84 patients followed over 2 years. All but 3 of the 84 patients were treated with oral prednisolone. Two patients were proven to be positive for anti-SRP antibodies ten year after the disease onset. They suffered from the progression of muscle weakness during the first 3–5 years, and then the disease activity stopped. The remaining patient developed a cerebral infarction soon after undergoing the muscle biopsy.

In addition to prednisolone, 62 (77 %) of 81 patients required additional immunotherapy, including intravenous