information was assessed based on records provided by the physicians.

We identified 295 alleles ranging in length from 180 to 258 bp by PCR amplification of the DM2 repeat. Heterozygosity was identified in 142 individuals (0.93). In the remaining 11 samples showing a single allele, Southern blot or repeat-primed PCR analysis showed no expanded CCTG repeats (Figure 1), indicating that all of them are homozygous for a single allele. Thus, in our extensive survey, no DM2-related CCTG expansion was detected.

Most DM patients in Japan have been considered to have DM1 (NIH Genetics Home Reference, http://ghr.nlm.nih.gov/condition/myotonic-dystrophy). Our study confirms that DM2 is an extremely rare cause of myotonic and/or LGMD patients in Japan. Although the spectrum of clinical presentation of DM2 is variable and only one Japanese DM2 patient has been reported to date, our data have important implications concerning the indications for genetic testing and counseling for DM2 in East-Asian populations. The origin of most DM2 mutations is estimated to be 200–540 generations ago in Europe, and DM2 has since spread into several European populations.[3] The rarity of DM2 in East-Asian populations may be because of a lack of founder effects or extinction of DM2 by genetic drift or selective causes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Liquori, C. L., Ricker, K., Moseley, M. L., Jacobsen, J. F., Kress, W ., Naylor, S. L. *et al.* Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* **293,** 864–867 (2001).
2 Harper, P. S. in *Myotonic Dystrophy* (W.B. Saunders, London, 2001).
3 Liquori, C. L., Ikeda, Y ., Weatherspoon, M., Ricker, K., Schoser, B. G., Dalton, J. C. *et al.* Myotonic dystrophy type 2: human founder haplotype and evolutionary conservation of the repeat tract. *Am. J. Hum. Genet.* **73,** 849–862 (2003).
4 Day, J. W., Ricker, K., Jacobsen, J. F., Rasmussen, L. J., Dick, K. A., Kress, W. *et al.* Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum. *Neurology* **60,** 657–664 (2003).
5 Bachinski, L. L., Udd, B., Meola, G., Sansone, V., Bassez, G., Eymard, B. *et al.* Confirmation of the type 2 myotonic dystrophy $(CCTG)_n$ expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. *Am. J. Hum. Genet.* **73,** 835–848 (2003).
6 Udd, B., Meola, G., Krahe, R., Thornton, C., Ranum, L., Day, J. *et al.* Report of the 115th ENMC workshop: DM2/PROMM and other myotonic dystrophies: 3rd Workshop. *Neuromuscul. Disord.* **13,** 589–596 (2003).
7 Coenen, M. J ., Tieleman, A. A., Schijvenaars, M. M., Leferink, M., Ranum, L. P., Scheffer, H. *et al.* Dutch myotonic dystrophy type 2 patients and a North-African DM2 family carry the common European founder haplotype. *Eur. J. Hum. Genet.* **19,** 567–570 (2011).
8 Saito, T., Amakusa, Y., Kimura, T., Yahara, O., Aizawa, H., Ikeda, Y. *et al.* Myotonic dystrophy type 2 in Japan: ancestral origin distinct from Caucasian families. *Neurogenetics* **9,** 61–63 (2008).
9 Young, N. P., Daube, J. R., Sorenson, E. J. & Milone, M. Absent, unrecognized, and minimal myotonic discharges in myotonic dystrophy type 2. *Muscle Nerve* **41,** 758–762 (2010).
10 Suominen, T., Bachinski, L. L., Auvinen, S., Hackman, P., Baggerly, K. A., Angelini, C. *et al.* Population frequency of myotonic dystrophy: higher than expected frequency of myotonic dystrophy type 2 (DM2) mutation in Finland. *Eur. J. Hum. Genet.* **19,** 776–782 (2011).

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Case report

Acid phosphatase-positive globular inclusions is a good diagnostic marker for two patients with adult-onset Pompe disease lacking disease specific pathology

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Abstract

Diagnosis of adult-onset Pompe disease is sometimes challenging because of its clinical similarities to muscular dystrophy and the paucity of disease-specific vacuolated fibers in the skeletal muscle pathology. We describe two patients with adult-onset Pompe disease whose muscle pathology showed no typical vacuolated fibers but did show unique globular inclusions with acid phosphatase activity. The acid phosphatase-positive globular inclusions may be a useful diagnostic marker for adult-onset Pompe disease even when typical vacuolated fibers are absent.
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Keywords: Pompe disease; *GAA*; Globular inclusion; Acid phosphatase

1. Introduction

Pompe disease (glycogen storage disease type 2; acid maltase deficiency; OMIM #232300) is an autosomal recessive disease caused by mutations in the gene encoding acid α-glucosidase (GAA, OMIM #606800), a lysosomal enzyme involved in glycogen degradation [1]. Based on age of onset and clinical severity, which depends on residual GAA activity, the disease can be classified into infantile, childhood-onset, and adult-onset forms.

Most of the infantile and childhood-onset forms exhibit disease-specific skeletal muscle pathology, which shows fibers occupied by huge vacuoles that contain basophilic amorphous materials. However, diagnosis of the adult-onset form is sometimes challenging due to clinical similarities to muscular dystrophy and the paucity of typical vacuolated myofibers. We diagnosed 37 patients with Pompe disease including 11 infantile, 16 childhood-onset, and 10 adult-onset forms in the muscle repository of the National Center of Neurology and Psychiatry (NCNP), Japan, based on a deficiency of GAA enzyme activity assayed using biopsied muscles, as previously described [2]. Among these 37 patients, two unrelated Japanese patients did not have disease-specific vacuolated muscle fibers but did have unique cytoplasmic inclusions. Here, we report the diagnostic utility of acid phosphatase (ACP)-positive globular inclusions for adult-onset Pompe disease.

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2. Case report

2.1. Clinical summary

Patient 1: A 44-year-old man had been well until the age of 41 years when he started having difficulty in running. He was admitted to the hospital because of progressive muscle weakness. His parents were first cousins, but there was no family history of neuromuscular disorders. He was clinically suspected to suffer from muscular dystrophy because of slowly progressive muscle weakness and elevated creatine kinase levels of around 800 IU/L (normal, <171 IU/L). On examination, he had grade 4-muscle weakness on medical research council (MRC) scale and marked atrophy in his thighs. He did not have apparent respiratory impairment. Electromyography (EMG) showed myopathic changes with fibrillation and increased polyphasic motor unit potentials (MUPs).

Patient 2: A 62-year-old woman first noticed difficulty in climbing stairs at the age of 35 years, and needed a stick to walk at 45 years. Muscle weakness gradually worsened predominantly in her proximal limbs, and she became wheelchair-bound at 55 years. A muscle biopsy was performed at the age of 61 years. On examination, she had muscle weakness and atrophy predominantly in the proximal upper and lower limbs at the grade 3–4 on MRC scale. Serum CK level was 70 IU/L (normal, <142 IU/L). An EMG showed myopathic changes with increased polyphasic MUPs and myotonic-like repetitive discharges. She had been on non-invasive positive-pressure ventilation since the age of 62 years when the respiratory insufficiency appeared.

2.2. Skeletal muscle pathology

The skeletal muscle pathology from the vastus lateralis of patient 1 and from the biceps brachi of patient 2 showed nonspecific myopathic changes with moderate fiber size variation, mild endomysial fibrosis, and some fiber splitting (Fig. 1A). No necrotic or regenerating fibers were seen. No vacuoles containing amorphous materials were observed. Importantly, both muscles contained red–purple globular inclusions on modified Gomori-trichrome (mGT) stain (Fig. 1A and B). The average percentages of fibers with globular inclusions in the whole mGT-stained section were 0.5% in patient 1 and 2% in patient 2. These inclusions were invariably highlighted by ACP stain but not stained by periodic acid Schiff (PAS) (Fig. 1C). Inclusions were stained only faintly on menadione-linked α-glycerophosphate dehydrogenase (MAG) without substrate (Fig. 3A). Fibers with ACP-positive globular inclusions were also found in 15 of 16 childhood-onset and seven of eight adult-onset patients with disease-specific pathology in varying proportions (0.1–10%). The rate of fibers with inclusions was not significantly different between the childhood-onset and adult-onset forms. Fibers carrying inclusions did not have typical vacuoles with amorphous materials inside. In the infantile cases, more than 90% of the fibers were vacuolated, whereas non-vacuolated fibers with inclusions were hardly recognizable.

Double immunostaining was performed using primary antibodies against a lysosomal marker, lysosomal associated membrane protein-2 (LAMP-2; Developmental Studies Hybridoma Bank (DSHB), Iowa City, IA, USA) and an autophagosomal marker, microtubule-associated protein 1 light chain 3 (LC3; Novus Biologicals, Littleton, CO, USA). In fibers with ACP-positive inclusions, immunoreactivity for LAMP-2 and LC3 were accumulated focally in inclusions and surrounding area (Fig. 1D). We also examined another samples from adult-onset patients with typical vacuoles. Fibers with typical vacuoles were entirely positive for LAMP-2 and LC3 (data not shown).

On PAS staining, performed on epon-embedded sections (Epon-PAS) to detect glycogen more sensitively, PAS was negative in globular inclusions but positive in the surrounding area (Fig. 1E).

Electron micrography was performed as previously described using a Tecnai spirit transmission electron microscope (FEI, Hillsboro, OR, USA) [3]. The inclusions consisted of homogeneous electron-dense globules surrounded by increased glycogen particles and autophagic vacuoles (Fig. 1F). The globules contained neither dotted glycogen particles nor a filamentous structure.

2.3. GAA enzymatic analysis and genetic analysis

Presence of globular inclusions led us to suspect Pompe disease, and GAA enzymatic activity analyses revealed 7.5% of normal control activity in patient 1 and 12.3% in patient 2.

Genomic DNA was extracted from peripheral lymphocytes or biopsied muscle using a standard protocol for mutational analysis of *GAA*. All exons and their flanking intronic regions of *GAA* were amplified by PCR and directly sequenced with an ABI PRISM 3100 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). Both patients carried the homozygous *GAA* mutation at the last codon of exon 2 (c. 546G > T). RT-PCR and direct sequencing were performed using RNA extracted from biopsied muscles. This novel mutation causes aberrant splicing by skipping exon 2 (Fig. 2). This homozygous c. 546G > T mutation was also found in another patient with the adult-onset form, whose muscle pathology showed typical skeletal muscle pathology with vacuolated fibers.

3. Discussion

ACP-positive globular inclusions were a good diagnostic marker for the two patients with adult-onset Pompe disease lacking typical vacuolated fibers. Among 12,103 muscle biopsies in the NCNP repository from 1979 to 2010, ACP-positive globular inclusions were not reported, except for Pompe disease.

The globular inclusions are most likely the same as "reducing body-like globular inclusions in late-onset Pompe disease" reported by Sharma et al., as the pathological features are

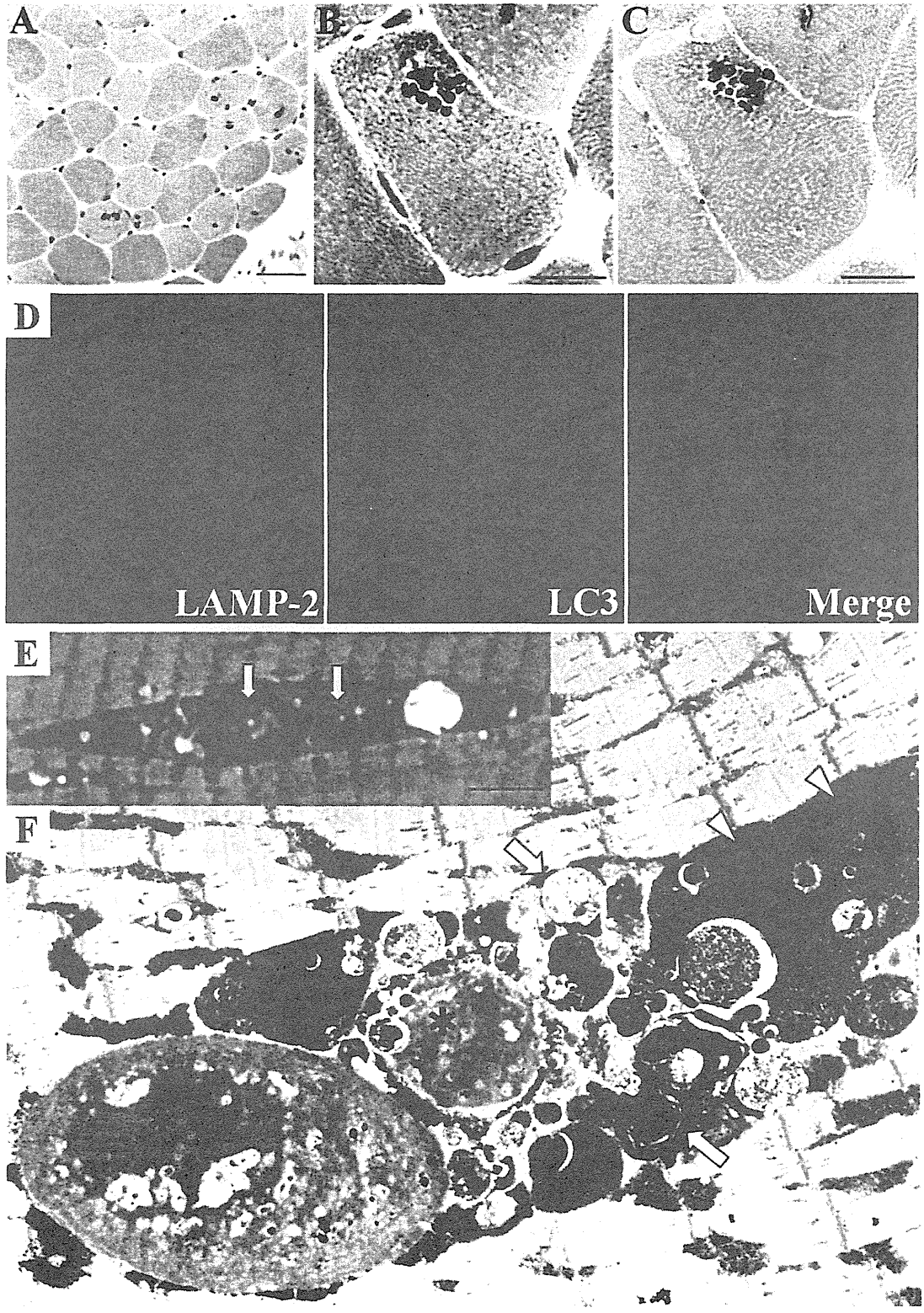

Fig. 1. Acid phosphatase-positive globular inclusions in patient 2. (A and B) Biopsied skeletal muscle showed nonspecific myopathic changes with scattered red–purple colored globular inclusions on modified Gomori-trichrome stain. (C) The inclusions have intense activity on acid phosphatase stain. Bar = 20 μm. (D) Double immunostaining for LAMP-2 (green) and LC3 (red) demonstrates colocalization of positive immunoreactions in the inclusions and surrounding area (B–D; serial sections). (E) On epon-embedded section, periodic acid Schiff stain is negative in inclusions (arrows). Bar = 5 μm. (F) On electron microscopy, globular inclusions (asterisks) lack Z-line structure, which differs from cytoplasmic bodies. Autophagic vacuoles (arrows) and glycogen particles (arrow heads) are seen in the vicinity of globular inclusions (12000×).

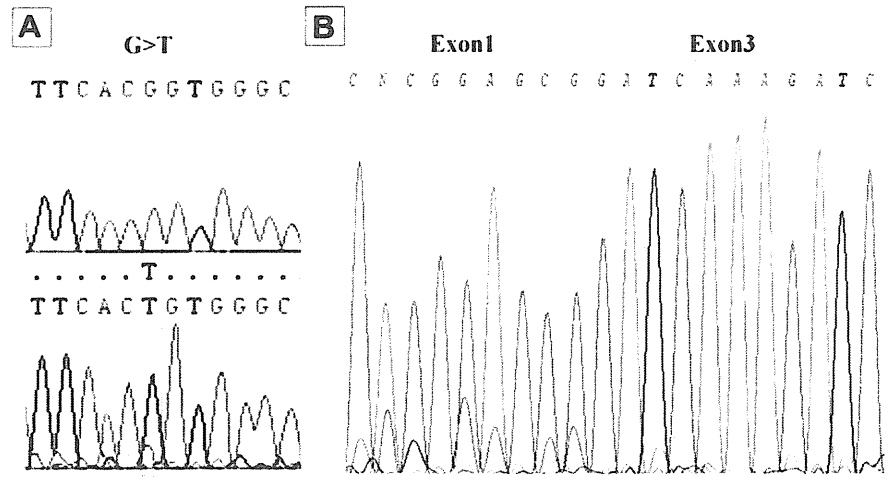

Fig. 2. Mutational analysis of *GAA*. Both patient have a homozygous c. 546G > T mutation at the last codon of exon2 (A upper: control, lower: patient), which creates mRNA with skipping exon 2 (B).

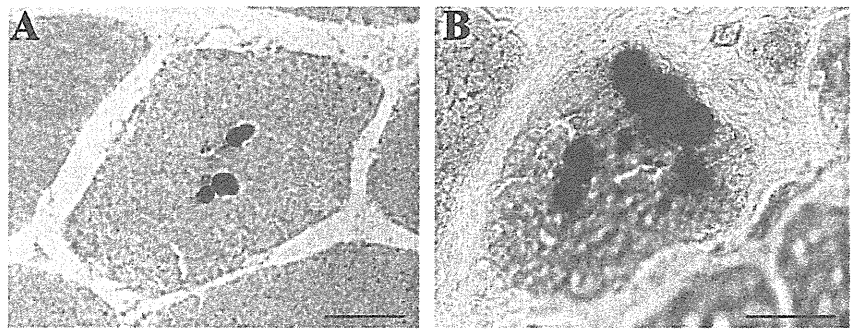

Fig. 3. Inclusions on menadione-linked α-glycerophosphate dehydrogenase (MAG) without substrate. Globular inclusions in Pompe disease (A) are only faintly stained comparing reducing bodies in reducing body myopathy with *FHL1* mutation (B). Bar = 20 μm.

rather similar [4]. However, globular inclusions showed much fainter staining on MAG without substrate than genuine reducing bodies seen in reducing body myopathy with *FHL1* mutations (Fig. 3). More importantly, ACP positivity has not been clearly described previously.

These globular inclusions are reminiscent of cytoplasmic bodies, which are nonspecific findings reflecting degeneration of the Z-disk in various neuromuscular diseases, particularly myofibrillar myopathies. However, the nature of the globular inclusions differs essentially from cytoplasmic bodies because of positive ACP staining and the lack of associated Z-disk components. Although it remains unclear how the ACP-positive globular inclusions are formed, the absence of glycogens in the globular inclusions suggest that they differ from glycogen accumulations in lysosomes. Fibers with typical vacuoles were diffusely positive for both lysosomal and autophagosomal markers as shown previously [5,6]. On the other hand, immunoreactivities of these markers accumulated more focally in fibers with inclusions. Further study should be needed to clarify what causes these pathological differences.

In conclusion, ACP-positive globular inclusions may be a hallmark of Pompe disease and a useful diagnostic marker for adult-onset Pompe disease lacking typical vacuolated fibers. Since enzyme replacement therapy is effective, albeit not fully, in adult-onset patients, early diagnosis is necessary for a better prognosis.

Ethical approval

All clinical materials used in this study were obtained for diagnostic purposes with written informed consent approved by the Ethical Committee of NCNP.

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References

[1] Hers HG. Alpha-glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). Biochem J 1963;86:11–6.
[2] Shanske S, Dimauro S. Late-onset acid maltase deficiency. Biochemical studies of leukocytes. J Neurol Sci 1981;50:57–62.
[3] Park YE, Hayashi YK, Goto K, et al. Nuclear changes in skeletal muscle extend to satellite cells in autosomal dominant Emery-Dreifuss muscular dystrophy/limb-girdle muscular dystrophy 1B. Neuromuscul Disord 2009;19:29–36.
[4] Sharma MC, Schultze C, von Moers A, et al. Delayed or late-onset type II glycogenosis with globular inclusions. Acta Neuropathol 2005;110:151–7.
[5] Raben N, Ralston E, Chien YH, et al. Differences in the predominance of lysosomal and autophagic pathologies between infants and adults with Pompe disease: implications for therapy. Mol Genet Metab 2010;101:324–31.
[6] Schoser BGH, Müller-Höcker J, Horvath R, et al. Adult-onset glycogen storage disease type 2: clinico-pathological phenotype revisited. Neuropathol Appl Neurobiol 2007;33:544–59.

ORIGINAL CONTRIBUTION

Myopathy Associated With Antibodies to Signal Recognition Particle

Disease Progression and Neurological Outcome

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Objective: To characterize the clinical course of myopathy associated with antibodies to signal recognition particle (SRP), or anti-SRP myopathy.

Design: Case series.

Setting: Keio University Hospitals and National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan.

Patients: We reviewed clinical features of 27 patients with anti-SRP myopathy and analyzed disease progression and neurological outcome.

Main Outcome Measures: Anti-SRP antibodies in serum were detected by RNA immunoprecipitation assay using extracts of K562 cells.

Results: Of the 27 patients, 5 (19%) showed chronic progressive muscle weakness as well as atrophy of limbs and trunk muscles from a younger age with more severe neurological outcomes compared with the other 22 patients (81%) with the subacute form.

Conclusion: A subset of patients with anti-SRP myopathy can show a chronic progressive form associated with severe clinical deficits.

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AUTOANTIBODIES AGAINST signal recognition particle (SRP) were first found in the serum of a patient with polymyositis and were listed as myositis-specific antibodies.[1] Myopathy associated with antibodies to SRP (anti-SRP myopathy) has recently been regarded as an immune-mediated necrotizing myopathy based on histological findings and has been clinically characterized by severe muscle weakness, marked elevation of serum creatine kinase (CK) levels, and poor response to corticosteroid therapy.[2-7] These observations were gathered mainly from patients with a clinical diagnosis of inflammatory myopathies. However, the clinical spectrum of anti-SRP myopathy may be broader.

The rapid progression of weakness is a characteristic clinical feature of anti-SRP myopathy.[2-7] The mean interval from its onset to diagnosis is 3 to 4 months, and clinical symptoms are usually progressive for 5 to 6 months.[3-5] In contrast, Dimitri et al[8] first described a 31-year-old man in whom weakness progressed for more than 3 years. Before the anti-SRP antibody was detected, he was diagnosed as having limb-girdle muscular atrophy. We also described a 32-year-old man with childhood-onset myopathy whose diagnosis alternated between inflammatory myopathy and muscular dystrophy for 21 years.[9] These results suggested that patients with anti-SRP myopathy can show chronic progression indistinguishable from muscular dystrophy. Herein, we analyzed the disease course and neurological outcomes in patients with anti-SRP myopathy.

METHODS

We chose 27 patients with myopathy with the anti-SRP antibody, including 10 previously reported cases.[9,10] The diagnosis of anti-SRP myopathy was based on clinical, electrophysiological, histopathological, and serological findings. Muscle weakness was assessed by manual muscle strength (Medical Research Council scale grade), and severe weakness was defined as grade 3 or lower. Muscle biopsy was performed in all 27 patients and showed fiber size variation as well as fiber necrosis and regeneration with or without lymphocyte infiltration. No patients had taken statins.

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Anti-SRP antibodies were detected by RNA immunoprecipitation assay using extracts of K562 cells as previously described.[11] Briefly, 10 μL of serum was mixed with 2 mg of Protein A Sepharose CL-4B (Pharmacia Biotech AB) in 500 μL of immunoprecipitation buffer (10mM TRIS hydrochloride, pH 8.0, 500mM sodium chloride, 0.1% Nonidet P40) and incubated for 2 hours. After washing 3 times with immunoprecipitation buffer, antigen-bound Sepharose beads were mixed with 100 μL of K562 cell extract (6×10^6 cell equivalents per sample) for 2 hours, and 30 μL of 3M 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 bound RNA. After ethanol precipitation, the RNA was resolved by using a 7M urea–8% polyacrylamide gel, and the gel was silver stained (Bio-Rad). Immunoprecipitated RNA located in the 7SL-RNA lesion was regarded as anti-SRP antibody. Other myositis-specific and myositis-associated autoantibodies were also detected by the RNA immunoprecipitation assay.

Neurological outcomes were assessed using the modified Rankin Scale (mRS).[12] This scale was principally used for evaluating function of patients with stroke; however, it was also applied to patients with myositis.[13] Neurological outcomes were divided into 3 groups: recovered, mild deficit, and severe deficit. Patients who responded optimally to the treatment and returned to their jobs (mRS score of 0-1) were defined as recovered. Patients who responded partially to treatment and resumed most activities of daily living (mRS score of 2-3) were defined as having a mild deficit. Patients who showed reworsening muscle weakness or re-elevation of serum CK levels after the treatment were also included in this group. Patients who responded minimally to the treatment and required support in daily activities (mRS score of 4) were defined as having a severe deficit.

This study was approved by the institutional review boards at Keio University and the National Center of Neurology and Psychiatry. Statistical analyses were performed using StatView version 5.0 statistical software (SAS Institute, Inc).

RESULTS

Figure 1 shows the distribution of periods between disease onset and the first examination. We divided 27 patients with anti-SRP myopathy into 2 subtypes (subacute and chronic forms) based on the clinical course. Of the 27 patients with anti-SRP myopathy in our study, 5 (19%) were considered to have the chronic form. The patients' demographic and clinical features are compared between those with the subacute and chronic forms (**Table 1**). Disease onset occurred at a younger age in those with the chronic form than in those with the subacute form (mean age, 15.4 vs 52.4 years, respectively; $P < .001$). No patients with the chronic form had a clear clinical history of antecedent infection, whereas 3 patients (14%) with the subacute form had antecedent infection. Despite a previous report,[5] seasonal occurrence was not clear in our series. Disease progression of the subacute form was usually rapid, and the mean duration between disease onset and the first examination was 3.1 months. In particular, 3 patients showed rapid disease progression in 2 to 3 weeks. In contrast, patients with the chronic form showed significantly slower progression, and the mean duration between disease onset and the first examination was 10.2 months ($P = .001$).

In our series, asymmetrical muscle involvement was seen in 2 patients, whereas the other 25 patients showed proximal-dominant symmetrical limb muscle weakness. Lower limbs were more severely affected than upper limbs. All 5 patients with the chronic form and about half of the patients with the subacute form showed severe muscle weakness and atrophy at the first examination. Several reports emphasized that dysphagia, but not dysarthria, was observed at a high frequency in 43% to 75% of patients with anti-SRP myopathy.[3,5,7] In our series, 7 patients (26%) had dysphagia and 3 (11%) reported it as the initial symptom. Previous reports also showed a high frequency of cardiac involvement,[2,5] while only 1 patient in our series had arrhythmias, which did not require treatment. Respiratory muscle involvement was detected in 3 patients. Myalgia was noted in 9 patients (36%) and tended to precede muscle weakness. Extramuscular manifestations were observed only in patients with the subacute form. Skin rash and interstitial lung disease, which were clinically suggestive of dermatomyositis, were observed in 2 and 4 patients, respectively. Serum CK levels were markedly elevated to more than 1000 IU/L (to convert to microkatals per liter, multiply by 0.0167) in all 27 patients; however, there was no difference between the subacute and chronic forms. Other autoantibodies were found in 6 patients with the subacute form, including Ro/SSA (3 patients), Th/To (1 patient), ribosome (1 patient), and U1RNP (1 patient).

All 27 patients were treated with oral prednisolone (1 mg/kg/d). Half of the patients were treated with additional immunosuppressive agents, including methotrexate (n = 5), azathioprine (n = 4), tacrolimus (n = 2), cyclophosphamide (n = 1), and cyclosporine (n = 1), or with intravenous immunoglobulin (n = 6). Although some patients required 2 to 3 months to respond to treatment, the patients with anti-SRP myopathy did not always respond poorly. The combination of oral prednisolone and intravenous immunoglobulin appears to be most effective for patients with the subacute form as the initial treatment. The neurological outcomes showed that 10 patients (45%) with the subacute form recovered. In contrast, all 5 patients with the chronic form had more severe neurological outcomes compared with the 22 patients with the subacute form ($P = .008$) (**Figure 2**).

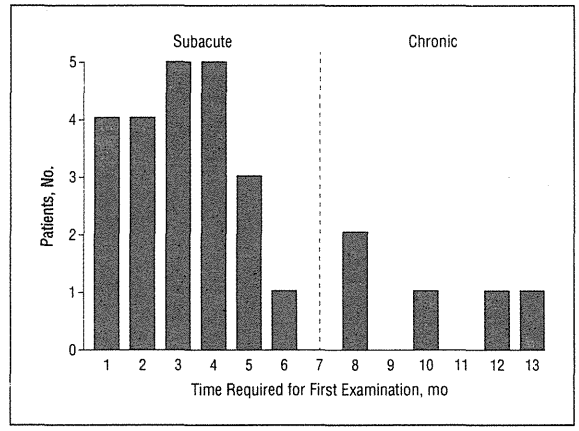

Figure 1. Period between disease onset and the first examination in 27 patients with anti–signal recognition particle myopathy. They were divided into 22 patients with the subacute form and 5 patients with the chronic form based on the clinical course.

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Table 1. Comparison of Clinical Features Between Subacute and Chronic Forms of Anti–Signal Recognition Particle Myopathy

Clinical Feature	Patients, No. (%)		
	Subacute (n = 22)	Chronic (n = 5)	*P* Value
Age at onset, mean (range), y	52.4 (14-82)	15.4 (5-32)	<.001[a]
Female	12 (55)	3 (60)	.78[b]
Antecedent infection	3 (14)	0	.93[b]
Time required for first examination, mean (range), mo	3.1 (1-6)	10.2 (8-13)	.001[a]
Muscle weakness			
Arms < legs	16 (73)	3 (60)	.98[b]
Arms > legs	6 (27)	2 (40)	.98[b]
Severe involvement	11 (50)	5 (100)	.12[b]
Laterality	1 (5)	1 (20)	.80[b]
Facial muscle involvement	1 (5)	1 (20)	.80[b]
Bulbar sign	6 (27)	1 (20)	.81[b]
Cardiac involvement	1 (5)	0	.80[b]
Respiratory failure	3 (14)	1 (20)	.73[b]
Neck weakness	9 (41)	4 (80)	.27[b]
Muscle atrophy	10 (45)	5 (100)	.08[b]
Myalgia	8 (36)	1 (20)	.86[b]
Extramuscular involvement			
Fever	4 (18)	0	.73[b]
Skin rash	2 (9)	0	.80[b]
Arthritis	1 (5)	0	.80[b]
Raynaud phenomenon	1 (5)	0	.80[b]
Interstitial lung disease	4 (18)	0	.73[b]
Associated disorder			
Cancer	1 (9)	0	.80[b]
Rheumatic disorder	1 (9)	0	.80[b]
Serum creatine kinase, mean (range), IU/L	6101 (1149-15 585)	4190 (2465-5725)	.08[a]

SI conversion factor: To convert serum creatine kinase to microkatals per liter, multiply by 0.0167.
[a] Statistical analysis by *t* test.
[b] Statistical analysis by χ^2 test.

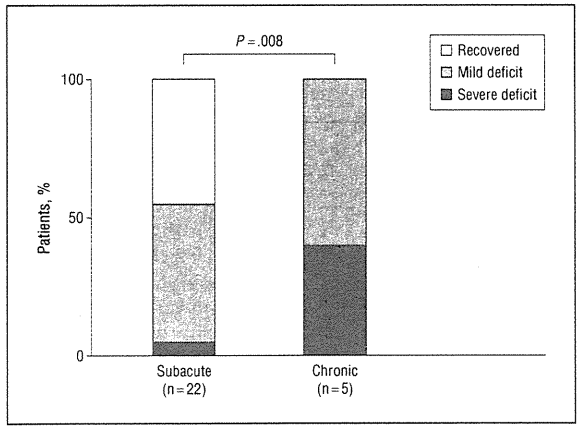

Figure 2. Neurological outcomes were assessed using the modified Rankin Scale[12] with some modifications and were compared between subacute and chronic forms of anti–signal recognition particle myopathy. The neurological outcomes were divided into recovered, mild deficit, and severe deficit. Differences between the groups were analyzed with the Mann-Whitney test. Five patients with the chronic form showed more severe outcomes than 22 patients with the subacute form (*P* = .008).

Detailed clinical features of 5 patients with the chronic form are summarized in **Table 2**. All patients had severe muscle weakness and marked atrophy in all 4 limbs and the trunk. Two patients (patients 2 and 5) noticed arm muscle weakness as the initial symptom. Importantly, scapular winging was noted in 2 patients (patients 2 and 3) at the first examination and was suspected to involve facioscapulohumeral muscular dystrophy. The serum CK level was decreased after treatment in patients with the chronic form, but muscle weakness gradually progressed and recovery of muscle strength was delayed. Three patients (patients 1, 2, and 3) became unable to walk independently, and 1 (patient 3) required mechanical ventilation. Because muscle biopsies were not suggestive of inflammatory myopathy, 1 patient (patient 3) was treated for only 3 months and 2 (patients 1 and 2) were treated after the detection of anti-SRP antibody. Of these younger patients, 2 (patients 2 and 3) became severely disabled, whereas the other 2 (patients 4 and 5) were treated soon after the muscle biopsy and responded partially to treatment.

COMMENT

There are 2 methods for detecting anti-SRP antibodies: the RNA immunoprecipitation assay we used and an immunoassay using the signal peptide–binding 54-kDa subunit of SRP (SRP54) as the antigen. Because SRP54 is regarded as the main antibody target, the immunoassay using SRP54 is easily conducted and the antibody level is also available.[1,2,14] However, epitopes of anti-SRP antibodies may also be located in other subunits of SRP proteins or 7SL-RNA.[7,15] In contrast, RNA immunoprecipitation assay, the standard method for detection of

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Table 2. Clinical Features of 5 Patients With the Chronic Type of Anti–Signal Recognition Particle Myopathy

Feature	Patient No. 1[a]	2[a]	3[a]	4	5
Sex	F	F	M	F	M
Age at onset	5 y 9 mo	9 y 8 mo	10 y 2 mo	20 y 10 mo	32 y 9 mo
Initial symptoms	Frequent falls	Difficulty raising arm	Difficulty running fast	Difficulty climbing stairs	Difficulty raising his child
Weakness and atrophy	Proximal limbs (U < L); trunk	Proximal limbs (U > L); trunk; scapular winging; left dominant; myalgia	Proximal limbs (U < L); trunk; scapular winging; facial, bulbar; respiratory	Proximal limbs (U < L); trunk	Proximal limbs (U > L); trunk; strenocleidomastoideus
Serum creatine kinase, IU/L	4629	2467	4180	3951	5725
Muscle images	Atrophy in proximal limbs and trunk	Left-dominant atrophy and edematous change in proximal limbs and trunk	Atrophy and edematous change in proximal limbs and trunk	Atrophy and edematous change in proximal limbs and trunk	Atrophy and edematous change in proximal limbs and trunk
Age at muscle biopsy	6 y 5 mo	10 y 4 mo	11 y 3 mo, 16 y 6 mo	21 y 8 mo	33 y 9 mo
Muscle biopsy					
Variation in fiber size	Scattered	Scattered	Marked	Marked	Marked
Fiber necrosis and regeneration	Moderate	Marked	Marked	Scattered	Marked
Lymphocyte infiltration	None	None	None	None	Perivascular
Endomysial fibrosis	Minimal	Mild	Marked	Minimal	Mild
Age at anti-SRP antibody detection	7 y 4 mo	10 y 9 mo	32 y 6 mo	21 y 10 mo	34 y 3 mo
Age at treatment start	7 y 4 mo	10 y 9 mo	11 y 6 mo	21 y 8 mo	33 y 9 mo
Treatment	PSL, MTX, MPR	PSL, MTX, IVCY, AZA, tacrolimus	PSL (3 mo)	PSL, MPR	PSL, MTX, IVIg, tacrolimus
Age at final follow-up	9 y 3 mo	13 y 10 mo	34 y 8 mo	23 y 3 mo	35 y 6 mo
Response and neurological outcome	Partial response; progression for 2 y; relapse; MMT grade 4; Gowers sign	Minimal response; progression for 2 y; MMT grade 2-3; walking 20 m; difficulty in holding dishes	No response; progression for 3 y; recovered from mechanical ventilation; MMT grade 2-3; wheelchair use	Partial response; progression for 1 y; MMT grade 4	Partial response; progression for 1.5 y; weakness recovered; relapse

Abbreviations: AZA, azathioprine; IVCY, intravenous cyclophosphamide; IVIg, intravenous immunoglobulin; L, lower; MMT, manual muscle strength; MPR, high-dose methylprednisolone sodium succinate; MTX, methotrexate; PSL, prednisolone; SRP, signal recognition particle; U, upper.

SI conversion factor: To convert serum creatine kinase to microkatals per liter, multiply by 0.0167.

[a]These patients were previously described.[9,10]

anti-SRP antibodies, has advantages in sensitivity and specificity.[1,2,4,6,9,11] The RNA immunoprecipitation assay can recognize the conformational epitopes of SRP, although the titer of antibodies is not available. Many studies showed that anti-SRP antibodies were principally specific to myositis or necrotizing myopathy except in a few patients with systemic sclerosis or rheumatoid arthritis.[1,2,4,6,9,11] In regard to myopathies, we demonstrated that anti-SRP antibody was not detected in patients with various types of muscular dystrophy, and it was useful for the differential diagnosis of myopathies using RNA immunoprecipitation assay.[9]

Anti-SRP myopathy can show a wider variety of clinical symptoms than was previously considered. When weakness progresses rapidly, within 2 to 3 weeks, with extremely high serum CK levels (>10 000 IU/L), acute rhabdomyolysis should be differentiated.[8] When patients experience progressive weakness within 2 to 6 months[2-7] accompanied by interstitial lung disease, skin rash, or associated rheumatic disorders, polymyositis or dermatomyositis should be considered. Because skin rash is observed in approximately 10% of cases of anti-SRP myopathy in the present and previous studies,[5] anti-SRP antibodies may be also detected in patients clinically diagnosed as having dermatomyositis. In fact, Hamaguchi et al[16] reported that anti-SRP antibodies were detected in 7 of 376 patients (2%) with dermatomyositis using a similar detection method.

In our series, 5 of 27 patients with anti-SRP myopathy (19%) showed chronic progressive muscle involvement. The mean age at onset in these 5 patients was significantly younger than that of the patients with the subacute form, and patients with the chronic form showed severe weakness and atrophy in limbs and trunk muscles as well as poorer outcomes. It was speculated that the poor outcome may be partially ascribed to the delay of the first examination or anti-SRP antibodies detection. Importantly, these clinical features may indicate the possibility of muscular dystrophy rather than inflammatory myopathy,[8-10] although the disease progression was faster than occurs in muscular dystrophy. In fact, facioscapulohumeral muscular dystrophy was initially suspected in 2 patients owing to prominent shoulder-girdle weakness.[9,10]

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It is well known that anti-SRP myopathy is usually resistant to treatment, resulting in severe disablement.[2-4,6,7] However, our observation suggested that patients with the subacute form had relatively good neurological outcomes. Early diagnosis by screening for anti-SRP antibodies is important for choosing intensive immunotherapy, which might contribute to better outcomes. In this regard, Hengstman et al[5] reported that the response to treatment for patients with anti-SRP myopathy did not differ significantly from that of myositis without anti-SRP antibodies. They reported that 75% of patients with anti-SRP myopathy could walk without any assistance after treatment. The severe outcomes of anti-SRP myopathy described in the previous studies may be attributable partly to results for patients with the chronic form. Rituximab therapy is potentially effective for patients with the chronic form.[7] Based on these findings, it may be useful to divide patients by disease progression to predict the neurological outcome.

An apparent question about the relationship between anti-SRP antibodies and muscle involvement is whether the anti-SRP antibodies themselves have any pathogenic effect against muscle. This hypothesis may be supported by several lines of data: (1) anti-SRP antibodies purified from patients' serum samples can inhibit the in vitro translocation of secretory proteins into endoplasmic reticulum[17]; (2) the levels of anti-SRP54 autoantibodies are closely associated with the levels of myolysis[14]; and (3) the removal of anti-SRP antibodies by plasma exchange improves muscle strength.[14,18] Nevertheless, the causal relationship between anti-SRP antibodies and muscle involvement is still not established, and further experiments such as passive transfer to animals are necessary to elucidate the pathogenesis of anti-SRP antibodies.

In conclusion, anti-SRP myopathy can show quite variable disease progression and neurological outcomes.

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REFERENCES

1. Reeves WH, Nigam SK, Blobel G. Human autoantibodies reactive with the signal-recognition particle. *Proc Natl Acad Sci U S A.* 1986;83(24):9507-9511.
2. Targoff IN, Johnson AE, Miller FW. Antibody to signal recognition particle in polymyositis. *Arthritis Rheum.* 1990;33(9):1361-1370.
3. Miller T, Al-Lozi MT, Lopate G, Pestronk A. Myopathy with antibodies to the signal recognition particle: clinical and pathological features. *J Neurol Neurosurg Psychiatry.* 2002;73(4):420-428.
4. Kao AH, Lacomis D, Lucas M, Fertig N, Oddis CV. Anti-signal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy. *Arthritis Rheum.* 2004;50(1):209-215.
5. Hengstman GJ, ter Laak HJ, Vree Egberts WT, et al. Anti-signal recognition particle autoantibodies: marker of a necrotising myopathy. *Ann Rheum Dis.* 2006;65(12):1635-1638.
6. Takada T, Hirakata M, Suwa A, et al. Clinical and histopathological features of myopathies in Japanese patients with anti-SRP autoantibodies. *Mod Rheumatol.* 2009;19(2):156-164.
7. Valiyil R, Casciola-Rosen L, Hong G, Mammen A, Christopher-Stine L. Rituximab therapy for myopathy associated with anti-signal recognition particle antibodies: a case series. *Arthritis Care Res (Hoboken).* 2010;62(9):1328-1334.
8. Dimitri D, Andre C, Roucoules J, Hosseini H, Humbel RL, Authier FJ. Myopathy associated with anti-signal recognition peptide antibodies: clinical heterogeneity contrasts with stereotyped histopathology. *Muscle Nerve.* 2007;35(3):389-395.
9. Suzuki S, Satoh T, Sato S, et al. Clinical utility of anti-signal recognition particle antibody in the differential diagnosis of myopathies. *Rheumatology (Oxford).* 2008;47(10):1539-1542.
10. Suzuki S, Ohta M, Shimizu Y, Hayashi YK, Nishino I. Anti-signal recognition particle myopathy in the first decade of life. *Pediatr Neurol.* 2011;45(2):114-116.
11. Okada N, Mimori T, Mukai R, Kashiwagi H, Hardin JA. Characterization of human autoantibodies that selectively precipitate the 7SL RNA component of the signal recognition particle. *J Immunol.* 1987;138(10):3219-3223.
12. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke.* 1988;19(5):604-607.
13. Danieli MG, Calcabrini L, Calabrese V, Marchetti A, Logullo F, Gabrielli A. Intravenous immunoglobulin as add on treatment with mycophenolate mofetil in severe myositis. *Autoimmun Rev.* 2009;9(2):124-127.
14. Benveniste O, Drouot L, Jouen F, et al. Correlation of anti-signal recognition particle autoantibody levels with creatine kinase activity in patients with necrotizing myopathy. *Arthritis Rheum.* 2011;63(7):1961-1971.
15. Satoh T, Okano T, Matsui T, et al. Novel autoantibodies against 7SL RNA in patients with polymyositis/dermatomyositis. *J Rheumatol.* 2005;32(9):1727-1733.
16. Hamaguchi Y, Kuwana M, Hoshino K, et al. Clinical correlations with dermatomyositis-specific autoantibodies in adult Japanese patients with dermatomyositis: a multicenter cross-sectional study. *Arch Dermatol.* 2011;147(4):391-398.
17. Römisch K, Miller FW, Dobberstein B, High S. Human autoantibodies against the 54 kDa protein of the signal recognition particle block function at multiple stages. *Arthritis Res Ther.* 2006;8(2):R39.
18. Arlet JB, Dimitri D, Pagnoux C, et al. Marked efficacy of a therapeutic strategy associating prednisone and plasma exchange followed by rituximab in two patients with refractory myopathy associated with antibodies to the signal recognition particle (SRP). *Neuromuscul Disord.* 2006;16(5):334-336.

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Heterozygous UDP-GlcNAc 2-epimerase and *N*-acetylmannosamine kinase domain mutations in the *GNE* gene result in a less severe GNE myopathy phenotype compared to homozygous *N*-acetylmannosamine kinase domain mutations

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Questionnaire
Natural history

ABSTRACT

Background: Glucosamine (UDP-N-acetyl)-2-epimerase/*N*-acetylmannosamine kinase (GNE) myopathy, also called distal myopathy with rimmed vacuoles (DMRV) or hereditary inclusion body myopathy (HIBM), is a rare, progressive autosomal recessive disorder caused by mutations in the *GNE* gene. Here, we examined the relationship between genotype and clinical phenotype in participants with GNE myopathy.
Methods: Participants with GNE myopathy were asked to complete a questionnaire regarding medical history and current symptoms.
Results: A total of 71 participants with genetically confirmed GNE myopathy (27 males and 44 females; mean age, 43.1 ± 13.0 (mean ± SD) years) completed the questionnaire. Initial symptoms (*e.g.*, foot drop and lower limb weakness) appeared at a mean age of 24.8 ± 8.3 years. Among the 71 participants, 11 (15.5%) had the ability to walk, with a median time to loss of ambulation of 17.0 ± 2.1 years after disease onset. Participants with a homozygous mutation (p.V572L) in the *N*-acetylmannosamine kinase domain (KD/KD participants) had an earlier disease onset compared to compound heterozygous participants with mutations in the uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase and *N*-acetylmannosamine kinase domains (ED/KD participants; 26.3 ± 7.3 vs. 21.2 ± 11.1 years, respectively). KD/KD participants were more frequently non-ambulatory compared to ED/KD participants at the time of survey (80% vs. 50%). Data were verified using medical records available from 17 outpatient participants.
Conclusions: Homozygous KD/KD participants exhibited a more severe phenotype compared to heterozygous ED/KD participants.

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1. Introduction

Glucosamine (UDP-N-acetyl)-2-epimerase/*N*-acetylmannosamine kinase (GNE) myopathy, also known as distal myopathy with rimmed vacuoles (DMRV), Nonaka myopathy (MIM: 605820) or hereditary inclusion body myopathy (HIBM; MIM: 600737), is an early adult-onset, progressive myopathy that affects the tibialis anterior muscle, but spares quadriceps femoris muscles [1,2]. The disease is caused by a mutation in the *GNE* gene, which encodes a bifunctional enzyme [uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) 2-epimerase (GNE) and *N*-acetylmannosamine kinase (MNK)] known to catalyze two rate-limiting reactions involved in cytosolic sialic acid synthesis [3–7]. Mutations in the *GNE* gene result in decreased enzymatic activity *in vitro* by 30–90% [7–10]. Therefore, hyposialylation is thought to

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contribute to the pathogenesis of GNE myopathy. This is supported by the myopathic phenotype associated with a mouse model expressing the human D176V mutant GNE protein (GNE−/−hGNED176V-Tg) [11]. Muscle atrophy and weakness are prevented by oral treatment with sialic acid metabolites in this mouse model [12].

A phase I clinical trial using oral sialic acid therapy has recently been performed in Japan for the treatment of GNE myopathy (ClinicalTrials.gov; NCT01236898). A similar phase I study is currently underway in the United States (ClinicalTrials.gov; NCT01359319). Natural history and genotype–phenotype correlations need to be established for a successful phase II clinical trial for the treatment of GNE myopathy. However, only a small number of studies have been conducted that review the natural course of this disease. In addition, the presence of genotype–phenotype correlations is controversial in GNE myopathy, with most reports denying significant correlations [7]. In fact, substantial heterogeneity is observed among participants who have the same mutations. For example, few subjects with p.D176V and p.M712T mutations exhibited a normal or very mild phenotype, with disease onset after the age of 60 [3,13]. Furthermore, only a limited number of studies that analyze compound heterozygous patients are available. Nonetheless, such studies report a variable degree of severity [14–17].

To clarify the potential relationship between genotype and clinical phenotype (*i.e.*, age at onset, disease course, and current symptoms) of GNE myopathy, we performed a questionnaire-based survey of participants with confirmed GNE myopathy.

2. Participants and methods

2.1. Study population

We obtained approval for this study from the Medical Ethics Committee of the National Center of Neurology and Psychiatry (NCNP). Seventy-eight participants with known GNE myopathy were seen at 8 hospitals specializing in muscle disorders in Japan and 83 participants (not all genetically diagnosed) from the Participants Association for Distal Myopathies (PADM) were recruited. Participants provided written informed consent prior to completing the questionnaire.

A total of 75 participants completed and returned the questionnaire. Of the 75 participants analyzed, 4 were found to have only one heterozygous mutation. Because single heterozygous mutations have not been confirmed to cause GNE myopathy, these 4 participants were excluded from this study.

2.2. Study design

The present study is a retrospective and cross-sectional analysis, which includes 71 participants with genetically confirmed GNE myopathy. Clinical information was collected from participants using a questionnaire and genetic information was acquired from available medical records.

2.3. Questionnaire

Participants completed a self-reporting questionnaire regarding 1) developmental and past symptoms, 2) past and present ambulatory status, and 3) information about diagnosis and medical services (Supplementary material, original version in Japanese).

To determine developmental history, we collected the following information: 1) trouble before and/or during delivery, 2) body weight and height at birth, 3) age at first gait, 4) exercise performance during nursery, kindergarten, or school, and 5) age at onset and signs of first symptoms. Participants were also asked about the onset of 1) gait disturbance, 2) walking with assistance (*i.e.*, cane and/or orthotics and/or handrails), 3) wheelchair use, 4) loss of ambulation, and 5) current gait performance. With regard to medical history, participants were asked about 1) age at the time of first hospital visit, 2) whether or not they had symptoms at the time of visit, 3) age at the time of final diagnosis, 4) how many hospitals/clinics were visited before final diagnosis, and 5) whether a biopsy was performed.

2.4. Medical record examination

To verify the accuracy of the information provided by each participant, available medical records from 17 participants (23.9%) seen at outpatient clinics at NCNP were examined (9 males and 8 females).

2.5. Data handling and analysis

All variables were summarized using descriptive statistics, which included mean, standard deviation (SD), median, range, frequency, and percentage. Each variable was compared against age, sex, genotype, and domain mutation (*i.e.*, within the UDP-GlcNAc 2-epimerase domain: ED or *N*-acetylmannosamine kinase domain: KD). Student's *t* test was used to compare the means for each participant group (ED/ED, ED/KD and KD/KD participants). Data from the two participant groups were calculated using chi-square contingency table analysis. The time from disease onset to walking with assistance, time from disease onset to wheelchair use, and time from disease onset to loss of ambulation were evaluated using the Kaplan–Meier method with log-rank analysis. Questionnaire reliability was tested using intraclass correlation coefficients (ICCs), and two-sided 95% confidence intervals (CIs) were calculated using a one-way random effects analysis of variance model for inter-rater reliability. All analyses were performed using SPSS for Macintosh (version 18, SPSS Inc., Chicago, IL).

3. Results

3.1. General characteristics

A total of 71 Japanese individuals (27 males and 44 females) participated in the study. The mean age at data collection was 43.1 ± 10.7 years. None of the participants showed developmental abnormalities during infancy or early childhood.

3.2. GNE mutations

Forty-one percent of study participants (n = 29/71) had homozygous mutations, while 59% (n = 42/71) had compound heterozygous mutations (Table 1). Among homozygous participants, 86.2% (n = 25/29) harbored the p.V572L mutation, while the remaining participants had other mutations. No homozygous participants for the p.D176V mutation were identified. Among compound heterozygous participants, 28.5% (n = 12/42) had p.D176V/p.V572L mutations, while the remaining participants had other mutations. With respect to allelic frequency, 50.0% (71/142) were p.V572L, 20.4% (29/142) p.D176V, 3.5% (5/142) p.C13S, 2.8% (4/142) p.M712T, and 2.1% (3/142) p.A630T. All other mutations accounted for 2%. A total of 18.3% (n = 13/71) of participants were homozygous with a mutation in the GNE domain (ED/ED), 39.4% (n = 28/71) of participants were compound heterozygous with a mutation in the GNE domain and one in the MNK domain (ED/KD), and 42.3% (n = 30/71) of participants had a mutation in the MNK domain in both alleles (KD/KD).

3.3. Past and present symptoms

Mean participant age at symptom onset was 25.2 ± 9.2 years (range, 12–58 years; median, 24.5 years). There was no significant difference between males and females for current age, age at disease

Table 1
Genotypes of the GNE myopathy patient population.

		Questionnaire	Outpatients
ED/ED	Total	13	4
	Homozygote	1	0
	p.C13S homozygote	1	
	Compound heterozygote	12	4
	p.C13S/p.M29T	1	1
	p.C13S/p.A631	1	1
	p.D176V/p.F233S	1	1
	p.D176V/p.R306Q	2	
	p.R129Q/p.D176V	1	
	p.R129Q/p.R277C	1	
	p.D27L/p.D176V	1	1
	p.B89S/p.D176V	1	
	p.D176V/p.R246W	1	
	p.D176V/p.R321C	1	
	p.D176V/p.V331A	1	
ED/KD	Total	28	8
	Compound heterozygote	28	8
	p.D176V/p.V572L	12	3
	p.C13S/p.V572L	1	1
	p.D176V/p.I472T	1	1
	p.D176V/p.L603F	1	1
	p.R177C/p.V572L	1	1
	383insT/p.V572L	1	1
	p.D176V/p.G708S	2	
	p.D187G/p.V572L	2	
	p.R8X/p.V572L	1	
	p.D176V/p.G568S	1	
	p.D176V/p.H626R	1	
	p.D176V/p.A630T	1	
	p.I276T/p.V572L	1	
	p.G295D/p.A631V	1	
	p.A600E/p.D176V	1	
KD/KD	Total	30	5
	Homozygote	28	5
	p.V572L homozygote	25	4
	p.M712T homozygote	2	
	p.A630T homozygote	1	
	Compound heterozygote	2	0
	p.V572L/p.R420X	1	1
	1756Gdel (stop)/p.V572L	1	

onset, age at walking with assistance, age at wheelchair use, and current ambulatory status. Initial symptoms included gait disturbance (66.2%, n = 47/71), other lower limb symptoms (26.8%, n = 19/71), easily fatigued (23.9%, n = 17/71), and weakness of hands and fingers (8.5%, n = 6/71). In addition, 21.1% (n = 15/71) had onset of symptoms before the age of 20. When specifically asked, 47.8% (n = 34/71) described themselves as slow runners during childhood, and 42.5% reported having had difficulty with physical exercise during school years.

3.4. Diagnosis

Mean participant age at diagnosis was 33.9 ± 12.6 years (median, 29.5 years; range 17 to 67 years). Mean participant age at first physician visit was 29.6 ± 10.4 years (median, 27 years; range, 12–62 years), and mean time between first visit and diagnosis was 4.4 ± 8.3 years.

3.5. Walking with assistance and wheelchair use

At the time of the survey, 52.0% (n = 37/71) were ambulant (41.3 ± 12.8 years); however, only 15.5% (n = 11/71, 40.0 ± 13.6 years) could walk without assistance, with the remaining 35.2% requiring assistance (n = 25/71, 41.8 ± 12.7 years). Only 7.0% of these participants (n = 5/71) could walk up stairs, while 49.3% (n = 35/71) were non-ambulant. Wheelchairs were used by 63.6% (23.9% partially bound and 43.7% totally bound) and an electric wheelchair was used by 41.9% (n = 31/71). Mean participant age of wheelchair users was 34.9 ± 11.7 years (range, 18–70 years). Wheelchairs were not used by 32.4% (n = 26/71) of participants. Current age of wheelchair-free participants was 39.4 ± 12.3 years (range, 21–61 years; median, 34 years) and that of wheelchair-bound participants was 42.8 ± 12.6 years (range, 21–71; median, 42 years).

Kaplan–Meier analysis revealed a median proportional age at walking with assistance of 30.0 ± 1.4 years. Median proportional age of wheelchair users was 36.0 ± 2.7 years, and that for loss of ambulation was 45.0 ± 4.2 years. The time from disease onset to walking with assistance was 7.0 ± 0.4 years, time from disease onset to wheelchair use was 11.5 ± 1.2 years, and time from disease onset to loss of ambulation was 17.0 ± 2.1 years.

3.6. Correlation between disease genotype and phenotype

To determine if a correlation between genotype and phenotype existed, we compared domain mutations (ED/KD, or both) available from medical reports to questionnaire answers (Table 2). Participants with KD/KD mutations (both homozygous and heterozygous) were younger and more severely affected compared to participants with ED/KD or ED/ED mutations. No significant difference in current age or age at disease onset between ED/ED and ED/KD participants was identified. Kaplan–Meier analyses revealed that the proportional time from disease onset to wheelchair use and from disease onset to loss of ambulation was significantly shorter in KD/KD compared to ED/KD participants. ED/ED participants exhibited a shorter time of disease onset to wheelchair use compared to ED/KD participants (Table 3, Fig. 1).

3.7. Comparison between p.V572L homozygous and p.D176V/p.V572L compound heterozygous participants

To compare clinical features in patients with the same mutations, we specifically analyzed data from those with p.V572L (n = 25/71, 35.2%) and p.D176V/p.V572L (n = 12/71, 16.9%) mutations, as these two were the most frequent mutations in our study population (Table 2). Age at disease onset of homozygous participants (p.V572L) was 21.3 ± 5.7 years (range, 12–32 years) and time from disease onset to wheelchair use was 11.3 ± 5.4 years (range, 3–21 years). Only 16.0% (n = 4/25) of these homozygous participants reported that they were not currently using a wheelchair. In contrast, the mean age at disease onset of heterozygous participants (p.D176V/p.V572L) was 35.5 ± 14.1 years (range, 13.5–57 years) and time from disease onset to wheelchair use was 17.9 ± 7.0 years (range, 11–28 years). A total of 66.7% of these compound heterozygous participants (n = 8/12) reported that they were not using a wheelchair.

3.8. Questionnaire response compared to medical records

Questionnaires from 17 participants (NCNP outpatient participants) were compared to available medical records (Table 2). Age at disease onset, age at onset of gait disturbance, age at walking with assistance, and age at loss of ambulation were assessed for inter-rater reliability. Age at disease onset, age at onset of gait disturbance, age at walking with assistance, and age at loss of ambulation were assessed for inter-rater reliability. ICC values were 0.979 (95% CI 0.941–0.992) for age at disease onset, 0.917 (95% CI 0.752–0.972) for age at onset of gait disturbances, 0.985 (95% CI 0.949–0.995) for age at walking with assistance, and 0.967 (95% CI 0.855–0.993) for age at loss of ambulation.

4. Discussion

The present study provides a detailed overview of disease severity and progression in 71 Japanese participants with genetically confirmed GNE myopathy. Questionnaire-based surveys have been used to study

Table 2
Comparison of disease course among genotypes.

		Total	ED/ED	ED/LD	KD/KD
Questionnaire	*n*	71	13	28	30
	Age (years old)	43.1 ± 10.7	44.2 ± 11.2	45.3 ± 13.4	40.6 ± 13.0
	Age at onset (years old)	25.5 ± 9.2	26.3 ± 7.3 [+]	29.8 ± 11.0 [*]	21.2 ± 5.5 [*,+]
	Age at walking with assistance	31.8 ± 10.0	34.0 ± 11.1	35.6 ± 10.9 [*]	27.8 ± 6.8 [*]
	Duration from onset to walking with assistance	8.4 ± 6.5	7.5 ± 7.3	9.2 ± 6.5	8.0 ± 6.6
	Wheelchair user (%)	48 (67.8)	10(76.9)	14 (50.0) [*]	24 (80.0) [*]
	Wheelchair use since (age)	37.6 ± 8.6	36.4 ± 12.0	43.0 ± 8.7 [*]	31.2 ± 9.3 [*]
	Number of patients with lost ambulation	35 (49.8)	6(46.2)	8 (28 6) [*]	21 (70.0) [*]
	Age at lost ambulation	33.6 ± 9.2	31.2 ± 6.0	39.7 ± 9.5	32.1 ± 9.3
	Duration from onset to loss of ambulation	12.2 ± 5.2	9.8 ± 3.5	13.8 ± 6.4	12.4 ± 5.1
NCNP outpatients	*n*	17	4	8	5
	Age (years old)	43.9 ± 14.1	53.5 ± 8.9 [+]	44.3 ± 16.3	35.6 ± 9.2 [+]
	Age at onset (years old)	25.8 ± 9.2	33.4 ± 9.2 [+]	29.6 ± 13.5	19.6 ± 4.2 [+]
	Duration from onset to walking with assistance	7.5 ± 4.2	8.9 ± 5.1	8.1 ± 4.7	5.2 ± 1.5
	Wheelchair user (%)	12 (70.6)	3 (75.0)	4 (50.0)	4 (100)
	Wheelchair use since (age)	33.3 ± 12.6	47.5 ± 17.7	35.2 ± 12.4	25.8 ± 6.3
	Number of patients with lost ambulation	9 (52.9)	3 (75.0)	3 (28.6) [*]	5 (100) [*]
	Age at lost ambulation	33.8 ± 9.3	40.0 ± 0.0	39.0 ± 16.5	31.0 ± 8.2
	Duration from onset to loss of ambulation	10.7 ± 4.2	11.2 ± 5.6	11.1 ± 7.8	6.2 ± 2.6

In the questionnaire group, age at onset and age at walking with assistance were significantly younger in KD/KD patients than in ED/KD patients. The number of wheelchair users and patients with loss of ambulation was significantly higher in the KD/KD group than in the ED/KD group. In contrast, with the exception of age at onset, there were no significant differences between ED/ED and ED/KD or KD/KD patients in these clinical parameters. The ED/ED patients were older than the others, and KD/KD patients tended to show the fastest progression.

[*] $p<0.05$ between ED/KD and KD/KD.

[+] $p<0.05$ between ED/ED and KD/KD.

the natural disease course of other rare neuromuscular disorders, such as Pompe disease [18] and spinal muscular atrophy type-1 [19]. It is difficult to establish the natural history of such rare disorders using medical records only because patients are typically seen in many different hospitals. In the present study, we used a self-reporting questionnaire and support its use for complementing medical records because it provides a more complete disease overview and establishes specific clinical trends or correlations. Indeed, our questionnaire demonstrates excellent inter-rater reliability against medical records and yields several findings regarding differences in disease progression among genetically distinct, GNE myopathy participants.

Only 15.5% of participants could walk and 7.0% could walk up stairs without assistance, which reflects the fact that GNE myopathy patients often require canes and/or leg braces at an early disease stage. This indicates that traditional six-minute walk or four-step walking tests often used to evaluate muscular dystrophies or myopathies can only be applied in a very limited number of cases, such as natural disease course studies or clinical trials. Therefore, alternate evaluation tools are required, which should include functional measurements that can be completed without canes or braces. For example, the Gross Motor Function Measure is a useful tool for evaluating mildly and severely affected patients [20].

The male to female ratio in our study population (27 males and 44 females) was skewed from the expected ratio for autosomal recessive inheritance. However, the male to female ratio of the 17 NCNP outpatient participants was 9:8. One possible explanation for the observed sex ratio in our study population is that female participants tend to be more enthusiastic toward questionnaire-based and/or PADM activities. There was no significant difference in age at survey and age at disease onset between male and female participants. However, in a mouse model of GNE myopathy, weight loss and muscle atrophy were more pronounced and occurred earlier in females compared to males [11].

We showed that KD/KD mutations are associated with a more severe phenotype compared to ED/KD mutations. Indeed, KD/KD participants had an earlier disease onset, a more rapid and progressive disease course, and a shorter time from disease onset to loss of ambulation. This was also observed in the 17 NCNP outpatient participants analyzed in our study. In contrast, ED/ED participants did not show significant differences across disease course parameters analyzed except for an earlier and later age at disease onset compared to ED/KD and KD/KD participants, respectively. Thus, ED/ED participants appear to have a disease severity intermediate between ED/KD and KD/KD participants. One possible explanation is that the major mutation, p.V572L, may be associated with a more severe phenotype. In general, the reasons for this earlier onset and disease progression remain unknown. Jewish GNE myopathy patients with homozygous p.M712T mutations have a milder phenotype compared to Japanese patients, as most of their quadriceps are spared and they usually become wheelchair-bound 15 years or more after disease onset [13,21]. Our study population included two women with homozygous p.M712T mutations: a 38 year-old ambulant and a 35 year-old non-ambulant participant. Although the two participants had a slightly later disease onset (ages 23 and 27 years, respectively) compared to KD/KD participants, the difference was not significant.

An asymptomatic patient with a p.D176V homozygous mutation was previously reported [3]. The study suggested that p.D176V homozygous patients may show a mild or late disease onset phenotype. The results presented here may support this observation as no p.D176V homozygous participants were present in our study

Table 3
Inter-rater reliability of the questionnaire.

	Onset	Age of gait disturbance	Age of gait with help	Age at loss of ambulant
Number of patients	17	17	13	9
ICC (95% CI)	0.979 (0.941–0.992)	0.917 (0.752–0.972)	0.985 (0.949–0.995)	0.967 (0.855–0.993)
p	0.000	0.000	0.000	0.000

Age at onset, age at onset of gait disturbances, age at walking with assistance, and age at loss of ambulation were assessed in a subgroup of 17 outpatients to evaluate the inter-rater reliability of the questionnaire.

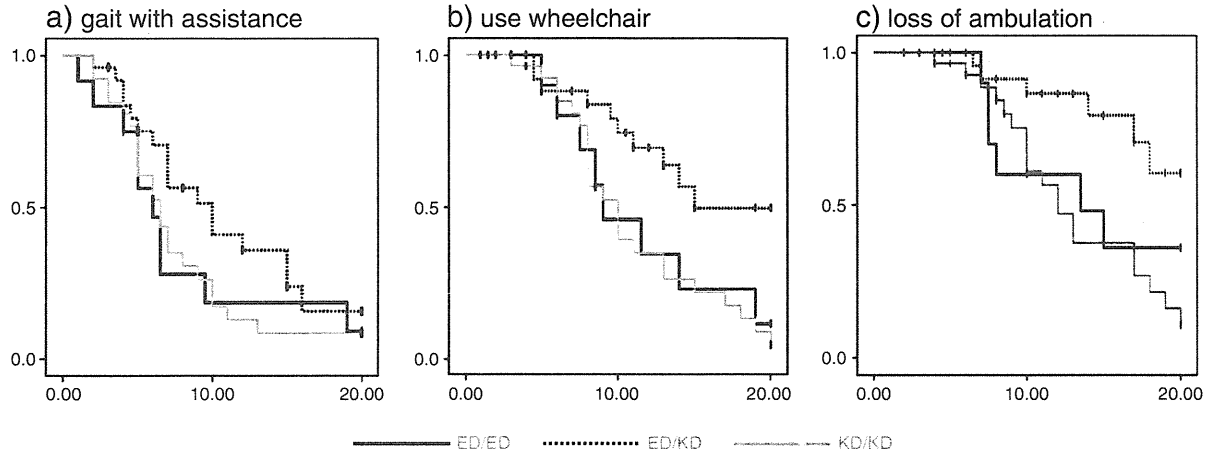

Fig. 1. Kaplan–Meier analysis of time from disease onset to (a) walking with assistance, (b) wheelchair use, and (c) loss of ambulation. Significant differences between ED/KD and KD/KD genotypes were identified. Age at disease onset was significantly different between ED/ED participants and ED/KD and KD/KD participants.

population, although p.D176V was the second most common mutation carried by 29 of our participants. In addition, a high variability was observed regarding age at disease onset and disease progression, underscoring the role of a yet-to-be identified factor(s) in determining disease phenotype.

The recruitment of participants from PADM and highly specialized neurology hospitals is a potential source of selection bias and thus a limitation of this study. These participants are likely to be more motivated because they are more severely affected compared to the general patient population. Furthermore, patients with lower disease severity may not yet be diagnosed with GNE myopathy. Therefore, our study may not accurately reflect the general patient population. Nevertheless, we believe our findings provide important information as our study population covers a broad range in age (22 to 81 years) and symptoms (minimal to wheelchair-bound). Finally, recall bias may also affect results presented in this retrospective study. Therefore, future studies should be performed with an emphasized prospective design.

In conclusion, our study shows that the KD/KD genotype (*i.e.*, p.V572L homozygous mutation) is associated with a more severe phenotype compared to compound heterozygous ED/KD mutations. Because only a small number of participants could walk, future studies should include ambulation-independent motor tests to yield a more comprehensive clinical overview in GNE myopathy patients with different genotypes.

Supplementary data to this article can be found online at doi:10.1016/j.jns.2012.03.016.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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References

[1] Nonaka I, Sunohara N, Satoyoshi E, Terasawa K, Yonemoto K. Autosomal recessive distal muscular dystrophy: a comparative study with distal myopathy with rimmed vacuole formation. Ann Neurol 1985;17:51–9.
[2] Argov Z, Yarom R. "Rimmed vacuole myopathy" sparing the quadriceps. A unique disorder in Iranian Jews. J Neurol Sci 1984;64:33–43.
[3] Nishino I, Noguchi S, Murayama K, Driss A, Sugie K, Oya Y, et al. Distal myopathy with rimmed vacuoles is allelic to hereditary inclusion body myopathy. Neurology 2002;59:1689–93.
[4] Eisenberg I, Avidan N, Potikha T, Hochner H, Chen M, Olender T, et al. The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. Nat Genet 2001;29:83–7.
[5] Kayashima T, Matsuo H, Satoh A, Ohta T, Yoshiura K, Matsumoto N, et al. Nonaka myopathy is caused by mutations in the UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase gene (GNE). J Hum Genet 2002;47:77–9.
[6] Keppler OT, Hinderlich S, Langner J, Schwartz-Albiez R, Reutter W, Pawlita M. UDP-GlcNAc 2-epimerase: a regulator of cell surface sialylation. Science 1999;284:1372–6.
[7] Malicdan MC, Noguchi S, Nishino I. Recent advances in distal myopathy with rimmed vacuoles (DMRV) or hIBM: treatment perspectives. Curr Opin Neurol 2008;21:596–600.
[8] Noguchi S, Keira Y, Murayama K, Ogawa M, Fujita M, Kawahara G, et al. Reduction of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase activity and sialylation in distal myopathy with rimmed vacuoles. J Biol Chem 2004;279:11402–7.
[9] Broccolini A, Gidaro T, De Cristofaro R, Morosetti R, Gliubizzi C, Ricci E, et al. Hyposialylation of neprilysin possibly affects its expression and enzymatic activity in hereditary inclusion–body myopathy muscle. J Neurochem 2008;105:971–81.
[10] Salama I, Hinderlich S, Shlomai Z, Eisenberg I, Krause S, Yarema K, et al. No overall hyposialylation in hereditary inclusion body myopathy myoblasts carrying the homozygous M712T GNE mutation. Biochem Biophys Res Commun 2005;328:221–6.
[11] Malicdan MC, Noguchi S, Nonaka I, Hayashi YK, Nishino I. A Gne knockout mouse expressing human GNE D176V mutation develops features similar to distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy. Hum Mol Genet 2007;16:2669–82.
[12] Malicdan MC, Noguchi S, Hayashi YK, Nonaka I, Nishino I. Prophylactic treatment with sialic acid metabolites precludes the development of the myopathic phenotype in the GNE myopathy mouse model. Nat Med 2009;15:690–5.
[13] Argov Z, Eisenberg I, Grabov-Nardini G, Sadeh M, Wirguin I, Soffer D, et al. Hereditary inclusion body myopathy: the Middle Eastern genetic cluster. Neurology 2003;60:1519–23.
[14] Tomimitsu H, Shimizu J, Ishikawa K, Ohkoshi N, Kanazawa I, Mizusawa H. Distal myopathy with rimmed vacuoles (DMRV): new GNE mutations and splice variant. Neurology 2004;11:1607–10.
[15] Yabe I, Higashi T, Kikuchi S, Sasaki H, Fukazawa T, Yoshida K, et al. GNE mutations causing distal myopathy with rimmed vacuoles with inflammation. Neurology 2003;12:384–6.
[16] Chu CC, Kuo HC, Yeh TH, Ro LS, Chen SR, Huang CC. Heterozygous mutations affecting the epimerase domain of the GNE gene causing distal myopathy with rimmed vacuoles in a Taiwanese family. Clin Neurol Neurosurg 2007;109:250–6.
[17] Ro LS, Lee-Chen GJ, Wu YR, Lee M, Hsu PY, Chen CM. Phenotypic variability in a Chinese family with rimmed vacuolar distal myopathy. J Neurol Neurosurg Psychiatry 2005;76:752–5.

[18] Hagemans ML, Winkel LP, Van Doorn PA, Loonen MC, Hop WJ, Reuser AJ, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. Brain 2005;128:671–7.
[19] Oskoui M, Levy G, Garland CJ, Gray JM, O'Hagen J, De Vivo DC, et al. The changing natural history of spinal muscular atrophy type 1. Neurology 2007;69: 1931–6.
[20] Sienko Thomas S, Buckon CE, Nicorici A, Bagley A, McDonald CM, Sussman MD. Classification of the gait patterns of boys with Duchenne muscular dystrophy and their relationship to function. J Child Neurol 2010;25:1103–9.
[21] Eisenberg I, Grabov-Nardini G, Hochner H, Korner M, Sadeh M, Bertorini T, et al. Mutations spectrum of GNE in hereditary inclusion body myopathy sparing the quadriceps. Hum Mutat 2003;21:99.

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Case series

Clinicopathological features of centronuclear myopathy in Japanese populations harboring mutations in dynamin 2

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ABSTRACT

Background: Missense mutations in dynamin 2 gene (*DNM2*) are associated with autosomal dominant centronuclear myopathy (CNM) with characteristic histopathological findings of centrally located myonuclei in a large number of muscle fibers.
Methods: To identify Japanese CNM caused by *DNM2* mutations (DNM2-CNM), we sequenced *DNM2* in 22 unrelated Japanese patients who were pathologically diagnosed with CNM. The clinical and pathological findings of DNM2-CNM in patients were reviewed.
Results: We identified 3 different heterozygous missense mutations (p.E368K, p.R369W, and p.R465W) in 4 probands from 4 families. Clinically, calf muscle atrophy and *pes cavus* are features that are highly suggestive of DNM2-CNM among all CNMs. Pathologically, all 4 DNM2-CNM patients showed a radial distribution of myofibrils in scattered fibers, type 1 fiber atrophy, type 1 fiber predominance, and type 2C fibers. None of the non-DNM2-CNM patients exhibited all the 4 abovementioned pathological features, although some patients showed radial distribution without type 1 fiber atrophy and/or type 2C fibers.
Discussion: These results indicate that the clinicopathological features of DNM2-CNM are rather homogeneous and can be distinguished from the features of non-DNM2-CNM.
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1. Introduction

Centronuclear myopathy (CNM) is a rare congenital myopathy named after its characteristic feature of centrally located nuclei in majority of the muscle fibers [1]. In autosomal dominant (AD) cases, muscular weakness and atrophy often begin in childhood or early adolescence [2,3]. CNM progresses slowly, and patients usually follow a mild course and can often expect a normal life-span. In muscle biopsy, a radial alignment of intermyofibrillar networks [1] is seen in nicotinamide adenosine dinucleotide-tetrazolium reductase (NADH-TR) preparations due to the presence of central nuclei; type 1 fiber atrophy is also often observed. Several families with CNM are found in Europe, the United States, Central Africa, Argentina, and Japan [2–6].

Thus far, 4 causative genes have been reported for CNM: myotubularin (*MTM1*), dynamin 2 (*DNM2*) [7], *hJUMPY* [8], and amphiphysin 2 (*BIN1*) [9]. Among them, *DNM2* mutations have been identified among patients in France, French Guiana, the United States, Belgium, Germany, Great Britain, Argentina, and Central Africa [6,10,11]. *DNM2* encodes a protein involved in endocytosis, membrane trafficking, actin assembly, and centrosome cohesion [12–14]. Thus, *DNM2* mutations cause a reduction of dynamin in transfected fibroblasts, leading to defects in centrosomal function.

Patients with CNM that is caused by mutation in the middle domain of *DNM2* (DNM2-CNM) present with a homogenous mild phenotype characterized by slowly progressing muscle weakness without cardiac or respiratory involvement [10]. Muscle computed tomography (CT) and MRI studies clearly show a relatively diffuse involvement in lower-leg muscles, while a selective pattern appears in thigh muscles [10,15,16]. Subtle mental impairment or peripheral nerve involvement was described in a previous report [17]. Mutations in the PH domain lead to an intermediate phenotype with mild respiratory failure and relatively severe weakness as compared to DNM2-CNM caused by middle-domain mutations [6]. Another study reported a more severe infantile form with hypotonia, weak suckling, and respiratory failure due to mutation in the PH domain of *DNM2* [11]. Although no *DNM2* mutations have

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been identified among Japanese patients, there have been reports of patients with evidently similar clinicopathological features [4,5], suggesting the possibility of the presence of DNM2-CNM in the Japanese population. We therefore aimed at detecting *DNM2* mutations among Japanese CNM patients.

2. Materials and methods

2.1. Patients

We retrospectively recruited patients who were diagnosed with CNM or myotubular myopathy at the National Center of Neurology and Psychiatry and analyzed their samples from a total of 9639 muscle biopsies obtained between 1978 and 2006. Inclusion criteria were the presence of more than 6% centrally nucleated fibers and the absence of characteristic findings indicating other muscle diseases upon muscle biopsy. Our cohort consists of 22 unrelated patients aged 1–72 years: 2 had an AD family history; 5 had affected siblings, and consanguinity was documented in one of the patient's families; and 8 were sporadic cases. No record of family history was available for 7 patients. Direct sequence analysis previously performed on these patients excluded CTG expansion in the *DMPK* gene and *MTM1* mutations. Their clinical history was carefully reviewed. Additional medical information from affected family members was obtained by the attending neurologist, when possible.

2.2. Sequence analysis of DNM2

'All 22 patients and 4 members of 1 family were examined for *DNM2* sequence variants. DNA was extracted from blood or muscle samples using standard protocols. We sequenced all the exons and the exon–intron boundaries of *DNM2*. Both strands of PCR products were sequenced directly using BigDye Terminator v1.1 Sequencing Standard Kit (Applied Biosystems) with an automated ABI 3100 DNA sequencer with custom-made primers (Supplementary Table).

3. Results

3.1. Genetic diagnosis

Among 22 patients, we identified 3 mutations in 4 probands: c.1102G>A (p.E368K), c.1105C>T (p.R369W), and c.1393C>T (p.R465W), all of which were previously reported [6]. We further confirmed the mutations in affected family members of 2 patients (Table 1). We did not identify mutations from the families with consanguinity.

3.2. Clinical features

The clinicopathological features of patients with *DNM2* mutations are shown in Table 1. Clinical information for Patient 1-1 was not available. He was autopsied at the age of 17 years, at which point the gastrocnemius muscle was taken as a sample for analysis (Fig. 1A). The inheritance pattern was compatible with AD transmission in families 2 and 3, while it was sporadic in Patient 4-1.

Patients 2-1 and 3-2 were previously reported to have AD CNM or myotubular myopathy (Fig. 2A) [4,5]. In brief, Patient 2-1 noticed an ankle contracture at the age of 10 years and started having difficulty in climbing stairs at the age of 30 years. Achilles tendon elongation was performed at the age of 37 years, during which this patient was found to have atrophy of facial and distal muscles, and diminished tendon reflexes. He had mild ptosis, but ophthalmoplegia was not observed. Creatine kinase (CK) levels were within the normal range. nEMG was myogenic. Muscle biopsy of the rectus femoris at the age of 42 years showed 68% centrally nucleated fibers and a scattered radial distribution (Fig. 1B). CT of the patient's hamstring, soleus, and gastrocnemius muscles showed atrophy and fatty changes. There was no cardiac or respiratory involvement. Nerve conduction velocities were normal except for low-median compound action potentials that could be explained by muscle atrophy. Patient 2-2 exhibited ankle contracture, *pes cavus* due to plantaris muscle atrophy, and distal atrophy since 10 years of age and also underwent Achilles tendon elongation for ankle contracture in his second decade. No ptosis or ophthalmoplegia was observed.

Patient 3-2 noticed progressive lower-leg weakness, atrophy, and ankle contracture when he was 15 years old and he underwent achillotenotomy at 18 years of age. He developed dyspnea at the age of 54 years that necessitated a tracheotomy at the age of 55 years. Neurological findings at the age of 55 years revealed mild ptosis, distal muscle atrophy and weakness, and mild facial muscle involvement including ptosis. CK level was 48 IU/L. nEMG was myogenic. Sural nerve biopsy was unremarkable. Muscle biopsy of the peroneus brevis showed centrally placed nuclei in 40% of the fibers (Fig. 1C). The patient unfortunately died at the age of 58 years, and the primary cause of death was undetermined. His children (Patients 3-6, 3-7, 3-8, and 3-9 (Fig. 2B)) were found to have *pes cavus* caused by plantar muscle atrophy and were slow runners in their childhood.

At the age of 20 years, Patient 3-6 could not appose his palms when his wrists were extended and at the age of 35 years, he had difficulty in walking. He developed bilateral ankle contracture, because of which he had to stand and walk tiptoed. When he was 50 years old, neurological examination showed distal muscle weakness and atrophy with ankle- and finger-joint contractures (Fig. 3A–D). His deep tendon reflexes were also diminished. He lost his left eye in an accident during his childhood, but neither ophthalmoplegia in his right eye nor ptosis was observed. No peripheral nerve involvement was found on normal nerve conduction study. nEMG was myogenic. Results of echocardiography, Holter ECG, and pulmonary function tests were normal. Muscle biopsy of the biceps brachii at the age of 50 years was compatible with the CNM diagnosis (Fig. 1D–G).

The daughters of Patient 3-6 (Patients 3-10 and 3-11) followed a similar clinical course. They did not have ophthalmoplegia nor ptosis (Fig. 3G). Muscle CT showed marked atrophy in the posterior compartment of the lower extremities (gluteus maximus, hamstrings, gastrocnemius, and soleus) and thigh abductors, while only moderate atrophy and fatty changes were observed in the paraspinal muscles (Fig. 3E). Patient 3-11 had muscle involvement limited to the biceps femoris, gastrocnemius, and soleus as shown on CT at the age of 19 years (Fig. 3F). Both Patients 3-10 and 3-11 showed myogenic changes on nEMG, and the findings of nerve conduction studies were normal.

Patient 4-1 had no obvious family history (Fig. 3C). He noticed ankle contracture at the age of 30 and had gait disturbance at the age of 40 years. He underwent muscle biopsy at the age of 55 years. He was ambulant but did not use a cane. nEMG was actively myogenic, and the results of nerve conduction studies were normal.

In all patients, *pes cavus* caused by plantar muscle atrophy was the earliest sign that appeared before the age of 10 years. Atrophy of calf and posterior thigh muscles was seen during the second decade, but could be detected by muscle CT even in early stages (Fig. 3E and F). The clinical course was relatively benign, except for that of 1 patient who died at the age of 16 years (Patient 1-1), although no detailed information on the cause of death was available. Neither cardiac nor respiratory failure occurred in any patient, except Patient 3-2 who underwent tracheotomy for dyspnea secondary to severe pneumonia. All the 3 patients who were above 50 years of age are still ambulant. With an exception of Patient 3-2,

Table 1
Clinicopathological features of DNM2-CNM.

	Family		1	2			3			4
Demographic data	Patient number		1-1	2-1	3-2	3-6	3-7	3-10	3-11	4-1
	Mutation		c.1102G > A (p.E368K)	c.1393C > T (p.R465W)	c.1105C > T (p.R369W)	c.1105C > T (p.R369W)	c.1105C > T (p.R369W)	c.1105C > T (p.R369W)	c.1105C > T (p.R369W)	c.1105C > T (p.R369W)
	Age/sex		16/M	42/M	55/M	50/M	47/F	22/F	19/F	55/M
Clinical features	Ability to walk		NR	Ambulatory	With cane	With cane	Ambulatory	Ambulatory	Ambulatory	Ambulatory
	Opthalmoplegia		NR	–	–	–	–	–	–	–
	Ptosis		NR	+	+	–	–	–	–	–
	MMT upper extremities	Proximal	NR	5	5	5	4	4	4	4
		Distal	NR	5	5	2	3	2	2	3
	MMT lower extremities	Proximal	NR	4	5	4	3	3	4	4
		Distal	NR	4	5	2	2	2	2	3
	Deep tendon reflexes		NR	–	NR	N	↓	–	NR	↓
	Joint contractures		NR	Elbow, wrist, ankle	Ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Ankle
	Muscle atrophy	Leg	NR	+	+	+	+	+	+	+
		Paraspinal	NR	NR	NR	+	+	+	+	+
		Plantar	NR	+	+	+	+	+	+	+
	Cardiovascular		NR	N	N	NR	NR	N	N	N
	Respiratory		NR	NR	Tracheotomy	Normal vital capacity	Normal vital capacity	Normal vital capacity	Normal vital capacity	NR
	Electromyography		NR	M	M	M	M	M	NT	M
	Nerve conduction studies		NR	*	N	N	N	N	N	NR
	Serum CK		NR	N	N	N	N	NR	N	N
	Muscle CT	Calf	NR	+	+	+	NR	NR	–	+
		Thigh	NR	2+	2+	2+	NR	NR	+	2+
Findings on muscle biopsy	% of centrally nucleated fibers		65	68	55	60				70
	Radial distribution of myofibrils		+	+	NT	+				+
	Type 1 predominance (%)		80	79	NT	88				90
	Type 1 atrophy		+	+	NT	+				+
	Type 2B deficiency		+	+	NT	+				+
	Type 2C fibers (%)		2	1	NT	2				5

Abbreviations: MMT, manual muscle testing; +, present; –, absent; N, normal; NR, no record; NT, not tested; ↓, decreased; EMG, electromyography; M, myogenic changes; and NCS, nerve conduction study. The CT scores are as follows: 1+: decreased signal density and 2+: decreased signal density with severe muscle atrophy.