を同定した個体由来の精子を用い、人工授精を行い、*sil1*変異体の発生期～孵化までの表現型を実態顕微鏡下で観察した。

（倫理面への配慮）

すべての動物実験は、（独）国立精神・神経医療研究センター神経研究所動物実験に関する倫理指針に従い行い、（独）国立精神・神経医療研究センター神経研究所動物実験管理委員会の審査・承認を得ている。研究に使用する際には、必要最小限度の動物を使用するとともに、動物に苦痛を与えないよう最大限の注意を払った。すべての組み換え DNA 実験は、カルタヘナ議定書に基づく「遺伝子組み換え生物等の使用等の規制による生物の多様性の確保に関する法律」と関係省令を遵守し、（独）国立精神・神経医療研究センター神経研究所組み換え DNA 実験安全委員会の審査・承認を得ている。

C. 研究結果

メダカ *sil1* 第 2 エクソンのスクリーニングの結果、68 番目のロイシンがグルタミンに置換している個体を同定した。ナンセンス変異を得るためさらに第 9、第 10 エクソンについても探索を行った結果、第 10 エクソン内でストップコドンを有する個体を見出すことができた。第 10 エクソンに変異を同定した個体由来の精子を用い、人工授精を行い、*sil1* ナンセンス変異体の発生期～孵化までの表現型を実態顕微鏡下で観察したが、少なくとも孵化までの胚発生および器官形成期においては顕著な表現型の変化は認められていない。

またシャペロン機能の改善に効果のある低分子化合物の簡便なスクリーニングを行う上で、必要な条件について、現在検討をすすめているところである。

D. 考察

メダカ*sil1*ナンセンス変異体は少なくとも孵化までの発生は正常に行われる。過去に報告されたマウス*Sil1*変異系統 *woozy*においても神経変性等の表現型は生後3カ月程度で認められることから、メダカにおける*sil1*欠損による生体への影響も、経過を追って調べていく必要があると考えられる。

E. 結論

メダカ変異体ライブラリーを用いて、*sil1*突然変異体のスクリーニングを行った結果、ナンセンス変異体を得ることができた。今後は、表現型の詳細な解析を行い、病態を引き起こす分子メカニズムの解明を行うとともに、治療法の開発に向けたシャペロン機能を改善する低分子化合物の探索に向けた取り組みを進めていく。

F. 健康危険情報

なし

G. 研究発表

1．論文発表

なし

2．学会発表

なし

H. 知的財産権の出願・登録状況（予定を含む）

1．特許取得

なし

2．実用新案登録
なし

3．その他
なし

Ⅲ.　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

発表者氏名：論文タイトル名．発表誌名．巻号：ページ，出版年
Tsuburaya RS, Monma K, Oya Y, Nakayama T, Fukuda T, Sugie H, Hayashi YK, Nonaka I, Nishino I: Acid phosphatase-positive globular inclusions is a good diagnostic marker for two patients with adult-onset Pompe disease lacking disease specific pathology. Neuromuscul Disord. 22(5): 389-393, 2012.
Suzuki S, Hayashi YK, Kuwana M, Tsuburaya R, Suzuki N, Nishino I: Myopathy associated with antibodies to signal recognition particle: disease progression and neurological outcome. Arch Neurol. 69(6): 728-732, 2012.
Mori-Yoshimura M, Monma K, Suzuki N, Aoki M, Kumamoto T, Tanaka K, Tomimitsu H, Nakano S, Sonoo M, Shimizu J, Sugie K, Nakamura H, Oya Y, Hayashi YK, Malicdan MC, Noguchi S, Murata M, Nishino I: 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. J Neurol Sci. 318(2012): 100-105, 2012.
Mori-Yoshimura M, Okuma A, Oya Y, Fujimura-Kiyono C, Nakajima H, Matsuura K, Takemura A, Malicdan MC, Hayashi YK, Nonaka I, Murata M, Nishino I: Clinicopathological features of centronuclear myopathy in Japanese populations harboring mutations in dynamin 2. Clin Neurol Neurosurg. 114(6): 678-683. 2012.
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Ⅳ. 研究成果の刊行物・別刷

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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.

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

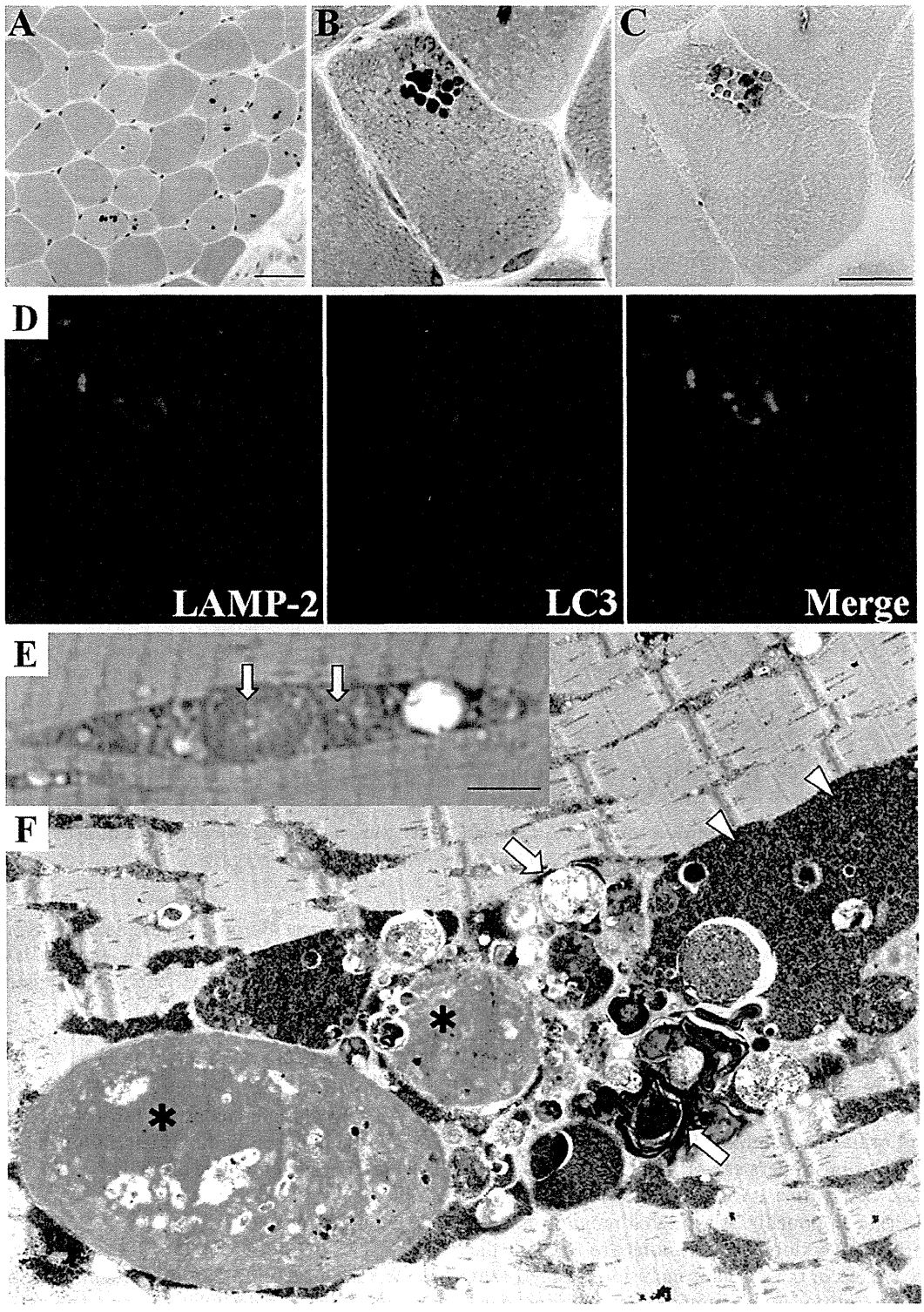

Fig. 1. Acid phosphatase-positive globular inclusions in patient 2. (A and B) Biopsied skeletal muscle showed nonspecific myopathic changes with scattered red–purple colored globular inclusions on modified Gomori-trichrome stain. (C) The inclusions have intense activity on acid phosphatase stain. Bar = 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×).

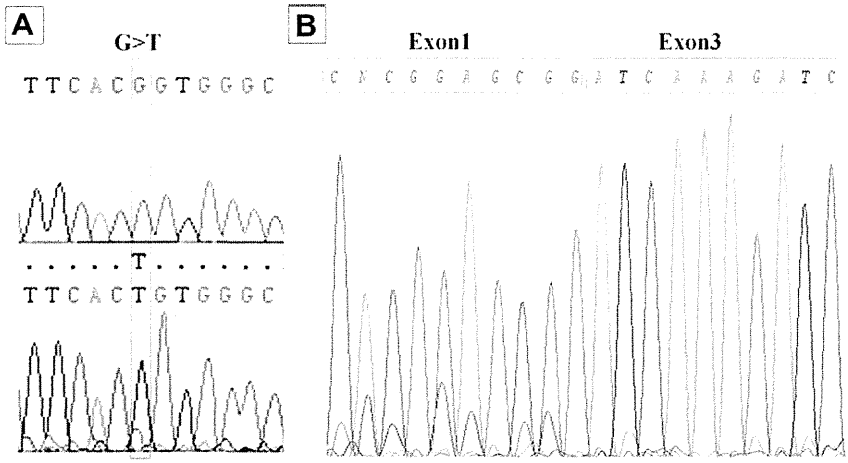

Fig. 2. Mutational analysis of *GAA*. Both patient have a homozygous c. 546G > T mutation at the last codon of exon2 (A upper: control, lower: patient), which creates mRNA with skipping exon 2 (B).

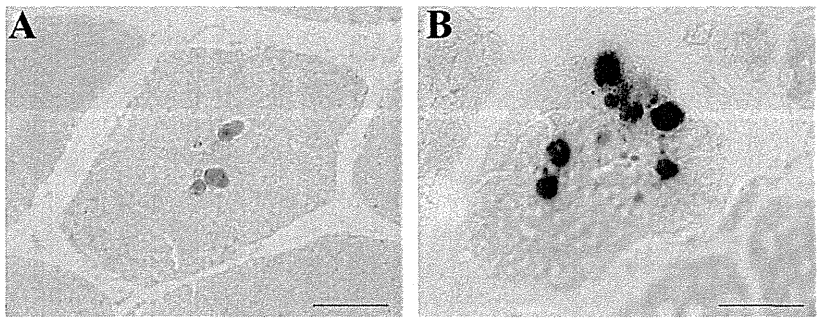

Fig. 3. Inclusions on menadione-linked α-glycerophosphate dehydrogenase (MAG) without substrate. Globular inclusions in Pompe disease (A) are only faintly stained comparing reducing bodies in reducing body myopathy with *FHL1* mutation (B). Bar = 20 μm.

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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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.

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**).

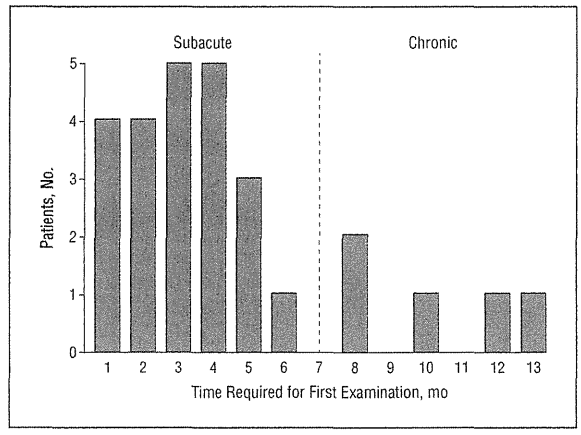

Figure 1. Period between disease onset and the first examination in 27 patients with anti–signal recognition particle myopathy. They were divided into 22 patients with the subacute form and 5 patients with the chronic form based on the clinical course.

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

Clinical Feature	Patients, No. (%)		*P* Value
	Subacute (n = 22)	Chronic (n = 5)	
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.

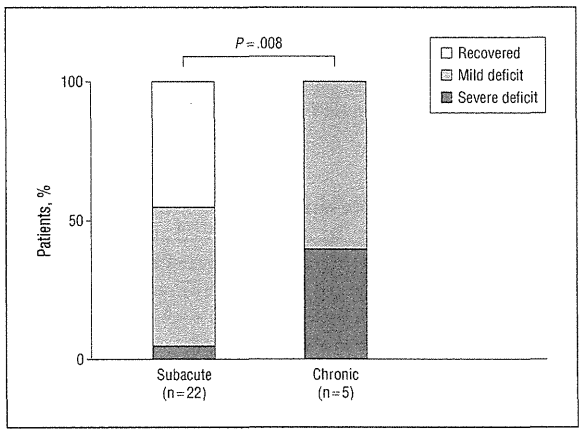

Figure 2. Neurological outcomes were assessed using the modified Rankin Scale[12] with some modifications and were compared between subacute and chronic forms of anti–signal recognition particle myopathy. The neurological outcomes were divided into recovered, mild deficit, and severe deficit. Differences 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

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]

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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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.

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.A63I	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.

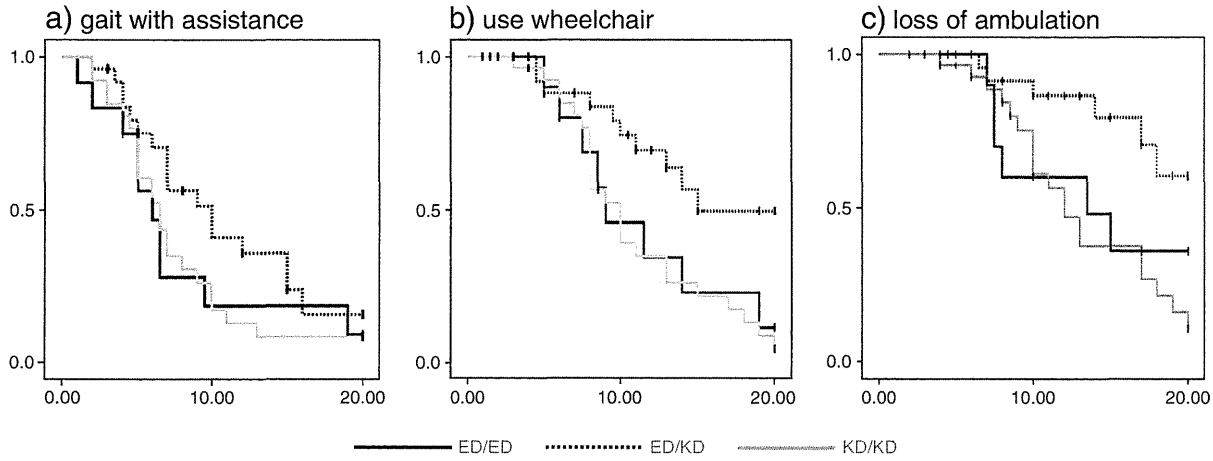

Fig. 1. Kaplan–Meier analysis of time from disease onset to (a) walking with assistance, (b) wheelchair use, and (c) loss of ambulation. Significant differences between ED/KD and KD/KD genotypes were identified. Age at disease onset was significantly different between ED/ED participants and ED/KD and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