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LETTER TO THE EDITOR

Absence of beta-amyloid deposition in the central nervous system of a transgenic mouse model of distal myopathy with rimmed vacuoles

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Keywords

Distal myopathy, rimmed vacuole, Gne knockout mouse, amyloid deposition

History

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Distal myopathy with rimmed vacuoles (DMRV) is an autosomal recessive disorder characterised by early adult-onset (15–40 years), slowly progressive myopathy with preferential weakness of the tibialis anterior and sparing of the quadriceps femoris muscles [1]. The muscle biopsy shows rimmed vacuoles containing deposits immunoreactive for β-amyloid (Aβ) and other proteins. DMRV is due to mutations in the *GNE* gene, which encodes a bifunctional enzyme (uridine diphosphate-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase) in the sialic acid synthetic pathway. A recently developed mouse model of DMRV that expresses the human *GNE* D176V mutation in a Gne knockout background reproduces the clinical, pathological and biochemical features of human DMRV [2]. As in the human disease the model exhibited muscle atrophy and weakness, and low levels of sialic acid in serum and solid organs. Moreover, Aβ was also found to be associated with the rimmed vacuoles and myofibrillar degeneration in the muscle fibres [2]. Because Alzheimer's disease (AD) is characterised by Aβ deposition in the brain, we examined the central nervous system of the DMRV mouse model for evidence of Aβ deposition and other pathological abnormalities to determine if there is any link between DMRV and AD.

Brain and skeletal muscle tissues from 30 DMRV mice older than 40 weeks were harvested, formalin-fixed, processed and paraffin embedded. Sixteen spinal cords were also available for examination. Tissues sections were stained by hematoxylin and eosin for light microscopy. Immunohistochemistry (IHC) to detect Aβ was performed using a mouse monoclonal primary antibody (clone 6E/10; Covance, Princeton, NJ) and the Envision method with minor modifications [3]. As a positive IHC control, we used tissues from an established mouse model of AD, the Tg2576 transgenic mouse (Taconic, Germantown, NY) that expresses the Swedish mutation of amyloid precursor protein. Brain tissue sections from a human case of AD were also included as a positive control. For negative controls, sections were incubated with normal goat serum to replace the primary antibody.

In the 30 aged DMRV mice examined there was no evidence of Aβ deposits in the brains or spinal cords (Figure 1B). However, Aβ deposits were detected in the skeletal muscles of all these animals. The deposits were usually linear, beaded and found in the central part of the fibres (Figure 1C, D). Occasionally, these deposits were associated with vacuoles (Figure 1C) corresponding to rimmed vacuoles as shown by the hematoxylin and eosin stains (data not shown). The three AD mouse brain positive controls showed plaque-like amyloid deposits in the cerebral cortex (Figure 1A). The human AD brain tissues showed Aβ deposition in the brain neuropil and around blood vessels (data not shown). There were no light microscopic abnormalities such as neurofibrillary tangles, amyloid angiopathy and other features of AD in the central nervous system of all test and control mice.

Although the aged DMRV mouse model demonstrated convincing Aβ deposition in skeletal muscles, there was no Aβ deposition in the CNS. This suggests that amyloidogenesis in AD and DMRV may be different. Amyloidogenesis in AD is mainly associated with post-translational proteolytic processing of amyloid precursor protein by α-, β- and γ-secretases. However, hyposialylation is an important factor in the pathogenesis of the disease and amyloidogenesis in DMRV. Hyposialylation of several important muscle glycoproteins

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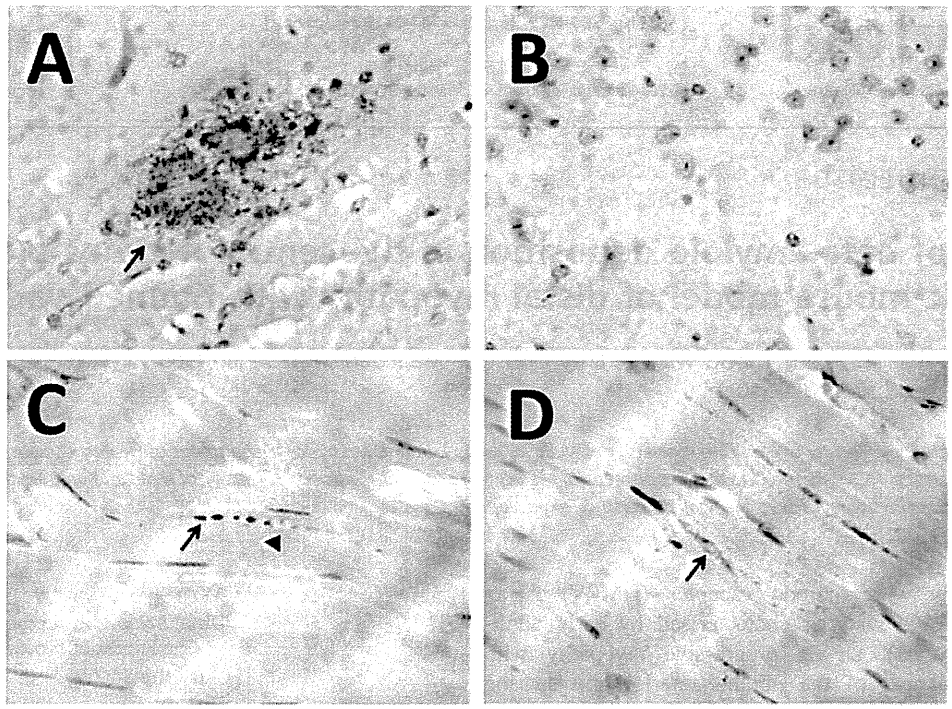

Figure 1. (A) Brain sections from Tg2576 transgenic mouse model of Alzheimer's disease shows plaque-like amyloid deposits (arrow). (B) β-amyloid deposition is not detected in brain sections of DMRV mouse. (C and D) Aβ deposits were detected in the skeletal muscle of DMRV mouse (arrow). (C) Occasionally these depositions were associated with vacuoles (arrow head).

such as neprilysin, a membrane proteinase involved with the normal breakdown of Aβ has been observed [4,5]. In DMRV, reduced muscle neprilysin activity may cause toxic Aβ accumulation in vulnerable fibres and a failure in repair or regeneration of muscle fibres possibly through modulation of the insulin-like growth factor I-dependent pathways [5]. In addition, it was demonstrated that reduced neprilysin activity in the DMRV mice was restored after treatment with sialic acid analogues [6]. Interestingly, the DMRV mouse brain showed normal sialylation levels [2,7], although the other organs showed hyposialylation. This is attributed to a strong sialic acid uptake mechanism in neuronal cells. It was suggested that sialin, a lysosomal sialic acid transporter protein, is involved in the uptake of exogenous sialic acid to maintain normal cellular sialylation in the brain [8]. This may be the reason there is no abnormal Aβ deposition in the DMRV mouse brain. This is also supported by the finding that sialic acid levels in the cerebrospinal fluid of the DMRV mice remain unaltered (Malicdan and Noguchi, unpublished data). In the DMRV mouse brain, normal levels of sialic acid could actually mitigate Aβ toxicity, if Aβ levels were to increase [6]. The level of sialic acid in brains of human DMRV is unknown and further investigations are needed. If there is no hyposialylation in the brains of DMRV patients, Aβ deposition may not occur. Nonetheless, based on current findings, we speculate that brain Aβ deposition is unlikely to be increased in human DMRV and hence, an increased incidence of AD in these patients is also probably unlikely.

Declaration of interest

The authors report no conflicts of interest. This study was partially supported by Intramural Research Grant (25-5) for Neurological and Psychiatric Disorders of NCNP, by Comprehensive Research on Disability Health and Welfare from the Ministry of Health, Labour and Welfare in Japan; JSPS KAKENHI (23390236) and University of Malaya HIR grant (H-20001-00-E000031).

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GNE myopathy: A prospective natural history study of disease progression

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Abstract

Mutations in the glucosamine (UDP-*N*-acetyl)-2-epimerase/*N*-acetylmannosamine kinase gene cause GNE myopathy, a mildly progressive autosomal recessive myopathy. We performed a prospective natural history study in 24 patients with GNE myopathy to select evaluation tools for use in upcoming clinical trials. Patient clinical conditions were evaluated at study entry and one-year follow-up. Of the 24 patients, eight (33.3%) completed a standard 6-min walk test without assistance. No cardiac events were observed. Summed manual muscle testing of 17 muscles, grip power, and percent force vital capacity (%FVC) were significantly reduced ($p < 0.05$), and scores for 6-min walk test and gross motor function measure were decreased ($p < 0.1$) after one year. The decrement in %FVC was significant among non-ambulant patients, whereas the decrement in grip power tended to be greater among ambulant patients. The 6-min walk test, gross motor function measure, manual muscle testing, grip power, and %FVC reflect annual changes and are thus considered good evaluation tools for clinical trials.

Keywords: GNE myopathy; Distal myopathy with rimmed vacuoles (DMRV); Natural history; Respiratory function

1. Introduction

GNE myopathy, also known as distal myopathy with rimmed vacuoles (DMRV), is an early adult-onset myopathy with slow progression that preferentially affects the tibialis anterior muscle and commonly spares the quadriceps femoris muscles [1,2]. The disease cause is a mutation in the *GNE* gene encoding a bifunctional enzyme [uridinediphosphate-*N*-acetylglucosamine (UDP-GlcNAc) 2-epimerase and *N*-acetylmannosamine kinase] that catalyzes two rate-limiting reactions in cytosolic sialic acid synthesis [3–7]. Oral sialic acid metabolite treatment has been shown to prevent muscle atrophy and weakness in a mouse GNE myopathy model [8].

A recent phase I clinical trial with oral sialic acid was performed in Japan (ClinicalTrials.gov; identifier: NCT 01236898), and a phase II study is currently underway in the United States and Israel (ClinicalTrials.gov; identifier:

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NCT01517880). A prospective natural history must be well understood prior to phase III clinical trials. We have identified genotype–phenotype correlations in our previous retrospective study [9], and found that a standard 6-min walk test (6MWT) might not be sufficient for evaluating most of patients, because the majority of Japanese patients were non-ambulant. On the other hand, respiratory function is impaired in patients with advanced GNE myopathy, and may serve as a useful evaluation tool especially among non-ambulant patients [10].

We performed a prospective study of confirmed GNE myopathy patients to assess the prospective natural history of GNE myopathy and obtain an appropriate evaluation tool. We aimed to identify evaluation items that can be used to detect disease progress within a year, with respect to observation duration of clinical trials.

2. Patients and methods

2.1. Study population and design

The present study included prospective data from genetically confirmed GNE myopathy patients who were evaluated twice (baseline and one-year follow-up) at a National Center of Neurology and Psychiatry (NCNP) hospital. All candidate patients were invited to participate in this study by mail and/or telephone. Patients who could not attend the follow-up visit were excluded from the study. The first patients were enrolled in April 2009, and last data were examined on November 25, 2013.

Approval for this study was obtained from the Medical Ethics Committee of the NCNP. Study objectives, design, risks, and benefits of participation were explained to all patients, and their written informed consent was obtained prior to enrollment.

2.2. Patients and Methods

A total of 27 Japanese patients (9 men, 18 women) participated in this study. Among them, 25 patients who completed 1-year follow up were included and two non-ambulant patients who could not visit annual evaluation were excluded. Of 25, one ambulant patient who got nephritic syndrome and resulted to 3 months bedrest and steroid therapy (maximal 1 mg/kg body weight) were excluded as it might have influenced the motor performance. A total of 24 Japanese patients (9 men, 15 women) participated in this study, of whom two women were siblings and the rest were unrelated.

Mean age at the time of data collection was 43.0 ± 12.9 years (mean ± SD). Mean age at disease onset was 25.9 ± 10.3 years (range, 15–58 years; median, 24 years). Of the 24 patients, 9 (36.0 %) were ambulant, 8 completed the 6MWT test without assistance, 1 required assistance (e.g., canes and ankle braces) and could not complete the 6MWT, and 15 (64.0 %) had lost ambulation. Among 9 ambulant patients, 4, 2, 1, and 1 patients used both cane and ankle brace, ankle brace only, cane only, and both walker and cane, respectively. Of 19 patients who used a wheelchair for transportation (4, part-time; 15, full time), 7 used wheelchair headrests, and 1 used a neck collar to prevent falling.

Medical complications and history were as follows: 3 patients had hypertension, 2 had obstructive sleep apnea syndrome, with 1 receiving treatment by continuous positive airway pressure, 2 had diabetes mellitus, hyperlipidemia, and past history of idiopathic thrombocytopenia, and 1 had atopic dermatitis, idiopathic thrombocytopenia, hypermenorrhea resulting anemia, and mastopathy. Occurrences of these diseases were similar to those of the general population of Japan.

All patients rested for more than two hours before each muscle strength test. Measurements using a hand held dynamometer of knee extension in sitting position (HHD, μ-Tas F-1®, Anima, Japan), grip power (Dynamometer®, TTM Japan), pinch power (PinchTrack™, JTECH, Japan), and occlusal force meter GM10® (NAGANO KEIKI, Japan) were repeated three times on both the right and left sides, and all six measurements were averaged for data analysis.

Muscle strength tests, including manual muscle testing (MMT) and gross motor function measure (GMFM, Japanese version; range, 0–100 [%]), were performed [11]. The following 17 muscle groups were examined: neck flexion, truncal flexion, shoulder abduction, shoulder adduction, shoulder flexion, shoulder extension, elbow flexion, elbow extension, wrist flexion, wrist extension, hip flexion, thigh adduction, thigh abduction, knee extension, knee flexion, ankle dorsiflexion, and planter flexion. Right and left MMTs were averaged, except for those corresponding to neck and truncal flexion. Summed MMT (range, 0–85) was obtained from the sum of the 17 muscle groups examined. The 6MWT was performed according to the American Thoracic Society guidelines [12] for patients who were able to walk without any assistance (canes or braces). Pinch and grip powers and MMT were measured by M.M.Y., and HHD measurement, GMFM, and 6MWT were measured by H.Y., assisted by other physiotherapists.

Patient condition was assessed by physical examination, electrocardiography (ECG), echocardiography (UCG; EF, ejection fraction; FS, fraction shortening), Holter ECG, percent force vital capacity (%FVC), lean body mass (whole body, arms, and legs by standard procedure) by dual-energy X-ray absorptiometry (DEXA; Discovery bone densitometer, Hologic, Bedford, MA), and skeletal muscle mass index (SMI) [13]. Blood tests included creatine kinase (CK) measurement. For activities of daily living (ADL) and quality of life (QOL), the Barthel index (BI, range, 0–100), modified Rankin scale (mRS, Japanese version; range, 1–5), and a 36-item short form survey (SF-36; Japanese version) were used [14,15].

Patients were asked simple question at 1-year follow up visit whether they felt any changes about their symptoms.

2.3. Data analysis

Data were summarized using descriptive statistics, including mean, standard deviation (SD), median, range, frequency, and percentage. Each variable for ambulant (including patients requiring assistance) and non-ambulant patients was compared using *t*-test. In correlation analysis, Spearman correlations were used to determine the association between each of the variables. The paired *t*-test was used to compare differences between baseline and one-year follow-up data. For this comparison, items with no significant abnormalities in all patients at baseline were excluded from annual examinations. Data under measurement (=0) were also excluded from the follow-up analysis. All analyses were performed using SPSS for Macintosh (Version 18; SPSS Inc., Chicago, IL).

3. Results

3.1. GNE mutations

A total of 37.5% (9/24) of the patients harbored a p.V572L homozygous mutation, and 25.0% (6/24) harbored a compound heterozygous mutation. Of these, 12.5% (3/24) exhibited the p.D176V /p.V572L genotype; the rest had a different mutation (Supplementary Table 1).

3.2. Baseline tests

Baseline data are shown in Table 1A. MMT revealed significant weakness both in hip adduction and ankle dorsoflexion, whereas knee extension was markedly preserved (Fig. 1). MMT of the baseline visit showed that knee extension was relatively spared, especially among ambulant patients (Supplementary Fig. 1). With respect to HHD, grip and pinch power, the number of patients who were too weak to complete measurement was 8, 8, and 6, respectively. Non-ambulant patients showed a significantly low %FVC (74.7 ± 19.3 vs. 110.5 ± 12.1, $p < 0.01$, Table 1B). Non-invasive positive pressure ventilation (NPPV) toward respiratory failure of GNE myopathy was used in two patients at night due to respiratory dysfunction and hypoxemia during hospitalization for baseline evaluation.

3.3. Cardiac functions

All patients underwent ECG, but 2 and 4 of 24 patients did not undergo UCG and Holter ECG, respectively, due to their schedules. Twenty-one patients had normal sinus rhythms on the ECG. Two right bundle branch blocks (one complete and one incomplete), a 1st degree atrioventricular block with sinus bradycardia due to beta-blocker use, and a non-specific ST-T change (but normal UCG) were observed. Wall motions on UCG were normal in all patients except in one who had a history of myocardial infarction. In addition, EF and FS were normal in all patients. Holter ECG showed normal ranges in 15 of 20 patients, whereas non-specific ST-T changes in 2, sinus tachycardia in 2, and bradycaldia in 1 were observed. Patients with ST-T changes had diabetes mellitus and/or hypertension.

3.4. Annual changes

During the study period, no patients suffered from a systemic disease or from trauma; moreover, none

Table 1
Patient characteristics and annual changes.

		n	Baseline	1 Year	*p*
Muscle testing	Summed MMT	24	36.0 ± 21.0	33.2 ± 21.0	<0.001
	6MWT (m)	8	321 ± 141.3	273.0 ± 130.6	0.061
	GMFM (%)	24	41.1 ± 39.0	39.6 ± 39.3	0.089
	HHD (N)	16	165.5 ± 98.1	165.5 ± 150.0	0.999
	Grip power (kg)	16	6.8 ± 6.3	5.3 ± 5.6	0.034
	Pinch power (N)	20	22.2 ± 18.6	20.6 ± 21.0	0.261
Pulmonary function	FVC (%)	24	88.1 ± 24.3	84.8 ± 25.7	0.03
DEXA	Whole-body lean body mass (kg)	24	31.0 ± 7.0	30.6 ± 7.4	0.226
	Arm lean body mass (kg)	24	2.6 ± 1.0	2.6 ± 1.0	0.345
	Leg lean body mass (kg)	24	8.5 ± 2.5	8.4 ± 2.6	0.97
	SMI	24	4.1 ± 1.1	4.1 ± 1.1	0.148
Laboratory data	CK (IU/L)	24	222.8 ± 220.5	191.3 ± 199.1	0.087
ADL	Barthel index	24	49.0 ± 39.6	48.1 ± 39.3	0.213
	mRS	24	3.6 ± 1.0	3.6 ± 1.0	–
SF-36	SF-36 PCS	24	10.9 ± 13.2	7.9 ± 10.7	0.148
	SF-36 MCS	24	56.7 ± 11.1	57.9 ± 9.3	0.53
	SF-36 RCS	24	46.3 ± 19.0	43.0 ± 21.6	0.241

The results of baseline and one-year follow-up evaluations for all patients are shown. A total of 33.3% (8/24) of the patients completed the 6-min walk test without assistance. Paired *t*-tests revealed significant reductions in summed MMT of 19 muscles, grip power, and %FVC after one year ($p < 0.05$), and reductions in 6-min walk test scores and gross motor function measure ($p < 0.1$).

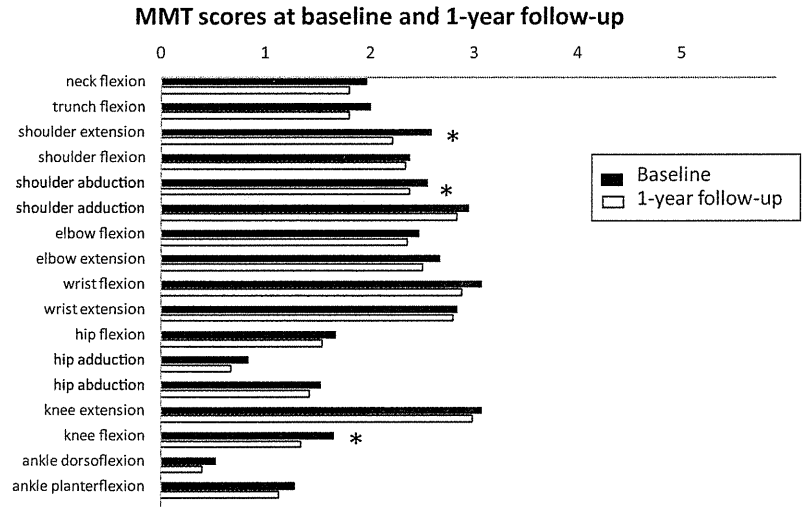

Fig. 1. MMT scores at baseline (black column) and 1-year follow-up (open column). Hip adduction and ankle dorsoflexion were markedly impaired, whereas knee extension was preserved among all the muscles examined. Shoulder extension ($p = 0.017$) and abduction ($p = 0.029$), and knee flexion ($p = 0.010$) showed significant annual decrements ($^{*}p < 0.05$).

required a major surgical intervention that could have influenced the natural course of the disease.

Of the 24 patients, the number of patients who were aware of worsening was 19 (79%). Among them, patients who were aware of worsening hand weakness, neck instability and weakness, gait disturbance, leg weakness, and/or arm weakness were 9, 7, 7, 6, and 2, respectively. Of the 8 ambulant patients not requiring assistance, 7 felt that their gait had become slower compared to the year before. In fact, one patient started using a wheelchair part-time during the one-year follow-up period. All patients who complained of neck instability and weakness were non-ambulant.

A significant reduction in summed MMT ($p < 0.01$), grip power ($p = 0.034$), and %FVC ($p = 0.030$), and a reduction in 6MWT ($p = 0.061$) scores, GMFM ($p = 0.089$), and CK ($p = 0.087$) were observed (Table 1). Among all the muscles examined, shoulder extension ($p = 0.017$) and abduction ($p = 0.029$), and knee flexion ($p = 0.010$) showed significant annual decrements (Fig. 1). Only one patient who succeeded in weight control and increased walking opportunity showed an improvement in 6MWT scores, while the results of other ambulant patients deteriorated in one year (Fig. 2A). Grip power decreased in ambulant patients (9.5 ± 6.9 to 7.1 ± 6.6, $p = 0.051$), but not in non-ambulant patients (3.3 ± 3.3 to 3.0 ± 3.0) (Table 2, Figs. 2D and E). On the other hands, changes in %FVC ($p = 0.034$) were greater in non-ambulant patients than in ambulant patients (Table 2, Figs. 2F and 2G). There were no significant changes in lean body mass, SMI, SF-36, BI, or mRS.

4. Discussion

To our knowledge, this is the first study to assess the prospective natural history of GNE myopathy. Patients with GNE myopathy were disseminated across Japan and were not concentrated around the specialized muscle center hospital, because most patients did not require specialized cardiopulmonary treatment, such as those with Duchenne muscular dystrophy. Accordingly, we selected evaluation items that are commonly accepted among physiotherapists (MMT, 6MWT, and GMFM), and measurement instruments that are relatively inexpensive (e.g., grip, pinch power, and HHD); therefore, the method presented here can be readily implemented by clinical trials and hospitals. For us, it was important that GMFM were validated in the Japanese population [10].

We found that summed MMT, grip power and %FVC were significantly changed in one year. Although statistical significance was lower in the 6MWT, a larger cohort may clearly detect deterioration, given that our study included only a small number of ambulant patients. Severity of Japanese patients is one reason for small number of ambulant patients. It was difficult for us to correct more patients, as ambulant patients were relatively small numbers in Japan. Multicenter study should be required to resolve if the 6MWT are effective tools for annual evaluation.

The 6MWT and summed MMT are important end-point item candidates for clinical trials because they can be used to determine annual changes in disease progression. Our study showed respiratory function decrement especially among non-ambulant patients, suggesting that %FVC can be a useful outcome measure for non-ambulant patients. On the other hand, the decrement in grip power was greater in ambulant patients. These results indicate that evaluation tools should be selected according to the ambulation status of patients.

On the other hand, we could not find annual changes in HHD, lean body mass, BI, mRS, and SF-36. As muscle

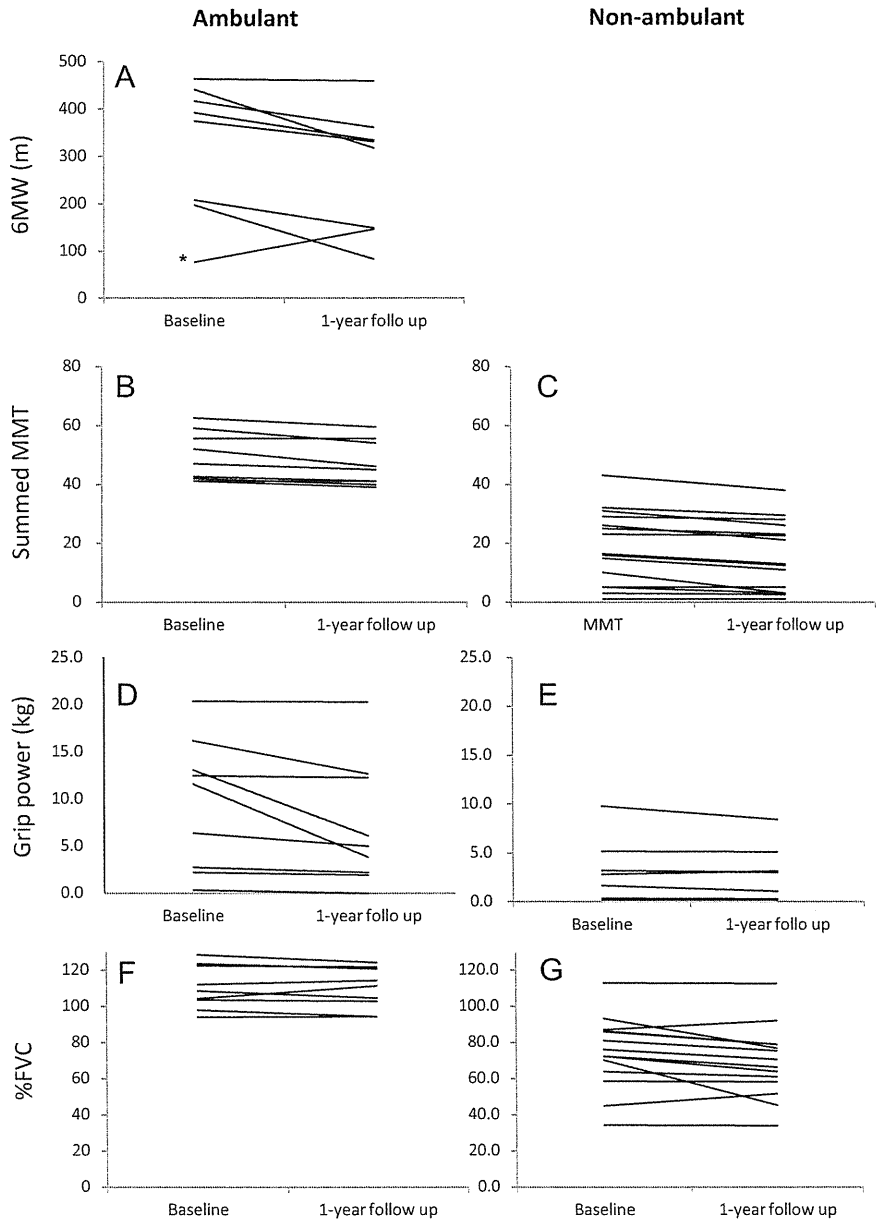

Fig. 2. Annual changes in motor functions. Right colomun: ambulant patients, left column: non-ambulant patients. A: 6MWT; B, C: summed MMT; D, E: grip power; F, G: %FVC. All patients but one (*) showed deterioration in 6MWT (A). Only one patient who showed an improved 6MWT had succeeded in weight control and had more opportunities to walk relative to baseline. Both ambulant (B) and non-ambulant (C) patient showed deterioration in summed MMT. The decrement in grip power was greater in ambulant patients (D, E), whereas the decrement in %FVC was greatern in non-ambulant patients.

strengths for knee extension were well preserved among patients with GNE myopathy, it may be difficult to detect disease progression during the one-year period. Indeed, MMTs of knee extension were preserved at follow-up evaluation. Weaker muscles, such as shoulder muscles or knee flexion muscles that showed deterioration over the one-year period, may be the possible candidates for evaluation. More detailed quantitative study must be carried out before the clinical trials. Although BI, mRS and SF-36 were unchanged, most of patients were aware of their symptoms, and indeed some parameters of muscle power were deteriorated. To detect disease progression, disease-specific QOL or ADL scales may be required.

Two patients started NPPV due to findings obtained during the study. They had been regularly followed by neurologists but had not been evaluated for respiratory function prior to the baseline visit. Both patients carried a V572L homozygous mutation with a more severely affected phenotype [9,16] and showed marked weakness, i.e., summed MMTs were under 5 and mRS was 5. It should be emphasized that patients with GNE myopathy are at risk of respiratory failure, and that physician

Table 2
Annual changes in ambulant and non-ambulant patients.

	Ambulant ($n = 9$)			Non-ambulant ($n = 15$)		
	pre	1 year	p	pre	1 year	p
Summed MMT	57.0 ± 9.5	55.0 ± 8.8	0.022	23.4 ± 14.8	20.1 ± 13.8	<0.001
GMFM (%)	87.6 ± 8.1	87.6 ± 7.9	0.933	13.2 ± 15.5	10.7 ± 11.9	0.078
HHD (N)	214.8 ± 83.2	221.6 ± 164.8	0.864	102.1 ± 80.9	93.2 ± 94.7	0.587
Grip power (kg)	9.5 ± 6.9	7.1 ± 6.6	0.051	3.3 ± 3.1	3.0 ± 3.0	0.179
Pinch power (N)	31.9 ± 18.5	30.4 ± 23.7	0.610	14.2 ± 15.2	12.6 ± 15.3	0.155
FVC (%)	110.5 ± 12.1	109.6 ± 11.5	0.624	74.5 ± 19.3	69.8 ± 19.2	0.034
CK (IU/L)	403.4 ± 273.8	343.9 ± 252.3	0.217	126.0 ± 117.0	108.3 ± 112.3	0.246

Annual changes according to ambulation status. Summed MMT showed a significant decrement in both ambulant and non-ambulant patients. On the other hands, %FVC tended to be preserved in ambulant patients, indicating that pulmonary functional impairment progressed only in non-ambulant patients. The decrement in grip power was also remarkable in non-ambulant patients.

should evaluate respiratory function if patients become non-ambulant and show advanced weakness.

Our study is the first to assess cardiac function in relation to GNE myopathy. However, we were unable to find any disease-related abnormalities in ECG, Holter ECG, and UCG even though cardiac involvement had been previously implicated in a mouse model [8]. Our data suggest that GNE myopathy does not involve cardiomyopathy.

There were limitations with our data analysis because of the small number of participants and short study period. Moreover, we are aware that recruitment of patients from NCNP, a national hospital highly specialized in muscle disease, is a potential source of selection bias, as they may be more severely affected than the general patient population. Japanese patients, especially those who carry a V572L homozygous mutation, show a more severely affected phenotype than previously reported [9,15]; in fact, no studies have ever reported on respiratory failure in GNE myopathy other than the one from Japan [10]. However, our study suggests that non-ambulant patients can be evaluated with %FVC, and that physician should pay attention to the yearly decrement in respiratory function. In rare diseases such as GNE myopathy, large-scale studies tend to be difficult. We have established a Japanese national GNE myopathy patient registry (Registration of Muscular Dystrophy; REMUDY, http://www.remudy.jp) to perform a broader investigation of associated conditions and for long-term observation of patients.

In conclusion, 6MWT, summed MMT, GMFM, grip power tests, and %FVC may be good clinical evaluation tools for trials and to correlate with disease progression, although %FVC and grip power should be used according to ambulation status. Our study revealed that both ambulant and non-ambulant GNE myopathy are basically progressive and do not involve cardiac abnormalities.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2014.02.008.

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RESEARCH PAPER

Mutation profile of the *GNE* gene in Japanese patients with distal myopathy with rimmed vacuoles (GNE myopathy)

Anna Cho,[1] Yukiko K Hayashi,[1,2,3] Kazunari Monma,[1] Yasushi Oya,[4] Satoru Noguchi,[1] Ikuya Nonaka,[1] Ichizo Nishino[1,2]

ABSTRACT

Background GNE myopathy (also called distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy) is an autosomal recessive myopathy characterised by skeletal muscle atrophy and weakness that preferentially involve the distal muscles. It is caused by mutations in the gene encoding a key enzyme in sialic acid biosynthesis, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE).

Methods We analysed the *GNE* gene in 212 Japanese GNE myopathy patients. A retrospective medical record review was carried out to explore genotype–phenotype correlation.

Results Sixty-three different mutations including 25 novel mutations were identified: 50 missense mutations, 2 nonsense mutations, 1 insertion, 4 deletions, 5 intronic mutations and 1 single exon deletion. The most frequent mutation in the Japanese population is c.1714G>C (p.Val572Leu), which accounts for 48.3% of total alleles. Homozygosity for this mutation results in more severe phenotypes with earlier onset and faster progression of the disease. In contrast, the second most common mutation, c.527A>T (p.Asp176Val), seems to be a mild mutation as the onset of the disease is much later in the compound heterozygotes with this mutation and c.1714G>C than the patients homozygous for c.1714G>C. Although the allele frequency is 22.4%, there are only three homozygotes for c.527A>T, raising a possibility that a significant number of c.527A>T homozygotes may not develop an apparent disease.

Conclusions Here, we report the mutation profile of the *GNE* gene in 212 Japanese GNE myopathy patients, which is the largest single-ethnic cohort for this ultra-orphan disease. We confirmed the clinical difference between mutation groups. However, we should note that the statistical summary cannot predict clinical course of every patient.

INTRODUCTION

GNE myopathy, which is also known as distal myopathy with rimmed vacuoles,[1] quadriceps sparing myopathy[2] or hereditary inclusion body myopathy (hIBM),[3] is an autosomal recessive myopathy characterised by skeletal muscle atrophy and weakness that preferentially involve the distal muscles such as the tibialis anterior. It is a progressive disease, whereby the symptoms of muscle weakness start to affect the patient from the second or third decade of life, and most of the patients become wheelchair-bound between twenties and sixties.[4] The characteristic histopathological features in muscle biopsy include muscle fibre atrophy with the presence of rimmed vacuoles and intracellular congophilic deposits.[4 5] GNE myopathy is caused by mutations in the gene encoding a key enzyme in sialic acid biosynthesis, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE).[6–8] Genetically confirmed GNE myopathy was initially recognised in Iranian Jews and Japanese,[7 9] but later appeared to be widely distributed throughout the world. More than 100 mutations in the *GNE* gene have been described up to date.

During the last decade, there has been extensive experimental work to elucidate the pathogenesis and to develop therapeutic strategies of GNE myopathy.[6 10–12] Better knowledge on the basis of those research achievements have currently enabled us to enter the era of clinical trial for human patients. At this moment, the identification of new GNE myopathy patients with precise genetic diagnosis and the expansion of global spectrum of *GNE* mutations are timely and important. Here, we report the molecular profile of Japanese GNE myopathy patients with a brief discussion of genotype–phenotype correlations.

METHODS

Patients

Two hundred and twelve patients from 201 unrelated Japanese families were included in this study. There were 117 female and 95 male patients. All cases were genetically confirmed as GNE myopathy. A retrospective medical record review was carried out to explore genotype–phenotype correlation. Informed consent was obtained for the collection of clinical data and extraction of DNA to perform mutation analysis.

Genetic analysis

DNA was extracted from peripheral blood leukocytes or skeletal muscle tissue. We used the previously described sequencing method to describe mutations at cDNA level.[7] All exons and splice regions of the *GNE* gene were sequenced. NM_005476.5 was used as a reference sequence. We screened 100 alleles from normal Japanese individuals to determine the significance of novel variations.

Pathological analysis

To evaluate histopathological phenotype according to genotype, we analysed muscle biopsies from two

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most common genotype groups in Japanese population. Each of the three age-matched and biopsy site-matched samples from c.1714G>C homozygous group and c.1714G>C/c.527A>T compound heterozygous group was compared. Muscle samples were taken from biceps brachii and frozen with isopentane cooled in liquid nitrogen. Serial frozen sections of 10 μm were stained using a set of histochemical methods including haematoxylin-eosin and modified Gomori trichrome.

Statistical analysis

Statistics were calculated using GraphPad Prism 5 software (GraphPad Software, La Jolla, California, USA). Between-group comparison for clinical data was performed using one-way analysis of variance with Dunnett's post-test. All values are expressed as means±SD. We performed two-sided tests with a $p<0.05$ level of significance.

Table 1 Allele frequency for GNE mutations in 212 Japanese GNE myopathy patients

	Allele frequency
Mutation type	
Missense	402 (94.8%)
Nonsense	3 (0.7%)
Insertion	1 (0.2%)
Small deletion	4 (0.9%)
Single exon deletion	2 (0.5%)
Intron	12 (2.8%)
Three most common mutations	
c.1765G>C (p.Val572Leu)	205 (48.3%)
c.578A>T (p.Asp176Val)	95 (22.4%)
c.38G>C (p.Cys13Ser)	15 (3.5%)
Total alleles	424

RESULTS

Mutation profile

We identified homozygous or compound heterozygous *GNE* mutations in all 212 patients (see online supplement 1). In total, 63 different mutations were found including 50 missense mutations, 2 nonsense mutations, 1 insertion, 4 deletions, 5 intronic mutations and 1 single exon deletion (figure 1). Twenty-five novel mutations were identified including 17 missense mutations, 4 small deletions, 3 intronic mutations and 1 single exon deletion (figure 1, see online supplement).

Twenty-one mutations were found to be shared between two or more unrelated families. The three mutations occurring most frequently in the Japanese population were c.1714G>C (p.Val572Leu), c.527A>T (p.Asp176Val) and c.38G>C (p.Cys13Ser); these comprised 48.3%, 22.4% and 3.5%, respectively, of the total number of alleles examined (table 1).

Genotype–phenotype correlations

The mean age of genetic analysis was 41.6±14.1 years (n=212), and the mean age of symptom onset based on the data available was 28.4±10.2 years (n=195). The earliest onset age was 10 and the latest was 61 years old in our cohort. Thirty-six among 154 patients (23.4%) were full-time wheelchair users at the point of genetic diagnosis with the average age at loss of ambulation being 36.8±11.3 years (n=36). The youngest wheelchair-bound age was 19, and the oldest ambulant age was 78. To investigate genotype–phenotype correlations in the major *GNE* mutations of Japanese population, we compared the age at symptom onset and loss of ambulation between the patients groups carrying either of the two most frequent mutations, c.1714G>C and c.527A>T (table 2). As with a previous report,[13] homozygous c.1714G>C mutations resulted in earlier symptom onset (23.9±7.1 years, $p<0.01$) and the majority of full-time wheelchair users were in this group. On the other hand, c.1714G>C/c.527A>T compound heterozygous patients first developed symptoms at a later age (37.6±12.6 years, $p<0.01$), and there were no wheelchair-bound patients at the time of genetic analysis in this group. Only three homozygous c.527A>T mutation patients were identified, and their average onset age (32.3±5.7 years) was also higher among total patients (28.4±10.2 years). All three patients were ambulant until the last follow-up visits (29, 40 and 44 years).

Among 212 cases, 80 patients underwent muscle biopsies. Overall pathological findings in our series were compatible with GNE myopathy. The characteristic rimmed vacuoles were observed in the majority (76/80, 95.0%) of the cases. Through the analysis of muscle biopsies from age-matched and biopsy site-matched samples, we found that the histopathological phenotypes were in line with these genotype–phenotype correlations (figure 2). Homozygous c.1714G>C mutations have led to much more advanced pathological changes with severe myofibre atrophy and increased numbers of rimmed vacuoles. Marked adipose tissue replacement was appreciated in a case with reflecting very advanced stage of muscle degeneration.

DISCUSSION

As shown in figure 1, mutations were located throughout the whole open reading frame of the *GNE* gene. The majority (94.8%, 402/424 alleles) of the mutations in our series were missense mutations (table 1), and there were no homozygous null mutations. These results are in accordance with previous reports[7 9] signifying that total loss of GNE function might be

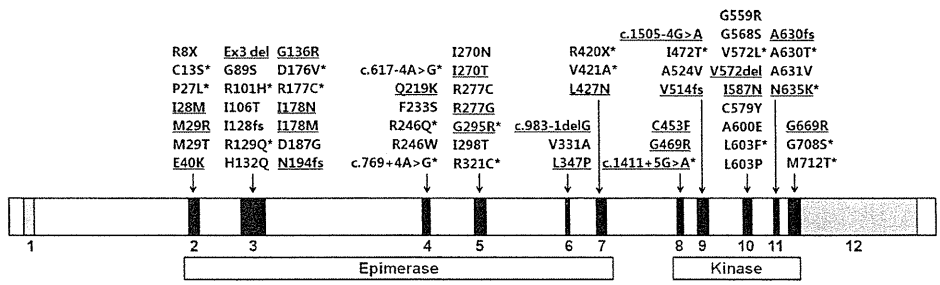

Figure 1 Mutation spectrum of *GNE* in the Japanese population. The mutations are located throughout the whole open reading frame. Twenty-five novel mutations are underlined, and 21 shared mutations are indicated with asterisks.

Table 2 Comparison of clinical course between two most frequent GNE mutations in Japanese population

Mutations	Age at exam (years)		Age at onset (years)		Age at WB (years)		Ambulant
c.1714G>C/c.1714G>C	38.6±13.4	(n=71)	23.9±7.1	(n=65)**	35.4±10.6	(n=28)	n=22
c.1714G>C/other	32.3±13.2	(n=25)	21.9±6.8	(n=22)*	37.0±8.6	(n=4)	n=16
c.1714G>C/c.527A>T	48.9±14.1	(n=38)	37.6±12.6	(n=35)**		(n=0)	n=29
c.527A>T/c.527A>T	37.7±7.7	(n=3)	32.3±5.7	(n=3)		(n=0)	n=3
c.527A>T/other	41.3±11.1	(n=51)	30.6±8.0	(n=46)		(n=2)	n=33
other/other	49.8±14.7	(n=24)	28.8±9.5	(n=24)		(n=2)	n=16
Total	41.6±14.1	(n=212)	28.4±10.2	(n=195)	36.8±11.3	(n=36)	n=118

Dunnett's multiple comparison test (control: total patients) *p<0.05, **p<0.01.
Other: a mutation other than c.1714G>C and c.527A>T; WB, wheelchair-bound.

lethal in human beings. The embryonic lethality of null mutation in *GNE* had also been proved in the mouse model.[14] Only three of total 212 patients carried a nonsense mutation; clinical data were available for two of them. Interestingly, one patient with compound heterozygous c.22C>T (p.Arg8X)/c.1714G>C (p.Val572Leu) mutations developed his first symptoms at the age of 15, while the other patient with c.1258C>T (p.Arg420X)/c.527A>T (p.Asp176Val) mutations developed her symptoms much later, at the age of 45. The similar difference was also observed in the phenotypes of patients with frame-shift mutations. A patient carrying c.383insT (p.I128fs) and c.1714G>C (p.Val572Leu) mutations developed his first symptom at the age of 13, whereas another two patients with c.1541-4del4 (p.Val514fs)/c.527A>T (p.Asp176Val) and c.581delA (p.N194fs)/c.527A>T (p.Asp176Val) mutations had later symptom onset, at the age of 30 and 32 years, respectively. This clinical variation can be explained as it reflects alternative missense mutations, because the two patients with very early onset shared the same missense mutation c.1714G>C, while the patients with the milder phenotype shared c.527A>T.

Among five intronic mutations identified in our series, c.617−4A>G and c.769+4A>G were previously reported as pathological mutations.[7 15] Three novel variants were located at splice junction of exon 6 (c.983–1delG), exon 8 (c.1411+5G>A) and exon 9 (c.1505–4G>A), raising the high possibility of relevant exons skipping. These variants were not detected in 200 alleles from normal Japanese individuals and also in the single nucleotide polymorphism (SNP) database.

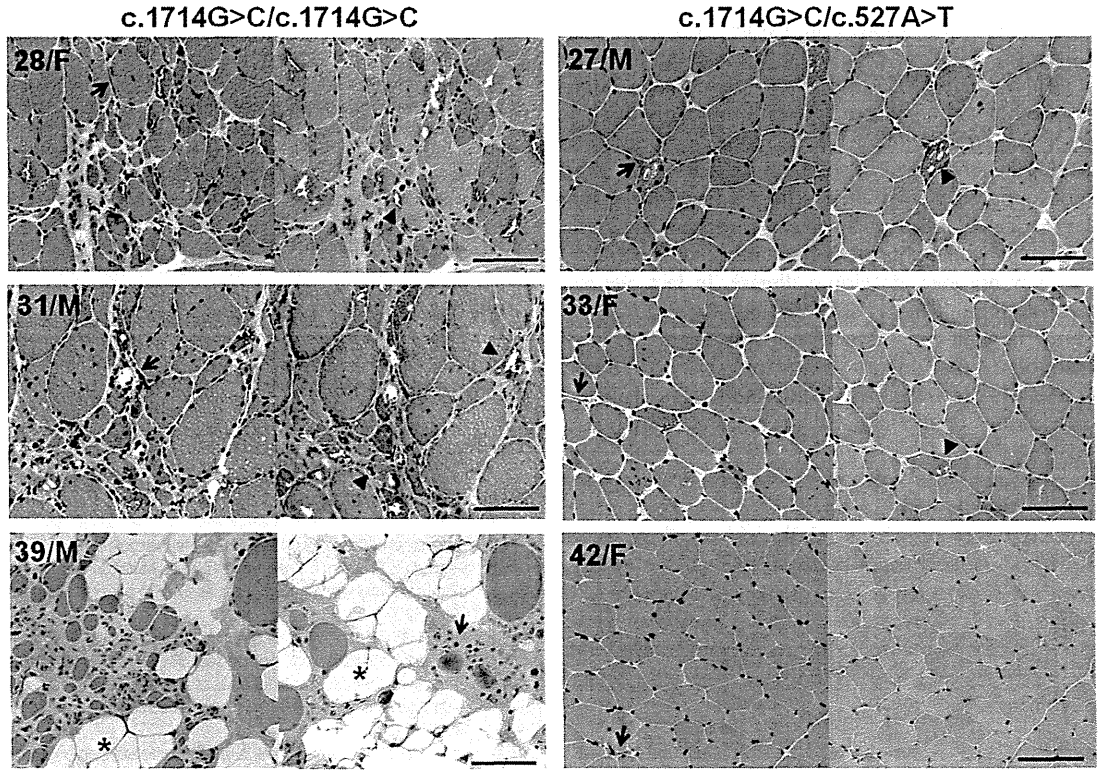

Figure 2 Comparison of muscle pathology between patients with homozygous c.1714G>C (p.Val572Leu) and with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Homozygous c.1714G>C (p.Val572Leu) mutations have led to much more advanced histopathological changes compared with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Haematoxylin-eosin (left) and modified Gomori trichrome (right) stains of muscle sections from age (c.1714G>C/c.1714G>C: 28, 31 and 39 years, c.1714G>C/c.527A>T: 27, 33 and 42 years) and biopsy site (biceps brachii muscles) matched samples. Bar=100μm; triangles: rimmed vacuoles; arrows: atrophic fibres; asterisks: adipose tissue.

As there are ethnic differences in *GNE* mutation frequencies,[9 16–19] establishing the mutation spectrum and defining predominant mutations in a certain population may be helpful for the diagnosis. Three most common mutations in the Japanese population and their allele frequencies (table 1) were in agreement with previous data.[7 13] The allele frequencies of top two mutations (c.1714G>C and c.527A>T) comprise more than two-third of the total number of alleles suggesting that founder effects are involved in the relatively higher incidence of GNE myopathy in Japan.

Although most of patients showed characteristic pathological features, the existence of exceptional cases with atypical biopsy findings implies that GNE myopathy cannot be totally excluded from the absence of rimmed vacuoles in muscle biopsies. On the other hand, we found 94 patients who were pathologically or clinically suspected but not had mutations in *GNE*. Several cases of VCP myopathy mutations in (*VCP*), myofibrillar myopathy mutations in (*DES*) and reducing body myopathy (*FHL1*) were later identified in this group, suggesting these diseases should be included as differential diagnosis of GNE myopathy.[20]

In terms of genotype–phenotype correlations, we confirmed that homozygosity for c.1714G>C (p.Val572Leu) mutation resulted in more severe phenotypes in clinical and histopathological aspects. In contrast, the second most common mutation, c.527A>T (p.Asp176Val), seems to be a mild mutation as the onset of the disease is much later in the compound heterozygotes with this mutation and c.1714G>C. Several evidences further strengthened the link between the more severe phenotype and c.1714G>C, and between the milder phenotype and c.527A>T. Compound heterozygosity for c.1714G>C and non-c.527A>T mutations resulted in earlier symptom onset (22.9±6.8 years, $p<0.05$) compared with the average onset age of the total group, whereas c.527A>T, both presented as homozygous and as compound heterozygous mutations, lead to slower disease progression (table 2). In addition, only three patients carrying this second most common mutation c.527A>T in homozygous mode were identified, which is much fewer than the number expected from high allele frequency (22.4%), raising a possibility that considerable number of c.527A>T homozygotes may not even develop a disease. In fact, we ever identified an asymptomatic c.527A>T homozygote at age 60 years.[7] Now he is at age 71 years and still healthy. Overall, these results indicate that different mutations lead to different spectra of severity. However, this is a result of a statistical summary that cannot predict clinical course of each individual patient.

Here, we presented the molecular bases of 212 Japanese GNE myopathy patients with 25 novel *GNE* mutations. Based on the current status of knowledge, sialic acid supplementation may lead to considerable changes in the natural course of GNE myopathy within near future. The ongoing identification of *GNE* mutations and further studies regarding the clinicopathological features of each mutation will provide better understanding of GNE myopathy and lead to accelerated development of treatment for this disease.

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Contributors AC had full access to all of the data in the study and wrote the manuscript; YKH supervised all aspects of this study including study design, data interpretation and manuscript preparation; KM and YO participated in collecting and analysing all the clinical and genetic data; SN, I Nonaka and I Nishino were involved in data analysis and interpretation and also supervised manuscript preparation.

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Competing interests None.

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Provenance and peer review Not commissioned; externally peer reviewed.

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Chapter 7

Autophagy in GNE Myopathy

Anna Cho and Satoru Noguchi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55223

1. Introduction

Muscle diseases represent specific muscle pathology. The characteristic features as hallmarks of diseases have been historically used to diagnose the patients. The "Rimmed vacuole (RV)" (Figures 1) is one of such characteristic features in certain groups of the diseases. This structure consists of the space (vacuole) and purple granules (rim) within myofibers, while the space is sometimes occupied with cytosolic contents indicating that the space is artificially produced during the staining process and the rims have the nature of this pathological hallmark. Ultrastructurally, as discussed later, many autophagic vacuoles and multi-lamellar bodies are observed in RVs.

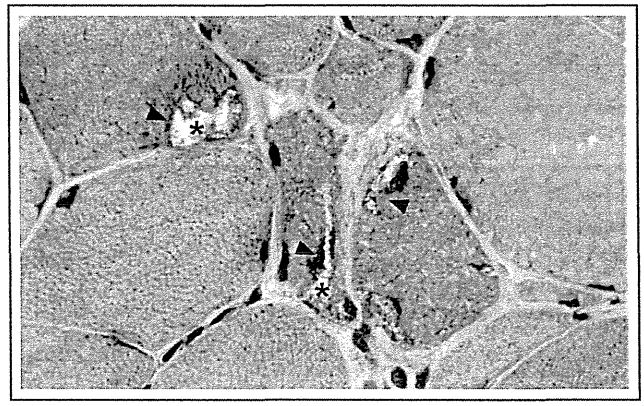

Figure 1. Rimmed vacuoles in a modified Gomori trichrome section. The purple granules (arrow heads) are surrounding vacuoles (asterisks).

Skeletal muscle represents 40~50% of the human body and is one of the most important sites for the control of metabolism. During catabolic conditions, muscle proteins are mobilized to provide alternative energy substrates for the other organs. The RV formation indicates dysfunction of autophagy and breakdown of energy homeostasis in diseased skeletal muscles. In addition, it also suggests the importance of autophagy in muscle functions. There is a group of muscle disease, generally referred to as autophagic vacuolar myopathies (AVM), which are characterized by the accumulation of autophagic vacuoles on skeletal muscle pathology.

In this review, we will give an outline of general knowledge and classification on AVMs and overview the molecular processes underlying autophagic vacuoles formation in rimmed vacuolar myopathies on the basis of our experimental evidences regarding GNE myopathy.

2. Autophagic vacuolar myopathies

Dysfunctional autophagy is associated with several neurodegenerative disorders [1-3]. As for muscle disorders, these are referred to as AVMs [4]. Since the autophagosomes are not observed in normal muscle fibers, autophagic vacuoles have been often recognized as pathologic hallmarks of numerous neuromuscular disorders. Two major categories in AVMs include lysosomal myopathies and rimmed vacuolar myopathies (Table 1) [4-6]. The former are associated with a primary defect in lysosomal proteins and the two best-described and genetically diagnosable AVMs, Pompe disease and Danon disease, are classified in this group. In contrast, autophagic vacuoles in rimmed vacuolar myopathies are secondarily caused by extra-lysosomal defects and usually observed at later stages of the disease. There are various kinds of rimmed vacuolar myopathies including sporadic inclusion body myositis (sIBM) and myofibrillar myopathies and most of them are clinically and etiologically heterogeneous disorders.

Disease	Causative Genes
Lysosomal Myopathy	
Acid maltase deficiency (Pompe disease)	*GAA*
Danon disease	*LAMP2*
X-linked myopathy with excessive autophagy (XMEA)	(identified)
Myopathy with rimmed vacuoles (RVs)	
Inclusion body myositis (sIBM)	?
Myofibrillar myopathy	*DES CRYAB MYOT ZASP etc*
GNE myopathy	*GNE*
Inclusion body myopathy, Paget's disease of bone, and frontotemporal dementia (IBMPFD)	*VCP*
Other myopathies often showing RVs	
Oculopharyngeal muscular dystrophy (OPMD)	*PABPN1*
Marinesco-Sjögren syndrome	*SIL1*
Myotonic dystrophy	*DMPK*

Table 1. Lists of autophagic vacuolar myopathies.

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2.1. Lysosomal myopathy

Two best known disorders in AVMs are associated with primary lysosomal protein defects, namely, Pompe disease [7] and Danon disease [8]. The former is caused by a deficiency of lysosomal enzymes within the vacuoles, whereas the latter is caused by a deficiency of lysosomal membrane structural protein [9].

2.1.1. Pompe disease

Pompe disease [7], also referred to as glycogen storage disease type II and acid maltase deficiency, is the best characterized lysosomal myopathy caused by a deficiency of acid α-glucosidase (GAA, also known as acid maltase). This enzyme defect results in lysosomal glycogen accumulation in multiple tissues and cell types, with skeletal and cardiac muscle cells the most seriously affected [10, 11]. The classic infantile form is a rapidly progressive disease with hypotonia, generalized muscle weakness, and hypertrophic cardiomyopathy, usually leading to death from cardiorespiratory failure or respiratory infection in the first year of life [12]. But enzyme replacement therapy with recombinant human GAA is now available, which can dramatically improve the clinical features and life expectancy of the infantile Pompe disease patients [13-15]. The late-onset type shows less progressive clinical characteristics and absence of severe cardiomyopathy; these phenotypical differences are related to residual enzyme activity [16]. The *GAA* gene is located on human chromosome 17q25.2-25.3 and more than 200 pathogenic sequence variations have been characterized up to date [17].

On muscle pathology, cytoplasmic vacuoles are so remarkable and large that these occupy most of the space in many muscle fibers (Figure 2). The vacuoles contain amorphous materials that are presumably glycogen because of the strong reactivity with periodic acid Schiff stain. Acid phosphatase staining also shows strong signals in these vacuoles, indicating high lysosomal content [6]. In terms of pathomechanism, the failure of the lysosomal degradation of glycogen leads to the accumulation of autophagic vacuoles, which may cause cellular dysfunction and abnormal cytoskeletal organization [18].

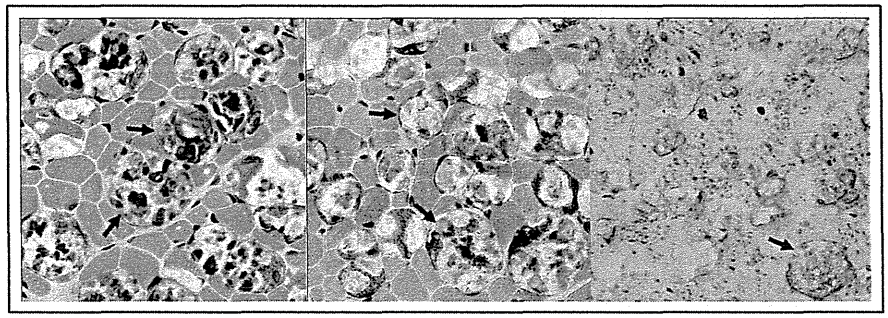

Figure 2. Pathologic findings in Pompe disease. Hematoxylin and eosin (left) and modified Gomori trichrome (middle) sections show pathognomonic vacuolar structures (arrows) in myofibers. These vacuolar structures are strongly stained by acid phosphatase (right).

2.1.2. *Danon disease*

Danon disease is an X-linked disorder caused by the primary deficiency of lysosome-associated membrane protein-2 (LAMP-2) [9]. Characteristic clinical features include skeletal myopathy, cardiomyopathy, and mental retardation. Male patients usually manifest the disease in their teens and die before their 30s from cardiac problems. LAMP-2 deficiency causes accumulation of autophagic vacuoles in a variety of tissues, including skeletal and cardiac muscles [5]. As LAMP-2 is required for the maturation of early autophagic vacuoles by fusion with endosomes and lysosomes, deficiency of LAMP-2 leads to a failure in the normal progression of autophagic maturation [6].

Muscle biopsy from the patients with Danon disease show scattered small basophilic granules in myofibers and lysosomal acid phosphatase activity is increased in these granules (Figure 3). Large vacuolar structures having sarcolemmal features with acetylcholine esterase activity are surrounding those lysosomal granules and these structures are known as autophagic vacuoles with sarcolemmal features (AVSF) [19]. This characteristic pathology in Danon disease (AVSF) is also seen in a number of diseases including X-linked myopathy with excessive autophagy (XMEA) [20], infantile autophagic vacuolar myopathy [21], and X-linked congenital autophagic vacuolar myopathy [22]. The list of this group of autophagic vacuolar myopathy is rapidly expanding [5] and they are expected to be related with lysosomal function because the pathologic features are quite similar to those in Danon disease.

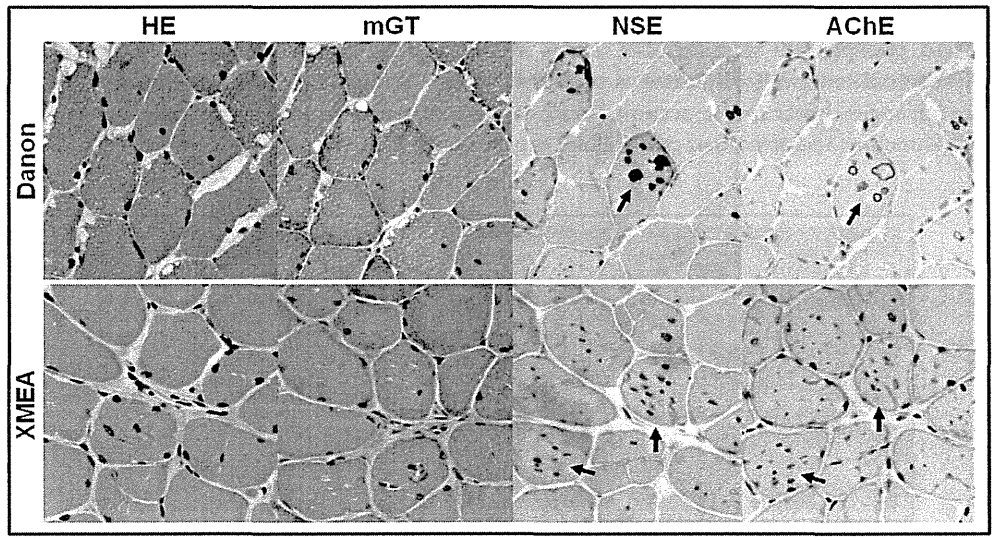

Figure 3. Pathologic findings in Danon disease and XMEA. Many AVSFs (arrows) are showing acetylcholine esterase and nonspecific esterase positivity. HE-hematoxylin and eosin; mGT-modified Gomori trichrome; NSE-nonspecific esterase; AChE-acetylcholine esterase.

2.2. Myopathy with rimmed vacuoles

Rimmed vacuolar myopathies comprise more various and heterogeneous disorders. The most common disease in this group is sIBM, which has been traditionally considered as an inflammatory myopathy. Myofibrillar myopathy, a group of chronic myopathies with a similar pathologic phenotype, is caused by several different genes. And VCP myopathy and GNE myopathy are well known single gene disorders which can be classified as hereditary inclusion body myopathies (hIBM). In addition, it is not uncommon that rimmed vacuoles are appreciated in numerous chronic myopathies which are not classically classified as rimmed vacuolar myopathies.

2.2.1. *Inclusion Body Myositis (sIBM)*

sIBM is the most common muscle disease in elderly patients [23-25]. Clinically, general progressive muscle weakness starts after age 50 years. The quadriceps muscle and finger flexors are usually affected early on. sIBM Patients may become unable to perform daily living activities and require assistive devices within 10 years of symptom onset. Muscle biopsy characteristically reveals rimmed vacuolar muscle fibers with endomysial T-cell inflammatory infiltrates. Although there still remains controversy whether sIBM is an autoimmune inflammatory myopathy or a primary degenerative myopathy with secondary inflammation, it is becoming more likely that abnormal myoproteostasis and muscle fiber degeneration with aging play primary pathogenic roles in this disorder [26].

Askanas and Engel [27] indicated that several phenomena observed in the degeneration of sIBM muscle fibers are similar to the neuronal degenerative processes occurring in both Alzheimer's disease and Parkinson's disease. Abnormal accumulations of various pathogenic proteins, posttranslational modifications of the accumulated proteins, abnormal protein disposal, and impaired autophagy and 26S proteasome function are common intracellular features of neurodegenerative disorders and thus suggest that sIBM is, like neurodegenerative diseases, a complex degenerative disorder caused by protein misfolding and associated with multiprotein aggregation [28].

2.2.2. *Myofibrillar myopathies*

RVs are often appreciated in large numbers of myofibrillar myopathies [29-31], which is a group of hereditary myopathies pathologically characterized as markedly disorganized myofibrils with cytoplasmic inclusion. Clinical symptoms of myofibrillar myopathies are very variable. The onset age rages from infancy to the eighth decade. Some patients show limb girdle muscle involvement, whereas others show distal myopathy. Cardiomyopathy is often involved and even can be seen in patients with no obvious skeletal muscle weakness. Seven disease-related genes have been identified (*DES*, *CRYAB*, *MYOT*, *ZASP*, *FLNC*, *BAG3*, and *FHL1*) up to date and all of them encode proteins closely related to Z-line. Electron microscopy findings imply that disintegration of myofibrils near Z-line causes accumulation of filamentous material and aggregation of membranous organelles and glycogen, leading to the entrapment of dislocated membranous organelles in autophagic vacuoles [31].

In the cardiomyocytes-restricted *CRYAB* over-expressed mice, autophagic activity is increased in response to protein aggregates and blunting autophagy *in vivo* dramatically worsen the disease progression [32]. Although myofibrillar myopathy includes various genetically and clinically heterogeneous disorders, accumulation of misfolded proteins is considered as a common pathological pattern and autophagy in myofibrillar myopathy is now becoming to be considered as an adaptive response.

2.2.3. Inclusion Body Myopathy, Paget's disease of the bone, and Frontotemporal Dementia (IBMPFD); Valosin-Containing Protein (VCP) myopathy

Inclusion body myopathy (IBM) with Paget's disease of bone (PDB) and frontotemporal dementia (FTD), now called IBMPFD or valosin-containing protein (VCP) myopathy, is a progressive autosomal dominant disorder caused by mutations in the *VCP* [33]. It is a rare multisystem degenerative disorder with three variably penetrated phenotypic features [34]. 90% of patients develop muscle weakness with a mean onset of 45 years of age and 50% of patients have osteolytic lesions consistent with PDB at the same mean age. About 30% patients develop a typical FTD manifested by apparent language and behavior dysfunction at fifties [35]. Other phenotypic features have been reported as well, including dilated cardiomyopathy, cataracts and sensory-motor neuropathy [36]. Muscle biopsy shows degenerating fibers with RVs and sarcoplasmic inclusions. While molecular pathogenesis in IBMPFD is unknown, the extensive accumulation of ubiquitin conjugates in affected tissues suggests impairment of protein degradation pathways in this disease. In addition, impaired maturation of ubiquitin-containing autophagosomes in cells expressing *VCP* mutants imply that defective autophagy also contributes to the pathogenesis of IBMPFD [37].

2.2.4. Other myopathies often related with rimmed vacuoles

Although they are not pathognomonic, RVs are often accompanied in various chronic myopathic conditions including Marinesco-Sjögren syndrome and oculopharyngeal muscular dystrophy (OPMD). It is interesting that these clinically and genetically different disorders are sharing a similar pathologic feature in skeletal muscles.

Marinesco-Sjögren syndrome is an autosomal recessive disorder clinically characterized by cerebellar ataxia, cataracts from infancy, mental retardation, and myopathy [38]. Loss of function mutations in *SIL1*, which encodes a nucleotide exchange factor for the Hsp70 chaperone BiP, was identified as a causative gene. Previous ultra-structural study showed that myofibrillar degeneration with autophagic phenomenon is prominent in Marinesco-Sjögren syndrome muscles [39]. In addition, it was demonstrated that increased ER stress and altered protein folding lead to neurodegeneration in *SIL1* knock-out mice [40], from which we can infer similar pathogenic process may occur in skeletal muscles of Marinesco-Sjögren syndrome.

OPDM is known to be caused by repeat expansion mutations in *PABPN1* [41]. It has recently become evident that autophagy has an important role in the pathogenesis of repeat expansion disease [42]. The role of autophagy has been extensively studied especially in the polyglutamine diseases such as Huntington's disease and spinocerebellar ataxia. Most of research