Table 2 Questionnaire administered in the main study

1	Have you ever been diagnosed with cataracts? If yes, when were you first diagnosed: () years old	Yes	No	Not sure
2	Can you whistle?	Yes	No	Not sure
3	When you eat food or beverages, do you feel a choking or sticking sensation?	Yes	No	Not sure
4	Can you lift up your head from a supine position without arm support?	Yes	No	Not sure
5	Can you sit up from a supine position without arm support?	Yes	No	Not sure
6	Can you easily unscrew the cap of a plastic bottle?	Yes	No	Not sure
7	Do you experience any difficulty in lifting the toes toward the shin or do you find that ? you often stub your toes against tiny bumps when you walk?	Yes	No	Not sure
8	Do you experience any muscle stiffness, such as difficulty opening your hand immediately ? after you grasp it strongly, or difficulty opening your mouth after you clench your jaw strongly?	Yes	No	Not sure
9	Do any of your blood-related kin have a muscular disorder?	Yes	No	Not sure

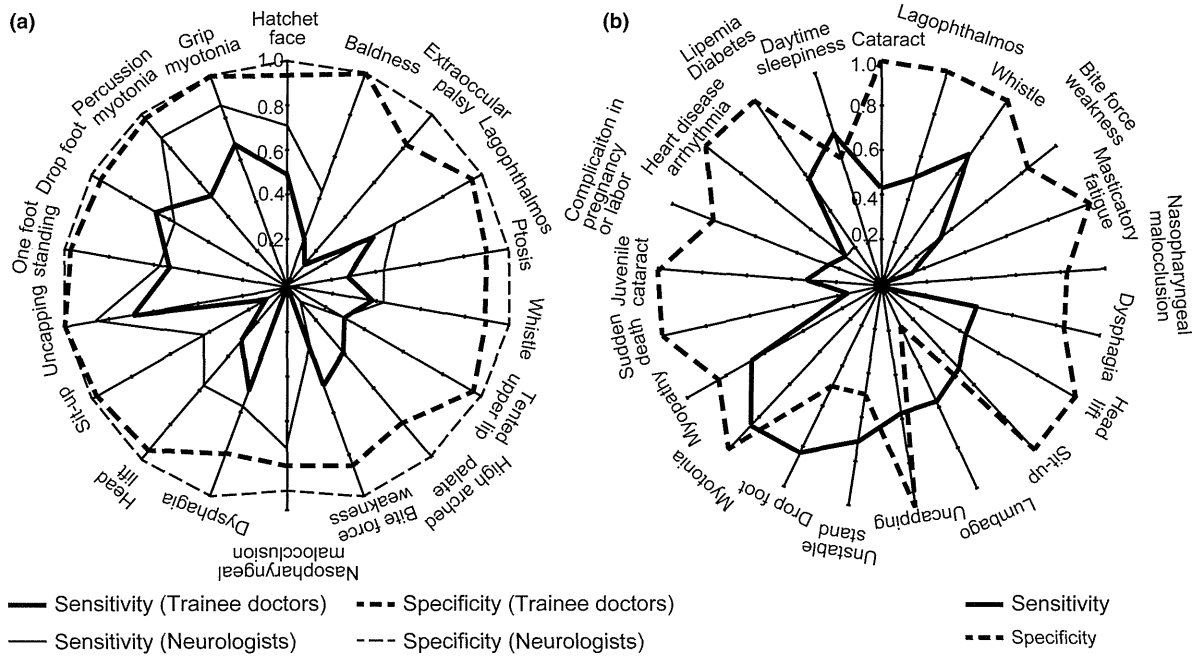


Figure 1 The specificity and sensitivity of each item included in (a) the physical examination and (b) the questionnaire in the pilot study.

not as good as in HC. In DC patients with mild disability, the specificity was over 70% for four items (cataract, difficulty lifting the head, difficulty unscrewing a bottle cap and myotonia; Table 3). The number of cases was not sufficient for statistical analyses, but it is possible that overlapping and/or confusing symptoms influenced the specificity of some items for some diseases. In mild muscular dystrophy, equinus foot could have been regarded as drop foot. In facioscapulohumeral muscular dystrophy, facial and abdominal weakness decreased the specificity of item 2 (difficulty whistling) and item 5 (difficulty sitting up), and in Parkinson's disease, akinesia, small shuffling gait and rigidity decreased the specificity of item 5 (difficulty sitting up), item 7 (drop foot), and item 8 (myotonia). However, confusion of morning stiffness with myotonia in patients with rheumatoid arthritis was not common.

The sensitivity of items was variable (Table 3). Although item 8 (myotonia) had the highest sensitivity, a considerable number (10/95) of patients reported no myotonia. Just three other items, item 5 (difficulty sitting up), item 6 (difficulty unscrewing a bottle cap) and item 7 (drop foot), had sensitivity over 70% (Table 3). For every item except item 8 (myotonia), sensitivity was lower when only participants with mild disability were considered than when all participants were considered (Table 3). Some DM1 patients were unaware of their symptoms, and responded in the negative even when a symptom was present. Although this might have impacted the sensitivity, the answers were not modified.

The largest AUC between mild DM1 and HC (0.998), and between mild DM1 and mild DC (0.915) was obtained by the combination of items 4 (difficulty lifting the head), 6

Table 3 Sensitivity and specificity of each item in the questionnaire

		Sensitivity	Specificity					
		DM1, % (%)	HC, %	All DC, % (%)	MD, % (%)	FSHD, % (%)	PD, % (%)	RA, % (%)
Item 1	Cataract	45.1 (36.5)	89.0	86.3 (84.6)	93.4 (84.6)	84.6 (100)	75.0 (66.7)	78.6 (81.2)
Item 2	Whistle	34.7 (28.1)	88.0	49.2 (67.3)	55.0 (84.6)	15.3 (22.2)	46.7 (83.3)	71.4 (72.7)
Item 3	Dysphagia	64.2 (57.8)	90.0	52.3 (61.5)	58.3 (61.5)	84.6 (77.8)	43.8 (66.7)	71.4 (81.8)
Item 4	Lift the head	69.5 (60.9)	97.5	39.7 (88.5)	23.0 (76.9)	46.2 (66.7)	56.3 (100)	78.6 (90.9)
Item 5	Sit up	81.1 (73.4)	92.6	27.5 (59.6)	16.4 (46.2)	23.1 (33.3)	18.8 (33.3)	78.6 (90.9)
Item 6	Unscrew a bottle cap	70.5 (60.9)	99.2	36.9 (75.0)	28.3 (69.2)	69.2 (77.8)	37.5 (66.7)	64.3 (81.8)
Item 7	Drop foot	78.3 (73.4)	82.6	34.5 (32.7)	37.3 (7.7)	30.8 (33.3)	0.0 (0.0)	50.0 (63.6)
Item 8	Myotonia	87.4 (92.2)	98.3	68.8 (78.8)	78.0 (76.9)	69.2 (77.8)	37.5 (50.0)	92.9 (100)

Item 9: family history of muscular disorders was excluded from statistical analysis as family members of myotonic dystrophy type 1 (DM1) patients were included in the healthy control (HC) group. The values in parentheses were obtained from patients with mild symptoms.
DC, disease control; FSHD, facioscapulohumeral muscular dystrophy; HC, healthy control; MD, muscular dystrophy; PD, Parkinson's disease; RA, rheumatoid arthritis.

(difficulty unscrewing a bottle cap) and 8 (myotonia; items 4 + 6 + 8). The second largest AUC between mild DM1 and HC (0.995), and between mild DM1 and mild DC (0.902) was obtained from the combination of items 4 (difficulty lifting the head), 5 (difficulty sitting up), 6 (difficulty unscrewing a bottle cap) and 8 (myotonia; items 4 + 5 + 6 + 8). The latter combination also showed the highest AUC between all DM1 and HC (0.996; Fig. 2). With both combinations, the optimal cut-off point for the number of positive items was one to discriminate mild DM1 from HC (items 4 + 6 + 8 sensitivity = 100% and specificity = 98.4%, and items 4 + 5 + 6 + 8 sensitivity = 100% and specificity = 93.4%), and two to distinguish mild DM1 from mild DC (items 4 + 6 + 8 sensitivity = 78.1% and specificity = 84.9%, and items 4 + 5 + 6 + 8 sensitivity = 89.1% and specificity = 81.1%).

A total of 51 participants (37 DM1, eight HC and six DC) reported the age of cataract onset. The age of cataract onset was younger in DM1 (42.1 ± 9.4 years) than in HC (60.4 ± 11.5 years, $P = 0.002$) and DC (56.2 ± 24.1 years, $P = 0.012$).

Although the answers to item 9 (family history) were excluded from the analyses, erroneous answers were noted. Some participants considered only relatives in ancestral/descendent generations, and some participants considered cohabiters when answering this question.

Discussion

Myotonic dystrophy type 1 is a multisystem disorder, and early introduction of multidisciplinary care is important for effective treatment. Non-motor symptoms often appear

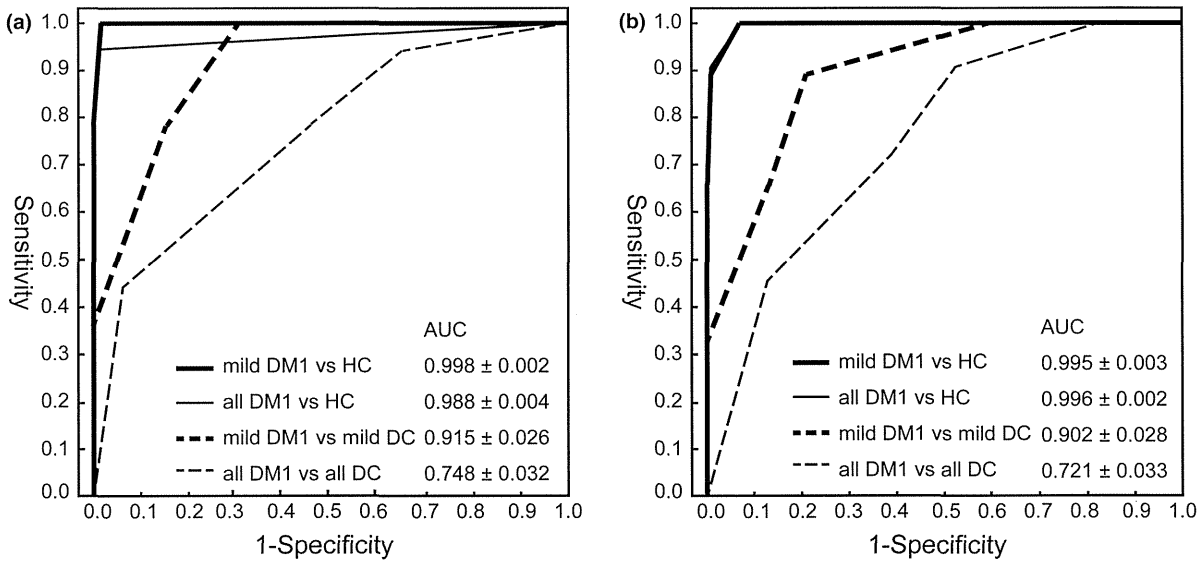


Figure 2 Receiver operating characteristic curve for (a) the combination of lifting the head, unscrewing a bottle cap and myotonia items (items 4 + 6 + 8) and (b) the combination of lifting the head, sitting up, unscrewing a bottle cap and myotonia items (items 4 + 5 + 6 + 8) in the main study. AUC, area under the curve; DC, disease control; DM1, myotonic dystrophy type 1; HC, healthy control.

before motor symptoms, and many patients pay little attention to motor symptoms.[1] Juvenile cataract is an example of a non-motor symptom, and the results of the present study show that the onset of cataract occurred at a younger age in DM1 patients than in HC and DC. DM1 patients often visit a variety of specialists before being correctly diagnosed. Making the correct diagnosis of such patients is important for proper medical management and prevention of complications. However, clinical symptoms of DM1 are variable, and there are no established clinical diagnostic criteria. It is therefore difficult for non-neurologists to identify DM1, particularly in mild cases. A simple screen for DM1 would therefore be of great significance. In the present study, we developed and tested a simple screen for DM1.

One purpose of the pilot study was to compare physical examination and questionnaire methods of screening. We examined mild cases of DM1, because this is the population that will benefit most from a screening tool. The specificity of items on the physical examination was generally high, but the sensitivity was variable, even when specialists carried out the exam. This is likely because the mild cases lacked

Self-questionnaires for screening of myotonic dystrophy

Please check the appropriate boxes(☑) of following questions

1 . Can you lift up your head from supine position without arm support?

☐ Yes ☐ No

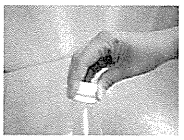
*Your head must take off from floor. You can place your arms beside your body however you must not push floor or grasp your clothes or body

2 . Can you sit up from supine position without arm support?

☐ Yes ☐ No

*You can place your arms beside your body however you must not push floor or grasp your clothes or body. You can stretch your legs.

3 . Can you unscrew caps of new plastic bottles using your fingers?



☐ Yes ☐ No

*You must use unopened plastic bottles. Please check "No" if you have to use any tools or body parts except your fingers.

4 . Do you feel delay of muscular relaxation after forced grasp of your finger, clench of your jaw, closure of your eyelids, and so on.?

☐ Yes ☐ No

This picture shows residual muscle contraction after forced grasp

5 . Do you have any patients of myopathies in your blood-related kins (brothers, sisters, parents, children, grandparents, grandchildren, uncle, aunts, cousins, etc.)?

☐ Yes ☐ No

* You can check "Yes" even if you do not know the accurate diagnosis. Your partners and blood-related kins of your partner are not included.

Thanks you for your cooperation

Figure 3 The final version of the self-report questionnaire to be used to screen for myotonic dystrophy type 1.

distinctive symptoms. The physical examination also required more time than the questionnaire, and required some tools.

The sensitivity and specificity of items on the questionnaire were also quite variable; however, the sensitivity and specificity of some items were equivalent to that of items in the physical examination. The short time and lack of equipment required to complete the questionnaire was a distinct advantage. We expected that a questionnaire would be able to identify patients with DM1 from the general population, and carried out a large study to determine the sensitivity and specificity of a questionnaire in a large group of participants.

To reduce the time and effort required of the participants, just nine items were included in the questionnaire used in the main study. In addition to DM1 patients and HC, we tested this questionnaire in DC to evaluate the capability of the questionnaire to distinguish DM1 from other disorders. In HC, the specificity of each of the eight items that were statistically analyzed was generally high. In DC, the specificity was poor, except for the item regarding cataract history; however, the specificity of four items exceeded 70% when only mild cases were considered. Overlapping symptoms appeared to influence the specificity of some items in some diseases. The sensitivity of each item was insufficient to identify DM1 by a single item. For all items except myotonia, the sensitivity was lower in mild DM1 patients than in all DM1 patients, likely because patients with mild disability were unaware of, or were free from, distinctive symptoms.

Receiver operating characteristic curve analyses showed that the combination of items 4 + 6 + 8 and items 4 + 5 + 6 + 8 had high detection capability for DM1. Both combinations showed an excellent capacity to detect DM1 from HC, and the AUC between mild DM1 and mild DC was over 0.9 for both combinations. The AUC between mild DM1 and HC, and between mild DM1 and mild DC was slightly higher for items 4 + 6 + 8 than for items 4 + 5 + 6 + 8, but one DM1 patient responded negative to all three items. In contrast, all DM1 patients had at least one positive item in the items 4 + 5 + 6 + 8 combination, and the sensitivity of at least two positive items was higher for items 4 + 5 + 6 + 8 than in items 4 + 6 + 8. Thus, we propose the use of the items 4 + 5 + 6 + 8 combination as a screen for DM1. In addition, we recommend including family history in the screen, because it would provide an opportunity to detect asymptomatic patients.

There were some instances where participants responded incorrectly to an item, either because they misunderstood the question or they were unaware of the presence of a symptom. To improve the quality of the questionnaire for future use, we have revised the text to use simple expressions and figures (Fig. 3). We hope that this screen will improve the diagnosis, and subsequent medical management, of DM1 patients. If an individual gives multiple positive answers, we recommend a consultation with a neurologist. In the case of only one positive answer, we recommend an examination of neurological findings, serum creatine kinase level and an electromyogram test. A detailed family history of muscular disorders and information about complications including juvenile cataracts, premature baldness and cardiac conduction block might be helpful. If any suspicious signs are detected, consultation with a neurologist should be considered. DM1 patients are high-risk for general anesthesia, and female DM1 patients are at high risk of abnormal pregnancy, congenital myotonic dystrophy and ritodrine-induced rhabdomyolysis.[1–5] Thus, if any operations requiring general anesthesia are considered, consultation with a neurologist is strongly recommended. If the female subject is expected to be married or pregnant, genetic counseling should be carried

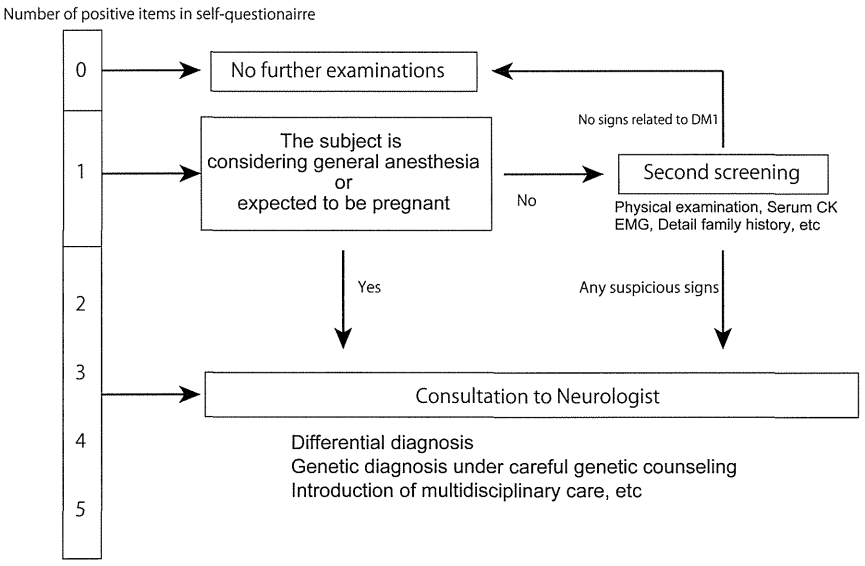


Figure 4 The flow chart of screening for patients with myotonic dystrophy type 1. CK, creatine kinase; DM1, myotonic dystrophy type 1; EMG, electromyogram.

out so that she understands the risk of her pregnancy and baby (Fig. 4).

The proposed screen should be used carefully in young children, elderly people and persons with disability. No children under the age of 10 years were included in the present study; therefore, we have no data for this population. The specificity of items was generally low in DC patients with severe symptoms; that is, patients requiring support for ADL. Thus, the capability of the screen to identify DM1 in these populations might be low.

In conclusion, a small, self-administered questionnaire can be a simple and efficient method to identify individuals with DM1. To determine the power of this tool, we aim to re-evaluate the sensitivity and specificity in clinical practice after dissemination to clinicians.

Acknowledgments

We thank all participants of this study. We also thank Dr Koichiro Takahi in the Department of Orthopedics at the National Hospital Organization Toneyama National Hospital for his contribution to the collection of data from patients with rheumatoid arthritis. This study was supported by a Research Grant for Intractable Disease from the Ministry of Health, Labor and Welfare to MPT, and an Intramural Research Grant for Neurological and Psychiatric Disorders of the National Center of Neurology and Psychiatry to MT and MPT. No authors have any conflict of interests in this study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Signs and symptoms of myotonic dystrophy type 1.

Table S2. Questionnaire administrated in the pilot study.

Table S3. Check-list for physical examination in the pilot study.

OPEN

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ORIGINAL ARTICLE

Clinical and genetic analysis of the first known Asian family with myotonic dystrophy type 2

Takahiro Nakayama[1], Harumasa Nakamura[2], Yasushi Oya[2], Takashi Kimura[3], Ichiro Imahuku[1], Kinji Ohno[4], Ichizo Nishino[5], Koji Abe[6] and Tohru Matsuura[6,7]

Myotonic dystrophy type 2 (DM2) is more common than DM1 in Europe and is considered a rare cause of myotonic dystrophies in Asia. Its clinical course is also milder with more phenotypic variability than DM1. We herein describe the first known Asian family (three affected siblings) with DM2 based on clinical and genetic analyses. Notably, two of the affected siblings were previously diagnosed with limb-girdle muscular dystrophy. Myotonia (the inability of the muscle to relax) was absent or only faintly present in these individuals. The third sibling had grip myotonia and is the first known Asian DM2 patient. The three DM2 siblings share several systemic characteristics, including late-onset, proximal-dominant muscle weakness, diabetes, cataracts and asthma. Repeat-primed PCR across the DM2 repeat revealed a characteristic ladder pattern of a CCTG expansion in all siblings. Southern blotting analysis identified the presence of 3400 repeats. Further DM2 studies in Asian populations are needed to define the clinical presentation of Asian DM2 and as yet unidentified phenotypic differences from Caucasian patients.
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Keywords: Asian; CCTG repeat; clinical spectrum; haplotype; limb-girdle muscular dystrophy; myotonia; myotonic dystrophy type 2 (DM2)

INTRODUCTION

Myotonic dystrophy (DM) is the most common adult-onset muscular dystrophy and is characterized by autosomal dominant progressive myopathy (muscle weakness), myotonia (the inability of the muscle to relax) and multiorgan involvement. Two genetically distinct forms of the disease with clinical similarities but distinct differences are known: DM type 1 (DM1) and type 2 (DM2). DM1 is caused by the expansion of a CTG repeat in the 3′-untranslated region of the dystrophia myotonica-protein kinase gene on chromosome 19p13.3,[1] while DM2 is caused by an expansion of a tetranucleotide CCTG repeat in the first intron of the cellular nucleic acid-binding protein (*CNBP*, formerly *ZNF9*) gene on chromosome 3q21.[2] The unprecedented number of expanded DM2 CCTG repeats (ranging from 75 to 11 000 with a mean of 5000) is highly unstable in intergenerational transmissions and varies in a tissue-specific, time-dependent manner.[2]

The clinical severity and spectrum of DM2 is highly variable, and this is not thought to be associated with the length of repeat expansions.[2,3] Most DM2 mutations have been identified in European Caucasians that originate from a single common founder and share an identical haplotype.[4–6] However, we previously identified the first Japanese DM2 patient carrying a haplotype distinct from that shared among Caucasians, indicating that DM2 exists in non-Caucasian populations and has separate founders.[7] Thus, it would be beneficial to investigate whether DM2 patients of different ethnicities and haplotypes have a comparable phenotype to the predominantly European patients with a common haplotype. To further characterize the variable clinical phenotype of DM2, we describe the clinical and molecular findings of the first known Asian family with DM2, including the previously reported Japanese female patient[7] and her two affected siblings.

SUBJECTS AND METHODS

The pedigree studied is shown in Figure 1. DNA was extracted from peripheral blood samples obtained from the three affected family members with their informed consent. Approval for the study was obtained from the ethics committees of Okayama University, Nagoya University Graduate School of Medicine and the National Center of Neurology and Psychiatry.

PCR products across the DM2 repeat (marker CL3N58)[2] in the first intron of *CNBP* were analyzed by capillary electrophoresis using an automated DNA sequencer (ABI 310A Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). The PCR reaction was performed in a 20 μl volume containing 100 ng genomic DNA as template, 1 × HotStarTaq *Plus* Master Mix (Qiagen, Valencia, CA, USA) and 0.2 μM each of the primers: 6FAM-fluorescent-labeled

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CL3N58-D F (5′-GCCTAGGGGACAAAGTGAGA-3′) and CL3N58-D R (5′-GGCCTTATAACCATGCAAATG-3′). The PCR conditions consisted of an initial denaturation at 95 °C for 5 min, then 30 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 30 s, with an additional extension at 72 °C for 10 min.

To detect the DM2 CCTG expansion, the repeat-primed PCR assay using an oligonucleotide primed within the DM2 CCTG repeat and Southern blotting analysis were performed as described elsewhere.[2,3,7,8] Briefly, repeat-primed PCR[8] was performed in a 20 μl volume containing 100 ng genomic DNA as template, 1 × HotStarTaq *Plus* Master Mix (Qiagen), 1 M betaine, 0.2 μM 6FAM-fluorescent-labeled CL3N58-D F (5′-GCCTAGGGGACAAAGTG AGA-3′), 0.05 μM reverse primer consisting of five CCTG repeats with an anchor tail: DM2-CCTG-for (5′-AGCGGATAACAATTTCACACAGGACCTGC CTGCCTGCCTGCCTG-3′) and 0.3 μM anchor primer corresponding to the anchor tail of the reverse primer: P3 (5′-AGCGGATAACAATTTCACACA GGA-3′). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, then 35 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C for 1 min, with an additional extension at 72 °C for 10 min. Fragment length analysis was performed on an ABI 310A Genetic Analyzer. Southern blotting was carried out with *Eco*RI-digested DNA (10 μg) separated on an 0.8% agarose gel, which was transferred to a positively charged nylon membranes (Roche, Indianapolis, IN, USA) and hybridized with a 474-bp *CNBP* probe.[2]

RESULTS

Genetic studies

Three patients with DM2 CCTG expansion were identified in this pedigree. Case 3 has been reported previously.[7] All cases showed one peak following PCR of the DM2 repeat and a characteristic ladder pattern by repeat-primed PCR,[3,7] confirming the presence of the CCTG expansion (Figure 2). DNA extracted from the Case 2 individual was degraded, so Southern blotting hybridization analysis was performed on the remaining two patients (Cases 1 and 3). This showed the presence of an 18.1 kb expanded DM2 allele, corresponding to 3400 CCTG repeats (Figure 3).

Case reports

Table 1 summarizes the clinical manifestations of three symptomatic siblings carrying the DM2 mutation. The siblings share common clinical features, including adult-onset proximal dominant progressive weakness, cataracts, diabetes, cardiac arrhythmias and hypercholesteremia, as described in the previous literature on DM2.[7] The degree of myotonia (the cardinal feature of DM) is variable, with two siblings having little or no myotonia. Interestingly, all three were asthmatic. The data of allergic and autoimmune tests are shown in Table 2. The other asymptomatic members of the pedigree were not assessed.

Case 1

A 69-year-old Japanese woman first presented at the age of 64 years with a slowly progressive difficulty in walking. Her calves had become

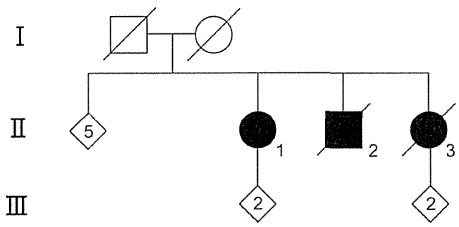

Figure 1 Pedigree of the sibling cases carrying the DM2 expansion. The parents did not suffer from muscle weakness and died at the ages of 67 and 72 years. The gender of the unaffected siblings and children of the affected cases is obscured to protect privacy.

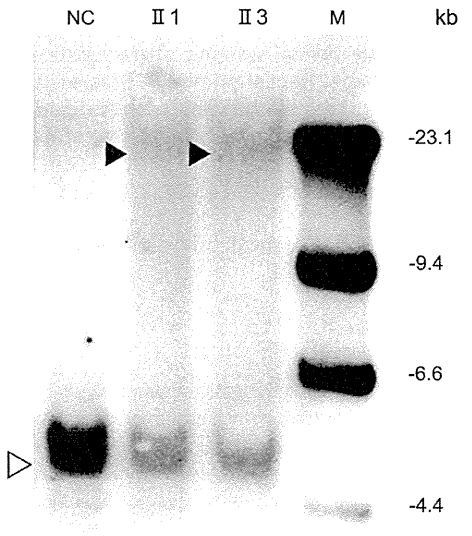

Figure 3 Southern blotting analysis of DM2. Closed arrowhead shows the expanded alleles in DM2. M, λDNA/*Hin*d III marker; NC, normal control; II1 and II3, Cases 1 and 3 showing an 18.1-kb expanded allele as well as a normal allele (open arrowhead).

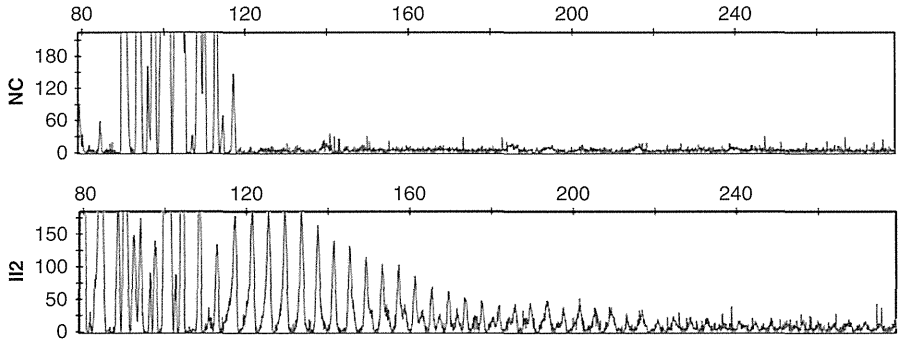

Figure 2 Repeat-primed PCR analysis specific for the DM2 expansion. Negative results from normal control (NC) are shown in the upper panel, whereas a characteristic continuous ladder from Case 2 (II2 in Figure 1), indicating the CCTG expansion, is detected in the lower panel. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Clinical features of DM2 patients in the Japanese pedigree

	DM2 individuals		
Characteristic	*Case 1*	*Case 2*	*Case 3*[7]
Age (years)/gender	69/F	61/M	59/F
Age at DM2 onset (years)	64	50	47
Skeletal muscle features			
Myotonia			
Grip/percussion	−/+	−/−	+/+
EMG diagnosis	−/+	−	+
Myalgia/stiffness	+	−/−	−/−
Muscle weakness/atrophy			
Facial muscles	+	−	+
Sternocleidomastoids	+	+	+
Limbs	Proximal dominant	Proximal dominant	Proximal dominant
Systemic features			
Cataracts, by history of extraction (age in years)	59	62	52
Diabetes, by history (age in years)	33	40	47
ECG	Normal	Normal	cRBBB
Holter monitoring	PVC	PVC	PVC
Other disorders	Asthma, HT, low IgG Hypercholesterolemia Hyperthyroidism	Asthma, HT Hypercholesterolemia	Asthma, low IgG Hypercholesterolemia
Initial clinical diagnosis	LGMD	LGMD	Myotonic dystrophy

Abbreviations: cRBBB, complete right bundle branch block; DM2, myotonic dystrophy type 2; EMG, electromyogram; ECG, electrocardiogram; F, female; HT, hypertension; IgG, immunoglobulin G; LGMD, limb-girdle muscular dystrophy; M, male; PVC, premature ventricular contraction.

Table 2 Profile of autoantibodies, IgE and eosinophils in this pedigree

	DM2 individuals		
	Case 1	*Case 2*	*Case 3*[7]
Antinuclear antiboby	Negative	Negative	Negative
Rheumatoid factor	Negative	Negative	Negative
Anti-thyroglobulin antibody	Positive	NE	Negative
TSH receptor antibody	Positive	NE	Negative
IgE (normal: $<250\,IU\,ml^{-1}$)	NE	1700	52.8
Eosinophils (normal: 0–5% of leukocytes)	0–25	10.4–13.9	3

Abbreviations: DM2, myotonic dystrophy type 2; IgE, immunoglobulin E; NE, not examined; TSH, thyroid stimulating hormone.

stiff over the last 2 or 3 years. There was no history of developmental milestone delay. She had a past medical history of taking asthma and hyperthyroidism medication. At 33 years of age, she had developed type 2 diabetes mellitus and hypertension. At 59 years, she had had both posterior subcapsular cataracts extracted. There was no complaint of myalgia. There was no known consanguinity or genetic admixture with other ethnicities in her family, as described elsewhere.[7] Neither of her parents, her three older brothers, two older sisters nor either of her two children had been referred with evident muscle symptoms. On examination, she was able to walk upstairs by holding onto a rail and walking on flat ground. She showed mild facial weakness as well as weakness and atrophy of sternocleidomastoid muscles. Motor examination showed slight symmetric muscle atrophy and predominant proximal weakness. Percussion myotonia could be slightly induced in the tongue and thenar eminence; however, grip myotonia was not present. Tendon reflexes were within the normal range. There were no cerebellar, sensory or autonomic disorders. Serum creatine kinase elevation was evident at $413\,IU\,l^{-1}$ (normal range: 37–$115\,IU\,l^{-1}$), and high hemoglobin A1c (7.6%) and total cholesterol levels ($248\,mg\,dl^{-1}$) were seen. The serum immunoglobulin G level was slightly decreased to $808\,mg\,dl^{-1}$ (normal range: 870–$1700\,mg\,dl^{-1}$). Holter monitoring detected premature ventricular contractions. Electromyography showed small motor unit potentials with occasional myotonic discharges in all the muscles examined. Nerve conduction studies were normal. Muscle computed tomography revealed diffuse muscle atrophy in the trunk and proximally in all limbs, whereas forearm and distal leg muscles were well preserved. Fluid-attenuated inversion recovery and T2-weighted brain magnetic resonance imaging showed non-specific periventricular white matter hyperintensities without significant cerebral atrophy.

Case 2

A 61-year-old man, the younger brother of case 1, began to notice difficulty lifting his knees when climbing mountains or stairs at the age of 50 years. He had had a normal birth and development. At the age of 55 years, he had started to hold onto something when rising from a supine or sitting position and to feel difficulty in lifting his right arm. At 40 years of age, he had developed type 2 diabetes mellitus and hypertension. At 62 years, he had a left posterior subcapsular cataract extracted. He had a past medical history of taking asthma medication. He was referred to our hospital by his primary physician because of an elevated creatine kinase level of

1094 IU l^{-1} (normal range: <195 IU l^{-1}). There was no complaint of muscle pain or stiffness.

Neurologically, the patient had normal language, speech and cognition on routine clinical evaluation. There were no cranial nerve abnormalities except for mild atrophy and weakness of the bilateral sternocleidomastoid muscles. Motor examination revealed predominant proximal muscle weakness and atrophy in all the limbs. In particular, atrophy of the right deltoid muscle and infraspinatus muscle and weakness of the right shoulder abduction and extortion were remarkable. There was no grip or percussion myotonia. Tendon reflexes were present but were hypoactive in both upper limbs. There were no cerebellar, sensory or autonomic disorders. There was slight serum creatine kinase elevation at 321 IU l^{-1} (normal range: 62–287 IU l^{-1}) and high hemoglobin A1c (7.2%) and total cholesterol levels (271 mg dl^{-1}). The serum immunoglobulin G level was 915 mg dl^{-1} (normal range: 870–1700 mg dl^{-1}). The electrocardiogram was normal, but 24-h Holter monitoring detected occasional premature ventricular contractions. Electromyography showed small motor unit potentials with early recruitment and complex repetitive discharges in all the muscles examined. No myotonic discharges were observed. Nerve conduction studies were normal. Muscle computed tomography revealed diffuse muscle atrophy in the trunk and proximally in all the limbs, whereas forearm and distal leg muscles were well preserved. The patient died from gastric cancer at the age of 66 years. Brain magnetic resonance imaging at the age of 65 years was normal without significant cerebral atrophy.

Case 3

The clinical presentation of Case 3 was previously described by Saito *et al.*[7] In addition, the patient had a past medical history of bronchial asthma, and hypercholesterolemia was detected (282 mg dl^{-1}) at the age of 59 years. The patient died suddenly at the age of 66 years of unknown causes.

DISCUSSION

This is the first known report of an Asian family with DM2. As expected for a dominantly inherited disorder, approximately 50% of first-degree relatives are at risk for DM2. However, given that clinical information is only available for three affected individuals among 13 members of a family of three consecutive generations, incomplete penetrance with asymptomatic carriers is the more favorable explanation of the pedigree. It could also reflect insufficient information about mild symptoms or signs in the past.

Notably, two of the affected members (Cases 1 and 2) had previously been diagnosed with limb-girdle muscular dystrophy because of the absence or subtlety of myotonia until their younger sister (Case 3) was genetically confirmed as the first Asian DM2 patient. Based on a report by Day *et al.*,[3] 90% of affected DM2 individuals from the European and American families have electrical myotonia and 75% have clinical myotonia. There is a possibility that Japanese/Asian DM2 individuals are underdiagnosed or misdiagnosed as another muscle disease because of the lack of myotonia[9] and the previous conception of the rarity of DM2 in Asian populations.[10] As seen in other repeat expansion disorders such as spinocerebellar ataxia type 2 (SCA2), Machado–Joseph disease/SCA3 and SCA10,[11–13] we also have to keep in mind the clinical differences between different ethnicities and geographical regions, which cannot be explained by the repeat size. A unique Japanese/Asian haplotype mutation[7] may also be related to low disease penetrance or the clinical presentation of mild DM2.

Multi-systemic features such as cataracts, diabetes, obesity, hypogammaglobulinemia, cardiac conduction block and arrhythmia are often seen in both DM1 and DM2 patients.[3,14] In addition, a strong association between Dutch DM2 patients and autoimmune diseases or autoantibody production was recently reported, in contrast with those with DM1 whose data were comparable with the general population.[15] Interestingly, all three patients in the present study were asthmatic, yet this association has not been previously described in the literature. To examine whether it is a true association or a mere coincidence, more families with different genetic backgrounds should be investigated. It is conceivable that the DM2 expansion directly affects the immune system or that the co-existence of a genetic change in flanking regions in linkage disequilibrium with the DM2 expansion confers susceptibility to allergic or autoimmune diseases.

It is well known that non-coding repeat expansion disorders, especially DM2, are associated with intra/inter-familial clinical variations and complex genotype–phenotype correlations.[2,3] Therefore, to achieve an improved diagnosis of DM2, clinicians should examine as many family members as possible in the same pedigree and be aware of its wide clinical spectrum, which is possibly influenced by racial and environmental factors. Although this study could not determine characteristic features in Asian DM2, further collection of additional Asian DM2 families is needed to investigate clinical and genetic differences between Caucasian and Asian patients with DM2. Comparison of the association between phenotype and ethnic/haplotype differences will be made possible in the future by the clinical and genetic assessment of DM2 subjects with different founders.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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短　報

舞踏運動を呈したdysferlin異常症の1例

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要旨：Dysferlin異常症は常染色体劣性遺伝形式をとり，三好型遠位型筋ジストロフィーや肢帯型筋ジストロフィー2B型を主な表現型とする．症例は53歳男性．31歳に筋力低下で発症し，37歳から不随意運動が徐々に進行した．左優位に近位筋力低下，病的反射，排尿障害，舞踏運動が認められた．Dysferlin遺伝子にc.2997G>T変異がホモ接合で認められた．舞踏運動をきたす他の疾患の合併は見出せなかった．Dysferlinの脳における働きやdysferlin異常症の中枢神経系の研究はほとんど行われていない．今後この分野の研究も必要と思われた．

(JMDD 2014; 24: 51–54)

緒　言

Dysferlin異常症は2番染色体に位置するdysferlin遺伝子の変異を原因とする．常染色体劣性遺伝形式をとり，三好型遠位型筋ジストロフィーや肢帯型筋ジストロフィー2B型を主な表現型とする．我々は筋ジストロフィーでありながら舞踏運動を呈したdysferlin異常症の1症例を経験し，2006年に報告[1)]した．今回，その後の研究報告の結果を考察に加え報告する．

症例提示

53歳男性．両親が血族婚．神経筋疾患や不随意運動の家族歴はない．31歳時に立ち上がる際の筋力低下に気付いた．37歳で肢帯型筋ジストロフィーと診断．同年妻が，症例の手が持続的に動いていることに気付く．筋力低下が徐々に進行，不随意運動は四肢に広がった．50歳時には本人も不随意運動が気になるようになった．49歳時から頻尿のためプロピベリン塩酸塩を服用していたが，1カ月の休薬で不随意運動は改善しなかった．

認知機能に問題なし．やや速めの話し方．著明な四肢近位優位の筋力低下と筋萎縮．肩の筋力が著明に低下し，頸，肘の屈曲，膝の伸展および足の底屈が次いで障害されていた．腸腰筋と大腿四頭筋は左がより弱かった．反射は上腕三頭筋と尺骨以外消失．伸展性足底反応でRossolimo反射は左が陽性．左優位に頸，肩と四肢に舞踏運動が見られた．計算や会話で増強した．

Key words：dysferlin，肢帯型筋ジストロフィー2B型，舞踏運動

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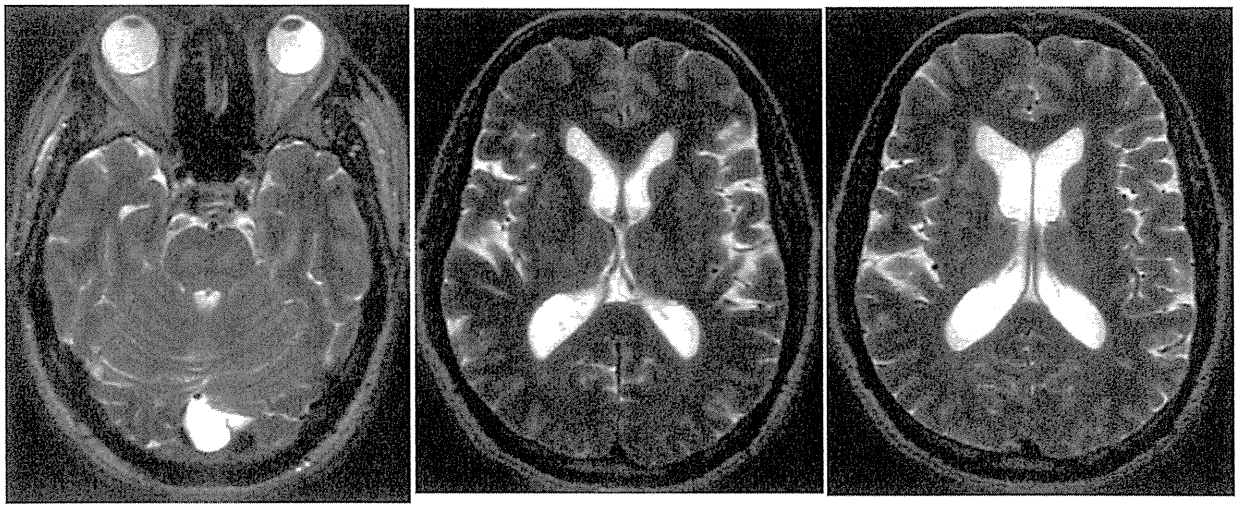

図1 症例の頭部MRIのT2強調像．橋，両視床，右尾状核頭近傍に高信号領域が認められた．くも膜嚢胞も小脳背側に見られた．（著作権の関係から文献1と異なる写真である．）

Mini-Mental State試験は28点，Wechsler成人知能検査改定版でIQ86．頭部MRIで軽度の所見が認められた（図1）．脊髄MRIは正常．CKが1,837 IU/l．髄液，末梢血像，赤沈，CRP，血清銅，セルロプラスミン，抗核抗体，抗リン脂質抗体，ASO，ASK，甲状腺ホルモンは正常．Dysferlin遺伝子にc.2997G>T変異がホモ接合で認められた．Huntington病，歯状核赤核淡蒼球ルイ体萎縮症の原因遺伝子は正常だった．

考 察

2006年の本症例報告後も，検索し得た範囲でdysferlin異常症が舞踏運動を呈した報告はない．我々は最近日本人40例の肢帯型筋ジストロフィー2B型の臨床的特徴を報告したが，その中にも舞踏運動を示したものは本例のみだった[2]．我々の検索で有棘赤血球舞踏病，Wilson病，全身性エリトマトーデス，甲状腺機能亢進症，Huntington病，歯状核赤核淡蒼球ルイ体萎縮症，プロピベリン塩酸塩の副作用，脳梗塞などは否定できる[1]ものと考えた．しかし舞踏運動を呈する疾患は他にも多数あり，今後も他の疾患の合併の徴候がないかは注意深く観察していく必要がある．

Dysferlin異常症に中枢神経合併症を呈した報告は，2006年以降2件見出すことができた．山中らは若年性脳梗塞をきたした39歳女性と脳梗塞とWillis動脈輪閉塞症を合併した42歳女性の姉妹例[3]を，和田らは認知症を併発した3同胞例のdysferlin異常症[4]を報告している．2006年時点では，dysferlin異常症の頭部MRI所見の報告は見出せなかった．上記の山中らの報告の39歳女性[3]と和田らの報告の発端者[4]では，合併症に見合う頭部MRI所見が報告されている．今回，自験例数例ではあるが，他のdysferlin異常症例の頭部MRIを見直してみたが，異常所見を見つけることはできなかった．なお，この他の頭部MRI所見の報告を見出すことはできなかった．

本症例報告の2006年，dysferlinは中枢神経系の錐体細胞や扁桃体の投射ニューロンの細胞質に局在する[5]との報告がみられた．しかし，dysferlinの脳における働きやdysferlin異常症の中枢神経系の研究はほとんど行われていない．今後この分野の研究も必要と思われた．

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Abstract

A case of dysferlinopathy presenting choreic movements

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Mutations in the dysferlin gene cause limb-girdle muscular dystrophy type 2B. We report the case of a 53-year-old man who had progressive muscle weakness and involuntary movements. He first noticed weakness at the age of 31. His wife first noticed involuntary movements when he was 37. The weakness and movements progressed slowly. Neurological examination revealed muscle weakness and atrophy in the limb-girdle, extensor plantar response, pollakisuria, and choreiform movements. The involuntary movements increased during mental tasks and speaking, and occured in the neck, the shoulders and all four limbs. A homozygous c.2997G>T mutation was identified in the dysferlin gene. The patient had no evidence of other causes of chorea. There have been few reports of the function of the isoform of the dysferlin gene, or of brain imaging or symptoms of the central nervous system in patients with dysferlinopathy. Further study is needed to determine the significance of the isoform of dysferlin in the brain and the involvement of the central nervous system in dysferlinopathy.

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Key words: dysferlin, limb-girdle muscular dystrophy type 2B, chorea

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

Nationwide patient registry for GNE myopathy in Japan

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Abstract

Background: GNE myopathy is a slowly progressive autosomal recessive myopathy caused by mutations in the *GNE* (glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase) gene. This study aimed to (1) develop a nationwide patient registry for GNE myopathy in order to facilitate the planning of clinical trials and recruitment of candidates, and (2) gain further insight into the disease for the purpose of improving therapy and care.

Methods: Medical records of genetically-confirmed patients with GNE myopathy at the National Center Hospital of the National Center of Neurology and Psychiatry (NCNP) were retrospectively reviewed in order to obtain data reflecting the severity and progression of the disease. We also referred to items in the datasheet of the nationwide registry of dystrophinopathy patients in the Registry of Muscular Dystrophies (Remudy). Items selected for the registration sheet included age, sex, age at onset, past history and complications, family history, body weight and height, pathological findings of muscle biopsy, grip power, walking ability, respiratory function, cardiac function, willingness to join upcoming clinical trials, and participation in patient associations. A copy of the original genetic analysis report was required of each patient.

Results: We successfully established the Remudy-GNE myopathy. Currently, 121 patients are registered nationwide, and 93 physicians from 73 hospitals collaborated to establish the registry. The mean age at onset was 27.7 ± 9.6 years, and 19.8% (24/121) of patients could walk without assistance. Mean presumed durations from onset to use of assistive devices (cane and/or braces) and a wheelchair, and loss of ambulation were 12.4, 15.2, and 21.1 years, respectively. Three patients had a past history and/or complication of idiopathic thrombocytopenia. To share the progress of this study with the community, newsletters were published on a regular basis, and included information regarding new phase I clinical trials for GNE myopathy. The newsletters also served as a medium to bring attention to the importance of respiratory evaluation and care for respiratory insufficiency.

Conclusion: The Japanese Remudy-GNE myopathy is useful for clarifying the natural history of the disease and recruiting patients with genetically-confirmed GNE myopathy for clinical trials.

Keywords: GNE myopathy, Distal myopathy with rimmed vacuoles (DMRV), Natural history, Remudy, Patient registry

Background

GNE myopathy, also known as distal myopathy with rimmed vacuoles (DMRV), Nonaka myopathy, or hereditary inclusion body myopathy (hIBM), is an early adult-onset myopathy with slow progression that preferentially affects the tibialis anterior muscles and commonly spares the quadriceps femoris muscles [1,2]. GNE myopathy is caused by mutations in the *GNE* gene encoding a bifunctional enzyme [uridine diphosphate-*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 supplementation prevents muscle atrophy and weakness in a mouse model of GNE myopathy [8]. While the incidence of GNE myopathy is unknown, more than 200 patients currently exist in Japan [9].

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Registries for rare diseases are broadly accepted for their usefulness in obtaining epidemiological data and patient recruitment for clinical trials [10] Translational Research in Europe–Assessment and Treatment of Neuromuscular Diseases (TREAT-MND ALLIANCE), a research network for neuromuscular disorders, developed a global database for patients with Duchenne muscular dystrophy (DMD) [11], spinal muscular atrophy, alpha-dystroglycanopathy with mutations in *FKRP*, and dysferlinopathy [12]. National registries for other muscular dystrophies and myopathies also exist. In 2009, we developed a national registry for neuromuscular diseases (Registry of MUscular DYstrophy; Remudy. http://www.remudy.jp/) in Japan in collaboration with the TREAT-MND ALLIANCE in order to aid in the recruitment of eligible patients for clinical trials, provide information regarding the natural history and epidemiology of diseases, and serve as a source of information on current clinical care [13]. Given that GNE myopathy is quite rare and the fact that clinical trials have already begun on this disease, the establishment of a patient registry is urgently needed, as it would allow for the early recruitment of patients in future clinical trials. Moreover, in addition to contributing to our knowledge on the natural history of GNE myopathy, accurate medical records also serve as a medium to judge clinical trial results. Remudy tentatively registered only male patients with dystrophinopathy. We intend to expand the registry to include patients with GNE myopathy.

Here, we describe the development of a national patient registry for GNE myopathy based on genetic diagnoses, analyze clinical and genetic characteristics of the disease, and provide etiological data important for clinical trials.

Methods

Institution, organization, registration method, data collection, and ethical approval

Remudy is supported by Intramural Research Grants (23-4/26-7) for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry (NCNP). Methodology used to establish the Remudy registry system was described previously [11,13,14]. Registry information was provided to interested individuals and their informed consent was obtained. Individuals whose data were included were informed that inclusion in the database confers no obligation to the patient, and that they may be removed from the registry immediately upon request. They were also told that refusal to participate would not affect the patient's subsequent medical care. This study was approved by 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.

Patients

Patients can join the registry via three routes: the Remudy homepage, attending specialists of neurology and myology, and patient associations (the Patient Association of Distal Myopathy, PADM; and the Japan Muscular Dystrophy Association, JMDA). This database includes mutation data confirmed by genetic analysis. Prior to launching the registry, members of SOCIETAS NEUOLOGICA JAPONICA (Japanese society of Neurology) were informed about the purpose of the registry, asked to inform their patients about the registry, and to cooperate when patients asked them to confirm medical information regarding the registry through leaflets.

Structure of the registry form

Based on our review of medical records and prospective natural history studies of GNE myopathy from the National Center Hospital of NCNP and questionnaires from previous studies [15-17], we concluded that walking ability and respiratory function might be important for evaluating disease status. Based on clinical information from patients with GNE and the basic form used in the registry for patients with dystrophinopathy in Remudy [13], we chose items required for registration.

Items in the registry form include past history, complications, family history, disease onset, ambulation status, results of muscle biopsy, and results of genetic analysis. Walking capability, grip power, cardiac and respiratory function, and serum creatinine kinase (CK) levels are also included in GNE myopathy natural history studies, given their relevance to the prognosis as well as their utility as outcome measures. A copy of the original report of the genetic analysis for *GNE* is required for registration, i.e., only patients with a diagnosis confirmed by a genetic report were included in the registry. Participants with only single heterozygous mutations in *GNE* were registered only when they had pathology results indicating the presence of rimmed vacuoles on muscle biopsy.

Data collection, curation, and accession

All patient data including clinical and genetic information were registered by patients. Each of the attending neurologists filled in information pertaining to past medical history, family history, and data from medical records (biopsy findings, laboratory and physiological data, and information regarding whether the patient had the capacity to understand the study objectives). The patients sent Case Report Forms, along with personal information (mailing address, phone number, and e-mail address), consent to use their information in clinical trials, participation consent for themselves and their attending physicians, and genetic diagnosis. As data were extracted from medical records, this study is a cross-sectional study for the purposes of the

present data, and a prospective study for the purposes of the annual data we are currently collecting. After the patient data were registered, medical and genetic curators cleaned up "tentative" data. Clinical curators are neurology specialists in myology at the NCNP, while genetical curator. is a neurologist and myological researcher responsible for genetic diagnosis of GNE myopathy at the NCNP. During the curation process, curators were able to ask the registrants and their attending neurologists to double-check the accuracy of information, with the agreement with both patients and their attending neurologists. As the Remudy-GNE registry utilizes a yearly renewable system to enable prospective data analysis, we asked registrants to renew their data at least once a year and with any change in physical status. All patient data provision is voluntary and data are not shared with any third party without the permission of the committee responsible for information disclosure. The structure of the Case Report Form and required registry items are shown in Table 1.

Table 1 Structure of the Case Report Form and registry items

Basic information	Date
	Hospital
	ID of hospital
	Name
	Date of birth
	Sex
	Address
	Phone
	E-mail
	Nationality
	Signed up for other registries?
	Attending any clinical trials?
Family history	Family history
	Consanguinity
Diagnosis	Muscle biopsy
	Genetic analysis* facility ( )
Patient status	Complications
	Body weight and height
	Age and symptoms at onset
	Walking capability and wheelchair use
	Grip power
	Respiratory function (VC,%VC, FVC,%FVC, mechanical support)
	Cardiac functions (EF, ES)
	Creatine kinase level

Gray cells are for patients to fill out, and white cells are to be filled out by physicians.
VC: vital capacity, FVC: forced vital capacity, EF: ejection fraction, FS: fraction shortening.

Medical record analysis

Medical records of all patients with genetically-confirmed GNE myopathy in the National Center Hospital of NCNP were retrospectively reviewed by M. MY.

Data analysis

Data were summarized using descriptive statistics, including mean, standard deviation (SD), median, range, frequency, and percentage. Each variable was compared using a t-test. Spearman's rank correlation coefficients were used to determine associations between variables. 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. All statistical analyses were performed using SPSS for Macintosh (Version 18; SPSS Inc., Chicago, IL).

Results

General characteristics at study entry

As of the end of October 2013, a total of 121 Japanese participants with GNE myopathy (55 men and 66 women) had registered (Table 2). Mean ages at data collection and disease onset were 44.9 ± 13.2 years (mean ± SD) (median, 43 years; range, 21–85 years) and 27.9 ± 9.6 years (median, 26 years; range, 12–61 years), respectively. The registry included participants from throughout Japan (38/47 prefectures) who were recruited through a collaboration with 92 attending physicians from 73 institutes (Figure 1). Among the 52 genetically-confirmed patients with GNE myopathy who had visited NCNP, 32 (62%) participated in the patient registry.

GNE mutations

Thirty-nine of 121 participants (32.3%) harbored a homozygous mutation in *GNE* and 64.5% (78/121) had a compound heterozygous mutation. Only single heterozygous mutations were found in four (3.3%) participants (Additional file 1: Table S1). Among participants with a homozygous mutation, 82% (32/39), 8% (3/39), and 5% (2/39) harbored p. V572L, p. C13S, and p. M712T mutations, respectively. Homozygous mutations of p. D176V and A630T were identified in only one participant.

Of those carrying two heterozygous mutations, 31% (24/78) had p. D176V/p. V572L mutations, while the remaining participants carried other combinations of mutations. The frequency of the p. V572L mutation was 46% (106/230), p. D176V was 25% (58/230), p. C13S was 4% (9/230), and each of p. M712T and p. A631V was 2% (4/230) (Additional file 2: Table S2). One patient with a single heterozygous mutation visited the NCNP Hospital, so we reviewed his medical records and noted that he was showing clinical symptoms of GNE myopathy as well as pathological features.

Table 2 Participant characteristics

		n	%	Mean ± SD	Minimum	Median	Maximum
Age		121	100	44.8 ± 13.0	21	43	85
Sex	Male	55	44				
	Female	66	55				
Age at onset		121	100	27.7 ± 9.6	15	26	61
Body mass index		121	100	21.4 ± 4.4	12.1	21	37.8
	Normal	65	54				
	Underweight	36	30				
	Obesity	20	16				
Walking capability	Ambulant without assistance	24	20				
	Ambulant with assistance	45	37				
	Non-ambulant	52	43				
Wheelchair use	Never	43	36				
	Part-time	28	23				
	Full time	50	41				
Respiratory function (%FVC)	Performed	79	65	88.4 ± 23.7	16.4	92.8	130
	Not performed	42	35				
	Normal (FVC ≥ 80%)	26	33*				
	Decreased	53	67*				
	Nocturnal NPPV	2	2				
Cardiac function	Performed	35	29				
(EF, ES)	Not performed	86	71				
	Normal	35	100*				
	Decreased	0	0*				
Grip power (kg)	Performed	96	79	5.5 ± 7.2	0	2.9	30
	Not performed	25	21				
	Grip power = 0	36	38*				
CK (Iu/L)	Performed	120	99	357.8 ± 209.0	11	209	459
	Not performed	1	1				

*ratio of examined/performed.

Family history

Thirty-nine of 121 participants (32.2%) had a family history of GNE myopathy. Eleven of 121 (9.1%) were from consanguineous parents. Among the 39 participants with homozygous mutations, 9 (23.1%) had consanguineous parents; 2 participants with a compound heterozygous mutation were from one family, as their mothers and fathers were siblings (i.e., these participants were double cousins).

Complications and past medical history

A detailed review of medical histories revealed that three participants had hypertension, two had diabetes mellitus, and two had hyperlipidemia. Two participants were diagnosed with obstructive sleep apnea syndrome, one of whom required continuous positive airway pressure. Atopic dermatitis and mastopathy were seen in one participant each. Of note, three participants had a past history of idiopathic thrombocytopenia (ITP). We obtained additional medical histories for three patients with histories of ITP. All three had experienced bleeding symptoms and had undergone intravenous and/or oral steroid therapy. Two of them were hospitalized for this therapy. Patients were unable to recall the platelet count or platelet-associated IgG (PAIgG). However, two patients presented with low platelet counts, one of whom was PAIgG-positive at the time of registration.

To clarify whether patients with GNE myopathy had thrombocytopenia, we reviewed blood counts of those with genetically-confirmed GNE myopathy. Among 52 patients with GNE myopathy in NCNP (including the three participants with a past history of ITP), mean platelet counts were 22.1 × 10^4/μl (normal range: 15–35 × 10^4/μl). Importantly, three patients, including two with a past history of ITP, had decreased platelet counts of 9.5, 10.3, and 7.1 × 10^4/μl, and

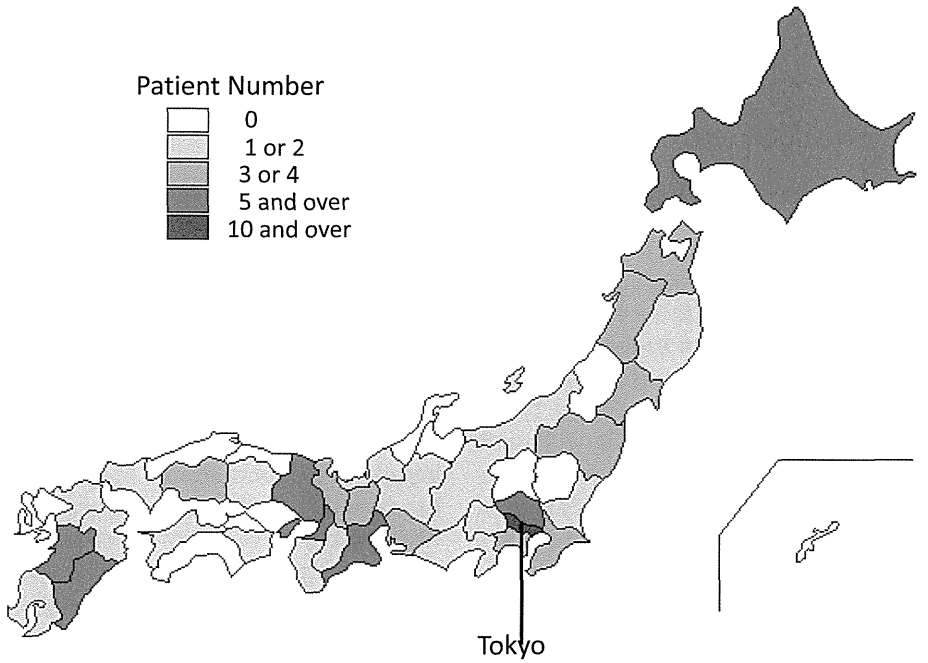

Figure 1 Participant distribution. Participants were distributed throughout Japan (38/47 prefectures), and 92 physicians in 73 institutes agreed to contribute to the registry.

carried *GNE* mutations of p. R420X/ p. V572L, 383insT/ p. V572L, and p. R8X/p. V572L.

Onset and ambulation status

Mean age at disease onset for the 121 registered participants was 27.7 ± 9.6 years (median, 27.5 years; interquartile range, 15–61). Initial symptoms were walking slowness and/or difficulty (65/121, 54%), stumbling (50/121, 41%), difficulty lifting toes (28/121, 23%), difficulty climbing stairs (12/121, 12%), difficulty running (9/121, 7%), difficulty lifting heels with a weakness of hands and/or fingers (5/121, 4%), and difficulty in thigh adduction and lifting the neck (2/121, 2%) (Figure 2). As weakness in the anterior parts is thought to be more prominent than that in the posterior calf in GNE myopathy, we reviewed medical records of the 62 patients who received treatment at NCNP hospitals and identified two patients for whom the first symptom was "difficulty lifting heels." Prominent calf weakness (MMT ankle dorsoflexion 5, plantar flexion 2) was evident in these patients, along with marked fat replacement in the calf muscles (Additional file 3: Figure S1).

Table 2 summarizes the clinical characteristics of participants included in the registry. A total of 20% (24/121) of participants were ambulant without assistance, 37% (45/121) required assistance (e.g., canes and/or braces), and 43% (52/121) had lost ambulation. Mean age at loss of ambulation was 35.4 ± 11.3 years. Kaplan–Meier analysis revealed a median time from disease onset to walking with assistance of 8.9 years (95%CI, 6.3-9.7), from disease onset to wheelchair use of 14.0 years (95%CI, 11.8-16.2), and from disease onset to loss of ambulation of 21.0 years (95%CI, 15.4-26.6) (Table 3).

Body Mass Index (BMI)

BMI of 65/121 (54%) participants were within the normal range in Japan (18.5-25) [13], whereas 36/121 (30%) were under the normal range and 20/121 (16%) were obese. Among the 20 obese participants, two were severely obese (>35%) by Japanese standards [18]. Mean BMI of

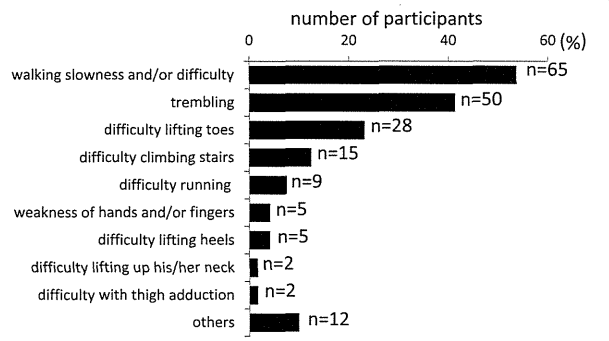

Figure 2 Initial symptoms. The most common initial symptom was walking slowness and/or difficulty (54%). Difficulty lifting toes (23%) due to foot drop was the third most common symptom, whereas certain populations had difficulty lifting heels (4%). Some participants had weakness in the hands and/or fingers (4%) and difficulty lifting the neck (2%) from the time of disease onset. The number of participants with the indicated symptoms are shown.

Table 3 Analysis of time from disease onset to walking with assistance, wheelchair use, and ambulation loss

									Incidence-free proportion	
	Mean	SD	95% CI		Median	Interquartile range	95% CI		10 years	20 years
Aassistive device use	12.4	1.3	10.0	14.9	8.0	5.0-13.0	6.3	9.7	0.38	0.18
Wheelchair use	15.2	0.8	13.6	16.9	14.0	8.0-22.0	11.8	16.2	0.64	0.29
Loss of ambulation	21.1	1.4	18.3	23.8	21.0	11.0-37.0	15.4	26.6	0.78	0.51

non-ambulant participants was higher than that of ambulant participants, although the difference was not significant (non-ambulant 22.0 ± 4.3 vs. ambulant 20.6 ± 4.6, $p = 0.077$). The number of participants who were underweight was greater than that of the normal population. Proportions of men and women who were underweight were 18.2% (n = 11; 16.4 ± 1.9; median, 17.2; range, 12.1-18.5) and 34.8% (n = 23; 16.9 ± 1.3; median, 17.1; range, 13.6-18.4), respectively, and were 4.7% and 9.1% among healthy men and women, respectively. There were fewer obese participants compared to the normal population (Figure 3) [18]. We identified no significant correlations between BMI and other items, with the exception of age ($r = 0.291$, $p = 0.001$).

Cardiopulmonary function

Information on pulmonary and cardiac function was available for 65% (79/121) and 34% (41/121) of participants, respectively. Of those examined, 33% (26/79) had respiratory dysfunction [% forced vital capacity (%FVC < 80)], and two were using nocturnal non-invasive positive pressure ventilation (NPPV).%FVC was significantly correlated with disease duration ($\rho = 0.479$, $p < 0.01$) and serum CK levels ($\rho = 0.573$, $p < 0.01$). None of the participants who underwent ultrasound cardiographic examination had cardiac dysfunction (ejection fraction, 50-82%; fraction shortening (FS), 25-50%). Mean serum CK level was 459.1 ± 355.0 IU/L (median, 202; range, 11–3133).

Bulletin, newsletter, and facilitation of participant recruitment through GNE myopathy registry

We have been publishing bulletins every three months and sending them to participants and doctors who join Remudy. The bulletin includes useful information regarding clinical care, translational medicine, and clinical trials, as well as articles introducing specialists and specialized hospitals for muscle diseases. These contents are also available on the Remudy homepage. Participant recruitment has also started for additional phase I clinical trials via the Remudy GNE myopathy registry homepage [19].

Discussion

To our knowledge, we describe the first patient registry for GNE myopathy in the world. This registry will contribute to the analysis of the natural history of GNE myopathy and aid in the recruitment of participants for clinical trials.

Participants with GNE myopathy were widely distributed throughout Japan, with 1.7 patients per hospital and 1.3 patients per physician in this study. In contrast, there were 5.8 patients with dystrophinopathy (60% of patients with DMD) per hospital and 3.6 per physician in the dystrophinopathy registry. Thus, while patients with GNE myopathy appeared to be dispersed throughout Japan, patients with dystrophinopathy were concentrated in specialized hospitals, given the need for cardiopulmonary care. This indicates that Remudy may serve a very important role in disseminating clinical information to patients with GNE myopathy and their doctors who are dispersed throughout Japan. The patient registry is also useful in that it allows for recruiting patients and resolving data deviation in comparison with analyses by isolated institutions. For example, the age at disease onset in the Remudy-GNE cohort was later than that determined from an analysis of medical records at the NCNP Hospital (26.8 ± 9.0 years). In our previous questionnaire-based study of core muscle disease center patients, we reported a median proportional duration from disease onset to walking with assistance, wheelchair use, and loss of ambulation of 7.0 ± 0.4 years, 11.5 ± 1.2 years, and 17.0 ± 2.1 years, respectively [14], which were all shorter than the durations determined in the present study. We speculate that this discrepancy may reflect the more advanced disease status of patients at neuromuscular disease-specialized center hospitals. Future improvement of Remudy-GNE registry may conclude why these bias were found in this study.

Three (2.5%) of 121 participants had a past history of ITP in our cohort. As the total number of patients with ITP is estimated to be 20,000 in Japan, with an annual occurrence of 3,000 [20], and the Japanese population was 1.27×10^8 in 2013, the prevalence of ITP is expected to be 15.7 per 100,000 (1.57×10^{-2}%). This means that the frequency of ITP among patients with GNE myopathy is 158 times higher than the general population, at least in our cohort.

UDP-GlcNAc 2-epimerase is a major determinant of cell surface sialylation in human hematopoietic cell lines and a critical regulator of the function of specific cell surface adhesion molecules [6]. Thus, alterations in platelets may occur in patients with GNE myopathy. For example, platelets from patients with ITP show increased electrophoretic mobility, reflecting increased sialic acid content [21]. Among the three participants with decreased platelet counts, two had ITP, raising the possibility of a causal