Downloaded from jnnp.bmj.com on July 14, 2014 - Published by group.bmj.com

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]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jnnp-2013-305587).

[1]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[2]Department of Clinical Development, Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, Japan
[3]Department of Neurophysiology, Tokyo Medical University, Tokyo, Japan
[4]Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan

Correspondence to
Professor Yukiko K Hayashi, Department of Neurophysiology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku, Tokyo 160-8402, Japan; yhayashi@tokyo-med.ac.jp

Received 4 June 2013
Revised 21 August 2013
Accepted 22 August 2013
Published Online First 11 September 2013

Linked

► http://dx.doi.org/10.1136/jnnp-2013-306414

CrossMark

To cite: Cho A, Hayashi YK, Monma K, *et al. J Neurol Neurosurg Psychiatry* 2014;**85**:912–915.

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

Downloaded from jnnp.bmj.com on July 14, 2014 - Published by group.bmj.com

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

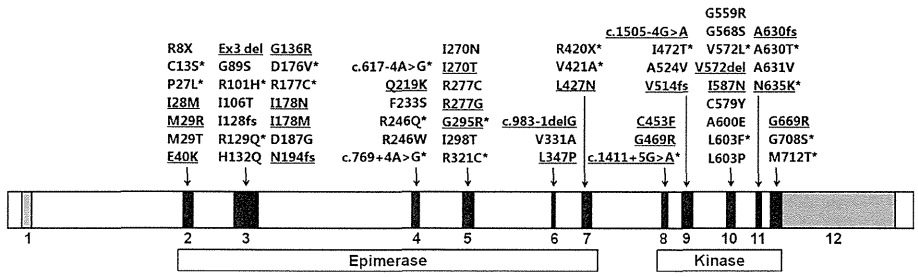

Figure 1 Mutation spectrum of *GNE* in the Japanese population. The mutations are located throughout the whole open reading frame. Twenty-five novel mutations are underlined, and 21 shared mutations are indicated with asterisks.

Downloaded from jnnp.bmj.com on July 14, 2014 - Published by group.bmj.com

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.

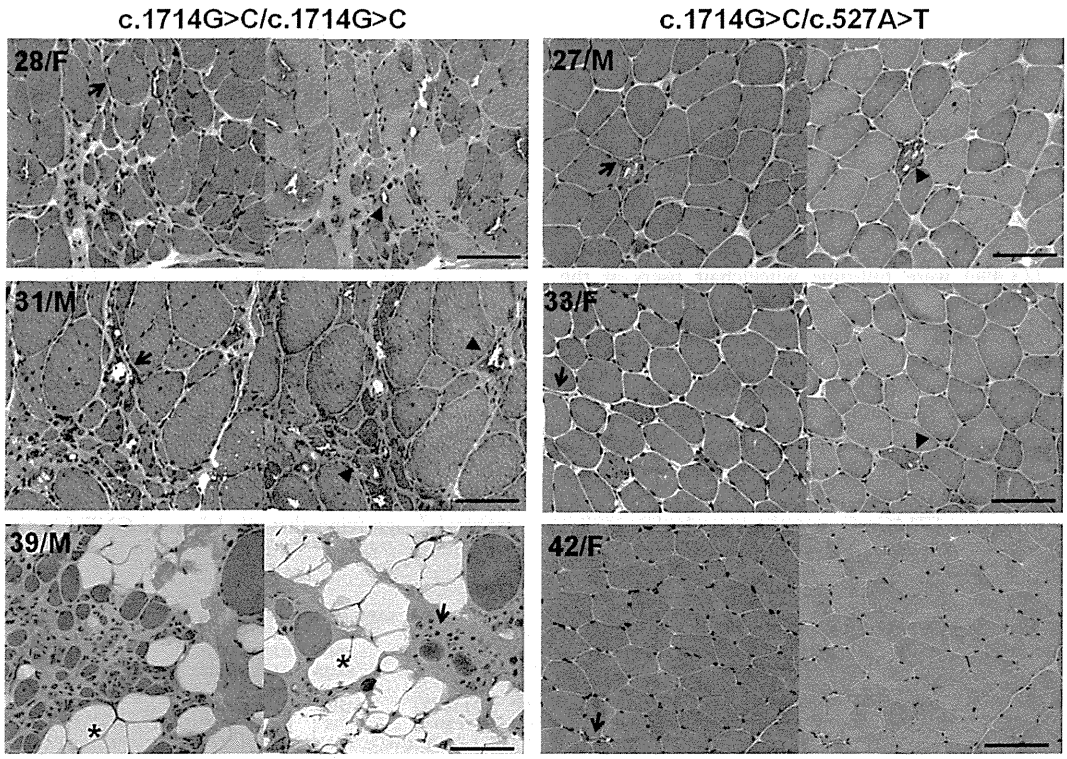

Figure 2 Comparison of muscle pathology between patients with homozygous c.1714G>C (p.Val572Leu) and with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Homozygous c.1714G>C (p.Val572Leu) mutations have led to much more advanced histopathological changes compared with 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.

Downloaded from jnnp.bmj.com on July 14, 2014 - Published by group.bmj.com

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.

Acknowledgements The authors thank Kanako Goto and Yuriko Kure for their invaluable technical support and assistant in genetic analysis.

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.

Funding This study was supported partly by Intramural Research Grant 23-4, 23-5, 22-5 for Neurological and Psychiatric Disorders of NCNP; partly by Research on Intractable Diseases, Comprehensive Research on Disability Health and Welfare, and Applying Health Technology from the Ministry of Health Labour and Welfare; and partly by JSPS KAKENHI Grant Number of 23390236.

Competing interests None.

Ethics approval This study was approved by the ethics committee of National Center of Neurology and Psychiatry.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Nonaka I, Sunohara N, Ishiura S, *et al*. Familial distal myopathy with rimmed vacuole and lamellar (myeloid) body formation. *J Neurol Sci* 1981;51:141–55.
2 Argov Z, Yarom R. "Rimmed vacuole myopathy" sparing the quadriceps. A unique disorder in Iranian Jews. *J Neurol Sci* 1984;64:33–43.
3 Askanas V, Engel WK. New advances in the understanding of sporadic inclusion-body myositis and hereditary inclusion-body myopathies. *Curr Opin Rheumatol* 1995;7:486–96.
4 Nonaka I, Noguchi S, Nishino I. Distal myopathy with rimmed vacuoles and hereditary inclusion body myopathy. *Curr Neurol Neurosci Rep* 2005;5:61–5.
5 Nishino I, Malicdan MC, Murayama K, *et al*. Molecular pathomechanism of distal myopathy with rimmed vacuoles. *Acta Myol* 2005;24:80–3.
6 Eisenberg I, Avidan N, Potikha T, *et al*. The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. *Nat Genet* 2001;29:83–7.
7 Nishino I, Noguchi S, Murayama K, *et al*. Distal myopathy with rimmed vacuoles is allelic to hereditary inclusion body myopathy. *Neurology* 2002;59:1689–93.
8 Keppler OT, Hinderlich S, Langner J, *et al*. UDP-GlcNAc 2-epimerase: a regulator of cell surface sialylation. *Science* 1999;284:1372–6.
9 Eisenberg I, Grabov-Nardini G, Hochner H, *et al*. Mutations spectrum of *GNE* in hereditary inclusion body myopathy sparing the quadriceps. *Hum Mutat* 2003;21:99.
10 Noguchi S, Keira Y, Murayama K, *et al*. Reduction of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase activity and sialylation in distal myopathy with rimmed vacuoles. *J Biol Chem* 2004;279:11402–7.
11 Malicdan MC, Noguchi S, Nonaka I, *et al*. A Gne knockout mouse expressing human *GNE* D176V mutation develops features similar to distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy. *Hum Mol Genet* 2007;16:2669–82.
12 Malicdan MC, Noguchi S, Hayashi YK, *et al*. Prophylactic treatment with sialic acid metabolites precludes the development of the myopathic phenotype in the DMRV-hIBM mouse model. *Nat Med* 2009;15:690–5.
13 Mori-Yoshimura M, Monma K, Suzuki N, *et al*. Heterozygous UDP-GlcNAc 2-epimerase and N-acetylmannosamine kinase domain mutations in the *GNE* gene result in a less severe GNE myopathy phenotype compared to homozygous N-acetylmannosamine kinase domain mutations. *J Neurol Sci* 2012;318:100–5.
14 Schwarzkopf M, Knobeloch KP, Rohde E, *et al*. Sialylation is essential for early development in mice. *Proc Natl Acad Sci USA* 2002;99:5267–70.
15 Ikeda-Sakai Y, Manabe Y, Fujii D, *et al*. Novel Mutations of the *GNE* gene in distal myopathy with rimmed vacuoles presenting with very slow progression. *Case Rep Neurol* 2012;4:120–5.
16 Li H, Chen Q, Liu F, *et al*. Clinical and molecular genetic analysis in Chinese patients with distal myopathy with rimmed vacuoles. *J Hum Genet* 2011;56:335–8.
17 Liewluck T, Pho-lam T, Limwongse C, *et al*. Mutation analysis of the *GNE* gene in distal myopathy with rimmed vacuoles (DMRV) patients in Thailand. *Muscle Nerve* 2006;34:775–8.
18 Kim BJ, Ki CS, Kim JW, *et al*. Mutation analysis of the *GNE* gene in Korean patients with distal myopathy with rimmed vacuoles. *J Hum Genet* 2006;51:137–40.
19 Broccolini A, Ricci E, Cassandrini D, *et al*. Novel *GNE* mutations in Italian families with autosomal recessive hereditary inclusion-body myopathy. *Hum Mutat* 2004;23:632.
20 Shi Z, Hayashi YK, Mitsuhashi S, *et al*. Characterization of the Asian myopathy patients with VCP mutations. *Eur J Neurol* 2012;19:501–9.

Downloaded from jnnp.bmj.com on July 14, 2014 - Published by group.bmj.com

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

Anna Cho, Yukiko K Hayashi, Kazunari Monma, et al.

J Neurol Neurosurg Psychiatry 2014 85: 914-917 originally published online September 11, 2013
doi: 10.1136/jnnp-2013-305587

Updated information and services can be found at:
http://jnnp.bmj.com/content/85/8/914.full.html

These include:

Data Supplement *"Supplementary Data"*
http://jnnp.bmj.com/content/suppl/2013/09/11/jnnp-2013-305587.DC1.html

References This article cites 20 articles, 4 of which can be accessed free at:
http://jnnp.bmj.com/content/85/8/914.full.html#ref-list-1

Email alerting service Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections Articles on similar topics can be found in the following collections

Muscle disease (226 articles)
Musculoskeletal syndromes (478 articles)
Neuromuscular disease (1151 articles)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

A nationwide survey on Marinesco-Sjögren syndrome in Japan

Masahide Goto[1,2], Mari Okada[3], Hirofumi Komaki[1], Kenji Sugai[1], Masayuki Sasaki[1], Satoru Noguchi[3,4], Ikuya Nonaka[3], Ichizo Nishino[3,4] and Yukiko K Hayashi[3,4,5*]

Abstract

Background: Marinesco-Sjögren syndrome (MSS) is an autosomal recessive multisystem disorder characterized by the tetralogy of cerebellar ataxia, congenital cataracts, intellectual disability, and progressive muscle weakness due to myopathy. MSS is extremely rare, and its clinical, pathological, and genetic features are not yet fully understood.

Methods: We conducted a nationwide, questionnaire-based survey on MSS in Japan and carefully reviewed the medical records of 36 patients suspected of having this disease. In addition, pathological examinations of muscles, sequence and haplotype analysis in *SIL1* were performed.

Results: The patients had been examined between the ages of 2 and 52 years. Delayed psychomotor development and cataracts from early childhood were observed in all patients, whereas no life-threatening events were observed. Mutations in *SIL1* were identified in 24 of the 27 patients tested, and 43 of the 48 chromosomes possessed the *SIL1* c.936dupG (p.Leu313fs) mutation. The haplotype analysis revealed that 31 of the 32 chromosomes (96.9%) with the c.936dupG mutation had the same haplotype.

Conclusions: The results of haplotype analysis suggested the presence of a founder effect. The clinical features of patients without *SIL1* mutations were indistinguishable from those with *SIL1* mutations, suggesting the genetic heterogeneity of MSS.

Keywords: Marinesco-Sjögren syndrome (MSS), *SIL1*, Founder effect, Cataracts, Intellectual disability, Ataxia, Rimmed vacuolar myopathy

Background

Marinesco-Sjögren syndrome (MSS; OMIM 248800) is an autosomal recessive multisystem disorder clinically characterized by the tetralogy of cerebellar ataxia, congenital cataracts, intellectual disability, and progressive muscle weakness due to myopathy [1-3]. Additional clinical features, including short stature, hypergonadotropic hypogonadism [4], and strabismus [5], are also observed. Mutations in *SIL1* (Gene ID: 64374) were reported to be causative for MSS [6,7]. This gene encodes SIL1, also known as BiP-associated protein (BAP), which is an endoplasmic reticulum (ER)-resident protein. Bip is an HSP70 chaperone family member located in the ER, and plays a key role in protein quality control. SIL1 regulates the ATPase cycle of BiP for proper protein folding [8,9]. SIL1-deficient woozy mutant mice exhibit progressive ataxia caused by the loss of Purkinje cells via ER stress [10].

MSS is an extremely rare disease, and very few cases have been reported. In this study, we performed a nationwide, questionnaire-based survey on MSS with the aim of characterizing its prevalence, clinical features, natural history, muscle pathological findings, and mutation status.

Methods

All clinical materials used in this study were obtained for diagnostic purposes with written informed consent. All surveys and experiments performed in this study were approved by the Ethical Committee of the National Center of Neurology and Psychiatry.

* Correspondence: yhayashi@tokyo-med.ac.jp
[3]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[4]Department of Clinical Development, Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, Japan
Full list of author information is available at the end of the article

BioMed Central

© 2014 Goto et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

The nationwide, questionnaire-based survey

To elucidate the clinical characteristics of MSS, we conducted a nationwide, questionnaire-based survey in Japan. The first set of questionnaires, which focused on the experience of treating patients suspected of having MSS, was sent to a total of 5,452 Japanese specialists for neurology or pediatric neurology. The second set of questionnaires, which focused on the clinical information of patients with suspected MSS, was sent to their attending physicians. These patients' medical records were carefully reviewed by 2 of our specialists (M.G., H.K.).

Histochemistry

Biopsied muscle specimens were flash-frozen in isopentane cooled in liquid nitrogen. Transverse serial frozen sections of 10 μm in thickness were subjected to various types of histochemical staining, including hematoxylin and eosin (H&E), modified Gomori-trichrome (mGT), and ATPases. We obtained biopsied skeletal muscles from a total of 17 unrelated patients clinically suspected of having MSS.

Sequence analysis of *SIL1*

Genomic DNA was extracted from either frozen muscle or peripheral blood lymphocytes using standard protocols. The PCR primers were designed to amplify all the exons of *SIL1* together with their flanking intronic regions. The primer sequences are available upon request. Direct sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing system and an ABI3100 automated Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequence data obtained were analyzed using the SeqScape (Applied Biosystems) program and compared with the genomic sequence of *SIL1* in the database (NM_022464). Genetic analysis was performed on DNA from 27 unrelated patients and the parents of 3 of them. Two hundred control chromosomes from healthy individuals were examined for each novel mutation in *SIL1* by direct sequencing.

Haplotype analysis of *SIL1*

For the haplotype analysis of the *SIL1* genomic region, we performed direct sequencing of the following 11 common single nucleotide variants (SNVs) in the Japanese population: rs11748097, rs929775, rs10045761, rs1433008, rs1 1958050, rs7717375, rs7722413, rs3763016, rs6596456, rs700629, and rs12653845 (http://www.ncbi.nlm.nih.gov/SNP/). Samples from 21 patients homozygous for the common c.936dupG mutation, the parents of 3 patients, and 92 control Japanese individuals were analyzed.

Results

Patients

A total of 1,875 responses (34.4% response rate) were received to the first set of questionnaires. The second set of questionnaires was sent to a total of 37 attending physicians (2.0%) who had treated patients suspected of having MSS. The detailed clinical records of a total of 36 patients were carefully reviewed. The shortfall in the number of patients was due to an overlap of 1 case.

Sequence analysis of *SIL1*

Frozen muscle or peripheral blood lymphocytes were obtained from 27 of the 36 patients suspected of having MSS, for genetic analysis. Mutations in *SIL1* were identified in 24 out of the 27 patients. Twenty-one patients were homozygous for the previously reported c.936dupG (p.Leu313fs) mutation in exon 9. Patient 4 was homozygous for the previously reported c.603_607del5 (p.Glu201fs) mutation in exon 6 [11]. Patient 12 was homozygous for the previously reported c.331C > T (p.Arg111X) mutation in exon 4 [6,7]. Patient 17 was a compound heterozygote for the novel c.617_618TC > AA (p.Leu206Glu) mutation in exon 6 and the c.936dupG (p.Leu313fs) mutation in exon 9 [12]. All mutations except for p.Leu206Glu are predicted to induce premature termination. Mutation of Leu206, which is highly conserved among species, was predicted to exert a deleterious impact on protein function by the Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/) and the Sorting Intolerant From Tolerant software (SIFT: http://sift.bii.a-star.edu.sg/). None of these nucleic acid changes were found in the 200 chromosomes from healthy Japanese controls or in the Japanese Single Nucleotide Polymorphisms database (http://snp.ims.u-tokyo.ac.jp/index_ja.html).

Haplotype analysis of *SIL1*

The *SIL1* c.936dupG mutation was identified in 43 of the 48 chromosomes (89.6%) in our cohort. The patients carrying this mutation were reported from different areas in Japan. To determine whether this was a result of a founder effect, we performed a haplotype analysis using 11 SNVs within or close to *SIL1*. The results revealed that 31 of the 32 chromosomes (96.9%) with the c.936dupG mutation had the same haplotype (P1-P24, Table 1). This haplotype was only found in 18 of the 184 chromosomes (9.8%) from the control group, suggesting a founder effect, although 1 chromosome from Patient 1 had a different haplotype.

Clinical features

Table 2 and Additional file 1: Table S1 show a clinical summary of the patients in our series.

The age at which the examination was performed in the 24 patients (10 men and 14 women) with *SIL1* mutations varied from 2 to 52 (mean = 20.1 ± 18.1) years. Bilateral cataracts requiring prompt surgical intervention had appeared and rapidly progressed in all 24 patients at the mean age of 3.5 ± 1.2 years. Strabismus was also

Table 1 Haplotype analysis

rs No	rs11748097	rs929775	rs10045761	*/#	rs1433008	rs11958050	rs7717375	@	rs7722413	rs3763016	rs6596456	rs700629	rs12653845
JPT	A: 0.24	G: 0.18	A: 0.15		C: 0.15	A: 0.76	A: 0.82		C: 0.11	G: 0.11	A: 0.13	A: 0.14	G: 0.11
P1	C/A	T/G	G	N	T	A/G	A	H	T/C	C/G	A	A	T
P2	C	T	G	N	T	A	A	H	T	C	A	A	T
P3	C	T	G	N	T	A	A	H	T	C	A	A	T
P5	C	T	G	N	T	A	A	H	T	C	A	A	T
P6	C	T	G	N	T	A	A	H	T	C	A	A	T
P7	C	T	G	N	T	A	A	H	T	C	A	A	T
P9	C	T	G	N	T	A	A	H	T	C	A	A	T
P10	C	T	G	N	T	A	A	H	T	C	A	A	T
P13	C	T	G	N	T	A	A	H	T	C	A	A	T
P14	C	T	G	N	T	A	A	H	T	C	A	A	T
P15	C	T	G	N	T	A	A	H	T	C	A	A	T
P16	C	T	G	N	T	A	A	H	T	C	A	A	T
P18	C	T	G	N	T	A	A	H	T	C	A	A	T
P20	C	T	G	N	T	A	A	H	T	C	A	A	T
P21	C	T	G	N	T	A	A	H	T	C	A	A	T
P24	C	T	G	N	T	A	A	H	T	C	A	A	T
P12	C	T	G	*	T	A	A	N	T	C	A	A	T
P4	C	T	G	#	T	A	A	N	T	C	G	C	T
P25	C/A	T/G	G/A	N	T/C	A/G	A/G	N	T	C	G	C	T/G
P26	C/A	T/G	G/A	N	T/C	A/G	A/G	N	T	C	G	C	T/G
P27	C	T	G	N	T	A	A	N	T	C	G	C	T

@: c.936dupG, *: c.331C > T, #: c.603_607del, JPT: Japanese frequency.
N: Normal, H: Homozygous.

Table 2 Clinical summary of patients

SIL1 mutations			Positive (n = 24)	Negative (n = 3)	Not examined (n = 9)
Ocular involvements		Cataracts	24/24 (100%), 2y-6y	3/3 (100%)	9/9 (100%)
		Strabismus	10/18 (56%)	1/3 (33%)	5/7 (71%)
		Muscle weakness	21/22 (95%), 2y-52y	3/3 (100%)	9/9 (100%)
Motor functions	Development	Head control	21/21 (100%), 4 m-18 m	5 m-8 m	4 m-7 m
		Sit	20/20 (100%), 10 m-36 m	12 m-18 m	12 m-36 m
		Stand with support	16/20 (80%), 1y-4y	15 m, 24 m	15 m-6y
		Walk with support	16/20 (80%), 2y-22y	2/2 (100%) 15 m, 24 m	3/3 (100%) 15 m-6y
		Loss of ambulation	5/16, 13y-28y		
Cerebellar signs		Hypotonia	21/24 (88%)	3/3 (100%)	9/9 (100%)
		Ataxia	16/24 (67%), 2y-52y	2/3 (100%)	6/8 (75%)
		Nystagmus	11/24 (46%), 2y-45y	0/3	5/8 (63%)
		Dysarthria	8/24 (33%), 2y-48y	2/3 (67%)	4/9 (44%)
	Psychomotor delay		20/22 (91%), IQ(DQ):24-100	3/3 (100%)	9/9 (100%)
	Hypogonadism		3/8 (38%)	0/1	2/3 (67%)
Skeletal abnormalities		Short stature	12/18 (67%),	1/3 (33%)	3/8 (38%)
		Spinal deformities	8/22 (36%)	1/3 (33%)	3/7 (43%)
		Flat foot	7/22 (32%)	0/3	1/7 (14%)
		Short fingers	5/22 (23%)	0/3	2/8 (25%)
Others		Serum CK (IU/L)	28-2000	144-3010	95-600
		Cerebellar atrophy	19/19 (100%)	3/3 (100%)	9/9(100%)
		Rimmed vacuoles in muscles	16/16 (100%)	0/1	2/6 (33%)

observed in 55.6% (10/18) of the patients. Cerebellar signs included hypotonia (21/24; 88%), ataxia (16/24; 67%), nystagmus (11/24; 46%), and dysarthria (8/24; 33%) were seen. Brain MRI demonstrated marked atrophy of the cerebellum, particularly the vermis, in all the patients examined (19/19). Mild to moderate intellectual disability, diagnosed by intelligence quotient/developmental quotient between 35 and 70, was seen in 91% (20/22) of the patients. Acquisition of meaningful words occurred at the age of 2.0 ± 0.8 years, and most of the patients had required special-needs education. Muscle weakness was observed in 95% (21/22) of the patients, with delays in motor milestones. Head control was first seen in all the patients at a certain time point between 4 and 18 (mean = 7.8 ± 3.7) months, and sitting at a certain time point between 10 and 36 (mean = 20.0 ± 8.7) months. Eighty percent (16/20) of the patients could stand with support at a certain point between the ages of 1 and 4 years, and walk with support at a certain point between the ages of 2 and 22 (mean = 5.8 ± 2.6) years; however, none of the patients acquired the ability to walk independently. Muscle weakness was slowly progressive and predominantly in the proximal muscles, with the patients becoming wheelchair-bound at a certain time point between the ages of 13 and 28 (mean = 17.4 ± 6.3) years. Serum creatine kinase levels were normal to moderately elevated (28–2000, mean = 389 ± 464; normal < 200 IU/L). Short stature (< −2 SD) was seen in 67% (12/18, mean = −3.6 SD) of the patients, and spinal deformity (8/22; 36%), flat foot (7/22; 32%), and short fingers (5/22; 23%) were also reported. Hypogonadotropic hypogonadism was seen in 3 of 8 (38%) patients (1 with microtestis, 2 with amenorrhea). No marked clinical differences were observed among patients with different *SIL1* mutations. No patient had cardiac and respiratory problems.

The 3 patients with no *SIL1* mutation (Patients 25, 26, and 27) and the 9 genetically unexamined patients showed clinical features indistinguishable from the patients with *SIL1* mutations, including cerebellar signs with cerebellar atrophy on brain images, intellectual disability, congenital cataracts, and muscle weakness. Elevation of serum CK levels was also seen in 2 patients (Table 2).

Pathological findings of skeletal muscles

Biopsied skeletal muscles were obtained from 16 patients with *SIL1* mutations and one patient without (Patient 27). All muscle specimens showed myopathic changes of variation in fiber size and endomysial fibrosis. A few necrotic and regenerating fibers were seen in some patients with *SIL1* mutations. No neurogenic changes, including fiber type grouping and grouped atrophy, were observed in any of the patients. Importantly, scattered rimmed vacuoles (RVs) were seen in all 16 patients with *SIL1* mutations, but not in the patient without (Figure 1).

Discussion

We conducted a nationwide, questionnaire-based survey to clarify the prevalence, clinical and pathological characteristics, and long-term course of MSS in Japanese patients. The total number of patients with MSS was only 36.

From a clinical point of view, it is important to carry out careful ophthalmological examination of MSS patients at a young age if visual acuity is to be preserved, as the cataracts characteristic of MSS usually appear abruptly and develop rapidly from an early age [13,14]. Indeed, all of the patients in our present series required early and prompt surgical intervention. Marked cerebellar atrophy on brain MRI is another characteristic of this disease, however cerebellar ataxia can be difficult to identify, especially in younger patients with muscle weakness. Skeletal muscle weakness is also a prominent characteristic. Almost all the MSS patients with *SIL1* mutations in this series had muscle weakness initially noticed as a delayed motor milestone, which was detected at an earlier age than cataracts, as reported previously [3,15,16]. Regarding muscle biopsy, myopathic changes, including RV formation are a characteristic of patients with *SIL1* mutations. RVs are not disease-specific, and are often seen in adult-onset chronic myopathies such as inclusion body myositis,

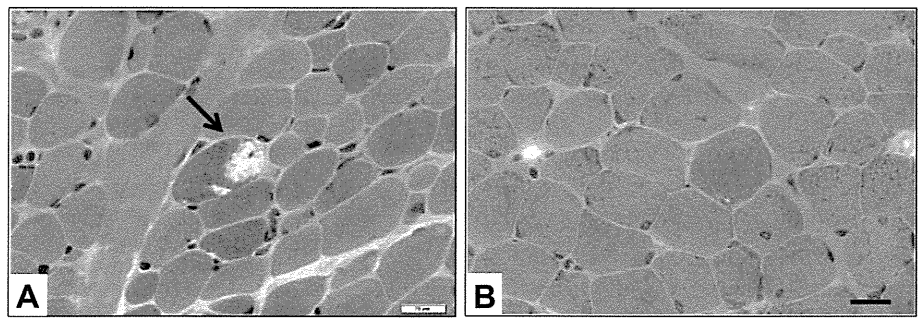

Figure 1 Modified gomori trichrome stain of the biopsied skeletal muscles. A muscle from a MSS patient with *SIL1* mutation shows rimmed vacuoles (arrow, **A**), whereas no vacuole is seen in a patient without *SIL1* mutation **(B)**. Bar = 20 μm.

distal myopathy with RVs, oculopharyngeal muscular dystrophy, and myofibrillar myopathies. They are rarely observed, however, in childhood-onset myopathies. The presence of RVs in muscle biopsy tissue can be helpful in formulating an early diagnosis of MSS, allowing the ophthalmologist to perform surgery for cataracts to prevent total visual loss. The life prognosis of MSS appears to be comparatively good, as respiratory, cardiac, and swallowing functions are well preserved, even in the patients who are over 50 years of age.

Most of the reported *SIL1* gene mutations have been predicted to induce premature termination and loss of function of SIL1. Based on a putative model of SIL1-BiP interaction, the C-terminal 5 amino acids of SIL1 are thought to play a key role in its association with BiP [17]. This concept is further strengthened by the fact that the p.Arg111X, p.Glu201fs, and p.Leu313fs mutations cause the generation of SIL1 proteins lacking the C-terminal region. Complete loss of function due to nonsense-mediated mRNA decay should also be considered. On the other hand, Leu206 in exon 6 is well preserved among species, and the novel nonsynonymous mutation p.Leu206Glu is predicted to exert a deleterious impact on protein function by both SIFT and PolyPhen2. The c.936dupG (p.Leu313fs) mutation in *SIL1*, which was first reported from Japan [12], is highly common in Japanese MSS patients. Haplotype analysis revealed that whereas 96.9% of chromosomes from MSS patients possessing the c.936dupG mutation had the same haplotype, less than 10% of the chromosomes of the controls did so, suggesting a founder effect.

The results of this study also strongly suggest the genetic heterogeneity of MSS. Three of the 27 patients (11.1%) had no *SIL1* mutation, but demonstrated the cardinal features of MSS, including congenital cataracts, ataxia, intellectual disability, and myopathy. We could not exclude the possibility of the mutation occurred in the promoter or other non-coding region of *SIL1* in these 3 patients. Previous reports also showed that approximately one-half of the MSS patients were genetically diagnosed as MSS from mutations in *SIL1* [7,16]. The absence of RVs in the muscle biopsy tissue of one patient with no *SIL1* mutation suggests the existence of a different disease mechanism(s) in such patients. Further analysis is required to identify the other causative genes for MSS.

Conclusions

MSS is an extremely rare disease, but a possible founder effect was present in Japan. The life prognosis of MSS is comparatively good, and early diagnosis is important for prevention of a total visual loss. Other causative genes for MSS can cause indistinguishable clinical features via different disease mechanisms.

Additional file

Additional file 1: Table S1. Clinical findings of each patient with or without *SIL1* mutations.

Competing interests

The authors declare that they have no competing of interest.

Authors' contributions

MG had full access to all the data in the study and wrote the manuscript; MO performed the mutation analysis; HK participated in analyzing all the clinical data; KS, MS, SN, I Nonaka, and I Nishino were involved in data interpretation and also supervised manuscript preparation. YKH supervised all aspects of the study, including study design, data interpretation, and manuscript preparation. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank Kanako Goto and Maki Takami for their invaluable technical support and assistance with the genetic analysis. The authors also thank Jeremy Williams (MA, Professor), Dr. Helena Popiel (MS, PhD, Lecturer), and Dr. Edward Barroga (DVM, PhD, Associate Professor) of the Department of International Medical Communications of Tokyo Medical University for editing and reviewing the manuscript.

Funding

This study was supported partly by grants for Research on Intractable Diseases, Comprehensive Research on Disability Health and Welfare, and Applying Health Technology, from the Ministry of Health, Labour and Welfare, Japan; partly by Intramural Research Grants 23–4, 23–5, 22–5 for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry; and partly by JSPS KAKENHI Grant Numbers 23390236 and 24390227.

Author details

[1]Department of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan. [2]Department of Pediatrics, Hitachiomiya Saiseikai Hospital, Ibaraki, Japan. [3]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan. [4]Department of Clinical Development, Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, Japan. [5]Department of Neurophysiology, Tokyo Medical University, Tokyo, Japan.

Received: 12 December 2013 Accepted: 14 April 2014
Published: 23 April 2014

References

1. Sjogren T: **Hereditary congenital spinocerebellar ataxia accompanied by congenital cataract and oligophrenia; a genetic and clinical investigation.** *Confin Neurol* 1950, **10**:293–308.
2. Herva R, von Wendt L, von Wendt G, Saukkonen AL, Leisti J, Dubowitz V: **A syndrome with juvenile cataract, cerebellar atrophy, mental retardation and myopathy.** *Neuropediatrics* 1987, **18**:164–169.
3. Superneau DW, Wertelecki W, Zellweger H, Bastian F: **Myopathy in marinesco-sjogren syndrome.** *Eur Neurol* 1987, **26**:8–16.
4. Skre H, Berg K: **Linkage studies on marinesco-sjogren syndrome and hypergonadotropic hypogonadism.** *Clin Genet* 1977, **11**:57–66.
5. Dotti MT, Bardelli AM, De Stefano N, Federico A, Malandrini A, Vanni M, Guazzi GC: **Optic atrophy in marinesco-sjogren syndrome: an additional ocular feature: report of three cases in two families.** *Ophthalmic Paediatr Genet* 1993, **14**:5–7.
6. Anttonen AK, Mahjneh I, Hamalainen RH, Lagier-Tourenne C, Kopra O, Waris L, Anttonen M, Joensuu T, Kalimo H, Paetau A, Tranebjaerg L, Chaigne D, Koenig M, Eeg-Olofsson O, Udd B, Somer M, Somer H, Lehesjoki AE: **The gene disrupted in marinesco-sjogren syndrome encodes SIL1, an HSPA5 cochaperone.** *Nat Genet* 2005, **37**:1309–1311.
7. Senderek J, Krieger M, Stendel C, Bergmann C, Moser M, Breitbach-Faller N, Rudnik-Schöneborn S, Blaschek A, Wolf NI, Harting I, North K, Smith J, Muntoni F, Brockington M, Quijano-Roy S, Renault F, Herrmann R, Hendershot LM, Schröder JM, Lochmüller H, Topaloglu H, Voit T, Weis J, Ebinger F, Zerres K:

Mutations in SIL1 cause marinesco-sjogren syndrome, a cerebellar ataxia with cataract and myopathy. *Nat Genet* 2005, **37**:1312–1314.
8. Zoghbi HY: **SILencing misbehaving proteins.** *Nat Genet* 2005, **37**:1302–1303.
9. Shomura Y, Dragovic Z, Chang HC, Tzvetkov N, Young JC, Brodsky JL, Guerriero V, Hartl FU, Bracher A: **Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange.** *Mol Cell* 2005, **17**:367–379.
10. Zhao L, Longo-Guess C, Harris BS, Lee JW, Ackerman SL: **Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP.** *Nat Genet* 2005, **37**:974–979.
11. Takahata T, Yamada K, Yamada Y, Ono S, Kinoshita A, Matsuzaka T, Yoshiura K, Kitaoka T: **Novel mutations in the SIL1 gene in a Japanese pedigree with the marinesco-sjögren syndrome.** *J Hum Genet* 2010, **55**:142–146.
12. Eriguchi M, Mizuta H, Kurohara K, Fujitake J, Kuroda Y: **Identification of a new homozygous frameshift insertion mutation in the SIL1 gene in 3 Japanese patients with marinesco-sjogren syndrome.** *J Neurol Sci* 2008, **270**:197–200.
13. Ishikawa T, Kitoh H, Awaya A, Nonaka I: **Rapid cataract formation in marinesco-sjogren syndrome.** *Pediatr Neurol* 1993, **9**:407–408.
14. Shimizu T, Matsuishi T, Yamashita Y, Koga Y, Ohtaki E, Kato H, Goto Y, Nonaka I: **Marinesco-sjogren syndrome: can the diagnosis be made prior to cataract formation?** *Muscle Nerve* 1997, **20**:909–910.
15. Horvers M, Anttonen AK, Lehesjoki AE, Morava E, Wortmann S, Vermeer S, van de Warrenburg BP, Willemsen MA: **Marinesco-sjogren syndrome due to SIL1 mutations with a comment on the clinical phenotype.** *Eur J Paediatr Neurol* 2013, **17**:199–203.
16. Krieger M, Roos A, Stendel C, Claeys KG, Sonmez FM, Baudis M, Bauer P, Bornemann A, de Goede C, Dufke A, Finkel RS, Goebel HH, Häussler M, Kingston H, Kirschner J, Medne L, Muschke P, Rivier F, Rudnik-Schöneborn S, Spengler S, Inzana F, Stanzial F, Benedicenti F, Synofzik M, Lia Taratuto A, Pirra L, Tay SK, Topaloglu H, Uyanik G, *et al*: **SIL1 mutations and clinical spectrum in patients with marinesco-sjogren syndrome.** *Brain* 2013, **136**:3634–3644.
17. Howes J, Shimizu Y, Feige MJ, Hendershot LM: **C-terminal mutations destabilize SIL1/BAP and can cause marinesco-sjogren syndrome.** *J Biol Chem* 2012, **287**:8552–8560.

doi:10.1186/1750-1172-9-58
Cite this article as: Goto *et al.*: A nationwide survey on Marinesco-Sjögren syndrome in Japan. *Orphanet Journal of Rare Diseases* 2014 **9**:58.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

BioMed Central

Environ Health Prev Med (2014) 19:452–458
DOI 10.1007/s12199-014-0405-7

SHORT COMMUNICATION

Three novel serum biomarkers, miR-1, miR-133a, and miR-206 for Limb-girdle muscular dystrophy, Facioscapulohumeral muscular dystrophy, and Becker muscular dystrophy

Yasunari Matsuzaka · Soichiro Kishi · Yoshitsugu Aoki · Hirofumi Komaki · Yasushi Oya · Shin-ichi Takeda · Kazuo Hashido

Received: 15 April 2014 / Accepted: 12 August 2014 / Published online: 24 August 2014
© The Japanese Society for Hygiene 2014

Abstract
Objectives Muscular dystrophies are a clinically and genetically heterogeneous group of inherited myogenic disorders. In clinical tests for these diseases, creatine kinase (CK) is generally used as diagnostic blood-based biomarker. However, because CK levels can be altered by various other factors, such as vigorous exercise, etc., false positive is observed. Therefore, three microRNAs (miRNAs), miR-1, miR-133a, and miR-206, were previously reported as alternative biomarkers for duchenne muscular dystrophy (DMD). However, no alternative biomarkers have been established for the other muscular dystrophies.
Methods We, therefore, evaluated whether these miR-1, miR-133a, and miR-206 can be used as powerful biomarkers using the serum from muscular dystrophy patients including DMD, myotonic dystrophy 1 (DM1), limb-girdle muscular dystrophy (LGMD), facioscapulohumeral muscular dystrophy (FSHD), becker muscular dystrophy (BMD), and distal myopathy with rimmed vacuoles (DMRV) by qualitative polymerase chain reaction (PCR) amplification assay.
Results Statistical analysis indicated that all these miRNA levels in serum represented no significant differences between all muscle disorders examined in this study and controls by Bonferroni correction. However, some of these indicated significant differences without correction for testing multiple diseases ($P < 0.05$). The median values of miR-1 levels in the serum of patients with LGMD, FSHD, and BMD were approximately 5.5, 3.3 and 1.7 compared to that in controls, 0.68, respectively. Similarly, those of miR-133a and miR-206 levels in the serum of BMD patients were about 2.5 and 2.1 compared to those in controls, 1.03 and 1.32, respectively.
Conclusions Taken together, our data demonstrate that levels of miR-1, miR-133a, and miR-206 in serum of BMD and miR-1 in sera of LGMD and FSHD patients showed no significant differences compared with those of controls by Bonferroni correction. However, the results might need increase in sample sizes to evaluate these three miRNAs as variable biomarkers.

Y. Matsuzaka, S. Kishi contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s12199-014-0405-7) contains supplementary material, which is available to authorized users.

Y. Matsuzaka · S. Kishi · K. Hashido (✉)
Administrative Section of Radiation Protection, National Institute of Neuroscience, Tokyo, Japan
e-mail: hashido@ncnp.go.jp

Present Address:
S. Kishi
Department of Pathology, Institute for Developmental Research, Kasugai, Aichi 480-0392, Japan

Y. Aoki · S. Takeda
Department of Molecular Therapy, National Institute of Neuroscience, Tokyo, Japan

Present Address:
Y. Aoki
Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK

H. Komaki
Department of Child Neurology, National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, Japan

Y. Oya
Department of Neurology, National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, Japan

Keywords microRNAs · Biomarker · Limb-girdle muscular dystrophy · Facioscapulohumeral muscular dystrophy · Becker muscular dystrophy

Introduction

miRNAs are approximately 19–23 nucleotides' long single-stranded non-coding RNAs, and the function is post-transcriptional regulation of target messenger RNAs (mRNAs) [1] dysregulation of miRNAs expression in skeletal muscle and myocardium is associated with muscle disorders [2, 3]. Interestingly, despite the high RNase activity within the circulating blood, a high concentration of these remarkably stable miRNAs has been found in various body fluids, including serum and plasma, as microvesicle-encapsulated [4] or RNA-binding protein-associated forms [5].

Muscular dystrophies are classified in accordance with their clinical and pathological features [6, 7]. Among them, DMD (OMIM310200) and BMD (OMIM 300376) are caused by various mutations in the *dystrophin* gene on the X chromosome, at Xp21.2 [8], and exhibit estimated prevalences of approximately one per 3,500 in DMD and 3–6 per 100,000 in BMD [9]. DM1 (OMIM160900), also known as Steinert's disease, represents an estimated prevalence of 5.5 per 100,000 Japanese [10], and is caused by expansion of a CTG repeat in the 3′ UTR of the DMPK (dystrophia myotonica-protein kinase) gene [11]. LGMD is caused by a total of twenty-two autosomal dominant or recessive causative gene mutations [12], and has an incidence of about one per 20,000 individuals. FSHD (OMIM158900) is caused by a loss of the D4Z4 microsatellite locus on chromosome 4 [13] with a prevalence of approximately one per 20,000 Japanese [6]. DMRV (OMIM605820), also called Nonaka myopathy, hereditary inclusion body myopathy (hiBM), and quadriceps sparing myopathy, is an autosomal recessive vacuolar myopathy of the distal muscles of the tibialis anterior, caused by mutations in the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) gene [14]. The prevalence of DMRV is approximately 300–400 patients within the Japanese population.

Serum CK values are commonly used as clinical blood-based biomarkers for these muscular dystrophies. However, there are some problems in using serum CK values for diagnostic evaluation of these disorders, i.e. CK levels are increased by vigorous exercise [15], decreased renal function due to aging, gender-dependent differences in skeletal muscle mass, pregnancy, and alcohol intake, and does not parallel motor ability in DMD [16, 17]. We, therefore, previously reported that three miRNAs, miR-1, miR-133a, and miR-206, in the serum were used as novel biomarkers in the dystrophin-deficient muscular dystrophy mouse models, as well as the canine X-linked muscular dystrophy in Japan dog (CXMDj) [18]. Furthermore, another group reported that an increase in miR-1, miR-133a, and miR-206 levels in the serum of DMD children patients was correlated with motor ability [17]. In other muscle diseases, however, stable and valuable biomarkers as an alternative to CK have not been established to date.

In this study, we measured and evaluated these three miRNAs, miR-1, miR-133a, and miR-206, in serum as possible stable and powerful biomarkers for DM1, LGMD, LGMAD2B, FSHD, BMD, and DMRV.

Materials and methods

Patients

A total of forty-eight unrelated Japanese patients with DMD, DM1, LGMD, LGMD2B, FSHD, BMD, and DMRV and each of the five age-matched controls were enrolled in this study (Table 1). DMD patients were divided into two groups by age (average ages and age ranges were 10.2 and 28.7 years of age, 5–18 and 27–31 years of age, respectively). LGMD patients were divided into two groups by whether they contained LGMD2B or not. Informed consent was obtained from the cases and controls by explaining the details of this study prior to collection of peripheral blood. The Research Ethics Committee for National Institute of Neuroscience, National Center of Neurology and Psychiatry approved the present study and all participants provided written informed consent.

Animals

All animals used in this study were housed in the National Center of Neurology and Psychiatry and treated in accordance with the guidelines provided by the Ethics Committee for the Treatment of Laboratory Animals of National Institute of Neuroscience, or the Ethics Committee for the Treatment of Laboratory Middle-sized Animals of National Institute of Neuroscience, which has adopted the three fundamental principles of replacement, reduction, and refinement.

RNA extraction and quantification of miRNA

Total RNA was extracted from 50 μl of serum using the mirVana miRNA isolation kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol and 50 μl of RNA eluate. Five μl of the RNA elute was reverse transcribed

Table 1 Muscle dystrophy patients

Disease	Sample no.	Average of age ± SD (y.a.)	Sex (male/female)	Sporadic/Familial	Average of onset ± SD (y.a.)	Serum CK (U/ml)
DMD	5	10.4 ± 4.2	5/0	3/2	3.8 ± 1.1	9,101 ± 6,282
DMD	7	28.7 ± 1.5	7/0	7/0	3.8 ± 0.7	220 ± 126
DM1	8	59.4 ± 17.4	5/3	4/4	18.4 ± 16.9	121 ± 66
LGMD	7	50.0 ± 19.8	5/2	5/2	15.5 ± 13.9	633 ± 661
LGMAD2B	4	51.0 ± 18.2	3/1	4/0	23.0 ± 5.7	2,713 ± 2,722
FSHD	8	52.3 ± 17.3	4/4	4/4	14.4 ± 10.8	162 ± 155
BMD	4	52.0 ± 12.9	4/0	4/0	14 ± 14.6	687 ± 562
DMRV	5	39.2 ± 7.3	0/4	4/1	22.2 ± 6.2	206 ± 177

y.a years of age

using the TaqMan miRNA Reverse Transcription kit (ABI, Foster City, CA, USA) and miRNA-specific stem-loop primers (part of TaqMan miRNA assay kit: Applied Biosystems) as previously reported [18]. For exosome and exosome-depleted supernatant, 5-fold diluted solutions of the RNA elute were used with distilled water. The expression levels of miRNA were quantified by real-time PCR using individual miRNA-specific primers (part of TaqMan miRNA assay kit: Applied Biosystems) with 7900HT Fast Real-Time PCR System (Applied Biosystems) according to the manufacture's protocol. Each samples were performed real-time PCR as triplicade. Each miRNA expression was represented relative to the expression of miR-16 used as an internal control. Data analysis was performed by SDS 2.1 real-time PCR data analysis software (Applied Biosystems). Expression data were given as median values obtained from three samples in conjunction with standard deviation. Statistical comparisons were performed by Mann–Whitney *U* test. Bonferroni correction was used to resolve a problem of multiple testing.

Creatine kinase activity

Serum creatine kinase (CK) levels were measured with the Fuji Dri-chem system (Fuji Film Medical Co. Ltd, Tokyo, Japan) according to the manufacture's protocol. Ten ml of serum was deposited on a Fuji Dri-chem slide and incubated at 37 °C. The increase in absorbance by the generated dye was measured for 5 min at 540 nm spectrophotometrically, and the activity was calculated according to the installed formula. Data were expressed as units per liter (U/l).

Exosome purification

Serum was harvested from the peripheral blood of DMD patients in tubes by centrifugation at 3,000×*g* for 15 min. Isolation of exosome from serum was performed by Exo Quick Exosome Precipitation Solution (System Biosciences, CA) according to the protocol provided by the manufacturer. Briefly, 63 µl of the Exo Quick Exosome Precipitation Solution was added to 250 µl of serum. The mixture was vortexed for 15 s and then incubated at 4 °C for 30 min. After centrifugation at 1,500×*g* for 30 min at room temperature, the supernatant was discarded. Again, the centrifugation and aspiration were repeated. The pellet including exosomes was resuspended in 1× phosphate-buffered saline (PBS).

CTX-induced skeletal muscle regeneration of mice

C57Bl/10SnSlc mice were obtained from Clea Japan Inc., and used at 7–8 weeks age. Hair from the bilateral hind limbs of diethyl ether-anesthetized animals was removed with a depilatory cream before the induction of injury. The tibialis anterior (TA) muscle of mice was injured by injection of 100 µl of PBS or cardiotoxin (CTX, 10 µM) (*Naja mossambica mossambica*, Sigma-Aldrich), a snake venom that selectively injures myofibers by disturbing calcium homeostasis at the neuromuscular junctions, followed by necrosis of muscle fibers [19]. The concentration of cardiotoxin ensures minimal damage to satellite cells and also to the nerves and blood vessels of the original muscles [20]. After 1, 3, and 5 days, whole body blood was collected from the abdominal aorta under anesthesia, and allowed to stand for about 30 min at room temperature before centrifugation at 1,200×*g* for 10 min at room temperature. The supernatant was used as serum to isolate miRNAs.

Results

miRNA levels in the serum of patients of various muscle diseases

To assess the validity of miRNAs as alternative serum biomarkers to CK for various muscle diseases, we analyzed the expression levels of three miRNAs, miR-1, miR-133a,

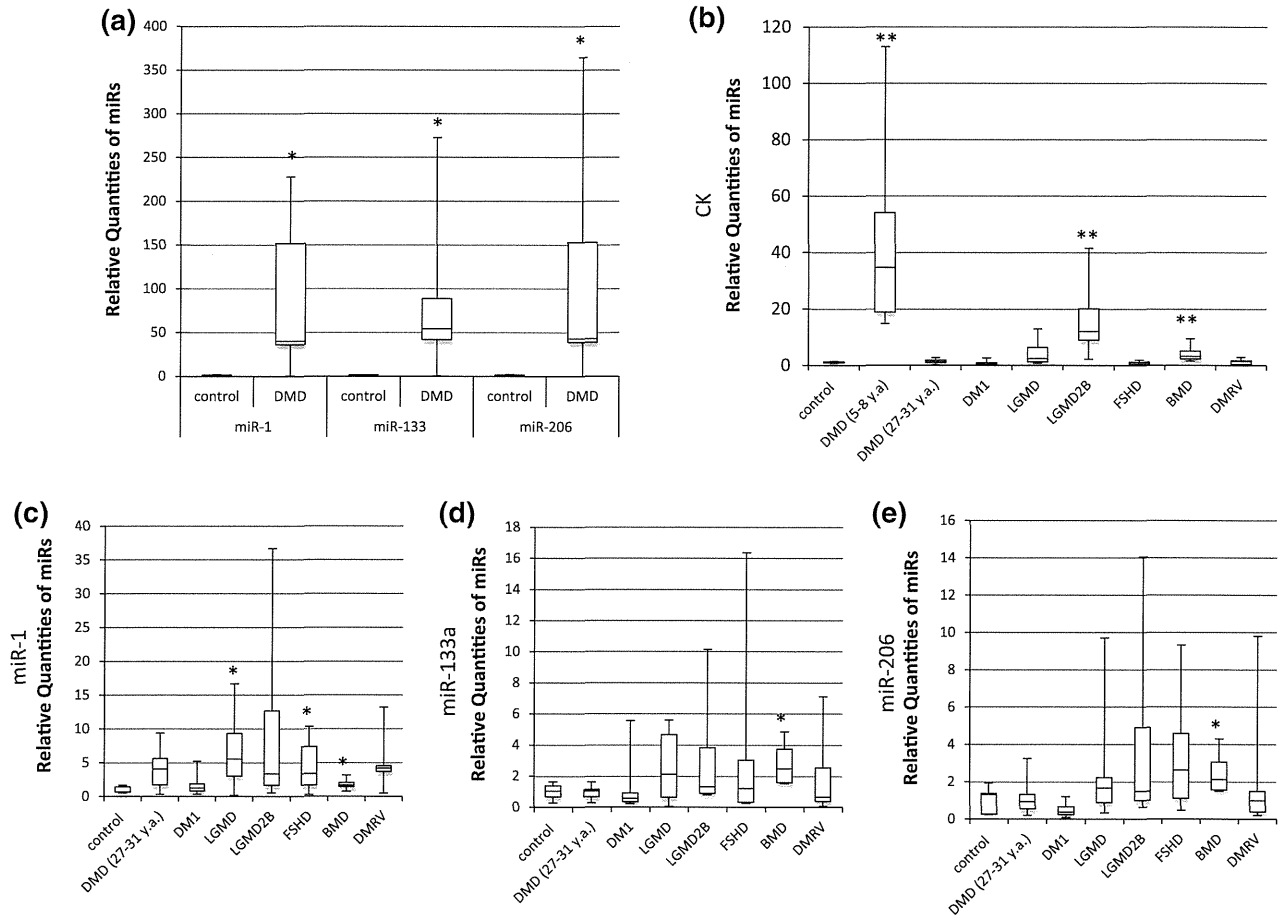

Fig. 1 Evaluation of miRNA levels in various muscular dystrophies indicated by *box plot*. **a** Expression levels of miR-1, miR-133a, and miR-206 in the serum of DMD (5–18 years of age) versus control children, evaluated by RT-pPCR. **b** CK activities in various muscular dystrophies. Expression levels of **c** miR-1, **d** miR-133a, and **e** miR-206 were examined using serum from patients with the indicated muscular dystrophies. Each *bar* represents mean ± SD. $^*P < 0.05$ versus control by Mann–Whitney *U* test

and miR-206, in DMD (5–18 and 27–31 years of age), DM1, LGMD, LGMD2B, FSHD, BMD, and DMRV patients and healthy controls. Although all these miRNA levels in sera of all muscle diseases tested in this study represented no significant differences with controls by Bonferroni correction, associations of these miRNA levels with some disorders of them were observed without multiple corrections. As previous reported, qRT-PCR showed that the median values of levels of all three miRNAs, miR-1, miR-133a, and miR-206, in the serum of DMD (5–18 years of age) were approximately 39.8, 54.0, and 43.0 compared with those of controls, 0.68, 1.03, and 1.32, respectively ($P < 0.05$, Fig. 1a). The median value of CK activity in the serum of DMD (5–18 years of age) was significantly increased compared with controls (cases verses controls; 34.6 verses 0.89, $P < 0.01$, Fig. 1b). Although LGMD2B and BMD patients also indicated high median value of CK activities compared with controls (LGMD2B, BMD verses controls; 12.1, 3.1 verses 0.89, $P < 0.05$, Fig. 1b). On the other hand, each median value of miR-1 levels in the serum of LGMD, FSHD and BMD patients was significantly increased compared with controls (LGMD, FSHD and BMD verses controls; 5.5, 3.3, and 1.7 verses 0.68, $P < 0.05$, Fig. 1c). As for miR-133a and miR-206, BMD patients presented significant increases in the median value of expression levels in the serum compared to controls (cases verses controls for miR-133a and miR-206; 2.49 verses 1.03, and 2.13 verses 1.32, $P < 0.05$, Fig. 1d, e). DM1 and DMRV showed no significant differences with controls for the three miRNAs. Next, we evaluated the three miRNAs as available biomarkers by receiver operating characteristics (ROC) analysis. These results indicated that area under the curve (AUC) in miR-1 displayed 0.83, 0.88, and 0.90 for LGMD, FSHD, and BMD, respectively, in spite of values below 0.8 for DMD (5–18 years of age), DM1, LGMD2B, DMRV (Supplemrntary Fig. 1a). Similarly, the AUC in miR-133a and miR-206 was 0.90 and 0.90 for BMD (Supplementary

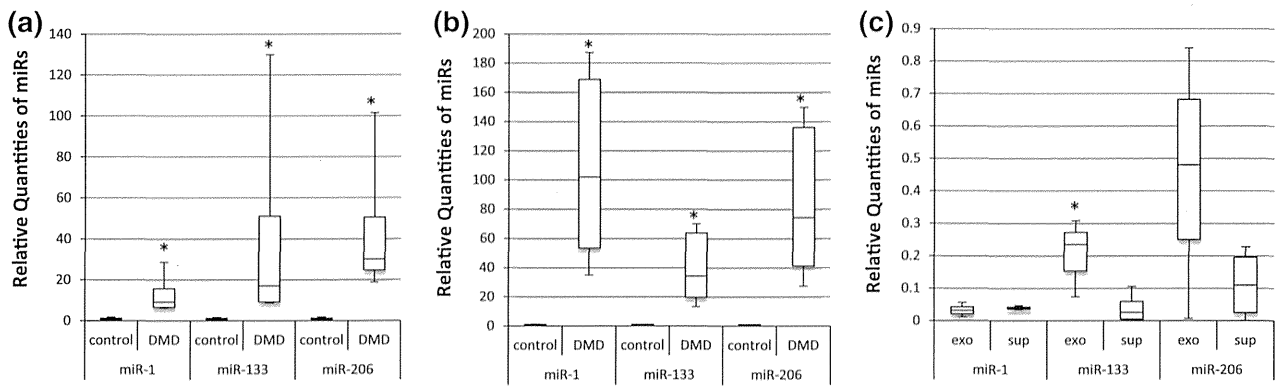

Fig. 2 Expression levels of miR-1, miR-133a, and miR-206 represented by *box plot* in (**a**) exosomes or (**b**) exosome-depleted supernatants extracted from the serum of DMD patients and controls, and analyzed by RT-qPCR. Relative expression of miR-1, miR-133a, and miR-206 is displayed as the difference in the threshold cycle number between miRNA from exosomes or the exosome-depleted supernatant extracted from the serum of DMD patients (**c**) in five times dilution. Each bar represents mean ± SD. $^*P < 0.05$ versus control by Mann–Whitney *U* test

Fig. 1b, c). miR-1 in LGMD and FSHD, and miR-1, miR-133a, and miR-206 in BMD may be useful novel biomarkers.

miRNAs expressions in exosome from the serum of DMD patients

To determine whether the up-regulation of miR-1, miR-133a, and miR-206 levels in serum of DMD patients resulted from inclusion of these miRNAs within exosome, we separated serums from patients and controls into exosome and exosome-depleted supernatant by Exo Quick Exosome Precipitation Solution. RNAs extracted from both sources were compared for the levels of the three miRNAs levels by RT-qPCR. The levels of the three miRNAs of serum of DMD patients in both fractions of exosome and exosome-depleted supernatant represented no statistically significant differences with those of controls by Bonferroni correction, but significant differences in these miRNA levels in serum between DMD patients and controls showed without multiple corrections. The median value of levels of each miR-1, miR-133a, and miR-206 in the RNAs within the exosome extracted from the DMD patients showed higher levels, 9.1, 17.0, and 30.1, compared with that of controls, 0.82, 0.77, and 0.99, respectively ($P < 0.05$, Fig. 2a). Furthermore, the median value of these miR-1, miR-133a, and miR-206 levels for RNAs from the exosome-depleted supernatant of DMD patients, 101.9, 34.1, and 74.2, exhibited high levels compared with that of controls, 1.0, 0.39, and 0.42, respectively ($P < 0.05$, Fig. 2b). To evaluate whether the majority of these miRNAs in the serum of DMD patients are concentrated in exosome or freely circulating in blood stream, the amount of the three miRNAs extracted from exosome and exosome-depleted supernatants was measured by RT-qPCR. All these miRNA levels exhibited no significant differences between exosome and exosome-depleted supernatants by Bonferroni correction. However, the levels of miR-1, miR-133a, and miR-206 in both fractions of exosome and exosome-depleted supernatant from the serum of DMD patients are remarkably increased compared with those in controls. The content of miR-133a within exosome is significantly higher than in the exosome-depleted supernatant without multiple corrections (Fig. 2c, $P < 0.05$).

miR-1, miR-133a, and miR-206 levels in mouse serum are up-regulated upon skeletal muscle regeneration

Next, to assess whether the three miRNA levels are affected in skeletal muscle regeneration in vivo, we induced skeletal muscle injury by injecting CTX into the TA of mice, and analyzed the expression of the three miRNAs by RT-qPCR at 1, 3, and 5 days after injection. The levels of miR-1, miR-133a, and miR-206 were increased dramatically by about 15-, 55-, and 15-fold in the serum of CTX-injured mice on day 1 compared with those of PBS-treated mice (Fig. 3a). However, the levels of the three miRNAs in the serum markedly decreased from day 3 to day 5 after CTX injury (Fig. 3a). On the other hand, CK activity in serum of CTX-injured mice was about 2.0-fold higher than controls between day 1 and day 3 (Fig. 3b). Our data indicate that the levels of miR-1, miR-133a, and miR-206 in serum are strikingly up-regulated by muscle injury.

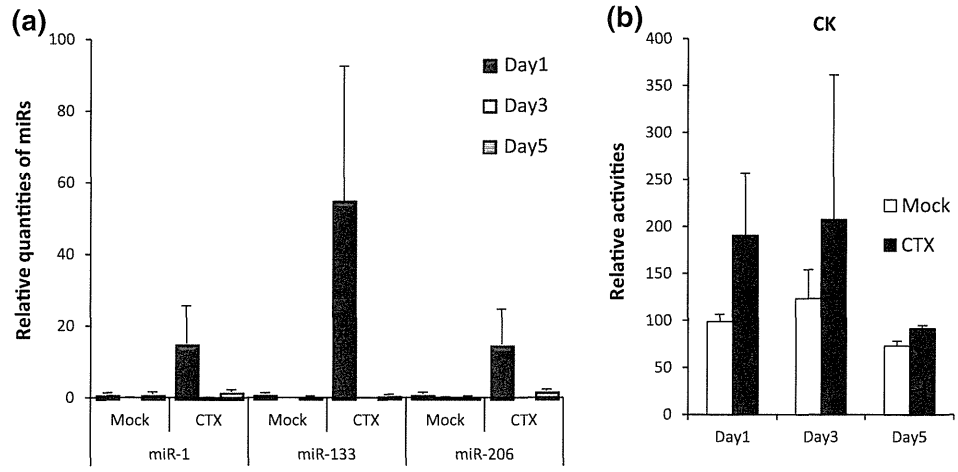

Fig. 3 **a** Expression levels of miR-1, miR-133a, and miR-206 and **b** CK activities in serum of mice on day 1, 3, and 5 after CTX ($n = 3$) injury or Mock ($n = 3$)

Discussion

In this study, miR-133a and miR-206 levels in exosome from the serum of DMD patients were increased compared to the exosome-depleted supernatant. However, it was reported that the miRNAs in *mdx* mice were more enriched in the supernatant fraction rather than in the exosome, and associated with Argonaute-2 (Ago-2) and Apolipoprotein A-1 (ApoA-1) [21]. This discrepancy may be depended on some differences for a degree of severe and progressive degeneration of affected tissues between human and mouse. It was recently reported that the miR-1, miR-133a, and miR-206 are up-regulated in both exosomes and a skeletal muscle cell line, C2C12 cells, and miRNA profiles in exosome alter during differentiation [22]. Furthermore, although the number of exosomes from C2C12 myotubes released into extracellular compartment by Dexamethasone was not changed, the abundance of the miR-1 in exosome was increased [23]. These findings represent that the up-regulations of miRNAs in serum of mice might be partly explained by their selective exports into exosomes induced by muscle degeneration and/or regeneration.

It was also reported that lack of miR-206 shows delay of muscle regeneration induced by CTX injury and more severe dystrophic phenotype in *mdx* mice due to impair differentiation of SC [24]. In our study, these miR-1, miR-133a, and miR-206 levels in the serum were up-regulated in response to CTX-induced injury. These suggested that excessive secretion of miRNAs might partly be a cause for muscle diseases.

In summary, we evaluated three miRNAs, miR-1, miR-133a, and miR-206, as novel biomarkers for muscle disorders. Although all diseases examined in this study exhibited no statistically significant associations with these miRNA levels in serum by Bonferroni correction, associations of miR-1 levels with LGMD, FSHD, and miR-133a and miR-206 with BMD showed significant differences without corrections of multiple test. However, additional studies on increasing sample size are required to further confirm its usefulness as novel biomarker for muscle disorders.

Acknowledgments We thank Dr. Jun Tanihata for valuable discussions and critical reading of the manuscript.

Conflict of interests The authors report no conflict of interests.

References

1. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet. 2011;12:861–74.
2. Eisenberg I, Eran A, Nishino I, Moggio M, Lamperti C, Amato AA, et al. Distinctive patterns of microRNA expression in primary muscular disorders. Proc Natl Acad Sci U S A. 2007;104:17016–21.
3. Eisenberg I, Alexander MS, Kunkel LM. miRNAS in normal and diseased skeletal muscle. J Cell Mol Med. 2009;13:2–11.
4. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654–9.
5. Yao B, Li S, Chan EK. Function of GW182 and GW bodies in siRNA and miRNA pathways. Adv Exp Med Biol. 2013;768:71–96.
6. Mercuri E, Muntoni F. Muscular dystrophies. Lancet. 2013;381:845–60.
7. Emery AE. The muscular dystrophies. Lancet. 2002;359:687–95.
8. Takeshima Y, Yagi M, Okizuka Y, Awano H, Zhang Z, Yamauchi Y, et al. Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy cases from one Japanese referral center. J Hum Genet. 2010;55:379–88.
9. Tsukamoto H, Inui K, Fukushima H, Nishigaki T, Taniike M, Tanaka J, et al. Molecular study of Duchenne and Becker muscular dystrophies in Japanese. J Inherit Metab Dis. 1991;14:819–24.

10. Yamagata H, Miki T, Sakoda S, Yamanaka N, Davies J, Shelbourne P, et al. Detection of a premutation in Japanese myotonic dystrophy. Hum Mol Genet. 1994;3:819–20.
11. Udd B, Krahe R. The myotonic dystrophies: molecular, clinical, and therapeutic challenges. Lancet Neurol. 2012;11:891–905.
12. Mitsuhashi S, Kang PB. Update on the genetics of limb girdle muscular dystrophy. Semin Pediatr Neurol. 2012;19:211–8.
13. van Deutekom JC, Wijmenga C, van Tienhoven EA, Gruter AM, Hewitt JE, Padberg GW, et al. FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. Hum Mol Genet. 1993;2:2037–42.
14. Ikeda-Sakai Y, Manabe Y, Fujii D, Kono S, Narai H, Omori N, et al. Novel mutations of the GNE gene in distal myopathy with rimmed vacuoles presenting with very slow progression. Case Rep Neurol. 2012;4:120–5.
15. Malm C, Sjödin TL, Sjöberg B, Lenkei R, Renström P, Lundberg IE, et al. Leukocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. J Physiol. 2004;556:983–1000.
16. Zatz M, Rapaport D, Vainzof M, Passos-Bueno MR, Bortolini ER, Pavanello Rde C, et al. Serum creatine-kinase (CK) and pyruvate-kinase (PK) activities in duchenne (DMD) as compared with becker (BMD) muscular dystrophy. J Neurol Sci. 1991;102:190–6.
17. Cacchiarelli D, Legnini I, Martone J, Cazzella V, D'Amico A, Bertini E, et al. miRNAs as serum biomarkers for Duchenne muscular dystrophy. EMBO Mol Med. 2011;3:258–65.
18. Mizuno H, Nakamura A, Aoki Y, Ito N, Kishi S, Yamamoto K, et al. Identification of muscle-specific microRNAs in serum of muscular dystrophy animal models: promising novel blood-based markers for muscular dystrophy. PLoS ONE. 2011;6:e18388.
19. Jia Y, Suzuki N, Yamamoto M, Gassmann M, Noguchi CT. Endogenous erythropoietin signaling facilitates skeletal muscle repair and recovery following pharmacologically induced damage. FASEB J. 2012;26:2847–58.
20. Couteaux R, Mira JC, d'Albis A. Regeneration of muscles after cardiotoxin injury I. Cytological aspects. Biol Cell. 1988;62:171–82.
21. Roberts TC, Godfrey C, McClorey G, Vader P, Briggs D, Gardiner C, et al. Extracellular microRNAs are dynamic non-vesicular biomarkers of muscle turnover. Nucleic Acid Res. 2013;41:9500–13.
22. Forterre A, Jalabert A, Chikh K, Pesenti S, Euthine V, Granjon A, et al. Myotube-derived exosomal miRNAs downregulate Sirtuin1 in myoblasts during muscle cell differentiation. Cell Cycle. 2014;13:78–89.
23. Hudson MB, Woodworth-Hobbs ME, Zheng B, Rahnert JA, Blount MA, Gooch JL, et al. miR-23a is decreased during muscle atrophy by a mechanism that includes calcineurin signaling and exosome-mediated export. Am J Physiol Cell Physiol. 2014;306:C551–8.
24. Liu N, Williams AH, Maxeiner JM, Bezprozvannaya S, Shelton JM, Richardson JA, et al. microRNA-206 promotes skeletal muscle regeneration and delays progression of Duchenne muscular dystrophy in mice. J Clin Invest. 2012;122:2054–65.

Journal of the Neurological Sciences 340 (2014) 94–98

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Congenital fiber type disproportion myopathy caused by *LMNA* mutations

Sachiko Kajino [a,b], Kayo Ishihara [a], Kanako Goto [a], Keiko Ishigaki [b], Satoru Noguchi [a,c], Ikuya Nonaka [a], Makiko Osawa [b], Ichizo Nishino [a,c], Yukiko K. Hayashi [a,c,d,*]

[a] Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[b] Department of Pediatrics, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan
[c] Department of Clinical Development, Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, Japan
[d] Department of Neurophysiology, Tokyo Medical University, Tokyo, Japan

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 24 February 2014
Accepted 26 February 2014
Available online 5 March 2014

Keywords:
LMNA-myopathy
CFTD
Fiber type disproportion (FTD)
ACTA1
TPM3
muscular dystrophy
congenital myopathy

ABSTRACT

A boy, who had shown muscle weakness and hypotonia from early childhood and fiber type disproportion (FTD) with no dystrophic changes on muscle biopsy, was initially diagnosed as having congenital fiber type disproportion (CFTD). Subsequently, he developed cardiac conduction blocks. We reconsidered the diagnosis as possible LMNA-myopathy and found a heterozygous mutation in the *LMNA* gene. This encouraged us to search for *LMNA* mutations on 80 patients who met the diagnostic criteria of CFTD with unknown cause. Two patients including the above index case had heterozygous in-frame deletion mutations of c.367_369delAAG and c.99_101delGGA in *LMNA*, respectively. Four of 23 muscular dystrophy patients with *LMNA* mutation also showed fiber type disproportion (FTD). Importantly, all FTD associated with LMNA-myopathy were caused by hypertrophy of type 2 fibers as compared with age-matched controls, whereas CFTD with mutations in *ACTA1* or *TPM3* showed selective type 1 fiber atrophy but no type 2 fiber hypertrophy. Although FTD is not a constant pathological feature of LMNA-myopathy, we should consider the possibility of LMNA-myopathy whenever a diagnosis of CFTD is made and take steps to prevent cardiac insufficiency.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Mutations in the gene encoding nuclear envelope proteins of A-type lamins (*LMNA*) cause several disorders referred to as laminopathies, which include skeletal and cardiac muscle disorders, lipodystrophy, peripheral neuropathy, and premature aging syndromes. Laminopathies predominantly affecting skeletal muscles (LMNA-myopathy) are clinically classified into three different phenotypes; Emery-Dreifuss muscular dystrophy (AD-, AR-EDMD), limb girdle muscular dystrophy type 1B (LGMD1B), and LMNA-related congenital muscular dystrophy (L-CMD). EDMD has distinctive clinical features including early joint contractures, humero-peroneal muscle weakness and dilated cardiomyopathy with conduction defects. LGMD1B is characterized by proximal muscle involvement and cardiomyopathy with conduction defects, but joint contracture is not prominent. L-CMD is an early onset form showing severe weakness of respiratory and neck muscles from infancy. Serum CK levels in LMNA-myopathy are normal to moderately elevated (2–20 times the upper limit of the normal range). Cardiac involvement, such as conduction blocks, dilated cardiomyopathy and sudden death, usually appears after the second decade of life. To minimize the risk of sudden cardiac death, early diagnosis and appropriate cardiac defibrillator implantation is recommended [1–3].

Pathologically, LMNA-myopathy is usually characterized by nonspecific dystrophic changes with variation in fiber size, mild necrotic and regenerating processes, and an increased number of muscle fibers with internalized nuclei. Both type 1 and type 2 fibers are affected. Nuclear abnormalities are common [4]. Interestingly, marked mononuclear cellular infiltrations mimicking inflammatory myopathy can be seen in some patients with the infantile onset form of LMNA-myopathy [5].

We recently experienced a patient with a *LMNA* mutation whose initial diagnosis was congenital fiber type disproportion (CFTD). This patient had shown muscle weakness, hypotonia, and unstable gait from early childhood with no dystrophic changes, but prominent fiber type disproportion (FTD) on his muscle biopsy performed at 4years of age. At his age of 16 years, he was pointed out to have atrial-ventricular conduction block and incomplete right bundle branch block. We thus reconsidered a possible diagnosis of LMNA-myopathy and identified a mutation in the *LMNA* gene.

CFTD is one of the congenital myopathies pathologically defined by smaller type 1 fibers, by at least 12%, than type 2 fibers without structural abnormalities such as nemaline bodies, cores, and central nuclei.

* Corresponding author at: Department of Neurophysiology Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan. Tel.: +81 3 3351 6141; fax: +81 3 3351 6544.
E-mail address: yhayashi@tokyo-med.ac.jp (Y.K. Hayashi).

http://dx.doi.org/10.1016/j.jns.2014.02.036
0022-510X/© 2014 Elsevier B.V. All rights reserved.