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Detection and management of cardiomyopathy in female dystrophinopathy carriers

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ABSTRACT

Regular health checkups for mothers of patients with Duchenne muscular dystrophy have been performed at National Hospital Organization Tokushima Hospital since 1994. Among 43 mothers participated in this study, 28 dystrophinopathy carriers were identified. Skeletal and cardiac muscle functions of these subjects were examined. High serum creatine kinase was found in 23 subjects (82.1%). Obvious muscle weakness was present in 5 (17.8%) and had progressed from 1994 to 2015. Cardiomyopathy was observed in 15 subjects (60.0%), including dilated cardiomyopathy-like damage that was more common in the left ventricular (LV) posterior wall. Late gadolinium enhancement on cardiac MRI was found in 5 of 6 subjects, suggesting fibrotic cardiac muscle. In speckle tracking echocardiography performed seven years later, global longitudinal strain was decreased in these subjects, indicating LV myocardial contractile abnormality. These results suggest that female dystrophinopathy carriers should receive regular checkups for detection and treatment of cardiomyopathy, even if they have no cardiac symptoms.

1. Introduction

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are diseases with X chromosome-linked modes of inheritance. A recent study in Japan showed that about 60% of DMD cases and 90% of BMD cases were inherited from mothers who were dystrophinopathy carriers [1]. Carriers develop disease symptoms such as muscle weakness, cardiomyopathy, and central nervous system manifestations at relatively low frequency, and are referred to as “manifesting carriers”. The phenotype could be related to the X-chromosome inactivation pattern [2], however, it is difficult to distinguish their symptoms and age of onset from conditions such as burden in daily life and aging at the moment. The reported prevalences of skeletal muscle damage, elevated serum creatine kinase (CK), and dilated cardiomyopathy are 3–46%, 53–100%, and 8–18%, respectively [3–8]. An improved prognosis is likely after treatment of carriers with dilated cardiomyopathy, but fatal cases have also been reported [9,10]; thus, appropriate management is important. Since the incidence of cardiomyopathy is thought to increase with age, detection of abnormalities in echocardiography after exercise loading is important [7], even

Abbreviations: BMD, Becker muscular dystrophy; BNP, brain natriuretic peptide; CK, creatine kinase; DMD, Duchenne muscular dystrophy; FS, fractional shortening; LGE, late gadolinium enhancement; LVDd, left ventricular end-diastolic diameter; MLPA, multiplex ligation-dependent probe amplification; NHO, National Hospital Organization; SPECT, single photon emission computed tomography; STE, speckle tracking echocardiography

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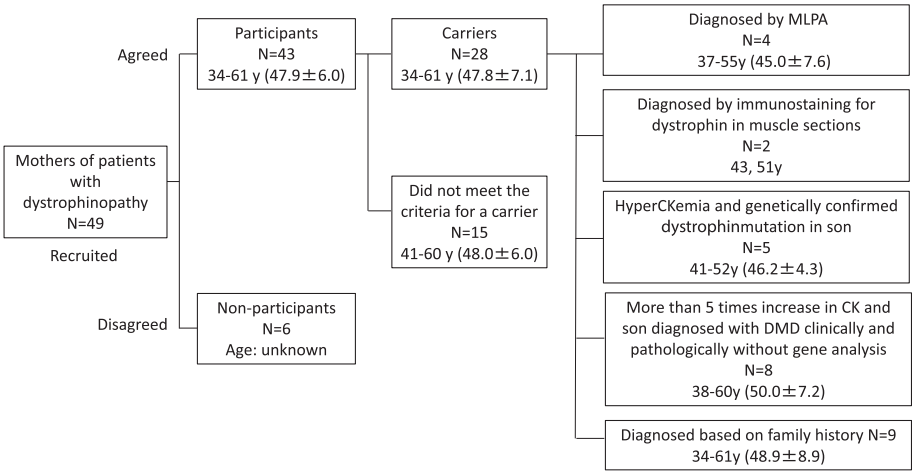

Fig. 1. Flowchart of study participation by mothers of patients with DMD. Among 49 mothers, 43 agreed to participate in checkups, of whom 28 manifested with muscle or cardiac symptoms and 15 had undefined symptoms. Diagnoses of the 28 subjects as carriers were made using the MLPA method for detection of a *dystrophin* mutation (exon 48–50, 49–50, 50, 50, all expected out-of-frame) (n = 4); by faint and patchy immunostaining of dystrophin protein on biopsied muscle sections (n = 2); by hyperCKemia with a genetically confirmed *dystrophin* mutation in sons (n = 5); by hyperCKemia of 5 times the normal level and sons diagnosed with DMD in the absence of immunostaining for dystrophin in muscle sections (n = 8); and by family history (n = 9).

in a case with a normal electrocardiogram and no skeletal muscle symptoms [11]. Aggravation or development of cardiomyopathy and skeletal muscle symptoms also occurs after pregnancy or delivery [12]. However, evidence for these events and accurate epidemiological data are insufficient.

In Japan, the life prognosis of patients with DMD has improved by almost 15 years with the introduction of multimodal treatment over the last 30 years [13]. The mean age at death is now ≥ 30 years old and many patients live to ≥ 40 years old. The number of patients who can live at home, even with a need for mechanical ventilation, has increased because of development of mobile medical devices and improvement of the medical and welfare systems. However, long-term home healthcare for patients requiring high-level nursing care increases the burden on aging mothers, who are the main caregivers in most cases. Therefore, maintenance of the long-term health of mothers is important for the quality of life of patients with DMD and their families, as well as for the quality of life of the mothers themselves. In this context, we have performed an annual health checkup of mothers of patients with DMD since 1994 to examine the conditions of skeletal and cardiac muscles. In this report, we describe skeletal muscle and cardiac symptoms based on data from these checkups, and we discuss the need for early detection and treatment, especially for cardiomyopathy.

2. Subjects and methods

We invited 49 mothers of patients with dystrophinopathy who were visiting or hospitalized at National Hospital Organization (NHO) Tokushima Hospital to a health checkup, in which 43 mothers finally participated from 1994 to 2016. The checkup included a history interview, general examination, neurological examination, manual muscle test, measurement of serum CK activity for screening of skeletal muscle damage, and CT scans and skeletal muscle biopsy performed when deemed necessary for diagnosis of muscle diseases during the mid-1990s. If significant muscle weakness or hyperCKemia was found, *dystrophin* gene mutation was analyzed by a southern blot or multiplex ligation-dependent probe amplification (MLPA) after genetic counseling and provision of informed consent, or immunostaining for dystrophin protein in muscle sections was performed as part of a pathological examination.

Cardiac function was evaluated by electrocardiography, echocardiography, measurement of plasma brain natriuretic peptide (BNP), single photon emission computed tomography (SPECT), and biopsy of cardiac muscle in the mid-1990s, as needed. Since 2008, a late gadolinium enhancement (LGE) test on cardiac MRI has been performed for early detection of cardiomyopathy [14,15], using a 1.5-Tesla MRI (General Electric, Signa Excite) and an 8-channel cardiac coil. The LGE test is performed 10–15 min after administration of a gadolinium contrast agent (TR 6 ms, TE 2 ms, inversion time 260–320 ms), using the inversion-recovery gradient echo method [10]. Since 2015, we have added speckle tracking echocardiography (STE) (Vivid q and EchoPac PC, GE Healthcare, Milwaukee, WI, USA), which enables quantitative assessment of regional myocardial deformation. It has been reported that this advanced technique is useful for early detection of cardiac fibrosis due to cardiomyopathy in patients with dystrophinopathy [15–18]. A respiratory function test was also performed in some participants in the general examination.

Female dystrophinopathy carriers in this report are defined based on meeting at least one of the following 5 diagnostic criteria: (1) heterozygous deletion or duplication of the *dystrophin* gene on MLPA or Southern blot, (2) mosaic-like muscle fibers or a patchy image in dystrophin immunostaining of skeletal muscle, (3) mothers with an increase in serum CK ≥ the normal level who have a child with a *dystrophin* mutation, (4) mothers with an increase in serum CK to ≥ 5 times the normal level who have a child with a clinical diagnosis of dystrophinopathy in the absence of immunostaining for dystrophin protein in muscle sections, and (5) genetically definite carriers. Mothers meeting criterion (3) or (4) are referred to as biochemical carriers.

The study protocol was approved by the ethics committee of NHO Tokushima Hospital and written informed consent was obtained from all subjects.

3. Results

3.1. Background of subjects

Among 49 mothers of patients with dystrophinopathy, 43 (age at first checkup: 34–61 years old, mean ± SD: 47.9 ± 6.0) agreed to participate in checkups between 1994 and 2015. Of these 43 mothers, 28 manifested with muscle or cardiac symptoms and 15 did not meet the criteria for a dystrophinopathy carrier in this study with ages at first checkup of 34–61 (47.8 ± 7.1) and 41–60 (48.0 ± 6.0) years old, respectively (Fig. 1). Diagnoses of the 28 carriers were based on MLPA detection of a *dystrophin* mutation (all out-of-frame mutations, deletion of exons 48–50, 49–50, 50, and duplication of exon 50) (n = 4); faint and patchy immunostaining of dystrophin protein on biopsied muscle sections (n = 2); hyperCKemia with confirmed *dystrophin* mutation in sons (all out-of-frame mutations, deletion of exons 44–52, 48–50, 48–52, and 50 in 2 cases) (n = 5), hyperCKemia with diagnosis of DMD in sons in the absence of dystrophin protein in biopsied muscle sections (n = 8); and a family history of dystrophinopathy in brothers or male relatives (n = 9). In the 9 subjects diagnosed by family history, CK levels were high in 5 (1270, 1608, 577, 241, and 312 IU/L; normal range: 30–200 IU/L) and normal in 4 (83, 119, 140, and 83 IU/L). Skeletal and cardiac symptoms of the 28 subjects are described below.

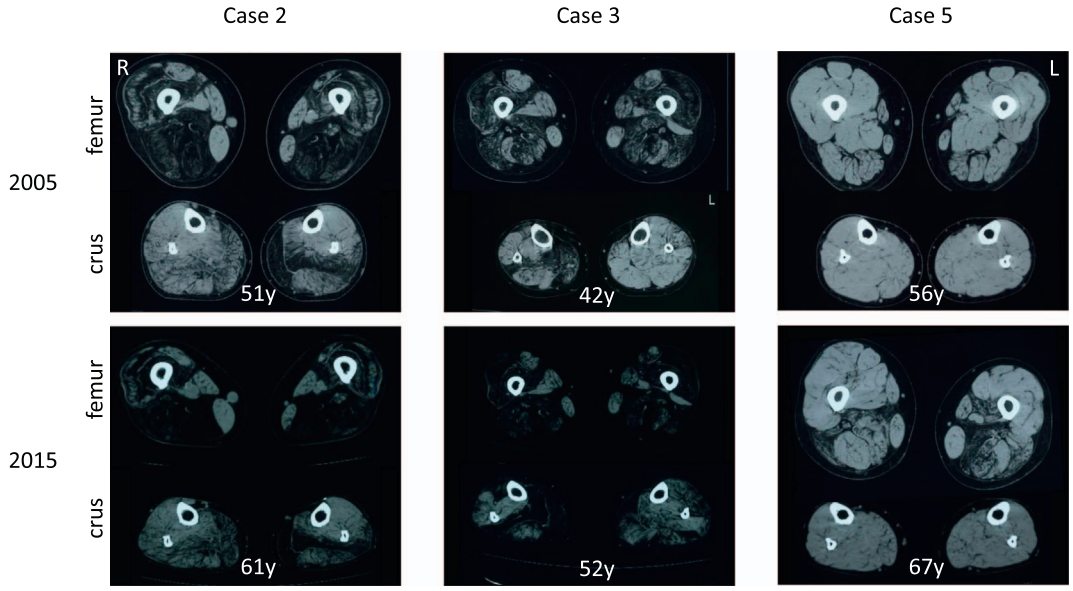

Fig. 2. Ten-year follow-up CT of the lower extremities of female dystrophinopathy carriers. CT images of cases 2, 3 and 5, described in Supplemental Table 2, showed progressive muscle atrophy.

3.2. Skeletal muscle symptoms and examinations

Five of the 28 subjects had weakness of the proximal lower extremity muscle and waddling gait. One subject could not stand up from a sitting position, 3 could barely stand up from a sitting position supported by her hands, and all of these subjects had obvious weakness of proximal muscles, including hamstring, iliopsoas, and gluteus maximus. In all 5 subjects, muscle weakness and atrophy progressed slowly during the observation period. Serum CK activity in the 28 subjects was 83–2925 IU/L (mean 916.1 ± 886.2 IU/L, normal < 200 IU/L), and only 5 subjects had normal values without obvious muscle weakness. Few changes over time occurred in subjects with low CK, but there were major changes in those with high CK. Of 24 subjects for whom skeletal muscle CT images were obtained, 11 showed amyotrophia, fat displacement, and bilateral differences. Lower extremity muscle CT values were decreased for the gracilis, quadriceps, semimembranosus/semitendinosus, caput mediale musculi gastrocnemii, and the biceps femoris most significantly. Over 10 years, CT values gradually decreased and correlated with muscle weakness, which was suspected to indicate progressive muscle degeneration (Fig. 2). Skeletal muscle biopsy was performed in 2 subjects in the mid-1990s. Hematoxylin-eosin (HE) staining for the deltoid muscle in a 51-year-old subject (shown in Supplemental Table 1), showed a mild difference in size and rounding of muscle fibers, but necrosis, degeneration, and regeneration were not observed in another subject (case 1, Supplemental Table 2). In immunohistochemical staining, dystrophin-positive normal fibers, patchy dystrophin-positive fibers (partially stained for dystrophin on the cell membrane), and dystrophin-negative fibers were found at rates of 18.2%, 79.3%, and 2.5%, respectively [11].

In summary, 5/28 (17.9%) had muscle weakness, 23/28 (82.1%) had elevated serum CK activity, 11/24 (39.3%) had abnormalities on skeletal muscle CT, and 2/2 (100%) had abnormal findings in muscle biopsy.

3.3. Cardiopulmonary symptoms and plasma BNP

Eight subjects (28.6%) showed subjective symptoms of cardiomyopathy, such as mild shortness of breath and cardiac palpitation, and two died due to cardiac failure. There were 4 subjects with mild hypertension (14.3%), 1 with mild hypotension (3.6%), and 23 with normal blood pressure (82.1%). One subject had a body mass index (BMI) < 18.5 kg/m^2 (below standard, 3.6%), 17 had BMI from 18.5 to 25 kg/m^2 (standard, 60.7%), and 10 had BMI from 25 to 30 kg/m^2 (mild obesity, 35.7%). There were no smokers among the subjects. Respiratory function tests were performed in 10 subjects (35.7%) and all had normal respiratory function. Plasma BNP in 18 subjects was 4–210 pg/mL (mean 42.9 ± 48.3 pg/mL, normal < 18.4 pg/mL), and 12 (66.7%) had high values.

3.4. Detection and evaluation of cardiomyopathy

Abnormal findings in electrocardiograms were found in 11 of 28 subjects: LV posterior inferior wall damage in 5, V1-induced high potential R wave in 2, deep Q wave of I, aVL and V5-6 in 3, and decreased ST of II, III and aVF in 3 subjects. Echocardiography in 25 subjects (89.3%) showed low EF (< 50%) in 8 (32.0%), including 6 with high CK and 2 with normal CK. In 21 subjects without obvious muscle weakness, 7 (33.3%) had a low EF. In 17 without subjective symptoms of cardiomyopathy, 5 (29.4%) had a low EF. Changes of EF with age are shown in Supplemental Table 1 and Fig. 4. Three subjects recovered from EF < 50% with cardioprotection therapy and 4 with EF < 50% were lost in follow up. Especially, of the 5 subjects with hyperCKemia among the 9 diagnosed based on family history (criterion 5), one had a low EF (45.9%) on echocardiography and four had values of 53.5, 59.2, 72.2 and 57.6%, respectively. Of the 4 subjects with normal CK, one had a low EF (36.0%) and three had values of 65.2, 78.9, and 55.1%, respectively. The LV end-diastolic diameter (LVDd) was measured in 22 subjects, with results of 38–62 mm (mean 50.1 ± 6.0 mm, normal < 50 mm). LV dilation was observed in 11 subjects, suggesting development of dilated myocardiopathy-like findings. LV fractional shortening (FS) in the 22 subjects was 39.9–9.4% (25.6 ± 8.6%, normal > 27%), and 14 (63.6%) had low values. The echocardiographic wall motion score was measured at individual sites of the left ventricle in 20 subjects. The mean of all sites was ≥ 1 point, and the highest mean score was at the basal posterior wall (1.7 points), followed by the mid-ventricular posterior wall (1.5 points), and the inferior wall (1.4 points).

In thallium-201 cardiac muscle SPECT performed in 2 subjects (ages 51 and 36), reduced blood flow in the LV posteroinferior wall was found in both. The 51-year-old female with this abnormality showed severe symptoms of cardiac failure, and cardiac muscle biopsy was performed for differential diagnosis of idiopathic cardiomyopathy in

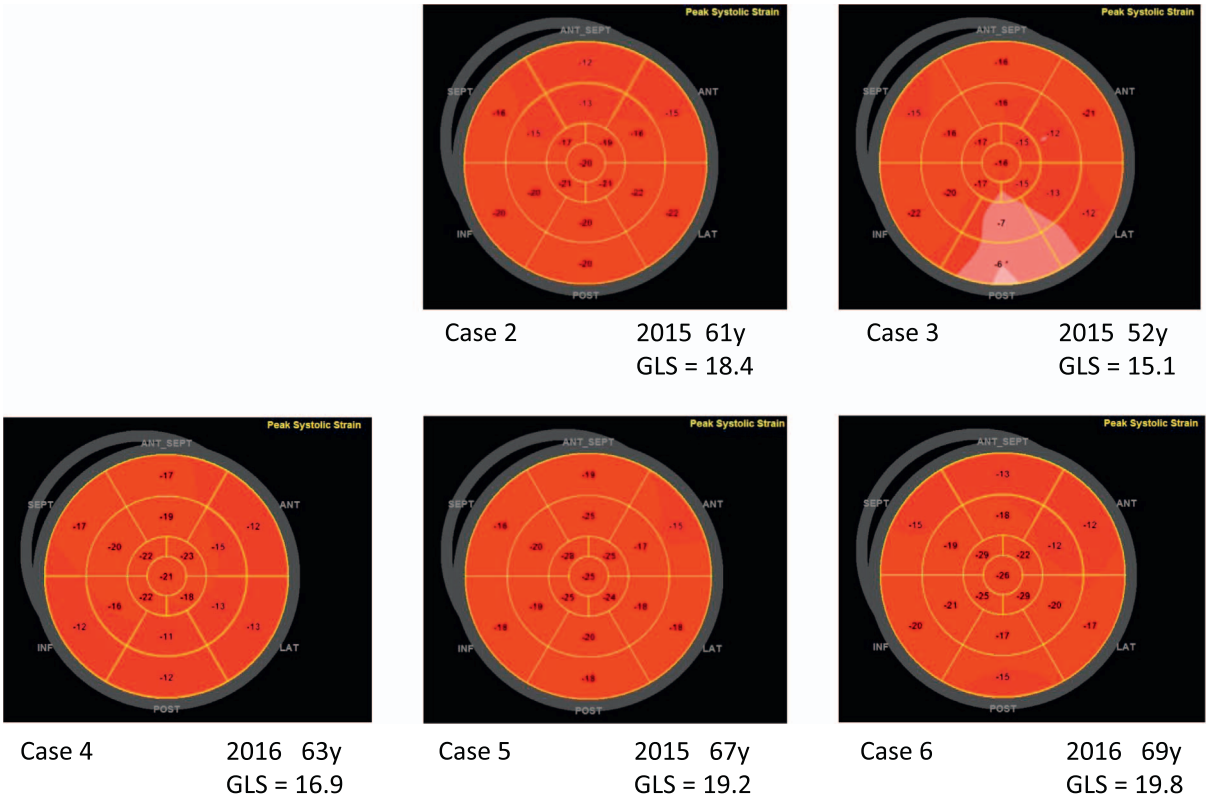

Fig. 3. Myocardial strain analysis by speckle-tracking echocardiography. Case 2, at age 61, EF 64%, GLS 18.4%. Case 3, at age 52, EF 48%, GLS 15.1%, Note low strain in the basal to mid-posterior wall indicating abnormal regional myocardial contractile ability. Case 5, at age 67, EF 62%, GLS 19.2%. Detail of each case were described in Supplemental Table 2.

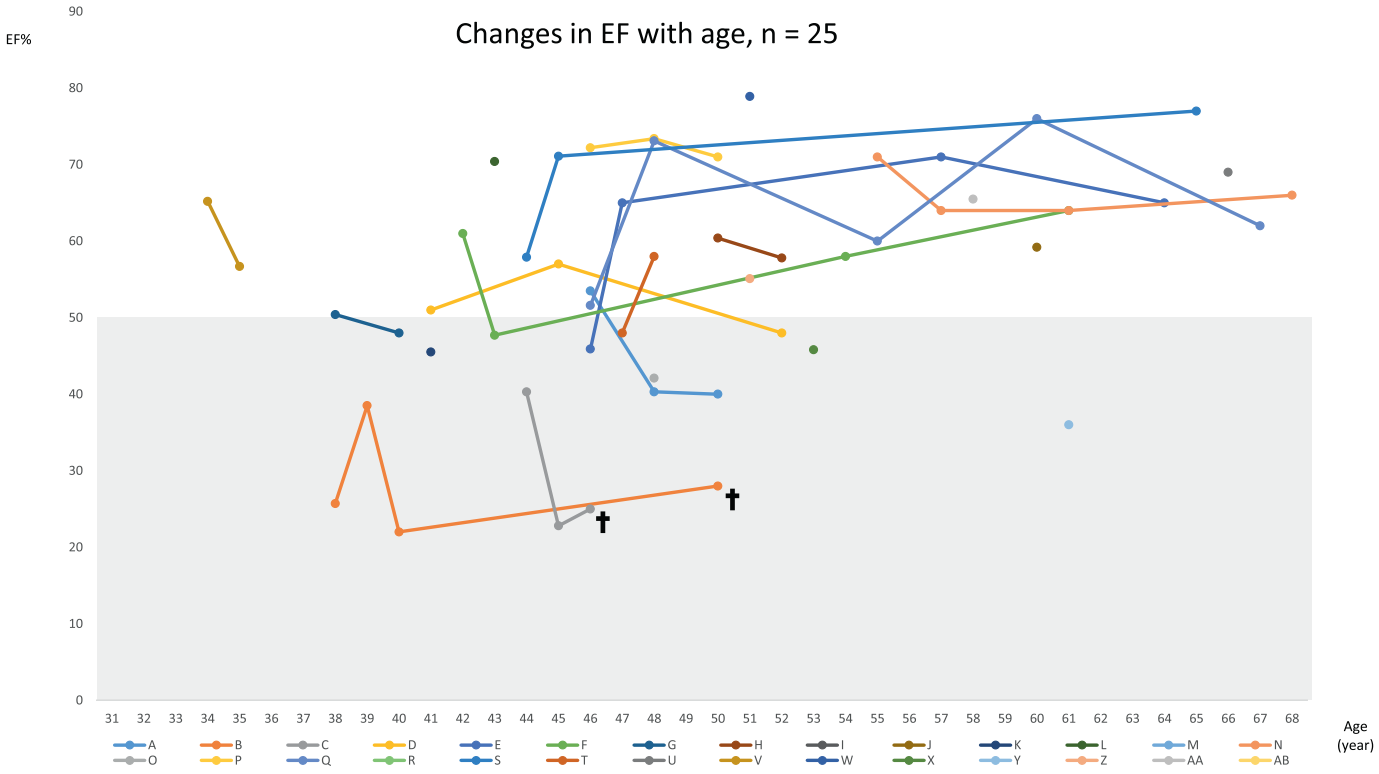

Fig. 4. Change of %EF with age in 25 of the 28 subjects. †Two subjects died at 53 and 49 years old. Three subjects recovered from EF < 50% with cardioprotection therapy, but one did not. Six subjects with EF < 50% were lost in follow up.

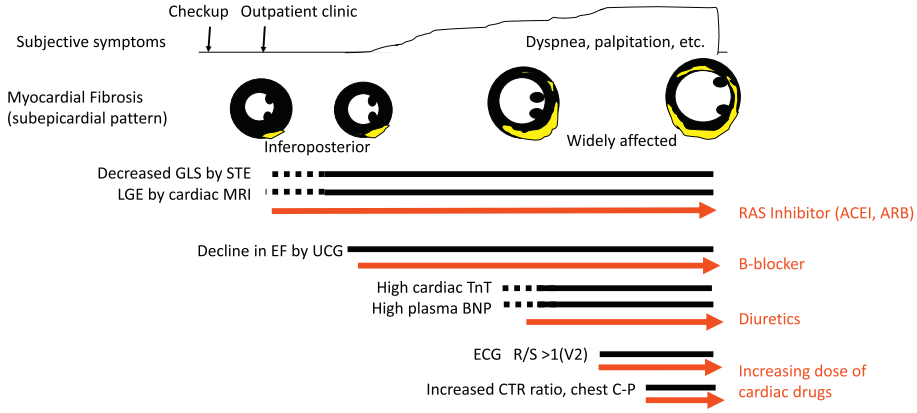

Fig. 5. Proposal for early detection and intervention of cardiomyopathy in female dystrophinopathy carriers. Before appearance of subjective cardiac symptoms, checkup and early consultation are useful for detection of subclinical cardiomyopathy by cardiac MRI and STE, which may then permit early management of cardiomyopathy.

1994. HE staining showed a mild difference in sizes of muscle fibers, and a slight increase in stromal tissues. Dystrophin staining showed normal dystrophin-positive cells, patchy dystrophin-positive cells, and dystrophin-negative cells at rates of 23.3%, 1.3%, and 74.4%, respectively [19]. Three subjects recovered from EF < 50% with cardioprotection therapy, but one did not. Two subjects died at 53 and 49 years old. Six subjects with EF < 50% were lost in follow up (Fig. 4). The two deaths due to cardiac failure, at ages 49 and 53, indicate the need for early detection and treatment of cardiomyopathy. Therefore, an LGE test in cardiac MRI was introduced in 6 subjects who had subjective cardiac symptoms and required a further detailed examination (age 45–62) in 2008 [15]. Clear LGE, suggesting fibrotic cardiac muscle, was found in 5 of these subjects, despite maintenance of LV contractile ability. In all cases, LGE was detected in the LV posterior wall, with subepicardial patterns in the epicardial aspect. Later examinations of cardiac muscle strain using STE in these patients in 2015 and 2016, excluding one patient who died (Supplemental Table 2), showed decreased global longitudinal strain (GLS) in 5 subjects, indicating clear development of myocardial contractile dysfunction (Fig. 3). Details of these cases are shown in Supplemental Table 2.

4. Discussion

Female carriers with a *dystrophin* mutation are not conventionally considered to be patients in Japan, but it is also well known that some have symptoms of cardiomyopathy and some may die due to cardiac failure. The reported prevalence of cardiomyopathy among carriers varies from 7% [17] to 75% [19] depending on the definition. In a cohort study of female dystrophinopathy carriers in Italy, Politano et al. reported dilated cardiomyopathy at a rate of 10.9% in those over 15 years of age, but with preclinical and clinical cardiac involvement of 84.3% and a significantly increased occurrence with age in an observation period of 3–10 years [3]. In a study of 99 carriers monitored over a median of 9 (7.0–10.6) years in the Netherlands, Schade van Westrum et al. [7] initially identified 11 carriers with dilated cardiomyopathy, including 1 who died due to cardiac failure and 9 who later developed dilated cardiomyopathy. The number of female carriers suffering from cardiomyopathy is expected to increase with age, but there is limited clinical information on this condition that is useful for following female dystrophinopathy carriers [18]. This is a particular concern in Japan due to ethical issues that persist for genetic rare diseases in a relatively conservative society (Kobayashi et al. in revision); thus, this type of study might have been viewed as a kind of taboo. This is the context in which we describe our experience of health checkups for dystrophinopathy carriers. In this report, we emphasize the importance of monitoring health conditions of carriers in regular checkups from the presymptomatic stage.

At NHO Tokushima Hospital, health checkups for skeletal and cardiac muscles in mothers of patients with DMD started in 1994, including mothers without subjective symptoms for 22 years. This occasion also provided the opportunity for psychological support for patients and families and peer support in their community. Muscle weakness was found in 5 of 28 subjects (18%) slowly but definitely progressed over 10 years. No subjects with normal CK had muscle weakness, but 2 had cardiac dysfunction (EF < 50%) and 3 had normal cardiac function (EF > 50%). Cardiac dysfunction resembling dilated cardiomyopathy was found in 42.9% of all 28 subjects and was more common in the LV posterior wall, as seen in DMD [16,20]. The subjects were relatively old (48.0 ± 7.5 years), thus, the prevalence was higher than that found previously. Two subjects died due to cardiac failure over 22 years. We recommend that adult dystrophinopathy carriers undergo echocardiography every 5 years in early adulthood, as suggested for patients with BMD [21,22], and that the interval should be shorter in later decades.

The apparent need for further examinations, countermeasures and early intervention caused us to add cardiac MRI to examine LGE for detection of cardiomyopathy [23,24] (Fig. 5). Detection of LGE can identify fibrotic cardiac muscle non-invasively in patients with BMD [25,26], and may be applicable to female dystrophinopathy carriers. In the small number of subjects examined in our study, the common site of fibrotic change in cardiac muscle was the LV posterior inferior wall, as seen for patients with DMD [16,20]. Asymptomatic cardiomyopathy was detectable by LGE earlier than by conventional echocardiography and with high sensitivity [15]. A mild case, case 5, without muscle weakness or atrophy on CT, presented with clear LGE, despite maintenance of LV contractile ability (Supplemental Table 2). A severe case, case 1, had LGE over a wide range from the anteroseptal area to the side and posterior wall, and this patient died due to cardiac failure 6 months later, despite cardioprotection therapy. Therefore, cardiac MRI might allow detection of early changes in cardiac muscle and evaluation of the severity of cardiomyopathy. In recent years, STE has been widely used to evaluate cardiac functions, and can be adapted to cardiomyopathy in female dystrophinopathy. Evaluation of cardiac muscle strain by STE also has advantages of a shorter examination time, no use of contrast agent, and reduced burden on the patient compared with detection of LGE in cardiac MRI. Subtle regional wall motion abnormalities, which cannot be detected visually, can be identified on STE. Overall, LGE and STE may be complementary for early detection of cardiomyopathy and evaluation of clinical conditions and therapeutic effects.

Treatment for cardiac failure was provided to LGE-positive subjects and those with decreased GLS on STE. A renin-angiotensin system inhibitor (ACEI or ARB) was used as first line therapy, with addition of a beta-blocker for patients with persistent cardiac symptoms [10,27]. LGE in cardiac MRI, evaluation of myocardial strain by STE, and regular cardiac function tests are required from an early phase. If an abnormality is detected, treatment for cardiac failure should be started with follow-up observation. In Japan and other East Asian countries, mothers are the main caregivers, and thus ADL of mothers can affect the

QOL of family members of the patient. Therefore, health management for mothers is particularly important for treatment and relief of patients, as well as for the mothers themselves.

There were some limitations in this study. First, data for checkups for mothers of patients with DMD at a single hospital in Japan were used, rather than data for all mothers or female siblings with a dystrophin abnormality, as well as cases of female dystrophinopathy caused by *de novo* mutations, which account for one-third of patients affected by dystrophinopathies. Only 4 of our cohort had a confirmed *dystrophin* mutation because genetic analysis of *dystrophin* in females was not common and not recommended in Japan 20–30 years ago, even though it has been covered by medical insurance from April 2006. In addition, sequencing analysis for *dystrophin* has been a costly self-paid medical examination until Remudy, a research based patient registry established in 2009, took care of male patients confirmed by muscle biopsy with immunostaining for dystrophin. This is likely to be a major reason why fewer participants in this study had a genetic diagnosis. Regarding diagnostic criteria for dystrophinopathy carriers, the study included 5 carriers (18%) with normal CK. As 6 mothers who did not agree to participate probably had no symptoms, and 15 subjects who did not meet the criteria for a dystrophinopathy carrier in this study also had no symptoms, it is possible that a subject with a *dystrophin* mutation was missed, and the rates of cardiac dysfunction and skeletal muscle damage in our cohort might be inaccurate. In the mid-1990s, diagnostic muscle biopsy was performed in 2 subjects with increased CK and muscle weakness because genetic analysis was not common at that time. It is recognized that muscle biopsy is a much more invasive procedure than genetic analysis, which is now considered to be the first step in diagnosis, but this choice was not available 30 years ago. In addition, there were differences in the respective examinations in each subject because participation in the health examination each year was based on convenience for the subjects themselves. Further, we did not collect information on cardiomyopathy in sons, and thus we cannot compare the onset and severity of cardiomyopathy between mothers and sons with DMD.

Despite these limitations, this report is important because of the little information on clinical manifestations of female dystrophinopathy carriers, who are generally not targets of health checkups. Some dystrophinopathy carriers now choose not to have a child due to increased access to genetic counseling and carrier diagnosis [28,29]. Carriers without children with DMD or BMD are unlikely to participate in health checkups, and thus may miss the chance of managing their health condition until appearance of cardiac or skeletal muscle symptoms. To inform such carriers of the physical risk and clinical options, a national database or registration system for future cohort studies is required to clarify the epidemiology of female dystrophinopathy carriers [18]. The current study can contribute to starting a discussion on collection of this information. We conclude that regular health checkups are important for protection of the health of female dystrophinopathy carriers before appearance of subjective cardiac symptoms.

Conflicts of interest

None

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