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Assessment of the upper limb muscles in patients with Fukuyama muscular dystrophy: Noninvasive assessment using visual ultrasound muscle analysis and shear wave elastography

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ABSTRACT

Fukuyama-type congenital muscular dystrophy (FCMD) is severe, childhood-onset muscular dystrophy. Recently, our group has discovered a potential treatment using antisense oligonucleotides. Therefore, an effective, reliable, and objective method of assessing muscle is needed. Ultrasound is a minimally invasive tool that can be applied without radiation exposure or pain. Evaluating tissue stiffness by shear wave elastography (SWE) has especially recently attracted attention. Here, we aimed to evaluate SWE value of the upper limb muscles: biceps brachii, triceps brachii, brachioradialis, abductor pollicis brevis, and abductor finger muscle in patients with FCMD. Upper extremity function was evaluated by visual muscle ultrasound analysis (VMUA) and SWE in 13 patients with FCMD and 20 healthy controls. The motor function evaluation tool was used to evaluate motor function, and the correlation with the dynamics of the SWE was determined. VMUA scaled using the Heckmatt scale was higher in patients with FCMD. SWE was also significantly higher and stiffer in the biceps brachii and brachioradialis in patients with FCMD. Furthermore, the severity of FCMD symptoms was correlated with muscle stiffness. We conclude that VMUA and SWE can be useful tools for monitoring muscle atrophy and upper limb function in patients with FCMD.

1. Introduction

Fukuyama-type congenital muscular dystrophy (FCMD; MIM253800) is the second most common childhood-onset muscular dystrophy in Japan [1,2]. It is an autosomal recessive disorder caused by variants in the *FKTN* gene [3]. FCMD is characterized by generalized muscle weakness and brain malformations, which cause movement disabilities, intellectual disabilities, and epileptic seizures [1]. Most patients with FCMD exhibit delayed psychomotor development in early infancy, usually identified by a lack of head control. In 75% of FCMD cases, motor function develops only up to the point of sitting or sliding on the buttocks, and 90% of patients never gain the ability to walk [4]. Peak motor function occurs between 2 and 8 years of age and then begins to deteriorate [1]. Movement disabilities are severe, but upper limb function (especially that of fingers) is relatively maintained compared with lower limb function [5], and finger movement is maintained until the end stage. Magnetic resonance imaging (MRI) and computed tomography (CT) are the standard imaging modalities for evaluating skeletal muscle disorders in

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Table 1
The modified Ueda classification.

Level 0	No head control
Level 1	Ability to keep the neck erect and the head still to a certain degree (the patient does not have to keep the head and neck erect from the horizontal position, so long as sufficient stability in keeping the neck erect is attained) but has no ability to maintain a sitting position without support
Level 3	Ability to turn around completely while maintaining a sitting position
Level 4	Ability to crawl on the knees, irrespective of the position
Level 5	Ability to stand by holding onto some form of support (the patient may use short leg or shoe-type braces but not long leg braces) or crawl on all fours
Level 6	Ability to walk on a flat surface by holding on to some form of support
Level 7	Ability to walk on a flat surface unaided
Level 8	Ability to ascend a staircase (use of braces or handrail allowed but supporting the body or leading the patient by the hand not allowed)

FCMD [6–9]. However, these radiographic examinations are not accessible for various reasons, including—but not limited to—exposure problems (CT), long-term rest, and sedation (MRI), and high costs. On the other hand, muscle ultrasound is simple, low-cost, minimally invasive, and can be evaluated in real time. The measurement of muscle mass by ultrasound has been found equivalent to X-ray imaging [10,13]. Furthermore, ultrasound enables easy general muscle tissue assessment [14], and ultrasonic elastography has recently attracted attention as a method of evaluating tissue elasticity. Shear wave elastography (SWE), specifically, is used to evaluate absolute elasticity by obtaining the elastic modulus from transverse waves propagating in tissue by aiming a pressure beam (acoustic radiation pressure, acoustic radiation force) produced by focusing ultrasonic waves. The elastic coefficient is obtained by capturing the propagation velocity of a shear wave caused by the resulting minute vibration [15–20]. As SWE could be used to reliably estimate muscle damage in patients with Duchenne muscular dystrophy (DMD) and cerebral palsy [21,22], we expected SWE to be a useful and minimally invasive evaluation tool in FCMD. In this study, SWE was used to assess upper limb muscle stiffness in patients with FCMD.

2. Patients and methods

2.1. Patients

Thirteen FCMD patients (8 boys and 5girls) and 20 healthy volunteers (9 boys and 11 girls) were enrolled in this study. All individuals were examined by visual muscle ultrasound analysis (VMUA) and SWE. This study was done as a pre-intervention study for FCMD patients.

FCMD was confirmed by genetic analysis of the genomic DNA of all patients. All patients had the SINE-VNTR-*Alu* retrotransposon (SVA) inserted in the causative gene. Twelve patients had homozygous SVA insertions, and one had compound heterozygosity with SVA insertions and point mutations in exon 3 [23].

2.2. Motor function analysis

One rehabilitation physician assessed motor function in all recruited patients using the Ueda classification (Table 1), the gross motor function measure (GMFM)[24], Hammersmith Motor Function Scale (HMFS)[25], and modified upper limb function scale. The modified Ueda classification divides the FCMD motor function into nine levels (Level 0 to 8) (Table 1), whereas HFMS is a simple and suitable scale for Spinal Muscular Atrophy patient [5,25]. The GMFM is a standardized observational instrument designed and validated to measure change in gross motor function over time in children with cerebral palsy, but now also applicable for FCMD patients [5,24]. The upper limb function measurement method reported by Matsuie (Table 2, *Matsuie Y. Rehabilitation of Duchenne muscular dystrophy. Sogo Riha 1987; 15:783–89. Article in Japanese*) is used to assess patients with DMD in Japan. This assessment classifies upper extremity motor performance into nine levels (Level 1 to 9) (Table 2). In this study, the FCMD motor function was organized into three groups. Patients who can stand or walk with or without support are considered mild, those who can sit or move in a sitting position without assistance were considered typical, and those who need help throughout life or with lack of head control were considered severe [25].

2.3. Muscle ultrasound examination method, Heckmatt scale, and quality indicator

All studies were performed by the same orthopedic surgeon, with 3 years of muscle ultrasound elastography experience. First, ultrasound intensity was evaluated in the longitudinal plane in B mode using an ultrasonic scanner (Aplio-500; Canon Medical Systems Corporation, Japan). Muscle ultrasound images were visually evaluated using a qualitative method, the 4-point Heckmatt scale for all FCMD patients muscle ultrasound data tested, which were developed by Heckmatt and Dubowitz, who proposed qualitative criteria in Duchenne muscular dystrophy [26]. Grade 1 represents normal echogenicity and grade 4 refers to an extremely fibrotic change, and appear hyperechoic with image qualities like bone and loss of bone reflection. The more closely muscle echotexture resembles bone under ultrasound, the greater this pattern represents infiltration of fat and fibrosis. Differentiating grades 2 and 3 as described is a major drawback of the original Heckmatt scale because a muscle may not be homogenously affected throughout its length, hindering precise distinctions [26]. In SWE mode, an ultrasonic scanner was used in combination with a linear transducer array (4–15 MHZ). This method quantifies the shear elastic modulus (stiffness) of a local region of tissue and produces a two-dimensional map of elasticity in real time (1 sample/sec). The color of the target site changes to blue, green, yellow, or red according to the muscle stiffness value indicated by SWE and approaches red as the muscle becomes stiffer [20].

All children were examined in a supine position and were instructed to lay down relaxed in a comfortable position during the examination. The examinee was accompanied by their guardians while enjoying music and videos. Capturing each muscle ultrasound image took approximately 5 seconds (Fig. 1A). A small amount of an ultrasound gel (Aquasonic 100, Parker Laboratories, New Jersey, USA) was used for better visualization. The probe was placed longitudinally to the long axis of each muscle.

Shear elastic modulus in the upper limb muscle was measured (right side): biceps brachii (BB); triceps brachii (TB); brachioradialis (BR); abductor pollicis brevis (APB); and abductor finger muscle (ADM). Briefly, in the BB, TB, and BR, the elbow was positioned at a 90° angle, and the hand was in the neutral position. For

Table 2
The Upper Limb Function Measure of Muscular Dystrophy.

1. Lift a weight ≥ 500 g vertically forward with the dominant hand
2. Raise a weight ≥ 500 g to the front 90 degrees with the dominant hand
3. Lift the dominant hand vertically forward without weight
4. Raise the dominant hand 90 degrees forward without weight
5. Flex the elbow of the dominant hand over 90 degrees without weight
6. Move the hand horizontally forward on the desk by extending the elbow
7. Move the hand horizontally forward on the desk by moving the trunk
8. Move the hand horizontally forward on the desk after extending the elbow by moving the trunk
9. Move horizontally forward only by hand motion on the desk

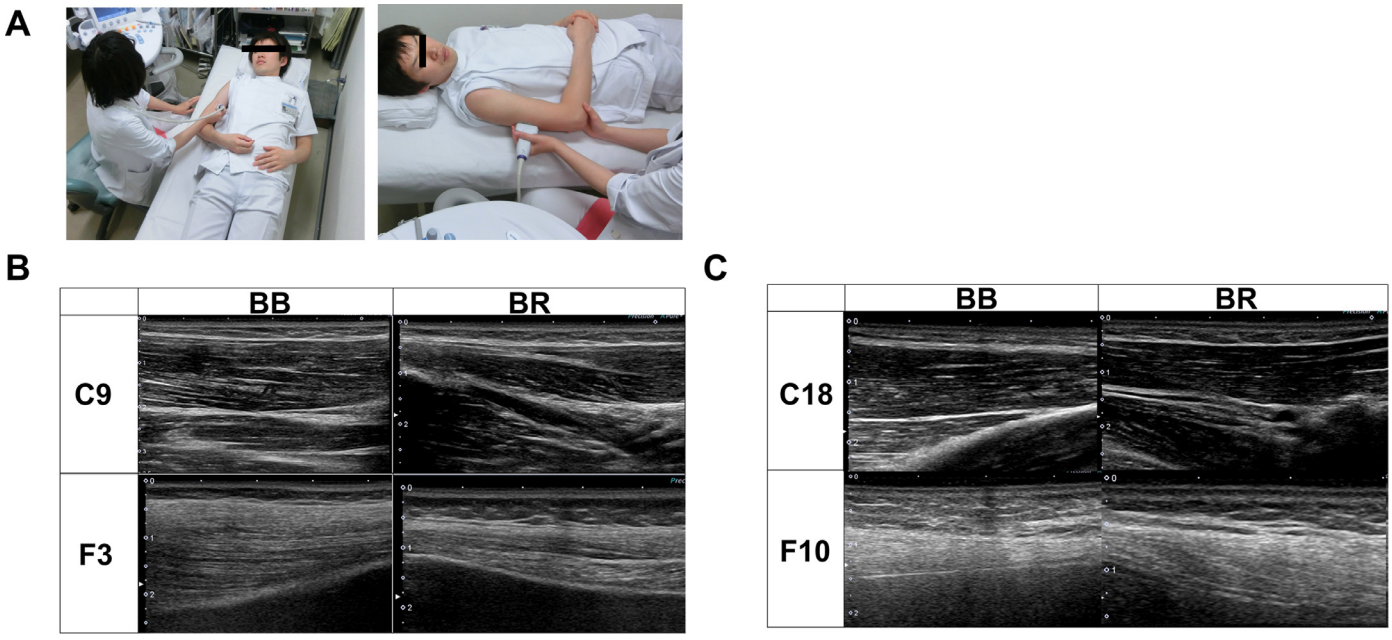

Fig. 1. Ultrasound examination
(A) Representative image of the performance of visual muscle ultrasound analysis with elastography. The left column shows the evaluation of the biceps brachii muscle. The right column shows the testing of the brachioradialis muscle. (B, C) Longitudinal grayscale muscle ultrasound image (B mode) of a healthy control (C) and patient with Fukuyama muscular dystrophy (FCMD) (F). BB, biceps brachialis; BR, brachioradialis. (B) Case C9: Imaging for a 7-year-old male healthy control. Case F3: Imaging for a 4-year-old male patient with FCMD. Longitudinal grayscale muscle ultrasound image of the right BB muscle. (C) Case C18: Imaging for a 15-year-old male healthy control. Case F10: Imaging of a 14-year-old male patient with FCMD. Longitudinal grayscale muscle ultrasound image of the right BB muscle. The muscle ultrasound images of muscles of patients with FCMD were brighter than those of the healthy controls.

APB and ADM, the elbow was flexed at 90°, with the wrist in the neutral position and with the hand open. (Fig. 1A). The measurements were registered in the widest and thickest part of the muscle belly—half-length in between the origin and insertion of each muscle [27,28]. For APB, since the muscle was not clearly identifiable probably due to muscle atrophy of the FCMD patients, we measured the thickest region of the muscle attached to the ulnar part of the first metacarpal bone. A region of interest (ROI) diameter of 5 mm was selected. No pressure was applied while probing the muscle. For each muscle, four-point measurements, triplicated, were averaged to obtain a representative value. 8 values out of 12 were averaged by removing outliers.

As a quality indicator (QI), IQR/median was used, and the score ≤ 30 % was assumed as reliable [29]. We mainly checked the quality of shear wave speed estimation using share wave quality map, in which we adopted the homogenous blue / green color (shown in Supplementary Fig. 1, left), and share wave propagation map, in which the lines were parallel to lines perpendicular to push pulse (shown in Supplementary Fig. 1, right), both of them indicate a reliable quality of shear wave speed estimation in the region of interest to measure shave wave velocity and shear modulus [30].

2.4. Statistical analysis

The Mann–Whitney U test was used for the comparison of continuous variables, and the chi-square test and spearman's rank correlation coefficient were used for comparison of categorical variables. P-values of <0.05 were considered statistically significant. Statistical analysis was performed using SPSS (version 19.0; IBM, Armonk, NY, USA).

2.5. Ethical considerations

The present study was approved by the Ethical Committee of our institute (Approval Number 1653-R4) and was conducted in strict adherence to the Declaration of Helsinki and Ethical Guidelines for Clinical Studies. Written informed consent for the tests were obtained from all patients or their guardians including those of the controls.

3. Results

Thirteen FCMD patients (median age, 9.6 years [range 1-17 years], including 8 boys and 5 girls) and 20 healthy controls

Table 3
Heckmatt scale, shear wave elastography, and motor functions of patients with Fukuyama muscular dystrophy.

	Age (yr)	Sex	Modified Ueda classifi-cation*	upper limb function score**	upper limb function	Hammer-smith function score	GMFM (total)	Heckmatt scale			Shear elastic modulus of the SWE															
											BB					BR					ADM				
					Weak (W) or Fair(F)		%	BB	BR	ADM	Ave.	SD.	MEDIAN	IQR	IQR/ median	Ave.	SD.	MEDIAN	IQR	IQR/median	Ave.	SD.	MEDIAN	IQR	IQR/ median
											(kPa)	(kPa)	(kPa)		(%)	(kPa)	(kPa)	(kPa)		(%)	(kPa)	(kPa)	(kPa)		(%)
F1	1	M	2	6	W	0	13.09	3	3	3	17.5	0.2	17.8	4.12 (16.01-20.13)	23.16	28.95	11.82	20.2	13.79 (11.67-25.46)	68.27	15.19	0.6	15.95	9.17 (9.78-18.94)	57.48
F2	3	F	4	4	F	16	21.9	4	4	3	22.38	0.52	20.45	7.91 (17.64-25.56)	38.7	35.81	1.31	37.36	17.55 (28.41-45.96)	46.98	25.25	1.67	27.11	24.34 (14.43-38.77)	89.8
F3	4	M	7	4	F	40	59.91	3	3	3	25.14	0.27	25.35	8.8 (20.59-29.39)	34.71	44.32	0.29	46.22	8.91 (40.06-48.97)	19.28	14.62	0.2	14.52	3.64 (12.34-15.98)	25.09
F4	4	M	1	5	F	2	9.25	3	3	3	20.51	0.49	20.6	11.06 (15.19-26.24)	53.68	42.55	0.27	42.76	7.02 (39.81-46.82)	16.41	32.08	1.42	33.7	24.76 (20.99-45.75)	73.46
F5	5	F	4	5	F	21	33.01	3	3	3	29.56	0.86	31.34	16.18 (22.44-38.62)	51.63	25.14	0.29	23.6	8.78 (21.51-30.29)	37.19	30.65	0.51	30.91	5.15 (26.5-31.64)	16.65
F6	6	M	4	2	F	25	35.55	3	4	3	23.04	0.93	19.78	15.06 (16.53-31.59)	76.1	49.94	0.63	50.78	18.99 (42.03-61.02)	37.39	22.96	0.16	23.86	4.45 (20.92-25.37)	18.65
F7	11	F	2	7	W	3	10.08	3	3	3	33.49	0.03	34.17	2.66 (32.38-35.04)	7.79	18.98	0.07	18.15	3.7 (17.72-21.43)	20.4	17.77	0.67	19.35	7.66 (12.16-19.81)	39.56
F8	13	M	0	8	W	0	0	4	4	3	101.66	0.19	104.08	5.98 (101.04-107.01)	5.74	84.87	0.88	79.41	8.8 (77.42-86.22)	11.08	18.86	0.16	17.57	3.57 (16.64-20.21)	20.33
F9	13	M	0	9	W	0	1.17	4	4	3	68.37	7.71	73.57	71.69 (35.53-107.22)	97.44	49.39	5.51	67.84	54.14 (24.29-78.43)	79.81	16.5	0.95	15.57	9.26 (11.33-20.59)	59.47
F10	14	M	0	9	W	0	0	4	4	3	50.86	0.41	46.45	15.28 (44.53-59.81)	32.9	49	0.96	47.88	14.86 (43.05-57.91)	31.04	49.76	0.24	50.68	9.65 (45.63-55.28)	19.04
F11	14	F	2	7	W	2	7.67	4	3	3	38.13	0.89	37.78	20.88 (28.14-49.03)	55.27	22.42	0.35	20.68	9.22 (18.6-27.82)	44.56	33.04	2.82	34.12	32.25 (18.27-50.52)	94.52
F12	14	M	4	4	F	11	14.77	3	3	3	17.1	0.16	17.35	4.64 (15.1-19.74)	26.73	18.06	0.04	18.23	2.27 (17.28-19.55)	12.43	15.27	0.4	15.97	7.13 (12.3-19.43)	44.64
F13	17	F	1	9	W	0	0.78	4	3	3	22.44	0.08	22.78	3.81 (20.05-23.86)	16.73	39.5	2.14	45.9	21.99 (29.8-51.79)	47.92	48.48	0.59	46.47	10.23 (43.27-53.5)	22.02

F1-13, Fukuyama-type congenital muscular dystrophy patient 1-13; M, Male; F, Female; Ave, Average; SD, Standard deviation; IQR, Interquartile ratio; BB, biceps brachii; BR, brachioradialis; ADM, abductor finger muscle.

(median age, 8.4 years [range 1-24 years], including 9 boys and 11 girls) volunteered to participate in this study (Tables 3 and 4). The age and sex of the patients in the two groups did not differ significantly ($P > 0.05$).

3.1. Motor function analysis of FCMD patients

The motor and upper limb functions of the patients were tested (Table 3). The patients with FCMD were divided into two groups according to the upper limb function score. Patients who could bend their elbows to the weight of their upper limbs were considered to have a "fair" phenotype (score: ≤5). Patients who were restricted only to movements on the desk were considered to have a "weak" phenotype (score: ≥6). The weak group showed a significantly lower Hammersmith Functional Motor Scale (HMFS) score compared with the fair group ($P = 0.018$, Table 3). Furthermore, patients in the weak group also had significantly lower GMFM scores ($P = 0.020$, Table 3), which was reported to be valid for measuring motor function of FCMD patients [5]. These results indicate that when the upper limb function is weak, the total motor function is lower compared with that in the fair group.

3.2. High ultrasound intensity by Heckmatt scale and SWE values in FCMD patients' skeletal muscle

The VMUA of muscles of patients with FCMD and healthy volunteers were compared. Patient F3 was able to walk, whereas patient F10 had difficulty sitting (Table 3) but both patients had significantly higher ultrasound intensity compared with the controls (Fig. 1B and 1C). Bedridden patients showed even higher ultrasound intensity, but all patients with FCMD had obscured muscle fibers and increased ultrasound intensity. The left columns of Fig. 2A and 2B show elastographic images of healthy controls and patients with FCMD, respectively. Muscular atrophy was very severe, as shown in Fig. 2B and muscle CT in Fig. 2.

We mainly followed the share wave quality map (left of Supplementary Fig. 1) and share wave propagation map (right of Supplementary Fig. 1) during the examination as a quality indicator (QI), we also checked the QI of SWE values using interquartile / Median scoring[29]. The shear modulus of elasticity of IQR/M was <30% in 66 % of the all values tested in healthy control (Table 4 and Supplementary Table 2), but 41 % in FCMD patients (Table 3 and Supplementary Table 1). In healthy controls, these scores of BB (90 %), BR (85 %) and TB (70 %) suggested high reliability, but, those of ADM (30 %) and APB (50%) had high range. In FCMD, these scores of BB (38%) and BR (38 %), TB (53 %), APB (30 %) and ADM (46 %) showed even wider range (54%). Elastography images for healthy volunteers were uniform and blue (Fig. 2A), but those for patients with FCMD had a slightly uneven color mixture of yellow, red, and green (Fig. 2B). Furthermore, the shear elasticity module of SWE in the FCMD group was higher than that in all healthy volunteers (Tables 3,4 and Supplementary Table 1, and 2). In particular, the SWE values for BB, BR, and APB were significantly higher than those of healthy volunteers (Fig. 3A). Furthermore, the SWE values for BB, TB, and BR were significantly higher in patients classified with a weak phenotype (Fig. 3B).

On the Heckmatt scale, all patients scored above 3 whereas all healthy volunteer scored 1 (Fig. 1 B, 1C, 2A, 2B, and Table 3, and Supplementary Table 1). A significant correlation was also found between the Heckmatt scale and GMFM ($r = 0.062$, $P = 0.024$).

When the correlation coefficient was obtained by the SWE value and each motor function scale, the correlation was recognized between the SWE value of BB and the Upper Limbs Functional Scale. (Supplemental Table 3A). The Mann–Whitney

Table 4
Visual muscle ultrasound analysis and shear wave elastography of healthy controls.

			BB					BR				ADM				
	Age (y)	Sex	Ave. (kPa)	SD. (kPa)	MEDIAN (kPa)	IQR	IQR/median (%)	Ave. (kPa)	SD. (kPa)	MEDIAN (kPa)	IQR	IQR/median (%)	Ave. (kPa)	SD. (kPa)	MEDIAN (kPa)	IQR	IQR/median (%)
C1	1	M	22.44	0.02	22.36	1.43 (21.59-23.02)	6.4	28.04	0.6	28.12	10.7 (22.82-33.52)	38.06	18.15	0.11	12.48	10.16 (10.84-21)	81.35
C2	1	M	19.74	0.15	19.89	4.06 (18.02-22.07)	20.4	16.8	0.16	17.65	4.47 (14.17-18.64)	25.34	14.92	0.13	0.16	0.06 (0.14-0.2)	39.06
C3	2	F	25.51	0.11	24.37	5.1 (23.31-28.41)	20.94	29.18	0.12	28.65	3.34 (26.73-30.07)	11.65	15.17	0.74	12.92	3.84 (11.94-15.78)	29.74
C4	3	F	12.08	0.2	13.04	1.97 (11.67-13.64)	15.1	27.05	0.07	26.3	5.01 (24.67-29.68)	19.04	18.25	0.18	0.23	0.09 (0.18-0.27)	38.04
C5	4	F	27.41	0.21	27.21	6.61 (24.03-30.64)	24.28	19.3	0.14	20.01	4.47 (17.4-21.87)	22.31	13.14	0.46	12.92	5.43 (9.91-15.34)	42
C6	5	M	23.65	0.27	23.52	4.62 (21.56-26.18)	19.65	16.85	0.14	17.01	4.65 (14.03-18.68)	27.32	21.57	0.58	20.7	10.3 (16.44-26.74)	49.75
C7	5	F	14.52	0.15	14.4	2.8 (13.2-15.99)	19.41	16.74	0.08	16.43	1.53 (15.94-17.47)	9.29	41.01	0.2	41.07	3.82 (39.2-43.03)	9.31
C8	5	F	35.58	0.29	36.02	7.82 (31.41-39.23)	21.71	19.7	0.14	19.15	5.33 (16.96-22.29)	27.83	22.75	0.41	0.25	0.18 (0.13-0.31)	72
C9	7	M	17.37	0.07	18.38	2.72 (15.81-8.53)	14.82	18.43	0.06	18.38	2.79 (17.29-20.09)	15.2	21.51	0.53	21.77	11.09 (16.02-27.11)	50.96
C10	7	M	38.15	1.4	39.01	17.46 (27.94-45.4)	44.75	31.91	0.18	32.18	6.14 (29.02-35.16)	19.07	21.95	0.16	22.97	6.92 (18.53-25.45)	30.13
C11	8	F	14.75	0.02	14.99	0.96 (14.43-15.39)	6.43	19.62	0.28	20.61	4.45 (18.2-22.65)	21.58	27.79	0.16	27.55	4.75 (25.15-29.9)	17.25
C12	8	F	30.15	0.57	28	10.4 (24.24-34.64)	37.13	20.34	0.44	18.3	10.2 (15.98-26.18)	55.72	12.2	0.56	12.46	5.31 (8.85-14.16)	42.63
C13	9	F	18.23	0.25	12.67	3.44 (11.09-14.53)	27.15	14.39	0.12	13.17	1.71 (12.33-14.04)	12.96	11.38	0.17	21.64	12.25 (16.5-28.75)	56.62
C14	9	M	12.9	0.16	17.79	4.8 (15.22-20.02)	26.95	13.12	0.06	13.81	4.32 (12.67-16.99)	31.32	22.65	0.7	11.12	3.23 (9.82-13.04)	29.01
C15	12	M	14.03	0.04	14.32	1.31 (13.68-14.99)	9.12	12.36	0.12	12.43	2.34 (10.92-13.26)	18.84	14.54	0.24	15.11	6.01 (12.18-18.19)	39.78
C16	13	F	16.2	0.16	16.29	4.45 (13.58-18.03)	27.33	17.5	0.26	16.57	2.7 (16.32-19.02)	16.29	23.79	0.35	23.6	10.55 (19.17-29.72)	44.71
C17	14	F	17.88	0.15	17.72	5.03 (15.26-20.29)	28.38	16.25	0.08	16.36	1.41 (15.73-17.14)	8.63	18.77	0.1	18.45	2.42 (17.71-20.13)	13.1
C18	15	M	18.14	0.27	18.49	5.49 (15.39-20.87)	29.67	15.39	0.04	15.19	2.41 (14.16-16.57)	15.86	17.84	0.19	17.43	2.75 (15.7-18.45)	15.79
C19	15	F	16.83	0.07	16.86	3.12 (15.16-18.27)	18.49	13.93	0.14	13.04	3.04 (12.3-15.35)	23.32	20.3	0.2	20.59	6.89 (16.89-23.77)	33.44
C20	23	M	38.93	0.09	39.42	4.35 (37.67-42.02)	11.04	10.41	0.06	10.11	1.85 (9.27-11.12)	18.32	28.41	0.69	28.62	14.35 (21.07-35.42)	50.13

C, healthy controls; M, Male; F, Female; Ave, Average; SD, Standard deviation; IQR, Interquartile ratio; BB, biceps brachii; BR, brachioradialis; ADM, abductor finger muscle.

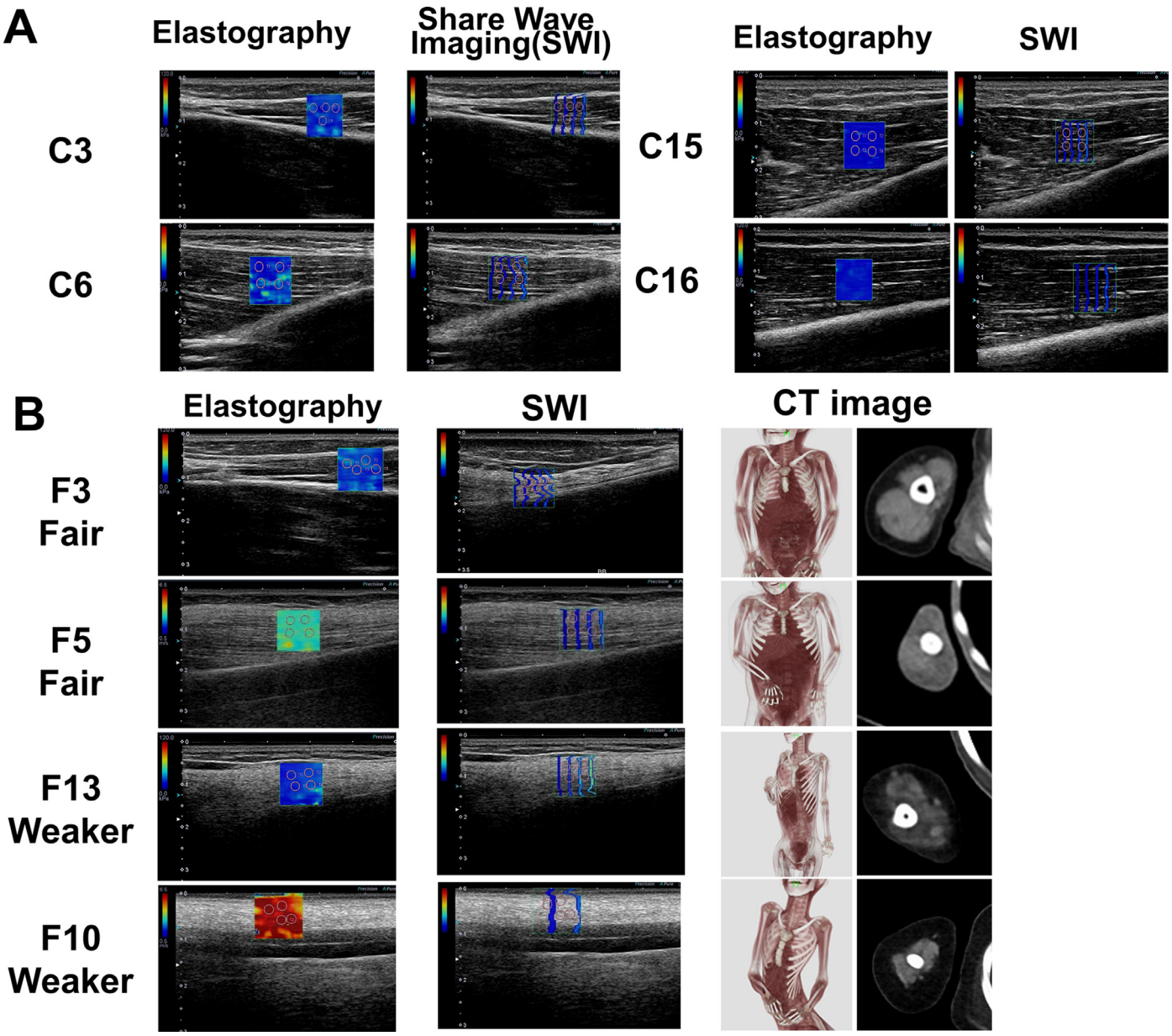

Fig. 2. Elastography and computed tomography imaging. (A) Elastography and shear wave imaging of healthy controls. The color of the target area was all blue (low elasticity). (B) Elastography, shear wave imaging, muscle computed tomography (CT), and arm CT of patients. The color of the target area was uneven and mixed. The two right columns show muscle CT and arm CT. Muscles of patients with FCMD were atrophic and became more atrophic in older patients.

U test of SWE scores between weak phenotype (W) and fair phenotype (F) also revealed a superiority difference in APB ($P = 0.022$) but none in BB, although the tendency was recognized ($P = 0.073$) (Supplementary Table 3B).

4. Discussion

In this study, we showed that ultrasound imaging and SWE can be useful in monitoring the progress of muscle atrophy and upper limb function in patients with FCMD. Since the upper motor function and the SWE score as well as Heckmatt scales was correlated, the evaluation of the upper limbs may help evaluate not only the muscle degeneration of present upper limbs but also the systemic motor function of each patient. Ultrasonography is a minimally invasive, low-cost tool without radiation exposure or pain; thus, it is a good tool for monitoring muscle condition or the effects of treatment in patients with FCMD, especially for patients with weaker muscle phenotype group. In our current experiment, patients' visual muscle ultrasound analysis and SWE were only measured once as a pre-intervention stage, but the follow-up examination of muscle ultrasound needs to continue to obtain the natural course of each patient data, which will be beneficial for clinical trial assessment. Moreover, we only assessed upper extremities. 90 % of FCMD patients are not able to walk, and the joint contractures are severer in the lower extremities, their echo intensity of lower extremity shows severe fat replacement from early infancy. In this study, we considered that the change of SWE of upper extremities were more comparable to detect changes between FCMD and control patients. However, including the US measurement of lower extremities will be also beneficial for clinical trial. The recovery of muscle tissue might be seen by checking the ultrasound imaging and SWE without having invasive muscle biopsy which is now required for these patients to assess the recovery.

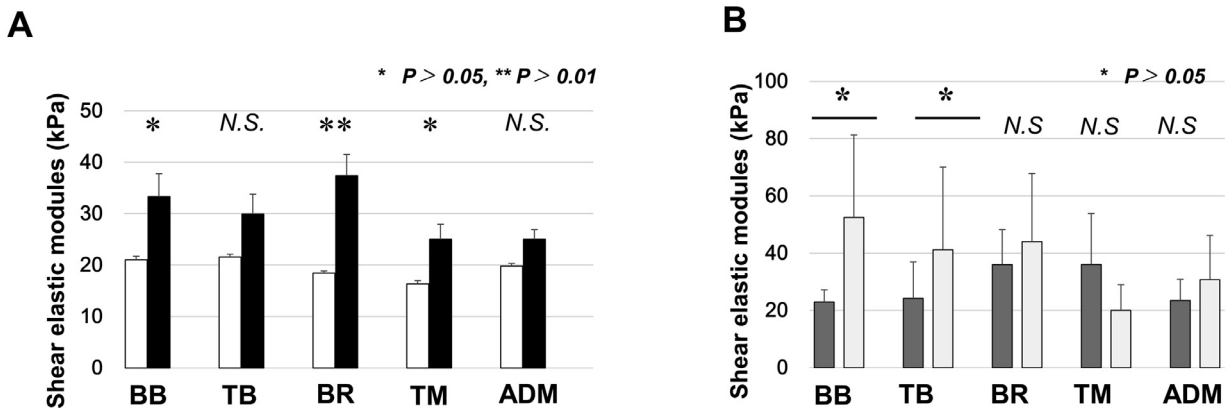

Fig. 3. Shear elastic modulus values of the upper limbs in healthy controls and patients with FCMD. (A) Shear elastic modulus values for BB, BR, and ADM in patients with FCMD were significantly higher than those in normal controls. (*P < 0.05, **P < 0.01). Error bar, standard deviation (S.D.); open bar, controls; black bar, patients with FCMD. (B) Shear elastic modulus values of the upper limbs in patients with FCMD. The shear elastic modulus values for BB and TB were significantly higher in the weak phenotype than in the fair phenotype. (*P < 0.05) Error bar, S.D.; gray bar, patients with fair phenotype (patients were able to flex their elbow against their upper limb weight); off-white bar, patients with a weaker phenotype (movement was limited to movement on the desk). BB, biceps brachii; TB, triceps brachii; BR, brachioradialis; ADM, abductor finger muscle.

Muscle ultrasound images of patients with FCMD clearly showed a higher ultrasound intensity and a higher Heckmatt scale score compared with healthy volunteers. The myofibers were not clearly arranged in longitudinal, parallel layers fiber, as observed in all healthy volunteers. Increased ultrasound intensity in FCMD muscle indicates increased muscle fat and fibrosis, since ultrasound intensity was affected by the stiffness of the tissue. In FCMD, muscle atrophy and retained tissue were replaced with fat infiltration fibrosis [31]. Patients with FCMD show severe degenerative atrophy of muscle fibers combined with fat and connective tissue replacement. The high intensity of the ultrasound may be the result of obscurity due to degenerative atrophy of muscle fibers, connective tissue, and fat infiltration. In fact, an increase in muscle ultrasound intensity is associated with muscle fat replacement [32]. Muscle ultrasound evaluation in muscular disease was also performed in DMD and revealed a higher ultrasound intensity than that in healthy controls [33]. Although quantification has a higher degree of agreement between observers and is more sensitive and suitable for screening, visual evaluation has a higher degree of specificity and is considered a useful method if the characteristics are understood. In addition, only one observer was involved in this study, and the error seems to be relatively small. The Ueda classification of the patient in Fig. 1A was mild type, but the ultrasound intensity was brighter than those of healthy volunteers. The results of this study suggest that degenerative muscular atrophy may have already progressed before the deterioration of motor function. Previous reports have also shown that muscle ultrasound can detect smaller structural changes in limb-girdle muscular dystrophy [34]. The evaluation of muscle ultrasound indicates that it may be useful for the early diagnosis and follow-up of patients with FCMD.

The SWE values of FCMD patients were mainly higher in the BB, BR, and APB. A study comparing the SWE levels in DMD patients and healthy volunteers noted that the muscles in patients with muscular dystrophy had higher SWE levels and higher muscle stiffness compared with muscles in healthy controls [21]. The same was true in children with cerebral palsy [22]. We hypothesize that this is because as the muscle mass decreases, the motor function decreases, joint contracture occurs, and muscle rigidity increases. This finding indicates that the same mechanism occurs in FCMD.

Regarding TB and ADM, patients with FCMD tended to have higher SWE values, but the differences were not statistically significant. This may be because the probe was not suitable due to contracture in patients' fingers. This study measured the elasticity of TB in the flexed position of the elbow joint with stretched muscles. The FCMD group showed higher values in both ADM and TB. Finger-friendly probes are thus required; however, the difference between patients with FCMD and controls may be more apparent with a larger study population. In addition, SWE values for BB, TB, and BR were significantly higher in patients with severe upper limb dysfunction in the FCMD group; thus, SWE values may be useful in monitoring upper limb dysfunction. No significant difference was found in the APB and ADM of patients and controls because this classification of the upper limb function did not reflect finger function. SWE is known to increase with age in adults and is slightly higher in women [35]. However, the values in children are unknown. In this study, no relationship was found between age and the SWE value and between sex and SWE value in the control group. Since FCMD is a rare disease, and we saw around fifty patients even in our facility where we see the second largest numbers of the FCMD patients in Japan. We chose 13 patients who were able to join our study (could visit us regularly and was cooperative to the study). However, due to the small study population, further studies involving a larger pediatric study population are necessary. Moreover, no difference was observed in the age and sex ratios between the FCMD and control groups, which indicates the low involvement of these factors.

Furthermore, examination using three motor function indexes in this study revealed a correlation between upper limb function and systemic motor function in patients with FCMD. Moreover, the evaluation of the biceps brachii and brachioradialis muscles was particularly useful in the assessment of SWE. That is, in patients with FCMD, the evaluation of the biceps brachii and brachioradialis muscle may help evaluate not only upper limb function, but also systemic motor function. The ease of assessing a single site rather than multiple sites also reduces the burden on the patient. Furthermore, ultrasound sonography can be repeated because it is painless.

FCMD is one of the most severe intractable diseases in infants. It is associated with muscle and central nervous system involvement and intellectual impairment. Therefore, a proper evaluation of motor function is often difficult, and cooperation with time-consuming medical examinations and imaging tests such as MRI can be challenging to obtain. Muscle ultrasound has been the most straightforward and safest imaging test to date. The time required to perform muscle ultrasound is as short as

≤10 minutes, and it does not require sedation. Muscle ultrasound analysis can be performed while observing the patient's condition and is exceptionally minimally invasive. Patients are often able to relax and be tested by their parents during the SWE measurement. It is a low-cost, minimally invasive, and repeatable quantitative evaluation; thus, it is an excellent tool for evaluating patients over time. It also enables the detection of a dynamic change in muscle condition. On the other hand, the examiner needs a certain amount of training to make an accurate evaluation, and differences among examiners cannot be denied. However, this dilemma will improve with developments in ultrasound technology, the spread of this method to general medicine, and increased proficiency of utilizing VMUA by examiners.

Our study has some limitations. First, the study population was relatively small. Second, the probe for measuring SWE was not designed for adults. Therefore, the SWE range was high, especially in small muscles. In FCMD, patients had severe joint contractures since the tension caused by the contracture might affect the SWE score more than the healthy controls. The high range of QI might be narrowed by appropriate probing or sedating patients to keep muscles relaxed. Third, we did not compare the errors in SWE measurements among multiple examiners. Fourth, the muscle tissue tested are restricted to a few muscle that are superficial, the image and SWE score could be affected by patients' postures or the probe directions more easily than MRI or CT

This study found that measuring muscle elasticity in FCMD patients using muscle ultrasound and SWE values may be useful in monitoring muscle pathology, upper limb dysfunction, and general motor function. FCMD is caused by a splicing abnormality, and the possibility of curative therapy for skeletal muscle using antisense nucleic acids to correct abnormal splicing has been reported [36,37]. A clinical trial for this antisense therapy has been just started. A tool that enables a more accurate and less invasive evaluation is essential for clinical trials. For the management of patients with FCMD, muscle ultrasound evaluation and SWE measurement may be applied for various purposes, from evaluating the patient's the condition to determining the effect of treatments administered. It may also be useful for other muscle disorders. Therefore, this study provides preliminary evidence of altered muscle stiffness in patients with FCMD. Further long-term research is needed to confirm the results.

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Declaration of Competing Interest

The authors declare no conflicts of interest in association with this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2022.05.004.

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