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Cardiomyopathy in children with mitochondrial disease: Prognosis and genetic background☆

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ABSTRACT

Background: Cardiomyopathy is a reported indicator of poor prognosis in children with mitochondrial disease. However, the association between prognosis and the genetic background of cardiomyopathy in children with mitochondrial disease has yet to be fully elucidated.
Methods and results: Of 137 children with mitochondrial disease whose genetic diagnosis was made between 2004 and 2018, 29 had mitochondrial cardiomyopathy (21%). After a median follow-up of 35 months, the overall survival rate was significantly lower in patients with cardiomyopathy than in those without ($p < 0.001$). Ten-year Kaplan-Meier estimates of overall survival were 18 and 67%, respectively. Among the 21 cardiomyopathy patients who died, two died within one month of birth (*COQ4* in one patient, and *COX10* in one patient), ten died within one year (*BOLA3* in three patients, *QRSL1* in two patients, large chromosomal deletions in two patients, *MT-ATP6/8* in one patient, *MT-TL1* in one patient, and *TAZ* gene in one patient), and nine died after one year (*MT-ND5* in three patients, *MT-TL1* in three patients, *ACAD9* in one patient, *KARS* in one patient, and *MT-TV* in one patient). In the three patients with mitochondrial DNA mutations whose cardiac tissues were available, high heteroplasmy rates in the cardiac tissue were observed for m.8528T>C (90%, died at 2 months of age) and m.3243A>G (90 and 80%, died at 12 and 13 years of age, respectively).
Conclusions: In children with mitochondrial disease, cardiomyopathy was common (21%) and was associated with increased mortality. Genetic analysis coupled with detailed phenotyping could be useful for prognosis.

1. Introduction

Mitochondrial diseases, or disorders of the mitochondrial respiratory chain (MRC) complexes, are caused by defects in oxidative phosphorylation (OXPHOS) [1,2]. The MRC complexes, embedded within the inner mitochondrial membrane, are composed of five enzyme complexes. Energy generated by MRC complexes is used to produce ATP through OXPHOS. Proteins of MRC complexes are under the control of both nuclear and mitochondrial DNA; defects in those can result in OXPHOS dysfunction [3]. The heart is highly dependent on oxidative metabolism, therefore, cardiac involvement in mitochondrial disease is commonly observed and can present as a major clinical symptom or as part of a multisystem disorder [4]. Among children with mitochondrial disease, those with cardiomyopathy have been reported to have a worse prognosis [5]. However, the association between prognosis and the genetic background of cardiomyopathy in children with

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mitochondrial disease has yet to be fully elucidated. Therefore, we investigated the prognosis and genetic background of cardiomyopathy in children with mitochondrial disease.

2. Methods

2.1. Patients

A total of 137 children under the age of 15 years old were included in this study. Between 2004 and 2018, pediatricians and neurologists throughout Japan referred patients with suspected mitochondrial respiratory chain complex deficiency, due to clinical manifestations compatible with mitochondrial disease, to Saitama Medical University or Chiba Children's Hospital for enzyme assay and/or genetic analysis. All patients had their diagnosis genetically confirmed with known pathogenic nuclear DNA mutations or mitochondrial DNA mutations, confirmed by Sanger sequencing, or large chromosomal deletions, confirmed by high-density oligonucleotide arrays. Written informed consent was obtained from the parents of each patient. Both institutions received approval for enzyme analysis and genetic analysis from their appropriate ethics review boards. Detailed clinical data, including present illness, family history, laboratory findings, and cardiovascular findings, were submitted by referral doctors. Among 137 patients, 29 children with mitochondrial disease (21%) were diagnosed with cardiomyopathy on cardiac ultrasound, or histopathological evaluation if the cardiac tissue was available.

2.2. Diagnosis of cardiomyopathy

Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), and left ventricular noncompaction (LVNC) were diagnosed based on echocardiography. Children with mitochondrial disease were considered to have HCM when the left ventricular myocardium was generally or regionally hypertrophic on two-dimensional echocardiography and the dimension of the left ventricular posterior wall in end-diastole and/or the intraventricular septum in end-diastole was ≥2 SD (standard deviation) greater than normal values, shown as a Z-score ≥ 2. Children were diagnosed with DCM if the end diastolic left ventricle dimension was ≥2 SD greater than normal, shown as a Z-score ≥ 2. Diagnosis of LVNC was based on two-dimensional echocardiography. For the patients with fetal onset, fetal echographic findings of HCM and LVNC were used for the diagnosis. For the autopsy cases, HCM was diagnosed with prominent hypertrophy of the left ventricle and endocardial fibroelastosis (EFE) was diagnosed based on the histological finding of a thickened endocardium.

2.3. Genetic analysis

All patients included in the study underwent at least one of the following genetic analyses:

(1) Investigation for common point mutations on mitochondrial DNA. This was performed on patients with clinical presentations consistent with Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, Stroke-like episodes (MELAS).
(2) Whole mitochondrial DNA screening. The detailed procedures for this screening have been previously reported [3].
(3) Investigations for mutations using a targeted gene panel of 241 genes known to cause mitochondrial diseases, as well as the whole mitochondrial genome.
(4) Whole-exome sequencing using next-generation sequencing for nuclear DNA mutations. Detailed procedures have been previously described [3].
(5) High-density oligonucleotide array for large chromosomal deletions, as previously described [3].

2.4. Measurement of mitochondrial DNA heteroplasmy rate

The heteroplasmy rates, or the rate of mutated mitochondrial DNA over total mitochondrial DNA, of the m.3243A>G mutations at available tissues were analyzed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) as described previously [6] with a modified protocol. Briefly, PCR was performed with patient DNA using ExTaq Polymerase (Takara, Shiga, Japan). Detailed PCR protocol was as follows: 94 °C for 2 min; 40 cycles of 94 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s; and 72 °C for 5 min. Primer sequences for PCR were CCTCCCTGTACGAAAGGACA and GGGCCTTTGCG TAGTTGTAT. The PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega, Fitchburg, WI, USA), and 300 ng were subjected to restriction enzyme *Apa*I digestion at 37 °C for 2 h. Fragment length and those molar concentration was measured by TapeStation2200 and D1000 ScreenTape (Agilent, Santa Clara, CA, USA). The heteroplasmy rate was calculated using the following formula: average of digested lower band/(digested lower band + remaining undigested band) and digested upper band/(digested upper band + remaining undigested band). The heteroplasmy rates of m.13513G>A, m.1644G>A, m.3302A>G were analyzed using a protocol similar to that used for m.3243A>G. Primer sequences used for PCR were: m.13513G>A, AGCTAG CTAGCTAGCTAGCTTTCCTCACAGGTTTCTACTCCAGA and CAGGCGTTTAATGGGGTTTA; m.3302A>G, CACCCAAGAACAGGGTTTGT and TAGATGTGGCGGGTTTTAGG; and m.1644G>A, GGTCGAAGGTGGATTTAGCA and GGGTTTGGGGCTAGGTTTAG. Restriction enzyme *Bsa*I (New England Biolabs Inc., Ipswich, MA, USA) was used to cut the wild type m.13513G>A sequence, *Apo*I (New England Biolabs Inc.) was used to cut the mutant m.1644G>A sequence, and *Dde*I (New England Biolabs Inc.) was used to cut the mutant m.3302A>G sequence. Using a previously described primer [7], the heteroplasmy rates of m.8528T>C mutations were measured as reported previously [8,9].

2.5. Biochemical analysis

The activity of MRC complexes I, III, and IV was measured in mitochondria isolated from skin fibroblasts, or in the crude supernatant following centrifugation at 600g from available tissue samples, using previously reported measures [10,11]. The enzyme activity of each complex was described as the percentage of normal control mean relative to appropriate reference enzyme activities, such as citrate synthase or MRC complex II. Enzyme activity was defined as being decreased at <40% in a cell line or <30% in a tissue, as previously described [12].

The cellular oxygen consumption rates (OCR) of fibroblast-derived cell lines were measured using microscale oxygraphy (Seahorse XF96 system; Seahorse Bioscience, Billerica, MA, USA) in cases with negative enzyme assay results. Material was prepared as previously described [13]. After measurement of the basal OCR, oligomycin, carbonyl cyanide phenylhydrazone, and rotenone were added sequentially, and OCR was recorded after each addition. Maximum respiration rate (MRR) corresponds to the OCR after the addition of carbonyl cyanide phenylhydrazone minus rotenone-insensitive OCR [14]. Samples were measured in a 96-well plate, using 16 wells for each sample. Each sample's data were normalized as 20,000 cells per well. We analyzed five control samples, measuring each one at least five times. Cells in passages five through nine were used for controls and patient samples. In each run, we measured one or two controls with patient samples. The OCR was expressed as percentage relative to the average of controls.

2.6. Statistical analysis

Differences in patient characteristics were compared with the Mann-Whitney *U* test for continuous variables and the chi-squared test for categorical variables.

The Z-scores for echocardiographic parameters were calculated from equations given in a previous study [15]. Overall survival was calculated and presented as Kaplan–Meier curves. Differences in survival rate were tested by log-rank test and $p < 0.05$ was considered significant. Comparison of baseline characteristics and all survival analysis calculations were made using IBM SPSS statistics version 22 (IBM, Armonk, NY, USA).

3. Results

3.1. Patient characteristics

A summary of clinical, genetic, and biochemical findings in 29 children with mitochondrial disease and cardiomyopathy is given in Table 1. Characteristics of the total study populations are summarized in Supplementary Table S1. Between patients with or without cardiomyopathy, sex did not significantly differ (48% and 56%, for males and females, respectively, $p = 0.49$). However, earlier age of onset is observed in patients with cardiomyopathy compared with those without cardiomyopathy (median 30 days [interquartile range: IQR, 1–135 days] and 240 days [IQR, 60–675 days], respectively, $p < 0.001$).

3.2. Diagnosis

Of 29 cardiomyopathy children with mitochondrial disease, 22 had HCM, five had DCM, one had LVNC and one had the dilated form of EFE. Among 29 patients with cardiomyopathy, three had a prenatal onset and the remaining 26 patients varied in time of onset from the day of birth to 11 years old (median onset of 1 month). For 12 patients, cardiomyopathy was the major symptom; among these patients, specific diagnosis of Barth syndrome with pathogenic mutations in *TAZ* gene was made in 4 patients. Of the remaining 17 patients, four were diagnosed with Leigh disease (LD), three with lethal infantile mitochondrial disease (LIMD), three with non-lethal infantile mitochondrial disease (NLIMD), six with mitochondrial cytopathy (MC), and one with combined malonic and methylmalonic aciduria (CMAMMA). Cardiac echographic findings are summarized in Table 2.

In addition to the 29 patients with cardiomyopathy, four children were diagnosed with other abnormal cardiac phenotypes. One patient (Pt0523), diagnosed as MELAS with m.3243A>G mutation, had Wolff-Parkinson-White (WPW) syndrome and is still alive (10 years and 6 months old). Another patient (Pt0587) was diagnosed with LIMD with a large chromosomal deletion (22q11.21del), had cardiac

Table 1
Clinical, genetic, and biochemical features of mitochondrial disease patients with cardiomyopathy.

ID (sex)	Clinical features					Enzymatic and genetic defects			
	DDx	Onset	Initial symptom	Outcome (age)	CM type	Enz (OCR)	Gene	Variants (heteroplasmy rate)	Ref
Pt0090ES (F)	NLIMD	d14	Tachypnea	Alive (10 y 8 m)	HCM	CI	ACAD9 (NM_014049)	c.1150G>A:p.V384M, c.1817T>A:p.L606H	[3]
Pt0090YS (F)	NLIMD	d01	Poor suckling	Dead (1 y 6 m)	HCM	CI	ACAD9 (NM_014049)	c.1150G>A:p.V384M, c.1817T>A:p.L606H	[3]
Pt0268 (F)	LIMD	d24	Lactic acidosis	Dead (11 m)	HCM	CC	BOLA3 (NM_212552)	c.287A>G:p.H96R, c.287A>G:p.H96R	[3]
Pt0314 (F)	CM	d83	Vomiting	Dead (3 m)	HCM	CC	BOLA3 (NM_212552)	c.287A>G:p.H96R, c.287A>G:p.H96R	[3]
Pt1366 (F)	LD	m06	Myocardial hypertrophy	Dead (11 m)	HCM	CC	BOLA3 (NM_212552)	c.A287G:p.H96R c.A287G:p.H96R	[3]
Pt0113 (F)	LIMD	Prenatal	Myocardial hypertrophy	Dead (1 d)	HCM	CC	COQ4 (NM_016035)	c.718C>T:p.R240C c.421C>T:p.R141X	[3]
Pt0223 (F)	CM	d03	Lactic acidosis	Dead (12 d)	HCM	CC	COX10 (NM_001303)	c.862G>A:p.G288R, c.1259C>T:p.P420L	[3]
Pt0459 (M)	MC	m04	Nystagmus	Dead (4 y 8 m)	HCM	CC	KARS (NM_005548)	c.1343T>A:p.V448D, c.953T>C:p.I318T	[3]
Pt0250EB (M)	CM	d02	Poor suckling	Dead (5 m)	HCM	CC	QRSL1 (NM_018292)	c.398G>T:p.G133V, c.398G>T:p.G133V	[3]
Pt0250YS (F)	CM	d01	Lactic acidosis	Dead (5 m)	HCM	CC	QRSL1 (NM_018292)	c.398G>T:p.G133V, c.398G>T:p.G133V	[3]
Pt0049 (M)	CM	d00	Hyperventilation	Alive (11 y 1 m)	DCM	CI	TAZ (NM_000116)	c.280C>T:p.R94C (hemizygous)	[18]
Pt0081 (M)	CM	m04	Failure to thrive	Dead (4 m)	EFE	CI	TAZ (NM_000116)	c.646G>A:p.G216R (hemizygous)	[16]
Pt1333 (M)	CM	Prenatal	Cardiac enlargement	Alive (4 y 5 m)	LVNC	–	TAZ (NM_000116)	c.109 + 6T>G (hemizygous)	[16]
Pt1492 (M)	CM	m04	Poor suckling	Alive (1 y 8 m)	DCM	ns (→)	TAZ (NM_000116)	c.589G>A:p.G197R (hemizygous)	[17]
Pt1719 (F)	CMAMMA*	y10	Arrhythmia	Alive (15 y 9 m)	DCM	ns (→)	TOP3A (NM_004618)	c.527C>T:p.A176V c.1072_1073dup:p.Y359fs	[21]
Pt0311 (F)	CM	d02	Lactic acidosis	Dead (2 m)	HCM	CI	MT-ATP6/8	m.8528T>C (90%(C)/86%(F)/88%(H,B)/91%(M))	[24]
Pt1809 (M)	MC	m06	Poor weight gain	Alive (1 y 8 m)	HCM	ns (↓)	MT-ATP6/8	m.8528T>C (15%(F)/59%(B))	[24]
Pt0248 (M)	MC	y11	Edema	Alive (21 y)	HCM	CI	MT-ND1	m.3302A>G (41%(M))	[24]
Pt0450 (M)	NLIMD	m04	Vomiting	Alive (7 y 5 m)	HCM	CC	MT-ND5	m.13513G>A (29%(F))	[24]
Pt0467 (M)	LD	m01	Poor suckling	Dead (2 y 5 m)	HCM	CI	MT-ND5	m.13513G>A (43%(B))	[24]
Pt0806 (M)	LD	m05	Epilepsy	Dead (9 y 10 m)	HCM	CI	MT-ND5	m.13513G>A (66%(M))	[24]
Pt1466 (F)	LD	m03	Exotropia	Dead (1 y 10 m)	HCM	CI	MT-ND5	m.13513G>A (26%(F)/49%(B))	[24]
Pt0556 (M)	MC	y04	Short stature	Dead (12 y)	HCM	CI	MT-TL1	m.3243A>G (90%(C)/70%(B))	[24]
Pt0856 (M)	MC	y01	Arrhythmia	Dead (13 y)	HCM	CC	MT-TL1	m.3243A>G (80%(C)/87%(K))	[24]
Pt1204 (F)	CM	Prenatal	Myocardial hypertrophy	Dead (2 m)	HCM	CC	MT-TL1	m.3243A>G (77%(F))	[24]
Pt1483 (F)	MC	m07	Developmental delay	Dead (3 y 6 m)	DCM	CI	MT-TL1	m.3243A>G (61%(F))	[24]
Pt0456 (F)	CM	d00	Asphyxia	Dead (3 y 10 m)	HCM	CC	MT-TV	m.1644G>A (14%(F))	[24]
Pt1324 (M)	LIMD	d00	Asphyxia	Dead (7 m)	HCM	ns (↓)	–	1p36.33del	[22]
Pt0452 (F)	CM	m01	Poor weight gain	Dead (1 y)	DCM	CIV	–	6q24.3-25.1del	[3]

DDx: diagnosis, CM: cardiomyopathy, Enz.: enzyme activity, OCR: oxygen consumption rate, Ref.: reference, F: female, M: male, ES: elder sister, YS: younger sister, EB: elder brother, NLIMD: non-lethal infantile mitochondrial disease, LIMD: lethal infantile mitochondrial disease, LD: Leigh disease, MC: mitochondrial cytopathy, *CMAMMA: combined malonic and methylmalonic aciduria due to ACSF3 (NM_174917) mutations (c.689G>A:p.W230X/c.1266delG:p.E422fs), HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, EFE: endocardial fibroelastosis, NC: left ventricular noncompaction, d: days, w: weeks, m: months, y: years, CI: complex I deficiency, CC: combined complex deficiencies, CIV: complex IV deficiency, ns: not significantly reduced, OCR (↓): decreased oxygen consumption rate, OCR (→): normal oxygen consumption rate, C: cardiac tissues, F: skin fibroblasts, H: hepatic tissues, M: skeletal muscles, B: blood, K: kidney.

anomalies (Ventricular septal defect (VSD), atrial septal defect (ASD), bicuspid aortic valve, tortuous pulmonary artery), and died at 5 months old. Another patient (Pt0695) was diagnosed with mitochondrial liver disease with a large chromosomal deletion (6q24.3-6q25.1del) and a cardiac anomaly (small VSD, patent foramen ovale (PFO), patent ductus arteriosus (PDA)); this patient died 23 days after birth. Patient (Pt1401) was diagnosed with Barth syndrome with a pathogenic mutation in the *TAZ* gene. This patient suddenly grunted without any previous symptom at 8 months and shortly developed ventricular fibrillation, which lead to his death after 3 days [16].

Table 2
Cardiac echographic findings of patients with cardiomyopathy.

ID (sex)	CM type	LVPWd (mm) (Z-score)	IVSd (mm) (Z-score)	LVOT obstruction	LVIDd (mm) (Z-score)	FS (%)	EF (%)	Valve insufficiency
Pt0090ES (F)	HCM	8.2 (4.9)	7.3 (2.9)	No	24.2 (1.0)	41.3	74.5	No
Pt0090YS (F)	HCM	5.3 (3.4)	4.6 (1.2)	No	18.1 (0.3)	40.3	74.3	No
Pt0113 (F)	HCM (fetal)	–	–	–	–	–	–	–
Pt0223 (F)	HCM (autopsy)	–	–	–	–	–	–	–
Pt0248 (M)	HCM	9.0 (5.0)	8.0 (3.0)	No	–	–	71.0	–
Pt0250EB (M)	HCM	1.0 (5.9)	–	No	–	–	70.0	–
Pt0250YS (F)	HCM	1.0 (6.1)	0.74 (3.1)	No	1.55 (−2.9)	41.0	80.0	–
Pt0268 (F)	HCM	9.1 (5.0)	9.4 (3.8)	No	29.1 (1.9)	–	41.1	No
Pt0311 (F)	HCM	11.0 (6.6)	6.0 (2.1)	No	19.0 (−0.8)	32.0	64.0	Moderate TR
Pt314 (F)	HCM	6.9 (4.1)	10.1 (4.4)	No	23.6 (1.1)	23.3	54.9	Moderate MR/PH
Pt0450 (M)	HCM	12.2 (5.8)	10.7 (3.9)	No	20 (−3.8)	–	87.5	–
Pt0456 (F)	HCM	9.5 (6.5)	9.9 (4.8)	No	13.1 (−2.8)	47.0	–	–
Pt0459 (M)	HCM	5.9 (2.6)	6.6 (2.0)	No	–	–	78.5	–
Pt0467 (M)	HCM	8.0 (3.6)	7.0 (1.9)	No	23 (−2.4)	27.0	55.0	–
Pt0556 (M)	HCM	9.1 (3.0)	–	No	43.7 (1.3)	16.0	34.0	–
Pt0806 (M)	HCM	7.4 (2.2)	7.6 (1.6)	No	–	–	66.3	–
Pt0856 (M)	HCM	18.9 (6.6)	12.9 (3.7)	No	45.0 (1.2)	10.0	–	Mild MR/TR
Pt1204 (F)	HCM	4.3 (2.5)	6.2 (2.7)	No	16.7 (0.1)	33.0	70.0	–
Pt1324 (M)	HCM	4.3 (2.4)	7.1 (3.3)	No	–	87.0	53.0	–
Pt1366 (F)	HCM	5.2 (2.3)	5.6 (1.5)	No	17.7 (−2.8)	36.0	–	–
Pt1466 (F)	HCM	10.5 (5.9)	8.2 (3.2)	Yes	18.4 (−2.3)	–	92.0	–
Pt1809 (M)	HCM	9.0 (5.0)	8.0 (3.0)	No	–	–	71.0	–
Pt0049 (M)	DCM	–	–	–	33.0 (3.4)	–	43.0	Moderate MR
Pt0452 (F)	DCM	3.0 (−0.2)	3.0 (−1.2)	–	46 (7.9)	–	25.0	Mild MR/AR
Pt1483 (F)	DCM	–	–	–	37.2 (2.6)	–	57.2	Mild MR
Pt1492 (M)	DCM	–	–	–	38 (5.3)	–	24.0	Moderate MR
Pt1719 (F)	DCM	5.1 (0.06)	5.1 (−0.04)	–	47.6 (2.2)	47.0	24.0	Severe MR
Pt0081 (M)	EFE	–	–	–	34 (5.0)	9.4	21.0	–
Pt1333 (M)	LVNC	6.7 (3.0)	6.7 (1.9)	No	22.8 (−1.8)	34.0	65.0	–

CM: cardiomyopathy, LVPWd: left ventricle posterior wall in diastole; IVSd: intra-ventricular septum in diastole, LVOT: left ventricle outflow tract, LVIDd: left ventricle inner diameter in diastole, FS: fractional shortening, EF: ejection fraction, F: female, M: male, ES: elder sister, YS: younger sister, EB: elder brother, HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, EFE: endocardial fibroelastosis, NC: left ventricular noncompaction, MR: mitral valve regurgitation, PH: pulmonary hypertension, TR: tricuspid valve regurgitation, AR: aortic valve regurgitation, –: data not available.

3.3. Genetics findings

Of 29 patients with cardiomyopathy, 15 patients had nuclear DNA mutations, two had large chromosomal deletions, and 12 had mitochondrial DNA mutations. Nuclear DNA mutations identified in the 15 patients were *ACAD9* (found in two siblings), *BOLA3* (three patients), *COQ4* (one patient), *COX10* (one patient), *KARS* (one patient), *TOP3A* (one patient), *QRSL1* (two siblings), and *TAZ* (four patients) [3,16–21]. Two patients had large chromosomal deletions at 6q24.3-6q25.1 and 1p36.33, respectively [3,22,23]. Among 12 patients with mitochondrial DNA mutations with confirmed pathogenicity in MITOMAP database [24], four had m.3243A>G mutations in *MT-TL1*, four had m.13513G>A mutations in *MT-ND5*, two had m.8528T>C mutations in the overlapped region of *MT-ATP6* and *MT-ATP8* [8,9], one had m.3302A>G mutation in *MT-ND1*, and one had m.1644G>A in *MT-TV*. Cardiomyopathy was observed in three of the five patients with pathogenic mutations in

BOLA3 (60%), four of six patients with pathogenic mutations in *TAZ* gene (67%), and four of 17 patients with m.3243A>G mutations in *MT-TL1* (24%).

3.4. Heteroplasmy rate of mitochondrial DNA mutations

For 12 patients with cardiomyopathy caused by pathogenic mitochondrial DNA mutations, cardiac tissues, skin fibroblasts, blood samples, or other tissues were used for measuring the heteroplasmy rate. Heteroplasmy rates of each pathogenic mutation in the available tissues of 12 patients are summarized in Table 1. Cardiac tissues were available for one patient with a m.8528T>C mutation in *MT-ATP6/8* and for two patients with m.3243A>G mutations in *MT-TL1*. Heteroplasmy rates of m.8528T>C mutations in Pt0311, who died at 2 months of age, were high in all available tissues: 90% in cardiac tissues, 86% in skin fibroblasts, 88% in blood samples, 88% in hepatic tissues, and 91% in skeletal muscles. Heteroplasmy rates of m.8528T>C mutations in Pt1809, who is alive at 1 year and 8 months of age, are relatively low: 15% in skin fibroblasts, and 59% in blood samples [8,9]. Heteroplasmy rates of m.3243A>G mutations were high in the cardiac tissues of two patients. Pt0556, who died at 12 years of age, had heteroplasmy rates of 90 and 70% in cardiac tissues and blood samples, respectively, and Pt0856, who died at 13 years of age, had heteroplasmy rates of 80 and 87% in cardiac tissues and kidney tissues, respectively. Although cardiac tissues were not available for two patients with m.3243A>G mutations, heteroplasmy rates in their skin fibroblasts were relatively high. Pt1204, who died at 2 months of age, had an m.3243A>G heteroplasmy rate of 77% in skin fibroblasts. Pt1483, who died at 3 years and 6 months of age, had an m.3243A>G heteroplasmy rate of 61% in skin fibroblasts. Heteroplasmy rates measured in the available tissues of the total study population are summarized in Supplementary Table S1.

3.5. Biochemical findings

Among 29 patients with cardiomyopathy, 28 patients were analyzed for MRC enzyme assay using available samples (skin fibroblasts, skeletal muscle, hepatic tissue, and cardiac tissue). For one patient, the tissue for biochemical assays was not available.

Of these, 24 assays exhibited decreased MRC activities (86%). Isolated complex I deficiency was most frequently observed, in 11 patients (46%), and isolated complex IV deficiency was observed in one patient (4%). Combined complex deficiencies were observed in 12 patients (50%). For four patients with normal enzyme assay results, oxygen consumption rate (OCR) was measured. Based on MRR distribution in our five controls, a reduction to <71.6% was considered a significant decline ($p < 0.05$). Two patients (Pt1324 and Pt1809) showed a significant decline, ranging from 59% to 68%, suggesting mitochondrial respiratory dysfunction, while two patients (Pt1492 and Pt1719) showed a normal OCR at 107% and 92%, respectively.

3.6. Clinical course and survival

During the median follow-up period of 35 months [interquartile range: IQR, 9–75 months], 21 of the 29 patients with cardiomyopathy died (72%). This is in contrast to the 27 deaths in 108 patients diagnosed with mitochondrial disease who did not have evidence of cardiomyopathy (25%). All of the 21 patients with cardiomyopathy died of cardiac deaths. The overall survival rate was significantly lower in cardiomyopathy patients than in those without cardiomyopathy ($p < 0.001$ by the log-rank test, Fig. 1), and ten-year Kaplan-Meier estimates of overall survival were 18 and 67%, respectively. The reason for censoring was that patients were alive at the end of the follow-up period. Fifty percent of patients with cardiomyopathy died within 1 year and 7 months of the follow-up period, while >60% of patients without cardiomyopathy are still alive at the latest follow-up period of 166 months.

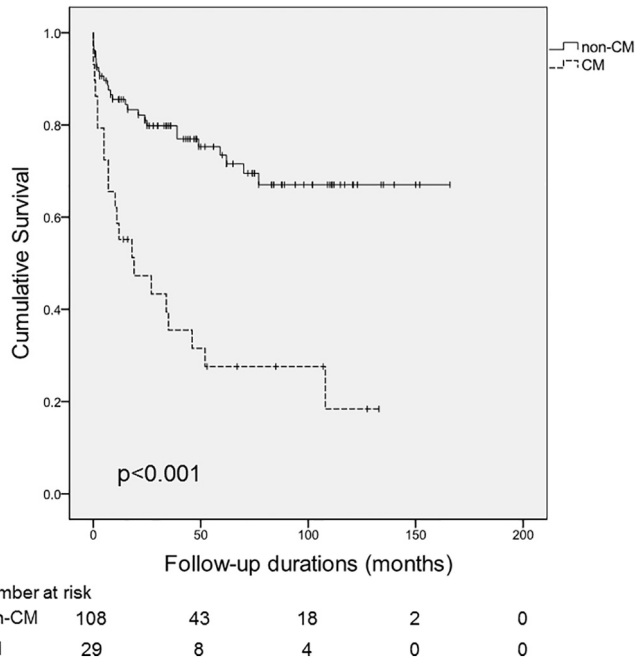

Fig. 1. Kaplan-Meier estimates of overall survival for mitochondrial disease patients with or without cardiomyopathy. Patients with cardiomyopathy had lower survival rates than did those without cardiomyopathy ($p < 0.001$, log-rank test). Numbers of patients at risk of death are indicated beneath the graph. non-CM: patients without cardiomyopathy, CM: patients with cardiomyopathy, black lines: tick marks for censored patients.

Among 29 patients with cardiomyopathy, the proportion of patients who died with nuclear DNA mutations or chromosomal deletions is 71% and that with mitochondrial DNA mutation is 75%. Among 21 patients with cardiomyopathy who died, 10 patients had nuclear DNA mutations, two had large chromosomal deletions, and nine had mitochondrial DNA mutations; two died within one month of birth (*COQ4* in one patient, and *COX10* in one patient), 10 patients died within one year (*BOLA3* in three patients, *QRSL1* in two patients, large chromosomal deletions in two patients, *MT-ATP6/8* in one patient, *MT-TL1* in one patient, and *TAZ* gene in one patient), and nine patients died after one year (*MT-ND5* in three patients, *MT-TL1* in three patients, *ACAD9* in one patient, *KARS* in one patient, and *MT-TV* in one patient).

Among 29 patients with cardiomyopathy, eight patients are alive (*TAZ* in three patients, *ACAD9* in one patient, *TOP3A* in one patient who underwent heart transplantation, *MT-ATP6/8* in one patient, *MT-ND1* in one patient and *MT-ND5* in one patient). Among the 27 patients who died without any evidence of cardiomyopathy, 26 died due to non-cardiac causes and one patient (Pt1401) died after sudden onset ventricular fibrillation without any previous cardiac symptoms.

4. Discussion

4.1. Epidemiology

Cardiomyopathy has been reported affect up to 20% of patients with mitochondrial disease [5]. Our results showed that cardiomyopathy affects 21% in children with mitochondrial disease, consistent with previous studies. Although this study was based on patients referred to our institutions, referral was made from pediatricians or neurologists across Japan and it may reflect the epidemiology of mitochondrial cardiomyopathy in Japan.

In this study, we only assessed cardiomyopathy patients, as a representative cardiac manifestation of mitochondrial disease. However,

our study populations also contained patients with cardiac arrhythmias (including lethal arrhythmia), cardiac anomalies, and apparent life-threatening events. Considering that not all children with mitochondrial disease receive autopsies in the event of sudden death, unrevealed cardiac manifestations may also be present in additional patients. Increased autopsy rates in children with mitochondrial disease will lead to more accurate assessments of cardiac involvement in mitochondrial disease. The fact that cardiac involvement other than cardiomyopathy was also seen reinforces the need for routine cardiac screening in children with mitochondrial disease.

4.2. Prognosis and genetic background

In our study population, the overall survival rate was significantly lower in patients with cardiomyopathy than in those without cardiomyopathy ($p < 0.001$, log-rank test), with ten-year Kaplan-Meier estimates of overall survival of 18 and 67%, respectively. This indicates that children with mitochondrial disease and cardiomyopathy have a poor prognosis, compared with children who have mitochondrial disease but do not have cardiomyopathy. Whether the fact that patients with cardiomyopathy showed earlier disease onset than those without cardiomyopathy affects prognosis should be assessed in future studies with larger numbers of patients.

Among 21 patients with cardiomyopathy who died, 10 patients had nuclear DNA mutations, two had large chromosomal deletion, and nine had mitochondrial DNA mutations. Among patients with cardiomyopathy, no significant difference is seen in the proportion of patients who died with nuclear DNA mutations or chromosomal deletions (71%) and that with mitochondrial DNA mutation (75%), given the relatively small numbers of cardiomyopathy patients in this study. Whether prognosis differs in patients with mitochondrial disease and cardiomyopathy caused by mitochondrial DNA mutations than in those caused by pathogenic nuclear DNA mutations or chromosomal deletions should be assessed in future studies with larger sample sizes.

Twelve patients with mitochondrial DNA mutations showed variation in prognosis, with nine patients dead (ranging from death at 2 months old to 13 years old) and with three patients alive. Severity of phenotypes in patients with mitochondrial DNA mutations depends on heteroplasmy rate. Therefore, it is important to assess the heteroplasmy rate of pathogenic mitochondrial DNA mutations in affected tissues if those tissues are available. Among our 29 patients with cardiomyopathy, cardiac tissues were available for heteroplasmy rate analysis for three patients who had either m.3243A>G or m.8528T>C mutations. All three patients had high heteroplasmy rates equal to or >80% and all had died by the age of 13 years. Cardiac involvement in patients with the m.3243A>G mutation greatly differs from non-cardiac symptoms to severe cardiomyopathy. Therefore, early detection of high heteroplasmy rates in the cardiac tissue of cardiomyopathy patients may be useful as a predictive indicator for prognosis or therapy. However, cardiac tissues are not available in all instances. Some of our patients showed high m.3243A>G mutation heteroplasmy rates in skin fibroblasts or blood. Therefore, future studies should be conducted to assess the relationship between the severity of cardiac phenotypes and the heteroplasmy rate in cardiac tissues and in other more easily available tissues such as blood, urine, or skin fibroblasts. The m.8528T>C mutation has been reported to be associated with infantile cardiomyopathy because it is located in the overlapping region of *MT-ATP6* and *MT-ATP8*, which are essential components of ATP synthase [7–9]. One of our patients with an m.8528T>C mutation showed high heteroplasmy rates in skin fibroblasts, blood, cardiac tissues, skeletal muscles, and hepatic tissues. These results indicate that for mutations with a great impact on infantile cardiomyopathy, such as m.8528T>C, assessing the heteroplasmy rate using more tissues easily obtained than cardiac tissues may be useful for prognosis determination. Building further evidence regarding the relationship between prognosis and genetic background of mitochondrial disease patients with cardiomyopathy, including heteroplasmy rate of mitochondrial DNA mutations, may be also useful for considering the indication of heart transplantation [7].

4.3. Study limitations

Cardiac biopsy is difficult to perform, especially in young patients or under unstable conditions. Therefore, not all children with cardiomyopathy underwent cardiac biopsy in this study. Among 29 patients with cardiomyopathy, cardiac tissues were available for 11 patients. Of 12 patients with cardiomyopathy caused by pathogenic mitochondrial DNA mutations, cardiac tissues were available for measuring heteroplasmy rates in three patients.

Increased endomyocardial biopsy or autopsy would provide more detailed classification in patients with cardiomyopathy, including the detection of EFE in Barth syndrome. Moreover, such biopsy or autopsy would help establish the relationship between heteroplasmy rate and the severity of cardiomyopathy. Although cardiac and other tissues were only available for measuring heteroplasmy rate in some of our cardiomyopathy patients with pathogenic mitochondrial DNA mutations, the relationship between prognosis and heteroplasmy rate in different tissues should be assessed in future studies. Routine cardiac echocardiography was not performed in all patients if they did not present any cardiac symptoms. Therefore, the proportion of patients with cardiac involvement may be underestimated.

5. Conclusions

Cardiomyopathy was seen in 29 out of 137 children (21%) with mitochondrial disease in our study. The overall survival rate was significantly lower in patients with cardiomyopathy than in those without cardiomyopathy ($p < 0.001$, log-rank test), with ten-year Kaplan-Meier estimates of overall survival of 18 and 67%, respectively. Analysis of the genetic background of cardiomyopathy in children with mitochondrial disease coupled with detailed phenotyping could be useful for prognosis.

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Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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