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ORIGINAL ARTICLE

Clinical manifestations and enzymatic activities of mitochondrial respiratory chain complexes in Pearson marrow-pancreas syndrome with 3-methylglutaconic aciduria: a case report and literature review

Takeshi Sato[1,5] · Koji Muroya[1] · Junko Hanakawa[1] · Reiko Iwano[1] · Yumi Asakura[1] · Yukichi Tanaka[2] · Kei Murayama[3] · Akira Ohtake[4] · Tomonobu Hasegawa[5] · Masanori Adachi[1]

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Abstract Pearson marrow-pancreas syndrome (PS) is a rare mitochondrial disorder. Impaired mitochondrial respiratory chain complexes (MRCC) differ among individuals and organs, which accounts for variable clinical pictures. A subset of PS patients develop 3-methylglutaconic aciduria (3-MGA-uria), but the characteristic symptoms and impaired MRCC remain unknown. Our patient, a girl, developed pancytopenia, hyperlactatemia, steatorrhea, insulin-dependent diabetes mellitus, liver dysfunction, Fanconi syndrome, and 3-MGA-uria. She died from cerebral hemorrhage at 3 years of age. We identified a novel 5.4-kbp deletion of mitochondrial DNA. The enzymatic activities of MRCC I and IV were markedly reduced in the liver and muscle and mildly reduced in skin fibroblasts and the heart. To date, urine organic acid analysis has been performed on 29 PS patients, including our case. Eight patients had 3-MGA-uria, while only one patient did not. The remaining 20 patients were not reported to have 3-MGA-uria. In this paper, we included these 20 patients as PS patients without 3-MGA-uria. PS patients with and without 3-MGA-uria have similar manifestations. Only a few studies have examined the enzymatic activities of MRCC.

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✉ Koji Muroya
kmuroya@kcmc.jp

Takeshi Sato
sato_tksjp@yahoo.co.jp

Junko Hanakawa
hanajun0624@yahoo.co.jp

Reiko Iwano
rosso-nero1996sapporo@hotmail.co.jp

Yumi Asakura
yumi79@mvb.biglobe.ne.jp

Yukichi Tanaka
ytanaka@kcmc.jp

Kei Murayama
kmuraya@mri.biglobe.ne.jp

Akira Ohtake
akira_oh@saitama-med.ac.jp

Tomonobu Hasegawa
thaseg@a6.keio.jp

Masanori Adachi
madachi@mars.sannet.ne.jp

[1] Department of Endocrinology and Metabolism, Kanagawa Children's Medical Center, Mutsukawa 2-138-4, Minami-ku, Yokohama 232-8555, Japan

[2] Department of Pathology, Kanagawa Children's Medical Center, Yokohama, Japan

[3] Department of Metabolism, Chiba Children's Hospital, 579-1 Heta-cho, Midori-ku, Chiba-shi, Chiba 266-0007, Japan

[4] Department of Pediatrics, Faculty of Medicine, Saitama Medical University, 38 Morohongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan

[5] Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Conclusion: No clinical characteristics distinguish between PS patients with and without 3-MGA-uria. The correlation between 3-MGA-uria and the enzymatic activities of MRCC remains to be elucidated.

What is Known:

- *The clinical characteristics of patients with Pearson marrow-pancreas syndrome and 3-methylglutaconic aciduria remain unknown.*

What is New:

- *No clinical characteristics distinguish between Pearson marrow-pancreas syndrome patients with and without 3-methylglutaconic aciduria.*

Keywords Pearson marrow-pancreas syndrome · Mitochondrial respiratory chain complexes · 3-Methylglutaconic aciduria · Organic aciduria

Abbreviations

ATP	Adenosine triphosphate
ATPase	Adenosine triphosphate synthase
CO	Cytochrome c oxidase
MRCC	Mitochondrial respiratory chain complexes
mtDNA	Mitochondrial DNA
NADH	Nicotinamide adenine dinucleotide
ND	Nicotinamide adenine dinucleotide dehydrogenase
PCR	Polymerase chain reaction
PS	Pearson marrow-pancreas syndrome
tRNA	Transfer ribonucleic acid
3-MGA-uria	3-Methylglutaconic aciduria

Introduction

Pearson marrow-pancreas syndrome (PS; OMIM #557000) is a rare disorder characterized by sideroblastic anemia, vacuolization of hematopoietic precursors, and exocrine pancreatic dysfunction [22]. Other clinical manifestations include failure to thrive, lactic acidosis, insulin-dependent diabetes mellitus, liver dysfunction, renal tubular dysfunction, and muscle and neurologic impairment. More than half of the patients die before 4 years owing to metabolic acidosis, severe infections, dehydration, and liver failure [16, 25, 34]. PS results from a defect in oxidative phosphorylation associated with deletions and rearrangements of the mitochondrial DNA (mtDNA) [26]. A common mtDNA deletion is 4977 bp, from nucleotide position 8483 to nucleotide position 13459 [2], frequently with a 9–13-bp direct repeat sequence flanking the deletion [9, 17, 25, 35]. No correlations between the clinical severity and the type, size, or location of the rearrangements have been identified [25].

The correlation of the deleted region of mtDNA and impaired mitochondrial respiratory chain complexes (MRCC) has not been fully investigated in PS. In addition, the correlation between the residual enzymatic activities of MRCC and the clinical course remains to be clarified. Previous studies indicate that impaired MRCC differ among individuals and organs. For example, biochemical analysis of muscle biopsy samples of PS patients revealed decreased enzymatic activities of MRCC I [13], MRCC I and III [11], MRCC I and IV [28], MRCC III [33], and MRCC II+III and IV [7, 9, 29]. On the other hand, one patient showed normal enzymatic activities of MRCC in the muscle, but reduced enzymatic activities of MRCC III and IV in the liver [11], while another patient showed reduced enzymatic activity of MRCC I in the muscle and reduced enzymatic activities of MRCC I, III, and IV in the liver [13].

3-Methylglutaconic aciduria (3-MGA-uria) is sometimes seen in mitochondrial disorders [36]. 3-MGA-uria is associated with single or combined MRCC deficiencies [36]. Although a subset of PS patients develop 3-MGA-uria [5, 12, 16, 23], the characteristic symptoms and impaired MRCC remain unknown.

Here, we report the clinical course of a 3-year-old girl with PS and 3-MGA-uria. We performed molecular and biochemical analyses on several organs. We also reviewed the literature on PS patients with and without 3-MGA-uria.

Case report

Our patient, a girl, was the second child of healthy, nonconsanguineous parents. The family history was unremarkable. A 1-year-old brother was healthy and developed normally. Oligohydramnios occurred in late pregnancy. She was born at term by cesarean section because of fetal distress. Birth weight was 2350 g (−1.5 SD), length 46.0 cm (−1.2 SD), and head circumference 32.5 cm (−0.4 SD). Pallor and respiratory distress were noticed soon after birth. Laboratory examinations revealed severe anemia (hemoglobin concentration, 5.5 g/dL) without evidence of hemolysis. She received a blood transfusion, and she was discharged 14 days after birth.

At 1 month of age, anemia recurred (hemoglobin concentration, 7.0 g/dL). The diagnosis of congenital hypoplastic anemia was confirmed by bone marrow examination showing erythroblastopenia. Erythropoietin therapy was initiated at 200 IU/kg twice a week subcutaneously. A month later, severe anemia persisted and we discontinued this treatment. She needed blood transfusions every 3 to 4 weeks. Then, she gained insufficient weight. At 7 months, her weight was 3.9 kg (−4.8 SD), height 62.0 cm (−2.4 SD), and head

circumference 40.0 cm (−2.4 SD). She could sit up without support, and then she regressed.

At 12 months of age, she was referred to our hospital. The second bone marrow examination revealed vacuoles in myeloblasts and no ringed sideroblasts (data not shown). She was diagnosed with pure red cell aplasia, and prednisolone therapy was initiated at 1–1.5 mg/kg/day orally. At 16 months, her weight was 5.0 kg (−5.0 SD), height 66.2 cm (−4.3 SD), and head circumference 43.0 cm (−2.2 SD). Re-evaluation revealed pancytopenia, hyperlactatemia, steatorrhea, insulin-dependent diabetes mellitus, mild liver dysfunction, renal Fanconi syndrome (glucosuria, hyperphosphaturia, proteinuria, and aminoaciduria), hypokalemia, and carnitine deficiency. We performed gas chromatography mass spectrometry analysis of organic acids in urine and evaluated the peaks of the substances compared to the peak of the internal standard (tropic acid): 3-methylglutaconic acid (0.44 and 0.36, reference <0.10), fumaric acid (0.97, reference <0.10), acetoacetic acid (0.12 and 0.84, reference <0.10), 3-hydroxybutyric acid (2.03, reference <0.10), and 3-hydroxydicarboxylic acids (3-hydroxysuberate 0.10, 3-hydroxysebacate 0.61, 3-hydroxydodecanedioate 0.38, reference <0.10) were elevated. 3-Methylglutaric acid (0.10, reference <0.10) and orotic acid (<0.10, reference <0.10) were not elevated. She received an alkalizer, potassium supplements, and insulin therapy. Our suspected diagnosis of PS was confirmed by Southern blot analysis of mtDNA from peripheral blood lymphocytes (Fig. 1a). She suffered from recurrent infections, and her renal function deteriorated. She was bedridden because of muscle weakness, and she babbled at 3 years of age.

At 3 years and 5 months of age, her weight was 5.0 kg (−5.2 SD) and height 71.0 cm (−6.9 SD). She was hospitalized because of dehydration with a large amount of isotonic urine, which was followed by fever and cough. Even after correction of dehydration, we could not discontinue fluid therapy. Thereafter, the patient needed continuous intravenous physiologic saline infusion, reaching 220 mL/kg/day.

At 3 years and 9 months of age, she vomited several times and presented with fever and cough. A chest radiography showed a right upper lobe infiltrate, consistent with aspiration pneumonia. We initiated ceftriaxone 120 mg/kg/day, intravenously. Her condition became better, but transiently. She developed lower abdominal petechiae and generalized convulsions. Computed tomography revealed bilateral occipital cerebral hemorrhages, right subdural hemorrhage, and a remarkable midline shift. In spite of desperate treatment, lactic acidosis deteriorated. She died from cerebral herniation. Consent for autopsy, excluding the brain, was obtained.

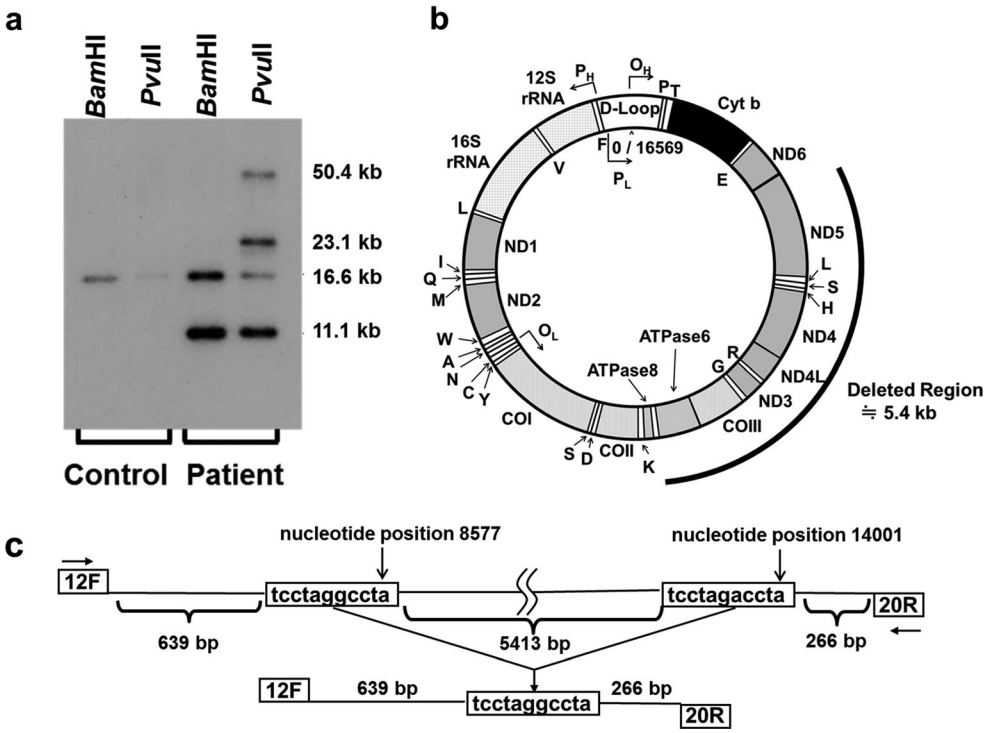

Fig. 1 Identification of mtDNA deletion. **a** Southern blot analysis of mitochondrial DNA from peripheral blood lymphocytes in a patient and a control subject. After digestion with *Bam*HI or *Pvu*II and hybridization, one band of 16.6 kb was seen in the control subject and the patient. Another band of 11.1 kb was seen in the patient. **b** Map of human mtDNA showing the extent of the deletion found in the patient. **c** Localization of the deletion. We amplified fragments of deleted mtDNA from the patient with the primers 12F and 20R. A 956-bp fragment was obtained from the patient's mtDNA. No PCR fragment was obtained from the control subject's mtDNA. We sequenced the 956-bp fragment and identified a 5424-bp deletion. Incomplete 11-bp direct repeats are indicated by *rectangles*

Materials and methods

Southern blot analysis

Genomic DNA was extracted from the peripheral blood lymphocytes of the control subject and the patient, using a standard protocol. Genomic DNA was digested with *Bam*HI (cleavage at position 14258) and *Pvu*II (cleavage at position 2652), separated by agarose gel electrophoresis, and blotted onto nylon membranes. A mtDNA probe, corresponding to nucleotides 3307 to 4519, was used for hybridization. The nucleotide numbers refer to the reference mtDNA sequence, according to MITOMAP (http://www.mitomap.org).

Detection of mtDNA deletion

According to a previous study describing 24 pairs of forward and reverse primers that cover the entire mtDNA sequence [24], we prepared the following seven primer sets to detect deletion: 12F and 15R, 12F and 16R, 12F and 17R, 12F and 18R, 12F and 19R, 12F and 20R, and 12F and 21R. A three-temperature touchdown polymerase chain reaction (PCR) program was used to amplify DNA: 95 °C for 10 min; 10 cycles at 94 °C for 20 s, 62 °C for 20 s (with temperature decreasing from 62 to 57 °C by −0.5 °C per cycle), 72 °C for 45 s; an additional 25 cycles at 94 °C for 20 s, 57 °C for 20 s, 72 °C for 45 s; and a final extension at 72 °C for 7 min. We sequenced the PCR products and examined them for deletions and rearrangements.

Measurement of the proportion of deleted mtDNA

We extracted total DNA from several organs, including the skin, pancreas, kidney, liver, muscle, and heart, using the standard protocol. We designed the following primer set, CF (5′-CAAACCCTGAGAACCAAAATGAAC-3′) and CR (5′-TGAGATTGCTCGGGGGAATAG-3′), positioned at nucleotides 8509–8532 and 14150–14170, respectively. Using CF and 12R primers, a fragment of 308 bp could be amplified from the normal mtDNA, but not from the deleted mtDNA. On the other hand, using CF and CR primers, a fragment of 238 bp could be amplified from the deleted mtDNA, but not from the normal mtDNA. On the basis of this assumption, we tried to amplify the normal and deleted mtDNA simultaneously by the abovementioned three-temperature touchdown PCR program with CF, 12R, and CR. We estimated the intensity of each band in the gel quantitatively.

Respiratory chain enzyme assay

The tissues for respiratory chain enzyme assay were sampled at the autopsy, 14 h after death. The enzymatic activities of MRCC I, II, III, and IV were assayed in skin fibroblasts, the liver, skeletal muscle, and heart as described previously [10, 20]. In these assays, citrate synthase was used as a mitochondrial enzyme marker.

Autopsy

Autopsy was performed 14 h after death. Examination of the brain was not permitted. Tissues were fixed in 10 % formalin and embedded in paraffin. Sections of 4-μm thickness were prepared for staining with hematoxylin-eosin and for immunohistochemical examination.

Literature review

A computerized literature search of PubMed was conducted. Literature searches were completed in March, 2015. We used the following search keywords: “Pearson syndrome” or “Pearson marrow-pancreas syndrome.” Language was restricted to English. We reviewed PS patients reported in the literature whose urine organic acid had been analyzed. We compared the clinical manifestations and impaired MRCC of patients with and without 3-MGA-uria.

Results

Molecular analysis of mtDNA

*Bam*HI and *Pvu*II cut the native circular mtDNA molecule at only one site and, thus, produce a linear molecule. Upon agarose gel electrophoresis and hybridization, a normal mtDNA molecule cleaved with *Bam*HI or *Pvu*II appears as a single sharp band, corresponding to a 16.6-kb mtDNA molecule. Such single bands were seen in the control subject and the patient (Fig. 1a). On the other hand, an intense band, corresponding to an 11.1-kb molecule, was seen in the patient (Fig. 1a). This indicated the presence of deleted and rearranged mtDNA, consistent with heteroplasmy. The other two bands seen in the DNA preparations with *Pvu*II may have resulted from uncut mtDNA or a deletion-dimer or a deletion-multimer (Fig. 1a), sometimes observed in PS [25].

Detection of mtDNA deletion

We detected fragments amplified with the following three primer sets: 12F and 19R, 12F and 20R, and 12F and 21R. We sequenced the fragments amplified with the 12F and 20R primers and compared the sequences to reference mtDNA sequences. We identified a 5424-bp deletion of mtDNA from nucleotide position 8578 to nucleotide position 14001 (Fig. 1b, c). Imperfect 11-bp direct repeats were present near the junction (Fig. 1c). According to MITOMAP, the

sequences encoding adenosine triphosphate synthase 6 (ATPase6) (coding sequence 8527–9207), cytochrome c oxidase III (COIII) (coding sequence 9207–9990), transfer ribonucleic acid (tRNA)-Gly (coding sequence 9991–10058), nicotinamide adenine dinucleotide (NADH) dehydrogenase 3 (ND3) (coding sequence 10059–10404), tRNA-Arg (coding sequence 10405–10469), ND4L (coding sequence 10470–10766), ND4 (coding sequence 10760–12137), tRNA-His (coding sequence 12138–12206), tRNA-Ser (coding sequence 12207–12265), and tRNA-Leu (coding sequence 12266–12336) were lost completely (Fig. 1c). The gene encoding ND5 (coding sequence 12337–14148) was partially lost (Fig. 1c).

Measurement of the proportion of deleted mtDNA

We obtained 308 and 238 bp fragments from normal mtDNA and deleted mtDNA, respectively, with CF, 12R, and CR (Fig. 2a). We found the proportion of deleted mtDNA higher in the pancreas, kidney, liver, and muscle (Fig. 2b).

Respiratory chain enzyme assay

The enzymatic activities of MRCC I and IV were markedly reduced in the liver and muscle and mildly reduced in skin fibroblasts and the heart (Table 1). Blue native polyacrylamide gel electrophoresis analysis revealed

Table 1 Respiratory chain enzyme assay

Percentage	Co I	Co II	Co II+III	Co III	Co IV	CS
Skin fibroblasts						
Crude activity	33	81	64	73	42	77
CS ratio	42	102	79	89	53	
Co II ratio	40		76	90	52	
Liver						
% of normal	41	229		216	50	367
CS ratio	11	62		58	13	
Co II ratio	18			94	21	
Skeletal muscle						
% of normal	10	63	62	45	5	63
CS ratio	16	98	96	72	8	
Co II ratio	16		97	73	8	
Heart						
% of normal	74	225	182	432	69	214
CS ratio	35	104	83	202	33	
Co II ratio	33		80	196	31	

Enzymatic activities, except for skin fibroblasts, are expressed as the percent of mean normal control activity relative to CS and Co II. Mitochondrial respiratory chain complex deficiency is defined as a CS ratio or Co II ratio less than 30 % in cultured cells and 20 % in tissues [3]

Co I complex I, *Co II* complex II, *Co III* complex III, *Co IV* complex IV, *CS* citrate synthase

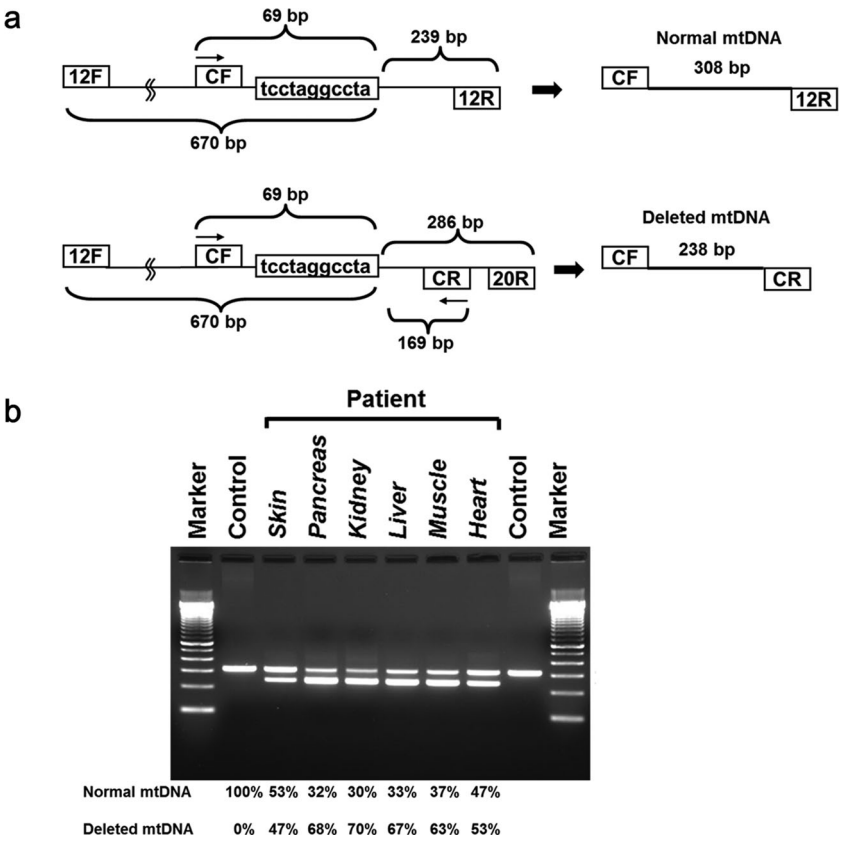

Fig. 2 Measurement of the proportion of deleted mtDNA. **a** Preparation of primers. We designed two primers, CF and CR, positioned at nucleotides 8509–8532 and 14150–14170, respectively. Using CF and 12R primers, a fragment of 308 bp could be amplified from the normal mtDNA, but not from the deleted mtDNA. On the other hand, using CF and CR primers, a fragment of 238 bp could be amplified from the deleted mtDNA, but not from the normal mtDNA. **b** Separation by gel electrophoresis. We obtained fragments with expected sizes of 308 and 238 bp. We estimated the intensity of each band in the gel quantitatively

markedly reduced expression of MRCC I in skin fibroblasts (data not shown).

Autopsy findings

The bone marrow showed normocellular marrow with some vacuolations of myeloid precursors and nuclear atypia of megakaryocytes (Fig. 3a). The pancreas showed marked fibrosis, fatty change, acinar atrophy, and decreases in the size and number of islets (Fig. 3b). Immunohistochemical examination revealed a decrease in insulin-positive cells (data not shown). The kidneys showed marked and global sclerosis of the glomeruli with tubular atrophy (Fig. 3c). Some tubules in the renal cortex showed vacuolation. Interstitial fibrosis and mixed inflammatory infiltrates were prominent. The liver showed periportal fibrosis with mild to moderate mixed inflammatory infiltrates in the portal areas and steatosis (Fig. 3d, e). Some hepatocytes contained vacuoles of various sizes. The heart muscle was normal (Fig. 3f).

Literature review

Urine organic acid analysis was performed on 29 PS patients, including our case (Table 2). Eight PS patients had 3-MGA-uria. Only one patient was shown to have no 3-MGA-uria [35], and the remaining 20 PS patients were not reported to have 3-MGA-uria. These 21 PS patients were referred to as PS patients without 3-MGA-uria in this paper. PS patients with and without 3-MGA-uria had similar manifestations of hematological, pancreatic, liver, renal, and neurological diseases (Table 2). Only a few studies examined the enzymatic activities of MRCC.

Discussion

We described a girl with PS and 3-MGA-uria who died of severe metabolic acidosis and cerebral hemorrhage at 3 years of age. She developed various manifestations, including failure to thrive, pancytopenia, lactic acidosis, insulin-dependent diabetes mellitus, liver dysfunction, chronic renal failure, renal Fanconi syndrome, and developmental delay. We identified a novel 5.4-kbp deletion of mtDNA from nucleotide position 8578 to nucleotide position 14001. Imperfect 11-bp direct repeats near the junction may have played an important role in rearrangement. The enzymatic activities of MRCC I and IV were markedly reduced in the liver and muscle and mildly reduced in skin fibroblasts and the heart.

The proportion of deleted mtDNA may at least partially determine the residual enzymatic activities of MRCC. Our results showed that the residual enzymatic activities of MRCC correlated inversely with the proportion of deleted mtDNA, consistent with a previous report [27]. In the liver and muscle, the proportion of deleted mtDNA was relatively high, and the enzymatic activities of MRCC were reduced. On the other hand, in the heart and skin, the proportion of deleted mtDNA was relatively low, and the enzymatic activities of MRCC were slightly reduced. Unfortunately, because we could not obtain a proper control of the pancreas and kidney, we did not evaluate the correlation between the proportion of deleted

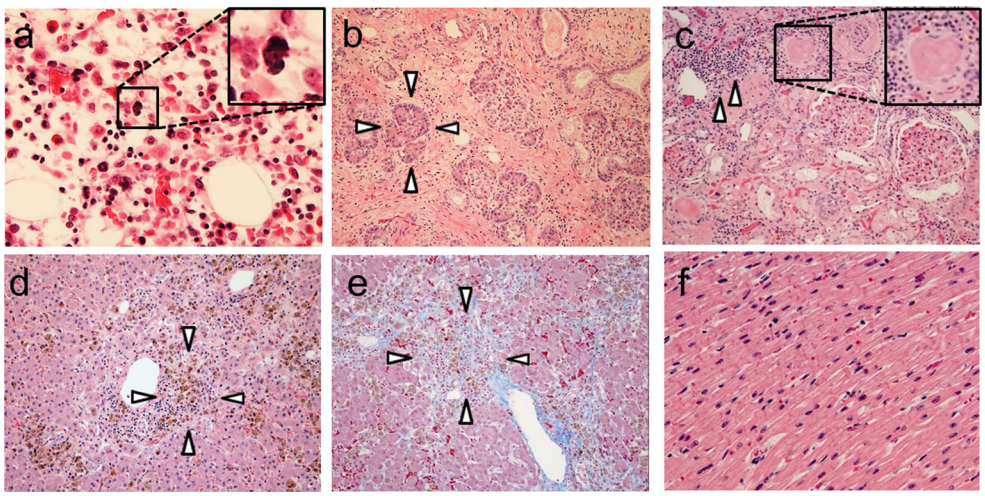

Fig. 3 Histopathological images. **a** The bone marrow, hematoxylin-eosin stain; original magnification ×400. The bone marrow showed normal cellularity, but megakaryocyte dysplasia (*inset*). **b** The pancreas, hematoxylin-eosin stain; original magnification ×100. Fibrosis and a reduced number of acini (surrounded by *arrowheads*) and islets were observed. **c** The kidneys, hematoxylin-eosin stain; original magnification ×100. The kidneys showed glomerulosclerosis (*inset*), atrophy of tubules, and mononuclear infiltrate (indicated by *arrowheads*). **d** The liver, hematoxylin-eosin stain; original magnification ×100. The liver did not show the features of fatty liver and cirrhosis, but the accumulation of hemosiderin (surrounded by *arrowheads*). **e** The liver, Masson stain; original magnification ×100. Mild to moderate fibrosis was observed (surrounded by *arrowheads*). **f**. The heart, hematoxylin-eosin stain; original magnification ×200. The heart muscle was normal

Table 2 Summary of clinical manifestations in patients with Pearson marrow-pancreas syndrome whose urine organic acid was analyzed

Ref.	Sex	Clinical manifestations							Outcome	3-MGA-uria	3-MGA-uria concentration (mmol/mol creatinine, reference)	Impaired MRCC/sampled organ(s)
		Anemia	Lactatemia or metabolic acidosis	Exocrine pancreatic dysfunction or diarrhea	Hyperglycemia/ diabetes mellitus	Liver dysfunction	Renal tubulopathy	Muscle weakness/ hypotonia				
Index case	F	+	+	+	+	+	+	+	Died at 3 yr 9 mo	+	0.44 and 0.36 (<0.10)[a]	I, IV/skin fibroblasts, liver, skeletal muscle, and heart
[5]	M	+	NL	+	NL	+	NL	NL	Died at 2 yr	+	80 (<9)	ND
[5]	F	+	NL	+	NL	−	NL	NL	Alive at 7 mo	+	32 (<9)	ND
[5]	F	+	+	+	−	NL	+	NL	Died at 3 yr 6 mo	+	80 (<9)	ND
[5]	M	+	+	+	NL	NL	NL	NL	Alive in his early teens	+	53 (<9)	ND
[12]	M	+	+	NL	−	−	−	NL	Alive at 2 yr	+	NL	-[b]/skeletal muscle
[16]	F	+	+	+	−	NL	+	+	Died at 3 yr	+	89 (<9)	ND
[23]	M	+	+	−	−	−	−	−	Alive at 3 yr	+	58–110 (<10)	ND
[35]	F	+	+	−	+	NL	+	NL	Died at 24 mo	−		ND
[1]	M	+	+	−	−	+	+	NL	Alive at 4 yr	NR		ND
[1]	F	+	+	NL[c]	NL	+	+	NL	Alive at 20 mo	NR		ND
[1]	M	+	+	NL[c]	NL	+	+	NL	Died at 13 mo	NR		ND
[1]	M	+	+	NL[c]	NL	+	+	NL	Alive at 7 yr	NR		ND
[1]	F	+	+	NL[c]	NL	NL[d]	+	NL	Died at 21 yr	NR		ND
[1]	F	+	+	−	NL	NL[d]	+	NL	Died at 30 mo	NR		ND
[1, 21]	F	+	+	NL[c]	NL	NL[d]	+	NL	Died at 10 yr	NR		-/circulating lymphocytes, and cultured fibroblasts
[4]	F	+	+	+	NL	+	+	+	Died at 23 mo	NR		ND
[4]	M	+	+	+	NL	+	+	+	Alive at 4 yr	NR		ND
[4]	F	+	+	+	+	+	+	+	Alive at 3 yr 5 mo	NR		ND
[4]	F	+	NL	NL	NL	+	NL	NL	Alive at 2 yr	NR		ND
[7]	NL	NL	+	NL	NL	NL	NL[e]	NL	Alive at 1 yr	NR		ND

Table 2 (continued)

Ref.	Sex	Clinical manifestations							Outcome	3-MGA-uria	3-MGA-uria concentration (mmol/mol creatinine, reference)	Impaired MRCC/sampled organ(s)
		Anemia	Lactatemia or metabolic acidosis	Exocrine pancreatic dysfunction or diarrhea	Hyperglycemia/ diabetes mellitus	Liver dysfunction	Renal tubulopathy	Muscle weakness/ hypotonia				
[13]	F	+	+	+	−	NL	+	NL	Alive at 5 yr	NR		ND
[15]	F	+	−[f]	−	NL	−	NL	NL	Alive at 8 yr	NR		ND
[18]	M	+	+	+	−	−	NL	+	Died at 18 mo	NR		ND
[31]	F	+	+	+	+	NL	+	+	Died at 19 mo	NR		ND
[32]	M	+	+	+	NL	+	+	NL	Died at 7 mo	NR		IV[g]/liver and skeletal muscle
[32]	F	+	+	+	−	+	+	NL	Died at 4 yr	NR		IV[g]/skeletal muscle
[33]	M	+	+	NL	+	NL	NL	NL	Died at 4 mo	NR		ND
[35]	M	+	+	−	−	NL	NL	NL	Alive at 5 yr	NR		ND

Ref., references; 3-MGA-uria, 3-methylglutaconic aciduria; MRCC, mitochondrial respiratory chain complexes; F, female; M, male; yr, years; mo, months; NL, not listed; ND, not determined; NR, not reported

[a] These values were obtained with gas chromatography mass spectrometry analysis

[b] Almost normal activities of respiratory chain enzymes were revealed by biochemical investigations

[c] Pancreatic dysfunction was reported

[d] No liver failure was reported

[e] Hyperaminoaciduria was reported

[f] Magnetic resonance spectroscopy revealed a large lactate peak

[g] Only cytochrome-*c*-oxidase staining was performed

mtDNA and the residual enzymatic activities of MRCC in these organs.

The histopathological findings seemed to correlate strongly with clinical manifestations in our patient. Microscopic examinations in our patient showed decreases in the size and number of the islets in the pancreas, mild fibrosis in the liver, and marked sclerosis of glomeruli with tubular atrophy in the kidney, consistent with insulin-dependent diabetes mellitus, mild liver dysfunction, and renal Fanconi syndrome. It is worth noting that the patient's liver dysfunction was mild, despite reduced enzymatic activities of MRCC in the liver. Such discrepancy between clinical manifestations and biochemical examinations was also seen in a previous study [27]. Organ dysfunction, associated with energy shortage, is thought to result from an imbalance between adenosine triphosphate (ATP) productivity and ATP demand. The liver has a large capacity for ATP production [30]. We speculated that our patient's liver, with reduced residual enzymatic activities of MRCC, produced substantial ATP.

In previous studies and our case report, PS patients with and without 3-MGA-uria have similar and typical manifestations of hematological, pancreatic, liver, renal, and neurological diseases. This implies that 3-MGA-uria is not a prognostic marker. On the other hand, 3-MGA-uria is reported to be a useful marker for the diagnosis of PS and mitochondrial DNA depletion syndromes (*POLG*, *SUCLG1*, *SUCLA2*, and *TWINKLE* mutations) [5, 36]. When we consider 3-MGA-uria along with the typical manifestations, the diagnosis becomes easier, even for clinical physicians unfamiliar with PS.

Only one previous study has reported on the enzymatic activities of MRCC in a PS patient with 3-MGA-uria [12]. Our patient had reduced enzymatic activities of MRCC I and IV, while the previous case had normal enzymatic activities of MRCC in the skeletal muscle [12]. In patients with other mitochondrial disorders and 3-MGA-uria, MRCC I and IV deficiency [19], MRCC I deficiency [36], and MRCC V deficiency [6] have been reported. Thus, we speculated that MRCC I and IV deficiency or MRCC I deficiency played an important role in the pathogenesis of 3-MGA-uria in our patient.

Reduced enzymatic activities of MRCC I and IV are common in mitochondrial DNA deletion syndromes [PS, Kearns-Sayre syndrome (KSS), and chronic progressive external ophthalmoplegia (CPEO)] and mitochondrial DNA depletion syndromes. Mitochondrial DNA depletion syndromes as well as PS often develop 3-MGA-uria. The origin of 3-MGA-uria in mitochondrial DNA depletion syndromes and PS remains unknown [5, 36]. Interestingly, 3-MGA-uria has not been reported in KSS and CPEO. In PS, KSS, and CPEO, differential tissue distribution of deleted mtDNA is probably a critical determinant of phenotype. Therefore, we speculate that other essential factors in the pathogenesis of 3-MGA-uria may include specific or multiple organ dysfunction due to the high percentage of deleted mtDNA or the depletion of mtDNA.

In summary, we reported the clinical course of a 3-year-old girl with PS due to a novel mtDNA mutation and 3-MGA-uria and reviewed the literature of PS patients with and without 3-MGA-uria. Our case report and previous studies suggest that no clinical characteristics distinguish PS patients with and without 3-MGA-uria. The correlation between 3-MGA-uria and the enzymatic activities of MRCC remains to be elucidated.

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Authors' contributions Takeshi Sato wrote the paper and reviewed the literature. Takeshi Sato and Koji Muroya revised the manuscript. Junko Hanakawa was an attending pediatrician and collected clinical information. Koji Muroya and Reiko Iwano performed the genetic analysis. Yumi Asakura, Tomonobu Hasegawa, and Masanori Adachi designed the study. Yukichi Tanaka performed the autopsy. Kei Murayama and Akira Ohtake performed the biochemical analysis.

Ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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