providing NAD^+ and reducing the lactate-to-pyruvate (L/P) molar ratio, which is high in cells with mitochondrial respiratory deficiency; as a result, ATP production by the glycolytic pathway would improve. In a preliminary study, they administered 5 g of sodium pyruvate to an adult patient with chronic progressive external ophthalmoplegia associated with mtDNA deletion. At 30 min after the administration of pyruvate, the blood lactate level decreased from 2.42 mM to 2.10 mM and the L/P ratio decreased from 25.65 to 16.29. No clinical improvement was, however, described in this report. So far, case reports on pyruvate therapy for mitochondrial diseases are very limited, and the efficacy of this treatment still remains inconclusive.

In the present report, we describe the clinical course of a one-year-old patient with myopathic MDS who was treated with sodium pyruvate, and discuss the efficacy of this newly proposed therapy for amelioration of the clinical manifestations of mitochondrial disorders.

2. Patients and methods

2.1. Patient

A one-year-old girl was born by Cesarean section (indication: breech presentation and placenta previa) to non-consanguineous parents at 37 weeks of gestation; the birth weight was 2970 g and the Apgar scores were 8 and 9. The family history was non-contributory. The infant began to have feeding difficulty on postnatal day 3 and developed respiratory failure and lactic acidosis (11.3 mM; normal range, 0.33–1.9 mM) on 10 days of age. She has been on a respirator ever since. The blood level of creatine phosphokinase was 3158 IU/L on postnatal day 3, but normalized later. There was no evidence of hepatomegaly and the blood levels of aspartate amino trasferase and alanine transaminase were mildly elevated (50 and 30 IU/L, respectively). Blood ammonia levels, acylcarnitine profile and urinary organic acids were normal. With improvement of the respiratory failure by mechanical ventilation, the blood lactate levels decreased, but remained between 3.0 mM and 6.5 mM, with high L/P ratios (between 36 and 97; normal <15), consistent with the diagnosis of a mitochondrial respiratory chain disorder. The lactate and pyruvate levels in the cerebrospinal fluid (CSF) were 4.2 mM and 0.18 mM, respectively with an L/P ratio of 23. Brain MRI at the age of 7 months showed mild dilatation of the lateral ventricles without any abnormal signals in the parenchyma. Treatment with coenzyme Q, thiamine, ascorbic acid and l-carnitine at the age of 3 months decreased the blood lactate levels (to between 1.4 mM and 3.1 mM), however, the L/P ratios remained high (between 16 and 45). The severe motor weakness and respirator dependence did not improve with this treatment.

Muscle biopsy performed at 10 months of age showed mild variations of the fiber size and predominance of the type 2A/2B fibers, comprising 71% of the fibers. A significant number of type 2C fibers were also found (22%). All fibers showed lipid droplets and glycogen accumulation. Ragged red fibers were found, however, strongly succinate dehydrogenase-positive vessels were not found. Cytochrome c oxidase staining was decreased, but not absent, in most fibers (Fig. 1).

Biochemical analysis of the respiratory chain enzymes in the muscle specimen revealed deficiencies of complex I (CoI), III (CoIII) and IV (CoIV), that were confirmed by the assay against citrate synthase (CS) or complex II (CoII) [11]: the activities of CoI, CoIII and CoIV relative to the activity of CS were 10.6%, 26.7% and 14.1%, respectively, and those relative to the activity of CoII were 6.5%, 16.4% and 8.8%, respectively (definite deficiency; <30% of CS or CoII).

Quantitative analysis of the mitochondrial DNA by real-time PCR [12] revealed that the ratio of the copy number of the mitochondrial NDI subunit relative to the nuclear CETR gene was 35.3% (normal; >40%), indicative of mitochondrial DNA depletion. Mutation analysis is underway.

The patient showed slowly progressive motor regression despite the treatment; by the age of 12 months, she lost the ability to smile, hold her arms above her chest against gravity or raise the lower *legs*, all of which she had been able to do until 8 months of age. At the age of 12 months, the patient was referred to our hospital for further treatment. Physical examination on admission showed severe

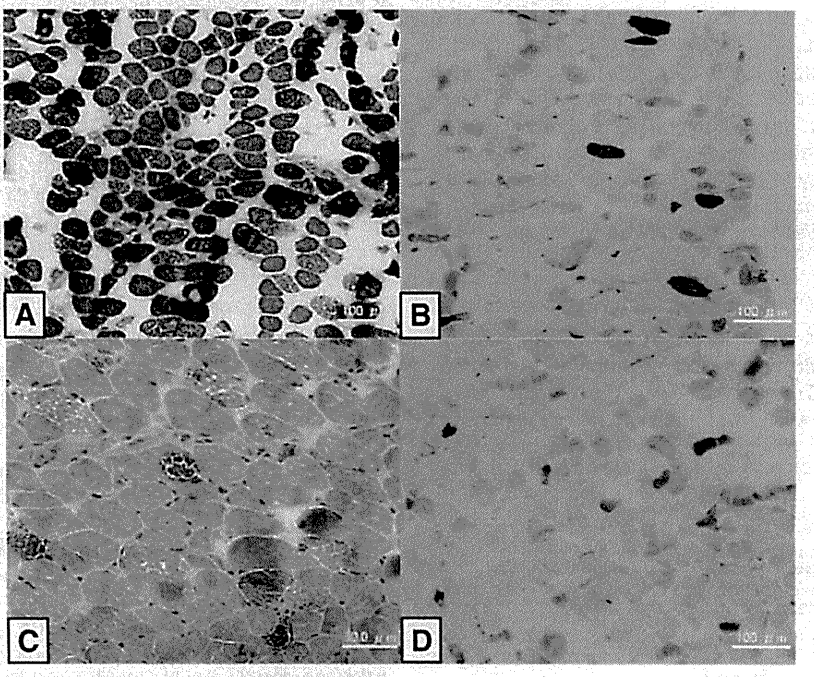

Fig. 1. Histochemistry of the biopsied muscle. ATPase staining shows type 2 fiber predominance at pH 10.6 (A) and an increased number of type 2C fibers at pH4.2 (B). The percentages of type 1, 2A/B and 2C fibers were 7%, 71% and 22%, respectively. Modified Gomori trichrome staining (C) shows scattered ragged-red fibers. Cytochrome c oxidase staining (D) shows decreased, but not absent, staining in most fibers.

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generalized hypotonia and muscle weakness. Echocardiography was normal. She had dysphagia and was fed via a nasogastric tube. Her cognition ability seemed normal despite the mild ventricular dilatation on MRI. Her hepatic dysfunction was limited to mild elevation of the serum transaminases. Therefore, the infant was diagnosed as having myopathic-type MDS.

2.2. Pyruvate therapy

The pyruvate treatment was approved by the ethics committee of Shiga Medical Center for Children and written informed consent was obtained from the parents. Sodium pyruvate (Musashino Chemical Laboratory, Tokyo), dissolved at 0.5 g/kg in water at the concentration of 0.06 g/ml was given through a nasogastric tube in three divided doses (although the recommended concentration of sodium pyruvate to avoid osmotic diarrhea is about 0.02 g/ml, we chose the higher concentration to avoid water overload). During the pyruvate therapy, other treatments, including vitamins and coenzyme Q, remained unchanged. Pyruvate was administered throughout the study period and the effects of the therapy were examined one month and two months after the initiation of the therapy.

2.3. Evaluation of the treatment effect

To evaluate the treatment effect, we used the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) for 0–24 months [13]. The measurements were performed on the day of the start of treatment before taking the first dose of pyruvate and one month and two months after the initiation of therapy with pyruvate. Considering that the motor disabilities of this patient were probably too severe for any changes to be detected by this scale, we also tried to evaluate the changes in the motor activities or muscle power by performing manual muscle testing (MMT) on the extremities as well as observing the patient's ability to perform tasks including pouting, pulling the corner of the mouth laterally, winking repeatedly, and tapping a toy xylophone with a stick by rotating the wrist while resting the arm on the floor. These tasks were the ones which her mother had let her do almost daily either as play or as a communication tool for more than two months before the initiation of the pyruvate therapy. We coaxed her to repeat the movements as many times as possible and counted the number of times she could repeat them. The measurement of each task was conducted only once because of development of fatigue. The examination was done on the day of initiation of the pyruvate therapy and one month and two months after the treatment initiation. During the treatment, the frequency of performance of the tasks which the infant's mother let her do almost daily was the same as that before the treatment, and the patient was not particularly trained to show better performance of the tasks.

2.4. Results

The pyruvate therapy did not cause any side effects, including diarrhea. The overall NPMDS score before the treatment initiation was 35, which decreased (improved) to 31 after one month of pyruvate therapy (Table 1). However, the improvement was only observed in the domain of the quality of life (section IV of the scale), which reflects the parent's subjective opinion. The scale measured two months after the initiation of therapy was the same as the one measured after one month of therapy. We also found that the patient became able to raise her forearms briefly by about 30° after one month of treatment, and by almost 90° after two months. She regained the ability to raise and hold the lower legs briefly by 2 months after the start of the therapy. She could move the wrist only horizontally before the treatment, but became able to also move it vertically after 1 month of the treatment. These observations indicated that the power of the biceps brachii, quadriceps femoris and brachioradialis muscles increased from grade 2 to grade 3 on MMT (Table 2). One month after the start of the pyruvate therapy, the number of times of pouting increased from 6 times to 15, winking from 6 times to 10, and tapping a xylophone from 5 times to 7. She could barely move the mouth corner before and until one month after the start of the therapy; however, she could move it 8 times by the second month (Table 2). Some other improvements which we observed, but could not measure quantitatively, included extended duration of each movement such as pouting and stretching of the mouth corner, increase in the speed and strength of the tapping, as well as more vivid facial expressions.

The blood lactate levels and L/P ratios did not change with the therapy. The lactate levels measured twice on separate days before the start of the treatment were 2.1 mM and 2.5 mM, with L/P ratios of 18 and 18, respectively. The lactate levels after one month and two months of pyruvate treatment were 2.7 mM and 2.3 mM, with L/P ratios of 18 and 18, respectively.

Table 1
Changes of the NPMDS scores with pyruvate therapy.

Section	Before Tx	1 month after Tx	2 months after Tx
I	7	7	7
II	6	6	6
III	5	5	5
IV	17	13	13
Overall	35	31	31

NPMDS, Newcastle Paediatric Mitochondrial Disease Scale; Tx, treatment.

3. Discussion

Tanaka et al. proposed several possible mechanisms by which pyruvate may improve the energy metabolism in respiratory chain-deficient mitochondria (Fig. 2) [10]: (a) Pyruvate reacts non-enzymatically with hydrogen peroxide to yield acetate, carbon dioxide and water, thereby eliminating hydrogen peroxide which is increased due to leakage of reactive oxygen species from the respiratory-chain deficient mitochondria. (b) In the presence of lactate dehydrogenase, pyruvate provides NAD^+ from NADH. NAD^+ is essential for oxidation of glyceraldehyde 3-phosphate by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to form 1,3-bisphosphoglycerate, which donates a phosphate group to ADP to produce ATP. Mitochondria with respiratory-chain disturbance are deficient in NAD^+, causing inhibition of the glycolytic pathway via GAPDH and an increase in the NADH-to-NAD^+ ratio, which is equivalent to the L/P ratio. Pyruvate supply reactivates the glycolysis which is impaired secondarily due to disturbance of the respiratory chain, and lowers the NADH/NAD^+ and L/P ratio. (c) Pyruvate dehydrogenase kinase (PDK) inhibits pyruvate dehydrogenase (PDH) activity, and pyruvate inhibits PDK activity. As a result, pyruvate activates PDH.

Table 2
Changes in motor function and lactate levels with pyruvate therapy.

	Before Tx	1 month	2 months
Lip pouting	6	15	ND
Winking	6	10	11
Pulling the mouth corner	None	None	8
Tapping a xylophone with a stick	5	7	ND
Raising the forearms from the bed floor	None	30°	90°
Raising the lower legs against gravity	Barely	Possible	Can hold
Flexing the wrists against gravity	Impossible	Possible	Possible
Blood lactate level	2.5 mM	2.7 mM	2.3 mM
Lactate-to-pyruvate ratio	18	18	18

The patient was asked to repeat the tasks as many times as possible. The number of times she could repeat the tasks was observed before, one month and two months after the start of the treatment. For raising the forearms, angles from the floor at which the arms could be raised were measured. Tx, treatment; ND, not done because the patient was not willing to perform.

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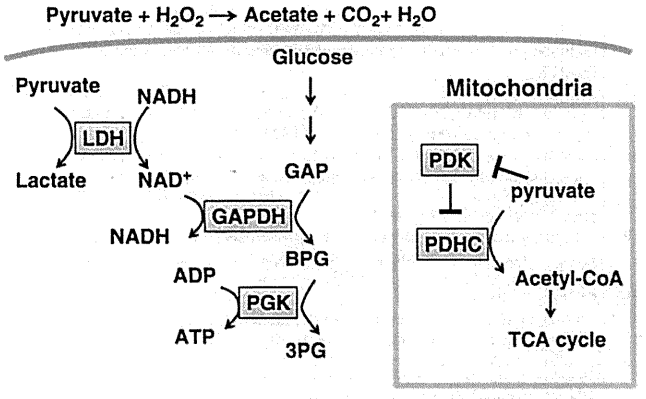

Fig. 2. Effects of pyruvate on energy metabolism and cell injury. Pyruvate eliminates hydrogen peroxide by a non-enzymatic reaction. Pyruvate provides NAD^+ from NADH with lactate dehydrogenase (LDH). In the presence of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), NAD^+ oxidizes glyceraldehyde 3-phosphate (GAP) to form 1,3-bisphosphoglycerate (BPG). BPG then provides its phosphate group to ADP to form ATP by phosphoglycerate kinase (PGK), and becomes 3-phosphoglycerate (3PG). In the mitochondrial matrix, pyruvate inhibits pyruvate dehydrogenase kinase (PDK) which inactivates pyruvate dehydrogenase complex (PDHC). As a result, PDH activates PDHC and provides acetyl-CoA, which enters TCA cycle.

The efficacy of pyruvate in improving the energy metabolism was observed in ρ^0 cells, which lack mtDNA. By adding pyruvate to the culture media, the ρ^0 cells survived, probably because of improved ATP production by pyruvate [14]. As the cells of the affected tissues in MDS are similar to the ρ^0 cells, it is reasonable to assume that pyruvate may be effective for ameliorating the clinical manifestations of MDS.

The weakness of the present study lies in the incomplete quantitative analysis of the treatment effect. Because of the patient's age and the severe weakness, it was not possible to measure the muscle strength accurately. As we anticipated, the NPMDS did not show any changes in scores in the domains that can show improvement in the muscle power, because the disability was too severe to allow detection of any improvement using this scale; in the domain for the current clinical assessment (section III), for example, the severity of myopathy is rated as severe when a patient is wheelchair dependent and the grade is defined as moderate when a patient has proximal weakness limiting functional movement. The improvement of the motor weakness in our patient was not sufficient to cause the rating to change from severe to moderate. However, even under this situation, the score for the quality of life showed improvement. One can argue that the improvement in the NPMDS score was due to the normal developmental process with age. However, the patient showed motor regression during the 11 months prior to the start of the treatment, and the parents noticed improvement by one month after the start of the treatment.

The tasks we chose to evaluate the muscle function can be influenced by skill rather than muscle strength. Therefore, the improvement in the performance of tasks could be simply due to a training effect, as the patient had been doing the same tasks daily. However, the patient had started to perform the tasks at least two months before the start of treatment, and no improvement was noticed during this pre-treatment period. On the other hand, improvement began to be noticed within a few weeks after the start of pyruvate therapy. Besides the improvement noted in the performance of these tasks which need skill, and may, therefore, be influenced by training, a significant increase in the muscle power in the biceps brachii, quadriceps femoris and brachioradialis muscles was observed; the patient became able to raise her forearms, lower legs and wrists against gravity, all of which she had become unable to do during the course of illness since 8 months of age. Our findings therefore suggest that the pyruvate therapy significantly improved the muscle strength and quality of life of the patient by a month after the start of treatment.

Contrary to the observed clinical improvement and the theory proposed by Tanaka et al., no significant changes of the blood lactate levels and L/P ratio were observed in this patient. One explanation for this discrepancy is that the blood lactate levels at the time of the therapy were too low (although higher than normal) to allow detection of any changes; the lactate level and the L/P ratio shortly before the start of pyruvate therapy were 2.5 mM and 18, respectively while those at the age of 3 months, by which time the patient was more active, were between 3.0 mM and 6.5 mM and 36 and 97, respectively. This apparent improvement in the *blood lactate levels* even before the start of pyruvate therapy might be due to the decrease in the muscle bulk as well as the severely weak muscle activity, which decreased the lactate production. Another factor which may have contributed to this discrepancy is the normal mitochondrial function in the liver. In myopathic MDS, mtDNA in the liver is not depleted; therefore, lactate released from the muscle might be metabolized in the liver, causing the blood lactate levels and L/P ratios to become near normal. On the other hand, when the lactate levels were very high at the age of 3 months, this factor did not contribute significantly. To prove that pyruvate does decrease the lactate levels and L/P ratios and increases the ATP production within the muscles, changes in these parameters in the muscles must be shown *in vivo*, possibly by magnetic resonance spectroscopy. We conducted no such evaluation in this study.

Thus, more clinical studies are necessary to precisely evaluate the efficacy of pyruvate therapy in patients with MDSs. However, there is only one published report, and several unpublished case reports on pyruvate therapy for mitochondrial diseases so far. Komaki et al. reported that an 11-year-old patient with Leigh syndrome associated with cytochrome c oxidase deficiency, who had easy fatigability and ataxic gait, became capable of participating in athletic games after treatment with oral sodium pyruvate at 0.5 g/kg [15]. They reported decrease of the blood lactate level from 2.3 mM to 1.1 mM and decrease of the L/P ratio from 18.1 to 11.7 in this patient. They also found an improvement in the cardiac dysfunction in the patient after one year's treatment. Other unpublished case reports include improvements in the MRI findings and cardiac dysfunction in a patient with Leigh syndrome (Wakamoto et al.) [15], cardiac improvement in another patient with Leigh syndrome (Koga et al.) [15], and activation of PDH activity which was estimated by measuring the $^{13}CO_2$ in exhaled air per unit time after administration of [1-^{13}C] pyruvate in two patients with PDH deficiency (Hamada et al. presented at the 52nd annual meeting of Japanese Society for Inherited Metabolic Diseases). We also treated a one-year old patient with Leigh syndrome associated with T9176C mutation in the mtDNA. The patient was severely disabled with tetraplegia at the time of the therapy, and showed no clinical improvement with pyruvate therapy. The severity of the symptoms at the time of pyruvate therapy may have differed between the patient treated by Komaki et al. and our own patient with Leigh syndrome. This finding highlights the limitation of this therapy and the possibly superior effects of the therapy in patients with an earlier stage of the disorder. No case reports on MDS are available.

Unlike non-physiological chemical drugs, such as dicholoroacetate, which can have some serious adverse effects, pyruvate is a physiological metabolite. The only possible side effects are sodium overload and osmotic diarrhea. Our patient did not develop diarrhea even though we did not dilute the sodium pyruvate as recommended, to avoid water overload. No serious adverse effects have been reported so far.

4. Conclusions

Oral (through a nasogastric tube) administration of 0.5 g/kg of sodium pyruvate improved the muscle power and quality of life of our patient with myopathic MDS. There are some case reports describing

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the efficacy of pyruvate in patients with different mitochondrial diseases. Considering that pyruvate can activate glycolysis even in cells without any mitochondria, as shown in ρ^0 cells, pyruvate therapy is a promising treatment for mitochondrial diseases. More clinical and biochemical studies are necessary to clearly prove the efficacy of this treatment.

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References

[1] C.T. Moraes, S. Shanske, H.J. Tritschler, J.R. Aprille, F. Andreetta, E. Bonilla, E.A. Schon, S. DiMauro, mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases, Am. J. Hum. Genet. 48 (1991) 492–501.

[2] T.H. Vu, M. Sciacco, K. Tanji, C. Nichter, E. Bonilla, S. Chatkupt, P. Maertens, S. Shanske, J. Mendell, M.R. Koenigsberger, L. Sharer, E.A. Schon, S. DiMauro, D.C. DeVivo, Clinical manifestations of mitochondrial DNA depletion, Neurology 50 (1998) 1783–1790.

[3] H.J. Tritschler, F. Andreetta, C.T. Moraes, E. Bonilla, E. Arnaudo, M.J. Danon, S. Glass, B.M. Zelaya, E. Vamos, N. Telerman-Toppet, et al., Mitochondrial myopathy of childhood associated with depletion of mitochondrial DNA, Neurology 42 (1992) 209–217.

[4] P.H. Ducluzeau, A. Lachaux, R. Bouvier, N. Streichenberger, G. Stepien, B. Mousson, Depletion of mitochondrial DNA associated with infantile cholestasis and progressive liver fibrosis, J. Hepatol. 30 (1999) 149–155.

[5] A. Suomalainen, P. Isohanni, Mitochondrial DNA depletion syndromes – many genes, common mechanisms, Neuromuscul. Disord. 20 (2010) 429–437.

[6] S. Alberio, R. Mineri, V. Tiranti, M. Zeviani, Depletion of mtDNA: syndromes and genes, Mitochondrion 7 (2007) 6–12.

[7] H. Yavuz, A. Ozel, M. Christensen, E. Christensen, M. Schwartz, M. Elmaci, J. Vissing, Treatment of mitochondrial neurogastrointestinal encephalomyopathy with dialysis, Arch. Neurol. 64 (2007) 435–438.

[8] M.C. Lara, B. Weiss, I. Illa, P. Madoz, L. Massuet, A.L. Andreu, M.L. Valentino, Y. Anikster, M. Hirano, R. Marti, Infusion of platelets transiently reduces nucleoside overload in MNGIE, Neurology 67 (2006) 1461–1463.

[9] M. Hirano, R. Marti, C. Casali, S. Tadesse, T. Uldrick, B. Fine, D.M. Escolar, M.L. Valentino, I. Nishino, C. Hesdorffer, J. Schwartz, R.G. Hawks, D.L. Martone, M.S. Cairo, S. DiMauro, M. Stanzani, J.H. Garvin Jr., D.G. Savage, Allogeneic stem cell transplantation corrects biochemical derangements in MNGIE, Neurology 67 (2006) 1458–1460.

[10] M. Tanaka, Y. Nishigaki, N. Fuku, T. Ibi, K. Sahashi, Y. Koga, Therapeutic potential of pyruvate therapy for mitochondrial diseases, Mitochondrion 7 (2007) 399–401.

[11] K. Murayama, H. Nagasaka, T. Tsuruoka, Y. Omata, H. Horie, S. Tregoning, D.R. Thorburn, M. Takayanagi, A. Ohtake, Intractable secretory diarrhea in a Japanese boy with mitochondrial respiratory chain complex I deficiency, Eur. J. Pediatr. 168 (2009) 297–302.

[12] L. He, P.F. Chinnery, S.E. Durham, E.L. Blakely, T.M. Wardell, G.M. Borthwick, R.W. Taylor, D.M. Turnbull, Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR, Nucleic Acids Res. 30 (2002) e68.

[13] C. Phoenix, A.M. Schaefer, J.L. Elson, E. Morava, M. Bugiani, G. Uziel, J.A. Smeitink, D.M. Turnbull, R. McFarland, A scale to monitor progression and treatment of mitochondrial disease in children, Neuromuscul. Disord. 16 (2006) 814–820.

[14] M.P. King, G. Attardi, Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation, Science 246 (1989) 500–503.

[15] H. Komaki, Y. Nishigaki, N. Fuku, H. Hosoya, K. Murayama, A. Ohtake, Y. Goto, H. Wakamoto, Y. Koga, M. Tanaka, Pyruvate therapy for Leigh syndrome due to cytochrome c oxidase deficiency, Biochim. Biophys. Acta 1800 (2010) 313–315.

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Original Article

Neonatal lactic acidosis with methylmalonic aciduria due to novel mutations in the *SUCLG1* gene

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Abstract ***Background***: Succinyl-coenzyme A ligase (SUCL) is a mitochondrial enzyme that catalyses the reversible conversion of succinyl-coenzyme A to succinate. SUCL consists of an α subunit, encoded by *SUCLG1*, and a β subunit, encoded by either *SUCLA2* or *SUCLG2*. Recently, mutations in *SUCLG1* or *SUCLA2* have been identified in patients with infantile lactic acidosis showing elevated urinary excretion of methylmalonate, mitochondrial respiratory chain (MRC) deficiency, and mitochondrial DNA depletion.

Methods: Case description of a Japanese female patient who manifested a neonatal-onset lactic acidosis with urinary excretion of methylmalonic acid. Enzymatic analyses (MRC enzyme assay and Western blotting) and direct sequencing analysis of *SUCLA2* and *SUCLG1* were performed.

Results: MRC enzyme assay and Western blotting showed that MRC complex I was deficient. *SUCLG1* mutation analysis showed that the patient was a compound heterozygote for disease-causing mutations (p.M14T and p.S200F).

Conclusion: For patients showing neonatal lactic acidosis and prolonged mild methylmalonic aciduria, MRC activities and mutations of *SUCLG1* or *SUCLA2* should be screened for.

Key words lactic acidosis, methylmalonic acid, mitochondrial respiratory chain, *SUCLA2*, *SUCLG1*.

Urinary excretion of methylmalonic acid is caused by a defect in the isomerization of L-methylmalonyl-coenzyme A to succinyl-coenzyme A. The reaction is catalyzed by L-methylmalonyl-coenzyme A mutase (MCM), an enzyme that requires adenosylcobalamin as a cofactor.[1] Methylmalonic acidemia/aciduria is mainly classified into two types: one resulting from a defect in the MCM apoenzyme and another resulting from a defect in the steps leading to adenosylcobalamin synthesis. In some cases, other causes of methylmalonic acidemia/aciduria have been reported. Recently, deficiency of the succinyl-coenzyme A ligase (SUCL) has been reported in cases of infantile lactic acidosis with mild urinary excretion of methylmalonic acid.[2]

Succinyl-coenzyme A ligase is a mitochondrial enzyme associated with the Krebs cycle, catalyzing the reversible conversion of succinyl-coenzyme A to succinate. The enzyme consists of two subunits. The substrate specificity for guanosine diphosphate (GDP) or adenosine diphosphate (ADP) is determined by the β subunit. The α subunit is encoded by the *SUCLG1* gene, whereas the β subunit is encoded by *SUCLA2* for the ADP-specific subunit and by *SUCLG2* for the GDP-specific subunit. *SUCLG1* is ubiquitously expressed, but its expression is particularly high in the heart, brain, kidney, and liver. The SUCLA2 protein is primarily present in the brain, skeletal muscle, and heart, and the SUCLG2 protein is present in the liver and kidney. More than 20 cases of deficiency in the α subunit (mutation in *SUCLG1*) or ADP-forming β subunit (mutation in *SUCLA2*) have been reported.[3–5] These patients have mitochondrial respiratory chain (MRC) deficiency, mitochondrial DNA (mtDNA) depletion, encephalomyopathy, and mild methylmalonic aciduria.[6–9]

Here, we describe the case of a Japanese female patient who presented with neonatal-onset lactic acidosis with urinary excretion of methylmalonic acid. *SUCLG1* mutation analysis showed that the patient was a compound heterozygote for disease-causing mutations.

Case report

In 1993 a female infant was born at 38 weeks gestation (birthweight, 2640 g; birth length, 47.3 cm). Her Apgar scores were normal. On the day after birth, she developed problems. Her blood sugar was lower than 1.1 mmol/L, and hence, continuous glucose infusion was started. Mechanical ventilation and peritoneal dialysis were started when the infant was 2 days old because of cyanosis, severe metabolic acidosis (pH, 6.638, base excess,

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Table 1 Laboratory data

	2 days	4 days	4 months
WBC (/μL)	46 500	19 400	
RBC ($\times 10^6$/μL)	4.50	4.59	
Hb (g/dL)	18.0	18.0	
Ht (%)	59.0	51.5	
Plt ($\times 10^3$/μL)		105	
Total bilirubin (mg/dL)		8.8	
γ-GTP (IU/L)		136	
AST (IU/L)	607	217	
ALT (IU/L)	125	128	
LDH (IU/L)	5400	3860	
CK (IU/L)		6370	
CK-MB (IU/L)		216	
Na (mBq/L)		140	
K (mBq/L)		3.0	
TP (mg/dL)		4.7	
BUN (mg/dL)	23	24	
Cr (mg/dL)		1.2	
pH	6.638	7.477	
HCO_3^- (mEq/L)		14.5	
Base excess	−26.8	−4.9	
NH_3 (mmol/L)	191	45	
Lactate (mmol/L)	11	8.1	
Pyruvate (mmol/L)		0.41	
BS (mg/dL)		101	
Urine (organic acids excretion)		High, lactate, pyruvate; Moderate, methylmalonate, methylcitrate; Slight, glutarate, fumarate, succinate, 3-methylglutaconate	
Acylcarnitine (dried blood spots)			increase in C3 and C4DC
Methylmalonic acid (serum)			13 μmol/L (control, not detected MCM-deficient patients, 220–2900)
Methylmalonic acid (urine)			321 mmol/molCr (control, mean [SD], 2.0 [1.2])
^{14}C-propionate fixation (cultured fibroblasts)			8% of control

ALT, alanine aminotransferase; AST. aspartate aminotransferase; BS, blood sugar; BUN, blood urea nitrogen; CK, creatine kinase; γ-GTP, γ-glutamyltransferase; Hb, hemoglobin; Ht, hematocrit; LDH, lactate dehydrogenase; MCM, L-methylmalonyl-coenzyme A mutase; Plt, platelets; RBC, red blood cells; TP, total protein; WBC, white blood cells.

−26.8), lactic acidemia (11 mmol/L), and hyperammonemia (191 μmol/L; Table 1). She was transferred to Tohoku University Hospital at 4 days old.

Upon admission there was a swelling in the liver 4 cm below the costal margin. The lactate and pyruvate levels were 8.1 mmol/L and 0.41 mmol/L, respectively (L/P ratio, 20). Gas chromatography and mass spectrometry of urinary organic acid showed high levels of lactate and pyruvate excretion; moderate methylmalonate and methylcitrate excretion; and slight glutarate, fumarate, succinate, and 3-methylglutaconate excretion.

Acidosis improved on the following day, and mechanical ventilation and peritoneal dialysis were stopped. She developed prolonged hypotonia. At 1 month of age, auditory brainstem response was absent, and severe hearing impairment was noted. Head computed tomography showed diffuse atrophy. At 4 months of age, mild cardiac hypertrophy was seen on echocardiogram. The patient could not balance her head.

Lactic acidemia (4–9 mmol/L) with an elevated L/P ratio (20–25) and mild urinary excretion of methylmalonic acid persisted. An acylcarnitine profile of dried blood spots showed an increase in C3 (propionylcarnitine) and C4DC (isomers of methylmalonyl carnitine and succinylcarnitine). The serum level of methylmalonic acid was 13 μmol/L (control, not detected; MCM-deficient patients, 220–2900 μmol/L). The urinary levels of methylmalonic acid and methylcitrate were 321 mmol/molCr and 81.7 mmol/molCr, respectively (control, mean ± SD, 2.0 ± 1.2 mmol/molCr and 2.0 ± 0.9 mmol/molCr, respectively). A ^{14}C-propionate fixation assay using cultured fibroblasts showed that propionate fixation in the patient was 8% of that in the control. Enzymatic analyses of the pyruvate dehydrogenase complex and pyruvate carboxylase were normal.

Histology of a liver biopsy specimen indicated moderate macrovesicular and microvesicular steatosis in the hepatic parenchyma. There was no active inflammation or fibrosis. On electron microscopy hepatocytes containing lipid droplets were seen. Mitochondrial abnormalities and other specific findings were not apparent morphologically. Muscle biopsy samples were stained with hematoxylin and eosin, reduced nicotinamide adenine

dinucleotide tetrazolium reductase, modified Gomori-Trichrome, succinate dehydrogenase, periodic acid–Schiff, and cytochrome oxidase. No particular abnormalities were noted in the muscle biopsy specimens.

At 6 months of age, the patient was discharged from hospital. She was able to follow objects with her eyes. Because of feeding difficulty, a naso-gastric tube was used. She developed a social smile at 13 months of age but did not have head control. At 20 months of age, she suddenly died at home. Autopsy was not performed.

Because her clinical course was similar to that of previously reported SUCL-deficient patients,[3,4] we restarted diagnostic analysis using fibroblasts and biopsied muscle samples that had been stored for 16 years in liquid nitrogen.

Methods

Blue native polyacrylamide gel electrophoresis and Western blotting

Expression levels of the MRC complex (Co) I, II, III, and IV proteins in cultured fibroblasts were assessed on Western blotting using blue native polyacrylamide gel electrophoresis (BN-PAGE) according to previously described methods.[10] Immunostaining was performed using monoclonal antibodies specific for the 39 kD subunit of Co I, 70 kD subunit of Co II, core 1 subunit of Co III, and subunit 1 of Co IV (Invitrogen, Camarillo, CA, USA).

Determination of enzyme activities

Activities of MRC Co I, II, III, and IV were assayed.[10] The activity of each complex was presented as a percentage of the mean value obtained from 20 controls. The percentages of Co I, II, III, and IV activities relative to that of citrate synthase (CS) as a mitochondrial enzyme marker or Co II activity were calculated. Deficiency of each complex is confirmed when either the CS ratio and/or the Co II ratio is <45% (fibroblasts) or 35% (muscle).

Quantitative polymerase chain reaction

The mtDNA was quantitatively estimated on real-time amplification of ND1 fragments in the mtDNA genome, as described previously.[10] To determine the overall abundance of mtDNA, the real-time amplification result of ND1 was compared with that of exon 24 of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, as nuclear DNA (nDNA).

Direct sequencing of the SUCLG1 and SUCLA2 genes

Genomic DNA was extracted from cultured fibroblasts using a Sepa Gene Kit (Sanko Junyaku, Tokyo, Japan). All coding exons, including flanking introns, in *SUCLG1* and the *SUCLA2* genes were amplified using polymerase chain reaction (PCR). To facilitate cycle sequencing analysis, M13 universal and reverse primer sequences were attached to the 5′ ends of sense primers and antisense primers, respectively. PCR products were directly sequenced using a Big Dye Primer Cycle Sequencing kit and an ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

The Ethics Committee of the Tohoku University School of Medicine approved the present study.

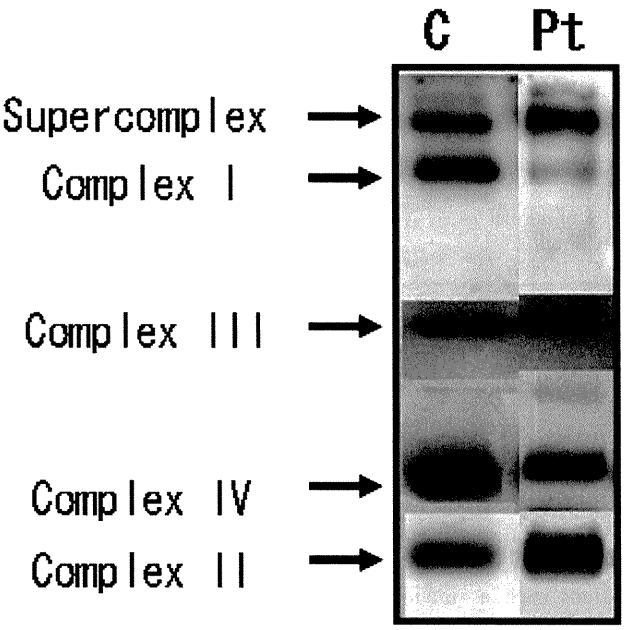

Fig. 1 Blue native polyacrylamide gel electrophoresis and subsequent Western blot analysis of mitochondrial respiratory chain complexes. The amount of assembled complex I was decreased. Complex I, anti-39 kDa subunit; complex II, anti-70 kDa subunit; complex III, anti-core 1 subunit; complex IV, anti-subunit 1.

Results

The amount of respiratory-chain complex in fibroblasts was determined on BN-PAGE Western blot. The intensity of the band corresponding to the assembled Co I of fibroblasts was decreased (Fig. 1). The intensity of the bands corresponding to Co II, III, and IV remained unchanged.

In fibroblasts, the enzyme activities of Co I and Co IV relative to that of Co II were decreased (<45%; Table 2). Even in the muscle biopsy samples, the ratios of (Co II + Co III)/CS, Co IV/CS, Co I/Co II, (Co II + Co III)/Co II, Co III/Co II, and CoIV/Co II were decreased.

Quantitative PCR showed that the ratio of mtDNA/nDNA of the fibroblasts did not decrease (72.9%; control, 76.4%). The ratio in the muscle biopsy specimen was also not decreased (270.1%).

Mutation analysis showed a heterozygous T-to-C substitution at position 41 in exon 1 of *SUCLG1* (c.41T > C; Fig. 2). This c.41T > C mutation changes the Met at position 14 to a Thr (p.M14T). Additionally, in exon 6, a heterozygous C-to-T substitution at position 599 in exon 1 of *SUCLG1* was found (c.599C > T). This mutation changes the Ser at position 200 to Phe (p.S200F). The p.M14T mutation was transmitted to the child from her mother; the other mutation (p.S200F) was transmitted to the child from her father (data not shown). Both substitutions were absent in the 100 alleles screened from healthy volunteers. No substitution was found in *SUCLA2*.

Table 2 Respiratory chain enzyme assay of the present patient

%	Co I	Co II	Co II + III	Co III	Co IV	CS
Fibroblasts						
% of normal	73	236	378	140	60	71
CS ratio	100	326	515	190	85	–
Co II ratio	**30**	–	158	58	**26**	–
Muscle						
% of normal	89	291	40	76	17	197
CS ratio	44	147	**20**	39	**8**	–
Co II ratio	**30**	–	**13**	**26**	**6**	–

Enzyme activities are expressed as % of mean normal control activity relative to protein, relative to CS, and relative to Co II. **Bold**, deficiency of the respective complex: <45% (fibroblasts) or 35% (muscle) of either CS ratio and/or Co II ratio. Reference range, fibroblasts 45–170; muscle 35–160.

Co I, complex I; Co II, complex II; Co III, complex III; Co IV, complex IV; CS, citrate synthase.

Discussion

The patient was identified to have a compound heterozygote mutation in *SUCLG1* (p.M14T and p.S200F). Clinical manifestations such as infantile lactic acidosis, mild methylmalonic aciduria, hypotonia, and hearing loss were compatible with symptoms previously reported in patients with *SUCLG1* or *SUCLA2* mutations.[3,6] The p.M14T and p.S200F mutations have not been reported previously. These substitutions were not found in the 100 alleles from healthy volunteers. p.M14 is located within the mitochondrial targeting sequence. Van Hove *et al.* reported a patient with a mutation at the same methionine (p.M14L) and speculated that the substitution of p.M14 would prevent proper translation initiation.[11] p.S200 is conserved across several species (Fig. 2). These data suggest that p.M14T and p.S200F are not polymorphisms but disease-causing mutations.

The amount of MRC complex I was decreased on BN-PAGE and Western blotting using fibroblasts, and multiple MRC defects were detected on enzyme assay. The ratios of mtDNA/nDNA of fibroblasts and muscle, however, did not decrease. Valayannopoulos *et al.* also reported that mtDNA depletion was not observed in two patients.[6] It is suggested that not all SUCL-deficient

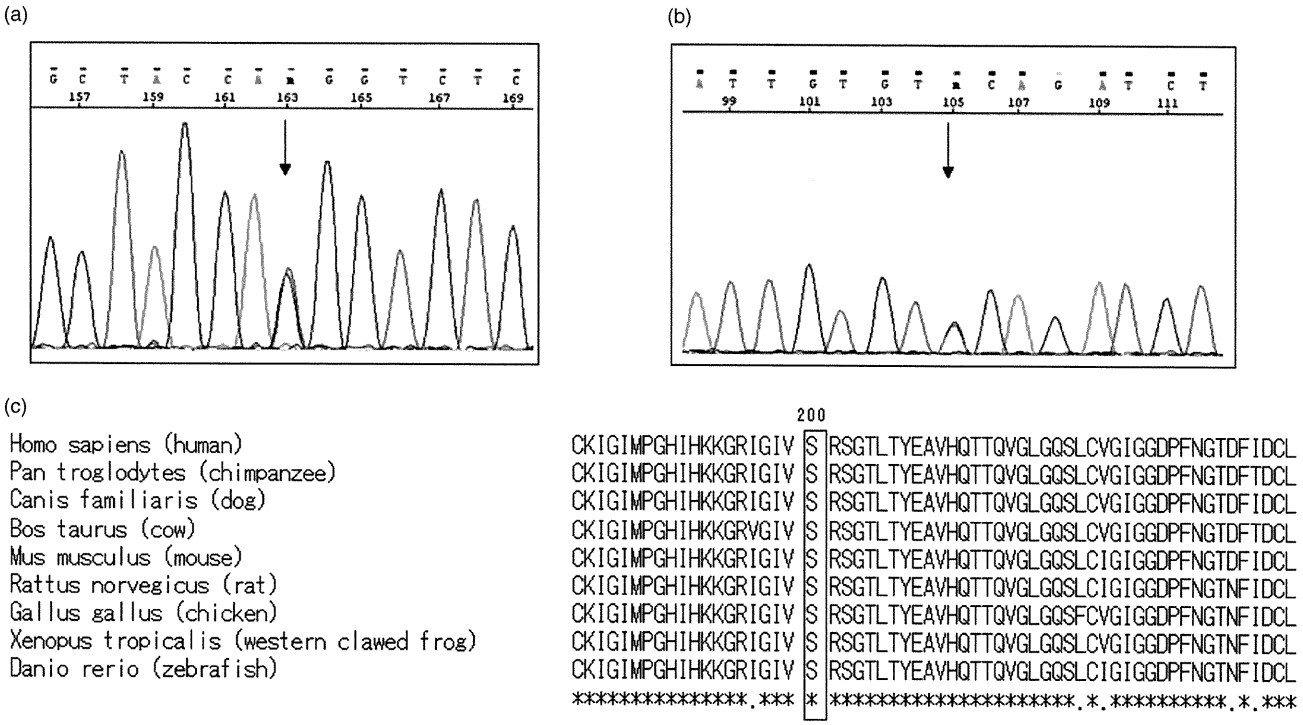

Fig. 2 (a) Heterozygous T-to-C substitution detected at c.41 in exon 1 of *SUCLG1*. This c.41T > C mutation changes the Met at position 14 to Thr (p.M14T). (b) Heterozygous C-to-T substitution detected at c.599 in exon 6 of *SUCLG1*. The c.599C > T substitution changes the Ser at position 200 to Phe (p.S200F). (c) Comparison of succinyl-coenzyme A ligase (SUCL) α subunits from several species. Serine at p.200 was conserved across all the species tested.

patients have mtDNA depletion, and that some mechanisms other than mtDNA depletion might participate in the multiple MRC deficiency observed in these patients.

In the present case, serum methylmalonic acid accumulation and low ^{14}C-propionate fixation capacity suggested disturbance of methylmalonic acid metabolism. Elevated methylmalonic acid may result from the accumulation of succinyl-coenzyme A under the assumption that accumulated succinyl-CoA inhibits the reaction catalyzed by MCM or causes an equilibrium shift, leading to the accumulation of methylmalonyl-coenzyme A, which is converted to methylmalonic acid. As usual, increased levels of C4DC are detected in patients with severe MCM deficiency during acute crises. It is suggested that the C4DC of the present patient was associated with an increased level of succinylcanritine due to accumulated succinyl-coenzyme A.

In conclusion, we identified two novel *SUCLG1* mutations in a Japanese female patient with neonatal lactic acidosis and prolonged mild methylmalonic aciduria. For patients showing these combined manifestations, MRC activities and mutations of *SUCLG1* or *SUCLA2* should be screened for.

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References

1 Fenton WA, Gravel RA, Rosenblatt DS. Disorders of propionate and methylmalonate metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*, 8th edn. McGraw-Hill, New York, 2001; 2165–93.
2 Ostergaard E. Disorders caused by deficiency of succinate-CoA ligase. *J. Inherit. Metab. Dis.* 2008; **31**: 226–9.
3 Elpeleg O, Miller C, Hershkovitz E *et al.* Deficiency of the ADP-forming succinyl-CoA synthase activity is associated with encephalomyopathy and mitochondrial DNA depletion. *Am. J. Hum. Genet.* 2005; **76**: 1081–6.
4 Ostergaard E, Christensen E, Kristensen E *et al.* Deficiency of the alpha subunit of succinate-Coenzyme A ligase causes fatal infantile lactic acidosis with mitochondrial DNA depletion. *Am. J. Hum. Genet.* 2007; **81**: 383–7.
5 Ostergaard E, Schwartz M, Batbayli M *et al.* A novel missense mutation in SUCLG1 associated with mitochondrial DNA depletion, encephalomyopathic form, with methylmalonic aciduria. *Eur. J. Pediatr.* 2010; **169**: 201–5.
6 Valayannopoulos V, Haudry C, Serre V *et al.* New SUCLG1 patients expanding the phenotypic spectrum of this rare cause of mild methylmalonic aciduria. *Mitochondrion* 2010; **10**: 335–41.
7 Rivera H, Merinero B, Martinez-Pardo M *et al.* Marked mitochondrial DNA depletion associated with a novel SUCLG1 gene mutation resulting in lethal neonatal acidosis, multi-organ failure, and interrupted aortic arch. *Mitochondrion* 2010; **10**: 362–8.
8 Randolph LM, Jackson HA, Wang J *et al.* Fatal infantile lactic acidosis and a novel homozygous mutation in the SUCLG1 gene: A mitochondrial DNA depletion disorder. *Mol. Genet. Metab.* 2011; **102**: 149–52.
9 Rouzier C, Le Guédard-Méreuze S, Fragaki K *et al.* The severity of phenotype linked to SUCLG1 mutations could be correlated with residual amount of SUCLG1 protein. *J. Med. Genet.* 2010; **47**: 670–76.
10 Kaji S, Murayama K, Nagata I *et al.* Fluctuating liver functions in siblings with MPV17 mutations and possible improvement associated with dietary and pharmaceutical treatments targeting respiratory chain complex II. *Mol. Genet. Metab.* 2009; **97**: 292–6.
11 Van Hove JL, Saenz MS, Thomas JA *et al.* Succinyl-CoA ligase deficiency: A mitochondrial hepatoencephalomyopathy. *Pediatr. Res.* 2010; **68**: 159–64.

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Living donor liver transplantation for ornithine transcarbamylase deficiency

Wakiya T, Sanada Y, Mizuta K, Umehara M, Urahasi T, Egami S, Hishikawa S, Fujiwara T, Sakuma Y, Hyodo M, Murayama K, Hakamada K, Yasuda Y, Kawarasaki H. Living donor liver transplantation for ornithine transcarbamylase deficiency. Pediatr Transplantation 2011: 15: 390–395. © 2011 John Wiley & Sons A/S.

Abstract: Ornithine transcarbamylase deficiency, the most common urea cycle disorder, causes hyperammonemic encephalopathy and has a poor prognosis. Recently, LT was introduced as a radical OTCD treatment, yielding favorable outcomes. We retrospectively analyzed LT results for OTCD at our facility. Twelve children with OTCD (six boys and six girls) accounted for 7.1% of the 170 children who underwent LDLT at our department between May 2001 and April 2010. Ages at LT ranged from nine months to 11 yr seven months. Post-operative follow-up period was 3–97 months. The post-operative survival rate was 91.7%. One patient died. Two patients who had neurological impairment preoperatively showed no alleviation after LT. All patients other than those who died or failed to show recovery from impairment achieved satisfactory quality-of-life improvement after LT. The outcomes of LDLT as a radical OTCD treatment have been satisfactory. However, neurological impairment associated with hyperammonemia is unlikely to subside even after LT. It is desirable henceforth that more objective and concrete guidelines for OTCD management be established to facilitate LDLT with optimal timing while avoiding the risk of hyperammonemic episodes.

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Key words: ornithine transcarbamylase deficiency – neonatal onset type – late onset type – treatment – liver transplantation

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OTCD is a urea cycle disorder seen in approximately one in every 40 000–80 000 births. It is the most common urea cycle disorder. Patients with OTCD have low OTC activity in the liver and thus experience repeated hyperammonemic episodes, leading to disorders of the central nervous system. It is not rare for impairment to be seen after such central nervous system disorders. This disease is occasionally fatal (1, 2). Generally, OTCD is an X-chromosome-linked disorder and is severe in hemizygous boys. Hemizygous boys often develop this disease during the neonatal period, and the patient often dies during this period (3). However, depending on the location of the gene mutation, the reduction in enzymatic activity can be relatively mild and the disease can develop in boys after the neonatal period with an initial symptom of sudden hyperammonemia. In heterozygous girls, enzymatic activity is various, ranging from complete deficiency to almost normal activity depending on the location of the gene mutation and because of random inactivation of the X chromosomes caused by lyonization. When lyonization is abnormal, girls can manifest symptoms. For this reason, clinical features of this disease in girls are quite diverse, ranging from symptoms appearing during the neonatal period to the absence of symptoms throughout the life (lifelong asymptomatic cases) (4).

If OTCD is treated medically, many cases require dietary restrictions and oral medications

Abbreviations: ACR, acute cellular rejection; DS, disease severity; GV, graft volume; HPS, hemophagocytic syndrome; LDLT, living donor liver transplantation; LT, liver transplantation; MS, metabolic status; NS, neurological status; OTC, ornithine transcarbamylase; OTCD, ornithine transcarbamylase deficiency; QOL, quality of life; SLV, standard liver volume ratio.

for the rest of their lives. In recent years, LT began to be adopted as a radical treatment for OTCD, yielding favorable outcomes (5–11).

We herein report a retrospective analysis of 12 patients with OTCD who underwent LDLT at our department. We evaluated the usefulness of LT in the management of OTCD and identified problems.

Patients and methods

Of the 170 children who underwent LDLT at our facility between May 2001 and April 2010, the 12 patients with OTCD (7.1%) were enrolled in this study. Their post-operative follow-up period ranged from three to 97 months.

We reviewed our 12 cases (Table 1) to collect the following data: gender, age at onset, peak plasma ammonium level (peak NH3), age at LDLT, body height at LDLT, body weight at LDLT, and inherited type of OTCD. Inherited type was classified into mutation and X-linked. We did mutation analysis of OTC gene to all recipients and their mothers. We classified it with X-linked when there was a common gene mutation to both. When only the recipient had the mutation, we classified it with mutation. We further investigated the following variables (Table 2): donor, ABO-blood type matching, GV/SLV ratio, post-operative complications, and outcomes of LDLT. DS, MS, and NS were assessed by accepted grading scales essentially following Morioka et al.'s study (5) as shown in Table 3. QOL was also classified into four subgroups as shown in Table 3. In addition, we compared the pre- and post-transplant states (Table 4), i.e., we examined chronological changes in DS, MS, NS, and QOL.

In all cases, elective LT was carried out using a segment 2+3 or 2+3+4 graft and there were no cases for which auxiliary partial orthotopic LT was carried out. Donor hepatectomy was determined according to recipient standard liver volume or body weight. The donor biliary anatomy was evaluated using intraoperative repeated real-time cholangiography or preoperative magnetic resonance cholangiography. Routine donor graft hepatectomy was performed with intraoperative ultrasonic guidance. If necessary, graft hepatic vein venoplasty was performed at bench surgery. For the recipient operation, total hepatectomy was performed with a portacaval shunt. After total hepatectomy, the recipient right, middle, and left hepatic veins were formed as a single orifice, which was then anastomosed end-to-end to the graft left hepatic vein. Portal vein was reconstructed between the recipient right or left portal vein branch patch and the graft left portal vein. Hepatic artery reconstruction was performed using microsurgical techniques. Biliary tract reconstruction was performed using a Roux-en-Y hepaticojejunostomy. Intraoperative color

Table 1. Characteristics of the 12 recipients

Case	Gender	Age at LDLT	Age at onset	Peak NH3 (μM)	BH at LT (cm) (Z score)	BW at LT (kg) (Z score)	Inherited type
1	F	2 yr 11 months	11 months	313	84.0 (−2.1)	11.9 (−0.7)	Mutation
2	M	1 yr 10 months	2 days	212	82.0 (−0.8)	10.0 (−1.3)	X linked
3	M	1 yr 1 months	2 days	1394	73.3 (−1.2)	11.0 (1.4)	X linked
4	F	8 yr 2 months	3 yr	234	117.1 (−1.6)	22.7 (−0.7)	X linked
5	F	3 yr 10 months	3 yr	191	94.7 (−0.9)	15.0 (0.1)	Mutation
6	M	3 yr 4 months	8 days	901	95.6 (−0.1)	16.0 (0.4)	X linked
7	F	3 yr 7 months	10 months	400	99.5 (0.8)	19.0 (2.5)	Mutation
8	F	11 yr 7 months	7 yr	302	145.4 (0.2)	38.5 (0.1)	X linked
9	M	1 yr 7 months	3 days	1136	72.0 (−3.0)	7.8 (−2.6)	X linked
10	F	3 yr 6 months	7 months	320	100.0 (1.1)	16.0 (1.0)	Mutation
11	M	1 yr 2 months	5 days	473	75.4 (−0.6)	9.0 (−0.7)	X linked
12	M	9 months	2 days	1632	68.4 (−1.3)	8.6 (−0.3)	X linked

Table 2. Outcomes of LDLT for OTCD

Case	Donor	ABO-blood type matching	GV/SLV ratio (%)	Postoperative complication	Survival outcome
1	Father	Identical	77.1	ACR	97 months, alive
2	Father	Identical	78.9	ACR, biliary complication	61 months, alive
3	Father	Imcompatible	74.6	Biliary complication	52 months, alive
4	Father	Imcompatible	62.6	HPS	3 months, died of HPS
5	Mother	Compatible	64.2	–	36 months, alive
6	Mother*	Identical	54.0	–	24 months, alive
7	Mother	Identical	42.4	ACR	23 months, alive
8	Father	Imcompatible	43.7	Biliary complication	23 months, alive
9	Father	Compatible	68.8	–	15 months, alive
10	Mother	Identical	63.6	–	10 months, alive
11	Father	Identical	71.1	–	4 months, alive
12	Father	Identical	95.6	–	3 months, alive

*Donor with heterozygotes.

Table 3. Grading scales to evaluate DS, MS, and NS, and classifications of QOL

Severity of the disease
- Grade 4: many episodes of severe hyperammonemia coma, some with NH3* >300 μM
- Grade 3: one to several episodes of hyperammonemic coma, no more than one with NH3* >300 μM
- Grade 2: one to few episodes of hyperammonemic coma, none with NH3* >300 μM
- Grade 1: only one episode of hyperammonemic coma, with NH3* <300 μM
- Grade 0: no episodes of hyperammonemic coma, no NH3* >100 μM

MS
- Grade 4: no improvement, severe hyperammonemia, need for constant, full doses of medication
- Grade 3: some improvement, moderate hyperammonemia, need for constant medication
- Grade 2: major improvement, moderate hyperammonemia, need for some medication for control
- Grade 1: almost complete correction, occasional hyperammonemia, with or without need for medication
- Grade 0: complete correction, no hyperammonemia, no need for medication

NS
- Grade 5: persistent coma or vegetative state
- Grade 4: responds to noxious stimuli, but no social interaction, no ambulation, no communication
- Grade 3: limited social interaction, no bipedal ambulation, limited communication through gestures
- Grade 2: definite social interaction, fair ambulation, though possibly limited by spasticity
- Grade 1: good social interaction, full ambulation but perhaps partially impaired gross and fine motor skills, use of language, mildly delayed development, only modest learning deficits
- Grade 0: seems to be normal spectrum for social interaction, motor skills, language development and learning

QOL
- Excellent: receiving one or no immunosuppressive drugs and all the above grading scales corresponding to a score of 0
- Good: receiving two or more immunosuppressive drugs and all the above corresponding to a score of 0
- Fair: regardless of the number of immunosuppressive drugs each patient received, one or more of the above scales corresponding to a score of 1
- Poor: with any episodes of graft dysfunction to necessitate frequent or long hospital stay regardless of their causes and/or one or more of the above scales corresponding to a score of 2 or more

*Plasma ammonium level.

Table 4. Chronological changes in DS, MS, NS, and QOL

Case	Pretransplant status (DS/MS/NS)	Status at the latest evaluation (DS/MS/NS)	QOL at the latest evaluation
1	3/3/0	0/0/0	Good
2	1/3/0	0/0/0	Excellent
3	3/3/2	0/0/2	Poor
4	2/3/0	–	Death
5	2/3/0	0/0/0	Good
6	3/3/0	0/0/0	Good
7	3/3/0	0/0/0	Good
8	3/3/0	0/0/0	Good
9	4/3/2	0/0/2	Poor
10	4/3/0	0/0/0	Good
11	3/3/0	0/0/0	Good
12	4/3/0	0/0/0	Good

Doppler ultrasonography was performed to assess the blood flow velocity and pattern after vascular reconstruction and during abdominal wall closure.

Tacrolimus and methylprednisolone were used as standard post-operative immunosuppression therapy. The target trough level of tacrolimus was 15–20 ng/mL during the first week, 8–12 ng/mL during the first month, 5–8 ng/mL during the first six months, 3–5 ng/mL during the first year, and 2–4 ng/mL thereafter. Methylprednisolone was administered at an initial dose of 20 mL/kg intravenously on the morning of the operation and before graft reperfusion. The methylprednisolone dose was gradually decreased to 3 mg/kg per day on post-operative day 1, 0.5 mg/kg per day on post-operative day 7, and 0.25 mg/kg per day at one month post-LT and was discontinued within one yr except in patients in whom immunosuppression could not be maintained with the lower dosage. Biopsy-proved acute rejection was treated with intravenously administered methylprednisolone, 20 mg/kg per day for three days. Mycophenolate mofetil was used when more potent immunosuppression was required, such as in ABO-incompatible recipients older than five yr, patients with steroid-resistant acute rejection episodes, and patients with liver dysfunction after cessation of methylprednisolone therapy.

Results

The study involved six boys and six girls with OTCD. In all of the boys, the disease had developed during the neonatal period (age at disease onset, 2–8 days) and LDLT was performed at ages between nine month and three yr four months. For the girls, the age at disease onset was seven months to seven yr and LT was performed at ages between two yr 11 months and 11 yr seven months. Before LT, all cases had developed hyperammonemia and medical treatment (oral medications and dietary restrictions) had been administered. Of the six boys presented in the neonatal period, two (cases 3 and 9) had preoperatively shown neurological impairment secondary to hyperammonemic encephalopathy. Preoperative peak plasma ammonium level was high in case 3 (1394 μM) and case 9 (1136 μM), each recorded at the time of the first attack after birth (Table 1).

The donor was the father in eight cases and the mother in four cases (Table 2). Transplantation from blood-type-incompatible donors was carried out in three patients (cases 3, 4, and 8), and the father served as the donor for all 3. Of the four patients with the mother serving as the donor, one (the mother of case 6) was an asymptomatic carrier having no history of hyperammonemic episodes and whose OTC activity was preserved based on preoperative needle liver biopsy. The post-operative follow-up period ranged from three to 97 months. The post-operative survival rate was 91.7%, with one patient (case 4) dying of HPS. As post-operative complications, ACR was seen in three cases and biliary complication in three others (Table 2). No patient developed hyperammonemic attack during the post-operative follow-up period. No case required resumption of dietary restrictions or oral medications. In the two cases in which neurological impairment secondary to hyperammonemic encephalopathy had been seen preoperatively, this impairment did not subside after LT. On post-operative evaluation, QOL was judged to have improved in all cases except for the patient who died and those in whom neurological impairment persisted (Table 4).

Discussion

OTCD is clinically classified into two types: neonatal onset and late onset. The neonatal onset type of OTCD develops within several days after birth and advances rapidly thereafter. The disease is severe in an overwhelming majority of cases with this type of OTCD, and many of these patients die within one year despite intensive care. In Japan, the five-yr survival rate is reportedly about 8.8% for patients with this type of OTCD and moderate to severe neurological impairments are often seen in long-term survivors (3, 12). The late onset type develops with initial symptoms resembling encephalopathy during infancy through adulthood following hyperammonemia triggered by infection, hunger, or fatigue. Unless treated immediately, encephalopathy advances, sometimes leading to death. When treated only medically, patients with this type of OTCD are likely to experience repeated cycles of remission and relapse of hyperammonemic episodes and the prognosis is poor, with the five-yr survival rate being 45% (3). At present, it is desirable to establish a valid strategy for treating OTCD (including radical treatment methods) to improve the survival of patients as well as QOL for patients and their family members.

LT has recently begun to be used as a radical treatment for OTCD, yielding favorable outcomes (5–11). In our facility, LDLT has been applied in 12 patients with OTCD, with the survival rate being 91.7%. A noteworthy finding from post-operative follow-up of these patients is that high-dose oral drug therapy, needed to control plasma ammonium level preoperatively, was no longer necessary after LT, and the hyperammonemic episodes that had occurred owing to resistance to medical treatment ceased after LT. Also, in the evaluation of these cases using the scale of Morioka et al., QOL improved post-operatively. Thus, LT was confirmed to be useful as a radical OTCD treatment at our facility.

Meanwhile, as reported by Maesteri et al., LT as a radical treatment is unlikely to alleviate the neurological impairment of OTCD (8, 9, 12). In our two patients in whom neurological impairment had been seen preoperatively, no alleviation of this impairment was achieved post-operatively, although LT avoided additional metabolic decompensations and made further dietary regimen unnecessary. Therefore, it is essential that hemodialysis is the first step to avoid brain edema in case of hyperammonemia. Furthermore, it is desirable to apply well-timed radical treatment before the appearance of neurological impairment.

With respect to the use of heterozygous donors in LDLT, Morioka et al. (13) reported no negative impacts of the use of heterozygous carriers as donors on either donors' or recipients' post-operative courses if their liver OTC activity was normal. The donor for our case 6, a heterozygous asymptomatic carrier who was employed because there were no other candidates, showed no apparent signs of hyperammonemia in the early post-operative period, and she has been doing well without hyperammonemic episodes in the post-operative follow-up period. Above all, her OTC activity was preserved based on preoperative needle liver biopsy. Her estimated residual liver volume was 81.8%. However, there are no long-term post-LDLT results using heterozygous donors, and both the donor and the recipient must be closely followed up. In addition, it is necessary to conduct worldwide multicenter studies.

Regarding the indications and timing of LT, a consensus is being reached over the view that the neonatal onset type of OTCD is absolutely indicated for LT and that early LT should be performed for cases with this type of OTCD (6, 10, 11). As Campeau et al. (14) recommended,

early transplantation in the first year of age has an excellent outcome. On the other hand, in LT for small infants, there is a high risk of post-operative complications and problems such as difficulties in perioperative management (15–17). Therefore, if we consider only perioperative safety to avoid various complications, it might be better to wait for the appropriate growth until we can perform subsequent LT. In the point to reduce the risk of hyperammonemic episodes and post-operative complications, we have a management dilemma for timing of LT. In our institutional policy, we performed LT after waiting to grow up to body weight 8 kg as long as we can keep favorable plasma ammonium control with medical treatment. If candidate will have acute liver failure and bad ammonium control with full medications, regardless of body weight, we decide to perform urgent LT.

No widely accepted view has been accepted regarding the late onset type of OTCD. At present, determination of the indications for and timing of LT in cases of late-onset-type OTCD utilizes only information about clinical symptoms and the course of the disease. In current practice, the indications for and timing of LT in these cases are determined based on a general assessment of the presence/absence of repeated hyperammonemic episodes resistant to medical treatment, presence/absence of marked growth retardation, presence/absence of QOL reduction owing to intense dietary restriction, presence/absence of signs of advanced disease revealed by diagnostic imaging (head MRI, etc.), and so on. However, as a result of the lack of widely accepted therapeutic guidelines, medical treatment is sometimes continued in cases requiring LT, resulting in repeated cycles of remission and relapse of hyperammonemic episodes. It is therefore advisable that LT be undertaken with optimal timing in cases indicated for this treatment. In cases in whom plasma ammonium level can be controlled with medical treatment, selection of LT is controversial and requires care, in view of the risks of surgery for both the donor and the recipient, post-operative complications, immunosuppression, and other factors.

It is desirable henceforth to establish more objective and concrete guidelines for the management of this disease to facilitate appropriate determination of the indications for and timing of LT to reduce the risk of hyperammonemic episodes and improve the survival of patients while avoiding neurological impairment. Pediatricians and surgeons both need to be involved in transplantation, cooperating to provide the best health care for patients with OTCD.

Conclusion

LT is absolutely indicated for neonatal-onset-type OTCD, and outcomes are favorable. However, because neurological impairment associated with OTCD is unlikely to subside even after LT, treatment at the first hyperammonemic episode is apparently very important. The LT results were also favorable in late-onset-type OTCD cases. When dealing with late-onset-type OTCD for which LT is relatively indicated, it is desirable to establish more objective and concrete treatment guidelines and to apply LT with optimal timing while avoiding the risk of hyperammonemic episodes.

Funding sources and conflict of interests

None.

References

1. Tuchman M. The clinical, biochemical, and molecular spectrum of ornithine transcarbamylase deficiency. J Lab Clin Med 1992: 120: 836–850.
2. Maestri N, Hauser E, Bartholomew D, Brusilow S. Prospective treatment of urea cycle disorders. J Pediatr 1991: 119: 923–928.
3. Uchino T, Endo F, Matsuda I. Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. J Inherit Metab Dis 1998: 21(Suppl. 1): 151–159.
4. Yorifuji T, Muroi J, Uematsu A, et al. X-inactivation pattern in the liver of a manifesting female with ornithine transcarbamylase (OTC) deficiency. Clin Genet 1998: 54: 349–353.
5. Morioka D, Kasahara M, Takada Y, et al. Current role of liver transplantation for the treatment of urea cycle disorders: A review of the worldwide English literature and 13 cases at Kyoto University. Liver Transpl 2005: 11: 1332–1342.
6. Mcbride KL, Miller G, Carter S, Karpen S, Goss J, Lee B. Developmental outcomes with early orthotopic liver transplantation for infants with neonatal-onset urea cycle defects and a female patient with late-onset ornithine transcarbamylase deficiency. Pediatrics 2004: 114: e523–e526.
7. Saudubray JM, Touati G, Delonlay P, et al. Liver transplantation in urea cycle disorders. Eur J Pediatr 1999: 158(Suppl. 2): S55–S59.
8. Wraith J. Ornithine carbamoyltransferase deficiency. Arch Dis Child 2001: 84: 84–88.
9. Whitington PF, Alonso EM, Boyle JT, et al. Liver transplantation for the treatment of urea cycle disorders. J Inherit Metab Dis 1998: 21(Suppl. 1): 112–118.
10. Hasegawa T, Tzakis A, Todo S, et al. Orthotopic liver transplantation for ornithine transcarbamylase deficiency with hyperammonemic encephalopathy. J Pediatr Surg 1995: 30: 863–865.
11. Ensenauer R, Tuchman M, El-Youssef M, et al. Management and outcome of neonatal-onset ornithine transcarbamylase deficiency following liver transplantation at 60 days of life. Mol Genet Metab 2005: 84: 363–366.
12. Maestri N, Clissold D, Brusilow S. Neonatal onset ornithine transcarbamylase deficiency: A retrospective analysis. J Pediatr 1999: 134: 268–272.
13. Morioka D, Kasahara M, Takada Y, et al. Living donor liver transplantation for pediatric patients with inheritable metabolic disorders. Am J Transplant 2005: 5: 2754–2763.

14. Campeau P, Pivalizza P, Miller G, et al. Early orthotopic liver transplantation in urea cycle defects: Follow up of a developmental outcome study. Mol Genet Metab 2010: 100(Suppl. 1): S84–S87.
15. Bekker J, Ploem S, De Jong K. Early hepatic artery thrombosis after liver transplantation: A systematic review of the incidence, outcome and risk factors. Am J Transplant 2009: 9: 746–757.
16. Ueda M, Oike F, Kasahara M, et al. Portal vein complications in pediatric living donor liver transplantation using left-side grafts. Am J Transplant 2008: 8: 2097–2105.
17. Chin K, Uemoto S, Takahashi K, et al. Noninvasive ventilation for pediatric patients including those under 1-year-old undergoing liver transplantation. Liver Transpl 2005: 11: 188–195.

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Patient Reports

Fatal case of mitochondrial DNA depletion with severe asphyxia in a newborn

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Key words asphyxia, mitochondrial DNA depletion, mitochondrial respiratory chain disorder.

Many factors, particularly maternal and placental conditions, are associated with asphyxia. However, the etiology of asphyxia is occasionally unclear. It has been shown that mitochondrial respiratory chain disorders (MRCD) probably cause severe asphyxia and sudden death in newborns.[1] Mitochondrial DNA (mtDNA) depletion is a prevalent cause of multiple respiratory chain deficiency during this period;[2] however, there is little information on the correlation between mtDNA depletion and asphyxia. We report a case of severe asphyxia caused by mtDNA depletion syndrome (MDS).

Case Report

A healthy male newborn was delivered by vacuum extraction to nonconsanguineous parents at 40 gestational weeks because of prolonged labor. His birthweight was 2.6 kg, which is <10th percentile according to Japanese reference data. His 37-year-old mother had no history of gestational or pregnancy complications, and gestation was achieved by intracytoplasmic sperm injection. Chronological clinical observation and echocardiography during pregnancy revealed no abnormalities. However the mother noticed paucity in his intrauterine movement. Immediately after birth, the boy was resuscitated for severe asphyxia, with Apgar scores of 2 and 5 at 1 and 5 min, respectively. He was admitted to our hospital 2 h after birth.

The infant was intubated immediately and given sodium bicarbonate intravenously for acidosis (pH 7.08, PCO_2 77.1, base excess −9.4). On admission, he presented with severe hypotonia, multiple arthrogryposis, and insufficient spontaneous respiration. Simultaneously, he was found to have bilateral undescended testes and cephalhematoma. Results of routine laboratory examinations were normal, except for a slightly elevated blood lactate level (25 mg/dL in the venous blood; normal 7–16 mg/dL); common enzymes, including serum creatine kinase, lactate dehydrogenase, and glutamic oxaloacetic transaminase were maintained at normal levels throughout the clinical course. A general X-ray and cardiac and cranial echograms showed normal results.

His vital signs were maintained within normal ranges by artificial ventilation. Magnetic resonance imaging (MRI) at days 9 and 18 of age showed no findings suggestive of ischemia, anomaly, or damage in the brain, except for subdural hematoma. However he remained hypotonic and unable to respire spontaneously. At 23 days of age, bradycardia occurred suddenly after tracheal suction; sudden death followed despite appropriate resuscitation.

The electrocardiograph showed sinus bradycardia, and laboratory findings showed normal electrolytes. His metabolic screening tests, such as amino acid profiles, acylcarnitines analysis, and urinary organic acids analysis, showed no findings suggestive of congenital metabolic disorders. The histological examinations of muscular tissues obtained at autopsy revealed marked muscular atrophy (Fig. 1).

Activities of respiratory chain complexes I, II, III and IV in the skeletal muscle, cardiac muscle, and liver were determined as described previously.[3] The activity of each complex was presented as a percent ratio relative to the mean value obtained from 35 healthy controls. The percent ratios of complex I, II, III and IV activities to the citrate synthase, that is, a mitochondrial enzyme marker, or complex II activity, were calculated for each patient.[4] All the respiratory chain complexes in the skeletal muscle showed low activities and respiratory chain complex I activity was low in the cardiac muscle. Complex I and IV activities in the skeletal muscle were strikingly low in contrast to those of other respiratory chain complexes (Table 1).

mtDNA was quantitatively estimated by real-time amplification of nicotinamide adenine dinucleotide dehydrogenase (ND) 1 fragments in the mtDNA genome, as previously described.[4,5] To determine the overall abundance of mtDNA, we compared the real-time amplification of ND1 with a single-copy nuclear reference gene (exon 24 of the cystic fibrosis transmembrane conductance regulator gene; selected because it lacks single-nucleotide

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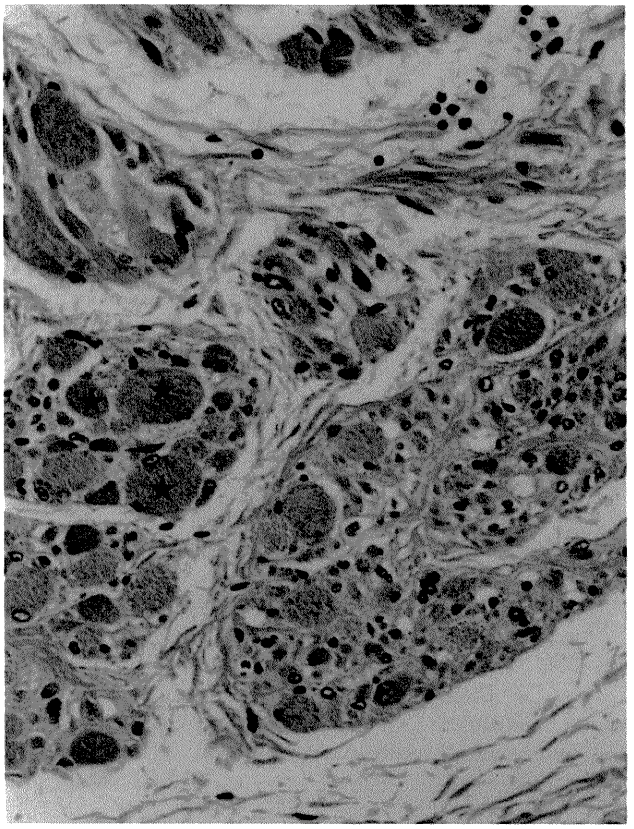

Fig. 1 Hematoxylin and eosin stain of skeletal muscle. Non-grouped muscular atrophy with marked variation in fiber size is noted (asterisks).

polymorphisms). Quantitative polymerase chain reaction revealed that mtDNA in both skeletal and cardiac muscles were markedly reduced in this patient (skeletal muscle, 14.4%; cardiac muscle, 9.9%; normal range, 40–150%). The infant was diagnosed with complex I and IV deficiency caused by mtDNA depletion. Genetic analyses of thymidine kinase 2 and ribonucleotide reductase M2 B for MDS revealed no abnormality. In addition, genetic analyses of neuronal apoptosis inhibitory protein and survival motor neuron, which were performed to confirm spinal muscular atrophy, also revealed no abnormality.

Table 1 Respiratory chain enzyme analysis in the skeletal muscle, cardiac muscle, and liver

	Co I	Co II	Co III	Co IV	CS
Skeletal muscle					
% of normal	2.1	9.4	10.8	1.4	11.3
CS ratio	18.6	88.3	99.1	12.2	
Co II ratio	21.8		115.8	14.6	
Cardiac muscle					
% of normal	20.8	40.9	57.2	35.4	69.0
CS ratio	28.7	59.7	80.9	49.6	
Co II ratio	43.0		124.1	75.3	
Liver					
% of normal	67.1	107.8	63.8	307.1	133.1
CS ratio	49.8	80.4	47.1	225.9	
Co II ratio	61.7		58.8	280.8	

Enzyme activities are expressed as % of mean normal control activity relative to protein, relative to CS and relative to Co II. Co I, complex I; Co II, complex II; Co III, complex III; Co IV, complex IV; CS, citrate synthase.

Discussion

MDS is characterized by a reduced mtDNA copy number.[6] The appropriate diagnosis of MDS would enable us to predict the risk of the recurrence in pregnancy because it forms part of a clinically and genetically heterogeneous group of autosomal recessive disease disorders. However, there is only limited information on MDS as the cause of asphyxia.

Common neonatal presentations of MRCD in newborns include a paucity of intrauterine movement, neonatal hypotonia or hypertonia, feeding and respiratory difficulties, and neonatal death.[1,7] Gibson *et al.*[8] reported that a combination of non-specific manifestations, such as prematurity and intrauterine growth retardation along with the above symptoms, should indicate the possibility of MRCD. Our case is clinically characterized by these manifestations, which are important clues for the diagnosis of mitochondrial respiratory disorders and mtDNA depletion. We established the diagnosis with respiratory chain disorders according to the modified diagnostic criteria reported by Bernier.[7]

Asphyxia may cause secondary mitochondrial impairments through hypoxia and ischemia, and they may influence the activities of respiratory chain complexes. Complex II is especially labile and estimation of its activity is difficult.[9] Nevertheless, our case showed a normal ratio of complex II activity to citrate synthase activity; this led us to consider a primary mitochondrial respiratory disorder, although it is impossible to completely exclude secondary mitochondrial impairments.

It is noteworthy that the levels of common enzymes, which are possible markers of asphyxia or predictors for neurological disorders in asphyxiated newborns,[10] were not elevated in our patient. Furthermore, he did not exhibit abnormal MRI findings reflecting the persistent symptoms during the clinical course. We speculate that this discrepancy may be a distinct and unique feature of asphyxia caused by mitochondrial respiratory disorders that is quite different from asphyxia caused by other maternal or placental factors.

In summary, neonatologists should be familiar with the mechanisms and diagnosis of mitochondrial disease, including mtDNA depletion, and consider it as a possible cause of asphyxia if the cause is unclear, suspected symptoms are persistent, and the symptoms and laboratory data and MRI findings are inconsistent.

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References

1 Sue CM, Hirano M, DiMauro S, De Vivo DC. Neonatal presentations of mitochondrial metabolic disorders. *Semin Perinatol.* 1999; **23**: 113–24.

2 Sarzi E, Bourdon A, Chretien D *et al.* Mitochondrial DNA depletion is a prevalent cause of multiple respiratory chain deficiency in childhood. *J Pediatr.* 2007; **150**: 531–4.
3 Kirby DM, Crawford M, Cleary MA, Dahl HM, Dennett X, Tourburn DR. Respiratory chain complex I deficiency. An underdiagnosed energy generation disorder. *Neurology* 1999; **52**: 1255–64.
4 Pagnamenta AT, Taaman JW, Wilson CJ *et al.* Dominant inheritance of premature ovarian failure associated with mutant mitochondrial DNA polymerase gamma. *Hum Reprod.* 2006; **21**: 2467–73.
5 He L, Chinney PF, Durham SE *et al.* Detection and quantification of mitochondrial DNA depletions in individual cells by real-time PCR. *Nucleic Acids Res.* 2002; **30**: e68.
6 Moraes CT, Shanske S, Tritschler HJ *et al.* mtDNA depletion with variable tissue expression: A novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet.* 1991; **48**: 337–41.
7 Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 2002; **59**: 1406–11.
8 Gibson K, Halliday JL, Kirby DM, Yaplito-Lee J, Thorburn DR, Boneh A. Mitochondrial oxidative phosphorylation disorders presenting in neonates: Clinical manifestations and enzymatic and molecular diagnoses. *Pediatrics* 2008; **122**: 1003–8.
9 Murray J, Taylor SW, Xhang B, Ghosh SS, Capaldi RA. Oxidative damage to mitochondrial complex I due to peroxynitrite: Identification of reactive tyrosines by mass spectrometry. *J Biol Chem.* 2003; **278**: 37223–30.
10 Lackmann GM. Influence of neonatal idiopathic respiratory distress syndrome on serum enzyme activities in premature healthy and asphyxiated newborns. *Am J Perenatol.* 1996; **13**: 329–34.

Prenatal lead poisoning due to maternal exposure results in developmental delay

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Key words developmental disability, language development disorder, lead poisoning, maternal exposure, neonatal disease.

Fetuses and young children are quite susceptible to the adverse effects of lead[1] but the prevalence of lead poisoning in the general population was largely unknown until the National Health and Nutrition Examination Survey I (NHANES I) collected data from over 20 000 individuals between 1976 and 1980 and correlated lead levels and symptoms. Subsequent data analyses identified three independent risk factors for an elevated blood lead level: age less than six years old; African-American; or city-dweller.[2] The Centers for Disease Control then reviewed several studies examining the effect of lead on intelligence and changed the definition of elevated blood lead level from 25 mcg/dL to 10 mcg/dL in 1991.[3] However, studies have shown that cognitive deficits and hearing loss can occur at levels below 10 mcg/dL.[4] We present a case of maternal pica behavior and the subtle cognitive effects upon the child. Our case has relevance given the continued use of leaded paint on children's toys leading to numerous product recalls and case reports of pediatric lead poisoning due to ingestion.[5]

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Case report

A 26-year-old G_4P_4 woman who had a history of domestic violence, treatment for meningitis, and poor prenatal care, stated during her delivery history and physical that she had been eating paint chips during the first trimester in a failed attempt to abort her fetus. She subsequently delivered a 38-week gestation male infant weighing 2835 grams with APGAR scores of 8 and 9. Her serum blood level was tested after delivery and found to be 7 mcg/dL. Her infant was admitted to the newborn nursery for monitoring and an initial cord blood lead level was 40.7 mcg/dL with a repeat serum blood level of 36.7 mcg/dL and hemoglobin of 17.4 g/dL. Our hematology consultant reviewed the case and recommended against chelation since there was not acute exposure. At three weeks old a repeat lead level was 34.5 mcg/dL. The infant was enrolled in the home infant stimulation program for at risk infants despite a normal four week neurodevelopmental evaluation.

At six weeks old the infant's hemoglobin was 11.6 g/dL without basophilic stippling or Howell-Jolly bodies and a long bone study showed lead lines 2 cm in width and 2 cm from each epiphysis consistent with an increased lead level (Fig. 1). At six months old, the lead level was 26.8 mcg/dL and hemoglobin of 11.2 g/dL with Mean Corpuscular Volume (MCV) of 73.2. Again, no developmental delays were noted at that visit.

At one year of age, the patient had a lead level of 20.3 mcg/dL and speech delay with a normal audiometric exam. An ophthalmological exam revealed iris heterochromia. A home visit found peeling wall paint and multiple paint chips both of

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Case report

Liver-specific mitochondrial respiratory chain complex I deficiency in fatal influenza encephalopathy

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Abstract

We report on a 4-year-old boy who died from influenza encephalopathy. The clinical course and microscopic findings of the autopsied liver were compatible with Reye's syndrome. We examined the mitochondrial respiratory chain function by blue native polyacrylamide gel electrophoresis (BN-PAGE), western blotting, and respiratory chain enzyme activity assays. The activity of liver respiratory chain complex (CO) I was markedly decreased (7.2% of the respective control activity); whereas, the other respiratory chain complex activities were substantially normal (CO II, 57.9%; CO III, 122.3%; CO IV, 161.0%). The activities of CO I–IV in fibroblasts were normal (CO I, 82.0%; CO II, 83.1%; CO III, 72.9%; CO IV, 97.3%). The patient was diagnosed with liver-specific complex I deficiency. This inborn disorder may have contributed to the fatal outcome. We propose that relying only on fibroblast respiratory chain complex activities may lead to the misdiagnosis of liver-specific complex I deficiency.

Keywords: Influenza encephalopathy; Reye's syndrome; Mitochondria; Complex I deficiency; Liver-specific

1. Introduction

Influenza encephalopathy is a critical complication of influenza infection. Although the pathological mechanism is poorly understood, mitochondrial malfunction is suggested to play a role in the pathogenesis [1]. We describe a boy with liver-specific mitochondrial respiratory chain complex I deficiency who developed fatal encephalopathy associated with influenza A infection. The possible contribution of the mitochondrial respiratory chain disorder to the clinical course is discussed.

2. Case report

A 4-year-old Japanese boy developed pyrexia. He was treated with acetaminophen once and visited the family doctor. Influenza A infection was diagnosed by nasal antigen test in a clinic and he was treated with oseltamivir. He was admitted to a nearby hospital due to a generalized seizure in the evening; then, he was transferred to our institute because of highly elevated serum transaminase. He was the first child born to healthy parents with no consanguinity. No other child had died in early

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infancy within three degrees of relationship. He had normal psychomotor development and had not been vaccinated against influenza.

On arrival, he was comatose and had a temperature of 38.9 °C, heart rate of 136 beats per minute, and blood pressure of 106/62 mm Hg. Neither arrhythmia nor cardiac hypertrophy was seen in the electrocardiogram or echocardiography. Blood examination showed marked liver dysfunction and ammonemia (aspartate aminotransferase, 4282 IU/l; alanine aminotransferase, 1750 IU/l; ammonia, 156 μg/dl). Blood gas analysis showed marked acidosis (pH 6.964, pCO_2 59.6 mm Hg, HCO_3 11.2 mol/l, BE −23.7 mmol, and lactate 9.0 mmol/l). Blood glucose was 128 mg/dl under intravenous infusion. Influenza encephalopathy was diagnosed and intensive therapy, including mechanical ventilation, steroid, and heart stimulants, was started. A few hours later, he developed cardio-pulmonary arrest and died 36 h after developing pyrexia. This clinical course led us to suspect Reye's syndrome and mitochondrial disorders. The parents consented to resection of the patient's liver and skin fibroblasts. Urine organic acid analysis, blood amino acid profile, and carnitine profile did not show any findings suggestive of congenital metabolic disorders. Microscopical finding showed microvesicular fatty droplets in hepatic cytoplasm in hematoxylin-eosin and oil red O staining (Fig. 1), that was compatible with Reye's syndrome. The grade of histological hepatic changes was milder than the fulminant clinical course.

The activities of respiratory chain complexes (Co) I, II, III, and IV were assayed in the crude post-600 g supernatant of the liver and in isolated mitochondria from skin fibroblasts as described previously [2]. The activity of each complex was presented as a percent ratio relative to the mean value obtained from 12 healthy controls. The activities of Co I, II, III, and IV were also calculated as the percent relative to citrate synthetase (CS), a mitochondrial enzyme marker, or Co II activity [2]. Liver respiratory chain complex I activities were very low, but CS, Co II, III, and IV activities were normal. In contrast to the liver, the fibroblast complex I activity was normal (Table 1).

The expression of the mitochondrial respiratory chain Co I, II, III, and IV proteins in the liver and fibroblasts were examined by Western blotting using blue native polyacrylamide gel electrophoresis (BN-PAGE) according to methods described previously [3]. The results of BN-PAGE are shown in Fig. 2. The band corresponding to Co I was not visible; while, the intensities of the Co II, III, and IV bands remained normal. Several base substitutions were detected by polymerase chain reaction, but there was no pathogenic mutation in the genomic DNA extracted from the autopsied liver tissue.

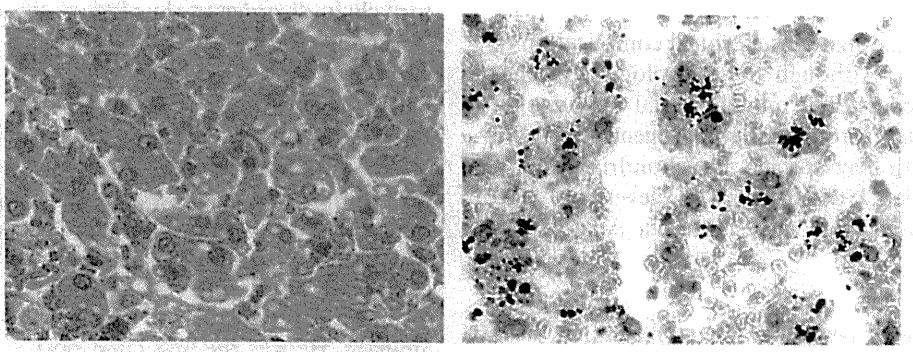
3. Discussion

Mitochondrial malfunction has been described in influenza encephalopathy. There are no reports of mitochondrial respiratory chain diseases, although disorders of fatty acid oxidation have been discussed [1]. Complex I deficiency was first recognized in 1979 by Morgan-

Table 1
Enzyme assay of respiratory chain complexes.

%	Co I	Co II	Co III	Co IV	CS
Liver					
% of normal	7.2	57.9	122.3	161.0	78.1
CS ratio	9.2	74.1	155.0	203.8	–
Co II ratio	12.3	–	212.2	272.2	–
Fibroblast					
% of normal	82.0	83.1	72.9	97.3	120.4
CS ratio	66.2	66.8	56.5	76.3	–
Co II ratio	98.2	–	83.7	112.5	–

Co I, complex I; Co II, complex II; Co III, complex III; Co IV, complex IV; CS, citrate synthase.
Enzyme activities are expressed as a % of the mean relative activity of the normal control and relative to CS and Co II.



A. Hematoxylin-Eosin staining (×400) B. Oil Red O staining (×400)

Fig. 1. Autopsy liver samples show preserved hepatic architecture with scattered distribution of micro-vesicular fatty droplets in the hepatic cytoplasm (A). Marked congestion, focal necrosis, and mild inflammatory cellular infiltration without fibrosis were noted. Fat deposition was also suggested with oil red O staining (B). The grade of histological hepatic changes was milder than the fulminant clinical course.