A case report of Leigh syndrome diagnosed by endomyocardial biopsy

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Background  
Leigh syndrome is a neurodegenerative disorder caused by mitochondrial dysfunction with both phenotypic and genetic heterogeneity. Mitochondrial impairments are usually demonstrated by skeletal muscle biopsy. We report a case of Leigh syndrome diagnosed by endomyocardial biopsy (EMB), not by skeletal muscle biopsy.

Case summary  
At aged 7 months, the patient had delayed motor development. He developed metabolic acidosis triggered by an infection with elevated lactate and pyruvate values in serum and cerebrospinal fluid when he was 1 year old. T2-weighted imaging on magnetic resonance imaging of the brain revealed bilateral hyperintensity in midbrain and dorsal pons. Biopsied skeletal muscle did not show evidence of mitochondrial disease. Left ventricular hypertrophy, bilateral putamen hyperintensity in T2-weighted imaging and a lactate peak in the right basal ganglia in single voxel spectroscopy, and a convulsive seizure appeared at the age of 12, 15, and 16, respectively. When he was 17 years old, biopsied myocardium showed cytoplasmic vacuolization and a marked proliferation of mitochondria within myofibrils pathologically. Respiratory chain enzyme activity of the biopsied myocardium showed decreased activity of complex I. Genetic testing revealed an m.14453 A>G mutation on the MT-ND6 gene. He was finally diagnosed with Leigh syndrome. Administration of oral 5-aminolevulinic acid reduced the frequency of seizures.

Discussion  
EMB led to the diagnosis of Leigh syndrome. Efforts to find and conduct the biopsy of affected organs are important to diagnose mitochondrial disease. EMB is a useful diagnostic method when there is a difficulty in diagnosing mitochondrial disease by skeletal muscle biopsy.

Keywords  
Leigh syndrome • Mitochondrial cardiomyopathy • Endomyocardial biopsy • Muscle biopsy • Case report

Learning points  
- Mitochondrial disease can present with cardiovascular complications in adulthood.
- Efforts to find and conduct the biopsy of affected organs are important to diagnose mitochondrial disease.
- Endomyocardial biopsy is a useful diagnostic method in cases with cardiomyopathy when there is a difficulty in diagnosing mitochondrial disease by skeletal muscle biopsy.

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Introduction

Leigh syndrome is a neurodegenerative disorder caused by mitochondrial dysfunction with both phenotypic and genetic heterogeneity.1 Leigh syndrome is characterized by: (i) neurodegenerative disease with variable symptoms due to (ii) mitochondrial impairment caused by a hereditary genetic defect accompanied by (iii) bilateral central nervous system lesions that can be associated with further abnormalities in diagnostic imaging.2 The term ‘Leigh-like syndrome’ should be used when these criteria are only partially met.2 Mitochondrial impairments are demonstrated by mitochondrial respiratory chain dysfunction, pathologically abnormal in skeletal muscle biopsy, or gene mutation.3 These abnormalities are usually found by skeletal muscle biopsy. We herein report a case of Leigh syndrome diagnosed by endomyocardial biopsy (EMB), not by skeletal muscle biopsy.

Timeline

| Age       | Events                                                                 |
|-----------|-------------------------------------------------------------------------|
| At birth  | Delivered vaginally at 38 weeks’ gestational age weighing 2348 g with no neonatal asphyxia. |
| 7-month-old | Delayed motor development appeared.                                       |
| 1-year-old | Metabolic acidosis triggered by a viral infection occurred.               |
| 12-year-old | Echocardiography showed left ventricular hypertrophy.                     |
| 15-year-old | T2-weighted imaging on MRI showed hyperintensity in bilateral putamen and right cerebral peduncle, and cerebellar atrophy. |
| 22-year-old | He can walk with no life-threatening complications.                        |

Case presentation

A 17-year-old boy was admitted to our hospital with left ventricular hypertrophy. Our patient was born from non-consanguineous parents, delivered vaginally at 38 weeks’ gestational age weighing 2384 g with no neonatal asphyxia. He had no family history of cardiomyopathy or sudden cardiac death. Manifestations of neurological illness appeared in 7 months with delayed motor development. He developed metabolic acidosis triggered by a viral infection on three occasions when he was 1 year old. At this point, lactate and pyruvate values in serum and cerebrospinal fluid were elevated. T2-weighted imaging revealed bilateral hyperintensity in midbrain and dorsal pons in brain magnetic resonance imaging (MRI) (Figure 1). Leigh syndrome was suspected based on clinical course and brain images. Although skeletal muscle biopsy was performed, ragged red fibres were not identified pathologically, and respiratory chain enzyme activity of the biopsied skeletal muscle was normal. Point mutations associated with mitochondrial diseases at the mitochondrial DNA position 3243, 8344, 8993, and 9176 in the biopsied skeletal muscle were not identified. As the evidence of mitochondrial dysfunction was not shown, he was diagnosed as ‘Leigh-like syndrome’. We started to supply thiamine and dichloroacetic acid as a treatment for mitochondrial impairments which was suspected at this time. The episode of metabolic acidosis did not occur after he was 4 years old. Although he had a delay in psychomotor development, he attended a school for disabled children and grew up with no regression. His activities of daily living such as functional mobility and self-feeding were independent, whereas bathing, dressing, and toilet hygiene needed partial assistance during childhood.

Left ventricular hypertrophy was identified on the electrocardiogram when he was 6 years old (Figure 2).4 Echocardiography showed left ventricular hypertrophy when he was 12 years old (Video 1).5–7 Although there were no cardiovascular manifestations such as dyspnoea or palpitation, mitochondrial cardiomyopathy was suspected based on these cardiological findings and clinical course. When he was 15 years old, T2-weighted imaging showed hyperintensity in bilateral putamen and right cerebral peduncle, and cerebellar atrophy in brain MRI (Figure 1). Single voxel spectroscopy demonstrated a lactate peak in the right basal ganglia (Figure 1). Although these MRI findings were suggestive of Leigh syndrome, a definitive diagnosis could not be made since we could not demonstrate evidence of mitochondrial dysfunction. The patient suffered a convulsive seizure when he...
was 16 years old. Levocarnitine supplementation as an additional treatment for suspected mitochondrial impairments and anticonvulsants were started.

When he was 17 years old, he was admitted to our hospital to investigate the cause of left ventricular hypertrophy. His blood pressure was 108/60 mmHg. Pulse rate was 53/min and regular. Heart sounds were regular, and he had no murmur. Neurological examination showed mild muscle weakness with normal deep tendon reflexes. Blood analysis showed normal brain natriuretic peptide value of less than 5.8 pg/mL (reference range: 0–18.4 pg/mL). EMB was performed by cardiac catheterization and biopsy samples were obtained at the ventricular septum in the right ventricle. Light microscopy of the biopsied myocardium showed cytoplasmic vacuolization (Figure 3), and electron microscopy showed a marked proliferation of mitochondria within myofibrils (Figure 3). Using electron microscopy with quantitative analysis, the volume density of mitochondria within...
cardiac muscle cells was 39%, which indicated the possibility of mitochondrial cardiomyopathy. Respiratory chain enzyme activity of the biopsied myocardium and cultured fibroblasts obtained from the biopsied skin showed decreased activity of complex I (Table 1). Oxygen consumption rate of skin fibroblasts measured by Seahorse XF96 apparatus was 92% of control in glucose medium and 64% of normal in galactose medium, which indicated oxidative phosphorylation impairment. Immunohistopathological analysis with respiratory chain enzyme antibodies of biopsied myocardium showed decreased signal intensity of complex I compared with complex II (22% of normal) and normal signal intensity of complex IV compared with complex II (116% of normal) (Figure 4). Genetic testing revealed an m.14453 A>G mutation on the MT-ND6 gene, with a heteroplasmy rate of the blood sample 60% and the cardiac tissue 83%. This mutation was not shown in his parents, suggesting that it was a de novo mutation. Association of mutations in MT-ND6 gene with Leigh syndrome has been reported previously. This m.14453 A>G mutation has been reported in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, whereas it has not been reported in Leigh syndrome previously. Taken together with clinical course and laboratory findings, this mutation was considered the causative agent. He was finally diagnosed with Leigh syndrome and mitochondrial cardiomyopathy. As the frequency of seizures gradually increased and his motor function gradually regressed, we started oral 5-aminolevulinic acid as a treatment when he was 21 years old. After the initiation of oral 5-aminolevulinic acid, the frequency of seizures decreased, whereas his motor function gradually continued to regress. In the most recent follow-up at the age of 22, he had no life-threatening complications and was able to walk slowly, whereas his activities of daily living such as functional mobility and self-feeding required partial assistance.

Discussion

Mitochondrial diseases are a genetically and clinically heterogeneous group of disorders due to dysfunctions of the mitochondrial oxidative phosphorylation. They can be caused by mutations in either mitochondrial DNA or nuclear genes. Mitochondrial DNA is usually all identical (homoplasmy), whereas individuals with mitochondrial diseases resulting from mutation of mitochondrial DNA may have a mixture of mutated and wild type mitochondrial DNA (heteroplasmy). In the presence of heteroplasmy, there is a threshold effect and clinical phenotypes may vary depending on the percentage level.

**Video 1** Cardiac movement in echocardiography at the age of 12. Left ventricular ejection fraction was 69%. Intraventricular septum thickness at end-diastole, left ventricular end-diastolic diameter, and left ventricular posterior wall thickness at end-diastole were 13.6 mm (Z-score +3.20), 52.9 mm (Z-score +1.94), and 10.6 mm (Z-score +2.70), respectively. Left ventricular mass and left ventricular mass index calculated using the Devereux equation was 258.8 g (Z-score +2.87) and 182.7 g/m², respectively. Mitral peak early filling (E) wave velocity, mitral peak atrial filling (A) wave velocity, and pulsed wave tissue Doppler imaging early diastolic (e') velocity at septal basal region were 106 cm/s, 29 cm/s, and 10 cm/s, respectively. E-wave deceleration time was 180 ms. E/A ratio and E/e' were 3.7 and 10.6, respectively.

**Figure 3** Pathological findings of biopsied right ventricle. Light microscopy of biopsied right ventricular myocardium showed cytoplasmic vacuolization (black arrow) in the myocardium (left panel). Electron microscopy of biopsied right ventricular myocardium showed a marked proliferation of mitochondria (white arrow) within myofibrils (middle and right panel).
of mutated mitochondrial DNA in each organ. Mitochondrial diseases may present at any age.18,19 Mitochondrial dysfunction is associated with a wide spectrum of diseases and clinical syndromes such as Kearns–Sayre syndrome, chronic progressive external ophthalmoplegia, MELAS, myoclonic epilepsy with ragged red fibres, neurogenic weakness with ataxia and retinitis pigmentosa, Leigh syndrome, or mitochondrial cardiomyopathy.12,20

The diagnostic criteria for mitochondrial respiratory chain complex disorder is widely used for the diagnosis of oxidative phosphorylation disorder.7 In this diagnostic criteria, ragged red fibres in pathological tissue of skeletal muscle are defined as diagnostic standard, whereas the diagnostic standard of pathological features in myocardial tissue is not defined. Therefore, in the case of mitochondrial cardiomyopathy alone, it is necessary to identify the decreased enzyme activity using myocardial tissue for diagnosis.

A clinical diagnosis of mitochondrial cardiomyopathy is usually made when cardiomyopathy appears as a complication of mitochondrial disease. A definitive diagnosis of mitochondrial cardiomyopathy is made by the trinity of genetic, pathological, and biochemical examination.8,20 Typical phenotypes of mitochondrial cardiomyopathy are hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, and left ventricular noncompaction.20 In practice, clinicians should be aware of mitochondrial cardiomyopathy as a differential diagnosis of unexplained cardiomyopathy, which can present for the first time in adulthood. The ‘red flag’ features which can point towards mitochondrial disease are unexplained cardiomyopathy, cardiac conduction disorder, maternal inheritance, diabetes mellitus, hearing impairment, neuromuscular features, and metabolic acidosis.

It has been reported that fusiform enlargement of affected myocytes around the peculiar region with cytoplasmic clearing and replacement of cross striae by fine granules in microscopy, and increased and larger mitochondria, abnormal cristae in the ultrastructural examination are found pathologically in cardiac specimens in mitochondrial cardiomyopathy.21 In our previous study, we revealed that the volume density of mitochondria within cardiac muscle cells above 30% strongly suggests the possibility of mitochondrial cardiomyopathy.8 Furthermore, immunohistopathological analysis with respiratory chain enzyme antibodies of biopsied myocardium of mitochondrial cardiomyopathy showed decreased signal intensity of complex I and/or complex IV compared with complex II.8 All these pathological findings were found in our case. These pathological features in the myocardium may also be added as a diagnostic standard of mitochondrial respiratory chain complex disorder.

Although we could not diagnose as Leigh syndrome for 17 years as the evidence of mitochondrial dysfunction was not identified by skeletal muscle biopsy, EMB led to the diagnosis of mitochondrial cardiomyopathy and Leigh syndrome in our case. In the case of Leigh syndrome, Lee et al.22 reported that muscle biopsies in 39 patients did not reveal any ragged red fibres and Sofou et al.23 reported that ragged red fibres occurred in muscle biopsies in 57 out of 107 patients. A biopsy of the affected organs is essential for the diagnosis of mitochondrial disease. Thus, if a skeletal muscle is not affected, skeletal muscle biopsy has no diagnostic capability as in our case. Efforts to find affected organs and conducting a biopsy of those organs are important when there is a difficulty in diagnosing mitochondrial disease. For cases in which mitochondrial disease is suspected but cannot be diagnosed by skeletal biopsy, the cardiac examination should be performed, and if there is evidence of cardiomyopathy, EMB should be considered.

The main treatments for mitochondrial cardiomyopathy are cardioprotective and symptomatic therapy.20,21 Cardiac manifestations

### Table 1  Respiratory chain activity of cultured skin fibroblasts and biopsied myocardium

|                | Co I | Co II | Co II + III | Co III | Co IV |
|----------------|------|-------|-------------|--------|-------|
| Fibroblasts CS ratio (%) | 26   | 53    | 45          | 41     | 131   |
| Heart CS ratio (%)       | 72   | 79    | 166         | 65     | 171   |

Respiratory chain activity showed defect in complex I (abnormal values are highlighted by underbars). Co I, complex I; Co II + III, complex II + III; Co II, complex II; Co III, complex III; Co IV, complex IV; CS, citrate synthase.

**Figure 4** Immunohistopathological analysis with respiratory chain enzyme antibodies of biopsied myocardium. Left, middle, and right panel shows immunohistopathological analysis of complex I, complex IV, and complex II, respectively. A signal intensity of complex I compared with complex II was 22% of normal and complex IV compared with complex II was 116% of normal.
vary from asymptomatic to severe heart failure or sudden death.\textsuperscript{24} As it may cause the fatal outcomes, accurate diagnosis is important and careful follow-up is necessary.

Recently, it has been reported that 5-aminolevulinic acid and sodium ferrous citrate improved mitochondrial functions in skin fibroblasts from patients with mitochondrial diseases, which may become a new treatment option for mitochondrial diseases.\textsuperscript{25} In our case, as the symptoms gradually worsened and there were no other specific treatment, we initiated 5-aminolevulinic acid as a treatment, which decreased frequency of seizures. Long-term follow-up is necessary to evaluate the efficacy of 5-aminolevulinic acid.

\section*{Conclusion}

In summary, we reported a case of Leigh syndrome diagnosed by EMB. Efforts to find and conduct the biopsy of affected organs are important to diagnose mitochondrial disease. EMB is a useful diagnostic method in cases with cardiomyopathy when there is a difficulty in diagnosing mitochondrial disease by skeletal muscle biopsy. Accurate diagnosis is important to prevent fatal outcomes and to perform suitable treatment in mitochondrial disease.

\section*{Lead author biography}

Dr Yuji Maruo received the MD degree from Hokkaido University Graduate School of Medicine (Sapporo, Japan) in 2015. He enrolled in the senior resident program of Department of Pediatrics, Hokkaido University Graduate School of Medicine in 2017. Since 2020, he has been working at the Department of Pediatrics, Japanese Red Cross Kitami Hospital as a paediatrician. His medical interest is in paediatric cardiology.
Supplementary material

Supplementary material is available at European Heart Journal - Case Reports online.

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Slide sets: A fully edited slide set detailing this case and suitable for local presentation is available online as Supplementary data.

Consent: The authors confirm that written consent for submission and publication of this case report including images and associated text has been obtained from the patient in line with COPE guidance.

Conflict of interest: none declared.

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References

1. Ruhoy IS, Saneto RP. The genetics of Leigh syndrome and its implications for clinical practice and risk management. Appl Clin Genet 2014;7:221–234.
2. Baertling F, Rodenburg RJ, Schaper J, Smeitink JA, Koopman WJ, Mayatepek E et al. A guide to diagnosis and treatment of Leigh syndrome. J Neural Neurosurg Psychiatry 2014;85:257–265.
3. Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. Neurology 2002;59:1406–1411.
4. Rijnbeek PR, Witsenburg M, Schrama E, Hess J, Kors JA. New normal limits for the paediatric electrocardiogram. Eur Heart J 2001;22:702–711.
5. Petterson MD, Du W, Skeens ME, Humes RA. Regression equations for calculation of z-scores of cardiac structures in a large cohort of healthy infants, children, and adolescents: an echocardiographic study. J Am Soc Echocardiogr 2008;21:922–934.
6. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57:450–458.
7. Foster BJ, Mackie AS, Mifsud E, Ali H, Mamber S, Colan SD. A novel method of expressing left ventricular mass relative to body size in children. Circulation 2008;117:2769–2775.
8. Takeda A, Murayama K, Okazaki Y, Imai-Oikazaki A, Ohtake A, Talakawa E et al. Advanced pathological study for definite diagnosis of mitochondrial cardiomyopathy. J Clin Pathol 2020;doi:10.1136/jclinpath-2020-206801.
9. Kirby DM, Crawford M, Cleary MA, Dahl HH, Dennett X, Thorburn DR. Respiratory chain complex I deficiency: an underdiagnosed energy generation disorder. Neurology 1999;52:1255–1264.
10. Murayama K, Nagasaki H, Tsuruoka T, Omata Y, Horie H, Tregoning S et al. Intractable secretory diarrhea in a Japanese boy with mitochondrial respiratory chain complex I deficiency. Eur J Pediatr 2009;168:297–302.
11. Invernizzi F, D’Amato I, Jensen PB, Ravaglia S, Zeviani M, Tiranti V. Microscale oxygenigraphy reveals OXPHOS impairment in MRC mutant cells. Mitochondrion 2012;12:328–335.
12. Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. Biochem J 2011;435:297–312.
13. Ogawa E, Shimura M, Fushimi T, Tajika M, Ichimoto K, Matsunaga A et al. Clinical validity of biochemical and molecular analysis in diagnosing Leigh syndrome: a study of 106 Japanese patients. J Inherit Metab Dis 2017;40:685–693.
14. Du M, Wei X, Xu P, Xie A, Zhou X, Yang Y et al. A novel mitochondrial m.14430A>G (MT-ND6, p.W82R) variant causes complex I deficiency and mitochondrial Leigh syndrome. Clin Chem Lab Med 2020;58:1809–1817.
15. Ravn K, Wilbrand F, Hansen JF, Horn N, Rosenberg T, Schwartz M. An mtDNA mutation, 14453G→A, in the NADH dehydrogenase subunit 6 associated with severe MELAS syndrome. Eur J Hum Genet 2001;9:805–809.
16. Ng YS, Turnbull DM. Mitochondrial disease: genetics and management. J Neural 2016;263:179–191.
17. Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 1990;46:428–433.
18. Leonard JV, Schapira AH. Mitochondrial respiratory chain disorders I: mitochondrial DNA defects. Lancet 2000;355:299–304.
19. Leonard JV, Schapira AH. Mitochondrial respiratory chain disorders II: neurodegenerative disorders and nuclear gene defects. Lancet 2000;355:389–394.
20. Takeda A. Mitochondrial cardiomyopathy. J Pediatr Cardiol Card Surg 2020;4:53–62.
21. Meyers DE, Basha HI, Koenig MK. Mitochondrial cardiomyopathy: pathophysiology, diagnosis, and management. Tex Heart Inst J 2013;40:385–394.
22. Lee JS, Kim H, Lim BC, Hwang H, Choi J, Kim KJ et al. Leigh syndrome in childhood: neurologic progression and functional outcome. J Clin Neurol 2016;12:181–187.
23. Sofou K, De Coo IF, Ischanni P, Ostergaard E, Naess K, De Meirleir L et al. A multicenter study on Leigh syndrome: disease course and predictors of survival. Orphanet J Rare Dis 2014;9:52.
24. Bates MG, Bourke JP, Giordano C, d’Amato G, Turnbull DM, Taylor RW. Cardiac involvement in mitochondrial DNA disease: clinical spectrum, diagnosis, and management. Eur J Heart J 2012;33:3023–3033.
25. Shimura M, Nozawa N, Ogawa-Tominaga M, Fushimi T, Tajika M, Ichimoto K et al. Effects of 5-aminolevulinic acid and sodium ferrous citrate on fibroblasts from individuals with mitochondrial diseases. Sci Rep 2019;9:10549.