



Case Report

Clinicopathological findings of a mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes/Leigh syndrome overlap patient with a novel m.3482A>G mutation in *MT-ND1*

Yuichi Hayashi,¹ Yasushi Iwasaki,² Nobuaki Yoshikura,¹ Megumi Yamada,¹ Akio Kimura,¹ Takashi Inuzuka,^{1,3} Hiroaki Miyahara,² Yuichi Goto,⁴ Ichizo Nishino,⁵ Mari Yoshida² and Takayoshi Shimohata¹

¹Department of Neurology, Gifu University Graduate School of Medicine, ³Department of Neurology, Gifu Municipal Hospital, Gifu, ²Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, Nagakute, Departments of ⁴Mental Retardation and Birth Defect Research, National Institute of Neuroscience and ⁵Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Japan

We report clinicopathological findings of a patient with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes/Leigh syndrome (MELAS/LS) associated with a novel m.3482A>G mutation in *MT-ND1*. A 41-year-old woman had experienced multiple stroke-like episodes since age 16. She developed akinetic mutism two months before admission to our hospital. Neurological examination revealed akinetic mutism, bilateral deafness, and muscular atrophy. Cerebrospinal fluid tests revealed elevated pyruvate and lactate levels. Fluid-attenuated inversion recovery images on magnetic resonance imaging showed hyperintense areas in the right frontal and both sides of temporal and occipital lobes, both sides of the striatum, and the midbrain. Muscle biopsy revealed strongly succinate dehydrogenase-reactive blood vessels. L-arginine therapy improved her consciousness and prevented further stroke-like episodes. However, she died from aspiration pneumonia. Postmortem autopsy revealed scattered infarct-like lesions with cavitation in the cerebral cortex and necrotic lesions in the striatum and midbrain. The patient was pathologically confirmed as having MELAS/LS based on two characteristic clinicopathological findings: presenting MELAS/LS overlap phenotype and effectiveness of L-arginine treatment.

Key words: L-arginine, Leigh syndrome, MELAS, mitochondrial encephalopathy, MT-ND1 gene.

INTRODUCTION

Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (OMIM #540000) is a neuromuscular syndrome. Approximately 80% of MELAS patients have an m.3243A>G in *MT-TL1*.¹ Other mutations in *MT-TL1* and other transfer RNA genes, including *MT-TF*, *MT-TV*, and *MT-TQ*, account for most of the remaining patients.¹ However, a small number of mutations in the MT-ND subunit genes of complex I have also been reported to cause MELAS, most notably in *MTND5*² and to a lesser extent in *MT-ND6*.³ MELAS associated with a mutation in *MT-ND1* is very rare.

In contrast, Leigh syndrome (LS) (OMIM #256000) is the most common pediatric presentation of mitochondrial disease. This neurodegenerative disorder is genetically heterogeneous, and, to date, pathogenic mutations have been identified in > 75 genes.⁴ Approximately 20% of LS patients have a mutation in mitochondrial DNA. The most common mitochondrial DNA in LS affects the MT-ATP6 gene.⁴ LS associated with a mutation in *MT-ND1* is also rare.

We report clinicopathological findings of a MELAS/LS overlap patient with a novel m.3482A>G mutation in *MT-ND1*.

CLINICAL SUMMARY

A 41-year-old woman had suffered from multiple stroke-like episodes since she was 16 years old. She had noted

Correspondence: Takayoshi Shimohata, MD, PhD, Department of Neurology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan. Email: shimohata@gmail.com

Received 30 May 2020; revised 28 August 2020; accepted 31 August 2020.

© 2020 Japanese Society of Neuropathology

progressive bilateral hearing loss in her 30s. The patient was diagnosed as having juvenile cerebral infarction at another hospital 25 years previously for which she received oral anti-platelet drugs. She developed akinetic mutism two months before admission to our hospital. She had normal motor and psychiatric milestones until age 16. The patient had no familial history of neuromuscular diseases, diabetes, or deafness. On admission, she presented with cachexia (body mass index: 14.2), and neurological examination revealed akinetic mutism, bilateral deafness, severe muscular atrophy, and exaggerated tendon reflexes in all extremities with Babinski signs. Laboratory tests revealed an elevated serum creatinine level (1.8 mg/dL) with

normal serum levels of fasting blood sugar, HbA1c, pyruvate, and lactate. Cerebrospinal fluid tests revealed normal cells, with elevated levels of protein (73 mg/dL), pyruvate (1.67 mg/dL; normal range: 0.63–0.77 mg/dL), and lactate (56.1 mg/dL; normal range: 13.7–20.5 mg/dL). Fluid-attenuated inversion recovery (FLAIR) images on magnetic resonance imaging (MRI) showed hyperintense areas in the white matter of the right frontal and both sides of temporal and occipital lobes, both the striatum, the thalamus, the midbrain, and the cerebellum (Fig. 1A–F). In addition, multiple cystic changes were found in the cerebral cortices. Their distribution did not correspond to the arterial perfusion territory. Magnetic resonance spectroscopy (MRS)

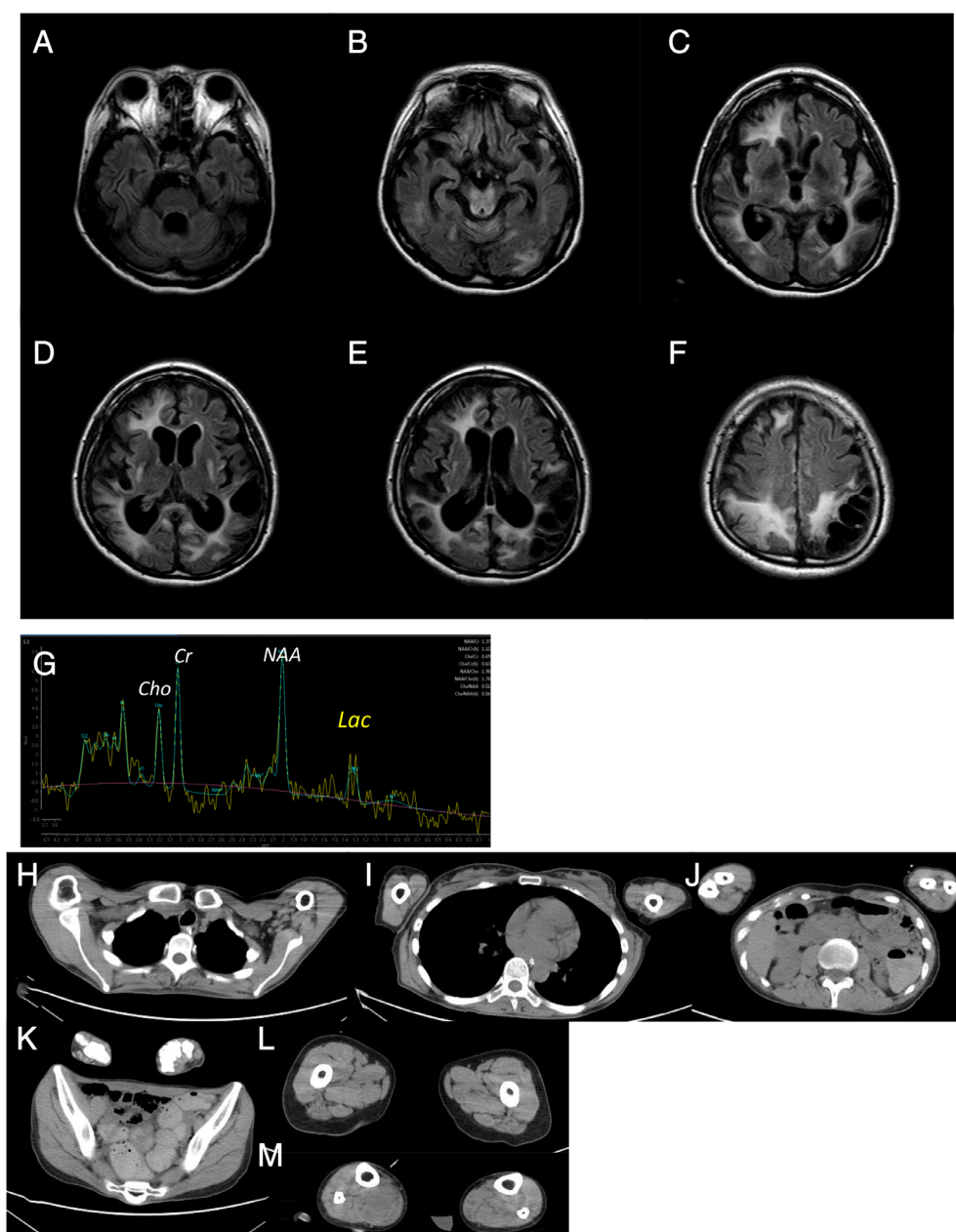


Fig 1 Images of the brain MRI and MRS and the muscle CT. (A–F) FLAIR images on MRI shows multiple hyperintense areas in the frontal, temporal, occipital, and parietal lobes, symmetrical striatum, thalamus, and midbrain. Multiple cystic changes are found in the left temporal, occipital, and parietal lobes (D–F). Their distribution does not correspond to the arterial perfusion. (G) MRS images show a lactate peak in the right occipital lesion. (H–M) Proximal-predominant, diffuse muscular atrophies are seen in muscle CT images.

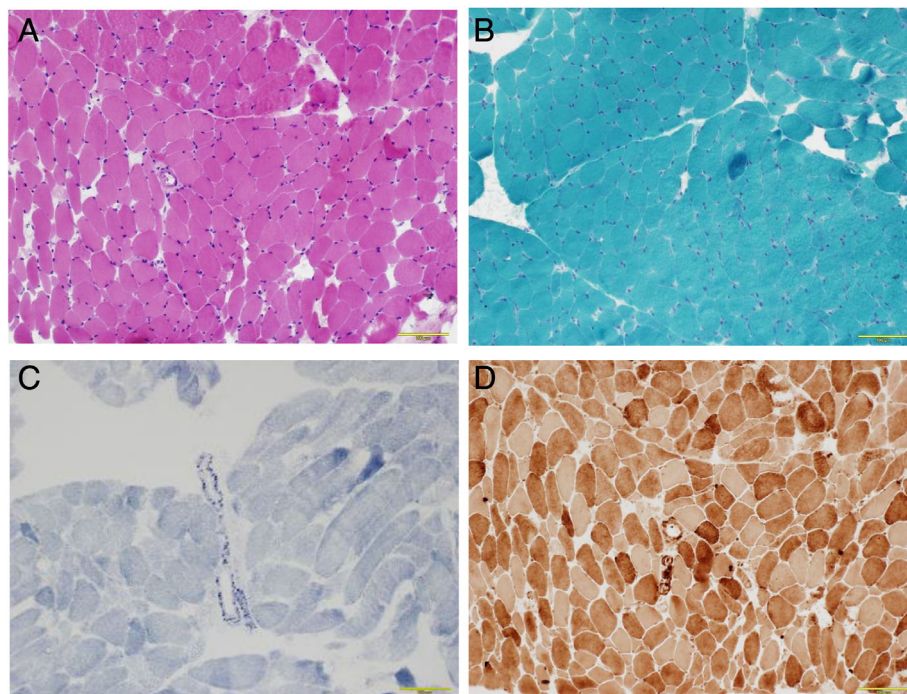


Fig 2 Pathological findings of the muscle biopsy specimens. (A) An HE-stained section shows uneven diameter of muscle fibers. (B) A modified Gomori-trichrome-stained section shows no ragged-red fibers. (C) Enzyme histochemistry for SDH reveals strongly SDH-reactive blood vessels. (D) Enzyme histochemistry for cytochrome oxidase reveals no significant alteration. Scale bars: 100 μ m (A–D).

revealed a lactate peak in the right occipital lobe lesion (Fig. 1G). Serial MRI and MRS images showed that symmetrical striatum lesions were found at least nine years before admission, and a midbrain lesion was firstly noted on admission (Fig. 1B). Proximal-predominant, diffuse muscular atrophies were found in muscle computed tomography (CT) images (Fig. 1H–M). Muscle biopsy of the left biceps revealed moderate uneven muscular cell diameter on sections stained with hematoxylin and eosin (HE) (Fig. 2A). No ragged-red fibers (RRFs) were found on sections stained with modified Gomori-trichrome (Fig. 2B). Strongly succinate dehydrogenase (SDH)-reactive blood vessels were evident by enzyme histochemistry (Fig. 2C). Enzyme histochemistry revealed no significant alteration in cytochrome oxidase (Fig. 2D). Gene analysis from the muscle revealed a novel m.3482A>G mutation (p.E59G) in *MT-ND1*, which was not found in the 200 health controls and not reported in the MITOMAP database (<http://www.mitomap.org/>, accessed August 2020) or in the mtSNP database (<http://www.mtsnp.tmg.or.jp/intsnp/index.html>, accessed April 2020).

Based on the multiple stroke-like episodes and radiological findings of the cerebral cortices, we clinically diagnosed the patient as having MELAS. Although she was in the chronic phase of MELAS, oral L-arginine therapy (0.5 g/kg/day)^{5,6} was initiated. The treatment was effective for her symptoms, which was apparent because she could reply to simple orders within four weeks after initiating therapy. At that time, she was transferred to a chronic hospital. Taurine or other supplements were not initiated.

The patient died from aspiration pneumonia five months after the diagnosis. She had no stroke-like attacks of MELAS for those five months. We obtained informed consent for autopsy from her family and transferred the patient's body to the Center for Pathology, Aichi Medical University.

PATHOLOGICAL FINDINGS

Macroscopic findings

An autopsy was performed 12 h after death. The patient's brain weighed 975 g before fixation. The brain was fixed in 20% buffered formalin. Her brain after fixation showed bilateral cerebral atrophy predominantly in the left occipital lobe (Fig. 3A) and cerebellar atrophy (Fig. 3B). The gross appearance of her brainstem was almost normal (Fig. 3B).

Microscopic findings

Histological examination was performed on 9- μ m-thick sections stained with hematoxylin and eosin (HE) and Klüver-Barrera. Multiple scattered infarct-like lesions with cavitation were observed in the cerebral and cerebellar cortices (Fig. 4A–E). Their distribution did not correspond to the arterial perfusion territory. These pathological findings were consistent with MELAS. In contrast, necrotic lesions were observed both sides of the striatum (Fig. 4F, G) and the midbrain (Fig. 4H, I). These necrotic lesions were consistent with LS. Therefore, the patient had

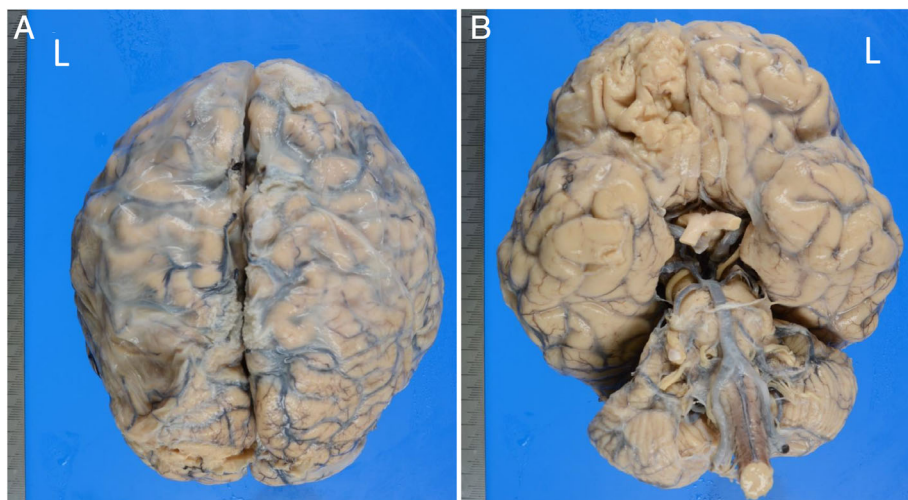


Fig 3 Gross appearance on convex (A) and basal (B) views of the autopsied brain after fixation. The brain shows bilateral cerebral atrophy predominantly in the left occipital lobe, as well as cerebellar atrophy. The brainstem looks almost normal. L, the left side.

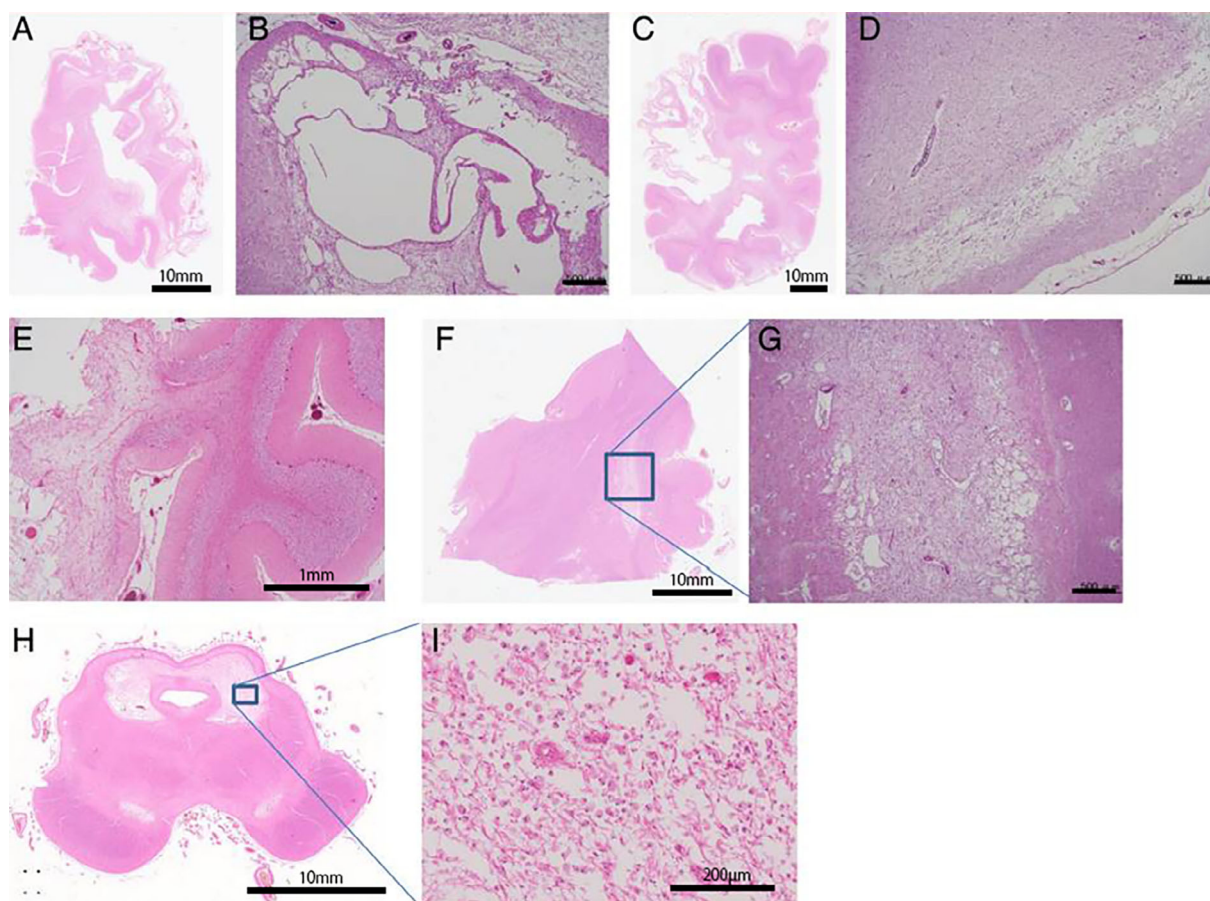


Fig 4 Histological findings of the brain sections stained with HE. The right frontal (A, B) and left occipital (C, D) lobes as well as the cerebellum (E) show scattered infarct-like lesions with cavitation, involving the cortex and white matter. Their distribution does not correspond to the arterial perfusion territory. The right basal ganglia (F, G) and midbrain (H, I) show focal necrotic lesions. Scale bars: 10 mm (A, C, F, H), 1 mm (E), 200 μ m (I), 500 μ m (B, D, G).

Table 1 Clinical characteristics of MELAS or Leigh syndrome patients associated with MT-ND1 mutation

Number	Author (year) [Reference]	Mutation (hetero- or homoplasmic)	Phenotype	Family history of mitochondrial disease	Onset of age (y)	Sex	Death of age (y)	Diagnosed age (y)	Disease duration (y)	stroke-like episodes	Muscular atrophy	Optic abnormalities	Deafness	Basal ganglia lesion (MRI/CT)	Brainstem lesion (MRI/CT)	L-arginine therapy		
1	Campos (1997) [12]	3308 T>C (hetero)	MELAS	+ (maternal inherited)	12	F	NA	22	>10	+	NA	NA	NA	+	(bilateral)	NA	NA	
2	Blakeley (2005) [13]	3376 G>A (hetero)	LHON/MELAS	-	20	F	NA	43	>23	+	NA	+	+	-	+	(midbrain)	NA	
3	Horvath (2008) [14]	3380 G>A (hetero)	MELAS	+ (deafness)	50	F	NA	62	>12	+	NA	NA	+	NA	NA	NA	NA	
4	Funalor (2003) [15]	3460 G>A (homo)	LHON/Leigh-like phenotype	+ (Visual loss)	34	F	NA	34	>5	NA	NA	+	NA	+	(bilateral)	+	(midbrain)	NA
4	Malfatti (2007) [16]	3481 G>A (hetero)	MELAS	NA	6	M	NA	6	>10 mo	+	NA	+	+	-	NA	NA	NA	
5	Moslemi (2008) [17]	3481 G>A (hetero)	LS	-	<1	F	2 y 10 mo	2	<3	-	NA	NA	NA	-	+	(pons)	NA	
6	Valente (2009) [18]	3688 G>A (homo)	LS	NA	10 mo	M	NA	NA	>4	-	NA	NA	NA	+	(bilateral)	NA	NA	
7	Kirby (2004) [19]	3697 G>A (hetero)	MELAS	NA	4	M	NA	10	>6	+	NA	-	-	+	(left)	NA	NA	
8	Danhelevska (2020) [20]	3697 G>A (hetero)	LS	NA	1 w	NA	7	NA	7	+	(stroke-like MRI findings)	NA	NA	-	+	(midbrain)	NA	
9	Danhelevska (2020) [20]	3697 G>A (hetero)	LS	NA	1 w	NA	1.8y	NA	1.8	-	NA	NA	NA	+	(bilateral)	+	(midbrain)	NA
10	Negishi (2014) [21]	3697 G>A (homo)	LS	+	1 y 6 mo	F	NA	9	>7.5	-	NA	NA	+	+	(bilateral)	-	NA	
11	Negishi (2014) [21]	3697 G>A (homo)	LS	+	2	M	NA	7	>5	-	NA	NA	NA	+	(bilateral)	-	NA	
12	Negishi (2014) [21]	3697 G>A (homo)	LS	+	1 y 6 mo	F	NA	5	>3.5	-	NA	NA	NA	+	(bilateral)	+	(midbrain)	NA
13	Wei (2018) [22]	3700 G>A (NA)	LS	NA	3	F	NA	NA	NA	NA	NA	NA	NA	+	(bilateral)	-	NA	
14	Kirby (2004) [19]	3946 G>A (hetero)	MELAS	+	5	F	NA	14	>9	+	NA	NA	+	NA	NA	NA	NA	
15	Danhelevska (2020) [20]	3946 G>A (hetero)	MELAS	NA	9	NA	NA	NA	>22	+	NA	NA	NA	NA	+	(midbrain)	NA	
16	Kirby (2004) [19]	3949 T>C (hetero)	MELAS	-	14	M	NA	16	>2	+	NA	NA	NA	-	NA	NA	NA	
17	Lin (2014) [23]	3959 G>A (hetero)	MELAS	-	17	M	NA	22	>5	+	NA	NA	-	NA	NA	NA	NA	
18	Lin (2014) [23]	3995 A>G (hetero)	MELAS	+	NA	M	NA	35	NA	+	NA	NA	NA	-	NA	NA	NA	
19	Pereira (2019) [24]	4142 G>T (hetero)	LS	-	2.5	M	NA	4	>1.5	-	NA	NA	NA	+	(bilateral)	NA	NA	
20	La Morgia (2014) [25]	4171 C>A (homo)	LHON/Leigh-like phenotype	+	12	M	NA	16	>4	NA	NA	+	NA	NA	+	(midbrain, pons)	NA	
21	The current patient	3482 A>G (hetero)	MELAS/LS	-	16	F	41	41	25	+	+	-	+	+	(bilateral)	+	(midbrain)	Effective

-, negative; +, positive; F, female; hetero, heteroplasmic; homo, homoplasmic; LHON, Leber's hereditary optic neuropathy; LS, Leigh syndrome; M, male; MELAS, mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; mo, months; NA, not available or not described; w, weeks; y, year-old or years.

pathologically confirmed MELAS/LS overlap. Calcification in the basal ganglia was very mild (data not shown). Aging pathology was very mild, as Braak stage I and AT8 stage I (data not shown).

DISCUSSION

This is the first report of a pathologically confirmed MELAS/LS overlap patient with a novel m.3482A>G mutation in *MT-ND1*. The patient showed two characteristic clinicopathological features. First, both sides lesions in the striatum and midbrain were seen on MRI. Second, oral L-arginine treatment ameliorated her neurological symptoms and prevented further stroke-like episodes.

The patient's clinical course was typical of MELAS with multiple stroke-like episodes. However, combined symmetrical lesions in both sides of the striatum and midbrain were unique for MELAS. Clinically, parkinsonism, dystonia, and other extrapyramidal signs were not observed in her disease course.

In contrast, brainstem lesions in mitochondrial disease are frequently caused by LS,^{4,7,8} and were also reported in patients with Leber hereditary optic neuropathy (LHON) plus syndrome⁸ or myoclonus epilepsy associated with ragged-red fibers (MERRF).⁹ Combined brainstem and striatal lesions were reported in a patient with LS.⁷ However, the clinical course and muscle biopsy findings of the current patient were not typical of LHON or MERRF. The clinical course of the current patient was unlike typical LS because LS usually presents subacute necrotizing encephalopathy in infancy, and the median age at death is just over two years. However, late-onset LS patients (onset > 2 years) and a small number of adult-onset LS patients have been reported.¹⁰ Their clinical characteristics were atypical,¹⁰ and prognosis of late-onset LS was not poor compared to that of early-onset LS.¹¹

Previously several mutations in *MT-ND1* are associated with MELAS or LS. For a better understanding, we reviewed the radiological findings of 21 patients with MELAS or LS associated with *MT-ND1* mutation, including the current patient (Table 1).^{12–25} According to Table 1, 11 patients presented with MELAS or LHON/MELAS, and 10 patients with LS or Leigh-like phenotype. Only one patient with early-onset LS having stroke-like MRI findings was previously reported; however, postmortem analysis was performed.²⁰

Interestingly, five of 11 patients (45.4%) with MELAS had additional LS-like radiological findings (Table 1). Striatum lesions were found in two patients: one showed bilateral lesions¹² and the other had only left-sided lesions.¹⁹ Symmetrical midbrain lesions were reported in two patients.^{13,20} However, combined striatum and brainstem lesions were only seen in the current patient. These

patients, except for our patient, were diagnosed with MELAS or LHON/MELAS; however, their LS-like radiological lesions were not pathological confirmed.^{12,13,19,20}

Pathological analysis in this patient revealed that necrotic lesions were found in lesions of both sides of the striatum and midbrain, and these findings were consistent with LS. Thus, MELAS/LS overlap phenotype was pathologically confirmed in the current patient.

Although RRFs were frequently found in muscle biopsy samples from MELAS patients, no RRFs were observed in the current patient. In contrast, RRFs were rarely seen in mitochondrial DNA-associated LS.²⁶ Moreover, it was reported that no RRFs were observed in a patient with MELAS/LS overlap.²⁷ Therefore, we considered that no RRFs may be associated with LS overlap.

Another notable finding is that oral L-arginine supplementation ameliorated neurological symptoms and prevented further stroke-like attacks in this patient, suggesting that the therapy is effective for MELAS with the m.3482A>G mutation. Previously, effectiveness of oral L-arginine therapy has mainly been reported in MELAS patients with an m.3243A>G mutation.^{5,6} Our case suggests that the therapeutic effect of oral L-arginine therapy should be confirmed for patients with MELAS, regardless of the mutation.

In summary, our clinicopathological findings indicate that the m.3482A>G mutation in *MT-ND1* caused a MELAS/LS overlap phenotype, and that oral L-arginine therapy may be effective for MELAS/LS patients with this mutation.

ACKNOWLEDGMENTS

This research was supported by JSPS KAKENHI Basic Research (C) grant number 20 K08196 (H. Miyahara). We thank Dr Muneharu Murakami, from the Department of Internal Medicine, Yamada Hospital, and Dr Akira Seko, from the Department of Internal Medicine, Sekigahara Hospital, for chronic follow ups.

DISCLOSURE

The authors declare that there are no conflicts of interest.

REFERENCES

- DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med* 2003; **348**: 2656–2668.
- Liolitsa D, Rahman S, Benton S, Carr LJ, Hanna MG. Is the mitochondrial complex I ND5 gene a hot-spot for MELAS causing mutations? *Ann Neurol* 2003; **53**: 128–132.
- Ravn K, Wibrand F, Hansen FJ, 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.
4. Lake NJ, Compton AG, Rahman S, Thorburn DR. Leigh syndrome: One disorder, more than 75 monogenic causes. *Ann Neurol* 2016; **79**: 190–203.
 5. Koga Y, Akita Y, Nishioka J *et al.* MELAS and L-arginine therapy. *Mitochondrion* 2007; **7**: 133–139.
 6. Koga Y, Povalko N, Inoue E *et al.* Therapeutic regimen of L-arginine for MELAS: 9-year, prospective, multicenter, clinical research. *J Neurol* 2018; **265**: 2861–2874.
 7. Finstere J. Leigh and leigh-like syndrome in children and adults. *Pediatr Neurol* 2008; **39**: 223–235.
 8. Bindu RS, Arvinda H, Taly AB *et al.* Magnetic resonance imaging correlates of genetically characterized patients with mitochondrial disorders: A study from South India. *Mitochondrion* 2015; **28**: 6–16.
 9. Ito S, Shirai W, Asahina M, Hattori T. Clinical and brain MR imaging features focusing on the brain stem and cerebellum in patients with myoclonic epilepsy with ragged-red fibers due to mitochondrial A8344G mutation. *AJNR Am J Neuroradiol* 2008; **29**: 392–395.
 10. Sakushima K, Tsuji-Akimoto S, Niino M, Saitoh S, Yabe I, Sasaki H. Adult Leigh disease without failure to thrive. *Neurologist* 2011; **17**: 222–227.
 11. Hong CM, Na JH, Park S, Lee YM. Clinical characteristics of early-onset and late-onset Leigh syndrome. *Front Neurol* 2020; **11**: 267.
 12. Campos Y, Martín MA, Rubio JC *et al.* Bilateral striatal necrosis and MELAS associated with a new T3308C mutation in the mitochondrial ND1 gene. *Biochem Biophys Res Commun* 1997; **238**: 323–325.
 13. Blakely EL, de Silva R, King A *et al.* LHON/MELAS overlaps syndrome associated with a mitochondrial MTND1 gene mutation. *Eur J Hum Genet* 2005; **213**: 623–627.
 14. Horváth R, Reilmann R, Holinski-Feder E, Ringelstein EB, Klopstock T. The role of complex I genes in MELAS: A novel heteroplasmic mutation 3380G>A in ND1 of mtDNA. *Neuromuscul Disord* 2008; **18**: 553–556.
 15. Funalot B, Reynier P, Vighetto A *et al.* Leigh-like encephalopathy complicating Leber's hereditary optic neuropathy. *Ann Neurol* 2002; **52**: 374–377.
 16. Malfatti E, Bugiani M, Invernizzi F *et al.* Novel mutations of ND genes in complex I deficiency associated with mitochondrial encephalopathy. *Brain* 2007; **130**: 1894–1904.
 17. Moslemi AR, Darin N, Tulinius M, Wiklund LM, Holme E, Oldfors A. Progressive encephalopathy and complex I deficiency associated with mutations in MTND. *Neuropediatrics* 2008; **39**: 24–28.
 18. Valente L, Piga D, Lamantea E *et al.* Identification of novel mutations in five patients with mitochondrial encephalomyopathy. *Biochim Biophys Acta* 2009; **1787**: 491–501.
 19. Kirby DM, McFarland R, Ohtake A *et al.* Mutations of the mitochondrial ND1 gene as a cause of MELAS. *J Med Genet* 2004; **41**: 784–789.
 20. Danhelovska T, Kolarova H, Zeman J *et al.* Multi-system mitochondrial diseases due to mutations in mtDNA-encoded subunits of complex I. *BMC Pediatr* 2020; **20**: 41.
 21. Negishi Y, Hattori A, Takeshita E *et al.* Homoplasmy of a mitochondrial 3697G>A mutation causes Leigh syndrome. *J Hum Genet* 2014; **59**: 405–407.
 22. Wei Y, Cui L, Peng B. Mitochondrial DNA mutations in late-onset Leigh syndrome. *J Neurol* 2018; **265**: 2388–2395.
 23. Lin J, Zhao CB, Lu JH *et al.* Novel mutations m.3959G>A and m.3995A>G in mitochondrial gene MT-ND1 associated with MELAS. *Mitochondrial DNA* 2014; **25**: 56–62.
 24. Pereira C, de Souza CF, Vedolin L *et al.* Leigh syndrome due to mtDNA pathogenic variants. *J Inborn Errors Metab Screen* 2019; **7**: e20180003.
 25. La Morgia C, Caporali L, Gandini F *et al.* Association of the mtDNA m.4171C>A/MT-ND1 mutation with both optic neuropathy and bilateral brainstem lesions. *BMC Neurol* 2014; **14**: 116.
 26. Thorburn DR, Rahaman J, Rahaman S. Mitochondrial DNA-associated Leigh syndrome and NARP [October 28, 2003; updated September 28, 2017]. In: Adam MP, Ardinger HH, Pagon RA *et al.*, (eds). *Gene Reviews*[®]. Seattle, WA: University of Washington, Seattle, 1993–2020.
 27. Wang Z, Qi XK, Yao S *et al.* Phenotypic patterns of MELAS/LS overlap syndrome associated with m.13513G>A mutation, and neuropathological findings in one autopsy case. *Neuropathology* 2010; **30**: 606–614.