CASE REPORT

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The A8344G mutation in mitochondrial DNA associated with stroke-like episodes and gastrointestinal dysfunction

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Abstract We report an unusual case of encephalo-entero-myopathy associated with the A8344G mutation in the tRNA^{Lys} gene of mitochondrial DNA (mtDNA). This patient had mitochondrial myopathy, multiple lipomatosis, mild hearing loss, stroke-like episodes, and paralytic ileus, but she lacked the canonical clinical features of MERRF, myoclonus, epilepsy, or ataxia. We conducted genetic, biochemical, histochemical, and immunohistochemical studies in skeletal muscle, brain, intestine, and lipoma tissue. The mutation was abundant in all tissues, and cytochrome c oxidase (COX) activity was selectively decreased in brain and small intestine. COX deficiency was also documented histochemically and immunohistochemically in the small intestine, suggesting that mitochondrial dysfunction played a role in the pathogenesis of paralytic ileus. This case illustrates an unusual and dramatic clinical phenotype of the A8344G mutation, characterized by stroke-like episodes and acute ileus.

Keywords MERRF \cdot Intestinal dysfunction \cdot Stroke-like episodes

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Introduction

Mitochondrial dysfunction has emerged as an important cause of neurological, endocrine, cardiac, and renal disorders, and point mutations in tRNA genes of mitochondrial (mt) DNA play a significant role in the pathogenesis of such disorders [1]. Specific mutations in tRNA genes have been associated with distinct clinical entities, including myoclonus epilepsy and ragged-red fibers (MERRF) and mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) [2, 3]. The typical clinical picture of MERRF includes myoclonus, generalized or partial seizures, myopathy, ataxia, and, less consistently dementia, neuropathy, hearing loss, and optic atrophy [4] However, MERRF is clinically heterogeneous and overlap syndromes between MERRF and MELAS have been reported [5, 6, 7].

We have studied a patient with the typical MERRF mutation in the tRNA^{Lys}(A8344G) gene, whose clinical presentation was strikingly different from typical MERRF because it was characterized by lipomatosis, stroke-like episodes and gastrointestinal dysfunction. We report molecular genetic, biochemical, and morphological findings in the most affected tissues, including brain, adipose tissue muscle, and small intestine.

Case report

This patient and her family history has been reported previously [8]. Briefly, this 39-year-old woman had familial lipomatosis and progressive exercise intolerance since age 20, and was diagnosed with MERRF-8344 at the age of 36 years. Neurological examination at that time showed only neurosensory hearing loss. During her stay in the hospital, she had the first stroke-like episode manifested by dysartria and dysphagia, followed by ventilatory insufficiency and lactic acidosis. Magnetic resonance imaging (MRI) of the brain showed two small bilateral frontal lesions. She survived the respiratory insufficiency, but had residual difficulties swallowing and speaking. Two years later, she was admitted to the emergency room because of abdominal distention. X-rays of the abdomen indicated paralytic ileus. Eight days later, she had a generalized tonic-clonic seizure followed by right hemiparesis and bilat-

70



Fig.1 a T2-weighted image showing scattered cerebral infarcts, particularly in the occipital lobes. **b** H&E stain illustrating an infarct region in the occipital cortex of the patient. **c** SDH and COX histochemistry on frozen sections of ileum from a control and from

the patient. Note the reduced stain for COX in all layers of the intestinal wall in the patient's sample (*H&E* hematoxylin and eosin, *SDH* succinate dehydrogenase, *COX* cytochrome *c* oxidase). **b**×55, **c**×2.5

eral cortical blindness. CT scan and MRI confirmed the presence of infarcts in the posterior part of the frontal lobes and showed new lesions in both occipital lobes (Fig. 1a). After 50 days in the intensive care unit, the patient developed aspiration pneumonia and died. An autopsy was performed 4 h postmortem.

Materials and methods

Pathological examination

The cerebrum was bisected, and the left part was fixed in formaldehyde, while the right part was frozen and kept at -70° C until analyzed. Samples from the small intestine (ileum) were also fixed in formaldehyde and frozen. Samples from lipoma tissue, non-lipoma abdominal fat, and skeletal muscle were also frozen for mtDNA studies. Routine samples from formaldehyde-fixed brain were processed for conventional neuropathological study.

Mutation analysis

Total DNA was extracted from frozen postmortem samples of brain (frontal, temporal and occipital lobes), muscle, lipomatous tissue, non-lipoma abdominal fat, and small intestine according to conventional methods. The previously studied muscle biopsy and lipoma tissue [8] were also processed simultaneously for DNA extraction to allow comparison with the corresponding postmortem samples. A PCR-based assay was used to determine the proportion of mutant and wild-type mtDNA. The primers used were as follows: reverse primer 5'-CTACCCCCTCTAGAGCCCAC-3', spanning from nucleotides 8278-8257; forward primer 5'-GTAG-TATTTAGTTGGGGCATTTCACTGTAAAGCCGTGTTGG-3', spanning from nucleotides 8386-8344 of mtDNA, with the "wildtype" AG dinucleotide replaced by a CC dinucleotide (underlined). The dinucleotide substitution in the forward primer creates a restriction site for the enzyme Bgl1 when the A8344G mutation is present. PCR amplification was carried out on a Perkin Elmer Cetus thermocycler using the following protocol: 1 min denaturation at 94°C, 1 min annealing at 60°C, and 1 min extension at 72°C, for 28 cycles. To quantitate the relative proportions of mutant and wild-type mtDNA, approximately 0.5 μ Ci [α -³²P]dATP was added immediately before the last cycle. The 108-bp PCR samples were digested with Bgl1 for at least 6 h and were run on a 12% acrylamide gel. Bgl1 digestion produces two fragments, one of 35 bp and one of 73 bp, which can easily be differentiated from the uncut wild-type fragment by gel electrophoresis. The gel was dried, exposed and analyzed on a PhosphorImager.

Biochemistry

Biochemical measurements of cytochrome c oxidase (COX; complex IV), NADH dehydrogenase (complex I), rotenone-sensitive NADH-cytochrome c reductase (complexes I and III), succinate-cytochrome c reductase (complexes II and III), and citrate synthase activities were performed in samples of brain and small intestine as described [4].

Histochemistry and immunohistochemistry

For histochemical studies, frozen samples of muscle and small intestine were cut into $6-\mu$ m-thick sections and stained for succinate dehydrogenase (SDH) and COX activities [9]. For immunohistochemical studies, paraffin-embedded blocks of small intestine from the patient and normal controls were cut into 4- μ m-thick sections and deparaffinized. Subsequently, the sections were stained immunohistochemically using the ABC method with monoclonal antibodies against the mtDNA-encoded subunits I of COX, and with polyclonal antibodies against the nuclear DNA (nDNA) encoded iron-sulfur subunit of complex III (FeS) [9]. Additional 4- μ m-thick sections were stained with hematoxylin and eosin (H&E) for routine histological evaluation of the small bowel.

Results

Histopathology of nervous system

By routine histological analysis, in the frontal, temporal and occipital lobes, we observed cortical infarcts of various sizes associated with different degrees of astrocytosis and capillary proliferation. The most severely affected region was the occipital lobe, where foci of infarction were widespread and had a patchy distribution (Fig. 1b). Spongiform degeneration, the neuropathological "hallmark" of mitochondrial encephalopathies [10], was remarkable in the cerebral cortex and in the subcortical white matter of temporal and occipital lobes. In addition, both cerebral cortex and white matter showed diffuse and moderate lymphocytic infiltration with mild cuffing around the blood vessels, but typical neuronophagia was not observed, and there were no obvious foci of infection. Neuronal loss, which is the most typical neuropathological feature of MERRF [10], was only very mild in the Purkinje cell layer of the cerebellar cortex, and absent in the dentate nucleus of the cerebellum, the red nucleus of the midbrain, and the olivary nucleus of the medulla.

Mutation analysis

Table 1 shows that tissues had high proportions of mtDNA carrying the A8344G mutation. The mutation was rather homogeneously distributed in the brain, ranging from 82% in the occipital lobe to 87% in the frontal lobe. Lipoma tissue carried 77% of mutants mtDNA, while non-lipoma abdominal fat tissue had 69%. In skeletal muscle and lipoma tissue, we did not observe any significant changes in the percentages of the mutation between biopsy and autopsy samples obtained 2 years apart (90% vs 89% in muscle and 81% vs 77% in lipoma). The small

Table 1	Mutation analyses				
		Tissue	Mutated mtDNA		
		Biopsy specimens Skeletal muscle Lipoma	90% 81%		
		Autopsy specimens Skeletal muscle	89%		
		Brain regions Frontal Temporal Occipital	87% 84% 82%		
		Adipose tissue	69%		
		Lipoma	77%		
		Small intestine	84%		

Table 2 Biochemical analyses. Activity is given in μ mol/min per g; normal control values are shown in parentheses [*COX* cyto-chrome c oxidase (complex IV), *SCCO* succinate cytochrome c

oxidase (complex II+III), *NADH-CCR* NADH-cytochrome *c* reductase (complex I+III), *NADH-D* NADH dehydrogenase (complex I), *CS* citrate synthase, *N/D* not determined]

	COX	SCCO	NADH-CCR	NADH-D	CS
Brain					
Frontal	0.16 (0.46)	0.45 (0.32)	0.90 (0.51)	6.92 (7.11)	4.15 (5.94)
Temporal	0.09 (0.46)	0.44 (0.40)	0.91 (0.31)	7.01 (6.54)	5.09 (4.09)
Occipital	0.16 (0.72)	0.36 (0.39)	0.56 (0.36)	6.23 (6.16)	12.8 (7.12)
Ileum	0.10 (0.27)	1.19 (1.18)	0.70 (N/D)	4.09 (N/D)	2.26 (3.40)



Fig.2a-e Histochemistry on serial sections of the patient's ileum. **a** H&E stain illustrates vacuolation (*black arrow*) of the arterial wall of a vessel in the submucosa. **b** SDH stain illustrates in the same artery regions of increased oxidative activity. **c** COX stain

shows reduced activity in the arterial wall. **d** The SDH stain of the smooth muscle layers and myenteric plexus, and **e** reduced COX stain in the muscle layers (*asterisk*) as well as in some of the nerve cells of the plexus (*arrow*) are shown. $a-e \times 180$



Fig.3a–d Immunohistochemistry on serial sections of the patient's ileum using the nDNA-encoded FeS subunit of complex III and the mtDNA-encoded subunit I of COX. The FeS immunoreaction is normal in the arterial wall of a submucosal vessel (**a**), and in muscle layers and nerve cells of the myenteric plexus (**c**). The COX I immunoreaction is reduced in the arterial wall (*arrow*) (**b**), and in the muscle layers (*asterisk*) and in some nerve cells of the myenteric plexus (*arrows*) (**d**). **a**, **b** ×180; **c**, **d** ×100

intestine showed an average mutation load of 84%. Samples of small intestine were taken from two different areas of the ileum, one that showed discoloration of the mucosa and thinning of the wall, and the other part with normal appearance. The former harbored 83% mutants mtDNA and the latter 85%.

Biochemistry

The results of biochemical analyses in postmortem samples from the brain and the intestine small bowel are shown in Table 2. The samples showed a selective decrease of COX activity, confirming the characteristic vulnerability of this complex to the A8344G mutation [4, 11]. Histochemistry and immunohistochemistry

Histochemical changes in the muscle biopsy have been previously reported [8]. Postmortem muscle showed scattered ragged-red fibers (RRF), group atrophy, and a few degenerating fibers with phagocytotic activity. All RRF were COX negative. Focally, the intramuscular arteries showed strong reactivity with SDH, while COX histochemistry revealed decreased enzyme activity in the same vessels (data not shown).

Sections from the ileum stained with H&E showed that the mucosa was generally thin and intestinal pits were shallow. In addition, the mucosal epithelial cells had lost their normal histological appearance, possibly due to postmortem changes. The lamina propria and the submucosal layers were fibrous. In the submucosa, the smooth muscle layers of some small arteries showed microvacuolation (Fig. 2a). However, we did not observe hyaline degeneration, necrotic changes, or inflammation in the blood vessel walls. In the circular and longitudinal muscle layers of the intestinal wall, there were no obvious degenerative changes, necrosis, or perforation.

Histochemical reactions on frozen sections from small bowel of the patient and that of a normal showed a general reduction of COX activity in the patient's muscle layers and in the submucosal connective tissue, including the blood vessels (Figs. 1c, 2c). Focal absence of COX stain was noted in some nerve cells of Meissner's and Auerbach's plexuses of the patient (Fig. 2e). In contrast, SDH enzymatic activity was homogeneous in all structures of the intestinal wall in both patient and control samples.

Immunohistochemical studies of the small bowel from normal controls, using anti-COX I and anti-FeS antibodies, showed that the immunostaining pattern was essentially the same with the two antibodies and consisted of finely punctuate immunoreactivity in the circular and longitudinal muscle layers. In addition, there was immunoreactive material in Meissner's and Auerbach ganglion cells as well as the walls of arterioles (data not shown). In the patient's sample, the immunostaining patterns differed widely with the two antibodies. With anti-FeS antibodies the pattern was similar to that of normal controls in all regions studied (Fig. 3a, c), whereas with the anti-COX I antibodies, immunoreactivity was markedly decreased. COX I deficiency involved all muscle layers of the intestinal wall as well as the walls of arterioles of the submucosa (Fig. 3b, d). In the enteric plexi, COX I deficiency was also evident in some ganglion cells (Fig. 3d).

Discussion

In its typical expression, MERRF is characterized by myoclonus, generalized seizures, mitochondrial myopathy, and cerebellar ataxia. Less common signs include dementia, hearing loss, optic atrophy, peripheral neuropathy, pigmentary retinopathy, ophthalmoparesis, and multiple lipomatosis [4]. Three point mutations in the tRNA^{Lys} gene, A8344G, A8356G, and G8363A, and a T7512C mutation in the tRNA^{Ser(UCN)} have been found in typical MERRF patients [12, 13, 14], although most patients harbor the A8344G mutation [2]. Although she harbored the A8344G mutation, our patient manifested an unusual clinical phenotype because she lacked the typical clinical features of MERRF. Instead, she presented with stroke-like episodes and ileus, which are seen more commonly in MELAS patients. In fact, cases of the MERRF/MELAS overlap syndrome have been associated with the tRNA^{Lys} T8356C and the tRNA^{Ser(UCN)} T7512C mutations [15, 16]. These patients, however, all had the clinical hallmark of MERRF, myoclonic epilepsy, variously complicated with stroke-like episodes or migrainous attacks.

Our patient was different from the previously reported MELAS/MERRF overlap cases because stroke-like episodes and ileus were not accompanied by any of the typical features of MERRF. In agreement with these observations, the neuropathology of our patient also failed to show the typical MERRF lesions; neuronal loss in dentate and olivary nuclei, a typical neuropathological feature of MERRF [10], was absent in our patient. Instead, brain pathology was characterized by foci of cortical infarctions and by spongiform degeneration, two features consistently observed in MELAS patients [10].

Gastrointestinal dysfunction is not uncommon in mitochondrial disorders associated with mtDNA mutations, including point mutations in tRNA genes and large-scale rearrangements [17, 18, 19, 20]. Two autopsy studies of

such cases showed microvacuolation, foci of necrosis, and abnormal mitochondria in the proper muscle of the intestine [21, 22]. In the present case, we observed a generalized reduction of COX histochemical stain in the intestinal wall, without any focal necrosis or degenerative changes of the muscle proper. We also noted focal COX deficiency in the smooth muscle cells of blood vessels and in the ganglion cells of the enteric plexi. These observations prompted us to study the steady-state levels of mitochondrial translation products by visualizing one mtDNAencoded polypeptide (COX I), and one nDNA-encoded polypeptide (FeS). We found decreased expression of COX I in all structures of the intestinal wall of our patient, in contrast with a normal immunoreactivity of FeS. The relative amount and distribution of wild-type mtDNA and mutant mtDNA in the different cellular components of the intestinal wall may account for the different immunoreactivity patterns between COX I and FeS, because the phenotypic expression of an mtDNA mutation is regulated by the "threshold effect," that is, the mutant phenotype (in this case, defective mtDNA translation) is expressed in heteroplasmic cells only when the relative proportion of mutant mtDNA reaches a certain minimal value [2, 3].

The enteric nervous system controls motility, exocrine and endocrine secretions, and microcirculation of the gastrointestinal tract [23]. The myenteric plexus (Auerbach's) primarily provides motor innervation to the circular muscle, and the submucosal plexus (Meissner's) innervates the muscularis mucosa, intestinal endocrine cells, and submucosal blood vessels [23]. The presence of focal COX deficiency in the ganglion cells of the enteric plexi in our patient strongly suggests that all of functions were disturbed. Although both mtDNA-encoded and nDNA-encoded subunits of the respiratory chain appear to be highly sensitive to denervation [24, 25], we assume that the global reduction of COX activity in the muscle layers of the intestinal wall and the focal COX deficiency of blood vessels in the submucosal layer were not due to denervation of the enteric nervous system. We base this assumption on immunohistochemical evidence that the nDNA-encoded FeS was well preserved in those same layers despite COX deficiency. We propose that COX deficiency in the smooth muscle of the blood vessels as well as of the enteric neurons results in disturbed regulation of the local blood circulation and dysmotility of the gut.

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References

- Smeitink J, Van den Heuvel L, DiMauro S (2001) The genetics and pathology of oxidative phosphorylation. Nat Rev Genet 2: 342–352
- 2. DiMauro S, Bonilla E (1997) Mitochondrial encephalomyopathies. In: Rosenberg RN, Prusiner SB, DiMauro S, Barchi RL (eds) The molecular and genetic basis of Neurological disease, 2nd edn. Butterworth-Heinemann, Newton, pp 201–235

- DiMauro S, Bonilla E, Davidson M, Hirano M, Schon EA (1998) Mitochondria in neuromuscular disorders. Biochim Biophys Acta 1366:199–210
- 4. Silvesti G, Ciafaloni E, Santorelli FM, Shanske S, Servidei S, Graf WD, Sumi M, DiMauro S (1993) Clinical features associated with the A to G transition at nucleotide 8344 of mtDNA (MERRF mutation). Neurology 43:1200–1206
- 5. Zeviani M, Montoni F, Savarese N, Serra G, Tiranti V, Carrara F, Maritotti C, DiDonato S (1993) A MERRF/MELAS overlap syndrome associated with a new point mutation in the mito-chondrial DNA tRNA^{Lys} gene. Eur J Hum Genet 1:80–87
- 6. Jaksch M, Klopstock T, Kurlemann G, Dorner M, Hofmann S, Kleinle S, Hegemann S, Weissert M, Muller-Hocker J, Pongrats D, Gerbitz K-D (1998) Progressive myoclonus epilepsy and mitochondrial myopathy associated with mutations in the tRNA^{Ser(UCN)} gene Ann Neurol 44:635–640
- 7. Nakamura M, Nakano S, Goto Y, Ozawa M, Nagahama Y, Fukuyama H, Akiguchi I, Kaji R, Kimura J (1995) A novel point mutation in the mitochondrial tRNA^{Ser(UCN)} gene detected in a family with MERRF/MELAS overlap syndrome. Biochem Biophys Res Commun 214:86–93
- Gamez J, Playan A, Andreu AL, Bruno C, Navarro C, Cervera C, Arbos MA, Schwartz S, Enriquez JA, Montoya J (1998) Familial multiple symmetric lipomatosis associated with the A8344G mutation of mitochondrial DNA. Neurology 51:258– 260
- Tanji K, Bonilla E (2001) Optical imaging techniques (histochemical, immunohistochemical, and in situ hybridization staining methods) to visualize mitochondria. In: Pon LA, Schon EA (eds). Mitochondria. Academic Press, San Diego, pp 311–332
- 10. Sparaco M, Bonilla E, DiMauro S, Powers J (1993) Neuropathology of mitochondrial encephalomyopathies due to mitochondrial DNA defects. J Neuropathol Exp Neurol 52:1–10
- 11. Lombes A, Mendell JR, Nakase H, Barohn RJ, Bonilla E, Zeviani M, Yates AJ, Omerza J, Gates TL, Nakahara K, Rizzuto R, Engel WK, DiMauro S (1989) Myoclonic epilepsy and ragged-red fibers (MERRF) with cytochrome c oxidase deficiency: neuropathology, biochemistry and molecular genetics. Ann Neurol 26:20–33
- 12. Shoffner JM, Lott MT, Lezza AMS, Seibel P, Ballinger SW, Wallace DC (1990) Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA^{Lys} mutation. Cell 61:931–937
- 13. Silvestri G, Moraes CT, Shanske S, Oh SJ, DiMauro S (1992) A New mtDNA mutation in the tRNA^{Lys} gene associated with myoclonic epilepsy and ragged-red fibers (MERRF). Am J Hum Genet 51:1213–1217
- 14. Ozawa M, Nishino I, Horai S, Nonaka I, Goto Y (1997) Myoclonus epilepsy associated with ragged-red fibers: a G-to-A mutation at nucleotide pair 8363 in mitochondrial tRNA^{Lys} gene in two families. Muscle Nerve 20:271–278

- 15. Zeviani M, Muntoni F, Savarese N, Serra G, Tiranti V, Carra F, Marittoti C, DiDonato S (1993) A MERRF/MELAS overlap syndrome associated with a new point mutation in the mitochondrial DNA tRNA^{Lys} gene. Eur J Hum Genet 1:80–87
- 16. Nakamura M, Nakano S, Goto YI, Ozawa M, Nagahama Y, Fukuyama H, Akiguchi I, Kaji R, Kimura J (1995) A novel point mutation in the mitochondrial tRNA^{Ser(UCN)} gene detected in a family with MERRF/MELAS overlap syndrome. Biochem Biophys Res Commun 214:86–93
- 17. Ashok V, Piccoli DA, Bonilla E, Berry GT, DiMauro S, Moraes CT (1997) A novel mitochondrial G8313A mutation associated with prominent initial gastrointestinal symptoms and progressive encephaloneuropathy. Pediatr Res 42:448–454
- Hiel JA, Verrips A, Keyser A, Jansen TL, Wesseling P, Coo R de, Gabreels FJ (1998) Ileus in mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes. Neth J Med 53:27–31
- 19. Cormier-Daire V, Bonnefont JP, Rustin P, Maurage C, Ogier H, Schmitz J, Ricour C, Saundubray JM, Munnich A, Rotig A (1994) Mitochondrial DNA rearrangements with onset as chronic diarrhea with villous atrophy. J Pediatr 124:63–70
- 20. Hirano M, Silvestri G, Blake DM, Lombes A, Minetti C, Bonilla E, Hays AP, Lovelace RE, Butler I, Bertorini TE, Threlkeld AB, Mitsumoto H, Salberg LM, Rowland LP, DiMauro S (1994) Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. Neurology 44:721–727
- 21. Kuroiwa T, Kuwata T, Nakayama T, Takemura T, Sakuta M, Ichinose S, Goto Y-I, Okeda R (1998) Mitochondrial encephalomyopathy showing prominent microvacuolation and necrosis of intestinal smooth muscle cells: a case diagnosed by rectal biopsy. Acta Neuropathol 96:86–90
- 22. Mori O, Yamazaki M, Ohaki Y, Arai Y, Oguro T, Shimizu H, Asano G (2000) Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) with prominent degeneration of the intestinal wall and cactus-like cerebellar pathology. Acta Neuropathol 100:712–717
- 23. Goyal RK, Hirano I (1996) The enteric nervous system. N Eng J Med 334:1106–1115
- 24. Hevner RF, Wong-Riley MTT (1993) Mitochondrial and nuclear gene expression for cytochrome oxidase subunits are disproportionally regulated by functional activity in neurons. J Neurosci 13:1805–1819
- 25. Bonilla E, Tanji K, Hirano M, Vu TH, DiMauro S, Schon EA (1999) Mitochondrial involvement in Alzheimer's disease. Biochim Biophys Acta 1410:171–182