A T-to-G Mutation at Nucleotide Pair 8993 in Mitochondrial DNA in a Patient With Leigh's Syndrome

Harumi Yoshinaga, MD; Tatsuya Ogino, MD; Shunsuke Ohtahara, MD; Ryoichi Sakuta, MD; Ikuya Nonaka, MD; Satoshi Horai

Abstract

We studied a patient with Leigh's syndrome using neurophysiologic, radiologic, enzymatic, biochemical, and molecular analysis. Her clinical course had started with acute encephalopathic symptoms at 7 months of age. With repeated remission and exacerbation, she developed hypotonia and symptoms of brainstem dysfunction, such as irregular respiration and swallowing difficulty. These symptoms were followed by epileptic seizures, including simple partial seizures and tonic spasms. Both serum lactate and serum pyruvate levels were elevated, and deficient activity was detected in cytochrome *c* oxidase in her quadriceps femoris muscle. From the early stages, we noted an abnormality in the auditory brainstem response and visual evoked potentials, and an abnormal symmetrical low-density area in the basal ganglia on the computed tomographic scan. We found a mitochondrial DNA point mutation at 8993 in blood samples from both the patient and her mother using a simple polymerase chain reaction method. The ratio of wild and mutant mitochondrial DNA calculated densitometrically on polymerase chain reaction products was 56.6% in the patient's blood cells and 8.4% in her mother's. This patient's disorder was thought to be maternally inherited Leigh's syndrome. Her brother had died of the identical clinical features at 1 year 9 months of age. (*J Child Neurol* 1993;8:129–133).

Leigh's syndrome is a degenerative disorder that shows characteristic pathologic findings in the brain, especially in the brain stem and basal ganglia. The fundamental feature of the disease is not yet well known, and its diagnosis during life is difficult.[1]

We describe one case of Leigh's syndrome with a maternally inherited mitochondrial DNA (mtDNA) mutation at position 8993, diagnosed by clinical, biochemical, histochemical, neurophysiologic, and computed tomographic (CT) scan findings.

Case Report

Clinical Summary

This patient is a girl, now 10 years 10 months of age, and the second child of a Japanese couple without consanguinity. The patient's brother was floppy, with delayed early development. He died of acute encephalopathy at age 1 year 9 months, but no autopsy was performed. No other particular family history was noted.

Our patient's prenatal course was uncomplicated except for threatened abortion in the later part of pregnancy. She was the 2370-g product of an uncomplicated labor and was delivered at 38 weeks of gestation. Soon after birth, she was noted to be hypotonic. She showed visual following skills and the ability to smile at 2 or 3 months of age, but had poor head control. She developed an upper respiratory infection at age 7 months, and after recovering from 2 days of fever, she showed feeding difficulty and could

Received July 20, 1992. Received revised Oct 12, 1992. Accepted for publication Oct 28, 1992.

From the Department of Child Neurology (Drs Yoshinaga, Ogino, and Ohtahara), Okayama University Medical School, Okayama, the Division of Ultrastructural Research (Drs Sakuta and Nonaka), National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, and the Department of Human Genetics (Mr Horai), National Institute of Genetics, Mishima, Japan.

Address correspondence to Dr Harumi Yoshinaga, Department of Child Neurology, 2-5-1 Shikatacho, Okayama 700, Japan.

cry only weakly. Ten days later, she became lethargic, and on the following day, she was admitted to the Fukuyama National Hospital in a comatose state. She was treated for an acute encephalopathy of unknown etiology. Her consciousness gradually improved after 2 days, and she left the hospital 2 weeks after admission. She was brought to the Okayama University Hospital for further examination at 8 months of age.

On admission to our hospital, she had near frog posture, showing severe generalized hypotonia with positive findings for scarf sign, double folding, and head-lag. Her patellar tendon reflex was bilaterally slightly hyperactive, and the Babinski reflex was present bilaterally. Her visual following skills were not impaired.

The patient was discharged from the hospital and soon began a cycle of improvement followed by relapse. She developed gradually, and at 11 months of age, she was able to hold her head up and roll over. However, at age 1 year 1 month, she suffered a febrile episode, and her progression ceased, she became hypotonic, and she lost the ability to hold her head up and roll over. This was followed a short time later by another period of improvement, and at age 1 year 7 months she again could hold her head up and roll over. Following this improvement, she developed a low-grade fever and became lethargic and unable to control her head or roll over and showed severe generalized hypotonia. At this point she reentered our hospital.

On the second admission, she had horizontal nystagmus, difficulty swallowing, irregular respiration in sleep, and hyperventilation, showing brainstem dysfunction, and also had choreoathetoid movement in her hands. By age 2 years 3 months, she developed focal convulsions, alternating between the right and left sides of the body, or only on her face. From 2 years 6 months of age, she also suffered from other epileptic seizures including tonic spasms. Her rigidospasticity progressed gradually, and she declined into a state of akinetic mutism at the age of 2 years 6 months.

Laboratory Findings

Fundus Oculi. Right macular degeneration had been found on her initial visit to our hospital, and at age 2 years 6 months bilateral optic disks showed yellow, waxy atrophy and retinal degeneration.

Biochemical Findings. Both serum lactate and serum pyruvate levels were elevated, 47 mg/dL (normal, 4 to 16 mg/dL) and 2.3 mg/dL (normal, 0.3 to 0.7 mg/dL), respectively. Serum levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, lactate dehydrogenase, and creatine phosphokinase were almost normal. Serum and urine screening tests for amino acids revealed no abnormality at 8 months of age. In arterial blood gas analysis, metabolic acidosis was noted with respiratory compensation, ie, pH 7.43, carbon dioxide tension 23.0 mm Hg, partial pressure of oxygen 95.5 mm Hg, bicarbonate 15.3 mEq/L, and base excess −7.7 mEq/L at age 2 years 6 months.

Enzymatic Investigation. The activities of lysozomal enzymes in leukocytes were all normal at 7 months. Thymidine triphosphate–adenosine triphosphate phosphotransferase inhibitor was absent in her urine. Pyruvate dehydrogenase, pyruvate carboxylase, phosphoenol-pyruvate carboxykinase, lipoamide dehydrogenase, and cytochrome *c* oxidase all showed normal enzymatic activity in the patients' cultured skin fibroblasts at age 2 years 6 months.

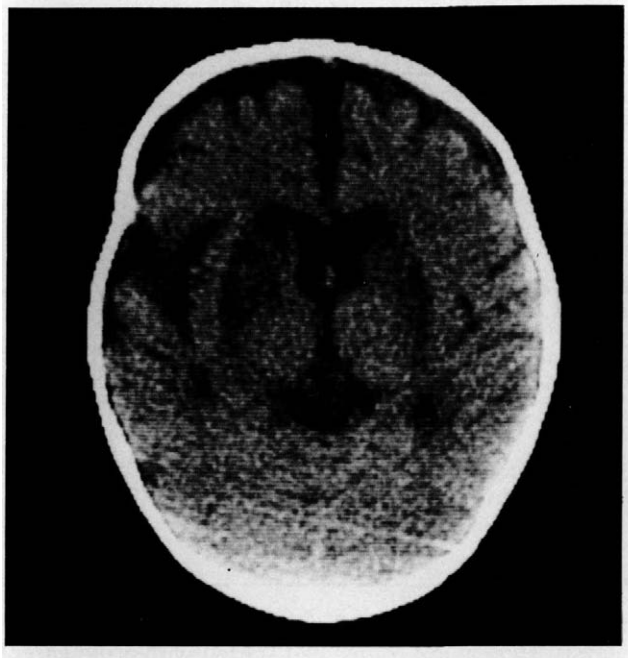

FIGURE 1
CT brain scans in the patient showed diffuse brain atrophy and bilaterally symmetrical low-density areas at the putamen and caudate nucleus.

CT Findings. Brain CT scans indicated diffuse brain atrophy and a bilateral symmetric low-density area in the basal ganglias (Figure 1).

Neurophysiologic Examination. Electromyography (bilateral anterior tibial muscle), sensory nerve conduction velocity, and motor nerve conduction velocity were normal at 8 months of age. At our initial examination, the electroencephalogram showed diffuse slow-wave dysrhythmia and multifocal spikes, and changed rapidly into a disorganized pattern by age 2 years 2 months, and to a flat pattern by age 2 years 6 months. Periodic lateralized epileptiform discharge was also noted transiently at about age 1 year 6 months.

At 2 years 3 months of age, an all-night polysomnogram revealed the existence of three types of apnea attacks—central, mixed, and obstructive, which appeared as irregular respiration during sleep.

Figure 2 shows a serial auditory brainstem response during the clinical course. In the early stages, the wave V amplitude was low and the III–V interpeak latency was prolonged, both of which indicated an upper brainstem dysfunction. Soon after, each wave component began to disappear progressively. The visual evoked potential was abnormal, with a prolonged wave IV latency appearing

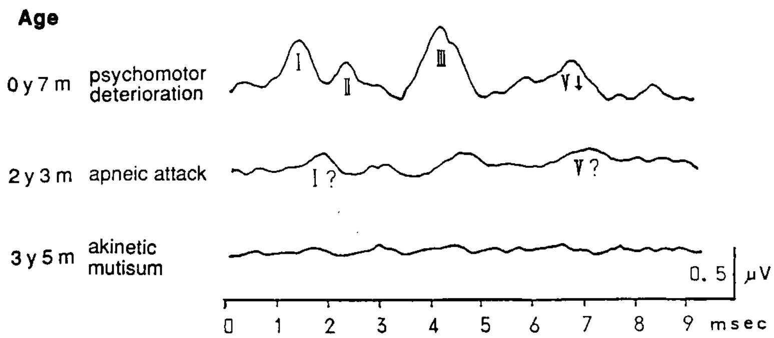

FIGURE 2
Subsequent auditory brainstem response studies in the patient. III–V interpeak latency was prolonged, and wave V amplitude was low at age 7 months. The auditory brainstem response deteriorated by age 2 years 3 months, revealing greatly prolonged wave V latency and poorer reproducibility. At age 3 years 5 months, loss of all wave components was demonstrated.

first, followed later by lower amplitude, and finally a total loss of components.

Muscle Biopsy

Biopsied quadriceps femoris muscle was divided into three pieces for histologic, biochemical, and DNA examinations. Serial frozen sections were stained with hematoxylin and eosin, modified Gomori trichrome, cytochrome *c* oxidase, and a battery of histochemical methods. The determination of enzyme activities in the mitochondrial respiratory chain on isolated mitochondria has been described previously by Koga, et al.[2] Mitochondrial DNA from muscle and blood cells bracketing the adenosine triphosphatase subunit 6 gene was amplified by polymerase chain reaction and digested with the restriction enzyme *Hpa* II.[3]

There was a mild variation in fiber size measuring from 20 to 50 μm in diameter (Figure 3B). No ragged-red fibers were seen. Cytochrome *c* oxidase activity was diffusely decreased and was almost absent in type 2 fibers

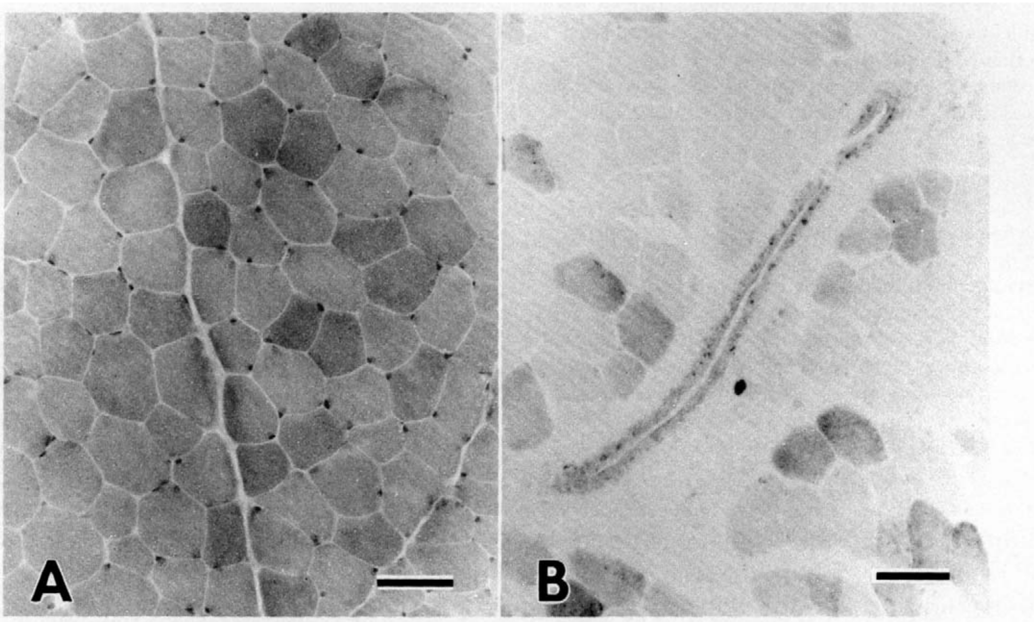

FIGURE 3A **FIGURE 3B**

FIGURE 3
In frozen cross-sections, there was a mild variation in fiber size. No ragged-red fibers were seen **(A)**. Cytochrome *c* oxidase activity was diffusely decreased but blood vessel had positive cytochrome *c* oxidase activity **(B)**. (**A**, modified Gomori trichrome; **B**, cytochrome *c* oxidase. Bar = 50 μm.)

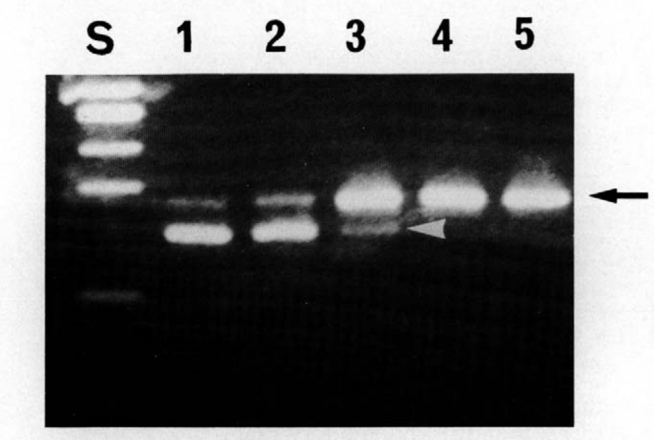

FIGURE 4
mtDNA within the ATPase 6 gene was amplified by polymerase chain reaction and digested with the restriction enzyme *Hpa* II. The 179-base polymerase chain reaction products (arrow) harboring the mutation were cleaved into 155-base (arrowhead) and 24-base fragments. Patient (1, muscle, and 2, blood), patient's mother (3, blood), Leigh's syndrome without mutation at 8993 (4), and normal control (5). Size-standard DNA (S) was ΦX 174 DNA digested by *Hae* III.

(Figure 3A). Type 2B fibers were predominant (70%) and occasionally aggregated, forming small fiber type grouping. Except for mildly decreased cytochrome *c* oxidase activity, no biochemical defects were found. We detected the 8993 heteroplasmic mutation with the *Hpa* II digestion in her muscle. An identical mutation was also found in her blood sample, as well as in her mother's, suggesting a maternal inheritance (Figure 4). The ratio of mutant mtDNA calculated densitometrically on polymerase chain reaction products was 56.6% in the patient's blood cells and 4.8% in her mother's.

Therapy

Based on clinical and laboratory findings, we suspected Leigh's syndrome. In the early stages, we tried vitamin B_1 therapy, L-glutamine therapy, and medium-chain triglyceride therapy, all without remarkable effects. The patient repeated the admission and discharge process several times, getting into respiratory trouble, with respiratory infection.

After a tracheotomy at age 8 years, her clinical course has now become stable. She is currently on course observation at home and is now 10 years 10 months of age.

Discussion

Leigh's syndrome is a group of heterogeneous disorders, and a number of inborn errors of energy metabolism in the mitochondria appear to be etiologically related. At first, because of the considerable resemblance of its lesions to Wernicke's encephalopathy, the theory of existence of thymidine triphosphate–adenosine triphosphate phosphotransferase inhibitor,[4] was suggested in such cases. Serious objection has recently been raised to this suggestion. Now, pyruvate dehydrogenase complex[5] deficiency and complex deficiency of the respiratory chain, such as nicotinamide adenine dinucleotide–coenzyme Q oxidoreductase[6] and cytochrome *c* oxidase,[7,8] are recognized as causative.

The most frequently reported etiology is cytochrome *c* oxidase deficiency, also shown in our patient.

Lately, the investigation of mtDNA has progressively advanced.[9,10] Specific mutations of mtDNA have been found for each syndrome, for example: myoclonus epilepsy associated with ragged-red fibers (MERRF) is associated with a mtDNA transfer RNA (alanine to glycine) mutation at 8344[11]; the syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS) is associated with mtDNA a mtDNA transfer RNA (alanine to glycine) mutation at 3243.[12] As for Leigh's syndrome, Lombes et al[13] reported that Southern analysis excluded any major deletion of a mtDNA-encoded subunit in three patients with Leigh's syndrome and cytochrome *c* oxidase deficiency. They discussed, however, that the level of resolution of this assay does not allow them to exclude small defects such as microdeletions or point mutations in the cytochrome *c* oxidase genes.

We found a heteroplasmic mtDNA point mutation (threonine to glycine) at position 8993 using polymerase chain reaction restriction-digest analysis in mtDNA region bracketing of the adenosine triphosphatase subunit 6 gene (from 8838 to 9017), which was isolated from the patient's and her mother's blood samples.

An mtDNA mutation at 8993 in heteroplasmic distribution was first described by Holt et al[14] in four members of a family with retinitis pigmentosa. Their cases showed variable neurologic disorders, such as developmental delay, dementia, seizures, proximal neurogenic muscle weakness, and their common clinical signs of retinitis pigmentosa and ataxia. Although all these features, except for retinitis pigmentosa, may be observed in cases of pathologically defined mitochondrial myopathy, we cannot categorize them into one identical syndrome because of the heterogeneity of their clinical features. However, one of these four patients was a child with prenatal onset, developmental delay, and emaciation. This child may be included as a case of Leigh's syndrome associated with this particular point mutation. On the other hand, Tatuch et al[15] reported a girl whose postmortem diagnosis was Leigh's syndrome with a mtDNA point mutation at 8993. A maternal aunt and uncle had died at age 1 year 5 months with similar clinical course, and another uncle with retinitis pigmentosa and ataxia remains alive. He is mentally retarded, as were those cases reported by Holt et al.

Tatuch et al[15] found an mtDNA point mutation at 8993 by polymerase chain reaction restriction-digest analysis of mtDNA isolated from the proband, her relatives, and from her healthy mother's blood cells. The percentage of abnormal mtDNA was more than 95% in the patient and her aunt, 78% in her uncle, and 39% to 71% in her mother, who showed variable heteroplasmy. Tatuch et al concluded that, according to the ratio of mutant type mtDNA, the mtDNA mutations at 8993 could produce the clinical phenotype of Leigh's syndrome in addition to the phenotype of ataxia and retinitis pigmentosa.

The clinical findings of our case support the diagnosis of Leigh's syndrome: the age of onset, the progressive course with brainstem disturbance as a main feature, correspondent neurophysiologic[16] and biochemical findings, and the symmetrical low-density area in basal ganglia on CT scan.[17,18] Our case is thought to be the third case of Leigh's syndrome with mtDNA mutation at 8993, and had 56.6% of mutant mtDNA. In addition, our case interestingly had retinal degeneration, as also shown in the reported by Holt et al,[14] case and in the proband and her uncle in the case by Tatuch et al.[15] This feature is unusual in Leigh's syndrome. This point mutation leads to an amino acid change from a highly conserved leucine to arginine in adenosine triphosphatase subunit 6, most likely causing failure in adenosine triphosphate synthesis.

Because its mutation is heteroplasmy, like another various point mutation, the clinical spectrum of disease is wide, including Leigh's syndrome, as exemplified by the case of Tatuch et al and ours. The clinical course of each patient will change according to proportionality of mutant and wild type mtDNA and according to what organs are affected most.

The fact that three cases discussed in this paper (those of Holt et al,[14] Tatuch et al,[15] and ours) were not always accompanied by the mitochondrial morphologic abnormality, indicate the usefulness of investigating the mtDNA using the polymerase chain reaction method. Common enzyme assay and histologic research cannot reveal the same type of results. The polymerase chain reaction method will enrich the etiologic diagnostic technique for inborn errors of mitochondrial energy.

Acknowledgments

We are indebted to the following doctors: Professor Shintaro Okada of Osaka University for lysosomal enzyme assays, Professor Yukinori Nishino of Kyoto University for analyzing thymidine triphosphate-adenosine triphosphate phosphotransferase inhibitor, and Professor Susumu Ito of Tohoku University for enzyme assays in cultured skin fibroblast.

References

1. Pincus JH: Subacute necrotizing encephalomyelopathy (Leigh's disease): A consideration of clinical features and etiology. *Dev Med Child Neurol* 1972;14:87–101.
2. Koga Y, Nonaka I, Sunohara N, et al: Variability in the activity of respiratory chain enzymes in mitochondrial myopathies. *Acta Neuropathol (Berl)* 1988;76:135–141.
3. Sakuta R, Goto Y, Horai S, et al: Mitochondrial DNA mutation and Leigh's syndrome. *Ann Neurol* 1992;32(4):597–598.
4. Cooper JR, Itokawa Y, Pincus JH: Thiamine triphosphate deficiency in subacute necrotizing encephalomyelopathy. *Science* 1969;164:74–75.
5. Kretzschmar HA, DeArmond SJ, Koch TK, et al: Pyruvate dehydrogenase complex deficiency as a cause of subacute necrotizing encephalopathy (Leigh disease). *Pediatrics* 1987;79: 370–373.
6. Robinson BH, De Meirleir L, Glerum M, et al: Clinical presentation of mitochondrial respiratory chain defects in NADH-coenzyme Q reductase and cytochrome oxidase: Clues to pathogenesis of Leigh disease. *J Pediatr* 1987;110:216–222.
7. DiMauro S, Servidei S, Zeviani M, et al: Cytochrome *c* oxidase deficiency in Leigh syndrome. *Ann Neurol* 1987;22: 498–506.
8. Van Coster R, Lombes A, De Vivo DC, et al: Cytochrome *c* oxidase-associated Leigh syndrome: Phenotypic features and pathogenetic speculations. *J Neurol Sci* 1991;104:97–111.
9. Anderson S, Bankier B, Barrell M: Sequence and organization of the human mitochondrial genome. *Nature* 1981;290: 457–465.
10. Rosing H, Hopkins L, Wallace D: Maternally inherited mitochondrial myopathy and myoclonic epilepsy. *Ann Neurol* 1985;17:228–237.
11. Shoffner J, Lott M, Lezza A: Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA $tRNA^{Lys}$ mutation. *Cell* 1990;61:931–937.
12. Goto Y, Nonaka I, Horai S: A mutation in the $tRNA^{Leu(UUR)}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1990;348:651–653.
13. Lombes A, Nakase H, Tritschler H: Biochemical and molecular analysis of cytochrome *c* oxidase deficiency in Leigh's syndrome. *Neurology* 1991;41:491–498.
14. Holt I, Harding A, Petty R: A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990;46:428–433.
15. Tatuch Y, Christodoulou J, Feigenbaum A: Heteroplasmic mt DNA mutation (T-G) at 8993 can cause Leigh disease when the percentage of abnormal mt DNA is high. *Am J Hum Genet* 1992;50:852–858.
16. Taylor M, Robinson B: Evoked potentials in children with oxidative metabolic defects leading to Leigh syndrome. *Pediatr Neurol* 1992;8:25–29.
17. Schwarz W, Hutchison H, Berg B: Computerized tomography in subacute necrotizing encephalomyelopathy (Leigh disease). *Neuroradiology* 1981;10:268–271.
18. Koch T, Lo W, Berg B: Variability of serial CT scans in subacute necrotizing encephalomyelopathy (Leigh disease). *Pediatr Neurol* 1985;1:48–51.