

Yuka Ueda · Atsushi Ando · Taeko Nagata ·
Hidehiko Yanagida · Kazuro Yagi ·
Keisuke Sugimoto · Mitsuru Okada ·
Tsukasa Takemura

A boy with mitochondrial disease: asymptomatic proteinuria without neuromyopathy

Received: 31 December 2002 / Revised: 5 August 2003 / Accepted: 6 August 2003 / Published online: 25 November 2003
© IPNA 2003

Abstract Mitochondrial disorder is a relatively rare disease during childhood. Previous studies concluded that renal complications in this disease most often occur in patients with mitochondrial encephalomyopathies. We describe a boy with mitochondrial disease who presented with proteinuria while lacking neuromyopathy. Proteinuria was detected at the age of 6 years, including large amounts of low-molecular-weight proteins such as β_2 - and α_1 -microglobulin. Renal functions were normal. Proximal tubular dysfunction and other renal manifestations were absent. Episodic neurologic problems such as migraine and nervous system diseases including epilepsy, depression, schizophrenia and amyotrophic lateral sclerosis (ALS) were found in the boy's family members. Renal tubular basement membrane atrophy and interstitial fibrosis with mononuclear cell infiltration were observed. Ultrastructural examination showed mitochondria, mainly in the proximal tubules, which varied in size and had disoriented cristae. Mutation analysis using mitochondrial DNA (mtDNA) extracted from renal tissues demonstrated a A→G point mutation at nucleotide position 3243 in the tRNA^{Leu(UUR)} gene, while there was no mutation found in mtDNA extracted from peripheral leukocytes. Awareness among pediatricians of mitochondrial disorders, detection of low-molecular-weight proteinuria, renal ultrastructural examination and mutation analysis of mtDNA obtained from renal tissues could be important for early diagnosis of this disease.

Keywords Mitochondrial disease · Proteinuria · β_2 -microglobulin · Proximal tubular cells · Ultrastructural renal pathology

Introduction

Mitochondria generate energy for cellular processes by producing adenosine triphosphate (ATP) via oxidative phosphorylation [1, 2]. Defects in mitochondrial function arising from mutations in mitochondrial DNA (mtDNA) including base substitutions in tRNA or rRNA genes, deletions, or duplications can lead to multisystemic organ dysfunction [1, 2, 3, 4], since mutant mtDNA produces defective oxidative phosphorylation subunits during mitochondrial protein synthesis [1, 2, 3]. For normal function, cells of the central nervous system, skeletal muscles, and the cardiac conduction system may require particularly large amounts of ATP, which is synthesized by mitochondria. These cells therefore are most strongly influenced by defects in mitochondrial function [5, 6]. The clinical categories of such defects include mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), Kearns-Sayre syndrome (KSS), and Wolff-Parkinson-White syndrome [1, 2, 3, 4, 7, 8, 9]. In a manner similar to these mitochondrial dysfunctions, mitochondrial diseases with renal involvement were recognized later [2, 4]. The most common renal manifestation of mitochondrial disease is proximal renal tubular dysfunction, representing the de Toni-Debre-Fanconi syndrome [10, 11]; this reflects the high energy requirements involved in the function of the proximal and distal convoluted tubules. Renal tubular acidosis [11], Bartter syndrome [12], tubulointerstitial nephritis [13], focal segmental glomerulosclerosis exhibiting nephrotic syndrome [14], and diabetic nephropathy [1, 2] have also become recognized as renal manifestations of mitochondrial disease in some patients. Previous studies concluded that these renal manifestations most often occurred in patients with mitochondrial encephalomyopathies [15, 16]. In the present study, we describe a boy with mitochondrial disease who presented with asymptomatic proteinuria while lacking neuromyopathic manifestations.

Y. Ueda · A. Ando · T. Nagata · H. Yanagida · K. Yagi ·
K. Sugimoto · M. Okada · T. Takemura (✉)
Department of Pediatrics,
Kinki University School of Medicine,
377-2 Ohno-Higashi, 589-8511 Osaka-Sayama, Japan
e-mail: tsukasa@med.kindai.ac.jp
Tel.: +81-723-660221 ext. 3231
Fax: +81-723-681566

Case presentation

A 12-year-old boy presented with an unremarkable prenatal and perinatal history. His parents were not related. The history of his family members showed that his mother had depression and epileptic seizure, two maternal aunts had schizophrenia or ALS, and his maternal grandmother had diabetes mellitus. Developmental delay was not noted in infancy (being unable to sit and roll over until 7–8 months of age and being capable of walking at 12 months of age). However, sensorimotor, language, and mental development were all normal, and neuromyopathic manifestations including muscle hypotonia, muscle weakness, seizures, stroke-like episodes, abnormal ocular movements and ataxia were all absent except for cataracts. Proteinuria (1+ by a dipstick method) was first detected when the patient was 6 years old by annual screening at his elementary school. Due to persistent proteinuria, he was referred to our hospital for diagnostic evaluation at the age of 12 years. At the time of admission, pertinent physical examination findings included body weight 34.5 kg (−1 SD), height 145 cm (−0.5 SD) and blood pressure 112/58 mmHg. Neither pitting edema of the lower extremities nor ascites were present. Laboratory examinations showed proteinuria (0.5 g/24 h) with large amounts of β_2 -microglobulin (3.1 mg/24 h) and α_1 -microglobulin (19 mg/24 h). Orthostatic proteinuria was ruled out by the force standing test. The serum creatinine concentration was slightly increased (1.5 mg/dl), but creatinine clearance was normal (96 ml/min/1.48 m²). Plasma concentrations of total protein (6.8 g/dl), albumin (4.3 g/dl), and

cholesterol (185 mg/dl) were all normal. Hematuria, glycosuria, aminoaciduria, kaliuresis, uricosuria renal tubular acidosis, decreased urinary osmolarity, lactic acidosis, and pancytopenia were all absent. There were no mutations in the gene encoding renal-specific chloride channel 5 (CLCN5) as the candidate gene for Dent disease. A renal biopsy specimen showed tubulointerstitial alterations including fibrosis and renal tubular basement membrane atrophy, suggesting tubulointerstitial nephropathy (Fig. 1A). No glomerular alterations including deposition of electron-dense material, thickening of glomerular basement membranes or fusion of the foot processes were observed (Fig. 1B). Electron-microscopic findings disclosed abnormal mitochondria with disoriented cristae accompanied by electron-dense bodies, mainly in the cytoplasm of proximal renal tubular epithelial cells (Fig. 1C, D). A mutation analysis using mtDNA extracted from renal tissues demonstrated A→G point mutation at nucleotide position 3243 in the tRNA^{Leu(UUR)} gene (Fig. 2). However, A→G substitution at this position of mtDNA obtained from peripheral leukocytes was not detected. Family members, including his mother, showed normal urinary β_2 -microglobulin levels without evidence of renal failure. A search for mutation of the mtDNA gene in his family members has not been carried out because consent for gene analysis could not be obtained.

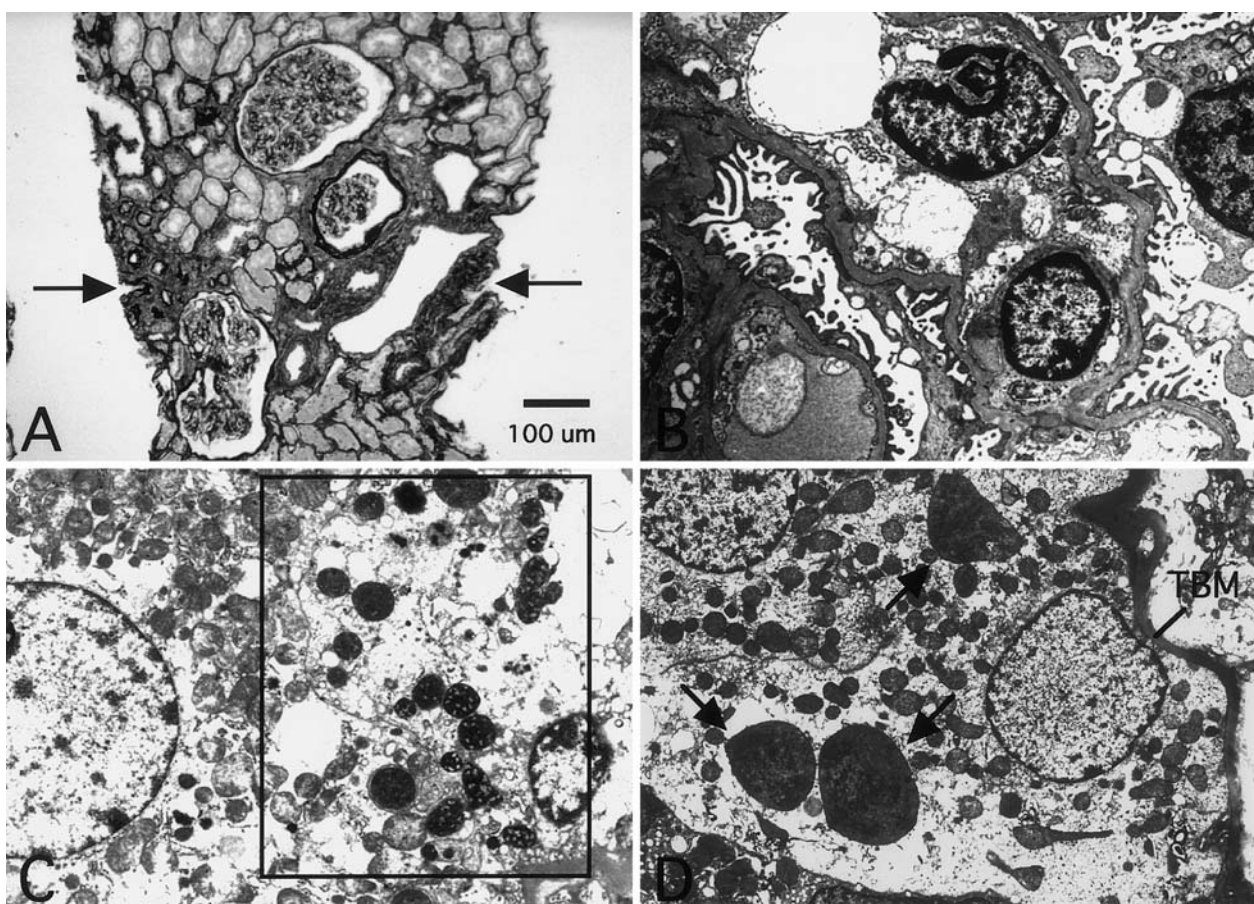


Fig. 1A–D Histologic ($\times 100$, methenamine silver stain) and ultrastructural features ($\times 3,000$) of renal tissue obtained from the patient. Interstitial fibrosis and collapse of renal tubular basement membranes are observed (A, arrows). Glomerular alterations are

minimal (B). Abnormal mitochondria showing disoriented cristae (C, closed box), and giant mitochondria with electron-dense bodies (D, arrow) are evident in the cytoplasm of proximal renal tubular epithelial cells

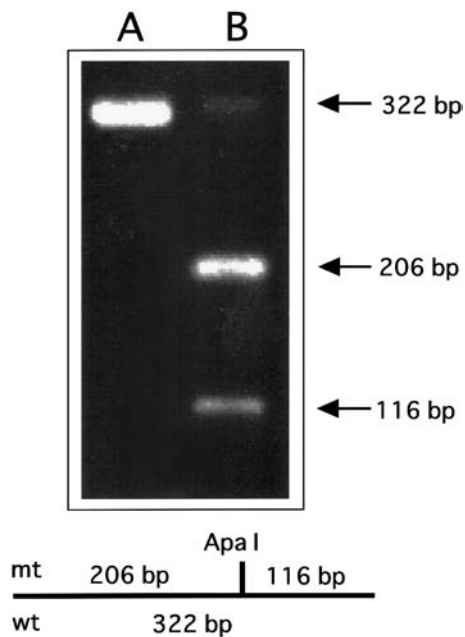


Fig. 2 Mutation analysis of the mtDNA at the nucleotide position 3243 in the tRNA^{Leu(UUR)} gene. mtDNA was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) from the mRNA obtained from renal biopsied tissue using specific primer pair, followed by digestion with *Apa*I. Replacement of adenine (A) with guanine (G) at the nucleotide position 3243 produces a new cleaved site by restricted enzyme *Apa*I. PCR fragments were then transferred to 3% SDS-polyacrylamide gel and visualized by ethidium bromide stain. In normal controls, a single band of a 322-bp fragment of mtDNA was only observed (A), while in this patient two additional cleaved fragments (206 bp and 116 bp) appeared after digestion by *Apa*I, suggesting a heteroplasmic pattern (B) (wt wild type, mt mutant)

Discussion

Each human cell contains numerous mtDNA copies. Normal and mutant mtDNA are inherited only from the mother, since mitochondria are transmitted from the cytoplasm of the ovum [17]. Since normal and mutated mtDNA can coexist within the same cell, expression of disease may be related to the number of mutant mtDNA molecules present in each tissue and may also be dependent on the energy requirement imposed by the functional activities of individual tissues, referred to as the threshold effect [18, 19]. The threshold effect may depend on the balance between oxidative supply and demand in each tissue.

Mitochondrial disease was first identified as affecting the central nervous system and skeletal muscle; hence the term “mitochondrial neuromyopathy” [20]. In family members of the boy described here, there were no symptoms suggesting mitochondrial neuromyopathy, although several neurologic and psychiatric manifestations including migraine headache, seizures and schizophrenia were present. Transient neurologic episodes such as migraine headache or incoherent speech or actions are commonly observed in mitochondrial diseases [21].

Therefore, such neurologic manifestations may be closely related to mitochondrial dysfunction. Mutation analysis of mtDNA in family members will be needed for final diagnosis. In the initial study using DNA extracted from peripheral leukocytes, we failed to find mutation of mtDNA in our patient, which is usually observed in some types of mitochondrial neuromyopathy, especially in MELAS [3, 4]. However, by using DNA extracted from renal tissues, we detected a mutation at nucleotide position 3243 in the tRNA^{Leu(UUR)} gene. Potential explanations include the inadequacy of a peripheral leukocyte sample for detecting mtDNA mutations, because the distribution of abnormal mtDNA is known to vary between tissues and organs [3]. Furthermore, the accuracy of the PCR method used in the present analysis is another possible explanation. Such individuals should be examined further using mtDNA obtained from renal tissues.

Previous studies have reported that renal involvement including acute renal failure, renal tubular acidosis, Fanconi syndrome, Bartter syndrome, focal segmental glomerular sclerosis (FSGS) or tubulointerstitial nephritis occurred in patients with MELAS, KSS, or Pearson syndrome [4, 11, 12, 13, 14, 15]. In the present boy, tubulointerstitial injuries including tubulointerstitial fibrosis and renal tubular basement membrane atrophy were partially observed. However, glomerular alterations were not evident, suggesting tubulointerstitial nephritis, which is supported by previous reports. In our patient, all aspects of development after birth were normal except for cataracts. Muscle weakness and hypotonia have not developed. This situation may be explained by the low percentage of abnormal mtDNA in these tissues given that the ratio of mutant to normal mtDNA often varies from tissue to tissue in mitochondrial disease [1, 3].

Hereditary and nonhereditary renal diseases, especially in FSGS, due to a mutation at nucleotide position 3243 in the tRNA^{Leu(UUR)} gene can occur in the absence of neuromuscular manifestations, as in our patient. Jansen et al. described four female patients showing this mutation in the mtDNA gene who had a medical history in which renal disease was predominant and diabetes developed secondary to steroid treatment after renal transplantation [22]. Similarly, it has also been reported that this mutation was found in some patients who had been initially diagnosed with familial FSGS or Alport-like syndrome [23, 24]. However, the present boy is still young, and degenerated or mutant mtDNA might accrue in other tissues with increasing age. Previous studies suggested that mitochondria in older organisms and older cultured cells show decreases in number, increases in size, and various morphologic and functional abnormalities [25]. Other investigations have suggested that deletions in mtDNA may be detected with increasing frequency as a function of age in tissues with high energy demands such as the brain and muscles [19]. Although the present patient essentially showed only asymptomatic proteinuria, careful follow-up will be required to detect the possible appearance of typical neuromyopathies in later years.

This study emphasizes the need for awareness of mitochondrial disorders among pediatricians and nephrologists. Detection of low molecular weight proteinuria, renal ultrastructural examination and mutation analysis using mtDNA extracted from renal tissues could be important for early identification of these patients.

Acknowledgements We are grateful to Ms. Naomi Jinno for preparation of the manuscript.

References

- Shoffner JM, Wallace D (1995) Oxidative phosphorylation diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) *The metabolic and molecular bases of inherited disease*, 7th edn. McGraw-Hill, New York, pp 1535–1575
- Johns DR (1995) Mitochondrial DNA and disease. *N Engl J Med* 333:638–644
- Kiechle FL, Kaul KL, Farkas DH (1996) Mitochondrial disorders, methods and specimen selection for diagnostic molecular pathology. *Arch Pathol Lab Med* 120:597–603
- Naudet P, Rotig A (1996) Renal involvement in mitochondrial cytopathies. *Pediatr Nephrol* 10:368–373
- DiMauro S, Moraes CT (1993) Mitochondrial encephalomyopathies. *Arch Neurol* 50:1197–1208
- Wallace DC, Lott MT, Torroni A, Shoffner JM (1991) Report of the committee on human mitochondrial DNA. *Cytogenet Cell Genet* 58:1103–1123
- Pavakis SG, Phillips PC, DiMauro S, DeVino DC, Rowland LP (1984) Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes: a distinctive clinical syndrome. *Ann Neurol* 16:481–488
- Egger J, Lake BD, Wilson J (1981) Mitochondrial cytopathy. A multisystem disorder with ragged red fibres on muscle biopsy. *Arch Dis Child* 56:741–752
- Kearns TP, Sayer GP (1958) Retinitis pigmentosa, external ophthalmoplegia and complete heart block. *Am Arch Ophthalmol* 60:280–289
- Moraes CT, Shanske S, Trischler HJ, Aprille JR, Andreetta F, Bonilla E, Schon EA, DiMauro S (1991) Mitochondrial DNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet* 48:492–501
- Matsutani H, Mizusawa Y, Shimoda M, Niimura F, Takeda A, Shimohira M, Iwakawa Y (1992) Partial deficiency of cytochrome c oxidase with isolated proximal renal tubular acidosis and hypercalciuria. *Child Nephrol Urol* 12:221–224
- Larsson NG, Holme E, Kristiansson B, Oldfors A, Tulinius M (1990) Progressive increase of the mutated mitochondrial DNA fraction in Kearns-Sayer syndrome. *Pediatr Res* 28:131–136
- Rotig A, Goutieres F, Naudet P, Rustin P, Chretien D, Guest G, Mikol J, Gubler MC, Munnich A (1995) Deletion of mitochondrial DNA in a patient with chronic tubulointerstitial nephritis. *J Pediatr* 126:597–601
- Rotig A, Lehnert A, Rustin P, Chretien D, Bourgeron T, Naudet P, Munnich A (1994) Renal involvement in the mitochondrial disorders. *Adv Nephrol* 25:367–378
- Hsieh F, Gohh R, Dworkin L (1996) Acute renal failure and the MELAS syndrome, a mitochondrial encephalomyopathy. *J Am Soc Nephrol* 7:647–652
- Hamman SR, Sweeny MC, Brickington M, Morgan-Hughes JA, Harding AE (1991) Mitochondrial encephalomyopathy: molecular genetic diagnosis from blood samples. *Lancet* 337:1311–1313
- Giles RE, Blanc H, Cann HM, Wallace DC (1980) Maternal inheritance of human mitochondrial DNA. *Proc Natl Acad Sci U S A* 77:6715–6719
- Wallace DC (1992) Disease of the mitochondrial DNA. *Annu Rev Biochem* 61:1175–1212
- Shoffner JM, Wallace DC (1990) Oxidative phosphorylation diseases: disorder of two genomes. *Adv Hum Genet* 19:267–330
- Pretty RKH, Harding AE, Morgan-Hughes JA (1986) The clinical features of mitochondrial myopathy. *Brain* 109:915–938
- Rothrock JF, Walicke P, Swenson MR, Lyden PD, Logan WR (1988) Migrainous stroke. *Arch Neurol* 45:63–67
- Jansen MM, Massen JA, van der Woude FJ, Lemmink HA, van den Ouweland JM, Hart LM, Smeets HJ, Bruijn JA, Lemkes HH (1997) Mutation in mitochondrial tRNA^{(Leu(UUR))} gene associated with progressive kidney disease. *J Am Soc Nephrol* 8:1118–1124
- Yamagata K, Nishiki K, Hagiwara S (2000) Mitochondrial gene mutation in patients with focal segmental glomerulosclerosis. *Nippon Naika Gakkai Zasshi* 89:144 (abstract in Japanese)
- Cheong HI, Chae JH, Kim JS, Park HW, Ha IS, Hwang YS, Lee HS, Choi Y (1999) Hereditary glomerulopathy associated with a mitochondrial tRNA^(Leu) gene mutation. *Pediatr Nephrol* 13:477–480
- Bittles AH (1989) The role of mitochondria in cellular aging. In: Warnes AM (ed) *Human, aging and later life*. Edward Arnold, London, pp 29–34