

Fumio Kurogouchi^a
Tomomasa Oguchi^a
Eiichirou Mawatari^a
Shuichi Yamaura^a
Kazuhiko Hora^a
Manabu Takei^a
Yoshiki Sekijima^b
Shu-ichi Ikeda^b
Kendo Kiyosawa^a

A Case of Mitochondrial Cytopathy with a Typical Point Mutation for MELAS, Presenting with Severe Focal-Segmental Glomerulosclerosis as Main Clinical Manifestation

^a Second and

^b Third Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

Key Words

Mitochondrial cytopathy
Mitochondrial encephalomyopathy
Mitochondrial cardiomyopathy
Focal-segmental glomerulosclerosis
Ragged-red fibers
Hypertrophic cardiomyopathy

Abstract

A 27-year-old female with short stature and mild hearing loss was diagnosed as having focal-segmental glomerulosclerosis by renal biopsy at our hospital. One year later she developed progressive renal dysfunction and cardiac failure and was admitted again to our hospital for evaluation. Though her only neurological disorder was mild hearing loss, her short stature and elevated lactate and pyruvate values in cerebrospinal fluid suggested mitochondrial cytopathy. A muscle biopsy specimen of the left biceps brachii, using modified Gomori trichrome stain, showed a typical image of ragged-red fibers, and an increased number of giant mitochondria with paracrystalline inclusions were visible by electron microscopy. Mitochondrial DNA from the skeletal muscle showed an A-to-G transition at 3243 of transfer RNA^{Leu(UUR)}, the common point mutation for mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. These data confirmed the diagnosis of atypical mitochondrial cytopathy with renal and heart involvement. Mitochondrial cytopathies are often associated with hypertrophic cardiomyopathy but rarely with renal disease. Among the few reported cases with associated renal disease, most included renal tubular disorders; few cases with focal glomerular sclerosis are known. The present case of atypical mitochondrial cytopathy was characterized by a unique clinical course and rare complications with focal-segmental glomerulosclerosis.

Introduction

Mitochondrial encephalomyopathies are clinically divided into three distinct subgroups: (1) cases of mitochondrial myopathy, encephalopathy, lactic acidosis, and stro-

kelike episodes (MELAS); (2) cases of myoclonus epilepsy associated with ragged-red fibers, and (3) cases of chronic progressive external ophthalmoplegia (CPEO), including Kearns-Sayre syndrome [1-3].

KARGER

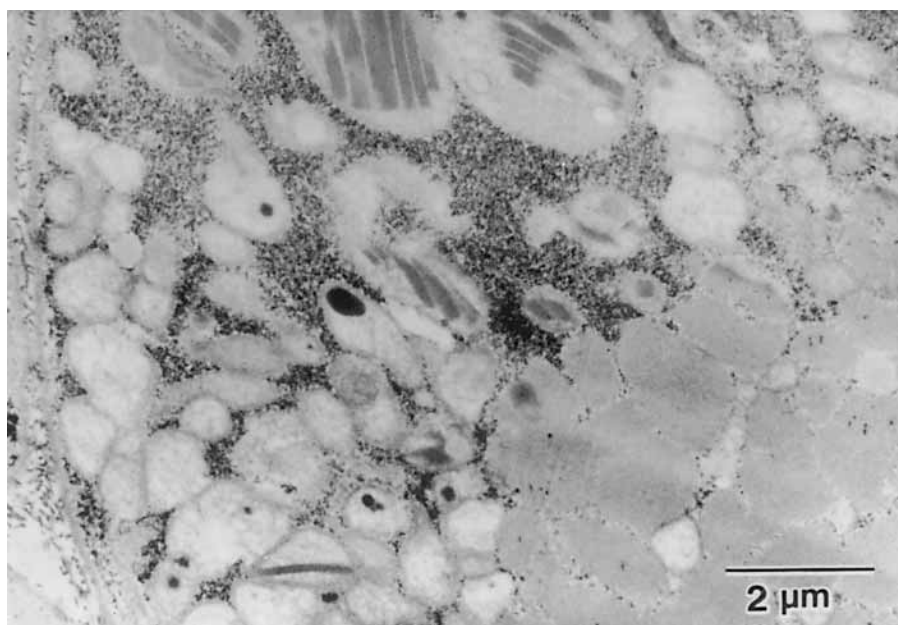
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 1998 S. Karger AG, Basel
0250-8095/98/0186-0551\$15.00/0

Accessible online at:
<http://BioMedNet.com/karger>

Fumio Kurogouchi, MD
2nd Department of Internal Medicine
Shinshu University School of Medicine
3-1-1 Asahi, Matsumoto, Nagano-ken, 390 (Japan)
Tel. +81 263 32 2632, Fax +81 263 32 9412

Fig. 1. Light microscopic findings of the renal biopsy specimen obtained in September 1995, showing global sclerosis in 10 of 12 glomeruli in the kidney tissue specimen and FSGS in 1 of the other 2 glomeruli. Severe tubular atrophy, interstitial fibrosis, and mononuclear cell infiltration were also observed. PAM. $\times 50$.



Recent studies have revealed mutations in the mitochondrial DNA of patients with these diseases. A large deletion ranging from 1 to 8 kbp has been shown in CPEO, including Kearns-Sayre syndrome [4, 5]; a single point mutation at 8344 in tRNA^{Lys(UUR)} has been shown in the tRNA^{Lys(UUR)} gene in myoclonus epilepsy associated with ragged-red fibers [6, 7], and an A-to-G transition has been shown at 3243 in tRNA^{Leu(UUR)} in MELAS [8, 9].

It remains uncertain, however, whether these mutations are specific to the diseases in which they were discovered. Inui et al. [10] detected an A-to-G transition at 3243 in tRNA^{Leu(UUR)}, a common point mutation for MELAS, in some MELAS patients with atypical and unclassifiable clinical symptoms. Mitochondrial cytopathies are, therefore, regarded as a heterogeneous group of multisystem disorders involving many organs [11].

We present an extremely rare case of mitochondrial cytopathy with a common point mutation for MELAS. The patient had severe renal dysfunction due to focal-segmental glomerulosclerosis (FSGS) and severe heart failure with a diffusely hypertrophic left ventricular wall, but manifested no neurological abnormalities other than mild hearing loss.

Case Report

A 27-year-old woman was referred to our hospital in June 1995 for further examination of proteinuria accompanied by progressive peripheral edema and common-cold-like symptoms. As a child the patient had no outstanding health problems until the age of 3, at which time it first appeared that her growth might be stunted. She undertook further examinations on her stature at a hospital, but no specific abnormalities were detected. At around the age of 18, she began to develop mild hearing loss, for which several institutes made complete examinations and concluded only that she had a sensorineural hearing loss of unknown etiology. During a routine high school checkup the same year, she was also found to have proteinuria. Intermittent peripheral edema followed, but the patient pursued no further examination or treatment. No specific hereditary disorders were known in her family's medical history.

Upon admittance to our hospital, the patient was well oriented and alert and showed no signs of mental retardation. Pitting edema of especially the bilateral lower limbs was noted. Head, neck, skin, chest, and abdomen were normal. Neurologically, no abnormalities were found except for a bilateral sensorineural hearing loss. Hematological analysis showed mild anemia: red blood cell count $410 \times 10^4/\mu\text{l}$, hematocrit 35.8%, and hemoglobin 12.5 g/dl. Blood chemical and serological analyses revealed normal values of renal function, liver function, and electrolytes. Urinalysis revealed ++ proteinuria (24-hour urinary excretion of protein 2 g), + hematuria, (1–2 erythrocytes and some hyaline casts per high-power field in the sediments), and otherwise normal findings. A chest radiograph showed mild cardiomegaly (a cardiothoracic ratio of 50%) without pulmonary congestion and pleural effusion. Light microscopic examination of a renal biopsy specimen performed in September 1995 showed global sclerosis in 10 of 12 glomeruli of the kidney tissue as well as segmental sclerosis in 1 of the other 2 glomeruli and severe tubular atrophy,

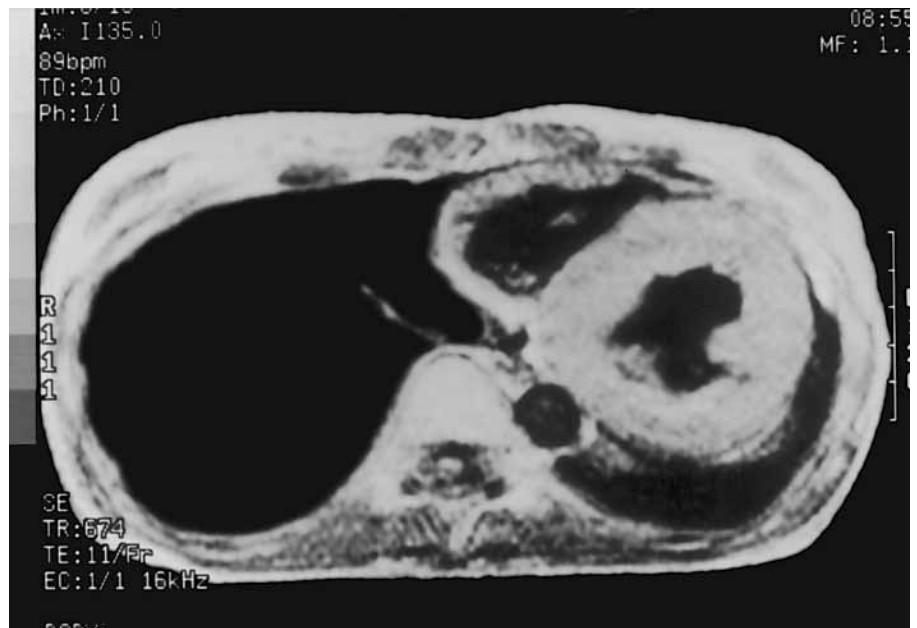


Fig. 2. T1-weighted MRI of the heart, demonstrating diffusely hypertrophied left ventricular wall and papillary muscle.

interstitial fibrosis, and mononuclear cell infiltration. Immunohistochemical examination revealed focal-segmental deposits of immunoglobulin M. These findings indicated a definite diagnosis of advanced FSGS (fig. 1). Electron microscopic examination demonstrated normal renal tubules and blood capillaries in the kidney. Glomeruli and vascular smooth muscle cells could not be examined electron microscopically. The patient was discharged upon disappearance of edema in September 1995.

Seven months later, peripheral edema, general fatigue, and dyspnea appeared, following common-cold-like symptoms; body weight gain, progressive cardiomegaly, and massive pleural effusion led to her second hospitalization in December 1996. On admission, the patient was well oriented and alert. Her body height was 143 cm, and her weight 34 kg, and her vital signs were as follows: blood pressure 150/76 mm Hg, heart rate 120 beats/min, body temperature 37.2°C, and the respiration rate 13/min. On physical examination, hypoactive breathing was audible over her lower lungs, a systolic murmur of 3/6 grade at her left sternal border, and an increased S2 sound and S3 gallop at the apex. An abdominal examination showed only hepatomegaly, and her extremities were covered with a massive pitting edema. Hematological findings showed mild anemia (red blood cells $341 \times 10^4/\text{mm}^3$, hemoglobin 10.8 g/dl, hematocrit 31.5%). Blood chemical analysis revealed renal dysfunction (blood urea nitrogen 46 mg/dl, serum creatinine 4.2 mg/dl) but otherwise normal findings. Serum lactate and pyruvate values were within normal limits. No other abnormalities were found. A cerebral spinal fluid examination showed substantial increases in the values for lactate and pyruvate; to 35 and 1.5 mg/dl, respectively. Echocardiography detected severe left ventricular systolic dysfunction (ejection fraction 20%) with diffuse hypertrophy of the left ventricular wall. Magnetic resonance imaging (MRI) of the heart showed diffuse hypertrophy of the left ventricular wall and papillary muscle with high intensity on both T1- and T2-weighted images (fig. 2). Due to the poor general health of the

patient, cardiac muscle biopsy was not performed. MRI of the brain demonstrated diffusely atrophic cerebral and cerebellar cortices and enlargement of the ventricles. No other abnormal intensities were noted.

In view of these data, the most likely diagnosis was mitochondrial cytopathy, with a resemblance to MELAS. A muscle biopsy specimen of the left biceps brachii showed moderate variation in fiber size and typical ragged-red fibers by modified Gomori-trichrome staining (fig. 3); NADH staining of the muscle gave specific evidence of mitochondrial abnormalities. Electron microscopic examination of the biceps brachii showed numerous enlarged abnormal mitochondria containing extremely proliferated cristae and paracrystalline inclusions (fig. 4).

The DNA samples extracted from leukocytes and muscle were analyzed for the point mutation associated with MELAS. Amplified DNA fragments encompassing that mutation site were digested with endonuclease *Apa I* using polymerase chain reaction. Primers 1 and 2 were dTTCACAAAGCGCCTTCCCCC for nucleotides 3153–3172 and dGCGATGGTGAGAGCTAAGGTC for nucleotides 3551–3531, respectively [9]. The restriction enzyme analysis of amplified mitochondrial DNA disclosed a mutant at nucleotide 3243 in the transfer RNA^{LEU(UUR)} gene.

These results confirmed the diagnosis of an atypical case of mitochondrial encephalomyopathy having the common point mutation for MELAS.

Discussion

It has been reported that each type of mitochondrial encephalopathy has several mitochondrial DNA mutations [1–9]. However, it is uncertain whether these muta-

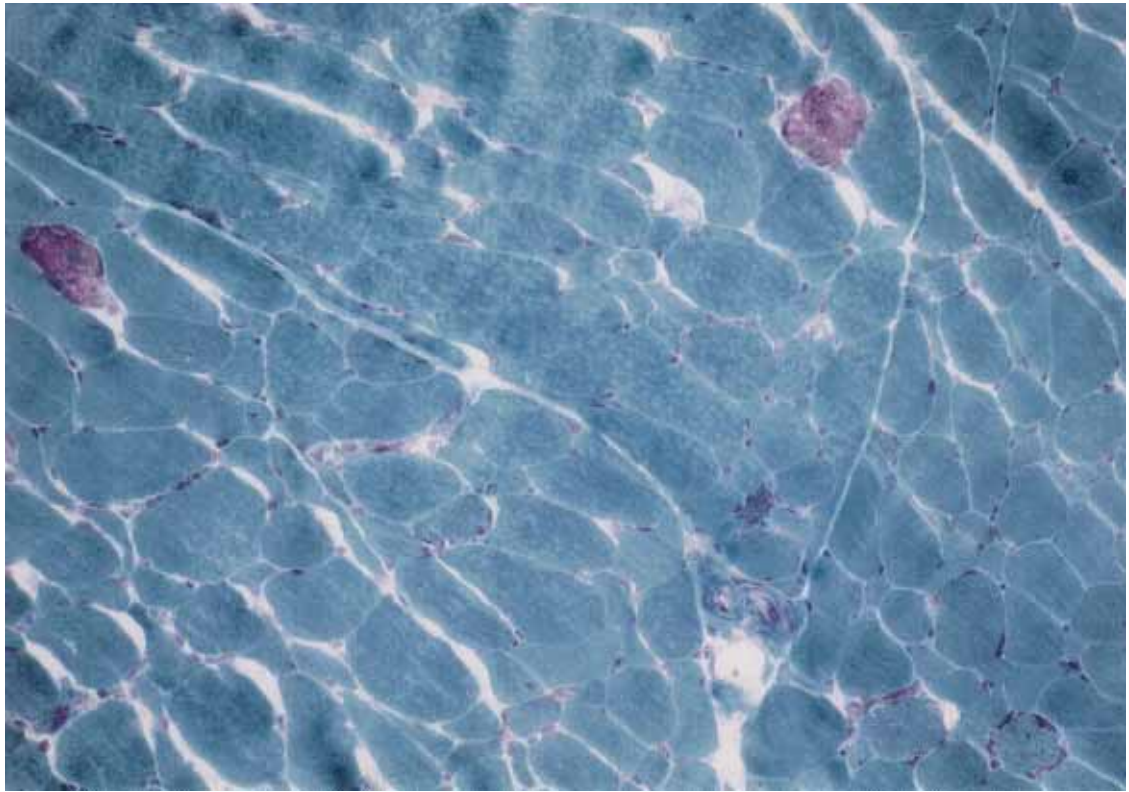


Fig. 3. Frozen sections from the biceps brachii muscle specimen, demonstrating a moderate variation in fiber size. Modified Gomori-trichrome staining shows numerous ragged-red fibers and scattered pathological fibers with mitochondrial clusters. No necrotic or regenerative fibers are visible. $\times 200$.

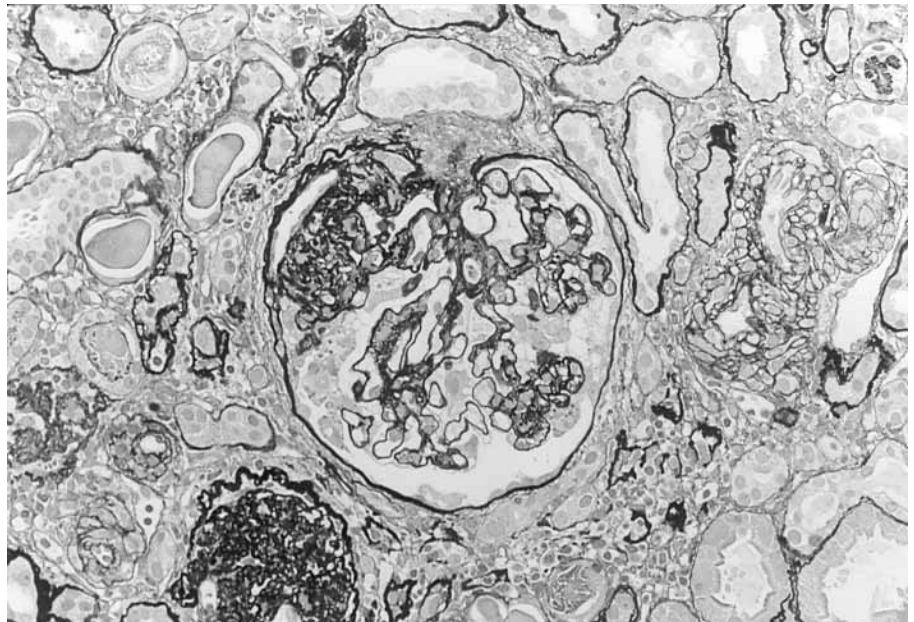


Fig. 4. Electron microscopic visualization of a specimen from biceps brachii, revealing some structural abnormalities of mitochondria, including numerous enlarged abnormal mitochondria containing extremely increased cristae and paracrystalline inclusions. Mitochondria with parallel arrays of cristae arranged in a circular fashion are also found. $\times 1,700$.

tions are specific for each type. Goto et al. [8] reported that an A-to-G transition at 3243 in the tRNA^{Leu(UUR)} gene mutation was detected in 84% of the patients with MELAS and in 3.4% of the cases with CPEO, suggesting that this mutation may cause clinical symptoms other than MELAS. Furthermore, Inui et al. [10] detected the same mutation of tRNA in some patients with atypical clinical symptoms that were not indicative of any known diseases. But the relationships between each of these mutations and the various organ disorders have yet to be determined.

The single quantitative alteration in the genotype, an A-to-G transition at position 3243 in the tRNA^{Leu(UUR)} gene, is pleiotropic, causing a number of small but distinct effects, each of which contributes in a quantitative way to the overall reduction in mitochondrial function, including respiratory chain [12]. But, the exact molecular mechanism by which the clinical distinction of MELAS occurs has not been established. In this patient, except for the existence of the point mutation described above and the normal findings of the karyotype, neither the qualitative and quantitative changes of proteins in mitochondria, including respiratory chain, nor mitochondrial and genomic DNA could be investigated.

The present case showed prominent clinical manifestations of chronic renal failure due to FSGS and chronic heart failure due to hypertrophic cardiomyopathy (HCM). Clinically, the only other symptoms were mild hearing loss and short stature without any other neurological or muscular insufficiencies.

With regard to diagnosis, these symptoms and test findings were not indicative of any of the major clinical categories of mitochondrial encephalomyopathy. However, the ragged-red fibers of the skeletal muscles and the A-to-G transition of tRNA^{Leu(UUR)} at 3243 suggested a diagnosis of atypical mitochondrial encephalomyopathy presenting with cardiac failure with HCM and severe renal failure as the main symptoms.

In a report by Anan et al. [13], 2 out of 3 patients with MELAS exhibited symmetric left ventricular hypertrophy. Obayashi et al. [14] detected an A-to-G transition at position 3243 in the tRNA^{Leu(UUR)} gene in 2 out of 6 patients with nonautosomal dominant HCM, suggesting a close relationship between this point mutation and nonautosomal dominant HCM. Thus this point mutation has been known to have close causal relations to HCM as well as to MELAS.

In the present case as well, there was an A-to-G transition at position 3243 in tRNA^{Leu(UUR)} in association with cardiomyopathy showing diffuse hypertrophic left ven-

tricular papillar muscle and wall on echocardiography and MRI of the heart. Furthermore, MRI findings of the heart demonstrated specifically a high intensity on both T1- and T2-weighted images. Though many reports have shown HCM accompanied by mitochondrial disease, the present characteristics of the left ventricular wall, as shown on the MRI, have not been reported. This unique intensity on MRI might result from the contents of the cardiac muscle, and specifically from the striking accumulation of lipids in the cardiac muscle. Because mitochondria play an active role in certain portions of lipid metabolism, mitochondrial dysfunction could result in lipid accumulation, as reported [15]. Therefore, these MRI findings might support the diagnosis of secondary HCM due to mitochondrial disease.

Renal involvements in mitochondrial encephalomyopathies have been reported in 21 patients [10, 16, 17]; the renal disease consisted of some form of tubular dysfunction, including Fanconi syndrome and renal tubular acidosis, in 8 cases [18, 19] and nephrotic syndrome in 4; renal dysfunction with proteinuria of unknown etiology and FSGS were observed in 6 and 3 patients, respectively. The age at onset ranged from 4 weeks to 37 years, with only 2 cases older than 20 years, as the present case [16]. Only 3 cases of mitochondrial cytopathy complicated by FSGS have ever been reported to the best of our knowledge. All 3 cases were clinically diagnosed as having MELAS, and in 2 of them an A-to-G transition at 3243 in tRNA^{Leu(UUR)} was confirmed [10, 15, 17].

Sakuta and Nonaka [20] showed that mitochondrial cytopathies, including MELAS, show striking abnormalities in blood vessels due to marked accumulation of giant mitochondria, and that these abnormalities might decrease the blood supply. Ban et al. [15] reported on a case of MELAS with FSGS and showed that the smooth muscle cells of the arterial capillaries in the kidneys were filled with increased numbers of enlarged mitochondria and cristae. Thus, these vascular changes in the kidneys might lead to renal hemodynamic insufficiency and might result in FSGS [21]. The relation between FSGS and the mitochondrial disorders might be explained by the mitochondrial abnormalities in the vascular smooth muscle cells in the kidney. Although no mitochondrial abnormalities were found in the kidneys in the present case, our examination was limited by an insufficiency of glomeruli and capillaries in the biopsy specimens, and this matter should be investigated further in the future.

Some primary HCMs without any other complications have been revealed to have specific mitochondrial DNA abnormality [14]; however, there have been no reports

concerning the relationship between mitochondrial abnormality and primary renal diseases. We do not conclude that FSGS in this patient was caused by mitochondrial abnormality. But considering the small size of the renal biopsy specimen in this case, the extremely rare complication of FSGS with mitochondrial cytopathy seems to indicate a close relation between mitochondrial abnormality and FSGS and furthermore between the A-to-G transition at 3243 in tRNA^{Leu(UUR)} and FSGS. For this reason, patients with renal disease involving FSGS should be carefully examined for possible mitochondrial

diseases, even when none of the neurological manifestations characteristic of mitochondrial cytopathy are present.

In summary, we have reported a case of atypical progressing mitochondrial cytopathy with HCM and FSGS, in association with no neurological abnormalities other than mild hearing loss. Though the association between FSGS and MELAS or the A-to-G transition at 3243 in tRNA^{Leu(UUR)} still remains obscure, this case suggests the close causal relation among them. To clarify this matter, further investigations should be done in the future.

References

- Pavakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP: Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes: A distinctive clinical syndrome. *Ann Neurol* 1984;16:481-488.
- DiMauro S, Bonilla E, Zeviani M, Nakagawa M, De Vivo DC: Mitochondrial myopathy. *Ann Neurol* 1985;17:521-538.
- Berrenberg RA, Pellock JM, DiMauro S, Schotland DL, Bonilla E, Eastwood A, Hays A, Vicale CT, Behrens M, Chutorian A, Rowland LP: Lumping or splitting? Ophthalmoplegia-plus or Kearns-Sayre syndrome? *Ann Neurol* 1977;1:37-54.
- Holt IJ, Harding AE, Morgan-Hughes JA: Deletions of mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 1988;331:717-719.
- Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, Rowland LP: Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988;38:1339-1346.
- Shoffner JM, Lott MT, Lezza AMS, Seibel P, Ballinger SW, Wallace DC: Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA^{Lys} mutation. *Cell* 1990;61:931-937.
- Yoneda M, Tanno Y, Horai S, Ozawa T, Miyatake T, Tuji S: A common mitochondrial DNA mutation in the rRNALys of patients with myoclonus epilepsy associated with ragged-red fibers. *Biochem Int* 1990;21:789-796.
- Goto Y, Nonaka I, Horai S: A mutation in the rRNA^{Leu(UUR)} gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1990;348:651-653.
- Kobayashi Y, Momoi MY, Tominaga K, Momoi T, Nihei K, Yanagisawa M, Kagawa Y, Ohta S: A point mutation in the mitochondrial tRNA^{Leu(UUR)} gene in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes). *Biochem Biophys Res Commun* 1990;173:816-822.
- Inui K, Fukushima H, Tsukamoto H, Taniike M, Midorikawa M, Tanaka J, Nishigaki T, Okada S: Mitochondrial encephalomyopathies with the mutation of the mitochondrial tRNA^{Leu(UUR)} gene. *J Pediatr* 1992;120:62-66.
- DiMauro S, Moraes CT: Mitochondrial encephalomyopathies. *Arch Neurol* 1993;50:1197-1208.
- King MP, Koga Y, Davinson M, Schon EA: Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the tRNA^{Leu(UUR)} mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. *Mol Cell Biol* 1992;12:480-490.
- Anan R, Nakagawa M, Miyata M, Higuchi I, Nakao S, Suehara M, Osame M, Tanaka H: Cardiac involvement in mitochondrial diseases: A study on 17 patients with documented mitochondrial DNA defects. *Circulation* 1995;91:955-961.
- Obayashi T, Hattori K, Sugiyama S, Tanaka M, Tanaka T, Itoyama S, Deguchi H, Kawamura K, Koga Y, Toshima H, Takeda N, Nagano M, Ito T, Ozawa T: Point mutations in mitochondrial DNA in patients with hypertrophic cardiomyopathy. *Am Heart J* 1992;124:1263-1269.
- Ban S, Mori N, Saito K, Mizukami K, Suzuki T, Shiraiishi H: An autopsy case of mitochondrial encephalomyopathy (MELAS) with special reference to extraneuromuscular abnormalities. *Acta Pathol Jpn* 1992;42:818-825.
- Suzuki T, Fujino T, Sugiyama M, Ishida M: A case of mitochondrial encephalomyopathy (MELAS). *Jpn J Nephrol* 1996;38:109-114.
- Mochizuki H, Joh K, Kawame H, Imadachi A, Nozaki H, Ohashi T, Usui N, Eto Y, Kanetsuna Y, Aizawa S: Mitochondrial encephalomyopathies preceded by de Toni-Fanconi syndrome or focal segmental glomerulosclerosis. *Clin Nephrol* 1996;46:347-352.
- Van Biervliet JPAM, Bruinvis L, Ketting D, De Bree PK, Heiden CVD, Wadman SK: Hereditary mitochondrial myopathy with lactic acidemia, de Toni-Fanconi-Debra syndrome, and a defective respiratory chain in voluntary striated muscles. *Pediatr Res* 1977;11:1088-1093.
- Minchom PE, Dormer RL, Hughes IA, Stansbie D, Cross AR, Hendry GAF, Johnes OTG, Johnson MA, Shorratt HSA, Turnbull DM: Fatal infantile mitochondrial myopathy due to cytochrome *c* oxidase deficiency. *J Neurol Sci* 1983;60:453-463.
- Sakuta R, Nonaka I: Vascular involvement in mitochondrial myopathy. *Ann Neurol* 1989;25:594-601.
- Rennke HG, Klein PS: Pathogenesis and significance of nonprimary focal and segmental glomerulosclerosis. *Am J Kidney Dis* 1989;13:443-456.