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# Hepatic Failure and Enhanced Oxidative Stress in Mitochondrial Diabetes

YUTAKA TAKAHASHI, KEIJI IIDA, RYOKO TAKENO, RIKO KITAZAWA*, SOHEI KITAZAWA*, HIDETSUNA KITAMURA**, YOSHIO FUJIOKA**, HIROYUKI YAMADA***, FUMIO KANDA, SHIGEO OHTA[#], KIYOMI NISHIMAKI[#], MASAYO FUJIMOTO*, TAKESHI KONDO*, GENZO IGUCHI, KENTARO TAKAHASHI, HIDESUKE KAJI[##], YASUHIKO OKIMURA[###] AND KAZUO CHIHARA

*Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chu-o-ku, Kobe 650-0017 Japan*

**Division of Pathology, Department of Pathology&Microbiology, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chu-o-ku, Kobe 650-0017 Japan*

***Division of Cardiovascular and Respiratory Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chu-o-ku, Kobe 650-0017 Japan*

****Division of Diabetes, Digestive, and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chu-o-ku, Kobe 650-0017 Japan*

*[#]Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School, Kosugi-cho, Nakahara-ku, Kawasaki, 211-8533 Japan*

*[##]College of Nursing Art and Culture 13-71, Kita-Oji-cho, Akashi, 673-8588 Japan*

*[###]Department of Basic Allied Medicine, Kobe University School of Medicine, 7-10-2, Tomogaoka, Suma-ku, Kobe 654-0142 Japan*

**Abstract.** Mitochondrial diabetes is characterized by diabetes and hearing loss in maternal transmission with a heteroplasmic A3243G mutation in the mitochondrial gene. In patients with the mutation, it has been reported that hepatic involvement is rarely observed. We demonstrated a case of hypertrophic cardiomyopathy and hepatic failure with mitochondrial diabetes. To clarify the pathogenesis we analyzed the mitochondrial ultrastructure in the myocytes, the reactive oxygen species (ROS) production in the liver and the status of heteroplasmy of the mitochondrial A3243G mutation in the organs involved. In cardiomyocytes and skeletal muscle, electron microscopic analysis demonstrated typical morphological mitochondrial abnormalities. Immunohistochemical analysis demonstrated enhanced ROS production associated with marked steatosis in the liver, which is often associated with mitochondrial dysfunction. Analysis of the A3243G mutation revealed a substantial ratio of heteroplasmy in these organs including the liver. The presence of steatosis and enhanced oxidative stress in the liver suggested that hepatic failure was associated with mitochondrial dysfunction.

*Key words*: Mitochondria, Mutation, Diabetes, Hepatic steatosis, Oxidative stress

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**MUTATIONS** in mitochondrial DNA (mtDNA) are associated with various diseases. In 1992, several families with diabetes and sensorineural hearing loss were described and were found to have inherited the heteroplasmic A3243G mutation in the gene of tRNAleu (UUR), the very same mutation of which is associated with systemic MELAS syndrome [1]. In clinical practice, mitochondrial diabetes can be type 1 or 2 in nature and generally presented via maternal transmission in conjunction with bilateral hearing impairment [2]. In the large majority of cases, mitochondrial diabetes is associated with the A3243G mutation in mtDNA, although a range of other mutations in mtDNA have also

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Correspondence to: Yutaka TAKAHASHI, Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe 650-0017 Japan

been implicated. The A3243G mutation is present in all tissues, but their particular heteroplasmy levels vary. In leukocytes, heteroplasmy levels are usually quite low, which hampers the detection of this mutation. Factors other than the heteroplasmy rate found within a cell or a mitochondrion, which contribute to the pathogenesis, are threshold effect, nuclear background, age, sex, and environment, resulting in a wide range of phenotypes [3].

Impaired hearing, reflected by a reduced perception of high tone frequencies, was highly characteristic for carriers of A3243G mutation. Hearing impairment generally preceded the onset of clinically manifest diabetes by several years [2]. It has been speculated that the cochlea is predominantly impaired in mitichondrial deafness [4]; however, it is not clear whether A3243G mutation in the auditory nerve itself is involved in the pathogenesis.

Although hepatic involvement is relatively rare in mitochondriopathy [5], Pearson syndrome has been characterized by refractory sideroblastic anaemia, exocrine pancreatic dysfunction and hepatomegaly with hepatic failure [6]. In these conditions, hepatic steatosis was frequently observed. Oxidative stress has been defined as impairment of cellular function by free oxygen radicals. Normally, the respiratory chain (RC) in mitochondria generates few free radicals. However, in the case of RC dysfunction, the level of free oxygen radicals markedly increases [6]. Mitochondrial dysfunction leads to RC dysfunction and produces free radicals that impair mitochondrial genome and protein, causing vicious cycles.

The present case demonstrated mitochondrial diabetes and cardiomyopathy with hepatic failure. We have analyzed the status of oxidative stress and heteroplasmy of the A3243G mutation to clarify the pathogenesis of hepatic failure.

## Patient and methods

*Subject*

In January 2005, a 59-year-old man was admitted to the hospital because of severe fatigue. He had diabetes for 11 years; during most of those years he had taken oral hypoglycemic agents and since 3 years before, he had insulin. He also had a hearing disturbance and hypertrophic cardiomyopathy for 11 years. At the first admission, muscle biopsy study identified the mitochodrial A3243G mutation and he was diagnosed as mitochondrial diabetes and cardiomyopathy. He had a past history of acute hepatitis when he was 18 years old. He had taken one bottle of beer daily. His laboratory finding has shown normal liver function until the admission. Ultrasonography and computed topography examination at the age of 53 years old demonstrated no abnormal finding in the liver. There was no family history of diabetes, liver or cardiac disease.

At admission, his laboratory findings revealed severe liver damage (alanine aminotransferase 3500 U/l, aspartate aminotransferase 6479 U/l, total bilirubin 2.5 mg/dl, and prothrombin time 16.6%). He had no hepatitis virus including A, B, C and E. Cardiac ultrasonographic examination at that time showed diffuse severe hypokinesis with an ejection fraction of 7%. He had received a treatment for cardiac failure, an infusion of 1500 mg of gabexate mesylate and 6–12 U units of fresh frozen plasma. On the third day, however, he died of hepatic failure.

*Quantitative detection of mtDNA with a A3243G point mutation*

Total DNA was prepared from peripheral blood cells and various tissues using the QIAamp DNA Blood Mini Kit and QIAamp DNA Mini Kit, respectively (Qiagen, Valencia, CA, USA) and stored at –20°C. For the quantitative detection of the mutation at nucleotide position 3243, PCR-RFLP was applied as previously described [7, 8]. Briefly, polymerase chain reaction (PCR) was performed using following primers; 5'AGGACAAGAGAAATAAGGCC3', covering positions 3130 to 3149, and 5'CACGTTGGGGCCTTT GCGTA3', covering positions 3423 through 3404. The 294-bp fragments of mitochondrial DNA were digested with ApaI, and analyzed by electrophoresis.

*Autopsy*

With written informed consent, from his family members, autopsy was performed 3 h after death. Autopsy specimens were fixed in 10% formalin and embedded in paraffin. Tissue sections 4 um in thickness were stained for hematoxylin and eosin as well as by the Azan-Mallory method.

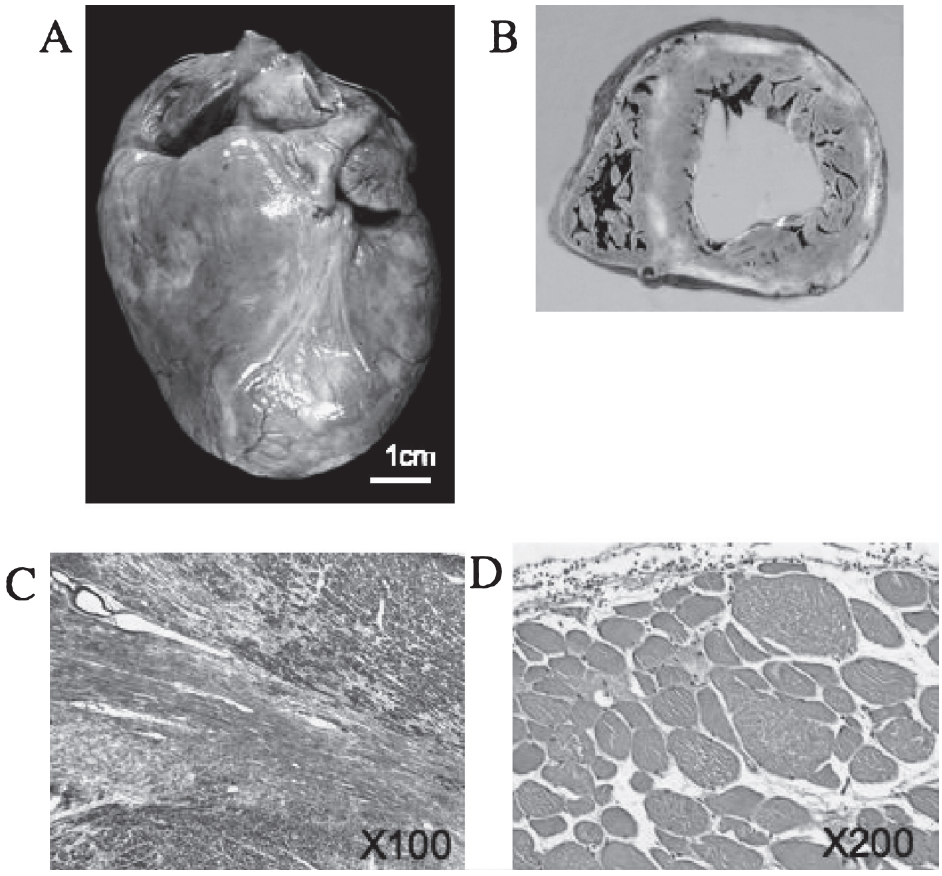


**Fig. 1.** Macroscopic features of the heart and histological analysis of cardiomyocyte and skeletal muscle. Cardiac enlargement (Fig. 1A), bilateral dilatation of both ventricles and hypertrophy in the left ventricle walls (Fig. 1B) were observed. Azan staining in the heart revealed marked fibrosis with degradation of the cardiomyocytes (Fig. 1C). In the skeletal muscle, muscle fibers were irregular in size (Fig. 1D).

*Immunohistochemistry and electronmicroscopy*

Sections were prepared as described above and incubated with monoclonal anti8-hydroxy-2'-deoxyguanosine/8-hydroxyguanosine (8OHdG/8OHG; 1 : 100; JaICA, Japan) and visualized by using standard immunohistochemical methods [9]. Electron microscopy analysis was performed as previously described [10].

## Results

Autopsy revealed marked cardiac hypertrophy (Fig. 1A, B), pancreatic degeneration (data not shown), hepatic congestion (Fig. 3A), steatosis (Fig. 3B) and necrosis (Fig. 3C). In the heart, cardiac enlargement (Fig. 1A), bilateral dilatation of both ventricles and hypertrophy in left ventricle wall (Fig. 1B) were ob-

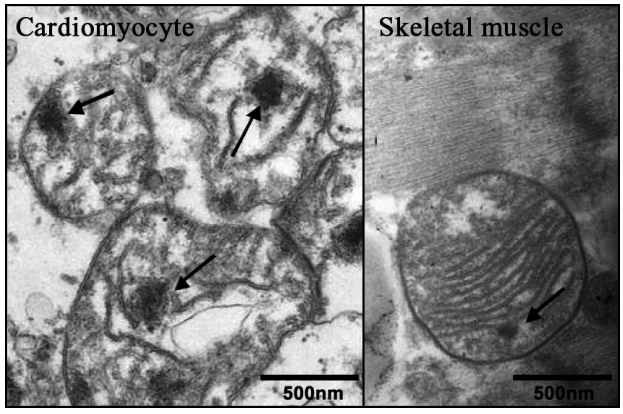


**Fig. 2.** Electron microscopic analysis of mitochondria in cardiomyocytes and skeletal muscle. Right; in the skeletal muscle, abnormal electron deposits (arrows) were seen in the mitochondria. Left; in the cardiomyocytes, disorganized cristae and similar electron deposits (arrows) were observed.

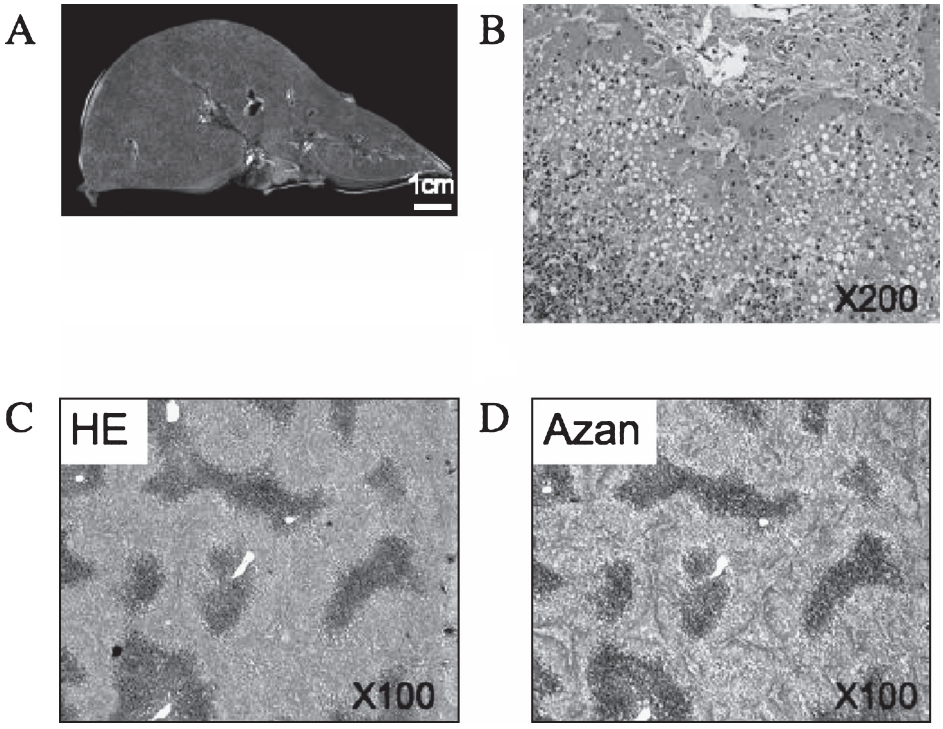


**Fig. 3.** Macroscopic features and histological analysis of the liver. A, Macroscopic features demonstrated congested liver. B, Severe steatosis was present predominantly in zone 3. C, H-E staining demonstrated massive necrosis surrounding the central vein. D, Azan staining revealed enhanced fibrosis in the liver.

served. Histological analysis in the heart revealed marked fibrosis with degradation of cardiomyocytes (Fig. 1C). In the skeletal muscle, muscle fibers were irregular in size (Fig. 1D).

Electron microscopic analysis in the cardiomyocytes and the skeletal muscle demonstrated morphological abnormalities in the mitochondria (Fig. 2). In the cardiomyocytes, the shape and the size were irregular and the structure in the cristae was disorganized (Fig. 2, left). Electron deposits, a characteristics feature of mitochondriopathy, were seen in the mitochondria. Also, in the skeletal muscle, disorganized cristae and similar electron deposits were observed (Fig. 2, right).

In the liver, macroscopic observation showed marked enlargement and congestion (Fig. 3A). In the histological analysis, hepatocytes showed severe steatosis (Fig. 3B). Massive necrosis was seen surrounding the central vein (Fig. 3C). Azan staining demonstrated fibrosis in Zone 3 (Fig. 3D). The status of oxidative stress in the liver was analyzed using immunohistochemistory with anti-8OHdG that was oxidative stress markers. Intriguingly, 8OHdG staining was strongly positive compared to control, demonstrating marked enhancement of oxidative stress in the liver (Fig. 4).

We further analyzed the status of heteroplasmy of A3243G mutation in various tissues using quantitative PCR methods [7]. Interestingly, a high ratio of heteroplasmy was identified in peripheral blood, heart, brain, muscle and auditory nerve (Fig. 5).

## Discussion

In the present case, typical manifestations of diabetes and hearing disturbance were observed. The subject also showed hepatic steatosis that is an unusual manifestation in A3243G mitochondrial mutation. In the cardiomyocyte and the skeletal muscle, electron microscopic analysis demonstrated morphological mitochondrial abnormality, suggesting that these organs were also involved in severe mitochondrial impairment. Enhanced ROS production in the liver suggested mitochondrial dysfunction. Finally, the analysis of heteroplasmy of the A3243G mutation revealed the presence of substantial heteroplasmy of the mutation

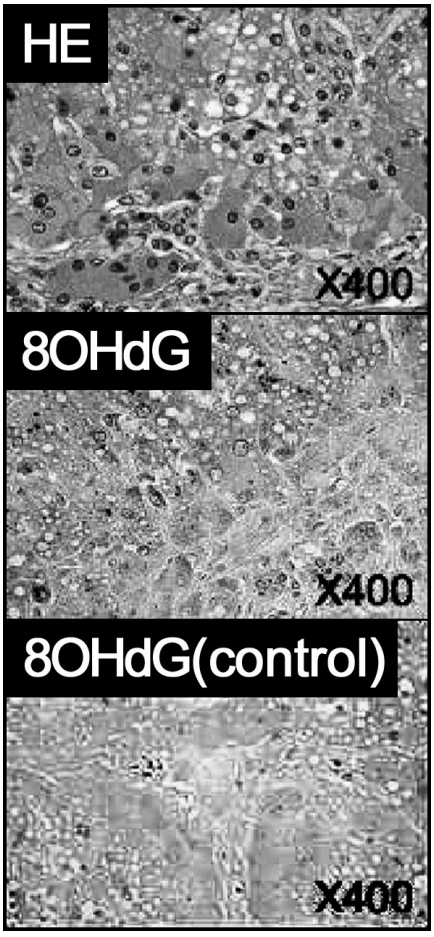


**Fig. 4.** Enhanced oxidative stress was observed in the liver. 8OHdG staining demonstrated oxidative stress in the nucleus. Normal liver tissue was used as a control.

in the liver.

Hepatic involvement in primary mitochondrial disorders rarely presents in adult life, but is a common feature in childhood respiratory chain disease, particularly in the neonatal period. Multiple complex defects in children with liver involvement, such as steatosis can be due to mitochondrial A8334G mutation or single deletion [11, 12]. In mitochondrial liver disease, histology usually shows steatosis, often accompanied by fibrosis, cholestasis and loss of hepatocytes [11]. In this case, the histological analysis showed steatosis and fibrosis that demonstrated striking similarity to that in mitochondrial liver disease. Although liver congestion caused by cardiomyopathy was present, it was speculated that the mitochondria mutation in the liver caused oxidative stress and cellular damage, and induced the hepatic failure in addition to the damage from liver congestion.

In terms of hearing loss, it has been generally speculated that the cochlea is predominantly impaired in mitochondrial deafness [4]. In this case, however, in light of the fact that neuronal tissue has a high susceptibility to mitochondria mutation, it was speculated that the high ratio of heteroplasmy in the auditory nerve may have led to mitochondrial dysfunction and the hearing disturbance.

In summary, we present a case of mitochondrial diabetes with hypertrophic cardiomyopathy and hepatic failure. It was speculated that the liver damage was related to the enhanced ROS production caused by mitochondrial mutation.

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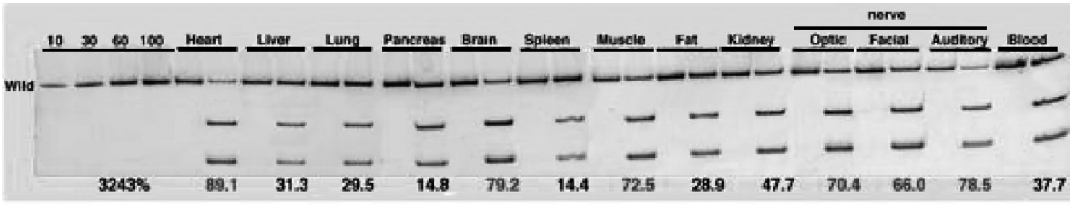


**Fig. 5.** Heteroplasmy status of mitochondrial A3243G mutation in various tissues. 3243% indicates the ratio of mutation, as quantified by densitometric analysis.

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