Cardiac Abnormalities in Diabetic Patients With Mutation in the Mitochondrial tRNA^{Leu(UUR)} Gene

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An A-to-G transition at position 3243 of the mitochondrial DNA is known to be a pathogenic factor for mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), diabetes and cardiomyopathy. This mutation causes dysfunction of the central nervous system in MELAS. Because the heart, as well as the brain and nervous system, is highly dependent on the energy produced by mitochondrial oxidation, these tissues are more vulnerable to mitochondrial defects. Cardiac abnormalities were assessed in 10 diabetic patients associated with this mutation using echocardiography and ¹²³I-metaiodobenzylguanidine (MIBG) scintigraphy, and compared with 19 diabetic patients without the mutation. Duration of diabetes, therapy, control of blood glucose and diabetic complications, such as diabetic retinopathy and nephropathy, were not different between the 2 groups. Diabetic patients with the mutation had a significantly thicker interventricular septum (16.8 ± 3.7 vs 11.0 ± 1.6 mm, $p < 0.001$) than those without the mutation. Fractional shortening was lower in diabetic patients with the mutation than those without it (30.7 ± 7.0 vs 42.5 ± 6.6 , $p < 0.001$). MIBG uptake on the delayed MIBG image was significantly lower in diabetic patients with the mutation than in those without the mutation (mean value of the heart to mediastinum ratio: 1.6 ± 0.2 vs 2.0 ± 0.4 , $p > 0.05$). In conclusion, left ventricular hypertrophy with or without abnormal wall motion and severely reduced MIBG uptake may be characteristic in diabetic patients with a mutation in the mitochondrial tRNA^{Leu(UUR)} gene. (*Jpn Circ J* 1999; 63: 877–880)

Key Words: Diabetes mellitus; ¹²³I-metaiodobenzylguanidine (MIBG) scintigraphy; Mitochondrial tRNA^{Leu(UUR)} gene

An A-to-G transition at position 3243 in the mitochondrial tRNA^{Leu(UUR)} gene was originally identified in patients with MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes)¹ This mutation was subsequently found in patients with diabetes mellitus (DM)^{2–4} Recently, several reports have suggested that the mutation in mitochondrial DNA, especially the substitution of guanine for adenine (A→G) at position 3243 of leucine tRNA may cause cardiac disorders.^{5,6} Although cardiac disorder is an important complication in diabetic patients, the effect of mitochondrial mutations on the heart in diabetic patients has not been systematically studied. Therefore, to clarify the characteristic features of cardiac abnormalities in diabetic patients with mitochondrial mutations, we clinically evaluated the cardiac structure and function in 10 diabetic patients with the mitochondrial tRNA^{Leu(UUR)} mutation and compared the results with those of 19 diabetic patients without the mutation.

Methods

Patients

We examined 10 diabetic patients with the mitochondrial tRNA^{Leu(UUR)} gene mutation. Mitochondrial DNA analysis was done as followed. In brief, using mitochondrial DNA extracts from the patients' platelets, we ampli-

fied the region encoding tRNAs (294-bp fragments of mitochondrial DNA: 3130–3423) by polymerase chain reaction and sequenced it directly, or subcloned into a plasmid vector and then sequenced.

We also studied 19 diabetic patients without the mutation. They were consecutively selected from DM patients who showed neither wall motion abnormalities nor valvular heart disease on echocardiograms. All study patients were negative on exercise ²⁰¹Tl myocardial scintigraphy.

Cardiac Examination

All patients underwent echocardiography and ¹²³I-metaiodobenzylguanidine (MIBG) scintigraphy. M-mode echocardiograms were recorded with a 3.75 MHz transducer with a Toshiba SSH 160 apparatus (Toshiba Medical Co, Tokyo, Japan). The left ventricular (LV) end-diastolic and end-systolic dimensions (LVDd and LVDs, respectively) were measured, and the LV fractional shortening (%FS) was calculated as (LVDd–LVDs)/LVDd.

¹²³I-MIBG Scintigraphy ¹²³I-MIBG at a dose of 111 MBq was intravenously injected at rest after a 3-h fast. The initial planar images were obtained 15 min after injection in the anterior and 45° left anterior oblique view over a 3-min interval, using a gamma camera system with a low-energy collimator (PRISM-2000XP, Picker). Single photon emission computed tomography (SPECT) was performed with data collections of 40 s/step, starting in the 45° right anterior oblique projection to the 45° left posterior oblique view. Both late planar and SPECT images were obtained 3 h after initial imaging. Visual analysis was performed on the early and delayed MIBG studies using the anterior planar images. For the semi-quantitative analysis, regions of interest (ROIs) over the whole heart and an area 10×10 mm in size over the upper mediastinum were drawn on the

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Table 1 Clinical Features

	With mutation (n=10)	Without mutation (n=19)	p value
Age (years)	48.6±13.6	69.0±6.8	<0.001
Gender (male/female)	6/4	6/13	
Height (cm)	153.8±9.2	154.0±10.1	NS
Body mass index (kg/m ²)	18.7±1.8	23.3±4.4	<0.05
Duration of (years) diabetes mellitus	11.8±9.3	15.2±6.0	NS
HbA1c (%)	7.9±2.1	8.1±1.6	NS
Treatment			
Insulin	5 (50%)	8 (42%)	NS
Oral hypoglycemic drugs	4 (40%)	8 (42%)	NS
Hypertension (≥ 140/90 mmHg)	1 (10%)	6 (31%)	NS
Retinopathy	3 (30%)	12 (63%)	NS
Neuropathy	5 (50%)	6 (31%)	NS
Hearing loss	8 (80%)	0 (0%)	<0.01

Table 2 Results of Echocardiography

	With mutation (n=10)	Without mutation (n=19)	p value
IVST (mm)	16.8±3.7	11.0±1.6	<0.001
LVPW (mm)	14.7±2.8	10.3±1.3	<0.001
LVDd (mm)	45.6±5.8	44.1±3.3	NS
LVDs (mm)	32.0±6.5	25.0±3.4	<0.005
%FS	30.7±7.0	42.5±6.6	<0.001

IVST, interventricular septum thickness; LVPW, left ventricular posterior wall thickness; LVDd, diastolic dimension of left ventricle; LVDs, systolic dimension of left ventricle; FS, fractional shortening.

Table 3 Results of ¹²³I-MIBG Scintigraphy

	With mutation (n=10)	Without mutation (n=19)	p value
H/M ratio			
MIBG-early	1.7±0.2	2.0±0.4	NS
MIBG-delayed	1.6±0.2	2.0±0.2	<0.05
%WR	23.7±13.3	30.1±7.2	NS

H/M ratio, heart-to-mediastinum ratio; WR, washout ratio.

early and delayed planar images to calculate the mean heart-to-mediastinum (H/M) ratio. After correction for the physical decay of ¹²³I, the washout rate of the tracer from the myocardium (%WR) was determined for the 3-h period.

Statistical Analysis

All values are presented as a mean values ± SD. Student t test was used for comparison of paired data and p<0.05 was considered significant.

Results

Clinical Results

Patients profiles are shown in Table 1. The 10 diabetic patients with the mutation were short in stature and leaner compared with those without the mutation (body mass index: 18.7±1.8 vs 23.3±4.4, p<0.001).

Duration of diabetes, therapy, control of blood glucose and diabetic complications, such as diabetic retinopathy and nephropathy, were not different between the 2 groups.

Hypertension was present in only 1 patient with the mutation and in 6 patients without the mutation. The sensory hearing loss often seen in patients with MELAS was found in 8 patients with the mutation (80%) compared with none of the 19 patients without the mutation (0%) (p<0.01).

Echocardiographic Findings

Diabetic patients with the mutation had a significantly

thicker interventricular septum (16.8±3.7 vs 11.0±1.6 mm, p<0.001) than those without the mutation.

Fractional shortening was lower in diabetic patients with the mutation than those without the mutation (30.7±7.0 vs 42.5±6.6, p<0.001). Impaired LV systolic function were seen in 3 patients with the mutation and 2 of them had the history of congestive heart failure.

Cardiac MIBG Uptake

The mean value of the H/M ratio on the delayed MIBG image was significantly lower in the diabetic patients with the mutation than in those without the mutation (1.6±0.2 vs 2.0±0.4, p>0.05). Although the diabetic patients with the mutation tended to have a lower H/M ratio on the early image and a lower %WR, no statistical significance was achieved.

Discussion

The present study using echocardiography, ²⁰¹Tl scintigraphy and ¹²³I-MIBG demonstrated left ventricular hypertrophy with or without abnormal wall motion and severely reduced MIBG uptake in diabetic patients with the 3243 mutation.

Left Ventricular Hypertrophy

Hypertrophic cardiomyopathy and left ventricular hypertrophy occurring in conjunction with mutation of tRNA^{Leu}

genes in patients with MELAS syndrome have been described previously in many reports.⁷⁻¹² Therefore, it is thought that left ventricular hypertrophy, with or without abnormal wall motion, is probably a characteristic clinical feature in MELAS. An A-to-G mutation at nucleotide 3243 in the tRNA^{Leu} gene was seen in all of the 10 patients in the present study who had the mitochondrial tRNA^{Leu(UUR)} gene mutation, which confirms the previous studies and extends these observations to diabetic patients with the 3243 mutation. The mechanism by which this mutation influences hypertrophy and dysfunction of the cardiac muscle remains obscure. However, light and electron microscopy of endomyocardial specimens in patients with MELAS and diabetic patients with the 3243 mutation has indicated abnormal mitochondria, with a marked increase in their number and size.^{6,13} Several factors have been reported to contribute to the left ventricular hypertrophy, including the role of hypertension with a concomitant increase in left ventricular mass,¹⁴ and the role of obesity and growth hormone.¹⁵ Although these factors can not completely excluded in the present study, they are unlikely because most of the patients were lean, short in stature and not hypertensive. Therefore, the left ventricular hypertrophy observed in diabetic patients with the 3243 mutation is more likely due to cellular hypertrophy caused mainly by the increased mitochondrial mass.

Reduced MIBG Uptake

MIBG participates in the same uptake and storage mechanisms as norepinephrine. Two types of uptake system for norepinephrine and MIBG have been identified in adrenergic tissues; the uptake one system (neuronal uptake) dominates at low concentration of the substrate and is sodium and ATP-dependent, whereas the uptake 2 system (extra-neuronal uptake) is a diffusion system that dominates at high concentration of MIBG.¹⁴⁻¹⁶ MIBG administered at the low doses used for clinical applications probably enters mainly through the uptake one system.¹⁶ There have been many reports of the abnormally low concentration of MIBG in the hearts of diabetics. Miyanaga et al showed a decreased concentration of MIBG (H/M ratio: 1.97 ± 0.27 in the early image) in diabetic patients with neuropathy and the MIBG abnormalities were especially obvious in the inferior wall.⁷ Nagamachi et al also demonstrated MIBG abnormality (H/M ratio: 1.9 ± 0.3 in the early image) in diabetic patients with autonomic neuropathy and the reduction in MIBG uptake was also seen in the inferior wall.¹⁸ Therefore, it is thought that a decreased concentration of MIBG, especially in the inferior segment, is a characteristic in diabetic patients. In the present study, diabetic patients with and without the mutation showed lower MIBG uptake in accordance with these observations. However, the H/M ratio in the diabetic patients with the 3243 mutation (H/M ratio: 1.71 ± 0.16) was lower than that in the DM patients without the mutation; that is, a more marked reduction in MIBG uptake was found in the diabetic patients with the mutation.

The exact mechanism or mechanisms by which MIBG uptake is reduced is incompletely understood, and the present study was not designed to address this issue. However, one possibility includes disturbed ATP-production caused by the mutation in mitochondrial DNA. As we mentioned before, the neuronal uptake function (uptake 1 system) is ATP-dependent. Thus, the decreased MIBG uptake found in the patients with the mitochondrial muta-

tion in the present study is likely to be related to an impairment of the neuronal uptake function (uptake 1 system) caused by dysfunction of ATP-production. However, we cannot rule out the possibility that decreased MIBG uptake could be the result of the neuropathy, because reduced MIBG uptake may be a general finding and DM intensifies this effect upon progression to diabetic autonomic nerve neuropathy. Although this possibility cannot be completely excluded, it is unlikely because diabetic complications, such as retinopathy and neuropathy, were relatively mild in the present patients. However, further investigations are required.

In conclusion, left ventricular hypertrophy with or without abnormal wall motion and severely reduced MIBG uptake may be characteristic of diabetic patients with mutation in the mitochondrial tRNA^{Leu(UUR)} gene.

References

- 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
- Van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, et al: Mutation in mitochondrial tRNA^{Leu(UUR)} gene in a large pedigree with maternally transmitted type I diabetes mellitus and deafness. *Nat Genet* 1992; **1**: 368
- Readon W, Ross RJM, Sweeney MG, et al: Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. *Lancet* 1992; **340**: 1376-1379
- Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakura R, Suzuki Y, et al: A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N Engl J Med* 1994; **330**: 362-368
- Zeviani M, Gellera C, Antozzi C, Rimoldi M, Morandi L, Villani F, et al: Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA tRNA^{Leu(UUR)}. *Lancet* 1991; **338**: 143-147
- Sato W, Tanaka M, Sugiyama S, Nemoto T, Harada K, Miura Y, et al: Cardiomyopathy and angiopathy in patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. *Am Heart J* 1994; **128**: 733-741
- Ito T, Hattori K, Tanaka M, Sugiyama S, Ozawa T: Mitochondrial cytopathy. *Jpn Circ J* 1990; **54**: 1214-1220
- Morgan-Hughes JA, Hayes DJ, Clark JB, Landon DN, Swash M, Stark RJ, et al: Mitochondrial encephalomyopathies: biochemical studies in two cases revealing defects in the respiratory chain. *Brain* 1982; **105**: 553-582
- Yoneda M, Tanaka M, Nishikimi M, Suzuki H, Tanaka K, Nishizawa M, et al: Pleiotropic molecular defects in energy-transducing complex in mitochondrial encephalomyopathy (MELAS). *J Neurol Sci* 1989; **92**: 143-158
- Kobayashi M, Morishita H, Sugiyama N, Yokochi K, Nakano M, Wada Y, et al: Two cases of NADH-coenzyme Q reductase deficiency: relationship to MELAS syndrome. *J Pediatr* 1987; **110**: 223-227
- Nemoto T, Satoh W, Harada K, Komatsu K, Gotoh A, Matsuno K, et al: Cardiac involvement in four cases of MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes). *Jpn J Pediatr* 1989; **93**: 1416-1421 [in Japanese with English abstract]
- Seyama K, Suzuki K, Mizuno Y, Yoshida M, Tanaka M, Ozawa T: Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes with special reference to the mechanism of cerebral manifestations. *Acta Neurol Scand* 1989; **80**: 561-568
- Shiotani H, Ueno H, Inoue S, Yokota Y, Yokoyama M: Diabetes Mellitus and Cardiomyopathy: Association With Mutation in the Mitochondrial tRNA^{Leu(UUR)} gene. *Jpn Circ J* 1998; **62**: 309-310
- Vanninen E, Mustonen J, Vainio P, Lansimies E, Uusitupa M: Left ventricular function and dimensions in newly diagnosed non-insulin-dependent diabetes mellitus. *Am J Cardiol* 1992; **70**: 371-378
- Levy D, Anderson KM, Savage DD, Kannel WB, Christiansen JC, Castelli WP: Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. The Framingham Heart Study. *Ann Intern Med* 1988; **108**: 7-13
- Gasnier B, Roisin MP, Scherman D, Coornaert S, Desplanches, Henry JP: Uptake of meta-iodobenzylguanidine by bovine chromaffin granule membranes. *Mol Pharmacol* 1986; **29**: 275-280

17. Miyanaga H, Yoneyama S, Kamitani T, Kawasaki S, Takahashi T, Kunushige H: Clinical usefulness of ^{123}I -Metaiodobenzylguanidine myocardial scintigraphy in diabetic patients with cardiac sympathetic nerve dysfunction. *Jpn Circ J* 1995; **59**: 599–607
18. Nagamachi S, Jinnouchi S, Nakahara H, Flores LG II, Ohnishi T, Hoshi H, et al: ^{123}I -MIBG myocardial scintigraphy in diabetic patients: Relationship to autonomic neuropathy. *Nucl Med Commun* 1996; **17**: 621–632