High Prevalence of Mitochondrial Diabetes Mellitus in Japanese Patients With Major Risk Factors

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To identify diabetes mellitus caused by the mitochondrial gene substitution at genomic nucleotide pair 3243 (M3243A → G) we selected 87 diabetic patients with high risk factors such as maternal inheritance and hearing loss. Total DNA was extracted from peripheral leukocytes, and mitochondrial DNA fragments containing M3243A → G were amplified by polymerase chain reaction (PCR). The amplified fragments were digested with a restriction endonuclease *Apa*1 and analyzed by agarose gel electrophoresis. The incidence of the M3243A → G mutation was 4.6% (four of 87) in diabetic patients with maternal inheritance and/or hearing loss. In a subgroup with both maternal inheritance and hearing loss, the incidence of the mutation was as high as 21.4% (three of 14). Cardiac disorders were also present in all four diabetic patients with the mutation. This study suggests that maternal inheritance and hearing loss are useful clinical findings to identify diabetic patients with the mutation, and that cardiac involvement is a high risk factor for the M3243A → G mutation.

DIABETES MELLITUS consists of heterogeneous disorders, and genetic and environmental factors may play important roles in it. Recently, several genetic abnormalities related to insulin,[1] the insulin receptor,[2] and glucokinase[3] have been identified in patients with diabetes, but these are rare.[4] In 1992, Ballinger et al[5] and van den Ouweland et al[6] reported diabetes associated with a 10.4-kb mitochondrial DNA deletion and a mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene, respectively. Oxidative phosphorylation in mitochondria may have a crucial role in insulin secretion from pancreatic β cells, as mitochondrial dysfunction may lead to impaired insulin release and subsequently the development of diabetes. Previous studies of the mitochondrial DNA mutation involving the substitution of guanine for adenine at position 3243 of leucine tRNA (M3243A → G) showed that as many as 1.5% of diabetic patients, regardless of phenotype, had this lesion.[7] The clinical characteristics of diabetes with the M3243A → G mutation include maternal inheritance, hearing loss, and the need for insulin because of an impaired release of endogenous insulin.[8-11] The identification of patients with mitochondrial gene disorders is helpful in terms of classification and treatment. We investigated the incidence of the M3243A → G mutation in diabetic patients with maternal inheritance and/or hearing loss to determine the usefulness of these risk factors as markers of this mutation. We also examined the clinical profiles of diabetic patients with the M3243A → G mutation.

SUBJECTS AND METHODS

Approximately 960 diabetic outpatients in our hospital and some affiliated hospitals were interviewed regarding whether they had hearing loss and their mother had diabetes mellitus. Eighty-seven diabetic patients (28 males and 59 females) had either maternal inheritance or hearing loss and were selected to investigate the M3243A → G mitochondrial gene mutation. Informed consent was obtained from all subjects.

Total DNA was extracted from peripheral leukocytes, and fragments of mitochondrial DNA encompassing position 3243 were amplified by polymerase chain reaction (PCR) with *Taq* polymerase. The forward primer was 5′TTCACAAAGCGCCTTCCCCC3′, covering positions 3153 to 3172, and the reverse primer was 5′GCGATGGTGAGAGCTAAGGTC3′, covering positions 3551 through 3531.[12] The resultant 399–base pair fragments of mitochondrial DNA were digested with restriction endonuclease *Apa*1 to identify any A to G mutation at nucleotide 3243, and analyzed by agarose gel electrophoresis. The nucleotide sequence was confirmed by sequencing the PCR products directly.

The presence of cardiac disorder in patients with the M3243A → G mutation was investigated using electrocardiography (ECG) and ultrasound cardiography (UCG).

The insulin secretory capacity of pancreatic β cells was evaluated by measuring 24-hour urinary C-peptide excretion. Impaired insulin release was defined as 24-hour urinary C-peptide excretion less than 20 μg/d.

We compared several clinical parameters, including body mass index (BMI), age at onset, current therapy, insulin secretory capacity, and frequency of cardiac disorder, between the two groups with or without the M3243A → G mutation in diabetic patients with both maternal inheritance and hearing loss.

Statistical Analysis

Fisher's exact test was used to determine the statistical significance of differences between group frequencies, and an unpaired Student's *t* test to compare mean values. *P* less than .05 was accepted as statistically significant.

RESULTS

The incidence of the M3243A → G mutation was 4.6% (four of 87) in diabetic patients with maternal inheritance and/or hearing loss. In a subgroup with both maternal inheritance and hearing loss, the incidence of the mutation was 21.4% (three of 14) (Table 1).

Clinical profiles of diabetic patients with the M3243A → G mutation are shown in Table 2. We could identify four diabetic patients with the mutation, and none of them suffered from mitochondrial myopathy, encephalopathy, lactic acidosis, or stroke-like episodes. All four were females and had cardiac

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Table 1. Prevalence of the M3243A → G Mutation in Japanese Diabetic Patients With Major Risk Factors

Phenotype	No. of Patients	Age, yr (mean ± SD)	Sex		Incidence	
			Male	Female	No.	%
Maternal inheritance only	30	61.1 ± 14.4	7	23	0	0
Hearing loss only	43	63.6 ± 14.3	16	27	1	2.3
Both maternal inheritance and hearing loss	14	53.0 ± 14.9	5	9	3	21.4
Maternal inheritance and/or hearing loss	87	60.5 ± 14.6	28	59	4	4.6

disorders detected by ECG and/or UCG. Cardiac studies with ECG revealed ischemic ST-T segment changes and left ventricular hypertrophy. UCG demonstrated moderate hypertrophy of the cardiac muscles and decreased left ventricular function. Two patients (no. 1 and 2) were insulin-deficient, and insulin therapy was required in three patients. Only one patient (no. 2) was positive for antibodies to glutamic acid decarboxylase (anti-GAD).

Among several clinical parameters, the frequency of cardiac disorder was shown to be statistically significant between the two groups with or without the M3243A → G mutation in diabetic patients with both maternal inheritance and hearing loss. All three patients with the mutation and two of 11 patients without the mutation had cardiac disorders ($P < .05$).

Two of three with the mutation and two of 11 without the mutation had impaired insulin release, and two of three with the mutation and four of 11 without the mutation were treated currently with insulin. BMI was 19.3 ± 2.3 and 21.9 ± 2.4 kg/m^2 and age at onset was 40.7 ± 14.2 and 43.3 ± 14.4 years in patients with or without the mutation, respectively. The frequencies of impaired insulin release and insulin treatment were higher and BMI and age at onset were lower in patients with the M3243A → G mutation versus those without it, although none of these trends were statistically significant.

DISCUSSION

We could identify mitochondrial diabetes mellitus effectively by selecting diabetic patients with high risk factors such as maternal inheritance and hearing loss. The identification of such patients is meaningful in terms of classification and treatment in the future, because they are likely to develop insulin dependence owing to impaired release of endogenous insulin, which may be related to mitochondrial dysfunction.[13] In the present study, three of four patients with the M3243A → G mutation required insulin therapy, and interestingly, the clinical characteristics of these patients are similar to those of patients with slowly progressive insulin-dependent diabetes mellitus.[14]

In Japan, we often encounter diabetic patients who are initially diagnosed as non–insulin-dependent diabetics and who develop insulin dependency not acutely but gradually. The present results suggest that mitochondrial gene mutation might be one of the causes of Japanese insulin-dependent diabetes mellitus.

The frequency of the M3243A → G mutation was approximately 1% among randomly selected Japanese diabetic patients,[15] and about twofold to threefold higher in cases with a family history of diabetes.[9,10,16] Compared with these previous data on this mutation in Japanese diabetic patients, the frequency of the mutation in the present study was much higher, owing to screening diabetic patients by a combination of high risk factors such as maternal inheritance and hearing loss.

Therapy designed to enhance mitochondrial function may be useful in patients with this mutation. Suzuki et al[17] reported that coenzyme Q10 therapy was effective in diabetic amyotrophy with the M3243A → G mutation.

In the present study, all four patients with this mutation had abnormalities in cardiac function such as ischemic ST-T segment changes, hypertrophy of the cardiac muscle, and impaired left ventricular function determined by ECG and/or UCG. These disorders may have resulted from mitochondrial cardiomyopathy.[18,19] Thus, the presence of cardiac disorder may also be an indicator of the mutation.

One patient with the M3243A → G mutation in the present study was positive for anti-GAD as a marker of autoimmunity. Oka et al[20] detected this mutation in three of 27 Japanese islet-cell antibody–positive, initially non–insulin-dependent diabetic patients. They suggested that islet-cell antibody might follow gradual β-cell destruction due to a mitochondrial gene mutation, but we cannot ascertain the relation between mitochondrial gene mutation and the presence of anti-GAD.

Diabetes mellitus represents heterogeneous disorders, and insulin release and insulin resistance probably play different roles. Diabetic patients with the M3243A → G mutation have been found to exhibit decreased release of insulin in the absence of insulin resistance.[21] The present results also demonstrated that most diabetic patients with this mutation had impaired insulin release, although insulin resistance was not evaluated.

Phenotypic variability of mitochondrial disease seems due to different expression of the mutant genotype in different tissues with different thresholds. It may be reasonable to presume that

Table 2. Clinical Profile of Diabetic Patients With the M3243A → G Mutation

Subject No.	Age (yr)	Sex	BMI (kg/m^2)	Onset (yr)	CPR*	Treatment	HbA_{1c} (%)	CD		Anti-GAD Antibodies	MI	HL
								ECG	UCG			
1	53	F	18	38	5-6	Insulin	8.5	+	+	−	+	+
2	40	F	18	28	7-15	Insulin	6.5	+	−	+	+	+
3	52	F	14	42	19-21	Insulin	9.9	+	+	−	−	+
4	65	F	22	56	40-50	Diet	6.9	+	−	−	+	+

Abbreviations: CPR, C-peptide immunoreactivity; CD, cardiac disorder; MI, maternal inheritance; HL, hearing loss.
*24-hour urinary C-peptide excretion (μg/d).

the M3243A → G mutation is a cause of impaired insulin release using pancreatic β cells themselves instead of peripheral blood cells, although obtaining pancreatic tissue specimens is difficult.

We conclude that maternal inheritance and hearing loss are useful clinical findings to identify diabetes with the M3243A → G mutation, and that coexistent cardiac involvement increases the occurrence of the M3243A → G mutation.

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