Markedly Different Clinical Features in 2 Diabetes Mellitus Patients with Extremely High Tissue Levels of the Mitochondrial DNA A3243G Mutation

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Key Words

Mitochondrial DNA • mtDNA A3243G mutation • Diabetes mellitus

Abstract

Background: Mitochondrial DNA (mtDNA) A3243G mutation is one of the major causative factors of mitochondrial diabetes mellitus. We found that tissues from 2 of 142 diabetes mellitus patients showed extremely high levels of the mutation. **Objective:** To investigate the level of the mutation in each tissue and to find the relationship between the mutation level and clinical features of the patients. **Methods:** Patient 1 was a 51-year-old woman, diagnosed as having diabetes mellitus at the age of 17, and was admitted to hospital because of cerebral infarction. Patient 2 was an 82-year-old woman who was admitted because of respiratory failure. mtDNA A3243G levels were measured in tissues collected at autopsy. **Results:** In patient 1, mtDNA A3243G levels were found to vary among the tissues. The patient's highest mtDNA A3243G value was 42% and the lowest value was 9%, whereas the level in most individuals is usually less than 1%. Although patient 2 did not exhibit serious clinical symptoms of diabetes mellitus, the mtDNA A3243G level was extremely high in all of the tissues surveyed (range 32–47%). **Conclusion:** Although both patients showed high levels of the

mtDNA A3243G mutation, their clinical conditions differed greatly. Thus, mitochondrial diabetes mellitus patients may show a wide variety of clinical features and large variations in life span.

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Introduction

There have been many reports on human disorders caused by mitochondrial DNA (mtDNA) mutations, most of which are caused by point mutations [1] and some of which are due to deletion [2].

Among the point mutations of human mtDNA that cause disorders or diseases, A3243G mutations are well known and have been widely described. This mutation is a major cause of several serious mitochondrial diseases such as MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) [3, 4], Leigh's syndrome [5], myoclonic epilepsy with ragged red fiber myopathy [6], chronic progressive external ophthalmoplegia [7, 8], hearing loss [9] and mitochondrial diabetes mellitus (MDM) [10, 11]. In patients with these disorders, mutated and normal mtDNA molecules coexist as heteroplasmy, and the incidence of the A3243G mutation is much higher than in normal individuals.

Position 3243, which is located in the tRNA (Leu) gene of mtDNA, may cause a conformational change of the tRNA (Leu) molecule, resulting in disordered synthesis of the protein [12]. Yasukawa et al. [13] reported that the presence of guanine at position 3243 blocked the base modification of the anticodon in the RNA, possibly resulting in mistranslation.

Recently, MDM has been intensively investigated and most cases appear to be caused by the mtDNA A3243G mutation [11, 14, 15]. Therefore, it is diagnostically important to measure the level of mtDNA A3243G in each diabetes mellitus (DM) patient in order to identify possible MDM patients and investigate the mechanism of the disease.

In this report, we describe the presence of the mtDNA A3243G mutation in 142 cases of DM. We also focus on 2 DM patients whose tissues showed extremely high levels of the mtDNA A3243G mutation, and discuss whether the mutation was the major factor responsible for the disease in each individual.

Patients and Methods

First, we examined mtDNA mutations of the esophageal epithelium in 142 autopsy cases (77 men, 65 women; age range 51–98 years; mean age 76 years). mtDNA mutation values in 140 of the 142 cases were less than 1%. These values were not different from the mutation ratio observed in white blood cells from controls, reported previously [16]. Corresponding values in the remaining 2 cases were 18.5% (patient 1) and 32.1% (patient 2). We therefore examined mtDNA mutation in all of the tissues from these 2 patients.

Patient 1

A 51-year-old woman with a history of type I DM was admitted to hospital because of cerebral infarction. She had been diagnosed as having DM at the age of 17 years. Although she had been treated with insulin since 19 years of age, her disease had not been well controlled. Due to heavy pleural effusion, she had been admitted to our hospital several times. By the age of 42 years, hemorrhage had occurred in her vitreous, and she had undergone hemodialysis due to diabetic nephropathy for 6 years. Her left heel had become gangrenous when she was 49 years old due to a DM-related circulatory disease. Her close family members included no other DM patients. The patient herself had not been diagnosed as having MDM.

Three months after hospitalization, she died due to myocardial infarction. At the time, her height was 157 cm and her weight was 40.2 kg. Autopsy revealed multiple cerebral infarctions and acute myocardial infarction superimposed on an old infarct. Her left ventricle was hypertrophic, and her heart weighed 450 g. The atrophic pancreas showed atrophy of the acini, interstitial fibrosis, and centroacinar and ductal dilatation. The cells of the islets of Langerhans were markedly reduced in number, with mild am-

yloid deposition, although we considered this change not to be specific to MDM. Diabetic nephropathy was observed and the kidneys were atrophic, weighing 70 and 80 g, showing marked hyalinization of glomeruli with mesangial and nodular sclerosis and fibrin caps. Levels of arteriosclerosis with calcification of the blood vessels were remarkable. The aorta showed atherosclerosis with ulceration and calcification. Pulmonary edema was evident, and the lungs weighed 527 and 725 g.

The patient's family history was unknown, although her 2 sons, aged 20 and 22 years, were free of DM.

Patient 2

An 82-year-old woman was admitted to hospital because of respiratory failure caused by a deformity in the vertebral column. The patient's blood sugar level was 265 mg/ml immediately after admission. Although she had been diagnosed as having DM at the age of 45 and admitted to hospital for instructions regarding self-care, she did not show any strong clinical signs of DM, and MDM had not been diagnosed.

She had not received any drugs for DM at any time in her life, and dietetic treatment had been the only intervention. She began using a hearing aid when she was 76 years old. She was admitted to hospital due to the possibility of cancer in the head of the pancreas at the age of 79. When she was 80 years old, she developed osteoporosis and was admitted again.

After her death, due to pneumonia after 3 months of hospitalization, an autopsy was performed. Extreme emaciation was evident (height 140 cm, weight 26 kg). Confluent pneumonia, probably aspiration pneumonia, was found in both lungs, which weighed 365 and 325 g. The pancreas was atrophic, with a reduced number of islet cells, and parts of the islets of Langerhans showed hyalinization. The heart showed brown atrophy and weighed 220 g. The liver also showed brown atrophy. Spondylosis deformans was observed in the vertebral column, and slight arteriosclerosis was observed in the kidneys (weight 120 and 150 g).

The patient had 2 brothers and 1 sister, who were healthy. Although she also had 2 sons and 2 daughters, 1 of her sons had died in infancy. All of her surviving children (a son aged 53, and daughters aged 45 and 47) were healthy.

Sample Collection

Family members of both subjects signed written consent forms for the educational and scientific use of the subjects' organs, including DNA analysis. The study protocol was approved by the Tokyo Metropolitan Institute of Gerontology.

Tissues were collected at autopsy for pathological analysis. The following fresh tissue samples were collected from patient 1: kidney, liver, cerebral cortex, lung, esophageal epithelium, myocardium, adrenal medulla, pancreas, thyroid gland, accessory thyroid, skeletal muscle (quadriceps femoris), abdomen skin, head skin, tongue, trigone of the bladder and upper side of the bladder. The following tissue samples were collected from patient 2: kidney, esophageal epithelium, myocardium and liver.

Tissues from the patients were stored at -80°C until use. DNA was extracted from each sample by the usual methods described previously [17].

Analysis of mtDNA A3243G Levels

Ratios of mtDNA A3243G variation were measured using the methods described by Takeuchi et al. [16] and Harihara et al. [18].

Fig. 1. mtDNA A3243G mutation levels in tissues taken from the internal organs of patients 1 and 2. Ratios of mtDNA A3243G variation were measured using the methods described by Takeuchi et al. [16] and Harihara et al. [18], using the ABI Prism 377 DNA Sequencer (PE Biosystems).

A 200-bp fragment containing position 3243 of the mtDNA was amplified for each sample by PCR, using specific primers. The upstream primer was labeled with 6-FAM (PE Biosystems, USA). PCR products were digested with restriction enzyme *Hae*III, and then quantitative analysis was conducted using an ABI Prism 377 DNA Sequencer (PE Biosystems) operated by the GeneScan program. The size of the labeled DNA fragment from the normal molecule was 157 bp, whereas the size of the DNA fragment from the mutated molecule was 83 bp. The peak heights of the 157-bp fragment (H1) and the 83-bp fragment (H2) were measured for each sample, and the ratio of the mtDNA A3243G homological mutation in each sample was obtained using the formula $H2/(H1 + H2)$.

Results and Discussion

Most of the regular autopsy cases had mtDNA mutation values of less than 1% in the esophageal epithelium.

All of the tissue samples from patient 1 showed extremely high values of mtDNA A3243G (fig. 1), varying widely from less than 10% to over 40%. The lowest value was 6.1% (skeletal muscle), the highest was 42.4% (cerebral cortex) and the mean ratio was $23.9 \pm 13.6\%$.

If this patient was suffering from MDM, an accumulation of abnormal molecules in the pancreas (mtDNA with the A3243G mutation) should be a major finding. However, in patient 1, the value for the pancreas was 11.1%, which was lower than the values for several other

organs. Asano et al. [19] also analyzed the heteroplasmic level of mtDNA A3243G in various tissues from 1 male DM patient, and showed that the level in the pancreas was relatively lower than in several other organs. Also in this study, a relatively lower level of mtDNA A3243G mutation may have caused the severe condition of the pancreas in patient 1, since the autopsy showed histological damage probably due to DM.

Ratios of the variation in mtDNA A3243G were also measured in samples from patient 2 (fig. 1). The ratio in each organ was extremely high, and varied from 32.1% (esophageal epithelium) to 46.6% (myocardium). In the myocardium, almost half of the mtDNA molecules harbored the mutation. The mean ratio for the subject's samples was $39.6 \pm 6.3\%$, and the variation in the value was much less than in patient 1.

Although we had no data for the level of mtDNA A3243G in the pancreas of patient 2, the pancreas itself showed little clinical evidence of DM-related damage since the autopsy showed the organ was atrophic, with a reduced number of islet cells, and parts of the islets of Langerhans showed hyalinization.

The mtDNA A3243G mutation is often found in MDM patients and may be the major cause of MDM. Both of the patients described in this report showed extremely high ratios of mtDNA A3243G mutation, and this was probably the clinical cause of DM. However, the clinical man-

ifestations and life spans of the 2 patients differed considerably.

Although the mean ratio of mtDNA A3243G in patient 1 was lower than that in patient 2, the clinical condition of the diabetes in the former patient was more serious than that in the latter. Patient 1 showed the typical manifestations of diabetes. She had been diagnosed as diabetic at the age of 17, her clinical symptoms were severe and the disease had not been well controlled by insulin. The autopsy also revealed the underlying serious conditions caused by the disease. Patient 2 had been diagnosed as diabetic at the age of 45. However, her diabetic condition was mild and no medical treatment had been necessary. At autopsy, no pathological change attributable to DM was evident in any organs, except for the pancreas.

MDM patients often develop deafness or difficulty in hearing [10]. Difficulty in hearing was not reported by patient 1, whereas patient 2 used a hearing aid. However, this patient's difficulty in hearing might be due to aging since she was 76 when she began using a hearing aid.

The levels of mtDNA A3243G mutation in the samples from patient 1 varied considerably among the organs (6.1–42.1%). Asano et al. [19] reported that the level of heteroplasmy differs considerably in the organs of MDM patients. It is difficult to conclude which organ is most likely to show a high level of the mutation.

As for the level of mtDNA A3243G among tissues of diabetic patients, Chinnery et al. [20] reported strict hierarchical distribution of the mutation among tissues of 5 individuals. However, the patients in their study belonged to 1 family, and the mutation may have been maternally inherited. It would be necessary to investigate samples from more diabetic patients in order to clarify whether some hierarchical order of distribution really does exist or whether observed variations in heteroplasmy are simply random.

In our previous study [18], we noted that the accumulation of the mtDNA A3243G mutation differed between the myocardium and esophageal epithelium, and suggested that this might be due to age-related change or tissue renewal, with the myocardium showing a higher value of the mtDNA A3243G mutation. In patient 2 of the present study, the level of mutation in the myocardium was higher than in esophageal epithelium and also several other organs. Asano et al. [19] also reported that the level of mtDNA A3243G in the myocardium was higher than in other tissues, and suggested that the mutation might be concentrated in the myocardium, though the mechanism was obscure.

Although the tissues of both of the present DM patients showed an extremely high level of the mtDNA A3243G mutation, which was the likely cause of their disease, they showed a marked difference in their clinical conditions. Patient 1 had the typical features of severe DM, and the autopsy also revealed serious disease-related damage to her organs, whereas patient 2 had rather mild clinical features of DM and died due to unrelated causes.

Various clinical conditions have been reported in relation to MDM [21], but the pathological mechanism responsible and the contribution of the mutation are still unclear. Although Yasukawa et al. [13] reported that the mtDNA A3243G mutation causes damage to normal protein synthesis, it is still difficult to account for the mechanism responsible for the variety of pathological and clinical features seen in MDM. Although the level of the mutation is undoubtedly one of the most important factors, it will be necessary to investigate other potential factors that cause or promote the pathological and clinical features and determine the life span of affected patients such as atherosclerosis that can cause diabetic nephropathy, retinopathy, neuropathy or coronary disorders.

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