Clinical Manifestations due to a Point Mutation of the Mitochondrial tRNA\textsuperscript{leu(UUR)} Gene in Five Families with Diabetes Mellitus


It has been shown that an adenine (A) to guanine (G) transition at position 3243 of the mitochondrial transfer RNA(tRNA)^leu(UUR) gene is associated with a subgroup of diabetes mellitus. Therefore, we screened for this transition in 86 patients with non-insulin-dependent diabetes mellitus (NIDDM) in which two or three generations were affected with diabetes, in 14 patients with insulin-dependent diabetes mellitus, and in 9 families with diabetes mellitus and/or associated disorders suggesting mitochondrial gene abnormalities. We failed to identify the mutation in 100 diabetic patients, 86 NIDDM and 14 insulin-dependent diabetes mellitus (IDDM). Out of the latter 9 families, we identified an A to G transition in 14 individuals in 5 families. Diabetes mellitus was shown to be maternally inherited in one family. In 9 of 14 patients with the mutation, insulin was required to treat diabetes mellitus, indicating impaired insulin secretion. A hyperglycemic clamp test performed in one subject revealed significant impairment of insulin secretion, whereas euglycemic clamp test showed normal insulin sensitivity in this patient. The heteroplasmy of the mutant mitochondrial DNA (mtDNA) in leukocytes does not appear to correlate with the severity of diabetes in terms of the insulin therapy required. Body mass index of the affected individuals was less than 23.3. In one family, in addition to diabetes mellitus and hearing loss, hypoparathyroidism was associated with the mutation, suggesting that hypoparathyroidism is caused by the impaired processing and/or secretion of proparathyroid hormone due to the mutation. In addition, the affected subjects presented with proteinuria at the time of diagnosis of diabetes mellitus which appeared not to be related with diabetic nephropathy.

Key words: mitochondrial DNA, maternal inheritance, diabetes mellitus, insulin secretion, hypoparathyroidism

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is caused by impaired insulin secretion, decreased insulin sensitivity in peripheral tissue and inappropriate regulation of hepatic glucose production (1). It is well-known from epidemiological studies that NIDDM is a heterogeneous syndrome in terms of its genetic predisposition. This concept has been underscored by recent investigations identifying the abnormalities of the candidate genes responsible for glucose metabolism including insulin (2), insulin receptor (3) and glucokinase (4). Recently, it has been reported that some form of diabetes mellitus could be caused by mitochondrial gene abnormalities; a large deletion (5) and an adenine (A) to guanine (G) transition at position 3,243 in the mitochondrial tRNA\textsuperscript{leu(UUR)} gene (6). The mitochondria is an indispensable organelle for oxidative phosphorylation. Thus, it is inferred that impaired oxidative phosphorylation in pancreatic beta cells leads to decreased
insulin secretion, thereby inducing diabetes mellitus (7). However, it is not clear whether decreased insulin sensitivity due to a mutant mtDNA in the muscle is involved (8).

An A to G transition at 3,243 nucleotide of tRNA\textsubscript{leu(UUR)} is associated with diabetes mellitus. This substitution was originally identified in neurological disorders including mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes (MELAS) and chronic progressive external ophthalmoplegia (CPEO) (9, 10). However, diabetes mellitus caused by this mutation is not always associated with neurological manifestations, except for deafness. This implies that the more common form of NIDDM, with a maternally linked genetic predisposition, may be caused by this mutation. To address this issue, we screened for an A to G transition at nucleotide 3,243 of tRNA\textsubscript{leu(UUR)} in NIDDM patients with a family history of diabetes mellitus, as well as in families with diabetes mellitus and associated disorders suggesting mitochondrial gene abnormalities. We investigated the clinical characteristics of diabetes mellitus associated with this mutation and the clinical phenotypes of the affected individuals. The present study indicates that the mutation results in pleiotropic clinical manifestations.

**Subjects**

We divided the subjects into three groups to screen for a mitochondrial gene mutation.

- **Group 1:** families of which a member is associated with diabetes mellitus, deafness and other clinical manifestations suggesting mitochondrial gene abnormalities including MELAS, cardiomyopathy and myopathy. (n=9)
- **Group 2:** patients with NIDDM in which two or three generations are affected. (n=86)
- **Group 3:** patients with NIDDM. (n=14)

Informed consent for this study was obtained from all subjects.

**Materials and Methods**

Genomic DNA was extracted from patients’ leukocytes as previously reported (11). In order to detect the A to G transition at the 3,243 nucleotide of tRNA\textsubscript{leu(UUR)}, DNA fragment encompassing tRNA\textsubscript{leu(UUR)} in mtDNA was amplified using a forward primer corresponding to base pair (bp)3,153–3,173 (TTC ACA AAG CGC CTT CCC CC) and a reverse primer corresponding to bp 3,551–3,531 (GCC ATG GTG AGA GCT AAG GTC) by polymerase chain reaction (PCR). The initial denaturation was conducted at 94°C for 2 minutes and then at 94°C for 1 minute. The annealing was performed at 60°C for 1 minute and extension at 72°C for 2 minutes for 25 cycles with a final extension at 72°C for 5 minutes (12). The amplified DNA fragment was then digested with a restriction endonuclease, Apa I. Digested DNA was subjected to 2% agarose gel electrophoresis and the gel was stained with ethidium bromide to determine the presence of a mutation. Furthermore, to identify a mutation precisely, the digested DNA was subjected to 5% polyacrylamide gel electrophoresis and the gel was stained with Silver Stain Plus (Bio-Rad, Hercules, California). Genomic DNA (100 ng) from the patients with a mutation were amplified by the same PCR condition and the products was run on 2% low melting agarose gel and extracted from the gel by Magic PCR Preps DNA purification system (Promega, Madison, Wisconsin). The extracted DNA was amplified using a forward PCR primer, fluorescent-dye dideoxy nucleotide terminators and Taq polymerase by thermal cycle and denatured at 96°C for 30 seconds with annealing at 50°C for 15 seconds and extension at 60°C for 4 minutes for 25 cycles. The nucleotide sequence was determined using a DNA automatic sequencer (model 373A, Applied Biosystems Inc, Foster, California).

**Euglycemic and hyperglycemic clamp tests**

Euglycemic and hyperglycemic clamp tests were performed according to a previous report (13). In brief, the euglycemic clamp test was performed by acutely raising the plasma insulin concentration with a priming injection of insulin and maintained by a continuous infusion of insulin at 1.2 mU/kg/min. The plasma glucose concentration was simultaneously held constant at fasting plasma glucose level by a variable glucose infusion. Glucose infusion rate was determined for the last 30 minutes during the steady-state period. In the hyperglycemic clamp test, the plasma glucose concentration was acutely elevated to 125 mg/dl above the fasting plasma glucose level by a priming infusion of glucose. Blood samples were collected for determination of insulin concentrations for 90 minutes after the hyperglycemic plateau was achieved. Plasma insulin levels were measured by a specific radioimmunoassay and total insulin secretion was calculated by determining the area under the curve.

**Results**

**Identification of an A to G substitution at nucleotide 3,243 of tRNA\textsubscript{leu(UUR)} gene**

We identified an A to G substitution in 14 individuals of 5 families in Group 1. Thirteen of them had diabetes mellitus and one had impaired glucose tolerance (IGT) according to NIH criteria (Fig. 1 and Table 1). However, we failed to identify the mutation in the 100 individuals including 86 patients with NIDDM (group 2) and 14 patients with IDDM (group 3).

Apa I digestion of PCR product amplified from genomic DNA of leukocytes revealed that mutant mtDNA exists in a heteroplasmic fashion. A heteroplasmic mutant DNA was also identified in the muscle obtained at autopsy from the proband in Family 5 (data not shown). Figure 2 shows a representative DNA sequence, indicating an A to G substitution at nucleotide 3,243 of tRNA\textsubscript{leu(UUR)} in a heteroplasmic form. Figure 3 A and B show the degree of heteroplasmy of mutant mtDNA from the subjects in Family 1 whose clinical data are available. A subject without diabetes mellitus (II-7) did not have heteroplasmic mutant DNA in her leukocytes and the mutant DNA from a subject with IGT (II-1) was much less heteroplasmic.
Family 1

I

II m

III nd nd nd

Figure 1. Pedigrees of 5 families with diabetes mellitus and an A to G transition at 3,243 position. Each subject is identified by the generations in Roman numerals and by Arabic number beyond the symbols. A subject (II-1) in Family 1 had impaired glucose tolerance (IGT). m and n indicate the individuals in which leukocytes proved the presence of mutation (m) and the absence (n).
## Table 1. Clinical Characteristics of the Subjects with Diabetes Mellitus and the Mutation

<table>
<thead>
<tr>
<th>Family</th>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Age at onset of Diabetes mellitus/Hearing impairment&lt;br&gt;(yrs)</th>
<th>Treatment&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Diabetic Complication</th>
<th>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>C-peptide Immunoreactivity&lt;br&gt;s-CPR (ng/ml)</th>
<th>u-CPR (µg/day)</th>
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<tbody>
<tr>
<td>1</td>
<td>II-1</td>
<td>73</td>
<td>M</td>
<td>73c/-g</td>
<td>OHA&lt;sup&gt;i&lt;/sup&gt;(16)→Insulin</td>
<td>PDR&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Proteinuria -g</td>
<td>23.3</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>II-4</td>
<td>68</td>
<td>F</td>
<td>40 / 60</td>
<td>OHA&lt;sup&gt;i&lt;/sup&gt;(14)→Insulin</td>
<td>PDR&lt;sup&gt;i&lt;/sup&gt;</td>
<td>-</td>
<td>15.3</td>
<td>0.68&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>II-6</td>
<td>64</td>
<td>F</td>
<td>42 / -</td>
<td>OHA&lt;sup&gt;i&lt;/sup&gt;(12)→Insulin</td>
<td>PDR&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Proteinuria +</td>
<td>16.7</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>III-5</td>
<td>33</td>
<td>F</td>
<td>14 / -</td>
<td>Insulin</td>
<td>-</td>
<td>15.3</td>
<td>1.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33.0&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>III-8</td>
<td>41</td>
<td>F</td>
<td>36 / -</td>
<td>OHA&lt;sup&gt;i&lt;/sup&gt;(1W)→Diet</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<td></td>
<td>III-9</td>
<td>37</td>
<td>M</td>
<td>35 / 27</td>
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<td>-</td>
<td>17.9</td>
<td>-</td>
<td></td>
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<tr>
<td>2</td>
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<td>35</td>
<td>F</td>
<td>23 / 19&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Insulin</td>
<td>-</td>
<td>14.5</td>
<td>5.4&lt;sup&gt;e&lt;/sup&gt;</td>
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</tr>
<tr>
<td>3</td>
<td>II-3</td>
<td>49</td>
<td>F</td>
<td>35 / -</td>
<td>OHA&lt;sup&gt;i&lt;/sup&gt;(6M)→Insulin</td>
<td>-</td>
<td>19.0</td>
<td>1.1&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>III-1</td>
<td>21</td>
<td>M</td>
<td>20 / 3</td>
<td>Insulin&lt;sup&gt;i&lt;/sup&gt;(1M)→Diet</td>
<td>-</td>
<td>15.8</td>
<td>1.5&lt;sup&gt;d&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>III-3</td>
<td>14</td>
<td>F</td>
<td>9 / -</td>
<td>Insulin</td>
<td>-</td>
<td>20.7</td>
<td>1.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>II-3</td>
<td>62</td>
<td>F</td>
<td>30 / -</td>
<td>Diet&lt;sup&gt;i&lt;/sup&gt;(8)→OHA</td>
<td>-</td>
<td>13.3</td>
<td>90.0&lt;sup&gt;f&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>III-3</td>
<td>31</td>
<td>M</td>
<td>22 / 21</td>
<td>OHA</td>
<td>-</td>
<td>16.0</td>
<td>70.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>II-1</td>
<td>55</td>
<td>F</td>
<td>35 / 38</td>
<td>Insulin</td>
<td>PDR&lt;sup&gt;i&lt;/sup&gt;</td>
<td>-</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II-2</td>
<td>48</td>
<td>M</td>
<td>35 / 45</td>
<td>Insulin</td>
<td>PDR&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Hemodialysis</td>
<td></td>
<td></td>
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</tbody>
</table>

<sup>a</sup>: Age at the onset of the symptom of hearing impairment is given.<br><sup>b</sup>: Number in parentheses is duration of the treatment [year or month(M), week(W)].<br><sup>c</sup>: Age at diagnosed as having impaired glucose tolerance.<br><sup>d</sup>: Fasting plasma C-peptide immunoreactivity value (s-CPR).<br><sup>e</sup>: Urinary excretion of C-peptide immunoreactivity value (u-CPR).<br><sup>f</sup>: She lost hearing completely at the age of 32.<br><sup>g</sup>: (+) and (-) indicate the presence or absence of hearing impairment and diabetic complications.<br><sup>h</sup>: OHA, oral hypoglycemic agents.<br><sup>i</sup>: PDR, proliferative diabetic retinopathy.

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**Clinical characteristics of the subjects in families with the mutation** (Fig. 1 and Table 1)

**Family 1**

The proband (III-5), her mother (II-4) and the proband’s cousin (III-9) had diabetes mellitus and hearing loss. Her aunt (II-6) and another cousin (III-8) had only diabetes. Her uncle (II-1) had IGT. Her grandmother was known to have only hearing loss. Nobody showed clinical evidence of optic atrophy, stroke-like episodes or myopathy. Diabetes mellitus and hearing loss was maternally inherited. The age of diagnosis of diabetes mellitus was 40 (II-4) and 42 (II-6) in generation II, and 14 (III-5), 36 (III-8) and 35 (III-9) in generation III, respectively. Nobody developed diabetic ketoacidosis except for III-8 who had ketoacidosis at the time of diagnosis. Late diabetic complications including retinopathy, nephropathy and neuropathy were found in II-4, II-6, III-5 and III-8. Four of five subjects with diabetes mellitus required insulin treatment in doses from 12 to 18 U per day. The sister of the proband was known to have cerebral paralysis since her birth. According to the laboratory examination at the previous admission into hospital, she showed neither glucosuria nor hyperglycemia. Body mass index (BMI) of five subjects with diabetes mellitus were from 15.3 to 20.9 and that of the proband’s uncle with IGT was 23.3 kg/m<sup>2</sup>.

**Family 2**

In this family the proband (III-4) and her cousin (III-1) had diabetes mellitus. The proband was diagnosed as having diabetes mellitus at the age of 23 year. Hearing disturbance was noted since the age of 19 year, and it deteriorated to complete loss in the right ear at the age of 23 year and in the left ear at the age of 32 year. She showed microalbuminuria without other complications. She had chronic nephritis, hypertrophic cardiomyopathy and glaucoma. Her brain magnetic resonance image (MRI) showed atrophy of cerebellum and mineralization of the striatonigral system. However, no clinical evidence suggesting MELAS was noted. Microscopic examination of her muscle-biopsy specimen revealed no abnormalities.
Diabetes with a Mitochondrial Gene Mutation

Figure 2. Nucleotide sequences surrounding the 3,243 position of the mitochondrial tRNA^{leu(UUR)} of the proband (III-5) in Family 1.

Family 3
In Family 3, the proband (III-1), his sister (III-3), his mother (II-3) and a sibling of his grandmother had diabetes mellitus. The proband was diagnosed as having diabetes mellitus at the age of 21 year with symptoms of polydipsia and polyuria. He had hearing loss at the age of 3 year. His sister complained of polydipsia and polyuria and was diagnosed as having diabetes mellitus at the age of 9 year. At the age of 4 year, oral glucose tolerance test indicated normal glucose tolerance. Their mother was diagnosed as having diabetes mellitus four days after the delivery of her third child (III-3) when hyperglycemia was first noticed. They had proteinuria when diabetes mellitus was diagnosed. They did not have retinopathy. Both the proband and his sister had primary hypoparathyroidism, short stature and retarded intelligence. Serum concentrations of intact parathyroid hormone (PTH) of the proband and his sister were <5 pg/ml and 9.0 pg/ml, respectively. Serum calcium and phosphorus of the proband and his sister were 8.0 mg/dl and 6.7 mg/dl, 7.8 mg/dl and 6.7 mg/dl, respectively. In addition, his sister had patent ductus arteriosus and minor external anomalies including lower hairline, hypertelorism and epicanthus. Muscle biopsy obtained from the proband showed only occasional ragged-red fibers. Computed tomography showed no abnormalities in the brain.

Families 4 and 5
In Family 4, the proband (III-3) noticed tinnitus at the age of 21 year. A year later he presented with episodic headaches with
convulsions, transient hemiplegia and high lactate levels in serum and cerebral spinal fluid (CSF). He was diagnosed as having MELAS. The morphological evidence of mitochondrial abnormalities were obtained from muscle biopsy specimens. He was diagnosed as having diabetes mellitus at the age of 22 year. His mother (II-3) and his uncle had only diabetes mellitus. His mother was diagnosed as having diabetes mellitus at the age of 30 year. The proband and his mother did not develop diabetic complications in spite of the duration of diabetes over 10 and 30 years, respectively.

In Family 5, the proband (II-1) was diagnosed as having diabetes mellitus at the age of 35 year and noticed hearing disturbance at the age of 38 year. Gait disturbance and akinesia were noted at the age of 53 year and deteriorated gradually. When she was admitted to the hospital at the age of 55 year, muscle atrophy, weakness and ophthalmoplegia were noted. Ophthalmoscopic examination revealed no pigmented degenerative changes in the retina. Biochemical and morphological examinations of the muscle obtained from biopsy showed mitochondrial abnormalities including decreased activities of complex I+III, II+III and ragged-red fibers. Computed tomography of her brain showed diffuse atrophy. She was diagnosed as having mitochondrial myopathy, but her condition did not fulfill the criteria either for MELAS or Kearns-Sayer syndrome. Her brother (II-2) was diagnosed as having diabetes at the age of 35. He presented with hearing disturbance, muscle weakness and ataxic gait at the age of 45. Two affected subjects in Family 5 developed advanced diabetic complications during the course of diabetes mellitus of 20 and 18 years, respectively.

**Insulin secretory capacity, insulin sensitivity of the subjects with the mutation** (Table 1)

Daily urinary C-peptide immunoactivity excretion (u-CPR) was low in 3 of 6 subjects measured (III-5 in Family 1, III-4 in Family 2 and III-3 in Family 3). Four of five patients with diabetes mellitus in Family 1 were required insulin treatment in doses of 12–18 U per day (0.5–0.6 U/kg body weight). Three of them developed NIDDM more than 20 years ago and they were treated with oral hypoglycemic agents (OHA) followed by insulin treatment about 10 years ago. In a subject (III-3 in Family 3) who was treated with insulin since the time of diagnosis, the insulin dose increased gradually to maintain proper glycemic control during the clinical course. It is likely that insulin secretory capacity was gradually impaired in these individuals.

In the proband in Family 3 (III-1), fasting serum level of CPR (s-CPR) and daily u-CPR were 1.5 ng/ml and 51.2 Jig/day, respectively, when good glycemic control was achieved. In Family 4, daily u-CPR in the subjects with diabetes mellitus (II-3, III-3) were 90 μg/day and 70 μg/day, respectively.

In the proband in Family 3 (III-1), the euglycemic and hyperglycemic clamp test was performed when the patient received dietary treatment only. In the hyperglycemic clamp test in which plasma glucose level was maintained at 204 ± 3 mg/dl, the area under the curve (AUC) indicating total insulin secreted was much lower (848 μU/ml-min) than that of normal subjects (4,660 ± 521 μU/ml-min; mean ± SD, n=7). In the euglycemic clamp test, the glucose disposal rate was 6.2 mg/kg/min, which was slightly low compared with normal values (7.4 ± 0.4 mg/kg/min; mean ± SD, n=7).

**Discussion**

We have identified an A to G substitution at the position 3,243 of tRNA^leu(UUR) in 14 individuals in 5 families. This substitution cosegregated with diabetes mellitus and deafness (6, 14–19) and other clinical manifestations including cardiomyopathy (20) and MELAS (10, 21, 22). In agreement with a mitochondrial gene mutation, these phenotypes appear to be maternally inherited as shown in Families 1, 3 and 4.

An A to G transition at nucleotide 3,243 of tRNA^leu(UUR) was shown to correlate with a defect of protein synthesis and a respiratory chain deficiency (23, 24). It is suggested that this mutation in the pancreatic beta cell gives rise to the decreased generation of ATP and causes decreased insulin secretion.

Nine of 14 subjects with diabetes mellitus and IGT who had the mutation required insulin treatment. After three subjects with diabetes mellitus in Family 1 had been treated with OHA for 12 to 16 years, insulin therapy was initiated for proper glycemic control, implying that the insulin secretion was gradually impaired. This is also true for one other subject (III-3) in Family 3.

Three subjects (III-5 in Family 1, III-4 in Family 2 and III-3 in Family 3) showed decreased u-CPR, which was measured when they received insulin treatment. It was reported that insulin secretion in NIDDM is suppressed by exogenous insulin in both the hyper- and euglycemic states (25). Thus, the decreased u-CPR in these subjects might be partly ascribed to insulin treatment.

We performed hyperglycemic and euglycemic clamp tests in one subject (III-1 in Family 3) at the time when he received only dietary treatment and did not show clinical signs indicating myopathy. A hyperglycemic clamp test showed that the insulin secretory capacity was greatly impaired. An euglycemic clamp test, however, revealed that the insulin sensitivity was not so impaired, suggesting that mutant mitochondrial DNA in the muscle did not induce insulin resistance in this individual.

In disorders with mitochondrial gene mutations, it is well-known that abnormal mtDNAs are present in different magnitudes (heteroplasmy) (9, 26, 27). Apa I digestion of PCR products from 14 individuals revealed a different degree of heteroplasmy of a mutant mtDNA. It is likely that the magnitude of heteroplasmy in leukocytes does not correlate with the severity of diabetes in terms of insulin treatment requirements. For instance, although two subjects (II-3 and III-3 in Family 4) showed a high level of heteroplasmy of a mutant mtDNA (data not shown), daily u-CPR was not greatly reduced. This suggests that the magnitude of heteroplasmy of a mutant mtDNA in leukocytes does not correlate with that of mutant mtDNA in pancreatic beta cells. An autopsy specimen is necessary to elucidate the relationship between the magnitude of heteroplasmy in beta cells and the insulin secretory capacity.
With respect to somatic development of the individuals with the mutation, it was found that BMI of each subject was less than 23.3, indicating that they are not grossly overweight. This finding is consistent with those of previous reports (17, 18, 19).

Three subjects with diabetes mellitus and the mutation in Family 3 (II-3, III-1 and III-3) developed proteinuria at the time of diagnosis of diabetes mellitus. It is, however, unlikely that the proteinuria found in two subjects (II-3, III-3) is due to diabetic nephropathy because the duration of their diabetes appeared to be short. In a case of Kearns-Sayer syndrome (28), it was suggested that a deletion of mtDNA detected in the biopsy-specimen of the kidney is responsible for the impaired renal tubular function. Some cases of fatal infantile mitochondrial myopathy have been reported to be associated with the Fanconi’s syndrome presumably due to the mtDNA abnormalities (29, 30). Thus, it may be possible that the proteinuria found in these subjects is caused by impaired renal tubular function.

In addition, 2 subjects in Family 3 (III-1 and III-3) presented with hypoparathyroidism. It was reported that hypoparathyroidism is associated with mtDNA abnormalities. These include an A to G substitution at nucleotide 3,252 of tRNA^{leu(UUR)} (31) and a large deletion (32), respectively. The former mutation is located close to the position 3,243 and both nucleotides are thought to lie within a possible transcriptional control region (33). Thus, it is likely that impaired oxidative phosphorylation in the parathyroid gland causes impaired processing and/or secretion of parathyroid hormone.

It is a consistent feature that tissues or organs whose functions are highly dependent on oxidative phosphorylation including the brain, heart and endocrine organs (“oxidative organs”) are frequently affected. As shown by the clinical descriptions of our families, it is evident that the magnitude of involvement of a given tissue is different. The degree of heteroplasmy of a mutant mtDNA in a given tissue may account for this (9, 26, 27). It is well-known that in MELAS patients, the affected individuals have the most degree of heteroplasmy in their muscles. However, this does not seem to be sufficient to account for the different involvement of a given tissue. It is reported that MELAS patients who have a similar degree of heteroplasmy of a mutant mtDNA in their muscles do not always have similar skeletal involvement. This may be true for the severity of diabetes mellitus due to a pancreatic mutant mtDNA. It is thought that the interaction with the products of the nuclear-encoded genes is necessary for the transcriptional of the genes coded for the enzymes responsible for oxidative phosphorylation and synthesis of tRNAs. It is likely that the different interaction between the mtDNA and nuclear-encoded factors may be involved in the different degree of clinical involvement in a given tissue.

Although it was reported that the prevalence of diabetes mellitus with an A to G substitution of tRNA^{leu(UUR)} is about 1% in both Caucasian and Japanese diabetic populations (16–18), we failed to identify the mutation in 100 individuals with either NIDDM or IDDM.

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