Mitochondrial Gene Mutations in the tRNA\textsuperscript{Leu(UUR)} Region and Diabetes: Prevalence and Clinical Phenotypes in Japan

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Background: Mitochondrial gene mutations play a role in the development of diabetes mellitus. We have assessed the frequency of the A3243G and other mitochondrial mutations in Japan and in the relationship to clinical features of diabetes.

Methods: DNA was obtained from peripheral leukocytes of 240 patients with diabetes mellitus (39 with type 1; 188 with type 2; 13 with gestational diabetes) and 125 control subjects. We used PCR-restriction fragment length polymorphism analysis (ApaI) for A3243G and PCR-single-strand conformation polymorphism analysis to determine the mutations in the mitochondrial gene including nucleotide position 3243.

Results: The A3243G mutation was found in seven patients, and an inverse relationship was observed between the degree of heteroplasmy and the age at onset of diabetes. A3156G, G3357A, C3375A, and T3394C were detected in addition. Those who shared the same mutation showed similar clinical characteristics, thus representing a putative clinical subtype. The patients with A3156G had a sudden onset of hyperglycemia and showed a rapid progression to an insulin-dependent state with positive anti-glutamic acid decarboxylase antibody. Those with T3394C showed a mild defect in glucose-stimulated insulin secretion, and hyperglycemia appeared after adding such factors as aging or obesity.

Conclusions: The identification of mitochondrial gene mutations allows preclinical diagnosis of diabetes and prediction of the age at onset by evaluating the degree of heteroplasmy in cases with A3243G. Mutation detection may also be important for patient management and identification of affected family members.

Mitochondria are organelles that play an important role in the energy production of a cell, and they also have extranuclear genes, which are transmitted maternally. Especially in pancreatic β cells, mitochondria are responsible for glucose-induced insulin secretion because the exocytosis of secretory granules is triggered by changes in the intracellular ATP and ADP concentrations and subsequent increase in the ATP/ADP ratio as a result of oxidative phosphorylation in the mitochondria caused by glucose metabolism (1). Mutations in mitochondrial genes may lead to disorders in ATP production, and thereby impair the glucose-induced insulin secretion.

The mitochondrial DNA is a ringed, double-stranded DNA, measuring 16 569 bp in overall length, and exists in the mitochondrial matrix. A cell contains $10^3$–$10^4$ molecules of mitochondrial DNA. The mitochondrial DNA encodes 13 subunits of the mitochondrial respiratory chain, 22 tRNAs, and 2 rRNAs. The mutations of mitochondrial DNA constitute one of the main causes of the diseases that involve abnormalities in the brain and muscular tissues. An impaired glucose metabolism is also a symptom that is often seen in patients with mutations of mitochondrial DNA. Among these diseases, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)\textsuperscript{4} is known to be a syndrome that...
frequently accompanies diabetes mellitus. The screening of the A-to-G mutation at nucleotide position (np) 3243, found in 1992 in patients with MELAS (2), has been applied recently among diabetic patients. As a result, a maternally transmitted clinical subtype of diabetes mellitus, called diabetes-deafness syndrome (OMIM 520000), has been revealed. Different mutations based on the substitution of one base pair have been found in this region of mitochondrial DNA (3–5), thus showing it to be a hot spot area for mutations. Patients with the A3243G mutation have been reported to comprise 1–3% of all diabetic patients in Japan (6–8). The clinical characteristics of the patients with the A3243G mutation include impaired insulin secretion, sensorineural deafness, and maternal inheritance of the disease. Once impaired insulin secretion becomes overt, most cases progressively develop a disease state of insulin dependence. Therefore, the early or preclinical diagnosis of this disease by detection of such a mutation might help to prevent or delay the onset of diabetes, while also allowing for the selection of optimal treatment, thereby possibly avoiding diabetic complications altogether.

We developed a rapid diagnostic system using PCR-restriction fragment length polymorphism (PCR-RFLP) and PCR-single-strand conformation polymorphism (PCR-SSCP) analysis for screening mutations in the tRNA^Leu(UUR)^ region of mitochondrial DNA. We determined the detection limit of our method, which was essential for applying this system as a screening test because mutant DNA and wild-type DNA could coexist in a single cell known as heteroplasmy. A total of 240 diabetic patients and 125 controls were examined by use of this screening method.

**Subjects and Methods**

**PATIENTS AND CONTROLS**

Peripheral blood was obtained from 240 unrelated Japanese with diabetes mellitus (188 with type 2; 39 with type 1; 13 with gestational diabetes). The mean age was 52.8 ± 16.2 years. Individual patients were considered to be affected if they were being treated for type 2 diabetes or if the results of a standard 75-g oral glucose tolerance test indicated diabetes mellitus. Type 1 diabetes had been clinically diagnosed at the onset based on WHO criteria (9). The nondiabetic control group consisted of 125 unrelated Japanese (mean age, 22.9 ± 2.7 years) who showed fasting blood glucose of <7 mmol/L and body mass index (BMI) of <24 kg/m². Informed consent, according to the medical ethics criteria of Fukuoka University, was obtained from all participants.

**MATERIALS**

A restriction enzyme, *Apa* I, was purchased from Takara. AmpliTaq DNA polymerase was from Roche Molecular Systems. SYBR Green I was obtained from FMC Bioproducts, and PhastGels, PhastGel Buffer Strips Native, and PhastGel DNA silver staining reagent sets were from Amersham Pharmacia Biotech. Plasmid vector p17Blue T was from Novagen. SepaGene™ was from Sanko Junyaku. Other reagents were of analytical grade.

**PREPARATION OF DNA AND PCR AMPLIFICATION**

Total DNA was extracted from peripheral leukocytes with SepaGene according to the manufacturer’s instructions. The fragments of mitochondrial DNA encompassing np 3243 were amplified by PCR with AmpliTaq DNA polymerase. PCR was carried out in a total volume of 100 μL containing 100 ng of extracted DNA, 200 μM each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 1.0 U of *Taq* polymerase. The forward primers were 5′-CGTTTGTCAACGATATTAAAG-3′ (MT1) and 5′-AGGACAAGAGAAATAAGGCC-3′ (MT3), and the reverse primers were 5′-AGCGAAGGGTGTAGTACGCC-3′ (MT2) and 5′-CACGTGGGGCGCTTGTGGA-3′ (MT4). The DNA was initially denatured at 94 °C for 5 min and subjected to 30 PCR cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. The PCR products were electrophoresed on 4% agarose gel and stained with ethidium bromide.

**RFLP ANALYSIS**

The 422-bp fragment amplified by the primers MT1 and MT2 was digested by *Apa* I to identify any A-to-G mutation at np 3243 and electrophoresed on 4% agarose gels. After electrophoresis, the gels were stained with ethidium bromide or SYBR Green I. Fluorescopic scans and analyses were performed with ChemiImager (Alpha Innotech).

**SSCP ANALYSIS**

After PCR with the primers MT3 and MT4, the mixture containing the 294-bp amplified fragment was diluted twofold with a solution containing 950 mL/L formamide, 20 mmol/L EDTA, 0.5 g/L xylene cyanol, and 0.5 mL/L bromphenol blue. The sample was heated to 95 °C for 5 min, and after cooling on ice, 1 μL was separated on 20% polyacrylamide gels with PhastGel native buffer strips (0.25 mol/L Tris, 0.88 mol/L L-alanine, pH 8.8) on the PhastSystem™ (Amersham Pharmacia Biotech) (10). After electrophoresis, the gel was fixed and stained with a PhastGel DNA silver staining reagent set.

**DNA SEQUENCING**

Mutations detected by PCR-RFLP (*Apa* I) and/or PCR-SSCP were confirmed by direct DNA sequencing of PCR products or DNA sequencing after cloning into plasmid vectors. The sequencing reactions were carried out by use of the BigDye terminator cycle sequencing method (Perkin-Elmer), and reaction products were analyzed on an ABI-Prism 377 automated sequencer (Perkin-Elmer).

**PREPARATION FOR CALIBRATORS OF THE PCR PRODUCTS ENCOMPASSING THE MUTATION OF A3243G**

To determine the detection limit of the A3243G mutation, we constructed the mixture of PCR products with known
proportions of amplified DNA that contained both the A3243G mutation and no mutation. The PCR products amplified with DNA from a patient with the heteroplasmic A3243G mutation, using primers MT1 and MT2, were subcloned into a plasmid vector, pT7Blue T. After cloning of the plasmid vector inserted with the mitochondrial DNA containing A3243G or no mutation, the inserted DNA was amplified with the primers MT1 and MT2 by PCR. The products containing only the A3243G mutation or no mutation were extracted with chloroform, precipitated with ethanol, resolubilized in distilled water, and quantified by measurement of the absorbance at 260 nm. The products from DNA containing the A3243G mutation or no mutation were diluted to a concentration of 150 ng/mL with distilled water and mixed at ratios of 0:100, 1:99, 2:98, 5:95, 10:90, 20:80, 40:60, 50:50, and 100:0 by volume. Each 150 ng of the mixed DNA was digested with 1 U of ApaI in 10 μL of the reaction mixture for 1 h at 37 °C. The digested DNA was electrophoresed on a 4% agarose gel and stained with ethidium bromide or SYBR Green I. Fluoroscopic scans and analyses were performed with ChemiImager. For PCR-SSCP, PCR products with or without mutation, amplified with the primers MT3 and MT4, were mixed as above and electrophoresed by use of the PhastSystem as described above.

STATISTICAL ANALYSIS
The statistical analysis was performed with the Stat View-J 4.02 program (Abacus Concepts). Fisher exact test or the χ² test was used for statistical analyses to compare the frequencies of mutations between the diabetics and controls. A regression analysis was performed to determine the correlation between the percentage of heteroplasmy and the age at onset of diabetes in the patients with A3243G.

Results
DETECTION LIMIT FOR A3243G MUTATION
The PCR product amplified by the primers MT1 and MT2 was 422 bp in length. After digestion with ApaI, it was separated into 210 bp or 212 bp if it had a substitution of A to G at np 3243 to constitute the recognition site for ApaI. When electrophoresed on a 4% agarose gel, both the 210 bp and 212 bp overlapped and appeared as a single band, thus leading to an improvement in the sensitivity of the PCR-RFLP analysis.

After analysis of the calibrators, which consisted of DNA fragments containing A3243G and no mutation in the various known ratios, it turned out that PCR-RFLP could detect the A3243G fragment at a percentage as low as 1.0% when stained with ethidium bromide and 0.2%
with SYBR Green I (Fig. 1). PCR-SSCP could detect 5.0% fragments of A3243G (Fig. 2). The detection limit of RFLP was lower than that of SSCP; however, the detection rates of the A3243G mutation among patients were the same between these two methods because all of the A3243G mutations showed heteroplasmy of >5%.

SCREENING OF THE MUTATIONS

Of 240 diabetic patients examined, five kinds of one-point mutations including A3243G were detected by PCR-SSCP (Figs. 3 and 4). They consisted of A3156G, A3243G, G3357A, C3375A, and T3394C mutations. Only A3243G is heteroplasmic, whereas the others are homoplasmic mutations.

A3243G, A3156G, and G3357A were found only in diabetic patients, whereas C3375A and T3394C were also detected in the controls. No statistically significant differences in the frequencies of mutations were observed between the diabetic patients and controls (Table 1), although the frequencies of the A3243G mutation tended to be higher in the diabetic patients than in the controls ($P = 0.085$).

The A3243G mutation was found in seven patients (one male and six females). The ages of the patients diagnosed to have A3243G mutations were 29–62 years. All patients showed hearing loss and a low height. The mean height of the six female patients was 146.3 ± 2.9 cm, and one male patient was 155 cm in height, all belonging to the lower 2.5 percentile of Japanese. Their clinical profiles are summarized in Table 2. The degree of heteroplasmy for A3243G was significantly correlated with the age at onset of hyperglycemia ($P < 0.05$; Fig. 5).

An A-to-G mutation at np 3156 is located in the 3’ portion of 16S ribosomal RNA. The patient carrying this mutation (case 1 in Table 3) showed the clinical features of type 1 diabetes. The same mutation was shared with her mother and younger sister, and her mother abruptly developed hyperglycemia with a high titer of anti-glutamic acid decarboxylase (GAD) antibody, fell into an insulin-dependent state, and was diagnosed as having type 1 diabetes mellitus 4 years after the detection of the A3156G mutation. The patient’s younger sister, who is 43 years of age, currently shows an impaired glucose tolerance.

G3357A, C3375A, and T3394C mutations are located in the coding region of ND1, the gene that encodes NADH dehydrogenase subunit 1. G3357A and T3394C are missense mutations (Met17Ileu and Tyr30His, respectively), and C3375A is a silent mutation. The patient with G3357A (case 2) had type 1 diabetes and was also positive for the anti-GAD antibody. The C3375A mutation was detected in two patients with type 2 diabetes. The T3394C mutation was found in six patients, all of whom had type 2 diabetes. Four patients (cases 5, 6, 7, and 8) were diagnosed with hyperglycemia between the ages of 51 and 63 years for the first time. They have all maintained relatively good insulin secretion and thus have only undergone diet therapy or drug therapy with hypoglycemic agents. The remaining two patients (cases 9 and 10) became diabetic in their youth and have been treated with insulin. These two patients also showed a relatively high BMI, and their clinical features are summarized in Table 3.

Discussion

Mitochondrial DNA is predominantly inherited maternally. Less than 0.1% of the mitochondrial DNA is contributed by a sperm. Each cell contains hundreds of mitochondria and thousands of copies of the mitochondrial DNA genome. Therefore, cells can harbor a mixture of mutant and wild-type mitochondrial DNAs, a phenom-
enon called heteroplasmy. Over multiple cell divisions, the proportion drifts toward either predominantly mutant or wild-type mitochondrial DNAs, leading to homoplasmy. The percentage of mitochondrial DNA with mutations varies from tissue to tissue, and is observed to be the highest in the affected tissues (7, 11, 12). In the case of diabetic patients with the A3243G mutation, which was responsible for a deterioration in insulin secretion, pancreatic β cells showed the highest percentage of heteroplasmy (13), and therefore β cells were considered the most suitable tissue for the examination. However, obtaining β cells by a biopsy of the pancreas is a hazardous and difficult procedure. The peripheral blood leukocytes are relatively easy to obtain and, therefore, are used most frequently to screen for gene mutations, although the observed percentages of heteroplasmy might be lower than those in the affected tissue (14, 15). In our diagnostic system, DNA is extracted from peripheral blood leukocytes, and then PCR is performed, followed by SSCP or RFLP. It takes 4.5–5.0 h to complete all processes, which makes it possible to diagnose mitochondrial DNA mutations the same day the sample is taken. The lower limit of detection for A3243G heteroplasmy by PCR-RFLP is 1.0% with ethidium bromide staining and 0.2% with SYBR Green I staining, whereas in PCR-SSCP, the lower limit of detection is 5.0% in our system. By the use of ligation-mediated PCR method, which can detect 0.01% heteroplasmy, only diabetic patients, and not controls, have been reported to show >0.01% heteroplasmy (16). As a result, the possibility of overlooking mutated mitochondrial DNAs if their number is <0.2% cannot be ruled out. However, ligation-mediated PCR takes a much longer time than PCR-RFLP or PCR-SSCP, and thus is not suitable for routine screening methods.

Among the 240 patients investigated, we identified 7 patients who had the A3243G mutation. The prevalence of this mutation was 2.9%, and this finding correlates with reports that the prevalence was within a range of 1–3% in Japanese diabetic populations (6–8, 17). In patients with the A3243G mutation, impaired insulin secretion tends to become overt between 20 and 30 years of age. Once a patient becomes diabetic, the amount of secretable insulin progressively declines. Thus, all cases in whom we detected the A3243G mutation had to receive insulin therapy. Among the six female patients carrying the mutation, four had experienced an abnormal pregnancy, leading to either still birth or premature birth. In these cases, it appears that hyperglycemia might have occurred during pregnancy for the first time. Among the population of Japanese women attending a diabetic pregnancy clinic, it was reported (18) that the prevalence of the A3243G mutation was higher than that observed in randomly selected Japanese diabetic patients. Moreover, the prevalence of spontaneous abortions in patients with A3243G was also higher than in those without this mutation.

### Table 1. Prevalence of mitochondrial gene mutations in diabetics and controls.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Diabetic patients, %</th>
<th>Controls, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3243G</td>
<td>2.9 (7/240)</td>
<td>0 (0/125)</td>
<td>0.057</td>
</tr>
<tr>
<td>A3156G</td>
<td>0.4 (1/240)</td>
<td>0 (0/125)</td>
<td>0.471</td>
</tr>
<tr>
<td>G3357A</td>
<td>0.4 (1/240)</td>
<td>0 (0/125)</td>
<td>0.471</td>
</tr>
<tr>
<td>C3375A</td>
<td>0.8 (2/240)</td>
<td>0.8 (1/125)</td>
<td>0.974</td>
</tr>
<tr>
<td>T3394C</td>
<td>2.5 (6/240)</td>
<td>3.2 (4/125)</td>
<td>0.706</td>
</tr>
</tbody>
</table>

### Table 2. Clinical characterization of the diabetic patients having the A3243G mutation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Type of diabetes mellitus</th>
<th>Therapy</th>
<th>Sex</th>
<th>Age, years</th>
<th>Age at onset, years</th>
<th>Height, cm</th>
<th>BMI, kg/m²</th>
<th>F-IRI,a pmol/L</th>
<th>F-CPR, pmol/L</th>
<th>Heteroplasmy of A3243G, %</th>
<th>Hearing loss</th>
<th>Cardiomyopathy</th>
<th>Abnormal pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Type 2</td>
<td>Insulin</td>
<td>F</td>
<td>62</td>
<td>58</td>
<td>150</td>
<td>14.2</td>
<td>60.0</td>
<td>0.50</td>
<td>7.4</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>Type 2</td>
<td>Insulin</td>
<td>F</td>
<td>51</td>
<td>27</td>
<td>147</td>
<td>13.9</td>
<td>34.8</td>
<td>0.30</td>
<td>15.0</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Type 2</td>
<td>Insulin</td>
<td>F</td>
<td>29</td>
<td>23</td>
<td>144</td>
<td>16.4</td>
<td>12.0</td>
<td>ND</td>
<td>38.9</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Type 1</td>
<td>Insulin</td>
<td>F</td>
<td>42</td>
<td>24</td>
<td>142</td>
<td>16.9</td>
<td>ND</td>
<td>&lt;0.10</td>
<td>18.9</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>Type 2</td>
<td>Insulin</td>
<td>F</td>
<td>42</td>
<td>30</td>
<td>148</td>
<td>15.5</td>
<td>23.4</td>
<td>0.36</td>
<td>18.2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Type 1</td>
<td>Insulin</td>
<td>M</td>
<td>49</td>
<td>30</td>
<td>147</td>
<td>15.3</td>
<td>ND</td>
<td>0.10</td>
<td>13.6</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Type 2</td>
<td>Insulin</td>
<td>M</td>
<td>45</td>
<td>37</td>
<td>155</td>
<td>14.6</td>
<td>ND</td>
<td>0.33</td>
<td>18.6</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*a F-IRI, fasting immunoreactive insulin; F-CPR, fasting reactive C peptide immunoreactivity; ND, not determined.

b +, positive; −, negative.
Therefore, the diagnosis of the A3243G mutation before a hyperglycemic state develops seems to be very important in female patients not only to prevent hyperglycemia, but also to prevent abnormal pregnancy. The percentages of heteroplasmhy for the A3243G mutation that were detected with amplified DNA from peripheral leukocytes were significantly associated with age at the onset of hyperglycemia (P < 0.05; Fig. 5). Although various percentages of heteroplasmhy were observed in the tissues and organs, heteroplasmhy in leukocytes was thought to be associated with heteroplasmhy in the pancreatic β cells. These findings may make it possible to predict the onset of hyperglycemia for the siblings and descendants of the patients who were unaffected at the time of diagnosis, while also allowing for a better control of blood glucose and the prevention of diabetic complications.

Regarding the etiology of impaired glucose tolerance, the A3243G mitochondrial gene mutation is thought to be a cause of impaired, glucose-induced insulin secretion. The clonal cybrid cells between the cells lacking mitochondrial DNA and fibroblasts from a patient with the A3243G mutation showed poor respiration, an increased ratio of lactate to pyruvate, and marked defects in the mitochondrial morphology and respiratory chain complexes I and IV activities (19). Janssen et al. (20) indicated that cells harboring patient-derived mitochondria with the A3242G mutation displayed a severe loss of respiration, whereas the rate of mitochondrial translation was not seriously affected despite the low degree of leucylation of tRNA\textsubscript{Leu} (UUR). Chomyn et al. (21) observed that cell lines that were nearly homoplasmic for this mutation exhibited a strong (70–75%) reduction in the degree of aminoacylated tRNA\textsubscript{Leu} (UUR), as well as a decrease in mitochondrial protein synthesis. They also revealed a reduced association of mRNA with ribosomes, possibly attributable to the presence of a tRNA\textsubscript{Leu} (UUR) aminoacylation defect. The cultured β-cell line MIN6 with knocked-out mtDNA (ρ\textsuperscript{0} MIN6) showed a loss of mitochondrial transcription, translation, and respiration activity and a glucose-stimulated increase in insulin secretion (22). Similar results were also obtained for the mitochondrial DNA-deficient ρ\textsuperscript{0} INS-1 cells established by Kennedy et al. (23).

In addition to the A3243G mutation, we detected four types of one-point mutations in this region of the mitochondrial gene. These mutations were A3156G, G3357A, C3375A, and T3394C. A3156G, G3357A, and C3375A were novel mutations. The np 3156 in the mitochondrial gene is located in the region close to the 3′ end of 16S rRNA, whereas 3357, 3375, and 3394 are located in the coding region of NADH dehydrogenase subunit 1 (ND1). The two mutations, G3357A and T3394C, induce an exchange of amino acids. In the case of G3357A, methionine is substituted for isoleucine and, in T3394C, tyrosine for histidine. It has not yet been determined whether these mutations cause mitochondrial dysfunctions, which reduce insulin secretion in the affected patients. However, some groups of the patients with the same mutation tended to show very similar clinical manifestations, including a sudden onset of hyperglycemia and a rapid progression to an insulin-dependent state with positive anti-GAD antibody in the case of the A3156G mutation, and a mild defect in glucose-stimulated insulin secretion and the appearance of hyperglycemia with aging or obesity in the cases of the T3394C mutation. These diabetic states are suggested to result from the same etiologies related to the same mutations in the mitochondrial gene.

The A3156G mutation was detected in three subjects in one family. The proband (case 1 in Table 3) and her mother became abruptly hyperglycemic and fell into an insulin-dependent state at 42 and 70 years of age, respectively, thus showing the typical clinical features of type 1 diabetes mellitus. The mother had anti-GAD antibody with a high titer at onset, whereas the proband was negative at the time of the investigation, 3 years after her own onset of the disease. Her younger sister had the same mutation and showed impaired glucose tolerance at the time of the investigation when the sister was 42 years of age. Although we could not complete the pedigree of the A3156G mutation because the extended family declined.

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation</th>
<th>Type of diabetes mellitus</th>
<th>Therapy</th>
<th>Sex</th>
<th>Age, years</th>
<th>Age at onset, years</th>
<th>BMI, kg/m(^2)</th>
<th>F-IRI(^a), pmol/L</th>
<th>F-CPR, pmol/L</th>
<th>Family history</th>
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<tr>
<td>1</td>
<td>A3156G</td>
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<td>Insulin</td>
<td>F</td>
<td>45</td>
<td>42</td>
<td>21.3</td>
<td>21.6</td>
<td>0.07</td>
<td>+(^b)</td>
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<td>Insulin</td>
<td>F</td>
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<td>16</td>
<td>23.9</td>
<td>ND</td>
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<td>+</td>
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<td>3</td>
<td>C3375A</td>
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<td>Diet</td>
<td>M</td>
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<td>66</td>
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<td>33.0</td>
<td>1.03</td>
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<td></td>
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<td>Diet</td>
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<td>8</td>
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<td>59</td>
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<td>34.8</td>
<td>ND</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
</tr>
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\(^{a}\) F-IRI, fasting immunoreactive insulin; F-CPR, fasting reactive C peptide immunoreactivity; ND, not determined.

\(^{b}\) +, positive; −, negative.
to participate in further studies, the examined subjects who demonstrated the A3156G mutation might represent a group of phenotypes with slowly progressive insulin-dependent diabetes mellitus (24, 25). Recent studies have shown that mitochondria are frequently involved in a variety of key events of apoptosis, including the release of such caspase activators as cytochrome c, changes in electron transport, a loss of mitochondrial transmembrane potential, altered cellular oxidation-reduction, and participation of pro- and antiapoptotic Bcl-2 family proteins (26, 27). The mitochondrial dysfunction, possibly resulting from the translational disorders produced by the mutated 16S rRNA with A3156G, could be associated with the apoptosis of β cells in islets, which is considered one of the causes of type 1 diabetes mellitus (28, 29).

Apoptotic features were observed in the muscle fibers of patients carrying a high percentage of single mtDNA deletions and tRNA point mutations including A3243G (30), but not in the islet β cells of patients with A3243G (13, 31).

A case of type 1 diabetes with the G3357A mutation (case 2) showed a high titer of anti-GAD antibody and was complicated with Down syndrome. Her mother showed impaired glucose tolerance by a 75-g oral glucose tolerance test.

The C3375A mutation was detected in two patients with type 2 diabetes. They showed a slight increase of blood glucose, and diet therapy has been introduced, thus leading to good control of the glucose concentration. The proband, an 8-year-old girl with the C3375A mutation (case 4) had an early onset of type 2 diabetes, whereas her mother showed normal glucose tolerance with a low insulin response after the 75-g oral glucose tolerance test. Her elder brother and sister also showed impaired glucose tolerance in their early teens.

The T3394C mutation has previously been reported to be implicated in Leber hereditary optic neuropathy (32, 33) and also in patients with type 2 (non-insulin-dependent) diabetes mellitus in the United Kingdom (34) and in Japan (35). All patients with the T3394C mutation who were detected by our screening process also showed the clinical features of type 2 diabetes. Among the four cases whose onset of disease started at more than 50 years of age, three showed a normal BMI and a mild disturbance in glucose tolerance and were treated with either dietary treatment or with acarbose or glibenclamide (cases 5, 7, and 8). On the other hand, patients who demonstrated diabetes before 40 years of age (cases 9 and 10) and had BMIs of 27.2 and 28.0, respectively, were placed on insulin therapy. In the case of this T3394C mutation, the disorder of insulin secretion might become overt when combined with such factors as aging or obesity. The prevalence of this mutation was 2.5% among diabetic patients and 3.2% among the controls according to our screening, whereas the prevalence was 4.9% among diabetic patients and 1.3% among the controls in another study on Japanese patients with non-insulin-dependent diabetes mellitus (35). Because the controls in our study were younger than the patients and were not age-matched to the patient group, there is a possibility that the control group might have contained some individuals who might become diabetic in middle age by aging or obesity. In addition, it is also possible that this T3394C mutation is a modifier locus for one or many other genes.

As a result of this screening method, we found both novel and known mutations of the mitochondrial gene and determined the potential clinical subtypes based on the site of the mitochondrial gene mutations. Our findings regarding mitochondrial gene mutations should provide valuable information related to the preclinical diagnosis of diabetes mellitus, the treatment and follow-up of such diabetic patients, and the identification of their relatives with maternally transmitted mitochondrial gene mutations.

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References
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