

# Diabetes With Mitochondrial Gene tRNA<sup>LYS</sup> Mutation

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**OBJECTIVE** — To solve a possible relationship between mtDNA mutation of tRNA<sup>LYS</sup>(8344) and diabetes, we have surveyed the tRNA<sup>LYS</sup> mutation, glucose intolerance, and insulin secretory capacity in a Japanese family with diabetes and myoclonic epilepsy with ragged-red fiber disease. Several lines of evidence suggested possible linkage between mtDNA mutation and diabetes (1–4).

**RESEARCH DESIGN AND METHODS** — DNA was isolated from peripheral lymphocytes. The polymerase chain reaction analysis for the tRNA<sup>LYS</sup>(8344) mutation of the mtDNA was conducted as described by Larsson (5). Insulin secretory capacity was assessed by 24-h urinary C-peptide immunoreactivity response (CPR) excretion and plasma CPR to glucagon administration.

**RESULTS** — We identified seven subjects with the tRNA<sup>LYS</sup> mutation as well as seven nonmutated members in the pedigrees. Oral glucose tolerance tests in the pedigree indicated that five of the mutated subjects were diabetic, one had impaired glucose tolerance, and one had normal glucose tolerance (NGT), whereas all nonmutated family members had NGT. The pedigree shows maternal transmission of diabetes and the tRNA<sup>LYS</sup> mutation over three generations. Twenty-four-hour urinary excretion of CPR was significantly reduced in the mutant subjects (mean  $\pm$  SD, 67.8  $\pm$  79.2 nmol/day,  $n = 6$ ,  $P < 0.001$ ) compared with the nonmutant members (276.6  $\pm$  41.8 nmol/day,  $n = 5$ ) and the age-matched normal control subjects (263  $\pm$  64.3 nmol/day,  $n = 12$ ). Plasma CPR 6 min after glucagon injection demonstrated a marked reduction in the mutant subjects (3.68  $\pm$  3.45 nmol/l,  $n = 5$ ,  $P < 0.001$ ) compared with the nonmutant members (19.4  $\pm$  1.17 nmol/l,  $n = 5$ ) and the normal control subjects (15.8  $\pm$  3.81 nmol/l,  $n = 12$ ). Bilateral neurosensory deafness was demonstrated in five of seven (71.4%) mutant subjects (five of five [100%] mutated diabetic patients), but not detected in six nonmutant members.

**CONCLUSIONS** — This observation is the first report of association of diabetes with the mitochondrial tRNA<sup>LYS</sup> mutation. Insulin secretory capacity was significantly lower in the mutant members than in the nonmutated members. These findings suggest that the pancreatic  $\beta$ -cell secretory defect of insulin might be one of the phenotypes of the mitochondrial tRNA<sup>LYS</sup> mutation.

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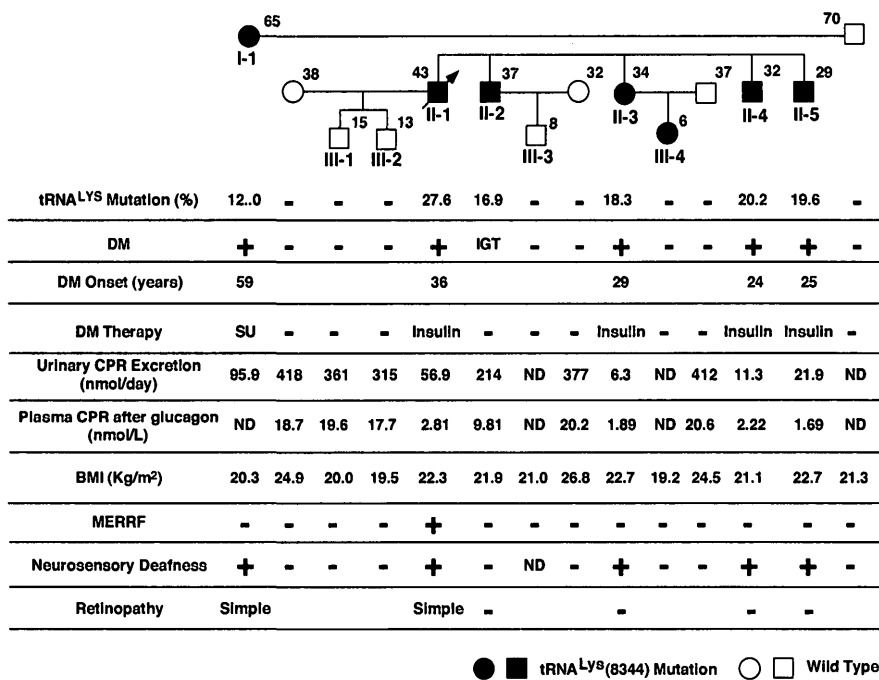
MERRF, myoclonic epilepsy with ragged-red fiber; ICA, islet cell antibody; CPR, C-peptide immunoreactivity response; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; IGT, impaired glucose tolerance; IDDM, insulin-dependent diabetes mellitus; PCR, polymerase chain reaction; bp, base pair; CCR, creatinine clearance; BMI, body mass index; CPEO, chronic external ophthalmoplegia; KSS, Kearns-Sayre syndrome.

Mitochondrial gene mutations, such as tRNA<sup>LEU(UUR)</sup>(3243), deletion, and duplication, are known to be associated with diabetes or glucose intolerance (1–4). Myoclonic epilepsy with ragged-red fibers (MERRF) is a neuromuscular disorder characterized by mitochondrial myopathy and progressive myoclonus epilepsy (6). A heteroplasmic A-to-G transition mutation in the mitochondrial encoded tRNA<sup>LYS</sup> gene at nucleotide pair 8344 has been suggested to be linked to the MERRF (6). We have investigated the mitochondrial mutations in a Japanese family, including a case with insulin-treated diabetes and MERRF and three diabetic patients. We have identified maternal transmission of diabetes and a point mutation of tRNA<sup>LYS</sup> over three generations. Possible linkage between diabetes and the tRNA<sup>LYS</sup> mutation in the pedigree is discussed.

## RESEARCH DESIGN AND METHODS

### Case report

The proband's pedigree is shown in Fig. 1. The proband (II-1) was diagnosed with diabetes at the age of 36 and controlled it with diet and an oral hypoglycemic agent until the age of 40, when he required insulin injection. He began to have a mild degree of hearing disturbance and optic atrophy at the age of 39. He had progressive muscle weakness and atrophy, mainly in the proximal muscles, and myoclonic seizures since the age of 41. Histological examination of muscle biopsy specimens demonstrated ragged-red fiber. Islet cell antibodies (ICAs) were negative at the age of 42. He was diagnosed with simple diabetic retinopathy at the age of 43. His insulin secretory capacity was considered low, based on low urinary C-peptide immunoreactivity response (CPR) excretion (56.9 nmol/day) (normal control subject, 263  $\pm$  64.3 nmol/day,  $n = 12$ ) and a low plasma CPR to glucagon (2.81 nmol/l) (normal con-



**Figure 1**—Pedigree of proband's family. Numbers on the shoulder of the symbols are subjects' ages during the study. tRNA<sup>LYS</sup> mutation was determined as described in METHODS. Twenty-four-hour urinary CPR excretion and plasma CPR 6 min after glucagon injection in the normal control subjects are  $263 \pm 64.3$  nmol/day and  $15.8 \pm 3.81$  nmol/l (mean  $\pm$  SD,  $n = 12$ ). ND, not determined.

control subject,  $15.8 \pm 3.81$  nmol/l,  $n = 12$ ). Two of the proband's children (III-1, III-2) and his wife had normal glucose tolerance (NGT). The proband's mother (I-1) was diagnosed with diabetes after an oral glucose tolerance test (OGTT) at the age of 59. She was treated with diet therapy for 2 years and then treated with an oral hypoglycemic agent until the age of 61. She was diagnosed with simple diabetic retinopathy at the age of 63. Her urinary CPR excretion (95.9 nmol/day) was moderately low. The proband's father had NGT. The proband's brother (II-2) was diagnosed with impaired glucose tolerance (IGT) at the age of 24. His insulin secretory capacity was considered normal, based on urinary CPR excretion (214 nmol/day) and plasma CPR to glucagon (9.81 nmol/l). His son (III-3) and his wife had NGT. The proband's sister (II-3) was diagnosed with ketosis-prone insulin-dependent diabetes mellitus (IDDM) at the age of 29 and then treated with inten-

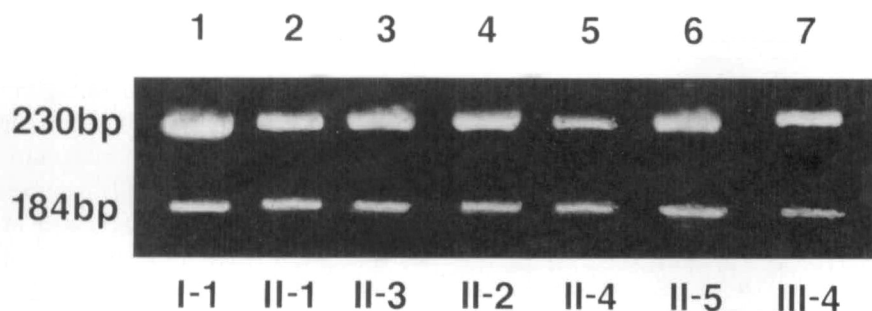
sive insulin therapy. She began to have sensory hearing loss at the age of 31. Her urinary CPR excretion (6.3 nmol/day) and plasma CPR to glucagon (1.89 nmol/l) were low. Her son (III-4) and her husband had NGT. The proband's brother (II-4) was diagnosed with diabetic ketoacidosis at the age of 24 and then treated with intensive insulin therapy. He recently had mild sensory hearing loss. His urinary CPR excretion (11.3 nmol/day) and plasma CPR to glucagon (2.22 nmol/l) were low. His brother (II-5) was diagnosed with IDDM at the age of 25 and then treated with intensive insulin therapy. He had episodes of diabetic ketoacidosis twice at the ages of 26 and 28. He began to have sensory hearing loss at the age of 28. His insulin secretory capacity was considered low, based on low urinary CPR excretion (21.9 nmol/day) and low plasma CPR to glucagon (1.69 nmol/l). No family members except the pro-

band had myoclonus, muscle weakness, optic atrophy, or MERRF symptoms.

Eight male and six female healthy, lean, age-matched individuals with no diabetic relatives formed the normal control group. All the proband's family members and the normal control subjects were investigated with fully informed consent. The research protocol was approved by the Tohoku University Institutional Review Board.

### Preparation of DNA and polymerase chain reaction (PCR) amplification

DNA was isolated from peripheral lymphocytes. The PCR analysis to detect the tRNA<sup>LYS</sup> mutation was conducted by the method of Larsson (5). The asymmetric primer method, with primers corresponding to nucleotides 8161–8180 (H81; 50 pmol) of the heavy strand and to nucleotides 8537–8556 (L85; 4 pmol) of the light strand of mtDNA, was used to generate single-stranded DNA for direct sequencing with a primer corresponding to nucleotides 8411–8430 (L84) of the light strand of mtDNA. A primer corresponding to nucleotides 8345–8390 (L83) of the light strand of mtDNA, with the normal AG dinucleotide at nucleotides 8352 and 8353 replaced by a CC dinucleotide, was synthesized. This primer creates a Bgl I restriction-enzyme site around nucleotide 8344 when mtDNA with the tRNA<sup>LYS</sup> mutation is amplified. The PCR reaction with the primers L83 and <sup>32</sup>P-end-labeled H81 was carried out for 30 cycles on a thermal cycler; denaturation was at 94°C for 1 min, annealing at 72°C for 1 min, and extension at 72°C for 1 min. Approximately 0.1–0.2  $\mu$ g of the resulting 230 base pair (bp) radiolabeled PCR fragment was digested overnight with 27 U of Bgl I and electrophoresed in 6% acrylamide gels. Two radiolabeled fragments, one of 230 bp, corresponding to normal mtDNA, and the other of 184 bp, corresponding to mtDNA with the tRNA<sup>LYS</sup> mutation, were detected.



**Figure 2**—PCR analysis of the pedigree. PCR amplification of DNA from peripheral leukocytes of the members and Bgl I digestion were done as described in METHODS. The upper band, 230 bp, is the fragment obtained from normal mtDNA. The lower band, 184 bp, is the fragment obtained from mtDNA with the tRNA<sup>LYS</sup> mutation.

### Assessment of insulin secretory capacity

At diagnosis, all patients fulfilled the World Health Organization criteria for diabetes and IGT (7). Insulin secretory capacity of pancreatic  $\beta$ -cells was evaluated by urinary CPR excretion for 24 h and plasma CPR 6 min after intravenous administration of 1 mg glucagon. Urinary excretion of CPR and creatinine clearance (CCr) for 24 h were estimated simultaneously. All studied subjects had normal CCr for 24 h. Plasma glucose was assayed using the glucose oxidase method. Plasma CPRs were assayed using radioimmunoassay.

### Statistical analysis

Statistical analysis was made by means of the unpaired Student's *t* test.  $P < 0.05$  was considered statistically significant.

**RESULTS**— We surveyed mtDNA mutation of tRNA<sup>LYS</sup> in a Japanese family with diabetes and MERRF. As shown in Fig. 2, we identified seven subjects with the tRNA<sup>LYS</sup> mutation as well as seven nonmutated subjects. We amplified the region encoding tRNA<sup>LYS</sup> by PCR, determined its sequence, and identified an A-to-G transition at nucleotide 8344 in the tRNA<sup>LYS</sup> gene (data not shown). The proband (II-1) showed normal and mutant genes of tRNA<sup>LYS</sup>. His mother (I-1), brothers (II-2, II-4, II-5), and sister (II-3) were also heteroplasmic for the tRNA<sup>LYS</sup>

mutation, whereas the proband's children (III-1, III-2) and his brother's child (III-3) were not mutant. His niece (III-4) was also heteroplasmic for the tRNA<sup>LYS</sup> mutation. OGTT demonstrated that his brother (II-2) had IGT. Five of the mutated members were diabetic, one had IGT, and one had NGT, but all nonmutated members were NGT, suggesting high association of the tRNA<sup>LYS</sup> mutation with glucose intolerance. Audiometry examination demonstrated bilateral neurosensory deafness in II-3, II-4, and II-5. Muscle weakness, myoclonus seizure, or other neurological signs were not shown in the mutated members, except for the proband.

Insulin secretory capacity of the family members with the mutation was compared with the nonmutated subjects and the normal control subjects. Ages of the mutant, the nonmutant members, and the normal control subjects were  $35.0 \pm 17.9$ ,  $38.4 \pm 21.3$ , and  $46.5 \pm 17.9$  years (mean  $\pm$  SD), respectively. Body mass indexes (BMIs) of the mutant, the nonmutant members, and the control subjects were  $21.5 \pm 1.3$ ,  $22.6 \pm 2.8$ , and  $22.5 \pm 2.2$  kg/m<sup>2</sup>, respectively. Ages and BMIs were not significantly different in the three groups. Twenty-four-hour urinary CPR excretion was also significantly reduced in the mutant members ( $67.8 \pm 79.2$  nmol/day,  $n = 6$ ,  $P < 0.001$ ) compared with the nonmutant members ( $276.6 \pm 41.8$  nmol/day,  $n = 5$ ) and the

age-matched normal control subjects ( $263 \pm 64.3$  nmol/day,  $n = 12$ ). Plasma CPR 6 min after glucagon injection demonstrated a marked reduction in the mutant subjects ( $3.68 \pm 3.45$  nmol/l,  $n = 5$ ,  $P < 0.001$ ) compared with the nonmutant members ( $19.4 \pm 1.17$  nmol/l,  $n = 5$ ) and the normal control subjects ( $15.8 \pm 3.81$  nmol/l,  $n = 12$ ). Bilateral neurosensory deafness was demonstrated in five of seven (71.4%) mutant subjects (five of five [100%] mutated diabetic patients), but not detected in six nonmutant members.

**CONCLUSIONS**— A heteroplasmic mtDNA mutation of tRNA<sup>LYS</sup> has been suggested to be linked to the MERRF syndrome (5,6). Holme et al. (8) reported a case of multiple lipomas with high levels of the tRNA<sup>LYS</sup> mutation in a carrier of MERRF. Neurosensory deafness has also been associated with the tRNA<sup>LYS</sup> mutation (6). This study demonstrates that tRNA<sup>LYS</sup> mutation appears to be associated with diabetes in this pedigree, and the tRNA<sup>LYS</sup> mutation and diabetes are maternally inherited. This study shows significant reduction in insulin secretion in the mutated subjects compared with the nonmutated members and the normal control subjects. It has been speculated that a mitochondrial mutation-related defect of oxidative phosphorylation in the endocrine cells might result in reduction of hormone secretion (9–14). Several studies described reduced insulin secretion in diabetes with chronic external ophthalmoplegia (CPEO) and Kearns-Sayre syndrome (KSS) (9–14). We have reported that insulin secretory capacity of pancreatic  $\beta$ -cells in the tRNA<sup>LEU(UUR)</sup> (3243) mutation was severely impaired, as evaluated by the insulinogenic index in OGTT, 24-h urinary excretion of CPR, and plasma CPR 6 min after intravenous administration of glucagon (15). Kadowaki et al. (16) and Awata et al. (17) demonstrated a significant reduction of maximal insulin secretory capacities and early secretion response of insulin to glu-

cose administration in diabetic patients with the tRNA<sup>LEU(UUR)</sup>(3243) mutation.

The proband was diagnosed with slowly progressive IDDM, and three mutated subjects (II-3, II-4, and II-5) in the pedigree were diagnosed with ketosis-prone IDDM. IDDM is known to be associated with CPEO and KSS (11). Oka et al. (18) reported that the tRNA<sup>LEU(UUR)</sup>(3243) mutation was detected in 3 of 27 ICA-positive patients who were initially diagnosed with non-insulin-dependent diabetes mellitus and then progressed to insulin dependency within a few years. They speculated that the tRNA<sup>LEU(UUR)</sup>(3243) mutation may cause gradual  $\beta$ -cell destruction. Although ICA was negative in the proband at age 42, the tRNA<sup>LYS</sup> mutation in the proband, II-3, II-4, and II-5 might cause gradual  $\beta$ -cell destruction to insulin dependency. Larsson et al. (5) reported that >92% of mtDNA with the tRNA<sup>LYS</sup> mutation in muscle is required to cause a respiratory-chain dysfunction that can be detected by biochemical methods. They demonstrated a positive correlation between the levels of mtDNA with the tRNA<sup>LYS</sup> mutation in lymphocytes and the levels in muscle. The levels of mutated mtDNA were lower in lymphocytes than in muscle. The mutated mtDNA levels in lymphocytes of the mutated subjects were 12.0–27.6%, as estimated by the method of Larsson (5). Because we could not determine the levels of mutated mtDNA in pancreatic  $\beta$ -cells, we do not have direct evidence that the tRNA<sup>LYS</sup> mutation causes a respiratory-chain dysfunction in the  $\beta$ -cells. We could not detect any direct correlation between the percentage of mutation in peripheral leukocytes and 24-h urinary CPR excretion or plasma CPR 6 min after glucagon injection. It is difficult to assess  $\beta$ -cell dysfunction as a primary failure or a secondary failure in the patients with long-standing diabetes. However, duration of diabetes in the diabetic patients in the family was <7 years. The fact that 24-h urinary CPR excretion and plasma CPR 6 min after glucagon injection were decreased in the mutated

IGT subject (II-2) and in the proband's siblings (II-3, II-4, and II-5) with ketoacidosis-onset of diabetes or ketosis-prone IDDM might suggest the presence of primary  $\beta$ -cell failure in these mutated diabetic patients. Further study is needed to resolve the pathological role of the tRNA<sup>LYS</sup> mutation on the insulin secretory defects in pancreatic  $\beta$ -cells.

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