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Pancreatic beta-cell secretory defect associated with mitochondrial point mutation of the tRNA$^{LEU(UUR)}$ gene: a study in seven families with mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)

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Summary Recent evidence suggests possible linkage between diabetes mellitus and mitochondrial gene mutation. We surveyed mitochondrial tRNA$^{LEU(UUR)}$ (3243) mutation in 7 mitochondrial encephalomyopathy, lactic acidosis and stroke-like episode (MELAS) families and identified 24 mutated subjects (7 MELAS probands and 17 non-MELAS relatives) as well as 11 non-mutant family members. An OGTT in the 24 mutant relatives revealed 14 diabetic subjects, 3 with impaired glucose tolerance and 7 with normal glucose tolerance and all non-mutant family members as having normal glucose tolerance. Insulinogenic index was significantly reduced in the mutant diabetic subjects and those with impaired and normal glucose tolerance in comparison with the normal control subjects and the non-mutant members. Urinary 24-h C-peptide immunoreactivity excretion was markedly reduced in the mutant diabetic subjects and those with normal and impaired glucose tolerance, compared with the control subjects and the non-mutant family members. Plasma C-peptide immunoreactivity 6 min after glucagon injection was markedly reduced in the mutant diabetic subjects and those with normal and impaired glucose tolerance compared with the control subjects and the non-mutant family members. Si, an index of insulin sensitivity of the four mutant subjects was within normal range. Islet cell antibodies were negative in sera of eight mutated diabetic subjects, 2 and 6 with impaired and normal glucose tolerance, respectively. Diabetic retinopathy and nephropathy were demonstrated in 7 (50%) and 12 (85.7%) of 14 mutant diabetic subjects, respectively. Neurosensory deafness was demonstrated in 12 (85.7%) of 14 mutated diabetic subjects, (66.7%) of 3 mutated impaired glucose tolerant subjects, but not detected in 6 mutated normal glucose tolerant subjects and 11 non-mutant family members. These findings suggest that the tRNA$^{LEU(UUR)}$ mutation is associated with pancreatic beta-cell secretory defect of insulin. [Diabetologia (1994) 37: 818–825]

Key words C-peptide, diabetes mellitus, insulin secretion, MELAS, mitochondrial gene mutation.

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Abbreviations: MELAS, Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; OGTT, oral glucose tolerance test; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; IRI, insulin-like immunoreactivity; CPR, C-peptide immunoreactivity; ICA, islet cell antibodies; mtDNA, mitochondrial DNA; CPEO, chronic external ophthalmoplegia, NIDDM, non-insulin-dependent diabetes mellitus; AER, albumin excretion rate; KSS, Kearns-Sayre syndrome; IDDM, insulin dependent diabetes mellitus; GAD-Ab, glutamic acid decarboxylase antibody; Kb, kilobase; bp, base pair; BS, blood sugar.

Oxidative phosphorylation in mitochondria possibly has a crucial role in insulin secretion from pancreatic beta cells in response to glucose and other nutrients [1]. The mitochondrial gene mutation is a possible candidate for the pancreatic beta-cell insulin secretory defect in diabetes mellitus. Four types of mutations in mtDNA have been identified as being a cause of maternally-inherited diabetes. Ballinger et al. [2] reported familial diabetes with deafness caused by a 10.4 kb deletion of mtDNA [2]. Rötig et al. [3] also reported on a family with maternally inherited duplication of the mitochondrial genome in a syndrome of diabetes, proximal tubulopathy and cerebellar ataxia. They reported a case of Wolfram syndrome with early-onset diabetes, optic atrophy and deafness caused by 7.6 kb

Table 1. Clinical characteristics of MELAS patients with mitochondrial mutation of $tRNA^{LEU(UUR)}$(3243)

Subject	A-II-1	B-II-1	C-II-2	D-II-1	E-II-1	F-II-1	G-I-1
Sex	Female	Male	Male	Male	Female	Female	Male
Height (cm)	137	158	158	151	154	149	161
Weight (kg)	36	41	31	39	40	46	50
MELAS	+	+	+	+	+	+	+
MELAS onset (years)	10	11	17	17	40	54	61
Stroke-like episode	+	+	+	+	+	+	+
Mental retardation	+	+	+	+	−	−	−
Sensory deafness	+	+	+	+	+	+	+
Serum lactic acid (mmol/l)	4.8	2.47	2.6	1.83	3.48	2.56	3.23
Serum pyruvic acid (mmol/l)	0.14	0.132	0.121	0.011	0.15	0.093	0.132
CSF lactic acid (mmol/l)	4.89	9.82	5.07	3.46	10.2	4.81	ND
CSF pyruvic acid (mmol/l)	0.14	0.29	0.14	0.03	0.23	0.19	ND
Ragged red fibers	+	−	−	−	+	+	+
Mitochondrial 3243 bp mutation	+	+	+	+	+	+	+
NADH-cytochrome c reductase	72.4	45.1	70.2	79.4	37.3	48.7	ND
Succinate dehydrogenase	110.5	ND	102	101.9	93.8	119.2	ND
Succinate cytochrome c reductase	144.6	127.1	75.8	101.8	158.1	147.2	ND
Cytochrome c oxidase	1442	ND	1671	1752	1244	1487	ND
Diabetes present	+	+	+	+	+	+	IGT
Diabetes onset (years)	16	22	26	24	26	53	−

Enzyme activites are expressed in $nmol \cdot mg^{-1} \cdot min^{-1}$. The range of values seen in age-matched healthy control individuals ($n = 8$) is as follows; NADH-cytochrome c reductase: 148.2 ± 25.5 $nmol \cdot mg^{-1} \cdot min^{-1}$; Succinate dehydrogenase: 116.6 ± 27.2 $nmol \cdot mg^{-1} \cdot min^{-1}$; Succinate cytochrome c reductase: 135.0 ± 51.7 $nmol \cdot mg^{-1} \cdot min^{-1}$; Cytochrome c oxidase: 1475 ± 390 $nmol \cdot mg^{-1} \cdot min^{-1}$. ND, Not determined

heteroplasmic deletion of mtDNA [4]. Van den Ouweland et al. [5] and Reardon et al. [6] have reported familial NIDDM with deafness associated with an A to G mutation of $tRNA^{LEU(UUR)}$ (3243). The 3243 bp mutation was originally identified in heteroplasmic form in patients with the MELAS syndrome [7]. The mutation is specific, but not exclusive to MELAS, because it has also been found in patients with CPEO [7], myopathy and cardiomyopathy [8], and maternally inherited diabetes with neurosensory deafness [5, 6]. Several reports have suggested possible linkage between diabetes and the mtDNA mutation [9–13]. However, clinical features of diabetes with the 3243 bp mutation have not yet been characterized.

In this paper, we have identified the 3243 bp mutation in seven MELAS patients and their families and investigated the incidence of diabetes or IGT. We found a high incidence of diabetes and reduced insulin secretion in the mutant subjects and characterized unique clinical features of diabetes. We also discuss the molecular mechanism of how the 3243 bp mutation induces diabetes.

Subjects and methods

Subjects. Seven MELAS patients and 28 subjects from their families were investigated for the mitochondrial gene mutation of $tRNA^{LEU(UUR)}$ (3243). Eight male and six female healthy, lean, age-matched individuals with no diabetic family members or the mitochondrial gene mutation, formed the normal control group. All the subjects gave fully informed consent. The protocol was approved by the Tohoku University Institutional Review Board.

Determination of $tRNA^{LEU(UUR)}$ (3243) mutation. Total DNA was isolated from blood and skeletal muscle. A 427 bp fragment encompassing the $tRNA^{LEU(UUR)}$ mutation site located at nucleotide 3,243 was amplified by polymerase chain reaction using [α-^{32}P]-dATP (1.7 $Ci \cdot mmol^{-1}$) with forward primer 5'-AAGGTTCGTTTGTTCAACGA (from 3,029 to 3,048) and reverse primer 5'AGCGAAGGGTTGTAGTAGCC (from 3,437 to 3,456) [5]. The radioactive fragment was digested with ApaI for 1 h at 37°C, followed by electrophoresis on a 5% nondenaturing polyacrylamide gel. Bands were visualized by autoradiography. The percentage of cleaved fragments was determined by densitometric analysis, as described by Shoffner et al. [14].

Assay of mitochondrial respiratory transfer enzymes. The activities of respiratory transfer enzymes, such as NADH-cytochrome c reductase, succinate dehydrogenase, succinate cytochrome c reductase and cytochrome c oxidase were assayed using freshly purified mitochondria from skeletal muscle biopsy specimens, as described by Miyabayashi et al. [15].

Assessment of insulin secretion capacity, insulin resistance and islet cell antibodies. At diagnosis, all the patients fulfilled World Health Organisation criteria for diabetes and IGT [16]. The insulin secretory capacity of pancreatic beta cells was evaluated using the following procedures: 1) insulinogenic index (ΔIRI/ΔBS(30')) in a 75-g, 2) 24-h urinary CPR excretion and 3) plasma CPR 6 min after i.v. administration of 1 mg glucagon. OGTT, urinary CPR measurement and glucagon tests were performed within 1 month. Measurement of 24-h urinary CPR excretion was started at 08.00 hours and finished at 08.00 hours in the next day. Si was assessed using Bergman's modified minimal model with an additional administration of insulin 20 min after the glucose bolus [17]. Plasma glucose was assayed using the glucose

Table 2. Clinical characteristics of the subjects with or without the mitochondrial mutation of $tRNA^{LEU(UUR)}$(3243) in the MELAS families

Subject		Age	Sex	Muscle mutation (%)	Blood mutation (%)	MELAS	Diabetes	Diabetes onset (years)	Diabetes duration (years)	Diabetes therapy	Insulin dose (IU)	HbA_{1c} (%)	Retinopathy	Nephropathy	Sensorineural deafness	ICA
A-II-1	Proband	18	F	54	ND	+	+	16	2	Diet	–	6.2	–	N	+	ND
A-I-1	Mother	41	F	ND	5	–	+	34	7	SU	–	6.5	SDR	M	+	ND
A-I-2	Father	44	M	ND	–	–	–	–	–	–	–	5	–	–	–	ND
B-II-1	Proband	24	M	59	14	+	+	22	2	Diet	–	6.1	–	N	+	–
B-I-1	Mother	49	F	ND	6	–	+	36	13	Insulin	32	7.2	SDR	P	+	–
B-I-2	Father	50	M	ND	–	–	–	–	–	–	–	4.9	–	–	–	ND
C-II-2	Proband	29	M	49	ND	+	+	26	3	Insulin	26	7.4	–	M	+	–
C-I-1	Mother	54	F	ND	5	–	IGT	–	–	–	–	6.2	–	–	+	–
C-I-2	Father	56	M	ND	–	–	–	–	–	–	–	5.4	–	–	–	ND
C-II-1	Sister-1	32	F	ND	9	–	–	–	–	–	–	5.2	–	–	–	–
C-II-3	Sister-2	26	F	ND	7	–	–	–	–	–	–	5.1	–	–	–	–
D-II-1	Proband	27	M	49	12	+	+	24	3	Diet	–	6.3	–	M	+	–
D-I-1	Mother	50	F	ND	7	–	+	41	9	SU	–	6.6	SDR	P	+	–
D-I-4	Father	56	M	ND	–	–	–	–	–	–	–	5.5	–	–	–	ND
D-II-2	Sister	24	F	ND	8	–	IGT	–	–	–	–	5.9	–	–	–	–
D-I-2	Aunt-1	57	F	ND	5	–	+	44	13	Insulin	22	7.3	SDR	P	+	ND
D-I-3	Aunt-2	53	F	ND	6	–	+	42	11	SU	–	7	SDR	P	+	ND
E-II-1	Proband	40	F	54	12	+	+	26	14	Insulin	28	6.7	–	M	+	–
E-II-3	Husband	45	M	ND	–	–	–	–	–	–	–	5.2	–	–	–	ND
E-I-1	Mother	65	F	ND	6	–	–	–	–	–	–	4.8	–	–	–	–
E-I-2	Father	69	M	ND	–	–	–	–	–	–	–	5.1	–	–	–	ND
E-II-2	Brother	38	M	ND	8	–	–	–	–	–	–	5.3	–	–	–	–
E-III-1	Son	8	M	ND	42	–	–	–	–	–	–	4.9	–	–	–	–
F-II-1	Proband	61	F	48	ND	+	+	53	8	Insulin	34	6.9	SDR	M	+	–
F-II-3	Husband	65	M	ND	–	–	–	–	–	–	–	5.2	–	–	–	ND
F-II-2	Brother	63	M	ND	9	–	+	63	0	Diet	–	6.2	–	M	–	ND
F-III-1	Daughter	26	F	ND	8	–	–	–	–	–	–	5.4	–	–	–	–
F-III-2	Son	21	M	ND	5	–	–	–	–	–	–	5.1	–	–	–	ND
F-I-1	Aunt	58	F	ND	10	–	+	42	16	Insulin	36	7.2	PDR	P	+	–
G-I-1	Proband	61	M	45	12	+	IGT	61	–	–	62	5.9	–	–	+	ND
G-I-3	Wife	57	F	ND	–	–	–	–	–	–	–	4.9	–	–	–	ND
G-II-1	Son	25	M	ND	–	–	–	–	–	–	–	5	–	–	–	ND
G-II-2	Daughter	22	F	ND	–	–	–	–	–	–	–	5.1	–	–	–	ND
G-II-3	Daughter	21	F	ND	–	–	–	–	–	–	–	4.8	–	–	–	ND
G-I-2	Brother	58	M	ND	6	–	+	57	1	SU	–	6.2	–	M	–	ND

SU, Sulphonylurea; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; N, normalbuminuria; M, microalbuminuria; P, proteinuria; ND, not determined

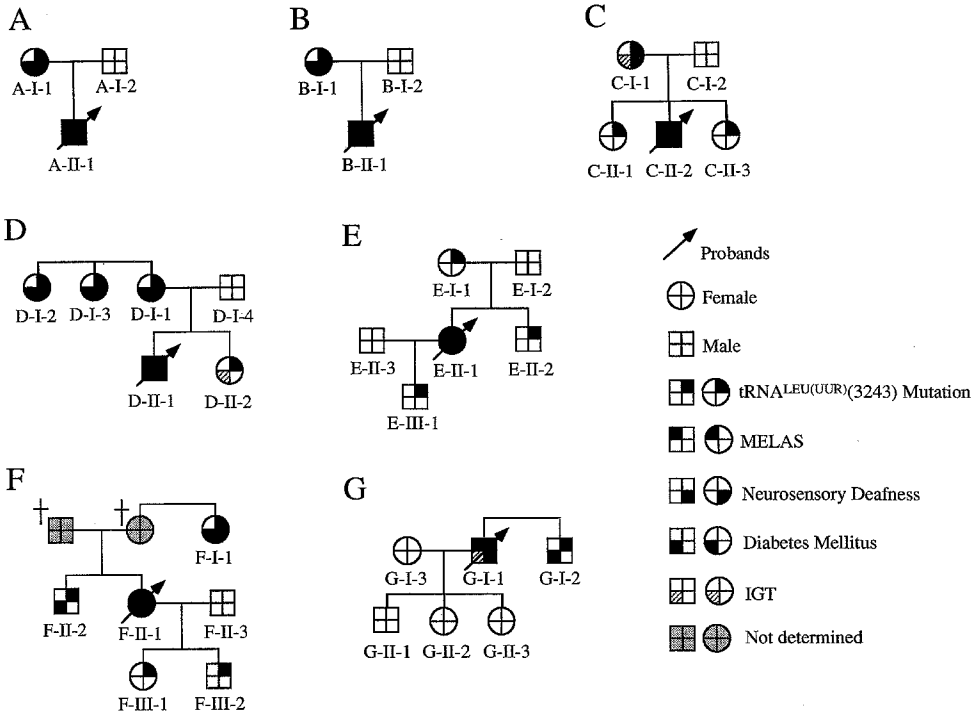

Fig. 1. The family trees of seven MELAS families

oxidase method. Plasma IRI and CPR were assayed using a radioimmunoassay. ICA were assayed by an immunoenzymatic method using fresh frozen sections of human pancreatic tissue, as described previously [18].

Evaluation of diabetic complications. Diabetic retinopathy was evaluated by ophthalmologists using direct fundscopy and classified according to the modified Airlie House System [19]. Diabetic nephropathy was assessed by 24-h AER. Normoalbuminuria, microalbuminuria and proteinuria were judged by AER under 20 μg/min, under 200 μg/min and over 200 μg/min, respectively. Deafness was assessed by pure tone audiometry examination.

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was made by means of the unpaired Student's *t*-test. A *p*-value of less than 0.05 was considered as statistically significant.

Results

Clinical characteristics of MELAS patients with the 3243 mutation. Clinical characteristics of the MELAS probands are shown in Table 1. All the MELAS probands were clinically diagnosed with proximal muscle atrophy, stroke-like episodes, neurosensory deafness, elevation of serum and cerebral-spinal-fluid lactate, as well as elevation of serum and cerebral-spinal-fluid pyruvate. Muscle biopsy showed ragged red fibers in four of seven MELAS patients. Mitochondrial 3243 bp mutation in muscle biopsy specimens was detected in all the probands. Rotenone-sensitive NADH-cytochrome c reductase activities were markedly reduced, but activities of the other enzymes were normal in the muscle mitochondria of MELAS patients, suggesting a significant reduction in mitochondrial respiratory complex I and III activities (Table 1). Six MELAS patients were diagnosed as diabetic and one MELAS patient with IGT. Three diabetic patients were treated with insulin and three were treated with diet therapy. In the younger patients (A-II-1, B-II-1, C-II-2 and D-II-1), MELAS proceeded diabetes by a few years, whereas diabetes proceeded MELAS in two adult-onset MELAS patients (E-II-1 and F-II-1).

Incidence of diabetes with the 3243 bp mutation. In the 35 subjects of the seven MELAS families, the 3243 bp mutation was detected in the 7 probands and the 17 relatives, but not shown in the 11 family members (Table 2). Family trees of the MELAS families are shown in Figure 1. Of 24 subjects with the 3243 bp mutation, 14 were diabetics (51.9%), 3 had IGT (12.5%) and 7 had NGT (29.2%). Treatment of the diabetic patients was as follows: insulin therapy, six patients; sulphonylurea therapy, four patients; and diet therapy, four patients. All six insulin-treated patients, who were initially thought to be NIDDM and treated with diet or sulphonylurea, progressed to insulin-dependence 2–8 years after the onset of diabetes. HbA_{1c} (%) in the mutant diabetic subjects was 6.70 ± 0.464 ($n = 14$), suggesting stable control of diabetes. HbA_{1c} (%) in the IGT, NGT, non-mutant members and the normal control subjects was 6.01 ± 0.17 ($n = 3$), 5.11 ± 0.21 ($n = 7$), 5.19 ± 0.39 ($n = 11$) and 5.21 ± 0.28 ($n = 12$), respectively. Ages of the mutant diabetic, IGT, NGT, the non-mutant family

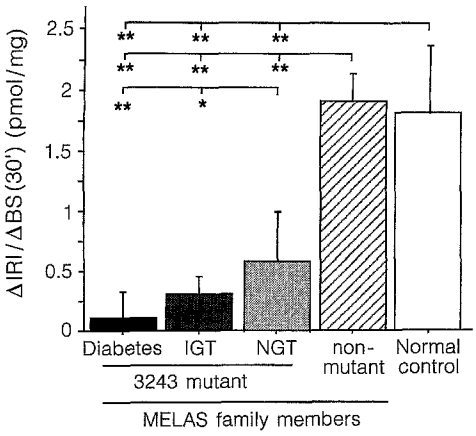

Fig. 2. Insulinogenic index, ΔIRI/ΔBS(30') on OGTT was compared between the mutant diabetes (8), IGT (3), NGT (7), non-mutant members (10) and the normal control subjects (11). Data are shown as mean ± SD. $^{*}p < 0.02$, $^{**}p < 0.001$

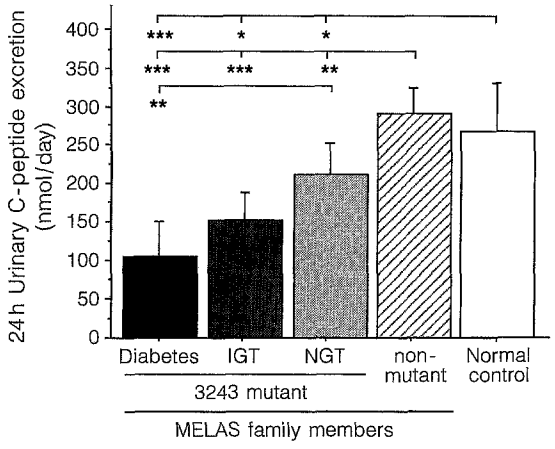

Fig. 3. Urinary 24-h CPR excretion was compared between the mutant diabetic (14), IGT (3), NGT (4), non-mutant family members (7) and the normal control subjects (12). Data are shown as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

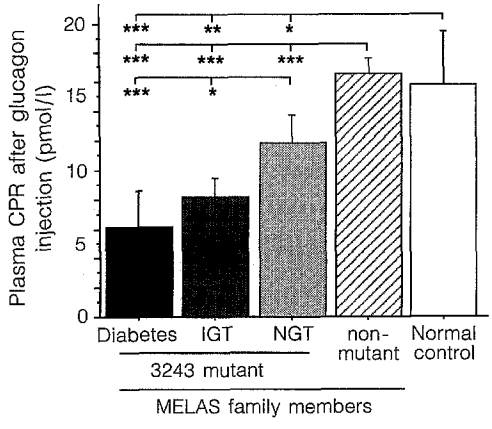

Fig. 4. Plasma CPR 6 min after glucagon injection was compared between the mutant diabetes (7), IGT (3), NGT (6), non-mutant family members (3) and the normal control subjects (12). Data are shown as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

members and the normal control subjects were 44.9 ± 15.1, 46.3 ± 19.7, 30.9 ± 17.7, 46.5 ± 17.9 and 42.4 ± 18.3 years, respectively. Body mass index (BMI) of the mutant diabetic subjects, IGT, NGT, the non-mutant family members and the normal control subjects were 20.4 ± 3.1, 21.0 ± 2.3, 21.6 ± 2.9, 22.4 ± 2.6 and 22.5 ± 2.2, respectively. Ages and BMI were not significantly different in these four groups. Absence of the mutation in the five fathers of the MELAS probands and in the son (G-II-1) and daughters (G-II-2, G-II-3) of the male proband (G-I-1), as well as presence of mutation in sons and daughters of the mutant mothers, suggest maternal transmission of the 3243 bp mutation. These 17 relatives did not have any eyelid ptosis, ophthalmoplegia, muscle weakness or stroke-like episodes.

Pancreatic islet beta-cell function, insulin sensitivity and islet cell antibodies in the subjects with the 3243 bp mutation. Insulin secretory response to oral glucose was evaluated in the mutant subjects, as shown in Figure 2. ΔIRI/ΔBS(30') was significantly reduced in the mutant NGT (0.523 ± 0.312 pmol/mg, $n = 7$), IGT (0.287 ± 0.106 pmol/mg, $n = 3$) and diabetic subjects (0.181 ± 0.105 pmol/mg, $n = 8$), as compared with the age-matched control (1.799 ± 0.633 pmol/mg, $n = 11$), as shown in Figure 2. As compared with the non-mutant members (1.910 ± 0.227 pmol/mg, $n = 10$), ΔIRI/ΔBS(30') was significantly reduced in the mutant NGT, IGT and diabetic subjects (Fig. 2).

Urinary 24 h excretion of CPR was significantly reduced in mutant NGT (211.5 ± 39.7 nmol/day, $n = 4$), mutant IGT (152.7 ± 35.5 nmol/day, $n = 3$) and mutant diabetic patients (104.5 ± 44.2 nmol/day, $n = 14$), as compared with the normal control subjects (263 ± 64.3 nmol/day, $n = 12$) (Fig. 3). Urinary 24 h CPR excretion was significantly reduced in mutant NGT, IGT and diabetic patients, as compared with the non-mutant members (288.3 ± 33.9 nmol/day, $n = 7$) (Fig. 3). Urinary 24 h CPR excretion was also significantly reduced in insulin-treated (52.1 ± 18.1 nmol/day, $n = 6$), sulphonylurea-treated (125.3 ± 13.8 nmol/day, $n = 4$) and diet only (151.0 ± 12.8 nmol/day, $n = 4$) mutant diabetic patients, as compared with the mutant NGT, the non-mutant family members and the normal control subjects (data not shown).

Plasma CPR 6 min after glucagon injection demonstrated a marked reduction in the mutant diabetic subjects (6.15 ± 2.46 nmol/l, $n = 7$), IGT (8.15 ± 1.32 nmol/l, $n = 3$) and NGT (11.79 ± 1.94 nmol/l, $n = 7$), as compared with the control (15.8 ± 3.81 nmol/l, $n = 12$) and the non-mutant family members (16.5 ± 1.06 nmol/l, $n = 7$) (Fig. 4). Si of C-I-1, C-II-1, C-II-3 and E-II-1 was 1.22, 1.31, 1.68 and 1.22 respectively, and was within normal range (mean ± SD, Si = 1.22 ± 0.42, $n = 6$) (data not shown). As shown in Table 2, ICA was not detectable in sera of the eight diabetic, two IGT and six NGT subjects with the 3243 bp mutation.

Diabetic complications in the subjects with the 3243 bp mutation. As shown in Table 2, diabetic retinopathy was demonstrated in 7 (50%) of 14 mutant diabetic subjects; 6 with simple retinopathy and one with proliferative retinopathy. Retinopathy was not shown in the mutant IGT, NGT or the non-mutant family members. Diabetic nephropathy was shown in 12 (85.7%) of 14 mutant diabetic subjects; 7 showed microalbuminuria and 5 proteinuria. Non-diabetic kidney diseases were not detected in the mutant diabetic subjects. Microalbuminuria or proteinuria were not shown in the mutant IGT, NGT or the non-mutant members. Pure tone audiometry examination demonstrated bilateral neurosensory deafness in 14 (58.3%) of 24 mutant subjects; 12 (85.7%) of 14 diabetic subjects, 2 (66.7%) of 3 IGT and none of 6 subjects with NGT.

Discussion

Several lines of evidence suggest that CPEO including Kearns-Sayre syndrome (KSS) is frequently associated with IDDM [20–22]. Recent reports also suggest that the MELAS-related mitochondrial gene mutation of $tRNA^{LEU(UUR)}$ (3243) associates with maternally inherited diabetes and with neurosensory deafness [5, 6, 9–13]. This study demonstrated the higher incidence of diabetes and IGT in the subjects with the 3243 bp mutation than the non-mutant members and the normal control subjects. Analysis using the insulinogenic index in OGTT, urinary 24-h CPR excretion and secretory response of CPR to glucagon injection provided the evidence that the mutant subjects had significant reduction in insulin secretory capacity, as compared with the non-mutant members and the normal control subjects. The mutant NGT subjects had lower insulinogenic index than the non-mutant NGT subjects and the normal control subjects, suggesting that the mutant NGT have a defect of early insulin secretion. The present study suggests that diabetes might be one of the major phenotypes of the mutation.

There are several possible explanations for how the mutation of $tRNA^{LEU(UUR)}$ (3243) affects glucose homeostasis and induces diabetes or IGT. The first is that the glucose sensing function and/or the insulin production capacity of the pancreatic islet beta cells might be affected by the partial defect of mutation dependent respiratory complex activities. Endocrinological abnormalities in mitochondrial encephalomyopathies did not only include diabetes [20–26], but also hypogonadism [24] and hypoparathyroidism [27]. Matuzaki et al. [28] reported a MELAS patient with the 3243 bp mutation and hypothalamic growth hormone deficiency. It has been speculated that the mitochondrial mutation-related defect of oxidative phosphorylation in the endocrine cells might result in reduction of hormone secretion. Insulin secretory capacity of pancreatic beta cells in the 3243 bp mutation was severely impaired as evaluated by insulinogenic index in OGTT, 24-h urinary CPR excretion and plasma 6-min CPR after i.v. administration of glucagon. Kadowaki et al. [9] and Awata et al. [10] demonstrated a significant reduction of maximal insulin secretory capacity and early secretion response of insulin to glucose administration in diabetic patients with the 3243 bp mutation. Several additional reports demonstrated reduced insulin secretion in diabetes with other mitochondrial diseases, such as CPEO and KSS [22, 25]. In contrast, Van den Ouweland et al. [5] and Sue et al. [13] showed substantial rises in insulin concentrations in several diabetic subjects with the mutation. The difference might reflect the degree of mutation-induced defect of mitochondrial oxidative phosphorylation in the pancreatic beta cells.

The second explanation could be that the impaired mitochondrial beta-cell function increases instability of pancreatic beta cells and induces autoimmune-mediated beta-cell destruction. Van den Ouweland et al. [5] and Tanabe et al. [22] suggested that the clinical phenotypes of the diabetic patients with mtDNA deletion tend to resemble IDDM. The mutant diabetic patients in our pedigrees have a phenotype of slowly progressive IDDM, insulin-requiring NIDDM or NIDDM. ICA can be detected in almost all IDDM patients within a few months after onset of the disease, but rapidly disappears during the course of the disease [29]. Oka et al. [30] report that the mitochondrial 3243 bp mutation was detected in 3 of 27 ICA-positive initially NIDDM patients. All three ICA-positive mutant patients progressed to insulin-dependency within a few years. At least in these three patients, the mitochondrial mutation may cause a gradual beta-cell destruction. However, Kadowaki et al. [9] and Schulz et al. [12] report that ICA was negative in two mutant diabetic patients. Vionnet et al. [31] report that Caucasian IDDM patients did not have the 3243 bp mutation. Glutamic acid decarboxylase antibodies (GAD-Ab) can be detected in 70–80% of newly-diagnosed IDDM patients [32]. GAD-Ab has been shown to appear several years before the onset of disease and tends to persist, compared to ICA. In this study, ICA was detected in sera of the eight diabetic patients, two impaired and six with normal glucose tolerance with the 3243 bp mutation. GAD-Ab determined by radioimmunoassay using pig brain 65,000-M_r GAD, was also negative in sera of the eight diabetic patients and two with impaired and six with normal glucose tolerance with the 3243 bp mutation (unpublished data). In addition, we did not find any of 32 IDDM patients presenting this mutation (unpublished data). These data suggest that the second hypothesis is not likely, but more clinical works need to clarify the contribution of the mitochondrial mutation to the autoimmunity to pancreatic islet beta cells.

The third explanation would be that the mitochondrial impairment increases glycolytic flux in the muscle and then gluconeogenesis in the liver might be enhanced via the Cori cycle. When gluconeogenesis is

suppressed by insulin, enhanced gluconeogenesis might result in hyperglycaemia. This study demonstrates normal insulin sensitivity in peripheral tissues of the mutant subjects using Bergman's minimal model analysis. Piccolo et al. [33] showed a low insulin secretion rate with normal insulin receptors in one diabetic patient with KSS and a mtDNA deletion. Insulin receptors were also found to be normal in six non-diabetic subjects with KSS. Tanabe et al. [22] showed reduced insulin secretion with absence of insulin resistance assessed by euglycaemic clamp technique in a diabetic patient with KSS. These data suggest that the mitochondrial malfunction might not induce peripheral insulin resistance. Additional clinical studies on these patients are in progress to elucidate the basis of the impaired glucose homeostasis in the mitochondrial diseases. These lines of studies provide further evidence that the molecular mechanism of diabetes in the 3243 bp mutation might be due to reduced insulin secretion resulting from abnormal energy metabolism in the pancreatic beta cells.

It is intriguing that the 3243 bp mutation can be involved in diabetes and deafness on one hand, and in MELAS, a more severe disorder, on the other hand. Several factors determine the way in which a particular mtDNA mutation results in a clinical phenotype, including the degree of heteroplasmy in different tissues and tissue-specific isoforms of some nuclear DNA-encoded subunits of respiratory chain enzymes. In MELAS patients, the percentage of mutant mtDNA exceeds 50% in muscle, 15% in peripheral blood cells, whereas the percentage of mutant mtDNA was below 10% in peripheral blood cells of non-MELAS individuals except E-III-1. Vionnet et al. [31] found no association between the heteroplasmy rate in blood and severity of diabetes. This study provides no correlation between the mutation rate in blood and insulin secretory defects.

Neurosensory hearing loss is known to be highly associated with mitochondrial encephalomyopathies. Neurosensory deafness has been known to be associated with diabetes [34, 35]. Malpas et al. [36] reported that neurosensory deafness was not linked to diabetic autonomic neuropathy. In this study, incidence of neurosensory deafness is higher in the mutant diabetics (85.7%) than the mutant NGT (0%). Complications of neurosensory deafness in mutant diabetes might be reflected by long-term diabetic condition. These studies demonstrated complications of diabetic retinopathy (50%) and nephropathy (85.7%) in the mutant diabetic patients. Peripheral neuropathies are known to be associated with mitochondrial myopathies [37] and are among the well-defined diabetic complications. There was higher incidence of peripheral neuropathy in the mutant diabetic patients than the mutant subjects with NGT (data not shown). More work needs to be done to clarify the pathogenesis of deafness and diabetic complications associated in mutant diabetes.

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