Diabetologia (1994) 37: 504–510

Diabetologia
© Springer-Verlag 1994

Mitochondrial diabetes mellitus: prevalence and clinical characterization of diabetes due to mitochondrial tRNA$^{Leu(UUR)}$ gene mutation in Japanese patients

H. Katagiri[1], T. Asano[1], H. Ishihara[1], K. Inukai[1], M. Anai[1], T. Yamanouchi[2], K. Tsukuda[3], M. Kikuchi[3], H. Kitaoka[4], N. Ohsawa[4], Y. Yazaki[1], Y. Oka[1]

[1] Third Department of Internal Medicine Faculty of Medicine, University of Tokyo, Tokyo, Japan
[2] Second Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan
[3] Institute for Adult Disease, Asahi Life Foundation, Tokyo, Japan
[4] First Department of Internal Medicine, Osaka Medical College, Osaka, Japan

Summary Mutations in the mitochondrial gene were recently identified in a large pedigree of diabetes mellitus and deafness. As the mitochondrial gene is maternally inherited, Japanese diabetic patients whose mothers were also diabetic were screened, using peripheral leucocytes, for an A to G transition at nucleotide pair 3243 of the mitochondrial gene, a tRNA$^{Leu(UUR)}$ mutation. This mutation was identified in four pedigrees from among 300 unrelated patients who were screened. Diabetes co-segregated with the mutation, except in one young subject, and was maternally inherited. The apparent onset of disease occurred between 11 and 68 years of age. Some of the affected members developed hearing impairment and congestive heart failure due to cardiomyopathy, though generally long after the onset of diabetes, and these patients had therefore not been diagnosed as having a specific form of diabetes. The duration of sulphonylurea treatment was not more than 8 years in these pedigrees and affected members were prone to progression to insulin-requiring diabetes. Thus, these patients were secondary sulphonylurea failures. Long-term follow-up revealed that the underlying disorder in affected members is a progressive impairment of insulin secretion. Some were initially diagnosed as having IDDM based on an apparent acute onset in youth and the clinical severity of their diabetes. Others were regarded as having MODY with an aggressive course. The mitochondrial gene mutation or diabetes is not transmitted to all offspring of the affected mothers. In conclusion, a mitochondrial tRNA$^{Leu(UUR)}$ gene mutation accounts for slightly more than 1 % of diabetic patients with maternally inherited disease and manifests a wide range of diabetic phenotypes, from the NIDDM phenotype to IDDM, in Japanese. [Diabetologia (1994) 37: 504–510]

Key words Insulin secretion impairment, secondary sulphonylurea failure, mitochondria, maternal inheritance.

Recently a large deletion in mitochondrial DNA was reported in a pedigree with maternally transmitted diabetes mellitus and sensorineural deafness [1]. Subsequently, an A to G transition at nucleotide pair (np) 3243, a conserved position in the mitochondrial gene for tRNA$^{Leu(UUR)}$, was reported in a pedigree with NIDDM and deafness [2, 3]. Since mitochondrial oxidative phosphorylation plays an important role in glucose-induced insulin secretion in pancreatic beta cells [4], we screened 300 diabetic patients with diabetic mothers for the mitochondrial gene mutation at np 3243 whether or not hearing impairment or symptoms of mitochondrial encephalomyopathy were present. This gene mutation was identified in four pedigrees and the clinical phenotypes of affected members are presented. This subset of diabetes is not particularly rare and therefore more attention is warranted.

Received: 15 September 1993 and in revised form: 3 December 1993

Corresponding author: Dr. Y. Oka, Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7–3–1, Hongo, Bunkyo-ku, Tokyo 113, Japan

Abbreviations: tRNA, Transfer RNA; NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; mtDNA, mitochondrial DNA; np, nucleotide pair; OGTT, oral glucose tolerance test; MODY, maturity-onset diabetes of the young

Subjects and methods

Patients

Three hundred unrelated diabetic patients were selected from those seen in our out-patient diabetes clinics. The only criterion for selection was that their mother also had diabetes based on patient interview. Other information such as type of diabetes, age, sex, the mode of treatment, and the presence of hearing impairment or symptoms suggesting mitochondrial encephalomyopathy were not considered. The study subjects consisted of 164 men (age 49.3 ± 10.5) and 136 women (age 47.7 ± 16.8), of whom 31 (10.3%) also had fathers with diabetes based on patient interview. Children are not usually followed at the clinics participating in this study. Furthermore, the incidence of IDDM is much lower in Japan than in Western countries. Consequently, the patients studied were all over age 20 and only six had been clinically diagnosed as having IDDM at the onset based on the criteria of the National Diabetes Data Group [5].

DNA studies

Mitochondrial DNA (mtDNA) was isolated from the peripheral leucocytes of patients and a mtDNA fragment surrounding the $tRNA^{Leu(UUR)}$ mutation site was amplified by polymerase chain reaction using sense primer 5'-AAGGTTCGTTTGTTCAACGA (np 3029–3048) and antisense primer 5'-AGCGAAGGGTTGTAGTAGCC (np 3456–3437) as previously reported [2]. The reaction conditions were: initial denaturation for 3 min at 94 °C then 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 3 min. These fragments were digested with restriction endonuclease ApaI, and subjected to agarose gel electrophoresis, followed by Southern blotting using polymerase chain reaction-amplified 214 bp fragments (np 3029–3242) as a hybridization probe. When ApaI digestion suggested the presence of the mutation [6], mtDNA fragments were subcloned into a plasmid vector using a TA cloning kit (Invitrogen, San Diego, Calif., USA) and were sequenced with ABI DNA sequencer. The presence of this mitochondrial gene mutation was studied in family members who were available for study.

Statistical analysis

Data are given as mean ± SD.

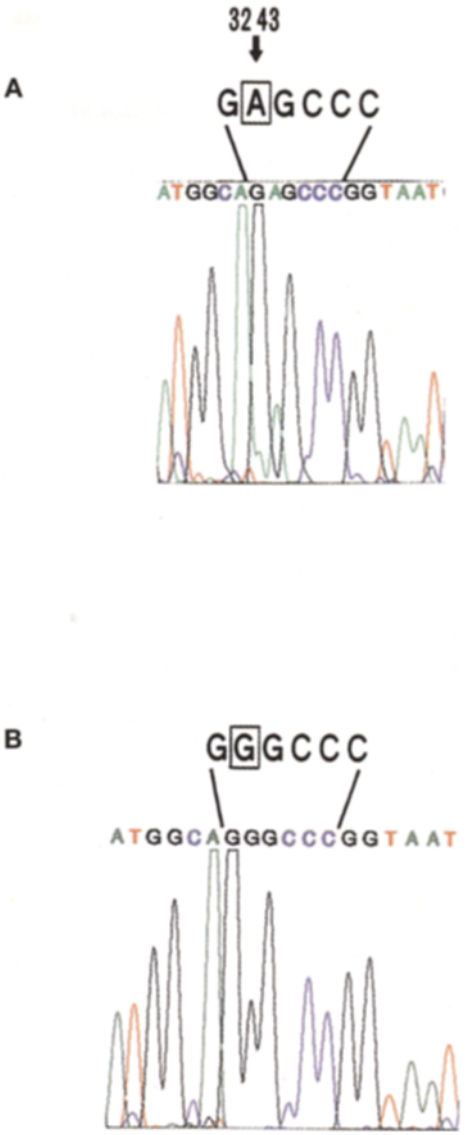

Fig. 1 A, B. mtDNA sequence surrounding the mutated site in mitochondrial $tRNA^{Leu(UUR)}$. **A**, normal mitochondrial gene in subject AIII-3; **B**, mutated mitochondrial gene in the same subject

Results

An A to G transition at np 3243 in the mitochondrial gene was identified (Fig. 1) in four of the 300 unrelated patients screened. The presence of diabetes and symptoms of hearing impairment were then ascertained in available family members. The family pedigrees showed maternal inheritance of diabetes and/or hearing impairment over two or three generations (Table 1 and Fig. 2). We found this mitochondrial gene mutation in 6, 4, 2, and 2 patients in pedigree A, B, C, and D, respectively (Fig. 2). All diabetic patients in the affected pedigrees were heteroplasmic for this mitochondrial gene mutation, which created a recognition site for restriction endonuclease ApaI [6] (inset, in Fig. 2). Subject AIII-1, the son of an affected male, exhibited normal glucose tolerance and no mitochondrial $tRNA^{Leu(UUR)}$ mutation was observed at np 3243.

Some of the clinical features of affected members are shown in Table 1. The apparent age of onset of diabetes ranged from 11 to 68 years and treatment also encompassed a wide range of modalities from diet only to insulin therapy. Thus, the clinical phenotypes of diabetic patients with the mitochondrial gene mutation appear to be very heterogeneous. However, several common clinical characteristics are noted among the diabetic patients in these pedigrees. First, although 11 of the 18 diabetic patients in these four pedigrees were or currently are treated with sulphonylurea, treatment has not lasted for more than 8 years. Secondly, the onset of diabetes appeared to be rather acute in most

Table 1. Clinical characteristics of the affected subjects

Subject	Age (years)	Age at onset (years)	Treatment[b]	Hearing[c] impairment	Symptoms at onset	C-peptide immunoreactivity
AII-1	65[a]	53	OHA(4)- > Insulin	–	+	
AII-2	51[a]	47	OHA(2)- > Insulin	+ 50	+	
AII-3	67	61	OHA	–	+	
AII-6	62	62	Diet	–	–	
AII-7	54	40	OHA(8)- > Insulin	–	+	1.4 ng/ml[d]
AIII-2	43	41	OHA	–	+	
AIII-3	32	20	OHA(6)- > Insulin	+ 29	+	1.9 ng/ml[e]
AIII-4	27[a]	12	Insulin	+ 23	+	
AIII-9	20	11	Insulin	–	+	1.3 ng/ml[d]
BI-1	73[a]	66	Diet	+ 69	– (+ ?)	
BII-1	51	39	Insulin	+ 44	+	
BII-2	40	36	Insulin(2 M)- > OHA	–	+	
BII-3	38	37	OHA	–	+	
BIII-1	22	19	Insulin(1 M)- > OHA	–	+	4.5 ng/ml[e]
CII-1	56	36	OHA(4)- > Insulin	+ 45	+	3.5 ng/ml[e]
CIII-2	30	24	OHA(3)- > Insulin	+ 24	+	47 μg/day[f]
DI-1	79[a]	68	Diet	+ 70	–	
DII-3	44	30	Insulin- > off- > Insulin	–	+	9.8 ± 5.3 (n = 6) μg/day[f]

[a] Subject deceased.
[b] Number in parentheses is duration of the treatment (year or month(M)).
[c] Age at the onset of the symptom of hearing impairment is given.
[d] Plasma C-peptide immunoreactivity value 30 min after breakfast;
[e] plasma C-peptide immunoreactivity value 6 min after intravenous injection of 1 mg glucagon;
[f] urinary excretion of C peptide immunoreactivity value.
OHA, Oral hypoglycaemic agents

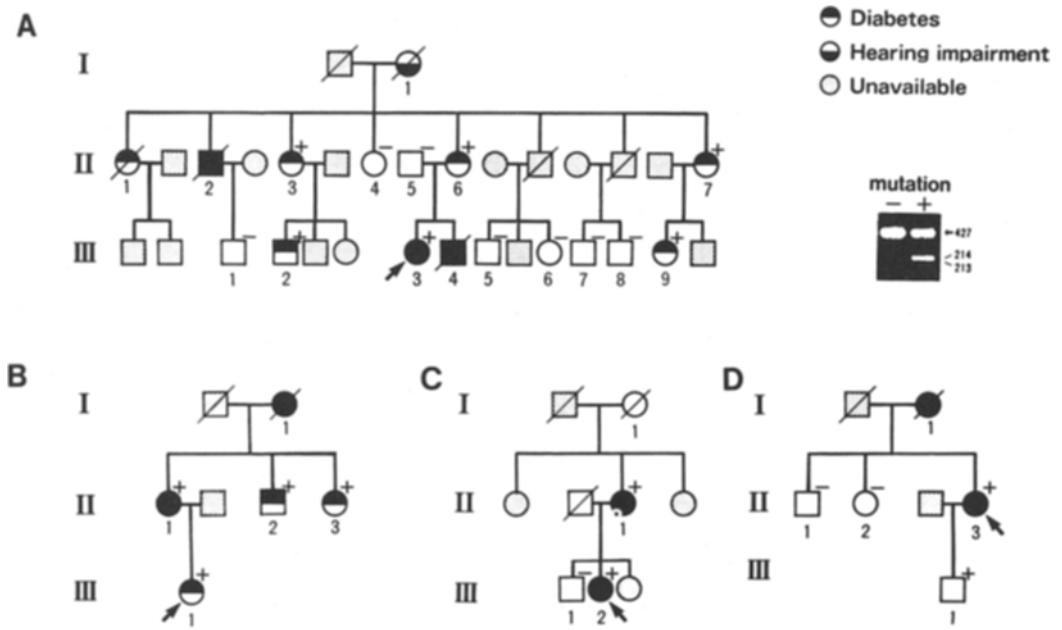

Fig. 2 A–D. Four family pedigrees. Roman and Arabic numerals indicate generation and identification numbers, respectively. Crossed symbols indicate deceased subjects. The arrow indicates the proband in each pedigree. (+) and (–) indicate the patients with and without the mitochondrial gene mutation at np 3243, respectively. An mtDNA fragment (from nucleotide 3,029 to 3,456) surrounding the $tRNA^{Leu(UUR)}$ mutation site (np 3243) was digested with ApaI, which cleaved the fragment derived from the mutant but not from the wild-type as shown in the inset

patients; the majority (15 of 18) of diabetic patients had classic symptoms of diabetes such as thirst and weight loss at the time of diagnosis (Table 1). These clinical features are not common in Japanese NIDDM patients, many of whom are diagnosed incidentally on routine examinations, have none of the classic symptoms and whose disease is well controlled with sulphonylurea for many years.

Subject AIII-3 is an example of such a patient. The clinical course and results of an OGTT are shown in Table 2. She had symptoms of thirst and polydipsia for 3 weeks at age 20 years and was diagnosed as having diabetes with a fasting blood glucose level of 12.7 mmol/l, which was measured after 2 weeks of diet therapy. The plasma insulin concentration 30 min after a 100-g oral glucose load was 28 mU/l and the peak

Table 2. Insulin secretion response in OGTT in the affected subjects

Age (years)		Time (min)						Glucose load	Treatment
		0	30	60	90	120	180		
Subject AIII-3									
20	Blood glucose	9.0	19.8	18.3	18.8	20.1	17.4	100 g	Diet
	IRI	11	28	39	39	48	39		
	CPR	2.0	3.1	4.1	4.4	6.2	5.5		
21	Blood glucose	7.0	12.6	14.3	14.0	13.3	11.6	100 g	Glibenclamide
	IRI	22	58	82	79	87	62		(1.25 mg)
22	Blood glucose	7.8	11.5	14.9	15.1	14.3	9.8	100 g	Glibenclamide
	IRI	9	15	34	32	41	21		(1.25 mg)
23	Blood glucose	5.4	10.7	12.1	10.4	10.2	9.7	100 g	Glibenclamide
	IRI	9	13	26	25	23	30		(2.5 mg)
24	Blood glucose	8.9	12.3	15.7	16.1	15.8	12.5	100 g	Glibenclamide
	IRI	7	12	25	23	26	25		(5.0 mg)
25	Blood glucose	11.0	15.0	18.9	20.6	21.7	20.3	100 g	Glibenclamide
	IRI	6	10	14	18	18	22		(7.5 mg)
Subject AIII-4									
15	Blood glucose	9.8	15.5	21.7	23.1	23.8	22.7	100 g	Insulin
	CPR	1.7	1.6	3.0	3.0	3.1	3.2		
Subject AII-1									
59	Blood glucose	10.5	17.0	21.3	24.7	27.3	25.8	100 g	Insulin
	CPR	1.1	1.7	2.4	2.7	3.2	3.7		
Subject AII-6									
50	Blood glucose	5.1	11.3	11.8	9.8	8.6	5.8	100 g	(Diet)
	IRI	14	61	118	113	56	17		
62	Blood glucose	7.0	12.3	15.7	13.8	13.6	8.8	75 g	(Diet)
	IRI	12	23	38	38	49	30		
Subject AII-7									
43	Blood glucose	9.0	12.2	16.6	18.2	19.9	20.0	100 g	Glibenclamide
	IRI	8	6	23	16	22	28		(5.0 mg)

Blood glucose values are given in mmol/l;
IRI, immunoreactive insulin, in mU/l; CPR, C-peptide immunoreactivity, in ng/ml

values of her plasma insulin and C-peptide levels were 48 mU/l and 6.2 ng/ml, respectively, 2 h after the load. Thus, although her insulin secretion response to a glucose load was slow, her pancreatic beta cells did have the capacity to secrete a considerable amount of insulin. Her fasting blood glucose concentration decreased to 5.6 mmol/l with 1.25 mg/day of glibenclamide. However, her fasting blood glucose concentrations gradually increased over the next 5 years despite the dose of glibenclamide being increased to 7.5 mg/day. Concomitantly, her insulin secretion capacity was decreased as evidenced by a peak plasma insulin value of only 22 mU/l after a glucose load (Table 2). One year later she started insulin injections. Currently (6 years after the start of insulin therapy) she is treated with 32 IU/day of insulin and an intravenous injection of 1 mg glucagon increased her plasma C-peptide concentration from 0.8 to 1.9 ng/ml, indicating that the capacity of her pancreatic beta cells to secrete insulin is not completely abolished at present.

We also had the opportunity to observe progressive insulin secretion impairment, though in much milder form, in subject AII-6 who was diagnosed as having impaired glucose tolerance 12 years ago prior to this study and had recently been diagnosed as having asymptomatic NIDDM (Table 2). The results of the OGTT reveal that her glucose-induced insulin secretion had decreased over the previous 12 years with concomitant development of diabetes consistent with World Health Organization criteria [7].

Two subjects from the affected pedigrees were clinically diagnosed with IDDM based on an apparent abrupt onset at an early age and disease severity including ketonuria and immediate requirement of insulin treatment; subjects AIII-4 and AIII-9 had acute onsets with symptoms of thirst and polydipsia and weight loss exceeding 4 kg in 1 month at ages 12 and 11 years, respectively. Nine years after onset, the postprandial plasma C-peptide level 30 min after breakfast was 1.3 ng/ml in subject AIII-9, indicating that some pancreatic beta cell function was preserved. After a 100-g OGTT, the peak C-peptide value was 3.2 ng/ml in subject AIII-4 3 years after onset (Table 2). He experienced a ketoacidotic coma episode at age 18 years. None of the

diabetic patients in these pedigrees showed instability of blood glucose concentrations.

The mutation in the mitochondrial gene is not always inherited by the offspring. A mitochondrial gene mutation at np 3243 was not detected by Southern blotting of ApaI-digested fragments in several offspring (subjects AII-4, CIII-1, DII-1 and DII-2) of the affected mothers. Furthermore, the amplified mtDNA fragments were subcloned into the plasmid vector in each of these subjects and 30 independent clones from each subject were sequenced. The mutation at np 3243 was not observed in any of these clones, indicating that this mitochondrial gene mutation was barely present, if at all. These subjects showed normal fasting blood glucose and glycated haemoglobin levels and two (CIII-1 and DII-1) were confirmed as having normal glucose tolerance with normal insulin secretion responses on OGTT. Interestingly, despite the mitochondrial gene mutation being present in leucocytes, subject DIII-1, at 19 years of age, has normal glucose tolerance based on normal fasting blood glucose (4.7 mmol/l) and normal HbA_{1C} (4.6%) levels, although normal glucose tolerance was not confirmed by OGTT.

None of the members of these pedigrees had clinical symptoms characteristic of mitochondrial encephalomyopathy except for hearing impairment which generally developed long after the onset of diabetes. No signs of retinitis pigmentosa were found in these patients. Two (AII-7 and DII-3) subsequently developed congestive heart failure, probably due to cardiomyopathy based on electrocardiographic and echocardiographic findings and history, after the mitochondrial gene mutation had been identified. Cardiac muscle biopsy was performed in DII-3 and the mitochondrial gene mutation was identified at np 3243 in the biopsy sample.

Discussion

The np 3243 mutation alters the dihydrouridine loop in $tRNA^{Leu(UUR)}$, leading to impairment of mitochondrial transcription termination, which may cause defects in mitochondrial protein synthesis [8]. It has been shown that inhibition of mitochondrial oxidative phosphorylation in pancreatic islets impairs insulin secretion [9] and a decrease in mitochondrial gene expression was associated with insulin deficiency in neonatally streptozotocin-treated rats [10]. Thus, it is very likely that mitochondrial gene defects decrease oxidative phosphorylation capacity in pancreatic beta cells, and impair glucose-induced insulin secretion in affected subjects. However, it is still not clear whether this is the sole mechanism of diabetes development in these patients, since sulphonylurea treatment would be able to ameliorate the defects, assuming that sulphonylurea acts on ATP-sensitive potassium channels in pancreatic beta cells [11].

The clinical phenotype of diabetic patients in the pedigree with a 10.4 kilobase mtDNA deletion tends to resemble that of IDDM [1], while all previously reported diabetic patients with the $tRNA^{Leu(UUR)}$ mutation had NIDDM and no history of ketoacidosis or ketonuria [2, 3]. This suggests that different changes in the mitochondrial genome result in different diabetic phenotypes [2]. However, the diabetic patients in our pedigree A were clinically diagnosed as having either NIDDM or IDDM. Diabetic ketoacidotic coma has been experienced by some affected subjects in other pedigrees, subjects BII-1 and DII-3. Thus, patients with the np 3243 mutation in their mitochondrial gene may develop various phenotypes of diabetes.

Some affected members, AIII-4 and AIII-9, had been diagnosed as having IDDM at the onset, because of the apparent abrupt onset in youth and the severity of the disease. However, the clinical course of the disease after onset appears to be slightly different from that observed in typical acute-onset patients whose IDDM is due to an autoimmune process. Some insulin secretion capacity persisted many years after onset in these patients with the mitochondrial gene mutation in contrast to the "total diabetes" often observed in IDDM caused by an autoimmune destructive process. It should be noted that minimal preservation of insulin secretion capacity is observed in slowly progressive IDDM patients, some of whom were recently shown to have the mitochondrial gene mutation at np 3243 [12].

Almost all diabetic patients in the previous reports also had hearing loss [1–3], whereas in our four pedigrees only some had hearing impairment. In addition, hearing loss generally developed long after the onset of diabetes in these patients. These results are based mainly on the symptoms of patients, and thus, the prevalence of hearing impairment may actually be higher in our pedigrees. Nevertheless, this feature is very important on clinical grounds, since it is impossible to recognize these diabetic patients as having a specific form of diabetes at the onset of disease or even during many years of follow-up.

The most interesting clinical feature is progressive insulin secretion impairment, as clearly demonstrated in subject AIII-3. Sulphonylurea treatment did not persist for more than 8 years in patients in these pedigrees. In other words, these patients are prone to progression to the insulin-requiring state; secondary sulphonylurea failure. It has been reported that islet cell antibody-positive patients more frequently develop secondary drug failure [13, 14]. Our results indicate that the mitochondrial gene mutation is also predictive. It is possible that glucose-toxicity and/or insulin resistance are related to development of diabetes in patients in these pedigrees. However, according to the serial data of OGTT in subject AIII-3, the insulin secretion did not improve even when glucose intolerance was improved at age 23 years with an increase in the dose of glibenclamide without an apparent change in body weight (45 kg). Thus, it is unlikely that glucose-toxicity is mainly responsible for the impairment of insulin secre-

tion. It was noted that rather high plasma immunoreactive insulin levels were observed in subject AII-6 at the beginning of the disease, although our assay for insulin had cross-reactivity with proinsulin. A possible relationship between insulin resistance and the defect in mitochondrial function should be further investigated, since the mutated mtDNA is likely to be present in muscle and fat tissues.

The gradual decrease in insulin secretion capacity might be due to a gradual increase in the percentage of mutated mtDNA in pancreatic beta cells, since a marked replicative advantage of mtDNA with np 3243 mutation was recently reported [15]. In addition, it has been shown that oxidative phosphorylation capacity declines with age in tissues such as skeletal muscle [16], which might reflect the accumulation of damaged mtDNAs. Thus, it is also possible that patients with the inherited mtDNA mutation start with a lower oxidative phosphorylation capacity and drop below the expression thresholds much earlier than do normal subjects [17]. It is likely that subjects AIII-4 and AIII-9 had very high percentages of mutant mtDNA in pancreatic beta cells, resulting in the onset of diabetes in youth, and that mitochondrial dysfunction in pancreatic beta cells may not have reached the threshold in subject DIII-1.

Another interesting point is that some of the pedigrees were regarded as having MODY. Although Tattersall and Fajans [18] described the inheritance of MODY as being autosomal dominant, only X-linked inheritance was ruled out, and involvement of the mitochondrial gene was not considered. Furthermore, autosomal dominant or maternal inheritance is hardly distinguished in small pedigrees. Subjects AIII-4 and BIII-1 are such MODY patients. MODY was originally described as being distinguishable from classical juvenile-onset diabetes, since patients from a MODY family exhibited a less aggressive clinical course [18]. However, subject AII-1 had severe diabetic nephropathy which required haemodialysis, and BII-1 is blind due to proliferative retinopathy and currently is on haemodialysis. These clinical features differ sharply from those with a glucokinase gene mutation recently identified in MODY pedigrees [19] and by screening of late-onset NIDDM patients [20], who can, in most cases, be managed with life-long dietary treatment and show minimal disease progression [21].

It should be stated that mutant mtDNA in the mother is not always inherited by the offspring. On the other hand, among the affected subjects, successive generations appear to develop diabetes at earlier age. It has been reported that the mitochondrial gene with the np 3243 mutation was selectively amplified through generations [22]. Analysis of mtDNA in Holstein cows showed that rapid segregation of populations of mtDNA may occur [23] and that polymorphic mtDNA may partition unequally among siblings [24]. Segregation to homoplasmic genotypes of bovine mtDNA could occur within two or three generations [25]. Thus, it was suggested that there must be a "bottleneck" where a few of the mtDNAs in every oocyte are selected to populate the organism and are transmitted to the progeny [23, 24], which yields the progeny with different levels of heteroplasmy and, in some instances, the progeny without the mutation.

In relation to this apparent inconsistent mode of inheritance of mitochondrial gene mutation, pedigree D should be kept in mind. This type of diabetic pedigree is often observed but attracts little attention with respect to the search for genetic causes of NIDDM. Our screening for mitochondrial gene mutations in diabetic patients whose mothers were also diabetic revealed four pedigrees, including this "ordinary" pedigree, among 300 unrelated patients. Thus, diabetes due to a mitochondrial gene mutation is not particularly rare and therefore merits more attention. This subset of diabetes, termed "mitochondrial diabetes", is characterized by maternal inheritance, progressive impairment of insulin secretion leading to the insulin-requiring state, and clinical picture of essentially NIDDM, though manifestation of IDDM at the onset is possible.

Acknowledgements. This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan and a grant for Diabetes Research from Ohtsuka Pharmaceutical Co..

References

1. Ballinger SW, Shoffner JM, Hedaya HV et al. (1992) Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. Nature Genet 1: 11–15
2. van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek K et al. (1992) Mutation in mitochondrial $tRNA^{LEU(UUR)}$ gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nature Genet 1: 368–371
3. Reardon W, Ross RJM, Sweeney MG et al. (1992) Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. Lancet 340: 1376–1379
4. Sener A, Malaisse WJ (1987) Stimulation by D-glucose of mitochondrial oxidative events in islet cells. Biochem J 246: 89–95
5. National Diabetes Data Group (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28: 1039–1057
6. Goto Y-I, Nonaka I, Horai S (1990) A mutation in the $tRNA^{Leu(UUR)}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 348: 651–653
7. WHO expert committee (1980) Second report on diabetes mellitus. WHO technical report series, No. 646. World Health Organization, Geneva
8. Hess JF, Parisi MA, Bennett JL, Clayton DA (1991) Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 351: 236–239
9. Yousufzai SYK, Bradford MW, Shrago E, Ewart RBL (1982) Characterization of the adenine nucleotide translocase of pancreatic islet mitochondria. FEBS Lett 137: 205–208

10. Welsh N, Pääbo S, Welsh M (1991) Decreased mitochondrial gene expression in isolated islets of rats injected neonatally with streptozotocin. Diabetologia 34: 626–631
11. Trube G, Rorsman P, Ohno-Shosaku T (1986) Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. Pfluegers Arch 407: 493–499
12. Oka Y, Katagiri H, Yazaki Y, Murase T, Kobayashi T (1993) Mitochondrial gene mutation in islet-cell-antibody-positive patients who were initially non-insulin-dependent diabetics. Lancet 342: 527–528
13. Irvine WJ, Gray RS, McCallum CJ, Duncan LJP (1977) Clinical and pathogenic significance of pancreatic islet cell antibodies in diabetics treated with oral hypoglycemic agents. Lancet I: 1025–1027
14. Groop LC, Bottazzo GF, Doniach D (1986) Islet cell antibodies identify latent type I diabetes in patients aged 35–75 years at diagnosis. Diabetes 35: 237–241
15. Yoneda M, Chomyn A, Martinuzzi A et al. (1992) Marked replicative advantage of human mtDNA carrying a point mutation that causes the MELAS encephalopathy. Proc Natl Acad Sci USA 89: 11164–11168
16. Trounce I, Byrne E, Marzuki S (1989) Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in aging. Lancet I: 637–639
17. Wallace DC (1992) Diseases of the mitochondrial DNA. Annu Rev Biochem 61: 1175–1212
18. Tattersall RB, Fajans SS (1975) A difference between the inheritance of classical juvenile-onset and maturity-onset type diabetes of young people. Diabetes 24: 44–53
19. Vionnet N, Stoffel M, Takeda J et al. (1992) Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. Nature 356: 721–722
20. Katagiri H, Asano T, Ishihara H et al. (1992) Nonsense mutation of glucokinase gene in late-onset non-insulin-dependent diabetes mellitus. Lancet 340: 1316–1317
21. Froguel P, Zouali H, Vionnet N et al. (1993) Familial hyperglycemia due to mutations in glucokinase–definition of a subtype of diabetes mellitus. N Engl J Med 328: 697–702
22. Kobayashi Y, Ichihashi K, Ohta S et al. (1992) The mutant mitochondrial genes in mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) were selectively amplified through generations. J Inher Metab Dis 15: 803–808
23. Hauswirth WW, Laipis PJ (1985) Transmission genetics of mammalian mitochondria: a molecular model and experimental evidence. In: Quagliarello E, Slater EC, Palmieri F, Sacconne G, Kroon AM (eds) Achievements and perspectives of mitochondrial research, vol. 2. Biogenesis, Elsevier Biomedical, Amsterdam, pp 49–59
24. Laipis PJ, Van de Walle MJ, Hauswirth WW (1988) Unequal partitioning of bovine mitochondrial genotypes among siblings. Proc Natl Acad Sci USA 85: 8107–8110
25. Ashley MV, Laipis PJ, Hauswirth WW (1989) Rapid segregation of heteroplasmic bovine mitochondria. Nucl Acid Res 17: 7325–7331