Mutation in the mitochondrial tRNA^{leu} at position 3243 and spontaneous abortions in Japanese women attending a clinic for diabetic pregnancies

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Summary Mitochondrial DNA is exclusively maternally inherited. We recently found the prevalence of diabetic patients with an A to G transition at position 3243 of leucine tRNA (3243 base pair (bp) mutation) to be nearly 1 % in randomly selected Japanese subjects. Here, we report the higher prevalence of diabetic patients with the 3243 bp mutation in a specific Japanese population of women attending a diabetic pregnancy clinic. Of 102 patients with non-insulin-dependent diabetes mellitus 6 (5.9 %) were positive for the mutation, 1 (8.3 %) of 12 patients with gestational diabetes and 2 (5.9 %) out of 34 borderline diabetic patients. In contrast, none of 64 patients (0 %) with insulin-dependent diabetes mellitus had the 3243 bp mutation. Moreover, there was a difference

in the prevalence of spontaneous abortions between patients with and without this mutation (27.3 vs 12.4 %). Among nine probands with the mutation, four had a history of one spontaneous abortion ($p = 0.0518$) and two had a history of two abortions ($p = 0.0479$). Two probands had a spontaneous abortion even while under strict diabetic metabolic control. The 3243 bp mutation thus may cause spontaneous abortion during pregnancy. [Diabetologia (1995) 38: 809–815]

Key words Mitochondrial DNA mutation, insulin-dependent diabetes mellitus, non-insulin-dependent diabetes mellitus, gestational diabetes mellitus.

It is well-known that one of the main causes of spontaneous abortion is an abnormality in the fetus rather than a problem in the mother [1]. The prevalence of abortion in diabetic women is higher in the high-titre HbA_{1c} group during the first trimester [2, 3]. Pre-pregnancy education courses are available in diabetic pregnancy clinics in Japan, and diabetic mothers are instructed to keep postprandial blood

glucose levels within the normal range prior to pregnancy. However, approximately 14 % of diabetic mothers have experienced spontaneous abortions compared with 10 % of non-diabetic mothers [2].

Mitochondrial DNA is exclusively maternally inherited and several case reports have suggested that mutations in this DNA, particularly if involving the substitution at guanine for adenine (A → G) at position 3243 base pair (bp) of leucine tRNA, may cause diabetes and deafness [4–7]. We recently reported a subtype of diabetes associated with the 3243 bp mutation in Japanese subjects [8]; we ascertained that approximately 0.9 % of Japanese diabetic patients have this mutation and there was no instance of this mutation in 200 control subjects with normal glucose tolerance [9]. There were no sex differences for this mutation and no differences of lifestyle in this mutation rate [8]. To our knowledge, the most frequent gene mutation in Japanese non-insulin-dependent diabetes mellitus (NIDDM) patients is the 3243 bp mito-

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Abbreviations: IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; PCR, polymerase chain reaction; PCDW, pregnancy clinic for diabetic women; GDM, gestational diabetes mellitus; GAD, glutamic acid decarboxylase; OGTT, oral glucose tolerance test.

Table 1. Clinical findings of 212 Japanese patients attending the pregnancy clinic for diabetic women

Diabetes type	n	Age at on-set (years) (range)	Present age (years) (range)	Family history of diabetes ^a	Diabetes with mutation
IDDM	64	19.1 ± 7.9 (2–28)	36.7 ± 7.9	14 %	0 (0 %)
NIDDM	102	25.0 ± 6.3 (14–39)	32.3 ± 5.4	49 %	6 (5.9 %)
GDM	12	32.0 ± 2.8 (25–35)	34.8 ± 4.1	67 %	1 (8.3 %)
Borderline	34	30.3 ± 3.6 (22–35)	31.4 ± 3.7	29 %	2 (5.9 %)

GDM exceeds two points of fasting blood glucose 5.5 mmol/l, 1-h blood glucose level 10 mmol/l, or 2-h blood glucose 8.3 mmol/l, and borderline is defined as a glucose tolerance test result of 1-h blood glucose $\geq$ 8.9 mmol/l and 2-h blood glucose $\geq$ 8.3 mmol/l and $<$ 11.1 mmol/l [29]. ^a Percentage of diabetic first-degree relatives

Fig. 1. Detection of the 3243 bp mutation of mitochondrial DNA by silver staining. The method of silver staining is described in Materials and methods. The DNA in lanes 1, 2, and 3 had 0.6, 1.9, and 7.4 % of the heteroplasmy for this mutation. Lanes 4 and 5 show the results of the DNA from the patients with IDDM and NIDDM, respectively. The DNA from the patients with NIDDM was positive for the mutation, whereas that from the IDDM patients was negative

chondrial gene mutation ($\sim$ 1 %) [9]. Damage to mitochondrial DNA has been implicated in the pathogenesis of age-associated degenerative diseases. Therefore, it is of interest to ascertain whether the 3243 bp mutation correlates with the higher prevalence of spontaneous abortion in diabetic pregnant women.

In this study, we identified nine diabetic probands with the mutation and examined the clinical features of their disease, in particular the prevalence of spontaneous abortion.

Subjects and methods

Patients. Our diabetes center at the Tokyo Women's Medical College has a pregnancy clinic for diabetic women (PCDW). Patients visiting our PCDW are referred from the clinic for children and adolescents in the diabetes center and/or by a primary physician. The 212 subjects in this study included 64 with insulin-dependent diabetes mellitus (IDDM), 102 with NIDDM, and 46 patients with abnormal glucose tolerance during pregnancy. Diagnosis of IDDM and NIDDM was made based on the World Health Organization criteria [10]. Of 46 pregnant women with abnormal glucose tolerance, 12 were diagnosed as having gestational diabetes mellitus (GDM) using the criteria of the Japanese Society of Obstetrics and Gynecology [11, 29] (Table 1), the remaining 34 women with abnormal glucose tolerance were diagnosed as "borderline" [12] (Table 1). Their mean ages were 30.3 ± 3.6 years during pregnancy. A family history of NIDDM in first-degree relatives was positive in 14 % of IDDM, 49 % of NIDDM, and 39 % of AGT patients (Table 1). In second-degree relatives there was a positive history in 33 % of IDDM, 67 % of NIDDM, and 48 % of abnormal glucose tolerance patients. A positive family history of diabetes and its type or therapy as well as associated symptoms such as sensory hearing disturbance and short stature were analysed either directly by examining family members or by questionnaire.

Definition of spontaneous abortion. Spontaneous abortion was defined as interruption of pregnancy within 21 weeks and 6 days. Premature delivery was defined as delivery from 22 weeks and 0 days to 36 weeks and 6 days.

Detection of the 3243 bp mutation. DNA was prepared from peripheral-blood leucocytes taken from the subjects. The 3243 bp mutation in the mitochondrial DNA was determined by the polymerase chain reaction (PCR) specific to this mutation [13] and digestion with restriction endonuclease Apa I followed by silver staining detection [14] after 10 % SDS-PAGE. The anti-GAD (glutamic acid decarboxylase) antibody was measured by radioimmunoassay using GAD purified from pig brain (Hoechst GAD kit; Hoechst, Tokyo, Japan) [15]. HLA serological typing was performed by the standard microlymphocyte method [16].

Statistical analysis

The chi-square test, with Yates' correction when necessary, was used to assess statistical significance.

Results

Detection of the 3243 mutation by silver staining method. We first determined the percentage of heteroplasmy of the 3243 bp mutation which could be detected by silver staining. Figure 1 shows representative results. The DNA in lanes 1, 2, and 3 had 0.6, 1.9, and 7.4 % of the heteroplasmy respectively for this mutation, was assessed by the method using radiolabelled primers described previously [7]. Lanes 4 and 5 show the results of the DNA from patients with IDDM and NIDDM, respectively. The DNA from the NIDDM patient was positive for the 3243 mutation, whereas that from the IDDM patient was

Table 2. Clinical characteristics of diabetes mellitus associated with the 3243 bp mutation

Patient	Onset age of diabetes (years)	Diabetes type	Present age (years)	Diabetic complication	Sensory hearing disturbance	MELAS	History of obesity	Short stature	Spontaneous abortion (n)	Premature deliveries (n)
T.O.	23	N (I)	41	–	+	–	–	–	2	0
K.H.	26	N (I)	39	–	+	–	–	–	0	2 ^b
O.T.	29	N (I)	41	–	+	–	–	–	1	1
K.K.	33	N (I)	35	–	–	–	–	–	0	0
J.K.	39	N (I)	35	–	–	–	–	–	0	0
M.F.	26	N (I ^a)	31	–	+	–	–	–	1	1
T.H.	25	GDM (I ^a)	25	–	–	–	–	–	0	0
Y.I.	30	Bo (D)	34	–	–	–	+	–	2	0
N.T.	26	Bo (D)	27	–	–	–	–	–	0	0

N, Non-insulin-dependent diabetes; GDM, gestational diabetes mellitus; Bo, Borderline; I, insulin-requiring;

^a Insulin-requiring only during pregnancy; ^b number of still-born deliveries

OHA, treated with oral hypoglycaemic agent; D, treated with diet only

negative. Thus, the silver staining method was able to detect 0.6 % of the heteroplasmy. Further experiments were performed by silver staining for DNA-band detection.

Prevalence of the 3243 bp mutation. DNA from the 212 subjects was used to detect the 3243 bp mutation (Table 2). The prevalence was 0 % for IDDM, 6 (5.9 %) for NIDDM, 1 (8.3 %) for GDM, and 2 (5.9 %) for borderline patients.

Clinical characteristics of the patients with the 3243 bp mutation. Of the 237 patients who attended the PCDW, nine were found to have the 3243 bp mutation (Table 2 and Fig. 2). None of them had mental retardation, ophthalmoplegia, or muscle weakness.

Patient T.O. had insulin-requiring NIDDM diagnosed at the age of 23 years and had a maternal family history of NIDDM; she had previously tested positive for proteinuria. Her mother had been treated with an oral hypoglycaemic agent. After the patient had started insulin treatment, although under strict glycaemic control, she experienced a second spontaneous abortion. After two abortions, she now has one son who, now 12 years old, has the 3243 bp mutation and has recently developed diabetes. Insulin secretion on meal tolerance tests was impaired before the second pregnancy and after the third pregnancy.

Patient K.H. was diagnosed with NIDDM at age 26 years and she experienced two premature stillbirths (at 37 weeks and 30 weeks). She attended the diabetes center and received pre-pregnancy education before becoming pregnant again. She was treated with a small dose of insulin from age 29 years. At the end of the second trimester of this third pregnancy, she noticed a hearing problem which was diagnosed at that time as sensory hearing disturbance. She possessed HLA-DR4/9 and DQ3. Her father had NIDDM and her mother was found to have a postprandial blood glucose level of 8.9 mmol/l. De-

spite diabetes of 14 years' duration, she did not show any diabetic complications. Her brother had insulin-requiring NIDDM, but his DNA had not been tested. An oral glucose tolerance test (OGTT) for her daughter (10 years) was in the normal range, but her DNA showed the 3243 bp mutation.

Patient O.T. was diagnosed with NIDDM at age 29 years. Insulin treatment began at age 32 years during her third pregnancy. She had a history of spontaneous abortion in her first pregnancy and a boy was delivered prematurely in her second pregnancy. Before her third pregnancy, she had pre-pregnancy education which resulted in her giving birth to a second mature baby. The 12-year diabetes duration did not cause any diabetic complications. She did not know whether or not her deceased parents had had diabetes.

Patient K.K. had insulin-requiring NIDDM with a maternal family history of this condition; her brother also had NIDDM. She began insulin treatment with pre-pregnancy education before her second pregnancy. The first pregnancy did not require any treatment, she now has two daughters and has had no abortions.

Patient J.K. had NIDDM diagnosed at age 29 years. At age 31 years, she consulted our clinic because of glycosuria and insulin treatment was required as part of her pre-pregnancy control. She has two daughters and has had no abortions. Her father had NIDDM, but she does not know whether or not her mother had diabetes.

Patient M.F. has NIDDM which has required insulin (maximum 40 IU/day) during three pregnancies. She underwent an abortion in her first pregnancy, and then gave birth to two children; the second son was premature weighing 700 g at birth, primarily due to pre-eclampsia during pregnancy. All the pregnancies were under strict glycaemic control ($HbA_{1c} \leq 6.0\%$). Her mother has NIDDM which is treated with insulin. After 5 years of diabetes, she has no diabetic complications, but does have pro-

Fig. 2. Pedigrees of the patients with the 3243 bp mutation

teinuria as a result of chronic glomerulonephritis. Her ECG revealed the Lown-Ganong-Levine syndrome.

During pregnancy at age 25 years, patient T. H. was diagnosed with GDM. She was found to have a maternal family history of NIDDM, and had been treated with insulin (maximum 8 IU/day) during her first pregnancy.

Patients Y. I. and N. T. were diagnosed with borderline diabetes, 26 weeks into a second pregnancy after a spontaneous abortion, and 22 weeks into a pregnancy, respectively. Their postprandial blood glucose levels were less than 6.7 mmol/l without insulin treatment during pregnancy, and their OGTTs reverted to normal after delivery. Y. I. experienced a second abortion after delivering one son while under strict glycaemic control; her mother has commenced insulin treatment. DNA from her peripheral blood did not show any band after Apa I digestion, and DNA from other tissues has not yet been evaluated. No family history of diabetes was reported in the questionnaire sent to the family of N. T.

Insulin secretory capacity of pancreatic beta cells. Table 3 shows the endogenous insulin secretory capacity of the nine probands with the 3243 bp mutation by OGTT or meal tolerance test at their first visit to our clinic and at present. Three NIDDM women (T. O., O. T., and K. K.) who required insulin treatment before or during pregnancy had insufficient endogenous insulin secretory capacity. Although K. H., J. K., and M. F. had adequate endogenous insulin secretory capacity before pregnancy, they were treated with a small dose of insulin to maintain postprandial blood glucose levels at 6.7 mmol/l. One GDM woman also had adequate endogenous insulin secretory capacity during pregnancy; however, she was treated with a small dose of insulin following GDM diagnosis at the end of her pregnancy to maintain her postprandial blood glucose level at less than 6.7 mmol/l. Y. I. and N. T. did not require insulin treatment during pregnancy. There were no probands with hyperinsulinaemia.

All patients with the 3243 bp mutation were negative for anti-GAD antibodies.

History of spontaneous abortion. Table 4 shows the prevalence of spontaneous abortion(s) in the pregnant diabetic woman with and without the 3243 bp mutation. Although it was not statistically significant, the prevalence of spontaneous abortions was higher in the population with the mutation than in the population without (27.3 vs 12.4 %).

Table 5 shows the prevalence of the pregnant women with and without the 3243 bp mutation. Thirty-seven of the subjects had a past history of spontaneous abortion, some of whom had been under strict metabolic control ($HbA_{1c} \leq 6\%$). The glycaemic control of the other patients is unclear because their abor-

Table 3. Oral glucose (GTT) or meal tolerance test (MTT), and anti-GAD antibody of probands

Patient	Age at GTT or MTT	B/D/A pregnancy	Blood glucose (mmol/l)				CRP(μg/ml) ^a /IRI (IU/ml)				Anti-GAD
			0	30	60	120	0	30	60	120	
T.O.	26 MTT	B	6.9–11.1–12.6–10.6				1.0–	1.9–	2.2–	2.1	< 4
	41 MTT	A	5.4– –13.1–12.4				0.7–	–	1.7–	2.1	
K.H.	28 MTT	B	5.9– 9.3– 8.5– 6.6				^a 5.2–25.0–38.9–29.0				< 4
	40 MTT	A	8.2– –12.7–12.1				1.0–	–	2.3–	2.0	
O.T.	33 MTT	D	6.4– 7.4–10.7– 6.6				0.3–	0.5–	0.9–	0.8	< 4
	40 MTT	A	6.5– 7.8– 6.5– 7.8				0.7–	0.8–	1.0–	1.3	
K.K.	34 MTT	B	5.7– 6.8– 7.8– 5.6				1.0–	1.7–	2.3–	1.6	< 4
	35 MTT	A	5.4– 7.6– 8.7– 5.1				0.9–	1.3–	1.8–	1.1	
J.K.	34 MTT	B	6.1– 6.7– 8.2– 9.3				1.5–	3.0–	4.4–	5.3	< 4
	35 MTT	A	4.8– 6.0– 5.9– 4.9				0.9–	1.8–	2.3–	2.0	
M.F.	30 MTT	B	7.1– 8.7–10.1– 7.6				3.4–	4.3–	6.2–	6.9	< 4
	31 MTT	A	4.8– 6.1– 6.4– 6.5				2.4–	2.6–	3.1–	4.2	
T.H.	25 MTT	D (29W)	4.1– 5.9– 7.2– 6.1				1.9–	4.1–	6.0–	5.3	< 4
	25 GTT	A (4D)	3.9– 8.4– 9.7– 7.2				1.4–	6.0–	8.3–	16.4	
Y.I.	30 GTT	D (26W)	4.3– 8.7–10.8– 5.8				^a 10.6–35.6–52.6–52.3				< 4
	34 GTT	A	5.2– 8.4– 6.6– 4.9				^a 8.3–44.9–49.2–25.8				
N.T.	26 GTT	D (22W)	4.2– 7.4– 9.2– 8.4				^a 4.4–31.3–40.6–47.7				< 4
	27 GTT	A	4.2– 5.9– 5.3– 5.4				^a 8.1–31.3–35.2–32.6				

^a IRI; Plasma immunoreactive insulin; CPR, C-peptide immunoreactivity;
B/D/A, before pregnancy, during pregnancy, or after delivery;
W, gestational week;

4D, 4th day after delivery;
< 4, anti-GAD antibody level within normal range

Table 4. Prevalence of spontaneous abortion experienced by diabetic women with and without the 3243 bp mitochondrial mutation

Diabetes type	With mutation abortion/total pregnancy (%)		Without mutation abortion/total pregnancy (%)	
IDDM	0/0		10/107	(9.3)
NIDDM	4/17	(23.5)	29/200	(14.5)
GDM	0/1	(0.0)	1/30	(3.3)
Borderline	2/4	(50.0)	8/49	(16.3)
Total	6/22	(27.3)	48/386	(12.4)

Table 5. Prevalence of diabetic women with and without the 3243 bp mitochondrial mutation who experienced spontaneous abortions

Diabetes type	With mutation		Without mutation	
	Abortion	No abortion	Abortion	No abortion
IDDM	0	0	8 (2) ^a	56
NIDDM	3 (1)	3	19 (4)	77
GDM	0	1	1 (0)	10
Borderline	1 (1)	1	5 (2)	27
Total	4 (2)	5	33 (8)	170

^a A total of two or more abortions shown in parentheses

tions occurred before they started to attend the clinic. There was no difference in age at the onset of diabetes between the groups with and without a history of abortion. Among the nine diabetic women with the 3243 bp mutation, four had a history of abortion (Table 2) compared with 33 of 203 women without the mutation ($p = 0.0518$). Although T.O. and Y.I. were under strict metabolic control, they each had undergone two spontaneous abortions ($p = 0.0479$) (Fig. 2). There was no relationship between spontaneous abortion and age at onset or diabetic metabolic control of the nine probands who consulted us which may support a possible role for the 3243 bp mutation in the abortion. Moreover, two other probands had experienced two premature deliveries. M.F. gave birth to a premature baby despite being under our strict metabolic control following a spontaneous abortion. The

nine probands with the 3243 bp mutation had a problem during their gestational and prenatal periods. The mothers of the nine probands had undergone no spontaneous abortions, or given birth to premature babies, or children with mental retardation as far as can be ascertained. The eight spontaneous abortions in the IDDM patients may have occurred as a result of causes other than the 3243 bp mutation.

Discussion

In this study we found that the nine probands (4.2 %) with the 3243 bp mutation from among the 212 subjects studied had either NIDDM, GDM or borderline diabetes, but none had IDDM. There was a higher prevalence of diabetic patients with the 3243 bp

mutation among women attending the PCDW than among the randomly selected diabetic patients [9]. This higher prevalence in the PCDW may be related to the greater frequency of family history of diabetes (NIDDM) compared with the previous report [8] and with the younger age at onset, which ranged from approximately 20 to 40 years. Of our IDDM subjects 14 % had a family history of NIDDM among first-degree relatives, though none of the IDDM patients had the 3243 bp mutation.

Another obvious difference was in the age at onset of IDDM between our IDDM subjects and those reported previously [8]. The IDDM patients in this study developed ketoacidosis with an abrupt onset at the age of 19.1 ± 7.8 years (range 2 to 28), while the previous IDDM subjects had an age of onset of 33.3 ± 13.3 years (range 2 to 69). This suggests that the 3243 bp mutation in the mitochondria may cause a subtype (subgroup) of IDDM with a relatively older age of onset. The result also indicates a heterogeneity in Japanese IDDM. On the other hand, all the probands in this study were negative for anti-GAD antibodies (Table 3), anti-thyroglobulin and anti-thyroid peroxidase test (data not shown). As 24 % of Japanese IDDM individuals were positive for anti-GAD antibodies even after a 10-year diabetes duration (unpublished data), negativity for anti-GAD antibodies may support the definition of NIDDM. Consistent with this, HLA-DR 4 or DR 9 was positive in only one proband. Taken together, the probands with insulin-requiring NIDDM associated with the 3243 bp mutation had no predictive marker of IDDM suggesting autoimmune destruction of pancreatic beta cells.

The five probands with NIDDM required insulin within 5 years after diagnosis to keep postprandial blood glucose levels at less than 6.7 mmol/l. The remaining four probands did not always require insulin treatment, although two of them with NIDDM and GDM required insulin treatment only during pregnancy. The two probands with GDM and borderline diabetes were found to have the 3243 bp mutation when they became pregnant. These findings raise two possibilities: as it has been shown that the degree of heteroplasmy in tissues and individuals is variable, the percentage of the 3243 bp mutation in pancreatic beta cells may be variable in individuals, thus causing variability in the defect in insulin secretion and glucose tolerance. Another possibility is that subjects with the 3243 bp mutation who do not develop diabetes in adolescence or in their twenties may develop this condition or impaired glucose tolerance when environmental factors, such as obesity or pregnancy, are added. This is supported by evidence that the OGTT pattern of Y.I., and N.T. who were diagnosed with GDM or borderline diabetes was almost within the normal range after delivery (Table 3). Such environmental factors might include infection, aging [17, 18] or great stress other than obesity and pregnancy, in

which there is an increase in pancreatic beta-cell demand.

Although GDM usually returns to a state of normal glucose tolerance after delivery, approximately 60 % of women with a history of GDM subsequently develop diabetes [19, 20]. On the other hand, less than 10 % of women with borderline type diabetes are reported to subsequently develop diabetes (unpublished data). Stoffel et al. [21] reported that about 5 % of American women with a history of GDM had the glucokinase mutation and Zaidi et al. [22] reported that 3.6 % of British women with a history of GDM had the glucokinase mutation. This mutation has been identified less frequently in the Japanese than in other races, however; only four patients with NIDDM have been identified as having glucokinase mutation to date [23–26].

Until the 1970's there was consensus that the prevalence of spontaneous abortion in diabetic women was almost equal to that in non-diabetic women [27, 28]. Several more recent reports stated that spontaneous abortion was more frequent in pregnant IDDM women with a high HbA_{1c} titre in the first trimester [2, 3]. However, the two probands with the 3243 bp mutation in this study had a spontaneous abortion even while under our strict diabetic metabolic control ($\text{HbA}_{1c} \leq 6\%$). This may suggest that the abortion occurred due to factors other than poor metabolic control. Gene defects and gene mutation in the fetus may be other possible explanations for spontaneous abortion induced by fetal abnormality [1]. The higher prevalence of abortion in subjects with NIDDM, GDM, and borderline diabetes associated with the 3243 mutation shown in this study may support a relationship between the abortion and the 3243 bp mitochondrial mutation.

The current study has raised another problems of spontaneous abortions unrelated to the 3243 bp mitochondrial mutation. It is possible that eight (12 %) spontaneous abortions in 64 IDDM patients may be not related to this mutation, and the prevalence of spontaneous abortion in the 64 IDDM patients was 10.3 %. We have previously reported that the prevalence of IDDM women who experienced spontaneous abortion from 1979 to 1987 was 20.0 % [2]. One reason for the difference in prevalence between this study and the previous study may be the introduction of pre-pregnancy education. The prevalence of non-IDDM women with abnormal glucose tolerance without this mutation who experienced spontaneous abortions was 21 % in this study, which was more than 10 % of the non-diabetic women. Because of slow onset and non-insulin dependency in NIDDM, the need for pre-pregnancy education could not always be anticipated. Therefore, metabolic control, especially in the first trimester, would be uncontrolled in addition to other unknown gene defects possibly related to development of NIDDM.

This study of subjects attending the PCDW in our center may provide an opportunity to learn more about the natural course of diabetes with the 3243 bp mutation by a follow-up of the metabolic state of the offspring. Further analysis of DNA from cells in amniotic fluid or placenta obtained from the abortion may show direct evidence of the relationship between the 3243 bp mitochondrial gene mutation and spontaneous abortion.

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