Heterogeneity of diabetes phenotype in patients with 3243 bp mutation of mitochondrial DNA (Maternally Inherited Diabetes and Deafness or MIDD)

PJ Guillausseau1, D Dubois-Laforgue2, P Massin3, M Laloï-Michelin1, C Bellanne-Chantelot4, H Gin5, E Bertin6, JF Blickle7, B Bauduceau8, B Bouhanick9, J Cahen-Varsaux10, S Casanova11, G Charpentier12, P Chedin10, C Derrien13, A Grimaldi14, B Guerci15, E Kaloustian16, F Olivier19, M Paques3, V Paquis-Flucklinger20, A Tielmans1, M Vincenot1, B Vialettes22, J Timsit2

GEDIAM, Mitochondrial Diabetes French Study Group

SUMMARY

Objective: In patients with maternally inherited diabetes and deafness (MIDD), due to 3 243 A > G mutation of mitochondrial DNA (mtDNA), diabetes may present with variable phenotypes.

Objective: To ascertain the existence of two distinct phenotypes, MIDD1 and MIDD2, in a series of patients with MIDD.

Design: Multicenter prospective study.

Patients: 77 patients with diabetes and the mtDNA 3243 mutation and 139 control patients with type 1 (T1D) or type 2 (T2D) diabetes, matched according to initial presentation of diabetes, age at onset, sex, and duration of diabetes (24 T1D and 115 T2D, including 55 treated with insulin).

Measurements: Anthropometric characteristics (height, body weight, body mass index [BMI], sex), family history of diabetes, and characteristics of diabetes (age at onset, treatment, hemoglobin A1c [HbA1c]), extrapancreatic manifestations.

Results: In 13 cases (17%, MIDD1), diabetes presented as insulin-dependent from the onset, with ketoacidosis in 6 cases. In 64 cases (83%, MIDD2), diabetes resembled T2D, and was treated with diet in 12 cases, oral hypoglycemic agents in 21 cases, or insulin in 31 cases. Compared with patients with MIDD2, patients with MIDD1 were characterized by lower age at onset of first manifestation of MIDD (25.4 ± 9.6 vs 33.7 ± 13.2 years, P < 0.0005), lower body weight (49.1 ± 7.4 vs 56.3 ± 10.9 kg, P < 0.0025), lower BMI (18.2 ± 2.3 vs 20.9 ± 3.6 kg/m², P < 0.0005), and higher HbA1c levels (9.5 ± 2.0 vs 7.5 ± 1.6%, P < 0.0005). Frequency of family history of diabetes and of extrapancreatic manifestations was the same in both MIDD subtypes. No difference was found within the MIDD2 subtype when comparing patients treated with or without insulin. Compared with matched controls, patients with MIDD had a lower BMI (MIDD1/T1D: 18.2 ± 2.3 vs 24.0 ± 3.6 kg/m² and MIDD2/T2D: 20.9 ± 3.6 vs 30.2 ± 5.9 kg/m², P < 0.0025). Lastly, male patients with MIDD had a shorter height than controls (MIDD1/T1D: 166.1 ± 3.2 vs 177.3 ± 6.6 cm and MIDD2/T2D: 168.4 ± 7.2 vs 173.6 ± 6.6 cm P < 0.025).

Conclusions: These results confirm the existence of two different phenotypes in MIDD, MIDD1 and MIDD2, which may be related to the severity of the mitochondrial disease. The role of other genetic and/or environmental factors in the variable phenotype of MIDD remains to be elucidated.

Key-words: Diabetes mellitus - Mitochondrial 3243mtDNA mutation - Maternally inherited diabetes and deafness - MELAS - Macular pattern dystrophy - Deafness - Height - Body weight - BMI - Type 1 diabetes - Type 2 diabetes - Phenotype.
RÉSUMÉ

Hétérogénéité phénotypique du diabète des patients atteints de diabète par mutation 3243 A > G de l’ADN mitochondrial (Maternally Inherited Diabetes and Deafness ou MIDD)

Chez les patients atteints d’un diabète par mutation 3243 A > G de l’ADN mitochondrial (Maternally Inherited Diabetes and Deafness ou MIDD), la présentation phénotypique du diabète pourrait être variable.

Objectifs : Confirmer dans une série de patients atteints de MIDD l’existence de deux phénotypes distincts, MIDD1 et MIDD2.

Type d’étude : Étude prospective multicentrique.

Patients étudiés : Soixante-dix sept patients atteints de diabète par mutation 3243 de l’ADN mitochondrial, et 139 témoins atteints d’un diabète de type 1 (DT1) ou de type 2 (DT2), apparus selon la présentation initiale du diabète, l’âge de début, le sexe, et la durée d’évolution du diabète (24 DT1 et 115 DT2, dont 55 insulino-traités).

Paramètres de l’étude : Caractéristiques anthropométriques (taille, poids, indice de masse corporelle [IMC], sexe), antécédents familiaux de diabète, caractères du diabète (âge de début, traitement, HbA1c), manifestations extrapancréatiques.

Résultats : Dans 13 cas (17 %, MIDD1), le diabète se présentait comme insulino-dépendant d’emblée, avec une acidoétose dans 6 cas. Dans 64 cas (83 %, MIDD2), le diabète ressemblait à un DT2, et était traité par régime dans 12 cas, hypoglycémiants oraux dans 21 cas. Comparés aux patients atteints de MIDD2, les patients atteints de MIDD1 avaient un âge plus précoce de survenue de la première manifestation du MIDD (25,4 ± 9,6 vs 33,7 ± 13,2 ans, P < 0,0005), un poids plus faible (49,1 ± 7,4 vs 56,3 ± 10,9 kg, P < 0,0025), un IMC plus bas (18,2 ± 2,3 vs 20,9 ± 3,6 kg/m², P < 0,0005), et des taux d’HbA1c plus élevés (9,5 ± 2,0 vs 7,5 ± 1,6 %, P < 0,0005). La fréquence des antécédents familiaux de diabète et des manifestations extrapancréatiques était la même dans les deux sous-groupes de MIDD. Nous n’avons pas trouvé de différence au sein du sous-groupe MIDD2 entre les patients insulino- et non-insulino-traités. Comparés à des témoins appariés, les patients atteints de MIDD avaient un IMC plus bas (MIDD1/DT1 18,2 ± 2,3 vs 24,0 ± 3,6 kg/m² et MIDD2/DT2 20,9 ± 3,6 vs 30,2 ± 5,9 kg/m², P < 0,025). Enfin, les patients de sexe masculin atteints de MIDD avaient une taille plus faible que celle des témoins (MIDD1/DT1 : 166,1 ± 3,2 vs 177,3 ± 6,6 cm, et MIDD2/DT2 : 168,4 ± 7,2 vs 173,6 ± 6,6 cm P < 0,025).

Conclusions : Ces résultats confirment l’existence dans le MIDD de deux phénotypes distincts, le MIDD1 et le MIDD2, qui pourraient refléter une sévérité variable de la maladie mitochondriale. Le rôle dans cette variabilité phénotypique d’autres facteurs génétiques et/ou environnementaux reste à élucider.

Mots-clés : Diabète sucré · Mutation 3243 de l’ADN mitochondrial · Maternally inherited diabetes and deafness · MELAS · Dystrophie maculaire réticulée · Sourde · Taille · Poids · Indice de masse corporelle · Diabète de type 1 · Diabète de type 2 · Phénotype.
Mitochondrial cytopathies due to mitochondrial DNA (mtDNA) abnormalities are responsible for multorgan diseases that may include diabetes mellitus. It is well recognized that a given point mutation or deletion of mtDNA can lead to various phenotypes. Clinical syndromes associated with a point mutation of mtDNA, an A to G transition at position 3243, encoding for transfer RNA leucine (tRNA_{Leu}^{UUR}) are good examples of such variability [1]. In some cases, this mutation cosegregates with a syndrome called mitochondrial encephalopathy, lactic acidosis, stroke-like episodes (MELAS), which is associated with diabetes in some instances [2], while, in other patients, the same mutation is associated with a less severe disease called maternally inherited diabetes and deafness (MIDD) [3-4].

We previously reported the MIDD phenotype in 54 patients from a large multicenter study from the GEDIAM (French Mitochondrial Diabetes Study Group). We showed that diabetes may present either as non-insulin-dependent, with secondary requirement for insulin therapy in almost half of patients, or as insulin-dependent from the onset [5]. This observation recalls that of Maassen and Kadowaki [6], who suggested that MIDD may comprise two different subtypes of diabetes. In a recent series of 113 Japanese patients, including 99 patients with MIDD and 14 with MELAS, diabetes was insulin-treated in 86.1% of cases, but no detailed analysis of phenotype heterogeneity was made [7].

The aim of the present study was thus to ascertain, in a large series of white patients with MIDD, the existence of two distinct phenotypes according to clinical presentation at onset of diabetes. In order to better delineate these phenotypes, patients with MIDD were compared with patients with "common" forms of type 1 and type 2 diabetes. This comparison was based on phenotypic presentation, since pathogenic mechanisms in MIDD are clearly distinct from those of type 1 and type 2 diabetes [6, 7].

**Methods**

The GEDIAM prospective study [5] was initiated in September 1995. By September 2003, 77 patients with MIDD (from 57 families) had been included in the study. As previously described, a structured interview and a standardized examination of the patients and of their relatives were used to ascertain a family history of diabetes, hearing loss, glucose tolerance disorders, treatment, and associated manifestations [5]. The 77 patients with MIDD were compared with a series of 139 unrelated patients with the "common" subtypes of diabetes (types 1 and 2) recruited from the Department of Clinical Immunology and Diabetology of Necker Hospital (Paris, France), and referred as to "control patients" in the present report. Among control patients, 24 had type 1 diabetes, and 115 had type 2 diabetes. In the latter, secondary diabetes and latent autoimmune diabetes of the adult were excluded. Control patients were matched to the patients with MIDD according to clinical presentation at onset of diabetes (ie, insulin-dependent or non-insulin-dependent), age at onset, sex, current treatment (diet and/or antidiabetic oral agents and/or insulin), and duration of diabetes. Diagnosis of diabetes was based on World Health Organization 1997 criteria [8]. Hemoglobin A_{1c} (HbA_{1c}) was assayed by high-performance liquid chromatography (HPLC). The study conformed to the principles of the Declaration of Helsinki, and subjects gave informed consent.

**Molecular studies**

The A3243G mutation detection was based on an allele-specific polymerase chain reaction (PCR), as previously described [9]. The common oligonucleotide and the two primers specific for the normal and mutant alleles were respectively defined at position 3077-3095 and 3243-3271 (Positions are given in reference to the mitochondrial sequence, accession number J01415). Two PCRs, each one with an allele-specific primer, were carried out in a 40-mL volume containing 100 ng genomic DNA, 1X PCR buffer (Applied Biosystems), 200 mM dNTPs, 75 ng of each primer, and 1 U of AmpliTaq DNA polymerase (Applied Biosystems). The PCR conditions were as follows: denaturation at 94 °C for 1 min followed by 26 cycles of denaturation at 94 °C for 10 s, annealing at 62°C for 10 s, and extension at 72 °C for 10 s with a final extension at 72°C for 6 min. An aliquot of each PCR product was loaded into agarose gel and stained by ethidium bromide for detection of the mutation.

**Statistical analysis**

Data are expressed as mean ± standard deviation, with range indicated in brackets. Differences between patient groups were assessed using the Student t-test, and chi-square test, and significance was set at P < 0.05.

**Results**

In the patients with MIDD (31 men, 46 women), diabetes was diagnosed by systematic screening in 37 patients (48%), and was revealed by clinical symptoms in 40 (52%). Age at diagnosis of diabetes was 35.3 ± 11.0 years (12-67), and in 30 patients (39%) diabetes was diagnosed before age 35. At the time of the study, patients were aged 48.6 ± 10.2 years (31-71), and known duration of diabetes was 11.0 ± 9.0 years (0-37). In 13 cases (17%), diabetes was insulin-dependent from the onset, with ketoacidosis in 6 (8%). This group is henceforth referred to as MIDD1 (Tab I). No difference was found between the age of diabetes onset of MIDD1 patients with ketoacidosis and that of MIDD1 patients without ketoacidosis (29.5 ± 6.6 vs 30.3 ± 6.6 years, NS). In the MIDD1 group, only 1 patient had an age of onset before 15 years. In 64 cases (83%), diabetes phenotype was close to type 2 diabe-
Islet cell antibodies (ICA, GAD 65, and/or IA2 antibodies) were not found in the 43 patients tested. The control groups (Tab II) included 24 patients with type 1 diabetes, and 115 patients with type 2 diabetes. Sixty type 2 diabetic patients were non-insulin-treated, and 55 were insulin-requiring, with a secondary failure of antidiabetic oral agents occurring 11.2 ± 7.1 years (1-26 years) after the diagnosis of diabetes (NS compared with insulin-requiring MIDD2 patients).

A first-degree family history of diabetes was present in 43/57 probands with MIDD (75%), and in 81/139 controls (58%) (P < 0.02). A first-degree maternal history of diabetes was present in 37/57 probands with MIDD (65%) and in 20/98 controls (20%) (P < 0.0005). No difference in the frequency of a maternal history of diabetes was observed between male patients (20/27, 74%), and female patients with MIDD (30/43, 69.8%).

As compared with patients with MIDD2, those with MIDD1 were characterized by an earlier onset of the disease, as shown by a lower age at onset of diabetes (by 9.1 years, on average), at onset of deafness, and at recognition of the first manifestation of the disease (Tab I). Age at onset of diabetes was less than 35 years in 77% of patients with MIDD1 and 39% of patients with MIDD2 (P < 0.02). MIDD1 patients also had a lower body mass index (BMI) than those with MIDD2, with a mean 2.7 kg/m² difference. This was related to a lower body weight, since height was not different between the two groups (Tab II). There was no difference between the two groups concerning the frequency of family history of diabetes and that of maternal history of diabetes, as well as the frequency of extrapancreatic manifestations of the disease. Lastly, HbA1c, at the time of the study was higher in patients with MIDD1 than in those with MIDD2 (Tab I).

Among patients with MIDD2, at the time of the study, diabetes was treated by diet alone in 12 cases, and with sulfonylureas and/or metformin in 21. In 31 patients (40%), secondary failure of a combination of maximally-dosed sulfonylureas and metformin had occurred 10.7 ± 6.8 years (1-28 years) after the diagnosis of diabetes. No difference was ob-

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<td>Main clinical characteristics of patients with maternally inherited diabetes and deafness (MIDD) with insulin-dependent diabetes at onset (MIDD1) or with noninsulin-dependent diabetes at onset (MIDD2). BMI, body mass index.</td>
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<td>Main characteristics of patients with maternally inherited diabetes and deafness MIDD1 and MIDD2, compared with patients with type 1 (T1D) and type 2 diabetes (T2D). BMI, body mass index; HbA1c, hemoglobin A1c.</td>
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erved between patients treated without or with insulin concerning age at onset of diabetes, age at onset of the first manifestation of the disease, sex ratio, height, body weight, BMI, and frequency of extrapancreatic manifestations. Mean HbA1c levels were higher in patients with insulin-requiring diabetes than in patients with non-insulin-treated diabetes (8.2% ± 1.7% vs 6.9% ± 1.1%, P < 0.025).

Finally, the clinical characteristics of MIDD1 and MIDD2 patients were compared with those of control patients with type 1 or type 2 diabetes, respectively (Tab II). We found two striking differences in the phenotype of the patients with MIDD compared with those with common types of diabetes, namely, lower BMI and shorter height. In patients with MIDD1, BMI was lower by 5.8 kg/m² than in those with type 1 diabetes. Mean body weight was dramatically lower in MIDD1 patients, by 23 kg on average. The height of male patients with MIDD was lower than that of male patients with common diabetes. Patients with MIDD1 were shorter by 11.2 cm on average than those with type 1 diabetes, and patients with MIDD2 were shorter by 5.2 cm than those with type 2 diabetes. No difference was observed between female patients among matched groups. Finally, mean HbA1c levels were significantly higher in patients with MIDD1 than in matched controls, while they were significantly lower in patients with MIDD2 than in matched controls.

Discussion

Several reports have shown that the coexistence of diabetes and extrapancreatic manifestations help diagnosis of MIDD [3-7]. However, since diabetes may be the first and/or main clinical manifestation of MIDD in some patients, we focused the present study on the clinical characteristics of diabetes. In this large series, we first show that two distinct phenotypes may be observed among patients with MIDD. We also confirm that some phenotypic traits clearly distinguish diabetes due to the mtDNA 3243 mutation from more “common” types of diabetes. Finally, we report the original observation that male patients with MIDD are shorter than patients with other types of diabetes.

In the present series, 17% of MIDD patients presented with insulin dependency from the onset, while in the others requirement for insulin therapy, if any, was delayed by 10 years on average. Although we may have introduced a selection bias by considering the patients according to treatment at onset of diabetes, we believe that the two phenotypes, ie, MIDD1 and MIDD2, represent subtypes of MIDD. Compared with patients with MIDD2, those with MIDD1 were younger at onset of diabetes by 9 years on average, and age at onset was below 35 years twice more often than in MIDD2. More generally, MIDD1 patients were younger at onset of the disease than MIDD2 patients, as shown by an age at first manifestation and an age at deafness lower by 8 years and 7 years, respectively. These data suggest that MIDD1 may be a severe form of the disease. Indeed, at the time of the study, patients with MIDD1 were also thinner than patients with MIDD2, even when the latter were treated with insulin.

We hypothesize that the very low BMI observed in MIDD1 is related, at least partly, to the severity of insulin deficiency. This is supported by the occurrence of ketoacidosis in half the cases, and by the high HbA1c value of MIDD1 patients, compared with MIDD2 patients, even those treated with insulin. The role of anti-β-cell autoimmunity is unlikely since, like others [7, 10, 11], we have not detected autoimmunity markers in any patient. The absence of obesity in MIDD fits well with the fact that diabetes is primarily due to a defect in insulin secretion [12], and that insulin sensitivity is normal [12, 13]. However, other mechanisms are likely involved in the low BMI observed in these patients, including loss of muscle mass and/or adipose tissue due to the severity of the mitochondrial disease, although this was not specifically assessed by us or other groups. The frequency of extrapancreatic manifestations of the disease was similar in the two subgroups, suggesting that patients with MIDD1 have a β-cell disease more severe and/or of earlier onset than those with MIDD2, rather than a different disease due to the same mutation.

Several mechanisms, related to environmental factors or to genetics, may be involved in the variable severity of MIDD. One interesting hypothesis is that exposure in utero to maternal diabetes may promote early onset of diabetes, as demonstrated in Pima Indians with type 2 diabetes [14], and more recently in patients with maturity-onset diabetes of the young-3 (MODY3) [15]. In a series of Japanese patients with MIDD, maternal diabetes was associated with an earlier age of onset of diabetes, lower glucagon-stimulated C-peptide levels, larger insulin doses, and higher heteroplasmic concentrations in leucocytes [7]. However, in the present series, the frequency of maternal history of diabetes was the same in MIDD1 and MIDD2 patients, making this mechanism unlikely. Two mechanisms involved in the expression of mitochondrial DNA diseases, ie, heteroplasmia and threshold effect [16], may directly account for the variable severity of MIDD. Several studies have shown that a high degree of heteroplasmia for the A3243G mutation is associated with an earlier age of onset of the disease [7, 18-19], although one conflicting report was published [20]. However, in these studies, heteroplasmia for the A3243G mutation was determined in leucocytes [18-20] and may not reflect the level of heteroplasmia in β-cells. Whether other genes may modulate the expression of diabetes, as suggested in the context of MODY3 [21], is unknown.

At variance with previous studies, we compared the phenotype of patients with MIDD to that of 139 patients with “common” subtypes of diabetes, ie, type 1 and type 2 diabe-
Diabetes mellitus, carefully matched for age, gender, duration and treatment of diabetes. We confirm previous results showing that patients with MIDD are much thinner than those with type 1 and type 2 diabetes [3, 7, 13, 22, 23]. This suggests a specific role of the mitochondrial disease in the very low BMI observed in patients with MIDD, as discussed above. Surprisingly, male patients with MIDD exhibited a shorter height than matched controls. This was not observed in a Japanese series, but, in that study, patients with MIDD were compared with subjects from the general population, not with patients with diabetes [7]. Our study does not provide an explanation for the short height of male patients. We do not think that diabetes itself is involved in this anomaly because, in the majority of the patients, diabetes occurred after puberty. Moreover, it has been shown that patients with type 1 diabetes have a normal height [24]. Since the shorter height was clearly dependent on male gender, we hypothesize that a sex-related mechanism may be involved in its occurrence.

In conclusion, the present study shows that among patients with MIDD diabetes may present with two distinct phenotypes that may be related to a variable severity of the disease. The mechanisms of this variation warrant further studies. We also show that male patients with MIDD are shorter than those with common types of diabetes, suggesting that other endocrine defects may be associated.

References