Maternally Inherited Diabetes and Deafness (MIDD) Syndrome: A Clinical and Molecular Genetic Study of a Taiwanese Family

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We report on a case of a 48-year-old woman presenting with maternally inherited diabetes mellitus and deafness (MIDD) syndrome. Molecular genetic analysis and clinical evaluation were conducted in the patient and her 4 children to investigate the interrelation between an MIDD-associated mitochondrial DNA (mtDNA) mutation and clinical manifestations. Various symptoms and markers of MIDD, including seizures, migraines, short stature, mental retardation, and stroke-like episodes, were reviewed. Diabetes mellitus (DM) was studied by oral glucose tolerance and glucagon stimulation tests. Hearing impairment was determined by standard hearing tests and a brainstem auditory evoked potential test. The A3243G and T3271C transitional mutations of mtDNA were investigated from muscle and/or leukocytes and hair follicles. Mitochondrial-related symptoms were not found in the children, although they all harbored a heteroplasmic A3243G transition of mtDNA, as detected in screened samples. For the patient, the proportion of mutant mtDNA was highest in muscle cells followed by hair follicles and then leukocytes. Moreover, the proportion of mutant mtDNA was also higher in hair follicles than in leukocytes for asymptomatic family members. This Taiwanese MIDD family was found to have the A3243G point mutation as revealed from molecular genetic studies of leukocytes, hair follicles, and muscle tissue. However, no correlation was found between the proportion of mutant mtDNA and clinical features of any family member. *(Chang Gung Med J 2004;27:66-73)*

Key words: **diabetes mellitus, deafness, mitochondrial DNA, A3243G mutation, family survey.**

Recent advances in molecular analyses have enabled the recognition of a specific diabetic syndrome characterized by the development of maternally inherited diabetes mellitus (DM) and sensorineural deafness. Furthermore, a point mutation at nucleotide pair (np) 3243 of the mitochondrial DNA (mtDNA) was identified from most diagnosed patients.[1] This syndrome was subsequently identified in diabetic patients from various racial origins and is referred to as a different phenotype of A3243G mutation-related mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episode (MELAS) syndrome.[2,3] It was later denoted as maternally inherited diabetes and deafness (MIDD) syndrome in order to signify its difference from the ordinary diabetic syndrome.[4] A recent multicenter prospective study of 54 patients with type 2 DM and the mtDNA 3243 mutation concluded the MIDD

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patients were young at diabetes onset and presented with a normal or low body mass index. Non-insulin-dependent diabetes, neurosensory hearing loss, macular pattern dystrophy, myopathy, cardiomyopathy, kidney disease, and neuropsychiatric symptoms were noted as clinical manifestations.[5] Relatively few cases of MIDD have previously been reported.[6] In this paper, we report on the diagnosis of typical MIDD syndrome in a Taiwanese patient. Clinical features and mtDNA abnormalities for the proband and family members were further investigated, and an attempt was made to establish a relation between the mtDNA mutations and clinical manifestations of this syndrome.

CASE REPORT

A 48-year-old woman of thin appearance and short stature (height, 146 cm; weight, 33 kg) had a history of diabetes mellitus with onset at age 32. Oral hypoglycemic agents had been used for diabetes control since that time. There was no history of seizures, myoclonus, migraines, or mental deficits, except for progressive hearing loss over the previous 5 years, and also a right cerebral infarction 1 year previous. Admission was due to poor blood sugar control. On evaluation, the patient appeared alert, with mild left-limb weakness but without Babinski's sign. A brain computed tomography (CT) scan revealed calcification over the bilateral basal ganglia and cerebellum. Brain magnetic resonance imaging (MRI) study revealed cerebral atrophy. The serum glycohemoglobin level was elevated (9.5%; normal < 6.2%), and serum immunoreactive insulin and C-peptide levels were lower than those for normal controls. Insulin antibody was not detected. Intravenous insulin and oral hypoglycemic agents for control of blood sugar and aspirin for antiplatelet therapy were prescribed. Elevated levels of serum creatinine kinase (270 U/L; control 15-130 U/L) and lactate dehydrogenase (176 U/L; control 47-140 U/L), and myopathic changes in electromyography suggested concurrent myopathy. A muscle biopsy was performed on the right vastus lateralis, which revealed ragged red fibers on modified Gomori-trichrome stain. An electron microscope study also revealed a large proportion of abnormal mitochondria of variable sizes in the subsarcolemmal region. Under the impression of mitochondrial encephalomyopathy, various tissues, including muscle and/or blood cells and hair follicles from the patient and family members, were sent for mtDNA analysis.

The phenotypic and genotypic characteristics of the patient and family members were studied. Information was recorded regarding stature, body weight, migraine, episodic vomiting, hemiparesis, optic atrophy, retinitis pigmentosa, seizures, myoclonus, ataxia, hearing impairment, and mental deficits. Special attention was given to DM and hearing impairment. To detect hearing impairment, a standard hearing test was conducted, and brainstem auditory evoked potentials were evaluated. In order to determine the diabetes subtype and severity of pancreatic β-cell dysfunction, the patient and her 4 children were subjected to an oral glucose tolerance test and an intravenous glucagon stimulation test, as described in 1989 by Juang et al.[7] Serial follow-up studies were also conducted 1 and 2 years later.

The patient had a history of diabetes mellitus and sensorineural-type hearing loss. The diabetes was classified as non-insulin-dependent diabetes mellitus (NIDDM) according to the National Diabetes Data Group (NDDG) criteria.[8] Furthermore, the patient's history included stroke-like episodes with left hemiparesis; however, no evidence of seizures, mental retardation, or lactic acidosis was determined. Both age at onset and body mass index (15.48 kg/m^2) were below typical figures (25.1 kg/m^2) for NIDDM patients.[9] None of the 4 children exhibited DM, hearing impairment, or other mitochondrial-related symptoms, except for 1 (II_2) who had been assessed as being mentally retarded. A study of the brain auditory evoked potentials revealed normal results for all 4 children, but not for the proband. Table 1 presents the demographic and clinical data for these family members.

Results of a series of family studies for β-cell function with glucagon stimulation are presented in Fig. 1. Levels of C-peptide were constant for the 4 children; however, the patient's level gradually decreased following glucagon stimulation at different times. The data indicated a progressive secretory defect of insulin for the pancreatic β-cells; however, secretion was normal in the children. Insulin antibody was not detected in any of the family members.

Mitochondrial DNA analysis

Total DNA was isolated from blood cells, hair

Table 1. Demographic and Clinical Data for a Taiwanese MIDD Family.

Subjects	I	II 1	II 2	II 3	II 4
Age (yr)/Gender	48/F	23/M	21/F	20/M	17/M
Short stature (< 150 cm)	+	−	−	−	−
Headaches	−	−	−	−	−
Episodic vomiting	−	−	−	−	−
Hemiparesis	+	−	−	−	−
Hemianopia	−	−	−	−	−
Stroke-like episode	+	−	−	−	−
Ophthalmoplegia	−	−	−	−	−
Optic atrophy	−	−	−	−	−
Retinitis pigmentosa	−	−	−	−	−
Seizure	−	−	−	−	−
Myoclonus	−	−	−	−	−
Ataxia	−	−	−	−	−
Mental deficits	−	−	−	+	−
Intracranial calcification	+	−	−	+	−
Hearing impairment	+	−	−	−	−
DM	+	−	−	−	−
Mitochondrial DNA mutation at 3243	+	+	+	+	+

F: female; M: male.

follicles, and/or muscle biopsies of the proband and her 4 children as previously described.[10] For analysis of the A3243G mutation, a 1159-base pair (bp) fragment of mtDNA ranging from np 2678 to 3836 was amplified by polymerase chain reaction (PCR) as described previously,[11] except that, here, for the last PCR cycle, 5 μCi of [α-^{33}P]- dATP was added to the reaction mixture to label the PCR products. The thermal profile consisted of 1 min of denaturation of DNA at 94˚C, 1 min of annealing with primers at 56˚C, and 1 min of primer extension at 72˚C. Amplification was usually done for 30 cycles, and a 10-μl aliquot of the PCR-amplified DNA fragment was digested overnight at 30˚C with 10 units of the restriction enzyme, *Apa* I, which recognizes the sequence GGGCCC resulting from the A3243G mutation. Two additional fragments of 591 and 568 bp were generated from the mtDNA fragment harboring the A3243G mutation. The *Apa* I-digested PCR products were then subjected to electrophoresis on a 1.5% agarose gel at 100 V for 2 hours.

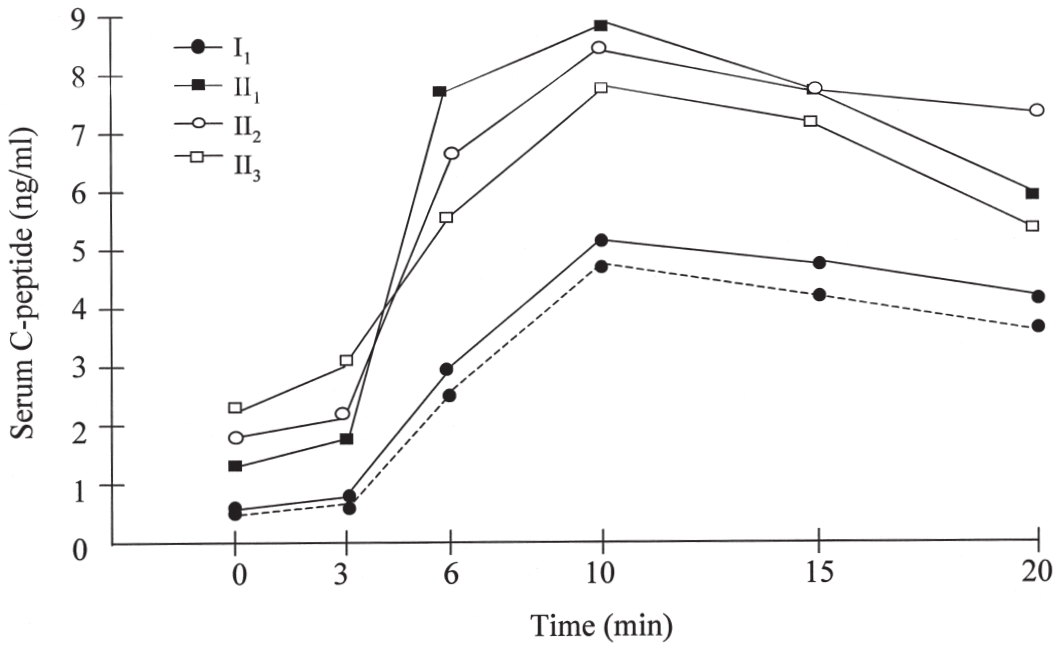

Fig. 1 Changes in serum C-peptide concentrations after intravenous administration of 1 mg of glucagon for the proband and her children. Serum levels of C-peptide gradually decreased from the original observation (—●—) to 2 years later (-●-) in the proband. Serum levels of C-peptide concentrations in her children remained constant (—■—, —○—, —□—).

Subsequent to electrophoresis, the gel was dried and subjected to autoradiography. The X-ray film was then scanned with a scanning densitometer (Personal Densitometer SI, Molecular Dynamics, Sunnyvale, CA). The mutant DNA and wild-type DNA (uncut) fragments were estimated by examining the ratio of their intensities on negative film.[12]

For analysis of the T3271C mutation, a 223-bp fragment was amplified from the mtDNA of each subject by a PCR technique using the primers: 5'-$_{3079}$GGAGTAATCCAGGTCGGT$_{3096}$-3' and 3'-$_{3272}$AATtcCAGTCTCCAAGTTAAGGAGAAGAAT$_{3301}$-5'. The small letters in the second primer indicate a G-to-T mismatch at np 3275 and a T-to-C mismatch at np 3276. These mismatches were specially designed to create a *Bfr* I (Afl II) recognition site following amplification of the DNA segment encompassing the putative T3271C point mutation in the mtDNA of MELAS patients. The 223-bp PCR product of T3271C mutant mtDNA was able to be cleaved by Bfr I into 197- and 26-bp fragments, and the digested DNA mixture was further processed and analyzed as described above.

Whole blood and hair follicles were obtained from the proband and her offspring, and a muscle specimen was also procured from the proband.

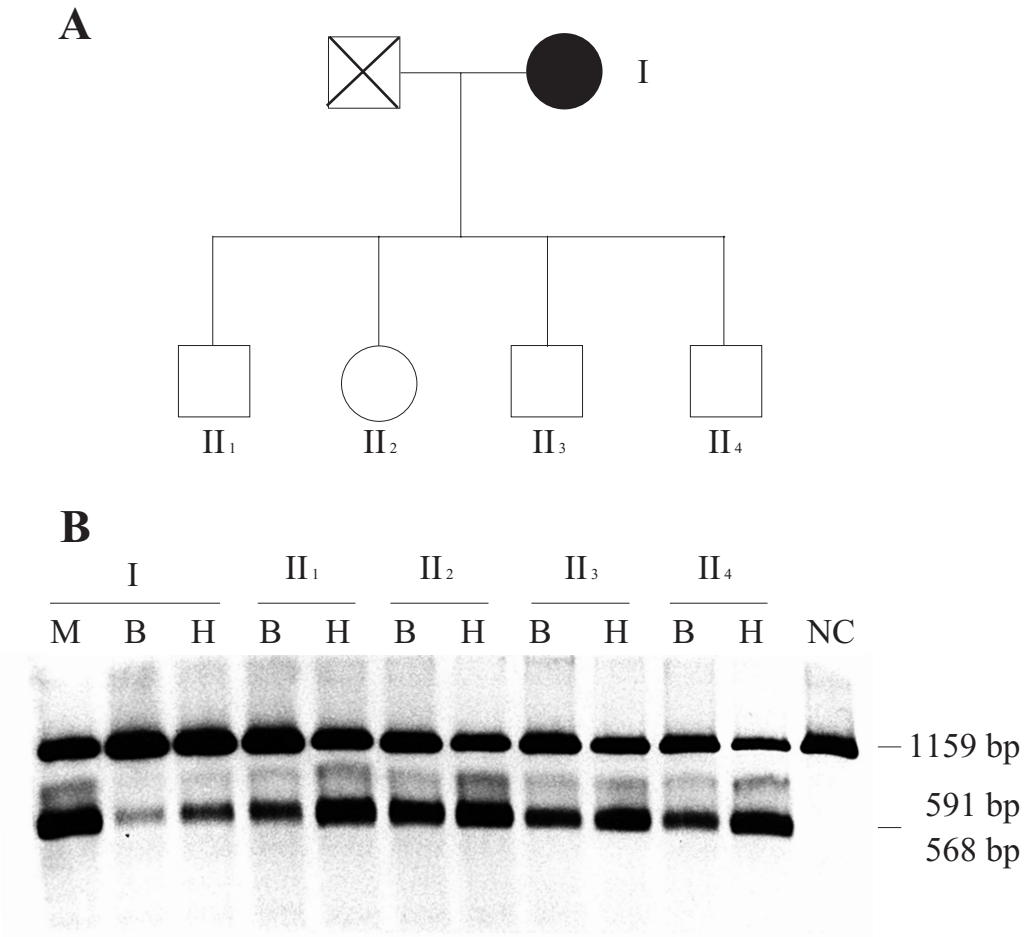

Fig. 2 Restriction analysis of the mtDNA from various tissue samples taken from MIDD family members. (A) Outline of the pedigree investigated in this study: filled circle, proband; open square and circle, generation II, children. (B) An 1159-bp polymerase chain reaction (PCR) product was cleaved into 2 fragments (591 and 568 bp) in all family members. Lanes 1-3 represent the PCR products from muscle, blood cells, and hair follicles of the proband; lanes 4-11 represent PCR products from the blood cells and hair follicles of the children; NC represents the PCR product of a healthy subject.

Upon digestion of the mtDNA PCR products with *Apa* I, 3 fragments were detected, including a wild-type undigested fragment (1159 bp) and 2 newly generated fragments (of 591 and 568 bp) from the mutant mtDNA in various tissues of the proband, and in blood cells and hair follicles of all of the children. This finding indicates that an A-to-G transition had occurred at np 3243 in the mtDNA of all family members. The mutant mtDNA was distributed heteroplasmically in different tissues, with the highest proportion found in muscle tissue (Fig. 2, Table 2). Furthermore, it was found that proportions of mutant mtDNA were higher in blood cells and hair follicles of the 4 children, as compared with the proband. Restriction analysis of the T3271C mutation revealed that the 223-bp fragments from various tissues of the proband and her family members were not cleaved by *Bfr* I, indicating an absence of the T3271C mutation in the individuals tested.

Table 2. Proportion of A3243G-Mutated mtDNA in Tissue Samples from the MIDD Family

Subjects	Proportion of A3243G mutated mtDNA (%)		
	Muscle	Blood cells	Hair follicles
I	50.4±1.0*	10.5±3.4	16.2±4.8
II-1	NA†	18.0±6.6	39.2±3.7
II-2	NA	33.8±5.2	50.7±1.2
II-3	NA	30.3±4.6	44.8±3.9
II-4	NA	30.1±6.6	56.2±6.2

*Data are presented as the mean±SEM of the results from 2 separate determinations. †NA indicates that the sample was not available from the indicated subject.

DISCUSSION

The acronym MIDD was first proposed by van den Ouweland et al. in 1994 for delineation of a compound syndrome, with concomitant maternally inherited diabetes and sensorineural deafness characterizing the condition.[3] The syndrome is associated with coexistent myopathy, mental subnormality, and/or short stature, seizures and/or endocrinal dysfunction.[4] It was originally considered part of the clinical manifestation of MELAS;[1] however, it has recently been identified as a distinct clinical entity,[13] initially generated by an mtDNA mutation at np 3243. Of the reported cases harboring the A3243G mtDNA mutation, DM was more frequently encountered in patients as well as their oligosymptomatic or asymptomatic family members harboring the A3243G mutation.[14] Diabetes mellitus is 1 of the common manifestations in patients harboring the A3243G mutation. A recent investigation found that DM is induced by many different transitional mutations of the nucleotide pairs from position 3271 to 3303 in the mitochondrial genome, which is the corresponding locus of the tRNA Leu(UUR) gene.[14] It has been suggested that this locus acts as a thrifty genome for diabetes.[15] To understand the mechanisms underlying mitochondrial DM and other NIDDM variants, an investigation expression of the mtDNA A3243G mutation its transcriptional messenger RNA or downstream protein may prove useful.

For patients diagnosed with the MIDD syndrome, characteristic features that have been noted include a thin build and short stature, no history of ketoacidosis or evidence of islet cell antibodies, earlier onset of diabetes compared to NIDDM, but later onset in comparison to insulin-dependent diabetes mellitus (IDDM).[14] Examination of our patient revealed all of the above characteristic findings, with NIDDM demonstrated at onset as well as subsequent deterioration in pancreatic β-cell function, eventually precipitating marked dependence on oral hypoglycemic agents. Although the data indicated rapid deterioration of pancreatic β-cell function, normal results were noted for the 4 children. Our investigation revealed features of mitochondrial diabetes in the patient compatible with those determined in 1995 by Gerbitz et al.[14]

An A3243G mtDNA mutation was noted in blood cells, hair follicles, and muscle tissues of the proband. The proportions of mutant mtDNA were highest in muscle cells followed by hair follicles and then leukocytes. Moreover, the presence of the mtDNA A3243G mutation was found in all 4 asymptomatic children, with a higher proportion of mutant mtDNA in hair follicles than in leukocytes. From our data, there is no correlation between the proportion of mutant mtDNA and the frequency of DM or hearing impairment. A non-random distribution of mutant mtDNA was noted in various tissues, with a higher proportion in muscle cells and a lower proportion in leukocytes, consistent with findings of previous studies.[1,2,16] Although as previously hypothesized, factors such as the percentage of mutant mitochondria in different tissues and the threshold for

failure of each organ may play an important role in the development of organ-specific symptoms,[17] age also seems to be a crucial factor for development of symptoms.[18] Additionally, we believe that environmental stress may also play an important role in the subsequent evolution of the diabetic condition.[19]

The incidence of sensorineural deafness for patients harboring the mtDNA A3243G mutation and DM is about 15%.[14] It has been suggested that a proportion of hearing impairment cases may be subclinical, and therefore the overall incidence for this dysfunction is underestimated.[14] In our investigation, hearing impairment was only found in the proband. There have been only a few reports regarding the pathogenesis of sensorineural deafness in MIDD, and it has been suggested that cochlear dysfunction is the primary pathology for hearing loss.[20] It has also been suggested that after the proportion of mutant mtDNA exceeds an expression threshold for deficiencies in mitochondrial protein synthesis and oxygen consumption, a drop in adenosine triphosphate level could lead to an imbalance of ion concentrations, resulting in accelerated and disproportionate cell death in the cochlea.[20] In addition, age and environmental stress may substantially influence the deterioration of mitochondrial function.[18,19] To gain a more complete understanding of the pathogenesis of MIDD, further large studies of patients with the A3243G mtDNA mutation are warranted.

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母系遺傳糖尿病及失聰症候群：一個台灣家族的臨床及分子生物學研究

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我們對一位48歲的女性母系遺傳糖尿病及失聰症候群 (MIDD) 之病人，及其4名子女進行臨床評估以及基因分析，以研究和MIDD有關之粒線體突變與臨床症狀間的關係。臨床上的評估包含是否有癲癇、偏頭痛、身材矮小、智能不足、或是類似中風發生等症狀，並進行血糖耐受性測驗、肝糖激素刺激試驗、聽力及腦幹誘發電位等檢查。分子生物學方面則從肌肉、白血球及毛囊取樣分析A3243G及T3271G粒線體DNA的點突變。結果顯示包括病人及其4位子女均有粒線體DNA A3243G基因點突變，然4位子女並無粒線體疾病相關症狀。從這個台灣MIDD家族的分析中可知，突變之出現及量的多寡與症狀的出現與否無相關性。(長庚醫誌 2004;27:66-73)

關鍵字：糖尿病，失聰，粒線體DNA，A3243G突變，家族研究。

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