Clinical and genetic characteristics in maternally inherited diabetes and deafness (MIDD) with mitochondrial m.3243A>G mutation: A 10-year follow up observation study of 1 family

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Introduction

Mitochondrial disease (MD) is a group of disorders caused by dysfunctional mitochondria, the organelles which play an important role in the production of ATP and exist in every human cell inthe body, except for the red blood cells (*1*). Mitochondrial DNA (mtDNA) is inherited maternally and affects organs dependent on high aerobic metabolism, such as the eye, inner ear, central nervous system, skeletal and cardiac muscle. Mitochondrial diabetes mellitus (MDM), also known internationally as maternally inherited diabetes and deafness (MIDD) syndrome, is a mtDNA mutation disease, with a progressive islet β cell secretory dysfunction (*2, 3*). MIDD is associated with early onset diabetes and sensorineural deafness, but there are various other systemic features, including cardiomyopathy, renal problems, and neuropsychiatric symptoms. It was first reported by J.A. Massen et al. in 1992, who found a large pedigree with mt.3243A>G mutation, suffering from diabetes with the presence of maternal transmission, in conjunction with bilateral hearing loss in most of the carriers (*4*). From then on, there has been growing scientific evidence that a range of other point mutations in mtDNA could contribute to the pathogenesis of MIDD, such as mt-tRNA encoding genes, including MT-TI, MT-TS1, and MT-TK, and mt-proteins encoding genes, incuding MT-ND1, MT-ND4, MT-COX2, and MT-COX3. In addition, deletion and depletion of nucleotides have also been described in patients with MIDD (*5*). These novel mutations, however, are extremely rare compared to the proportion of mt.3243A>G, where there is an A to G substitution at position 3243. MIDD is currently found to be the most common type of monogenic diabetes mellitus, accounting for about 1% of all diabetes mellitus, and the incidence in China is approximately 0.6% (*6*). Because of its low incidence and irrespective of its complex clinical phenotype, MIDD is often misdiagnosed as type 1 or type 2 diabetes. Nonetheless, its treatment is different from the common types of diabetes, and incorrect diagnosis and treatment will accelerate the disease process and the occurrence of complications. Therefore, correct genetic diagnosis and treatment are crucial for patients with MIDD. This study discusses the clinical manifestations and treatment process of a typical patient with MIDD, and summarizes its clinical characteristics through a comprehensive review of the literature, with the scope of improving clinicians' cognition of the disease and reducing misdiagnosis rate.

Materials and methods

Participants

Age of at diagnosis was estimated by the patient's recall, as well as the review of medical records and a detailed medical and family history was obtained. We collected this large family of four generations, ranging in age from 20 to 80 years, including 15 females and 9 males. Classification and diagnosis of diabetes were in accordance with ADA's latest guidelines (7). This study was approved by the ethics committee of the Central Hospital of Wuhan in China. Peripheral blood was collected from the proband, his mother and two daughters. Written informed consent was obtained from all participants.

Sample preparation and whole mitochondrial DNA sequencing

Total DNA was extracted from the peripheral blood samples of the participants (the proband, his mother and his both daughters) using a TIANGEN DNA extraction kit (TIANGEN, Beijing, China). Sample preparation and pretreatment for next generation sequencing were performed using the SureSelect XT Low Input Reagent Kit Agilent (G9703A). MtDNA sequencing was carried out on an HiSeq2500 system (Illumina), including MT-DLOOP, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, MT-CYB, MT-CO1, MT-CO2, MT-CO3, MT-ATP6, MT-ATP8, MT-TA, MT-TR, MT-TN, MT-TD, MT-TC, MT-TE, MT-TQ, MT-TG, MT-TH, MT-TI, MT-TL1, MT-TL2, MT-TK, MT-TM, MT-TF, MT-TP, MT-S1, MT-TS2, MT-TT, MT-TW, MT-TY, MT-TV, MT-RNR1 and MT-RNR2. The resulting sequences were compared with the updated Cambridge sequence (GenBank accession number: NC_012920). Blast homology searches were performed using the programs available at the National Center for Biotechnology Information website and were compared with the wild-type sequence. Areas containing putative novel variations were amplified and sequenced again on both strands to exclude possible polymerase chain reaction (PCR) artifacts.

Sanger sequencing

The presence of candidate variants in the proband and his family was confirmed by direct Sanger sequencing of the PCR products, after which the variants co-segregated with with diabetes were selected for further analysis. The presence of mt.3243A>G variant in the proband and his family(mother) was also confirmed by direct Sanger sequencing of the PCR products. The MT-TL1 gene was amplified in a PCR assay using the forward primer 5'-CCGGAGTAATCCAGGTCGGT-3' and thereverse primer 5'-CAGCATTCCCCCTCAAACCT-3'. The PCR assay conditions were as follows: 95 °C for 5 min; 35 cycles, at 95 °C for 1 min, 60 °C for 30 sec, and 72 °C for 1 min; and then 72 °C for 10min. The PCR products were assessed by direct Sanger sequencing (Xiangyin Medical Testing Center, Hangzhou, China).

Medication plan adjustments

Patients' treatment plans were adjusted based on genetic testing results and previously reported findings. For example, insulin should be administered to the proband, and melbine should never be used.

Results

Medical history

The proband was a 33-year-old man, who was hospitalized for symptoms such as poor glycemic control and deafness for 11 years and abdominal pain and vomiting for 1 day. The patient had a diagnosis of type 1 diabetes at the age of 22 years. During the clinical course of the disease, the patient repeatedly experienced dry mouth, polydipsia, polyuria, fatigue, weight loss, limb convulsions, abdominal pain, vomiting, and repeated hospitalizations for ketosis. His previous hospital records did not indicate conventionally-assessed islet autoantibodies, and oral hypoglycemic drugs were found to be ineffective. Figure 1 shows a pedigree chart of the proband's family. His family history indicated that his mother had diabetes and his father was a healthy individual without any family history of diabetes. His grandmother (I-2), grandaunt (I-3), granduncle (I-4), mother (II-1), five aunts (II-2, II-3, II-4, II-5, and II-8), one uncle (II-6), and one cousin (III-5) experienced similar diseases, but the offspring of the granduncle (II-9, II-10, II-11, and II-12), his grandfather (I-1), one uncle (II-7), and five cousins (III-2, III-3, III-4, and III-6) did not have diabetes. The proband's family had more women than men, and the offspring of the men did not have diabetes. These findings conclude that an extensive history of early-onset adult diabetes exsits within the maternal lineage.

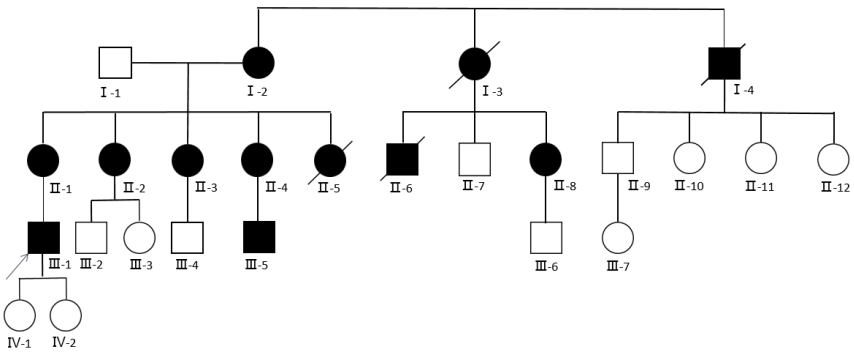

Figure 1. Pedigree chart of the patient's family. Generations are indicated on the left in Roman numerals, and the numbers under the individuals represent identification numbers for each generation. Squares represent males, and circles represent females. Black arrow indicates the proband (III-1). Individuals with diabetes are indicated as black symbols, and oblique line indicates death. The proband (III-1) had a diagnosis of type 1 diabetes mellitus at the age of 22 years, and his grandmother (I-2), grandaunt (I-3), granduncle (I-4), mother (II-1), five aunts (II-2, II-3, II-4, II-5, and II-8), one uncle (II-6), and one cousin (III-5) were diagnosed with type 2 diabetes mellitus at the age of 40, 40, 45, 50, 50, 30, 40, 40, 50, 40, and 28 years, respectively.

Clinical characteristics

The clinical characteristics of the proband's family are presented in Table 1 and Table 2. A total of five medical records of the proband were collected during 2013-2022. The proband's laboratory analysis results revealed poor glycemic control, and he was always hospitalized for diabetic ketosis or ketoacidosis. The patient was lean, with a weight of 52 kg and a height of 1.70 m with a body mass index (BMI) of only 17.99 kg/m^2, in 2022. His fasting and 2-h C-peptide levels were 0.6 and 3.0 ng/mL, respectively, in 2014, which decreased to 0.32 and 0.63 ng/mL, respectively, in 2022, indicating that the patient had progressive islet β-cell secretory dysfunction. He tested negative for all diabetes autoantibodies in 2022. His blood lipid profile slightly elevated or generally normal, but uric acid levels were high, possibly because of ketoacidosis. In 2022, ultrasound examination of the liver, kidney, pancreas, bladder, ureter, spleen, and gall bladder showed normal organ morphology, but complications of the eye and lower extremity artery showed faster progression. He was had a diagnosis of glaucoma in 2013 and diagnosed with diabetic retinopathy in 2014 after the diagnosis of glaucoma was ruled out. The patient reported as being diagnosed with type 1 diabetes mellitus (T1DM) at the age of 22 years, and his grandmother (I-2), grandaunt (I-3), granduncle (I-4), mother (II-1), five aunts (II-2, II-3, II-4, II-5,and II-8), one uncle (II-6), and one cousin (III-5) were diagnosed with type 2 diabetes mellitus (T2DM) at the age of 40, 40, 45, 50, 50, 30, 40, 40, 50, 40, and 28 years, respectively. The proband and his mother corrected the diagnosis of MIDD until 2022. Although errors were observed in the classification of diabetes in this family, we could still observe that all patients had early-onset diabetes and a few of them (I-2, II-1, and II-8) could achieve glycemic control with oral hypoglycemic drugs alone. In addition to the proband (III-1), his grandmother (I-2), grandaunt (I-3), granduncle (I-4), mother (II-1), and two aunts (II-4 and II-8) had hearing impairment, and his mother stated that the disease onset was very early. Only the proband (III-1), his grandmother (I-2), and his mother (II-1) had myoclonus, but some patients had diabetes alone (II-2, II-3, II-5, II-6, and III-5). Combined symptoms suggested that the occurrence of diabetes in this family was distinctive, and this type of diabetes was inherited maternally, often together with other symptoms, but the disease phenotype was often different.

Table 1. Physical and laboratory examination findings of the proband

Year	2013	2014	2019	2021	2022
Symptoms					

BMI, kg/m^2	17.30	17.30	17.30	17.30	17.99
Random urine glucose	Unknown	3+	3+	1+	4+
Ketones in urine	Unknown	3+	2+	2+	Neg
Fasting plasma glucose, mmol/L	-	6.95	-	-	10.60
Fasting C-peptide, ng/mL	-	0.6	-	-	0.32
2-h fasting C-peptide, ng/mL	-	3.0	-	-	0.63
HbA1c, %	10.9	13.1	15.4	12.8	11.8
Albumin, g/L	Normal	Normal	Normal	Normal	38.6
Globulin, g/L	Normal	Normal	Normal	Normal	25.2
Alanine aminotransferase, U/L	Normal	Normal	Normal	Normal	11.3
Aspartate aminotransferase, U/L	Normal	Normal	Normal	Normal	11.1
Creatinine, μmol/L	Normal	Normal	Normal	137.0	69.0
Uric acid, μmol/L	Normal	441.4	595.3	648.0	424
TG, mmol/L	Normal	Normal	3.11	5.00	1.88
TC, mmol/L	Normal	Normal	Normal	6.38	3.77
HDL-C, mmol/L	Normal	Normal	0.83	Normal	1.00
LDL-C, mmol/L	Normal	Normal	Normal	3.125	2.22
CRP, mg/L	-	Normal	-	Normal	0.03
Anti-GAD	-	-	-	-	Neg
Anti-IAA	-	-	-	-	Neg
Drug therapy	Lispro mix 25+Acarbose	BIAsp30	BIAsp30	Apidra+IDegAsp +Voglibose	IDegA

The results of biochemical indicators when the proband was admitted to the hospital. -: no detection; unknown: tests were conducted but the results are unknown.

Abbreviations: BMI, body mass index; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; CRP, C-reactive protein; GAD, glutamic acid decarboxylase antibody; IAA, insulin autoantibodies; Neg, negative; +, positive.

Table 2. Disease characteristics of different patients in the family

	Age at onset (years)	Gender	Symptom	Therapies	Ending
I-2	40	F	Diabetes, lean, hearing impairment, myoclonus	OAD	Alive
I-3	40	F	Diabetes, hearing impairment	Unknown	Dead
I-4	45	M	Diabetes, hearing impairment	Unknown	Dead
II-1	50	F	Diabetes, lean, hearing impairment, myoclonus	OAD	Alive
II-2	50	F	Diabetes	Insulin injection	Alive
II-3	30	F	Diabetes	Insulin injection	Alive
II-4	40	F	Diabetes, hearing impairment	Insulin injection	Alive
II-5	40	F	Diabetes	Unknown	Dead
II-6	40	M	Diabetes	Unknown	Dead
II-8	50	F	Diabetes, hearing impairment	OAD	Alive
III-1	23	M	Diabetes, lean, hearing impairment, myoclonus	Insulin injection	Alive
III-5	28	M	Diabetes	Insulin injection	Alive

Abbreviations: OAD, oral antidiabetic drug.

Genetic analysis

Depending on the clinical symptoms and maternal clustering characteristic in the proband's family, we performed sequencing analysis of the whole mtDNA in the proband. The proband exclusively had a het-

eroplasmic m.3243A>G mutation in the MT-TL1, with a mutation frequency of 31.46% (Figure 2a and Table 3). This mutation was detected in his mother (II-1) but absent in the other two family members (his daughters IV-1 and IV-2) through Sanger sequencing (Figure 2b, 2c, and 2d). Genetic testing confirmed that the aforementioned family members belonged to the MIDD subtype.

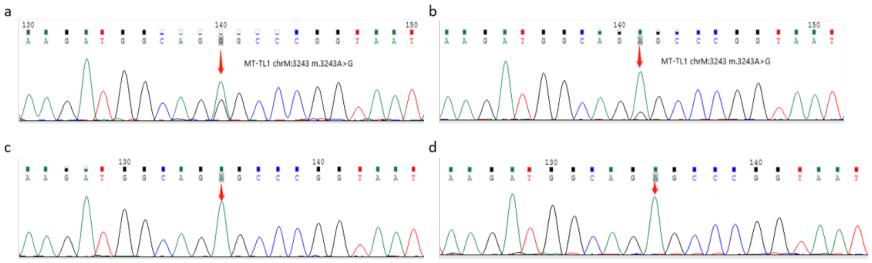

Figure 2. Sanger sequencing chromatogram. Arrows indicate the changed position of the mutation in the MT-TL1 gene. (a) Proband (III-1), mutant type; (b) the proband's mother (II-1), mutant type; (c) the proband's daughter (IV-1), wild type; and (d) the proband's daughter (IV-2), wild type;

Table 3. Detection details of the proband mt.3243A>G mutation site

Chr	Mutation	Gene	Group	Homo/hete	sum_depth	alt_depth	alt_rate
ChrM	m.3243A>G	MT-TL1	tRNA	hete	5000	1573	34.16%

Precise treatment based on the patient's genotype

In this study, a patient had a re-diagnosis of MIDD after 10 years' suffering, and an m.3243A>G mutation was identified in the MT-TL1 gene in his family. Although the diagnosis is quite clear, the clinical management of MIDD remains a complicated issue. This information, combined withthe results reported in the literature, led to the recommendation that everybody in his family needed individual-based management. At this condition, the patient himself needed insulin administration because he had defective insulin secretion by pancreatic β-cells with m.3243A>G mutant mtDNA. According to the literature, we added coenzyme Q10 to improve themitochondrial function, and drugs that may weaken mitochondrial function, such as metformin and statins, had to be avoided. During follow-up, the incidence of diabetic ketoacidosis was found to be significantly reduced, and fasting blood glucose and HbA1c levels evidently improved. Cochlear implantation was recommended for deafness. For maternal relatives of patients with MIDD, long-term follow-up is advisable and a careful examination of mutant mtDNA is preferred.

Discussion

MIDD is a group of clinical heterogeneous diseases caused by dysfunction of the mitochondrial respiratory chain and oxidative phosphorylation, and it manifests as multiorgan damage (*8*). MIDD often causes early-onset diabetes and sensorineural deafness, but various other organs systems are involved, including ophthalmic disease (*9, 10*), myopathy, encephalopathy, cardiac disease, gastrointestinal diseases (*11*), renal disease (*12*), and so on. Because of mitochondrial DNA heteroplasmy, a great difference in clinical manifestations is observed among patients with MIDD. Even the same mutation can exsit in different phenotypes or can be"silenced" eternally. Although the mutation was screened only in four individuals and two people were found to carry the mutation in this family, based on the genetic characteristics of MIDD, we can reasonably suspect that other people with diabetes carry the mutation, and asymptomatic carriers cannot be ruled out. Of the 12 people with diabetes in our study, only three were males. Seven patients had hearing impairment, three presented myoclonus, three were lean, and five had diabetes only. In this family, diabetes started at the age approximately 40 years, and for a few people, the onset age was earlier. According to

his mother, the disease onset was very early. In this family, the proband (III-1), his grandmother (I-2), and his mother (II-1) had myoclonus. The proband, whom we did not encounter reported that, myoclonus always occurred in the lower extremities, less frequently, and often accompanied by pain after exercises, this symptom is consistent with that reported in the literature. The patient had a correct diagnosis of the disease through genetic technology after 10 years. The proband had a diagnosis of T1DM because of his younger onset age (<30 years old), lean body, poor glycemic control, low fasting C-peptide level, and repeated ketoacidosis. The more important reason was that his hearing impairment was progressive, so much so that when the patient was repeatedly hospitalized for life-threatening ketoacidosis, his hearing impairment was ignored by both the patient and the physician. Through careful inquiry of the patient's history and physical examination, the onset time of hearing problem and diabetes was found to be the same, and many of the maternal relatives experienced the same situation. Further genetic testing confirmed our suspicion that the patient had MIDD with mt.3243A>G mutation. In conclusion, the clinical features of MIDD are as follows: (1) maternal inheritance. Almost all offspring of female patients carry the mutation, but not all of them are affected by the disease. The offspring of male patients generally do not carry this mutation. (2) A vast majority of people have diabetes at onset age before 40 years, and most of them have diabetes onset in adulthood, rarely in adolescents. (3) Most patients had normal or lean body mass, with a mean BMI of less than 20 kg/m^2. (4) Mostly negative results were obtained for islet autoantibodies. (5) Abnormalities in glucose metabolism combined with multiorgan damage. Special attention should be paid to the occurrence of early-onset diabetic retinopathy in the proband. According to literature, the most common ophthalmic feature is typical macular retinal dystrophy(*13*). Most patients with macular disease have a history of diabetes for more than 5 years and the onset is always after the age of 40 years. Early-onset diabetes and poor glycemic control may contribute to diabetic retinopathy progression.

During a suspected diagnosis of MIDD in the clinical setting, the diagnosis needs to be clearly confirmed by genetic testing such as Sanger sequencing, gene chip sequencing, and high-throughput gene sequencing. Blood leukocytes are mostly used for detection, but compared with blood leukocytes, muscle, urine(*13*) and buccal mucosa(*14*) are better as testing samples because of a higher heterogeneity. Reports have suggested that the patient had an apparent phenotype and family history, and the family carried the mt.3243A>G mutation, but the blood test results of the patient showed no mutation, which may be attributed to the heterogeneity in the specimen.

In addition to the clinical phenotype, heteroplasmy plays an important role in considering the severity of MIDD (*15*). Mt.3243A>G occurs in a wide range of mitochondrial encephalomyopathies including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke) or MELAS/MERRF (myoclonic epilepsy and red ragged fibers), and overlap syndrome (*16*). The proband's grandaunt (I-3), granduncle (I-4), one aunt (II-5), and one uncle (II-6) died ofdiabetic complications. Whether the decreased patients had these severe phenotypes leading to death is an open question. Compared with his mother, the proband had an earlier onset age and severe disease, and the same mutation (A3243G) was observed. Considering a large-scale study in Finland, A3243G levels may gradually increase over subsequent generations (*17*), we suggest that the heterogeneous mutation frequency in his mother is lower than that in the proband(34.16%). The mt.A3243G heterogeneity level is negatively correlated with age, which suggests that his mother has a lower heterogeneous mutation frequency than the proband (*18*). A meta-analysis showed that mitochondrial heterogeneity of A 3243G is significantly associated with the incidence of diabetes, that is, the greater the heterogeneity, the greater the probability of MIDD development, and the younger the age of onset in offspring (*19*). Our clinicians should note that genetic counseling plays a crucial part in such a family, which can help asymptomatic patients achieve early diagnosis and prevent complications. Unfortunately, we only screened the mt.3243A>G mutation in the patient's mother and children. His other family members should be urged to screen this mutation owing to special inheritance. Early screening of mutation sites, especially for patients with low mutation heterozygosity, may improve treatment and prognosis and delay the occurrence of complications.

Because of the heterogeneity of MIDD, treatment for MIDD is highly challenging. Patients with MIDD tend to be lean and have insufficient energy synthesis; hence, they are unsuitable for a too strict diet to avoid

malnutrition or aggravation of the disease process. Endurance training can lead to a decrease the proportion of the mutant mitochondrial DNA; therefore, an appropriate level of exercise can improve mitochondrial function (*19*). However, too strenuous exercise can easily lead to severe lactic acidosis, a fatal condition, in these patients. As for drug therapy, the most important aspect to remember is that individualized treatment should be provided. Among the patients in this family, five were treated with insulin and three were treated with oral hypoglycemic drugs. The use of different glycemic control regimens may be attributed to the different mutation load in each patient. Although some patients do not need hypoglycemic agents temporarily or need only oral hypoglycemic agents, we still recommend starting insulin therapy as early as possible because mtDNA mutations may aggravate β-cell apoptosis to impair insulin secretion. As mentioned above, we added coQ10 (*20*) to our patient during follow-up, which proved that the drug could improve mitochondrial energy metabolism. Other drugs can also enhance mitochondrial function, such as lipoic acid (*21*) and thiamine. On the contrary, many common drugs should be avoided in these patients, such as tetracycline, chloramphenicol, sodium valproate, phenytoin, phenobarbital (*22*), antiretrovirals (*23*), statins, and metformin (*24*). Because of the rapid progression of diabetic vascular complications in the proband, we specifically advised the patient not to use statins to avoid acute complications, and patients taking oral hypoglycemic drugs were instructed to avoid metformin. However, we need to know that the above treatment was administered only to control symptoms, and only gene therapy is the fundamental treatment. Recombinant RNA molecules have been designed such that they are imported into the mitochondria of human cells to decrease the proportion of the mutant mitochondrial DNA (*25*). However, this technique has not been applied to clinical practice, necessitating further exploration.

In conclusion, MIDD is a complex syndrome with variable clinical features and is often misdiagnosed. Our findings indicate that whole mtDNA sequencing analysis can be used to detect m.3243A>G mutation in the mtDNA. In clinical practice, the patient's medical history, especially accompanying symptoms and family history, should always be carefully recorded. Once the patient has a suspected diagnosis, genetic testing should be performed as soon as possible to make a timely diagnosis and standardize individualized treatment.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Shasha Zheng and Juanjuan Wang contributed equally to this work. Wei Shi and Zhongzhi Zhang gathered patient medical records from outside hospitals. Hongmei Zhang and Zhongjing Wang conceived and designed the experiments. Pei Wang, Minxian Sun, Juanjuan Wang and Shasha Zheng analyzed the data. Shasha Zheng and Juanjuan Wang wrote the paper.

Acknowledgments

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