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Maternally transmitted diabetes mellitus associated with the mitochondrial tRNA$^{Leu(UUR)}$ A3243G mutation in a four-generation Han Chinese family

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Abstract

We report here the characterization of a four-generation Han Chinese family with maternally transmitted diabetes mellitus. Six (two males/four females) of eight matrilineal relatives in this family exhibited diabetes. The age of onset in diabetes varies from 15 years to 33 years, with an average of 26 years. Two of affected matrilineal relatives also exhibited hearing impairment. Molecular analysis of mitochondrial DNA (mtDNA) showed the presence of heteroplasmic tRNA$^{Lue(UUR)}$ A3243G mutation, ranging from 35% to 58% of mutations in blood cells of matrilineal relatives. The levels of heteroplasmic A3243G mutation seem to be correlated with the severity and age-at-onset of diabetes in this family. Sequence analysis of the complete mitochondrial genome in this pedigree revealed the presence of the A3243G mutation and 38 other variants belonging to the Eastern Asian haplogroup M7C. However, none of other mtDNA variants are evolutionarily conserved and implicated to have significantly functional consequence. Thus, the A3243G mutation is the sole pathogenic mtDNA mutation associated with diabetes in this Chinese family.

Keywords: Diabetes mellitus; Mitochondrial DNA; tRNA$^{Leu(UUR)}$ gene; A3243G mutation; Chinese

Diabetes mellitus is a heterogeneous disorder, affecting about 5% of the world population. The diabetes can be caused by acquired factors, inherited factors or interaction between genetic and environmental factors. In some cases, the mode of inheritance of diabetes shows a predominantly maternal transmission [1,2], suggesting that mutation(s) in mtDNA is one of the molecular bases of this disorder [3]. The landmark discoveries of mitochondrial diabetes were the identification of a 10.4 kb deletion in mtDNA associated with diabetes and deafness syndrome [4] and the tRNA$^{Leu(UUR)}$ A3243G mutation associated with diabetes and deafness in a large pedigree [5]. Since then, numerous point mutations, deletions, and duplications in mtDNA have been associated with diabetes worldwide [6,7]. Of these, the A3243G mutation appears to be the most common diabetes-associated mtDNA mutation, accounting for between 0.5% and 3% of all type II diabetes in various ethnic populations [8–10]. This mutation, which is present in heteroplasmic form in patient cells, exhibited a remarkable variability of its clinical manifestation. When 70% of total mtDNA in cells carried the A3243G mutation, this mutation does not cause diabetes, but instead causes more severe symptoms, including short stature, cardiomyopathy, CEPO and mitochondrial encephalomyopathy,

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lactic acid and stroke-like episodes (MELAS) syndrome [11]. When present at relatively low levels (10–30%) in the patient's blood, the patients may manifest only diabetes with or without sensorineural hearing loss [5,12,13]. The age-at-onset of diabetes in patients with the A3243G mutation varied, with average at the age of 35 years [12,14].

However, less has been known about the pathogenesis of diabetes in the Chinese populations. Recently, a systematic and extended mutational screening of the mitochondrial genome has been initiated in the large clinical population of Endocrinology Clinic at the Wenzhou Medical College, China. In the present study, we report the clinical, genetic, and molecular characterization of a four-generation Han Chinese family with maternally transmitted diabetes. Molecular analysis has led to identification of the heteroplasmic A3243G mutation in $tRNA^{Leu(UUR)}$ gene in this family. To elucidate the role of mitochondrial haplotype in the phenotypic expression of the A3243G mutation, we performed PCR-amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of this family.

Materials and methods

Patients. We ascertained a four-generation Han Chinese family (Fig. 1) through Department of Endocrinology, the First Affiliated Hospital Wenzhou Medical College, China. Diagnosis of diabetes was based on the criteria of the World Health Organization [15]. Informed consent, blood samples, and clinical evaluations were obtained from all participating family members, under protocols approved by the Cincinnati Children's Hospital Medical Center Institute Review Board and the Wenzhou Medical College Ethics Committee. Members of this pedigree were interviewed at length to identify both personal or family medical histories of diabetes and other clinical abnormalities.

Mutational analysis of the mitochondrial genome. Genomic DNA was isolated from whole blood of participants using the Puregene DNA Isolation Kits (Gentra Systems). The presence of the A3243G mutation was examined as detailed elsewhere [11]. Briefly, affected individuals' DNA fragments spanning the mtDNA mutation were amplified by PCR using oligodeoxynucleotides corresponding to mtDNA at positions 3152–3550 [16]. For the detection of the A3243G mutation, the amplified PCR segments were digested with a restriction enzyme *Apa*I [11]. Equal amounts of various digested samples were then analyzed by electrophoresis through 6% polyacrylamide gel. The proportions of digested and undigested PCR product were determined by using a PhosphorImager (Molecular Dynamics) and the IMAGE-QUANT program after ethidium bromide staining to determine the amount of the A3243G mutation in these subjects.

The entire mitochondrial genome of an affected patient III-6 was PCR amplified in 24 overlapping fragments using sets of the light (L) strand and the heavy (H) strand oligonucleotide primers as described previously [17]. Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. These sequence results were compared with the updated consensus Cambridge sequence [16]. DNA and protein sequence alignments were carried out using seqweb program GAP (GCG).

Results and discussion

To elucidate the molecular basis of diabetes, we have performed a mutational analysis of the mitochondrial genome in a cohort of Chinese subjects, who were diagnosed as diabetics by Endocrinology Clinics at the Wenzhou Medical College, China. First, we examined the commonly known diabetes-associated mtDNA A3243G mutation by PCR amplification and subsequent restriction enzyme digestion analysis of PCR fragments derived from each proband of those families. As shown in Fig. 2, one subject with diabetes carried the heteroplasmic A3243G mutation. To confirm the presence of the A3243G mutation, these PCR-amplified segments were then purified and subsequently analyzed by DNA sequencing. In fact, the sequence analysis confirmed the presence of A3243G mutation. A comprehensive history and clinical examinations were performed to identify any clinical abnormalities, and genetic factors related to the diabetes in all available members of this Chinese family carrying the A3243G mutation.

The proband (III-6) was given a diagnosis of type 1 diabetes after an oral glucose-tolerance test at the age of 15

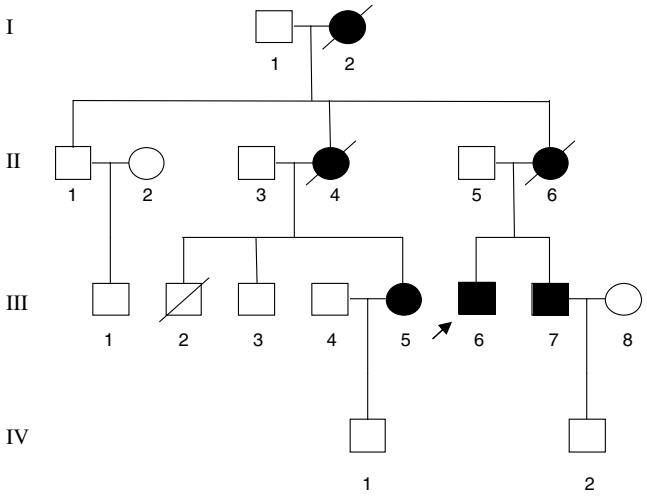

Fig. 1. One four-generation Han Chinese pedigree with diabetes. Affected individuals are indicated by filled symbols. Arrow denotes proband.

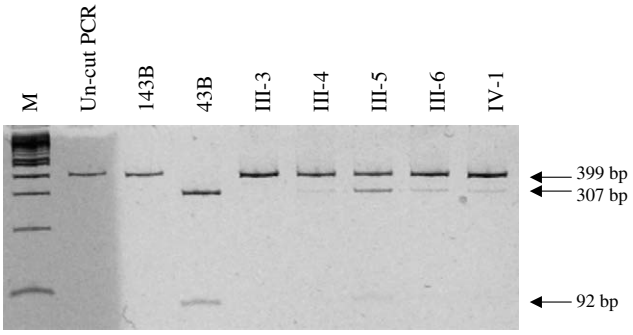

Fig. 2. Quantification of the A3243G mutation in the $tRNA^{Leu(UUR)}$ gene of mutants and control subjects derived from the Chinese family. PCR products around the A3243G mutation were digested with *Apa*I and analyzed by electrophoresis in a 6% polyacrylamide gel stained with ethidium bromide. Patients and control individuals are indicated. 143B is a Caucasian control and 43B was a positive control derived from a MELAS subject [18].

years. He received insulin therapy at the age of 17, when he became prone to ketoacidosis. He experienced hearing impairment at the age of 25. Audiological evaluation showed that he exhibited moderate bilateral hearing impairment (45 dB at both ears). In addition, he had mild neuromuscular abnormality. However, he does not have other significant medical history. Members of this family are living in Zhejiang Province in Eastern China. As shown in Fig. 1, this familial history is consistent with a maternal inheritance. None of the patrilineal relatives suffered from diabetes, while six (two males/four females) of nine matrilineal relatives exhibited diabetes as one of clinical symptoms. The age-at-onset of diabetes in three affected matrilineal relatives of this family varied from 15 years to 33 years, with an average of 26 years. Of these matrilineal relatives, III-4 exhibited a moderate hearing loss (55 dB at

Table 1
Summary of clinical and molecular data of several members in one Chinese pedigree with diabetes mellitus

Subjects	Gender	Age at test (years)	Age at onset (years)	BMI (kg/m^2)	Fasting glucose (mmol/L)	Hearing loss	Ketoacidosis	Heteroplasmy of A3243G (%)
III-3	M	45		26.0	5.8	No	No	0
III-4	F	42	30	21.0	18.4	Yes	No	37.8
III-6	M	36	33	22.9	10.6	No	No	36.7
III-5	M	28	15	21.5	10.8	Yes	Yes	58.1
IV-1	M	21		20.0	5.7	No	No	35.4

Table 2
mtDNA variants in one Chinese family with diabetes mellitus

Gene	Position	Replacement	Conservation[a] H/B/M/X	Previously reported[b]
D-Loop	73	A to G		Yes
	146	T to C		Yes
	263	A to G		Yes
	310	T to CTC		Yes
	489	T to C		Yes
	16038	A to C		No
	16189	T to C		Yes
	16223	C to T		Yes
	16266	C to A		Yes
	16295	C to T		Yes
	16519	T to C		Yes
12S rRNA	750	A to G	A/A/G/–	Yes
	1438	A to G	A/A/A/G	Yes
16S rRNA	2706	A to G	A/G/A/A	Yes
$tRNA^{Leu(UUR)}$	3243	A to G	A/A/A/A	Yes
ND1	3483	G to A		Yes
	4071	C to T		Yes
ND2	4769	A to G		Yes
	4850	C to T		Yes
	5442	T to C (Phe to Leu)	F/F/M/L	Yes
CO1	6455	C to T		Yes
	7028	C to T		Yes
A6	8701	A to G (Ala to Thr)	A/V/V/T	Yes
	8860	A to G (Thr to Ala)	T/A/A/T	Yes
CO3	9540	T to C		No
	9824	T to C		Yes
ND3	10398	A to G (Thr to Ala)	T/T/T/A	Yes
	10400	C to T		Yes
ND4	10873	T to C		Yes
	11350	A to G		Yes
	11665	C to T		No
	11719	G to A		Yes
	12091	T to C		Yes
ND5	12705	C to T		Yes
Cytochrome *b*	14783	T to C		Yes
	14942	A to C (Ile to Leu)	I/V/V/V	No
	15043	G to A		Yes
	15301	G to A		Yes
	15326	A to G (Thr to Ala)	T/M/I/I	Yes

[a] Concervation of amino acid for polypeptides or nucleotide for rRNAs, in human (H), bovine (B), mouse (M) and *Xenopus laevis* (X).
[b] See http//www.mitomap.org.

both ears). Comprehensive family medical histories of other individuals showed no other clinical abnormalities, including muscular diseases, hearing impairment, vision loss, and neurological disorders.

To further determine the presence and amount of the A3243G mutation in other three matrilineal relatives, the 399 bp PCR segments corresponding to mtDNA at positions 3152–3550 were first digested with the restriction enzyme *Apa*I and separated them by electrophoresis using a 6% polyacrylamide gel. As can be seen in Fig. 2, there was detectable wild type DNA, indicating that the A3243G mutation is present in heteroplasmy in the matrilineal relatives of this Chinese family. Qualification of the A3243G mutation, as shown in Table 1, revealed the presence of variable levels in matrilineal relatives of this family: 37.8% in III-5, 58.1% in III-6, 36.7% in III-7, and 35.4% in IV-1. The levels of heteroplasmic A3243G mutation in those matrilineal relatives seem to correlate with the severity and age-of-onset.

To determine the role of mitochondrial haplotype in the phenotypic manifestation of the A3243G mutation, the DNA fragments spanning the entire mitochondrial genome of an affected patient III-6 were PCR amplified and each fragment was purified and subsequently analyzed by direct sequence. The comparison of the resultant sequences with the Cambridge consensus sequence [16] identified a number of nucleotide changes as shown in Table 2. Indeed, this mitochondrial genome belongs to eastern Asian haplogroup M7C [19]. Of these mtDNA variants, there are two known variants in the 12S rRNA gene and one previously identified variant in the 16S rRNA gene [7]. Those variants are apparently present in homoplasmy in this affected matrilineal relative. Of other nucleotide changes, there were 11 polymorphisms in the D-loop region and 24 variants in protein encoding genes. Of these, the A16038C variant in the D-loop region, the T9540C variant in the CO3 gene, the C11665T variant in the ND4 gene, and the A14942C (I66L) variant in CytB gene are novel polymorphisms. These variants in the RNA gene and protein encoding genes were further evaluated by phylogenetic analysis of these mtDNA variants and mtDNAs from other organisms including mouse [20], bovine [21], and *Xenopus laevis* [22]. None of variants was located at site that is highly evolutionarily conserved. This suggests that the A3243G mutation is the sole pathogenic mtDNA mutation associated with diabetes in this Chinese pedigree.

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