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Case Report

Molecular genetic analysis of Type II diabetes associated m.3243A>G mitochondrial DNA mutation in a Pakistani family

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ABSTRACT

Background: Type II diabetes is the most often considered as maternally inherited disease and A>G transition at position 3243 of mitochondrial DNA (m.3243A>G) in the encoding $tRNA^{Leu\ (UUR)}$ gene is thought to be strongly responsible for the pathogenesis of the disease in number of cases.
Aim: Current study was conducted to investigate the family prevalence of m.3243A>G mutation in a Pakistani family with the context of insulin sensitivity and B-cell function.
Subject and method
This study was carried out in 6 suspected diabetic members of a Pakistani family. Mitochondrial DNA was extracted from saliva and after polymerase chain reaction, amplified DNA was subjected to direct sequencing.
Results and conclusion: Sequencing results revealed absence of most common mtDNA m.3243A>G mutation in 6 subjects with devotedly congenital mitochondrial diabetes phenotype. Conclusively, the m.3243A>G mutation in mitochondrial $tRNA^{Leu}$ gene was not found to be a frequent cause of Type II diabetes mellitus in a Pakistani (haripur) family. Our finding suggests that there might be some underlying genetic cause for Type II diabetes mellitus in the subjects under study.

1. Introduction

Diabetes is multifactorial disorder characterized by inappropriate secretion of insulin leading to chronic hyperglycemia. Pancreatic B-cells are key player in maintaining normal glucose homeostasis by secretion of insulin. There are number of genetic and non-genetic risk factors associated with diabetes. Generally mitochondrial diabetes is an unremarkable form of diabetes with familial clustering. Type II diabetes has maternal inheritance pattern and in majority of the cases m.3243A>G heteroplasmic mutation encoding $tRNA^{Leu\ (UUR)}$ gene is thought to be responsible for the disease phenotype [1,2].

Underlying molecular mechanism include gradual B-cell failure beside impaired insulin sensitivity [3]. In m.3243A>G mutant individual there is impaired insulin secretory capacity of pancreatic B-cell due to impaired glucose sensing mechanism [4]. Clinical manifestation of m.3243A>G point mutation in Type II maternally inherited diabetes also include, obesity, hearing and visual impairment, renal dysfunction, maternal inheritance and association with progressive neurosensorial deafness. This mutation is also reported in association with the MELAS syndrome (encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome) [5–9].

The m.3243A>G mutation leads to the dimerization of the mutant tRNA molecule and impaired amino acylation [10]. The precise consequences for biochemical processes within the mitochondrion are a matter of debate but seem to encompass distorted mitochondrial proteome. Furthermore, high heteroplasmy levels lead to mitigated oxygen consumption, depicting functional impairment of the respiratory chain [7]. Five different point mutations i.e. m.3156A>G, m.3243A>G, m.3357G>A, m.3375C>A, and m.3394T>C were reported among 240 diabetic subjects in Japanes population [11]. Apart from these some other mitochondrial point

Abbreviations: mtDNA, mitochondrial DNA; tRNALeu, transfer RNA leucine; MELAS, encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome; *MT-TL1*, mitochondrial transfer RNA leucine 1.

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mutation in maternally inherited diabetic individuals are; m.3252A>G, m.3260A>G, m.3265C>T, m.1888G>A, m.4216T>G andm.4917A>G. Mitochondrial mutations m.3357G>A (Met → Ile) and m.3394T>C (Tyr → His) showed strong association with Type II diabetes [9]. Conclusively the previous data revealed that m.3243A>G is primary cause of maternally inherited Type II diabetes in approximately 1.5% of diabetic individuals from Japan's and the Netherlands population [12]. Given the enormous challenge of diabetes mellitus epidemic in the coming years on one hand and the lack of resources on the other, it is mandatory to initiate proactive measure on urgent basis [13]. In the present study we sought to determine whether this m. 3243A>G variant is significantly associated with proven Type II diabetes in a Pakistani family.

2. Subjects and methods

2.1. Subjects

Prior to the start of the study, approval was obtained from the Hazara University Institutional Review Board and written informed consent was obtained from all the 6 patients (2 female and 4 male) and control individuals of the same family.

2.2. Samples collection

All patients underwent a standardized clinical and laboratory evaluation. Next, samples were collected from the 6 suspected diabetic members of the same family (Fig. 1). We collected 1.5–2.0 mL of saliva from each participant to extract adequate amount of DNA. Each sample was held on ice and freeze at −20 as soon as possible.

2.3. DNA extraction, Library preparation and sequencing

Genomic DNA was isolated from saliva following a standard protocol, quantified by spectrophotometric measurement of optical density at 260 nm and diluted to 40 ng/mL for amplification by polymerase chain reaction (PCR). To screen for mutations, all coding exons and exon/ intron splice junctions of the *MT-TL1 gene* were PCR amplified from genomic DNA using primers designed from intronic sequences of the gene (Fig. 2). To amplify the PCR fragment containing *tRNA^{LEU}* gene, the following primers were used: 5′-TCCAATAACTTGACCAACGGAACAAG-3′ (forward), 5′-AAC

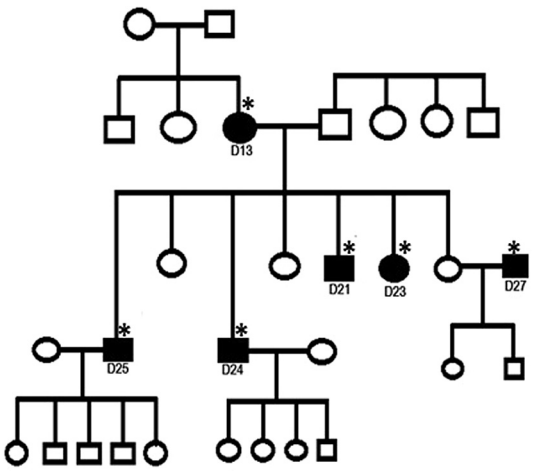

Fig. 1. Pedigree of patients with Type II maternally inherited diabetes mellitus. Affected members are shown by filled symbols. Numbers are given for family members who provided the sample and informed consent for the study. In the pedigree each proband is indicated by asterisk.

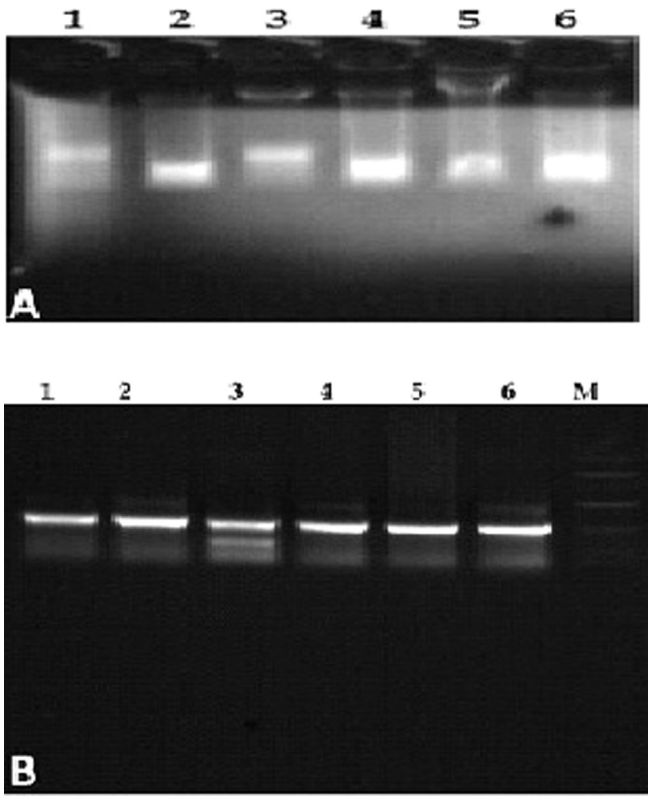

Fig. 2. (A) Agarose gel electrophoresis photograph of isolated DNA. (B) Agarose gel electrophoresis photograph of PCR amplified DNA fragments.

GTTGTGGTCTTTGCGTAGTTGTA-3′ (reverse). The PCR products were purified using the Gene JETTM PCR purification kit (# K0701) and were sent for sequencing to Macrogen Korea (www.macrogen.com). Sequence variants were identified using Bioedit; a sequence alignment editor version 6.0.7 (http://www.mbio.ncsu.edu/bioedit/bioedit).

3. Results and discussion

3.1. Clinical finding

All the probands of the family were underwent clinical and laboratory examination. Probands with Type II diabetes of maternal history showed some shared clinical characteristics e.g. dry mouth, weight loss and muscular pain (Table 1).

3.2. Mutation analysis of MT-TL1 gene

The tRNA$^{Leu(UUR)}$ gene in 6 probands of the family were sequenced as well as their flanking regions. After thorough sequence analysis of the probands we were unable to identify homozygous A>G transition at nucleotide position 3243 located in the tRNA^{LEU} gene. Bidirectional DNA sequencing further validated our sequencing results. Next, we performed comparative analysis of the patient's sequences with the standard sequences obtained from NCBI (https://www.ncbi.nlm.nih.gov). Comparison of diabetic individuals with control (Fig. 3) further validated the sequencing data.

Mutations of mtDNA, particularly in *tRNA^{LEU}* gene are emerging surprisingly as a major cause of maternally inherited Type II diabetes mellitus in developed countries. The prevalence of mtDNA *MT-TL1* gene mutations in the Pakistani family is yet to be explored. In the present study, we selected a family with 6 probands having maternally inherited Type II diabetes mellitus

Table 1
Sampling details of diabetic individuals with maternal history.

Tube No.	Names	Age	Family history	Duration of disease	Symptoms	Medicines
D13	ZahidaBibi	65	Mother	14 Years	Thirst, Pain in foots, Frequent Urination	Minidiab.
D21	AteeqAslam	48	Son, Brother	7 Years	Dry Mouth, Weight loss	Glucophage
D23	ShaheenaBibi	47	Daughter, Sister	6 Years	Frequent Urination, Hunger	Getformin
D24	Parvez Iqbal	45	Son, Brother	4 Years	Dry Mouth, Weight loss	Neodopar
D25	Ali Asghar	43	Son, Brother	2 Years	Muscular Pain	Daomil
D27	TanveerIqbal	40	Sister husband	1 Year	Dry Mouth, Weight loss	Insulin

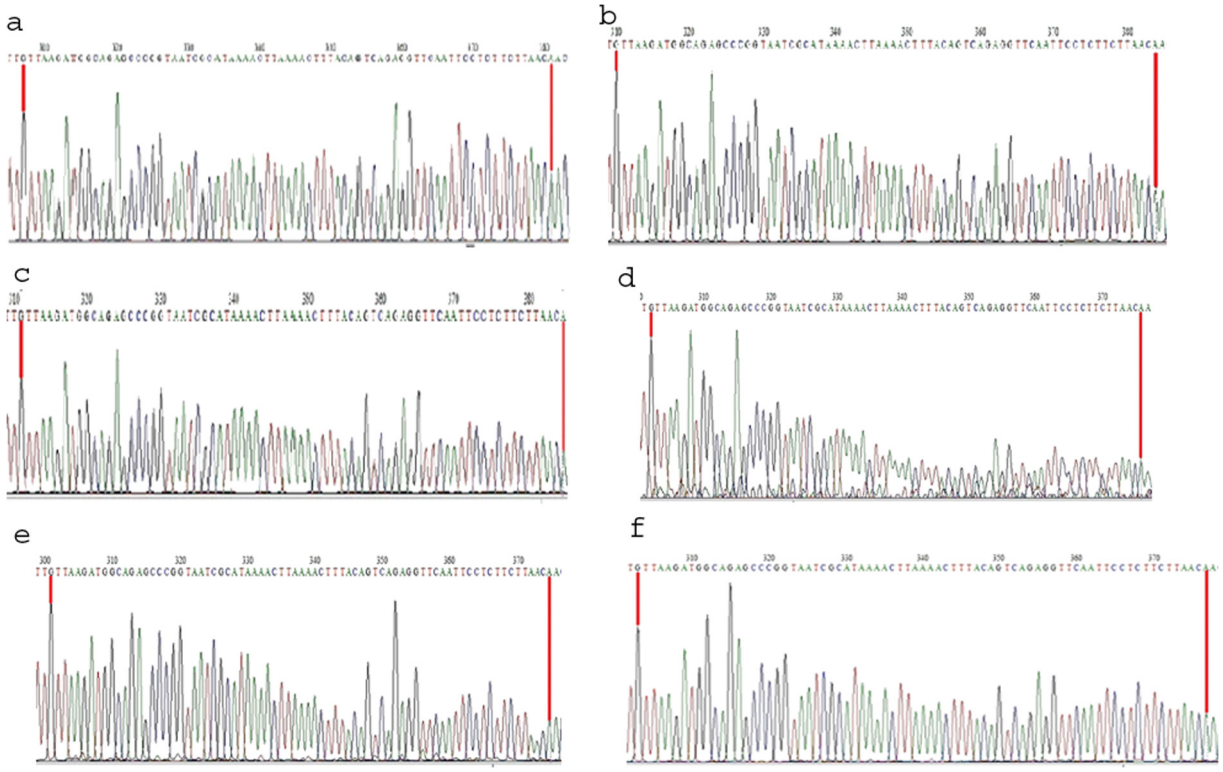

Fig. 3. Sequencing peaks of six patients: **(a) D13,** Sequence peak of tRNA$^{(LEU)}$gene from 307 to 381 **(b) D21,** Sequence peak of tRNA$^{(LEU)}$gene from 310 to 384 **(c) D23**, Sequence peak of tRNA$^{(LEU)}$gene from 311 to 385 **(d) D27** Sequence peak of tRNA$^{(LEU)}$gene from 302 to 376 **(e) D25,** Sequence peak of tRNA$^{(LEU)}$gene from 301 to 375 **(f) D24,** Sequence peak of tRNA$^{(LEU)}$gene from 303 to 377.

phenotype. Molecular genetic analysis revealed no significant variant of *MT-TL1* gene in a Pakistani family. Parallel researches conducted in Argentina [14], Poland [15] and Indonesia [16] were found unproductive in discerning an m. 3243A>G mutation. Thus our findings in a Pakistani family further strengthen the previous studies in context of m. 3243A>G variant.

Conclusively, although previous clinical observations and studies have suggested some role of mitochondrial dysfunctions in the progression of Type II diabetes mellitus but the mutations in mitochondrial *MT-TL1* gene cannot be labeled as a major contributing etiological factor of Type II diabetes mellitus in a Pakistani family. But we cannot exclude the significance of other genetic variants in the etiology of maternally inherited diabetes. Mitochondrial genome is susceptible to pathogenic mutations, hence comprehensive and thorough detection of mitochondrial DNA mutations requires sequencing of the entire mtDNA for investigation of underlying molecular and genetic factors.

Conflict of interest

Authors declare no conflict of interest.

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