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Effect of tRNALys DNA m.8360 A>G Human Mitochondria Mutation on Diabetes

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Abstract : This study points out for the first time that mitochondria are energy production sites and other metabolic functions mitochondria DNA(mDNA) is only maternal inherited. Mutations in(mDNA) are associated with many human mitochondrial diseases linked with disorders in the oxidation production of energy. It can lead to diabetes. this study is designed to investigate the mitochondria $tRNA^{Lys}$ DNA m.8360 A>G mutation in Iraq families with type 2 diabetes mellitus using microgenealliment sequencing technique. Eleventh Iraqi families underwent the experiment.Genomic DNA was isolated from whole blood samples following a standard protocol and was measured by a Nanodrop device, where the purity of DNA was 1.8-1.9 at 260 nm and its concentration was 22.4ng. Eight mothers out of eleven (72.7%) have this mutation for this result it can concluded that the A8360G mtDNA mutation of the tRNALys and associated with maternal diabetes.

Keywords: Diabetes, Mitochondria,DNA$tRNA^{Lys}$

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Introduction

Diabetes is a multifactorial disorder characterized by inappropriate insulin secretion leading to chronic hyperglycemia. Pancreatic B cells are a key player in maintaining normal glucose homeostasis by secreting insulin. There are a number of genetic and non-genetic risk factors associated with diabetes (1,2). In general, mitochondrial diabetes is an unremarkable form of diabetes with familial clustering. Type 2 diabetes has a maternal inheritance pattern and in most cases, AD. The heterozygous mutation 8295-8364G encoding the tRNALys gene is thought to be responsible for the disease phenotype (3,4).

Human mitochondrial tRNA has received attention due to the association between point mutations in tRNA genes and various neuromuscular and neurodegenerative disorders (5). However, in other cases, mutations occurring in mRNA genes can damage the respiratory chain, leading to mitochondrial dysfunction, which greatly contributes to the development of diabetes. Initially, tRNA mutations were reported in individuals with mitochondrial encephalopathy, lactic acidosis, and stroke-like episode.

At the beginning of the research, work was done on the A8343G mutation, as the homozygous A8343G mutation affects the first adenine in the TψC ring of tRNALys (position 54). Nucleotides at this position are often

modified and thus play an important role in the structure and function of tRNA.(6) This mutation may affect the amino acid tRNA ability and binding affinity for the mitochondrial elongation factor Tu, which is critical for mitochondrial protein synthesis. (7,8) Therefore, the m.A8343G mutation is pathogenic in PCOS-IR and has no significant effect on diabetes. After that, we moved to the m.A8360 G mutation, as this mutation affects the association of tRNALys with amino acid through an enzyme.

Martial and methods

Subjects

The study was conducted at the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies at Baghdad University, where venous blood samples were collected from 100 donors from 25 families.

Samples collection

All individuals underwent standardized clinical and laboratory evaluation. After that, samples were collected from 100 individuals and the cumulative blood sugar level was examined to confirm the presence of diabetes in some members of the same family. We collected 4 ml of blood from each participant to extract a sufficient amount of DNA. Each sample was placed on ice and frozen at −20 as quickly as possible.

DNA extraction, Library preparation, and sequencing

Genomic DNA was isolated from whole blood samples following a standard protocol and was measured by a Nanodrop device, where the purity of DNA was 1.8-1.9 at 260 nm and its concentration was 22.4ng. To detect mutations, the scone method was used after amplifying the tRNAlys gene fragment through the primer that was designed by the NCBI BLAST website. And the primer is 5-GGTCAAATGCTCTGAAA3TCTGTGGAG-3(forward).And Primer Reverse3-AGATTTTCGTTCATTTTGGTTCTC-5.

Results

Clinical finding

All samples taken from families underwent laboratory examination. Probands with a history of mothers with type 2 diabetes showed some common clinical characteristics, e.g. Dry mouth, weight loss, and muscle pain as shown in (Table 1).

Table (1): Comparison between DM and non-DM individuals according to FBS and HbA1c

Group	Means ±SE	
	F.B.S. (mg/dl)	HbA1c (mg/dl)
DM Mothers (23) and DM offspring (6)	168.84 ±8.89	7.64 ±0.32
Non-DM offspring (50)	101.28 ±0.80	5.18 ±0.04
T-test	12.719 **	0.460 **
P-value	0.0001	0.0001

** (P≤0.01).DM: FBS: HbA1c: DM Mothers (23) and DM offspring (6)
Non-DM offspring (50)

Glycated hemoglobin test (HbA1c) is recommended as a standard of care for testing and monitoring diabetes, specifically the T2DM (World Health Organization, 2011). Non-diabetes usually falls within the 4.0%–5.6% HbA1c range. The pre-diabetes usually has the HbA1c levels as 5.7%–6.4%, while those with 6.4% or higher HbA1c levels have diabetes (American Diabetes Association, 2021).

Mutation analysis of tRNAlys gene

The tRNAlys gene present in 4 domains of the family as well as their flanking regions was sequenced. After comprehensive sequence analysis of the probes, we were unable to identify the A>G homology transition at nucleotide position 8360 located in the tRNAlys gene. Bidirectional DNA sequencing has dated our sequencing results. Next, we performed a comparative analysis of the patient sequences with standard sequences obtained from NCBI (https://www.ncbi.nlm.nih.gov). Comparison of diabetic individuals with controls (Figure 1 and 2) validated the sequencing data.

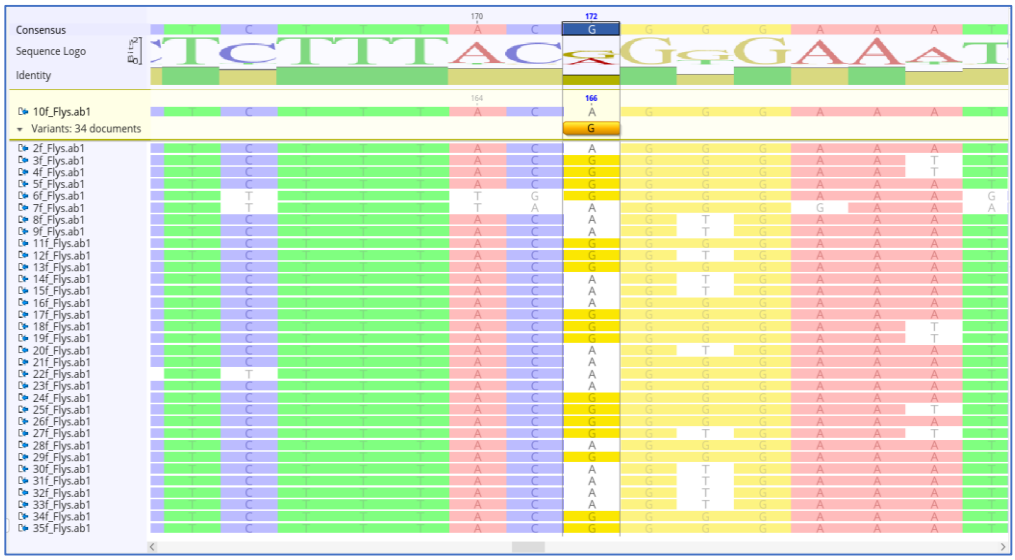

Fig (1): Comparison of diabetic individuals with controls Validity of sequencing data.

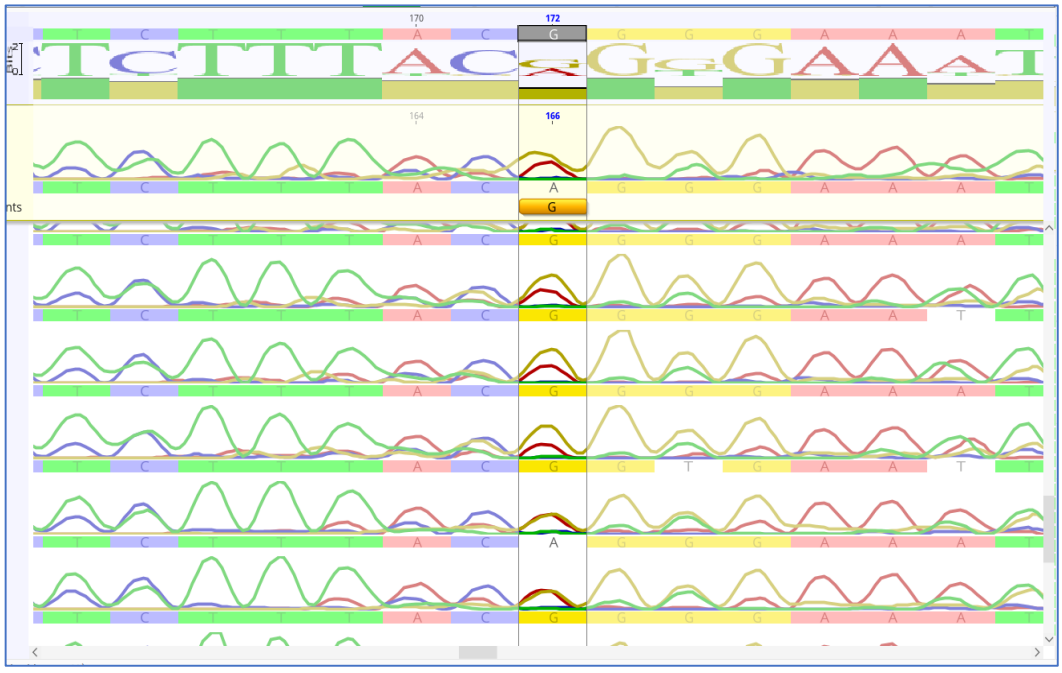

Fig (2): Comparison of diabetic individuals with controls Validity of sequencing data..

4.Discussion

Phenotype. Molecular genetic analysis revealed no significant difference in the tRNAlys gene in Iraqi families. Parallel research conducted in Argentina (9), Poland (10), Indonesia (11) and Pakistan (1) was found fruitless in distinguishing M. 8360>G mutation. Thus, our findings in Iraqi families further strengthen previous studies in the context of AD. 8360A>G variant. Conclusively, although previous observations and laboratory studies have indicated some role for mitochondrial dysfunctions in the development of type 2 diabetes, mutations in the mitochondrial tRNAlys gene cannot be classified as a major causative factor for type 2 diabetes in Iraqi families. But we cannot rule out the importance of other genetic variants in the etiology of maternally inherited diabetes. The mitochondrial genome is vulnerable to disease-causing mutations, so comprehensive and comprehensive detection of mitochondrial DNA mutations requires sequencing of the entire mitochondrial DNA to investigate the underlying molecular and genetic factors.

Conclusions

The many underlying molecular abnormalities in common diabetes etiology are highlighted in this work. We have introduced a novel variation, G8360 A, as the pathogenic change, although none of the previously identified mtDNA mutations have been discovered. This mutation is causally related to diabetes, according to many pieces of evidence. One potentially dangerous alteration found in individuals' mitochondrial DNA was G8360 A. Two, it affects a gene whose variations have been linked to diabetes in the past. (3) It disrupts a tightly conserved base-pairing connection in the aminoacyl acceptor stem of the encoded tRNALys, resulting in impairment of tRNALys function. (4) Neither healthy individuals nor those with any other condition have shown any signs of it. (5,12) Lastly, G8360A may improve the efficiency of glucose consumption by the mitochondria, which is a hallmark of muscle mitochondrial dysfunction(13).

The modest mutation levels seen in the mother's blood are in line with the theory of maternal inheritance. Interestingly, this goes against the existing standards for genetic counseling in diabetes, which state that the A8360 G mutation in the blood should be taken into account to lower the chance of having children.

Mutation mapping in the mitochondrial tRNALys gene is consistent with a unique function for this translational switch in the development of diabetes. Nevertheless, our understanding of the pathogenic processes that cause its expression remains incomplete. Even though it has been shown for cells with A8344G and G8356A that all tRNALys mutations eventually disrupt mitochondrial protein synthesis,15 the impact on tRNA function has only been investigated for A8344G. This is because A8344G does not have oscillatory rule modulation, a defect that leads to impaired codon and anticodon interaction and decreased mitochondrial translation simultaneously. It is unclear how G8361A affects tRNA function, however, as it binds to the aminoacyl acceptor stem instead of the TC domain. code and Country)

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