Research article

A study of ATPase gene variants of mitochondrial DNA in patients with Type 2 diabetes mellitus

Ali Jumaah Alhussona[1], Ali Habeeb Al-Musawi[2], Hayder Hussein Jalood[3], Sawsan S. Hamzah[4]

[1,3]Ministry of Education, General of Directorate of Education Thi Qar, Iraq
[2]College of Education for women, University of Thi- Qar, Iraq
[4]Al-Farahidi University, College of Dentistry, Baghdad, Iraq

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Corresponding author: **Ali Habeeb Al-Musawi.** Email: alim.bio93@gmail.com; ali.habeeb2020@utq.edu.iq

ABSTRACT

Introduction and Aim: Diabetes mellitus is a complex disease that is a major global health problem. It is defined as a chronic metabolic disease characterized by high blood glucose levels. Diabetes risk has been linked to genetic variations within mitochondrial DNA. The current study sought to identify and compare genetic variation (mutations) in ATPase genes in diabetic patients and healthy individuals.

Materials and Methods: This study included 100 individuals (50 T2DM patients and 50 healthy volunteers). Peripheral blood samples were collected from each individual and DNA extracted. The mitochondrial mtATPase genes were amplified using specific primers by polymerase chain reaction. The amplified products were sequenced, and the sequences obtained analyzed for nucleotide changes.

Results: The ATPase gene sequence analysis revealed nine nucleotide changes, three of which (A9039G, G9092A, and G9100T) are classified as polymorphisms in the human mitochondrial genome database. Furthermore, we presented the first description of mutations in ATPase genes such as (A8586G, C8637T, G8813A, A8953G, C8981T and T9024G). The A9039G mutation changed the amino acid isoleucine to glycine, the G9100T mutation changed the amino acid serine to isoleucine, and the C8981G mutation changed the amino acid threonine to isoleucine. Most patients were found to predict haplogroup H2a (H2a2), in addition to having H1c (H1c3) and L1c(L1c2). The presence of genetic variations in mtATPase genes may be an inheritable risk factor for type 2 diabetes mellitus pathogenesis.

Conclusion: Variants in ATPase mitochondrial genes may be one of the risk factors associated with type 2 diabetes. Hence, documenting these mutations is clinically important in the possibility of diagnosing diabetes, as well as the likelihood that these mutations could be pathogenic.

Keywords: Type 2 diabetes mellitus; ATPase genes; mtDNA; haplogroup.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by high blood sugar levels because of complete or relative deficiency of insulin hormone or the presence of anti-insulin agents (1). Diabetes mellitus is associated to several risk factors which includes genetic or functional factors resulting in high level of glucose in the blood plasma (2). Diabetes has been classified into many types, the most common among them being insulin-dependent type 1 diabetes and non-insulin-dependent type 2 diabetes, the rise in glucose levels is caused by a relative or absolute lack of insulin hormone secretion or action, or both, resulting in disruptions in the metabolism of important nutrients such as carbohydrates, fats, and proteins, all of which result in an increase in blood sugar from normal (3).

Diabetes is a result of lifestyle changes in dietary habits as well as lack of mobility, all of which have led to the worldwide spread of this disease (4). Furthermore, diabetes may develop because of an increase in oxidative stress, which could be a temporary or chronic condition in the body (5) disrupting the normal functioning of the cells and the release of oxidative stress enzymes (6).

Mitochondria are essential intracellular organelles where ATP and reactive oxygen species are formed by the electron transport chain (5). Several studies have indicated that mitochondrial dysfunction plays an important role in causing type 2 diabetes, mitochondrial dysfunction in beta-pancreatic cells leading to impaired release of glucose-catalyzed insulin. It is also associated with reduced oxidative phosphorylation and fatty acid oxidation in insulin-sensitive tissues. As an ATP-generating site, mitochondria play an important role in insulin secretion and variation in mitochondrial DNA has been linked to an increased risk of diabetes. Studies have associated polymorphisms within the mitochondrial DNA to be related to T2D, among which the most common mutation A3243G in the

tRNA gene (Leu, UUR) is closely related to diabetes, affecting oxidative phosphorylation and poor insulin secretion (7). The present study aimed to determine the genetic variation (mutations) in *ATPase* genes in diabetic patients and compare them with healthy individuals.

MATERIALS AND METHODS

Sample collection

Peripheral blood samples were collected from diabetic patients (n=50) who were chosen at random from the Diabetes and Endocrine Center in Thi-Qar province. Their ages ranged from 34 to 71 years. Blood was also draw from 50 healthy individuals (controls) with no diabetes. Ethical clearance was obtained from the Ethics Committee of the Diabetes and Endocrine Center in Thi-Qar Governorate prior to the study (No. 6789, 12-2-2022).

DNA extraction and amplification

DNA was extracted from blood using a DNA Mini kit (gSYNCTM, Taiwan) as per the manufacturer's instructions. The mitochondrial mtATPase genes was amplified using primers (Macrogene, South Korea). The forward primer 5'-ATGCCCCAACTAAAT ACTACCGT-'3 a reverse primer 5'-CTTGGATT AAGGCGACAGGCG-'3 was used to amplify a 790bp of the mitochondrial gene (8). Amplification was carried out using a thermocycler (Bioneer company, South Korea) under the following conditions: the first cycle of 95 Cº for 5 min (primary denaturation), followed by 30 cycles at 95ºC for 45 seconds (denaturation), 60ºC for 30 seconds (annealing), 72º C for 30 seconds (extension) and one cycle of final extension at 72ºC for 10 minutes. The PCR method has many medicinal uses, including the identification of MRSA (9), the genotyping and evaluation of some parameters in toxoplasmosis (10), the diagnosis of adenocarcinoma (11), *Brucella melitensis* (12), and *Clostridium perfringens* strains (13). Further, the PCR method can be applied to diagnosis of CML (14). The products were subjected to electrophoresis on 2% agarose gel, stained with ethidium bromide (0.5 µL) and the bands visualized under UV light. The PCR products were excised, purified, and outsourced for sequencing (Macrogene, Seoul, South Korea). The sequences obtained were subjected to a BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search available at the National Center Biotechnology Information (NCBI) website. Haplogroup was predicted using the tool Mitomaster (www.mitomap.org/foswiki/bin/view/MITOMASTER).

Statistical analysis

Data were analyzed statistically using the SPSS where the ANOVA variation analysis was tested to compare the different age groups of the study aggregates. Data was considered statistically significant at a p-value $p \leq 0.05$.

RESULTS

This study investigated the mitochondrial DNA variations, and the extent to which these variations are associated in patients with type 2 diabetes and in healthy control groups. All participants in the study underwent systematic evaluation for data including family history, age, treatment method and other medical conditions which is presented in Table 1.

Table 1: Age, gender and clinical aspects of individuals in the study group

Parameters		**Patients**	**Healthy**	**P- value**
		N (%)	N (%)	
Age group (years)	≤ 50	26 (52)	20 (40)	0.236
	> 50	24 (48)	30 (60)	
Mean ± SD		56.90 ± 10.63	45.95 ± 9.43	
Gender	Male	33 (66)	35 (70)	0.526
	Female	17 (34)	15 (30)	
Family history	Yes	22 (44)	-	0.273
	No	28 (56)	-	
Comorbidities	Hypertension	12 (24)	-	0.008
	Heart disease	13 (26)	-	
	Kidney failure	10 (20)	-	
	No comorbidities	15 (30)	-	
Treatment	Insulin	39 (78)	-	0.06
	Diet or regulator	11(22)	-	

P-value ≤ 0.05 mean significant

Table 2: Amino acid variations in mtATPase sequence

Variation	Codon change	Amino acid change	Originality	Predicted Haplogroup	Treatment
G8587A	CAG to CAA	Glycine to Glycine	Novel	L1c (L1c2)	Insulin
A9039G	TAC to TGC	Tyrosine to Cysteine	Mitomap	H2a (H2a2)	Insulin
T9024G	CAT to CAG	Histidine to Glycine	Novel	H2a (H2a2)	Insulin
G9092A	CGC to CAC	Arginine to Histidine	Mitomap	H2a (H2a2)	Diet
G9100T	GCT to TCT	Alanine to Serine	Mitomap	H2a (H2a2)	Insulin
A8953G	TAA to TAG	Stop to stop	Novel	H1c (H1c3)	Insulin
C8981T	CTC to TTC	Leucine to Phenylalanine	Novel	H21(H21)	Diet +Fortamet
C8637T	ACC to ATC	Threonine to Isoleucine	Novel	H2a (H2a2)	Diet +Fortamet
G8813A	CCG to CCA	Proline to Proline	Novel	H2a (H2a2)	Diet+Fortamet

Novel refers to mutation identified in this study

Analysis of the mtATPase gene sequence identified nucleotide changes at nine positions (Table 2). Three of these mutations (A9039G, G9092A, and G9100T) were like those identified and documented in the human mitochondrial genome database for diabetic patients. In this study, we identified six novel mutations (G8587A, C8637T, G8813A, A8953G, C8981T, and T9024G) within the ATPase gene sequence which has not been reported previously (Table 2). The amino acid change and originality of mutation are given in Table 2. Haplogroup analysis for *ATPase* revealed most patients to belong to the haplogroup H2a (H2a2). The other haplogroups observed were L1c(L1c2), H1c (H1c3) and H21(H21) (Table 2).

The most common mutation type found in the patients was A8953G (TAA to TAG), followed by A9039G (Tyrosine to Cysteine) and C8981T (Leuine to Phenylalanine) in 16.7%, 10%, and 6.7% of the patients, respectively. Except for A8953G, which was seen in one individual, no mutations were found in healthy individuals.

We also studied whether these amino acid changes altered the RNA secondary structure of the ATPase protein. The RNA structure was predicted using the Vienna RNA Package ver. 2.0 (15). A comparison of structural models obtained showed the mutations at sites T9024G, G9092A, A8953G and G9100T alters the secondary structure of the molecule (Fig.1A) as compared to control group (Fig.1B).

DISCUSSION

Type 2 diabetes is influenced by several genetic actors, one among them being the adenosine triphosphate (ATP)-dependent potassium channels (16). The ATP gene is a highly conserved gene within the mitochondrial DNA, mutations to which impairs the secretion of insulin (16, 17).

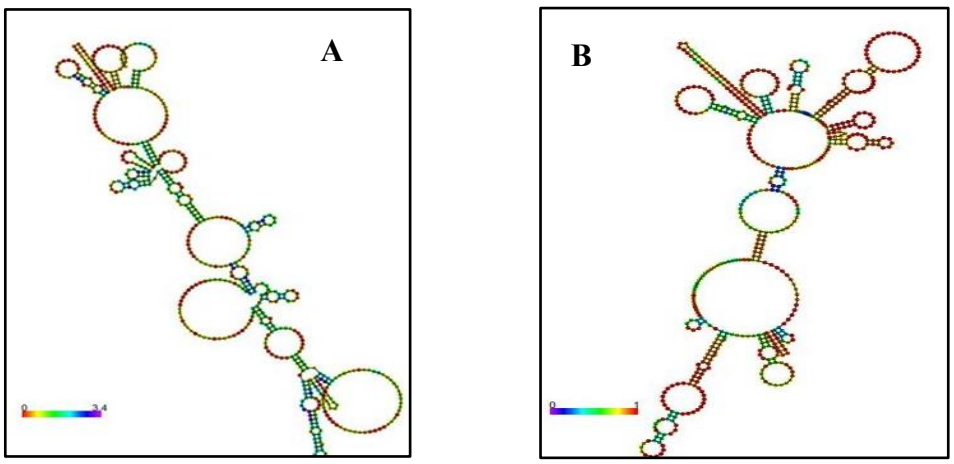

Fig. 1: Secondary structure of ATPase (A): Mutations T9024G, G9092A, A8953G and G9100T (B): Control group.

The association of certain genetic mutations in the ATPase complex among diabetics was investigated in this study. Approximately 83.3% of patients with type 2 diabetes were found to have pathogenic mutations in the ATPase complex, indicating the importance of mtDNA defects in causing type 2 diabetes. Our studies are consistent with earlier studies wherein mutations A3243G, C16270T and C16320T were shown to be strongly associated with increased risk of type 2 diabetes (18,19). Mitochondrial DNA mutations associated with type 2 diabetes mellitus were reported in the Chinese population (19). A study also showed T8551C mutation in the overlapping region in the MT-ATP8 gene to be associated to diabetic mellitus (20). In this study, several of the mutations observed were of substitution type which altered the amino acid. Such alterations could lead to dysfunction of the ATPase enzyme activity leading to decreased enzyme production (21,22).

A mtDNA variant 16189T>C reduces the association convergence of the single-strand DNA binding protein (mtSSB) and is shown to be associated with type2 diabetes among Asians (23). In this study several of the mutations seen caused alterations to the secondary RNA structure which probably indicates the pathogenic nature of these mutations. Further, the mutation, mtDNA 3243A>G, is known to be a cause of maternal diabetes and common mtDNA variations such as mtDNA 16189T>C and many mtDNA haplogroup, are also associated with an increased risk of diabetes (23). A correlation between type 2 diabetes and mitochondrial DNA haplogroups was studied, which showed that the haplogroup effect is unlikely to be significant in the causes of the disorder (24). Whether or not the haplogroups observed in this study has any association to diabetes mellitus requires further study.

CONCLUSION

The presence of variants in ATPase mitochondrial genes might be one of the causes of risk factors for type 2 diabetes. A basic perception of genetic variances can be developed in people with type 2 diabetes, since documenting these mutations is clinically important in the possibility of diagnosing diabetes, as well as the likelihood that these mutations will be pathogenic.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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