# Forensic Application of ATP6 Gene Haplogroups in a Cohort of Obese Saudis

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## Abstract

Blood samples from 108 obese individuals (73 males and 35 females) and 106 healthy ones (79 males and 27 females) were collected from a cohort of Saudis inhabiting Riyadh province. ATP6 mitochondrial gene was amplified and sequenced to identify obesity-related SNPs and the mitochondrial DNA haplogroups for forensic purposes. A novel site (A8660G) was found while C8655T and T9103C were obesity- related sites. The haplogroups H2a and H1a were common as they were found in 70.4% and 66% of obese and non-obese individuals, respectively. L2 was recorded in the obese samples only and H21 was recorded in the obese males only. The J2a haplohroup was more common in obese individuals. Constructing mtDNA database for people inhabiting different regions of Saudi Arabia for forensic purpose and to affirm the utility of H21 and L2 in identifiying obesity is necessary.

***Keywords:*** *haplogroups, obesity, ATP6 gene, Saudi Arabian*

## Introduction

The mitochondrial genome (mtDNA) plays an important role in determining human haplotype as its SNPs could determine the population pattern of ethnic groups[1,2]. It can also be used to calibrate the migration events of human ancestors and their origins as it expresses their maternal inheritance[1]. MtDNA might be, therefore, used to determine the population structure of a particular society.

MtDNA played an important role in identifying obesity as mitochodria plays a key role in cellular energy metabolism. ATP6, cytochrome b (cytb) genes and d-loop are the most effective mtDNA segments in this perspective. ATP6 gene controls the production of ATP molecules while cytb gene plays a role in electron transport during the oxidative phosphorylation and d-loop controls the heavy strand replication of the mtDNA. Researchers used these fragments in identifying obesity from differnt ages and sexes[3-7].

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Studies investigated the association of obesity and metabolic disorderes to the mtDNA haplogroups[8-11]. Demir et al.[6] found obesity-related SNPs in ATP6 and cytb genes in a cohort of obese Turkish children. Knoll et al.[5] revealed a significant correlation of ATP6 gene and d-loop SNPs to obesity in W haplogroup. The d-loop acquired 5 SNPs related to abdominal fat accumulations and diabetes and blood pressure[12]. Similar investigations have been conducted for Turkish, Caucasian and Arabian ethnic populations[7, 13-15].

Few studies have been conducted so far on mtDNA of Arabian populations[2,7,10]. The mitogenome of over 500 random individuals from different 5 geographical Saudi Arabian regions have been targeted and showed that Saudi Arabians are belonging to 5 main haplogroups L, M1 U6, U, M[10]. Amer et al.[7] used cytb gene for a population from the western Saudi Arabia and found 4 haplogroups H2a, JT, U5a and R0a. Eaaswarkhanth et al. [2] detected d-loop variants and haplogroups identifiable to obesity in a Kuwait population. The present study amplified and sequenced ATP6 gene to identify obesity-related SNPs and haplogroups for a cohort of Saudis inhabiting Riyadh city for forensic purposes.

## Materilas and Methods

### Sampling

Blood samples of 110 obese (75 males and 35 females) and 104 normal (74 males and 30 females) adults (age > 21 and < 52 years) were collected from randomly selected Saudis inhabiting Riyadh city under the supervision of a specialized clinician. According to WHO[16], obese inidivduals acquired BMI >30 kg/m$^2$ and the normal ones acquired BMI between 20 kg/m$^2$ and 25 kg/m$^2$. Individuals with chronic diseases were excluded. Those who had BMI >25 ~ <30 or BMI <19 kg/m$^2$ and those with age <18 years were also excluded. Demographic data and family history were collected from donors in a consented questionnaire.

### DNA extraction, PCR and sequencing

300 µl blood samples were pippetted into 1.5 ml sterilized tube. DNA extraction was conducted using Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Polymerase chain reaction was conducted in 50 µl reaction mixtures containing 2 µl DNA template, 25 µl GoTaq Green Master Mix (Promega Corporation, Madison, WI 53711-5399, USA), 22.6 µl nuclease free water and 20 pmoles of each primer (forward primer: 5'-TCT GTT CGC TTC ATT CAT TG -3' and the reverse primer: 5'- TGA AAA CGT AGG CTT GGA-3'). The PCR conditions were according to Amer et al.[17]. Purified products were sequenced from both strands using The Koryean Macrogen company facilities.

## Statistical Analysis

Sequnces were prepared by DNASIS v. 3.5 and MacClade V. 4.10 software[18] and were aligned with the revised Cambridge Reference Sequence[19] (RSRS). Data were deposited in DDBJ/NCBI Genbank database (MN427654 - MN427867). ATP6 haploroups were predicted by using MitoMaster program using HaploGrep2 with Phylotree 17 for haplogroup determination (Topic revision: r3 -02. May2017, MarieLott).https://www.mitomap.org/foswiki/bin/view/MITOMASTER/WebHome).

## Results and Discussion

ATP6 gene variants of 214 adult indivduals from both sexes were investigated by direct sequencing of 531-bp fragment between nucleotide position 8615 and 9145. Polymorphisms with a frequency 5³% in controls or obese cases were assumed to be relevant and further analyzed[14]. Table 1 shows the ratios of 7 recorded variants, 5 of which were between pyurines and 2 were between pyrimidines. They were C8655T, A8664G, G8697A, A8701G, A8860G, G8994A and T9103C. The variant C8655T was recorded in 5.6% of obese patients, while it was recorded in 3.8% controls. The two variants A8664G (2%) and G8994A (0.9%) were recorded in the patients only in spite of being irrelevant (below polymorphism threshold). The frequencies of the G8697A (8.3% vs 8.5%), A8701G (25% vs 22.6%) and A8860G (100% vs 100%) were high in both obese and controls, respectively. The last variant was considered to be a novel as it did not appear in the reference sequence. The variant T9103C was recorded in 7.4% patients and in 4.7% controls. Among the 7 variants, 3 were non-synonymous substitutions and the other 4 were silent (C8655T, A8664G, G8697A and G8994A). The variants A8701G and A8860G exhibited exchange of thrionine to alanine at the potions Thr$^{59}$Ala and Thr$^{112}$Ala, respectively. At T9103C, the amino acid changed from phenylalanine to leucine at the position Phe$^{193}$Leu.

Haplogroups were predicted using HaploGrep2[20] with PhyloTree mtDNA tree Build 17[21]. Seven haplogroups (H2a, H1a, H21, T, J2a, L0 and L2) were identified, most of which are belonging to three macro-haplogroups H, T, L and J. Table 2 and Table 3 show haplogroup percentages and their prevalence. H2a was common exhibiting 51.9% in the obese samples and 46.2% in the controls. H1a was prevalent in 18.5% patients and 18.8% in the controls. The haplogroup T was equally prevalent (7.5%) in obese and controls. J2a was found in obese samples with 8.3% and in controls with 4.7% being higher in obese females (14.2%). In obese males, the haplougroup H21 appeared with 1.9%, while L2 appeared in 2.8%. The predicted haplogroups refelected the results shown by Abu-Amero et al.[10] that the Arabian Peninsula has received substantial gene flow from Africa, detected by the presence of a major L lineage (62%) being migrated from the Northern hemisphere (H, T and J).

By comparing this study to the previous ones (Table 4), 5 novel variants associated with obesity were found (8655C>T, 8664A>G, 8697G>A, 8994G>A and 9103C>T). The two variants 8701A>G, 8860A>G were recorded also in obese Turkish[8] and Japanese[3,4] juveniles. However, these two variants could be associated to ethnicity rather than obesity since they appeared in a larger healthy Saudi population[10] as well.

It could be therefore possible to use this gene in forensic purposes.

An individual mtDNA haplogroup can be used as a reference when analyzing the mitochondrial genome data of an unknown sample since a human race to which this sample belongs can be predicted. Rodriguez-Flores et al.[22] suggested that the difference in mtDNA haplogroups may be also due to the heterogeneous marriage from outside and inside the population. The present study, therefore, used random samples from both sexes to excute a preliminary mtDNA database for an ethnic group inhabiting Riyah city. H, R[23] and L[24] are associated with some genetic disorders. In the present study, the sub-haplogroup H21 (2.7%) was associated with obesity in males, while J2a was associated with obesity in females (14.2%).

The 7 predicted sub-haplogroups herein belong to 4 main haplgroups (H, T, J, L) and all of which were recoreded in Sadui Arabian populations inhabiting diffrerent geographical regions[7,25,26]. By sharing haplogroups (H, T and L) with those predicted by Abu-Amero et al.[10], the present study might confirm that Arabian Pinnensula received human races from diffrernt ethnic groups of Africa, Asia and Europe. Meanwhile, the haplogroup H (H2a, H1a and H21) was dominant in the studied population as it showed high prevalence in both pateints (71.2% ♂ and 74.2% ♀) and controls (63.2% ♂ and 74% ♀). In spite of its low prevalence among Saudis[10], H haplogroup is common in the middle region (Riyadh) from which the samples of the present study were collected. Roostalu et al.[25] also recorded the major prevalence of H2a followed by H1a in the middle region of Arabian Pinnensula. Khubrani et al.[27] predicted the Y-chromosome haplogroups for 500 Saudis inhabiting 5 distant geographical regions and found that the haplogroup J is common in the midde region. The present study recorded this haplogroup too.

**Table 1. Synonymous ATP6 gene SNPs and their frequency % in obese and control Saudis.**

| Synonymous substitution | Gender % | | | | | |
|---|---|---|---|---|---|---|
| | Control=106 | | | Obese =108 | | |
| | F | M | ∑ | F | M | ∑ |
| C8655T | 3.3 | 2.7 | 6.0 | 5.7 | 5.3 | 5.5 |
| A8664G | 0 | 2.7 | 2.7 | 0 | 1.3 | 1.3 |
| G8697A | 6.6 | 9.5 | 16.1 | 2.9 | 10.7 | 13.6 |
| G8865A | 0 | 0 | 0 | 0 | 2.7 | 2.7 |
| G8994A | 0 | 0 | 0 | 0 | 2.7 | 2.7 |
| T9042C | 2.9 | 1.4 | 4.3 | 6.7 | 4 | 10.7 |

**Table 2. Non-synonymous ATP6 gene SNPs and their frequency % in obese and control Saudis.**

| Nonsynonymous substitution | Amino acid subsitition | Gender % | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control =106 | | | Obese=108 | | |
| | | F | M | ∑ | F | M | ∑ |
| A8701G | Thr59Ala | 30 | 22.9 | 52.9 | 22.9 | 29.3 | 52.2 |
| A8836G | Ala104Val | 0 | 2.8 | 2.8 | 0 | 2.8 | 2.8 |
| A8860G | Thr112Ala | 100 | 100 | 100 | 100 | 100 | 100 |
| A8887G | Ile121Val | 0 | 2.7 | 2.7 | 0 | 0 | 0 |
| G9055A | Ala177Thr | 0 | 4.1 | 4.1 | 0 | 4 | 4 |
| T9103C | Phe193Leu | 3.3 | 5.3 | 8.6 | 14.3 | 6.6 | 20.9 |
| T9116C | Ile193Thr | 0 | 0 | 0 | 0 | 2.7 | 2.7 |

**Table 3. Prevalence % of the predicted ATP6 gene haplogroups.**

| Predicted haplogroup | Gender | | | | Total | | Variants |
|---|---|---|---|---|---|---|---|
| | Female | | Male | | | | |
| | control | obese | control | obese | obese | control | |
| H2a | 48 | 60 | 45.5 | 48 | 51.9 | 46.2 | A8860G |
| H1a | 26 | 14.2 | 17.7 | 20.5 | 18.5 | 19.8 | A8701G/A8860G |
| H21 | 0 | 0 | 0 | 2.7 | 1.9 | 0 | A8860G/G8994A |
| T | 3.7 | 2.8 | 8.8 | 9.5 | 7.4 | 7.5 | G8697A/A8860G |
| J2a | 3.7 | 14.2 | 5 | 5.5 | 8.3 | 4.7 | A8860G/T9103C |
| L0 | 3.7 | 5.7 | 2.5 | 1.4 | 2.8 | 2.8 | A8860G/A8701G/C8655T/ C9042T |
| L2 | 0 | 2.8 | 0 | 2.8 | 2.8 | 0 | A8860G/A8701G/C8655T |

**Table 4. Comparison between the present and previous studies for the recorded SNPs.**

| Ethnic group | Sample size | Number of detected substitutions | non-synonymous variations |
|---|---|---|---|
| Japan (Fuku et al., 2002) | Young obese (n=96) Controls (n=0) | n=26 novel=17 | 8584G>A, 8563A>G, 8701A>G, 8764G>A, 8950G>A |
| Japan (Guo et al., 2005) | Young obese n=96 Type 2 diabetes (n=96) | obese group: n=26 | 8584G>A, 8563A>G, 8701A>G, 8764G>A, 8950G>A |
| Saudi Arabia (Abu-Amero et al., 2008) | 553 healthy unrelated individuals | n=2 | 8557G>A,8860A>G |
| Turkey (Demir et al., 2014) | Obese children (n=100) Controls (n=100) | obese group: n=39 novel=1 | 8557G>A, 8584G>A, 8684C>T, 8701A>G, 8764G>A, 8812A>G, 8836A>G, 8843T>C, 8860A>G, 8867T>C, 8869A>G, 8950G>A, 8962A>G, 8966T>C, 8975T>C, 9007A>G, 9055G>A |
| This study | Obese Adult (n= 108) Controls Adult (n= 106) | n= 7 novel= 5 | 8655C>T, 8664A>G, 8697G>A, 8701A>G, 8860A>G, 8994G>A, 9103C>T |

## Conclusions

Seven ATP6 gene variants were recorded in the studied samples among which, 5 were related to obesity and 2 were related to ethnicity and therfore can be used for forensic purpose. Building mtDNA database for human population inhabiting a specific gegraphic area could be used as a forensic reference since a human race to which this sample belongs can be predicted.

**Ethical Clearance:** 214 Saudi individuals gave written informed consent before participating in this

study. Ethical clearance was also obtained from Internal Security Forces Hospital in Riyadh.

**Conflicts of Interest** : Authors declare that there are no conflicts of interest regarding the publication of this paper.

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