Journal of Forensic Research

Amer et al., J Forensic Res 2015, 7:1
http://dx.doi.org/10.4172/2157-7145.1000318

Research Article Open Access

Analysis of the Cytochrome b Gene in Some Obese and Non Obese Saudi Arabian Populations

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Abstract

Forty six Saudi samples were collected from obese and non-obese males aged between 25 and 35 years old. Approximately 1000 bp of the mitochondrial cytochrome b gene for these samples were amplified and sequenced as an initial effort to determine the sequence variation within this mtDNA coding region. The sequenced fragment was aligned with its counterparts from 100 healthy African and Asian humans found in the Genbank database. The polymorphisms of these sequences were analyzed for forensic purposes. A total of 12 polymorphic sites and 25 variations were noted. Sixteen out of the 25 variations were synonymous and the other 9 encoded different amino acids (non-synonymous). The haplogroup of all samples was determined using Mitomaster software. A total of 5 common different haplogroups were observed (H2a, U5a, JT, L3 and R0a). The recorded SNPs were tribe other than obesity-dependent except one common SNP (C15452A) which seemed to be an obesity marker, however, it was also found in the non obese individuals. Although the polymorphisms of cytochrome b gene were observed in Saudi populations and could be considered as a powerful forensic marker, however, more samples must be analyzed to investigate the unique distribution for forensic applications.

Keywords: Cytochrome b; Saudi population; SNPs; Forensic application

Introduction

The hyper variable regions in the d-loop of the mitochondrial genome were used in forensic purposes [1-8]. Besides this d-loop region, the cytochrome b gene was also used for individual identification in case the conventional STR typing is unavailable based on the effect of environmental factors, including soil acidity and composition, heat, and humidity [9] or on the nature of changes during evolution [10]. These changes can contribute to the fragmentation of DNA molecules, thereby preventing two-copy nuclear markers to persist over time. It is well known that, cytochrome b is among the mitochondrial DNA genes being useful in species discrimination. Lee et al. [11] found a total of 30 polymorphic sites distributed along cytochrome b gene in 98 unrelated Koreans. Mishmar et al. [12] also reported that environmental factors may cause regional mtDNA variation in humans. Kong et al. [13] reported that the polymorphisms of cytochrome b sequence were very informative for defining the haplotype status of East Asian mtDNAs.

There are two major populations living in Saudi Arabia, one is the indigenous population included many Arabian tribes (Hothali, Harbi, Thaqafi, Zahrani, Qurash etc…) and the other included the immigrating Asian and African populations. In this study, samples from random populations were collected and the polymorphisms of 1000 bp of cytochrome b gene were analyzed to show the distribution variations between these tribes or populations. Unique SNPs observed could be valuable for either obesity diagnosis or for investigation of evidence origin on forensic applications.

Materials and Methods

Samples and DNA extraction

Obese and non obese Saudi male donors from different tribes were randomly chosen based on the body mass index (BMI). The BMI of all samples were above 30 indicating that the collected samples were all obese. Six non obese samples (BMI was between 18 and 25) which were relatives to some tribes were also used. These obese samples were named from H1 to H39 while the control non obese samples were named from H40 to H46. Swabs were carefully taken and placed in sterilized tubes. Total genomic DNA was extracted from the swab samples by using Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, USA). The extraction method was performed according to the manufacturer's instructions (www.promega.com/protocols). The final extracts were dissolved in DNA rehydration solution. DNA concentration and quality were determined spectrophotmetrically at 260/280 nm and the samples were stored in 4°C for later use.

Amplification, sequencing and analysis

In order to amplify 1000 bp from the mitochondrial cytochrome b gene (nt14747-15887), the rCRS of Andrews et al. [14] sequence has been used to design the following primer sets: cytbF: 5'-CCCCAATACGCAAAATTAACCC-3' cytbR: 5'-GTATAGTACGGATGCTACTTGTC-3'. PCR was conducted in a final volume of 50 µL containing 2 µL DNA templates, 2 µL of 10 picomolar from each of forward and reverse primers, 25 µL PCR master mix (Promega Corporation, Madison, WI) and 19 µL autoclaved deionized distilled water. Amplification was conducted in a Thermo scientific thermo cycler with the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and then 72°C for 5 min for further extension. The PCR products were analyzed in 1% agarose gel electrophoresis in TAE buffer (40 mm Tris, 40 mM acetic

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Received November 06, 2015; **Accepted** January 06, 2016; **Published** January 12, 2016

Citation: Amer SAM, AlHothali BRA, Tubaigy SMA (2016) Analysis of the Cytochrome b Gene in Some Obese and Non Obese Saudi Arabian Populations. J Forensic Res 6: 318. doi: 10.4172/2157-7145.1000318

acid and 1 mM ethylenediamine-tetra acetic acid) with ethidium bromide staining. 100bp DNA ladder (Biolabs) was used as a molecular marker. Then PCR products bands were visualized under UV light and photographed. The PCR products were then excised from agarose gels and purified using spin column (BioFlux, Tokyo, Japan) according to the manufacturer's instructions.

The purified PCR products were sequenced in an ABI PRISM ABI3730xl sequencer (Applied BioSystems) and BigDyeTM Terminator Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems) following the protocols supplied by the manufacturer. Sequences were compared with Andrews reference sequence using the Mitomaster software through MitoWeb program (http://mitomap.org/MITOMAP) [15] to reveal the nucleotide variations and to define the haplogroups.

Results and Discussion

In this study, DNA was obtained from all studied Saudi samples and 1000 bp of their cytochrome b gene were amplified and sequenced successfully. The sequenced fragments were deposited in the NCBI Genbank database with their corresponding accession numbers (KT215436-KT215473 and KT248511-KT248517). The variable sites were identified by aligning all sequences with their counterpart of the Andrews reference sequence. A total of 12 polymorphic sites, found in at least 2 individuals, and 25 variations were noted (Table 1). In concordance to the Malay people [16], several frequently mutable sites were revealed, A15326G, G15301A and G15043A. In this protein-coding gene, the recorded changes were nucleotide substitutions and neither deletion nor insertion was found. Base substitutions were commonly transitions with a transition:transversion ratio of 25:2 as revealed in several investigations [16-18].

Based on the genetic code of vertebrate mitochondrial DNA, 16 out of the 25 variations (Table 2) were synonymous (do not cause an amino acid change) and the other 9 encoded different amino acids (non-synonymous). A total of 5 common different haplogroups were observed (H2a, U5a, JT, L3 and R0a, Table 3). Due to absence of a standard for assignment of haplogroup, we called each different sequence as an individual haplogroup and thus 20 haplogroups were found. The most similar sequence to the reference was that of H19 that was different just in one nucleotide at a position 15148 (G to A) which was also synonymous. The sequences H9, H17 and H34 were entirely identical to the reference if the position 15326 (A to G), which is considered as an obesity marker, is not counted.

There is a debate for the strength of cytochrome b gene in forensic purposes. Farghadani and Babadi [16] stated that since the power of the discrimination using cytb gene is lower than those of hyper variable regions in the D-loop [19,20], this gene by itself is probably not adequate for routine forensic investigation. However, Tsai et al. [10], Hwa et al. [21] and Ablimit et al. [22] revealed that this gene is a good candidate for forensic investigation. As the argument of Farghadani and Babadi [16] was based on a small segment of cytb gene (402 bp) and the other investigations, beside ours, were based on nearly the complete gene sequence, the present investigation strongly recommended that the cytb gene by itself is a candidate marker in forensic casework. The small haplogroup frequency in this study could be overcome in future investigation by collecting more samples from wide different Saudi Arabian tribes. The genetic diversity (D=0.8975) as calculated from the estimated haplogroup frequencies (Table 3) [23] was shown to be comparable to that calculated for the hypervariable regions (D=0.964) [8] and this finding approved the efficiency of cytochrome b gene in forensic investigations.

With respect to the obesity markers, few individual mutations were recorded as shown in the haplogroups of H15 and H27 at position G15431A where non-synonymous substitution was recorded in which alanine amino acid was substituted with thrionine. Similar substitution was recorded in the obese Japanese people with different ages exposed to sudden cardiac death caused by cardiomyopathy [24]. The body mass index of H15 and H27 were 39.7 and 44.8, respectively. We may consider this as a weak marker for obesity because other individuals

Nucleotide												Individuals
15784	**15679**	**15674**	**15607**	**15452**	**15431**	**15326**	**15301**	**15257**	**15218**	**15043**	**14905**	
T	A	T	A	C	G	A	G	G	A	G	G	rCRS
.	.	.	G	A	.	G	.	.	.	.	A	H6, H33
.	.	.	.	.	A	G	.	.	.	.	.	H27
.	.	.	.	.	.	G	A	.	.	A	.	H11, H15
.	.	.	.	.	A	G	.	.	.	.	.	H15
.	.	.	.	.	.	G	A	.	.	.	.	H18
.	.	.	.	.	.	G	.	.	G	.	.	H26, H32
.	G	.	.	A	.	G	.	A	.	.	.	H10, H16
.	.	.	.	.	.	G	A	.	.	.	.	H1, H2, H3 H5, H8, H14, H29, H35, H36, H37, H38, H39, H41, H42, H46
.	.	.	.	A	.	G	.	.	.	.	.	H4, H20, H21, H22, H31, H33,
.	.	.	.	A	.	.	.	.	.	.	.	H40, H43, H44, H45
	.	C		.		G	.	.	.	.	.	H24, H25, H28
C	.	.	.	.	.	G	.	.	.	.	.	H14, H26, H41, H42

Table 1: Sequence variations of the Saudi cytochrome b with respect to Andrews sequence. The variations are listed when they were polymorphic (found in more than one individual). The reference stands for the Andrews sequence is in bold. The numbers on the top indicate the nucleotide position in the mitochondrial genome and dots (.) represent the matches with the reference sequence.

Synonymous	Position in the codon	Amino acid	Individuals	Substitution	Reference nucleotide position
+	3	Met-met	H33	G-A	14905
-	1	Ile-Leu	H27	A-C	14981
+	3	Gly-Gly	H11, H15	G-A	15043
-	1	Ala-Thr	H18	G-A	15110
+	3	Gly-Gly	H27	C-T	15136
+	3	Pro-Pro	H19	G-A	15148
+	3	Gly-Gly	H18	G-A	15217
-	1	Thr-Ala	H26, H32	A-G	15218
+	3	Val-Val	H32	T-C	15229
+	3	Trp-Trp	H8	A-G	15235
-	1	Asp-Asn	H10, H16	G-A	15257
+	3	Ser-Ser	H29, H46	T-C	15262
+	3	Leu-Leu	H1-H3, H5, H8, H11, H14, H15, H18, H29, H35-H39, H41-H42, H46	G-A	15301
-	1	Thr-Ala	all except H19	A-G	15326
+	3	Gly-Gly	H5	A-G	15358
+	3	His-His	H29, H46	T-C	15388
-	1	Ala-Thr	H15, H27	G-A	15431
-	1	Leu-Ile	H4, H6, H10, H16, H20-H22, H31, H33, H40, H43-H45	C-A	15452
+	3	Met-met	H31, H40	G-A	15466
+	3	Tyr-Tyr	H8	T-C	15514
+	3	Lys-Lys	H6, H33	A-G	15607
-	1	Ser-Pro	H24, H25, H28	T-C	15674
+	3	Lys-Lys	H10, H16	A-G	15679
-	1	Ile-Val	H13	A-G	15746
+	3	Pro-Pro	H14, H26, H41, H42	T-C	15784

Table 2: Amino acid changes as a result of 25 nucleotide substitutions.

Haplogroup	Number of individuals per haplogroup	Haplogroup frequency
H2a	6	0.15
JT	5	0.125
U5a	5	0.125
L3	3	0.075
R0a	3	0.075
T	2	0.05
HV1a	2	0.05
J2a	2	0.05
L0a	1	0.025
L3i	1	0.025
L3f	1	0.025
L2b	1	0.025
L2a	1	0.025
M30	1	0.025
M	1	0.025
R30b	1	0.025
B4a	1	0.025
X2i	1	0.025
J1b	1	0.025
J2b	1	0.025
Genetic diversity	$1-\Sigma p^2$	0.8975

Table 3: Haplogroups and their frequencies for the studied populations. The genetic diversity for the analyzed DNA fragment was calculated according to the formula $D=(1-\Sigma p^2)$ [23].

with BMI higher than that of haplogroups H15 and H27 did not show this substitution. Meanwhile, individuals (both obese and non obese) from the same mother showed the same forensic SNPs like H8 and H41, H43 and H44, H29 and H46 and H20, H21, H22 and H45 (Table 2).

We therefore concluded that, for Saudi Arabian populations, cytb gene could be applied as a forensic candidate to a certain limit and its application in obesity diagnosis is questionable. The application of this gene in both trends is still week and needs more investigations on hundreds of closely related obese and non obese people.

References

1. Cann R, Stoneking M, Wilson A (1987) Mitochondrial DNA and human evolution. Nature 325: 31-36.
2. Harihara S, Saitou N, Hirai M, Gojobori T, Park K, et al. (1998) Mitochondrial DNA polymorphism among five Asian populations. Am J Hum Genet 43: 134-143.
3. Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson A (1991) African populations and the evolution of human mitochondrial DNA. Science 253: 1503-1507.
4. Torroni A, Schurr TG, Yang CC, Szathmary EJE, Williams RC, et al. (1992) Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. Genetics 130: 153-162.
5. Monsalve M, Hagelberg E (1997) Mitochondrial DNA polymorphisms in Carib people of Belize. Proc R Soc Lond B Biol Sci 264: 1217-1224.
6. Al-Zahery N, Semino O, Benuzzi G, Magri C, Passarino G, et al. (2003) Y-chromosome and mtDNA polymorphisms in Iraq, a crossroad of the early human dispersal and of post-Neolithic migrations. Mol Phylogenet Evol 28: 458-472.
7. Al-Zahery N, Pala M, Battaglia V, Grugni V, Hamod MA, et al. (2011) In search of the genetic footprints of Sumerians: a survey of Y-chromosome and mtDNA

variation in the Marsh Arabs of Iraq. BMC Evol Biol 11: 288.

8. Hameed IH, Gebor MA, Kareem MA (2015) Forensic analysis of mitochondrial DNA hypervariable region HVII (encompassing nucleotide positions 37 to 340) and HVIII (encompassing nucleotide positions 438-574) and evaluation of the importance of these variable positions for forensic genetic purposes. African J Biotechnol 14: 365-374.
9. Gabriel M, Huffine E, Ryan J, Holland M, Parsons T (2001) Improved mtDNA sequence analysis of forensic remains using a "mini-primer set" amplification strategy. J Forensic Sci 46: 247-253.
10. Tsai L-C, Lee JC-I, Lin AC-Y, Su C-H, Chen S-J, et al. (2009) Analysis of the cytochrome b gene in Taiwanese populations. Forensic Sci J 8: 29-34.
11. Lee S-D, Lee Y-S, Lee J-B (2002) Polymorphism in the Mitochondrial cytochrome B gene in Koreans. An additional marker for individual identification. Int J Legal Med 116: 74-78.
12. Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark A-G, et al. (2003) Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci USA 100: 171-176.
13. Kong Q-P, Yao Y-G, Liu M, Shen S-P, Chen C, et al. (2003) Zhang Y-P. Mitochondrial DNA sequence polymorphisms of five ethnic populations from northern China. Human Genet 113: 391-405.
14. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, et al. (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23: 147.
15. http://www.mitomap.org.
16. Farghadani R, Babadi AA (2015) Nucleotide variation of the mitochondrial cytochrome B gene in the Malay population. Rom J Leg Med 23: 57-60.
17. Tzen CY, Wu TY, Liu HF (2001) Sequence polymorphism in the coding region of mitochondrial genome encompassing position 8389-8865. Forensic Sci Int 120: 204-209.
18. Lee J-C, Hsieh H-M, Huang L-H, Kuo Y-C, Wu J-H, et al. (2009) Ivory identification by DNA profiling of cytochrome b gene. Int J Legal Med 123: 117-121.
19. Van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, et al. (2003) Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am J Hum Genet 72: 804-811.
20. Wong HY, Tang JSW, Budowle B, Allard MW, Syn CKC, et al. (2007) Sequence polymorphism of the mitochondrial DNA hypervariable regions I and II in 205 Singapore Malays. Leg Med (Tokyo) 9: 33-37.
21. Hwa H-L, Ko T-M, Chen Y-C, Chang Y-Y, Tseng L-H, et al. (2010) Study of the Cytochrome b Gene Sequence in Populations of Taiwan. J Forensic Sci 55: 167-170.
22. Ablimit A, Qin W, Shan W, Wu W, Ling F, et al. (2013) Genetic diversities of cytochrome B in Xinjiang Uyghur unveiled its origin and migration history. BMC Genet 14: 100.
23. Jones DA (1972) Blood samples: probabilities for discrimination. J Forensic Sci Soc 12: 355-358.
24. Kobayashi M, Nakamura S, Murakami C, Maeda K, Irie W, et al. (2011) Mutational analysis of the mitochondrial DNA detected in sudden cardiac death caused by cardiomyopathy. Kitasato Med J 41: 127-135.

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