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Brief Communication

Possible Application of Cytochrome b Gene for Human Identification

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Abstract

The mitochondrial cytochrome b gene (cytb) has been partially amplified and sequenced in order to identify the characteristic SNPs (single nucleotide polymorphism) for some Saudi Arabian tribes. Approximately 1 kbp from this gene has been sequenced and aligned with the same fragment of the revised Cambridge Reference Sequence (rCRS). The polymorphic sites and the haplotypes of all studied individuals were identified. Commonly, three main SNPs (G15301A, A15326G and T15674C) and 6 haplotypes (H, L, JT, U5a, R, J) were found. Most of the recorded SNPs and haplotypes were tribe dependant. Therefore, cytb gene could be considered as a powerful forensic marker; however, more samples must be analyzed to investigate the unique distribution for forensic applications.

Key words: Mitochondrial DNA, Saudi tribes, Single Nucleotide Polymorphism SNPs, Forensic Marker.

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التطبيقات المحتملة لاستخدام الموقع الوراثي سيتوكروم ب Cytochrome b لتحديد الهوية البشرية

المستخلص

تم في هذه الدراسة إجراء عملية تكثير جزئي وتحديد للتسلسل النيوكليتيدي للموقع الوراثي سيتوكروم بي Cytochrome b (cytb)، وذلك من أجل التعرف على النيوكليتيدات المميزة في مواقع تعدد أشكال النيوكليتيدة الواحدة Single Nucleotide Polymorphism (SNPs) لبعض القبائل العربية السعودية. حيث تم تحديد التسلسل النيوكليتيدي لما يقارب من واحد كيلو من القواعد النيتروجينية ومقارنتها مع الجزء نفسه من تسلسل كامبردج النيوكليتيدي المرجعي المعدل (revised Cambridge Reference Sequence (rCRS.

وقد تم تحديد المواقع التي يوجد بها تعدد لأشكال النيوكليتيدة الواحدة والـ Haplotypes لجميع الأفراد الذين أجريت الدراسة عليهم. على نحو مُشتَرك، وجدنا ثلاثة مواقع رئيسية لتعدد أشكال النيوكليتيدة الواحدة وهي (G15301A, A15326G، T15674C)، و ستة مواقع لـ Haplotypes وهي (H, L, JT, U5a, R, J).

وكان معظم مواقع تعدد الأشكال التي تم تسجيلها ومواقع الـ Haplotypes مميزة في كل قبيلة. لذلك، يمكن اعتبار الموقع الوراثي (cytb) موقع يمتلك قوة تمييز في المجال الجنائي يمكن من خلاله تحديد هوية الأشخاص. وبالرغم من ذلك، لا بد من عمل تحليل للمزيد من العينات لتحديد التوزيع الفريد لهذه العلامات والذي يمكن تطبيقه في المجال الجنائي.

الكلمات المفتاحية: الحمض النووي للميتوكوندريات، القبائل، تعدد أشكال النيوكليتيدة الواحدة (SNPs)، العلامات الجنائية.

Introduction

Besides the forensic application of the d-loop region in the mitochondrial genome [1-4], the cytochrome b gene has also been used for human identification [5,6], particularly for samples which may have been subjected to abnormal environmental factors.

Within the d-loop of the mitochondrial DNA, there are many SNPs which are accumulated in the hyper-variable region 1 (HV1) and the hyper-variable region 2 (HV2) [7]. The identification of key SNPs in these regions are considered for forensic purposes but with insufficient results [8]. Therefore, analyses of coding region SNPs within this genome were developed [9]. Among the coding regions, the cytochrome b gene is 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 [2], or on the nature of changes during evolution [10]. Lee et al found a total of 30 polymorphic sites distributed along the cytochrome b gene in 98 unrelated Koreans [6]. Mishmar et al reported that environmental factors may cause regional mtDNA variation in humans. Kong et al reported that the polymorphisms of the cytochrome b sequence were very informative for defining the haplogroup status of East Asian mtDNAs [11].

There are many Arabs inhabiting Saudi Arabia besides other immigrating Asian and African tribes. In this study, swab samples from random human individuals were collected, and the characteristic features of their cytochrome b gene were analyzed in order to show their specific SNPs which could be valuable for forensic purposes.

Materials and methods

Forty-six Saudi males with ages ranging between 25 and 35 years old that are belonging to 20 different tribes and inhabiting the western region of Saudi Arabia were randomly chosen. The common tribes (from which at least 3 samples were available) were Malki, Jazani, Zahrani, Hothali and Otaibi. These individuals were ethically requested to provide their swabs that were carefully placed in sterilized tubes. Total genomic DNA was extracted from the swab samples by using the Wizard® Genomic DNA extraction Kit (Promega).The extraction method was performed according to the manufacturer's instructions. The final extracts were dissolved in DNA rehydration solution. DNA concentration (50 ng) and quality were determined spectrophotmetrically at 260/280 nm and the samples were stored at 4°C for PCR experiments.

In order to amplify 1000 bp from the mitochondrial cytochrome b gene, the rCRS sequence of the cytochrome b gene was used to design the primer pairs: cytbF: 5'- CCCCAATACGCAAAATTAACCC -3' cytbR: 5'- GTATAGTACGGATGCTACTTGTC -3' [12]. PCR was conducted in a final volume of 50 μL according to the procedures detailed in Amer et al. [13]. The PCR products were visualized on the agarose gel electrophoresis and purified as also described by the above-mentioned authors.

The amplified 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. The obtained sequences were treated statistically with DNASIS and MacClade v. 4.1 programs for gene identification, alignments and analyses. First, the sequences were checked for their accuracy by sequencing the forward and the reverse strands. Then, the collected data were examined by Sequencher v.4 for correcting the bias nucleotides. The corrected data were then saved in the DNASIS files which is

Table 1- *The recorded SNPs in the cytochrome b gene for some Saudi tribes as compared to the reference (Andrews) sequence. The reference sequence is in bold. represent the matches with the reference sequence [12].*

Tribes	Individuals	Nucleotide		
	rCRS	15301	15326	15674
		G	A	T
Malki, Jazani and Zahrani	H5, H2, H3, H29, H1, H14, H37, H36, H35, H39, H38, H8	A	G	
Hothali	H20, H21, H33, H31, H4, H22		G	
Otaibi	H28,H25, H24 Otaibi		G	C

Table 2- *Amino acid changes of the three common nucleotide mutations.*

Reference nucleotide position	Substitution	Amino acid	Position within codon	Synonymous
15301	G - A	Leu-Leu	3	+
15326	A - G	Thr-Ala	1	-
15674	T - C	Ser-Pro	1	-

accessible to the alignment MacClade program. Sequences were also compared with the rCRS using the Mitomaster software through the MitoWeb program (http://mitomap.org/MITOMAP) to reveal the nucleotide variations.

Results and discussion

In this study, 1 kbp of the cytochrome b gene was amplified and sequenced successfully using the same PCR primers for all selected samples. We selected only male donors since the mitochondrial DNA is maternally inherited and false relationships could be obtained when we use samples from mixed sexes. The variable sites were identified by aligning all sequences with their counterpart in the Andrews reference sequence. Among the collected tribes, we selected and studied only those with at least three samples per tribe [12]. A total of three polymorphic sites were

Table 3- *Haplotypes and their frequencies for the studied tribes.*

	Expected Haplotype	Sample size	Haplotype frequency
Common haplotypes	H	8	0.20
	L	8	0.20
	JT	5	0.125
	U5a	5	0.125
	R	4	0.10
Non-common haplotypes	J	4	0.10
	T	2	0.05
	M	2	0.05
	B4a	1	0.025
	X2i	1	0.025
Genetic diversity		$1- \Sigma p^2$	0.8975

distributed in 5 tribes (Malki, Jazani, Zahrani, Hothali and Otaibi). These three SNPs were G15301A, A15326G and T15674C, and all the studied individuals differed from rCRS in the position A15326G (Table 1). In concordance with the Malay people [14], 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 were found. Base substitutions were common transitions with a transition:transversion ratio of 25:2 as revealed by the investigations of Tzen et al, Lee et al and Farghadani and Babadi [6, 14, 15].

As shown in Table 2 and based on the genetic code of vertebrate mitochondrial DNA, G15301A did not cause an amino acid change (a synonymous change) while the other 2 (A15326G and T15674C) were non-synonymous showing different amino acids from the rCRS. A total of 6 common different haplotypes were observed (H, L, JT, U5a, R, J).

There is a debate regarding the use of cytochrome b gene for forensic purposes. Farghadani and Babadi revealed that since the discrimination strength of the cy-

tochrome b gene is lower than that of the D-loop hyper variable regions, this gene by itself is probably not suitable for routine forensic investigation [14, 16, 17]. However, Hwa et al. and Ablimit et al. revealed that this gene is a good candidate for forensic investigation [18,19]. As the argument of Farghadani and Babadi was based on a small segment of the cytochrome b gene (402 bp) and the other investigations, beside ours, were based on nearly the complete gene sequence, the present investigation strongly recommended that the cytochrome b gene by itself is a candidate marker in forensic casework [14]. The small haplotype frequency in this study can be improved in a further study by collecting more samples from a wider selection of different tribes. In order to highlight on the genetic structure of the studied population, the genetic diversity (D= 0.8975) was calculated according to estimated haplotype frequencies (Table 3). It was shown to be comparable to that calculated for the hypervariable regions (D= 0.964) supporting the effectiveness of the cytochrome b gene analysis in forensic investigations [4]. In conclusion, the cytochrome b gene could be a possible forensic marker if more samples, more tribes and the entire gene sequence are considered.

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