Rom J Leg Med [23] 57-60 [2015]
DOI: 10.4323/rjlm.2015.57

Nucleotide variation of the mitochondrial cytochrome B gene in the Malay population

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Abstract: Identification of coding region polymorphism in addition to the hypervariable regions is helpful to increase the value of mitochondrial DNA polymorphic data in forensic genetics. In this study, DNA sequence of the mitochondrial cytochrome b gene was analysed among unrelated ethnic Malays as an initial effort to determine the sequence variation within the cytochrome b gene of mtDNA coding region. Totally 13 different sequence types containing 15 polymorphic sites were found. Sequence positions with high frequency and a novel polymorphism site were observed. The results suggest that sequence polymorphisms of the human mitochondrial DNA cytochrome b gene can be applied as an additional marker in individual identification for forensic casework and may be valuable in anthropological and population studies. However, more samples must be analysed.

Key Words: coding region, cytochrome B gene, forensic science, Malay population, mitochondrial DNA, nucleotide variation.

Human mitochondrial DNA is a closed circular double-stranded molecule with 16,569 base pairs in length which its sequence first reported by Anderson *et al.* in 1981 [1]. It is composed of the coding region, which codes for 2 ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA) and 13 proteins, and a noncoding region or displacement loop (D-loop) making about 1.1 kb of mtDNA [2]. Mitochondrial DNA has several unique characteristics compared to the nuclear DNA. MtDNA is only inherited through mother with no recombination event [3] and each cell contains hundreds to thousands of mtDNA copies, making it easier to recover and sequence. In addition the mutation rate of mtDNA is much higher than that of the nuclear DNA occurring 5-10 times faster [4] due to the lack of the intron, protective proteins such as histones and efficient repair system as well as it is exposed to the oxygen radicals generated in the inner membrane of mitochondria [5]. Therefore, these properties of mtDNA make it as a useful tool for forensic application and anthropology studies.

In order to provide additional genotyping information and improve forensic discrimination, assessing variations happening in coding region can be very useful [6, 7]. That's why, the mitochondrial DNA coding region polymorphisms have become in attention not only in the case of pathogenic mutation [8-10], but also in the forensic application [11]. In this regard, there are an increasing number of studies related to the

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polymorphism identification of different parts in the mtDNA coding region [11, 12]. Cytochrome b gene (MTCYB) within mtDNA coding part is widely applied in species identification and evolutionary studies [13]. Moreover, the human cyt b gene encompassing 1142 bp of mtDNA undertaken several changes through evaluation has been evaluated to be used as an additional individual identification marker in forensic casework and the sequence polymorphisms in different populations have been present [7, 14].

The Malays represent approximately 50.4% of the entire Malaysian population, but mitochondrial DNA data on this population is so far very scarce and existing mtDNA data are limited to the non-coding region [15, 16]. However, one limitation of the effective use of mtDNA in forensic casework is the lack of the relevant database [17]. Therefore, the mtDNA cytochrome b (CYTB) gene within the coding region of mtDNA has been studied in the Malay population as an initial effort in order to identify variable sites and evaluate the possible usefulness of these sequence polymorphisms in forensic genetics.

MATERIALS AND METHODS

Subjects and DNA extraction

Unrelated Malay donors from different locations of Peninsular Malaysia were randomly chosen in the UTM University. The 3 ml of fresh peripheral bloods were collected into anticoagulant tubes containing EDTA to prevent coagulation.

Total genomic DNA was extracted from fresh blood samples by using Wizard® Genomic DNA Purification Kit (Promega).The extraction method was performed according to the manufacturer's instructions. The final extracts were dissolved in DNA rehydration solution and stored in 4°C for later use.

PCR Amplification

In order to amplify the mitochondrial cytochrome b gene (nt14747-15887), Anderson sequence [1] has been used to design the following primer sets:

F14742: 5'- CACCAATGACCCCAATACGC-3'

H15894: 5'-CAAGGACAGGCCCATTTGAG-3'

The PCR reaction was carried out in 50 µl of reaction mixture containing 1.25 U of Taq DNA polymerase, 0.2 µM each primer, 0.2 mM of each dNTP and 10x PCR buffer (PCR Core Kit, Qiagene).Amplification was conducted in a Thermo scientific thermo cycler with the following conditions: initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and then 72°C for 10 min for further extension. After amplification, the products were confirmed using 1% agarose gel electrophoresis and purified using HiYield™ Gel/PCR DNA Mini Kit (Real Biotech Corporation, RBC).

DNA Sequencing and Sequence analysis

Sequencing of PCR products was performed using the primer F 14742 and the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).The DNA sequence was detected with an ABI3730xl DNA Analyzer (Applied Biosystems).

Sequences were compared with the Anderson reference sequence using the BioEdite software v7.1.6.0 through ClustalW multiple alignment to reveal the nucleotide variations.

RESULTS AND DISCUSSION

DNA from all samples were amplified successfully and sequenced. All the sequences were aligned with the

Table 1. Sequence variations with respect to the Anderson sequence

	14783	15043	15172	15235	15236	15301	15326	15340	15458	15553	15565	15613	15650	15663	15666
Reference / Sequence type	**T**	**G**	**G**	**A**	**A**	**G**	**A**	**G**	**T**	**G**	**T**	**A**	**G**	**T**	**T**
Type 1	.	.	A	.	.	A	G	A	.	A	.	G	.	.	.
Type 2	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.
Type 3	C	A	.	.	G	A	G	.	.	.	.	.	A	.	A
Type 4	.	.	.	.	.	A	G	A	.	.	.	.	.	.	.
Type 5	.	A	.	.	.	A	G	.	.	.	.	.	.	.	.
Type 6	C	A	.	.	.	A	G	.	.	.	.	.	.	.	.
Type 7	C	A	.	.	.	A	G	.	.	.	.	.	A	C	C
Type 8	.	.	.	.	.	.	G	.	.	.	C	.	.	C	.
Type 9	C	A	.	.	.	A	G	.	C	.	.	.	.	.	.
Type 10	C	A	.	.	G	A	G	.	.	.	.	.	.	C	.
Type 11	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
Type 12	C	.	.	.	.	.	G	A	.	.	.	.	.	.	.
Type 13	.	.	.	G	.	.	G	.	.	.	.	.	.	.	.

Table 2. Amino acid alterations as a result of 16 nucleotide substitutions

Anderson nucleotide site	Nucleotide change	Result	Position	Synonymous
14783	T C	Leu-Leu	1	Yes
15043	G A	Gly-Gly	3	Yes
15172	G A	Gly-Gly	3	Yes
15235	A G	Trp-Trp	3	Yes
15236	A G	Ile -Val	1	No
15301	G A	Ile-Val	1	No
15326	A G	Thr-Ala	1	No
15346	G A	Leu-Leu	3	Yes
15458	T C	Ser- Pro	1	No
15553	G A	Lys-Lys	3	Yes
15565	T C	Tyr-Tyr	3	Yes
15613	A G	Gly-Gly	3	Yes
15650	G A	Ala-Thr	1	No
15663	T C	Ile-Thr	2	No
15666	T A T C	Leu-His Leu-Pro	2 2	No No

Anderson reference sequence in order to identify variable sites in the mitochondrial cytochrome b gene. A total of 15 polymorphic sites and 16 variations were noted (table 1), among which 14 sites were previously registered in the human mitochondrial genome database, mitomap (www.mitomap.org). Several frequently mutable sites were revealed, A15326G (100%), G15301A (70%), G15043A (50%) and T14783C (50%). All the changes noted were nucleotide substitutions and neither deletion nor insertion was found. The frame-shifting mutation in this coding region would induce severe consequences which would not occur in healthy individuals [14].

In Table 1, the newly identified polymorphism site is bold. The reference stands for the Anderson sequence. The numbers on the top indicate the nucleotide position in the mitochondrial genome and dots (.) represent the matches with the reference sequence.

As previously reported [6, 7, 14], Transitions of nucleotide substitution were more common than transversion types with a transition:transversion ratio of 15:1. According to the mitochondrial genetic code, 8 variations out of 16 found in this study do not cause an amino acid change (synonymous polymorphism) and others encode different amino acid, non-synonymous. Table 2 shows the change of amino acid resulted from nucleotide substitution.

In this study, a total of 13 different haplotypes (genotypes) were observed. Anderson haplotype was not observed. The most similar sequence to original one was sequence type 11 that was different just in one nucleotide at position 15326 (A to G). Forensic parameters were calculated. Haplotype (genetic) diversity, the extent of variation in a given population, was calculated by $h = (1 - \Sigma xi^2)\ n / n-1$ where n is the sample size and xi is the frequency of i-th haplotype. In the present study the genetic diversity (h) was 0.9263. In addition genetic identity (p) was calculated by Σxi^2 [12]. Consequently, the probability of two unrelated individuals that were randomly selected sharing the same mtDNA haplotype was 0.12. Since the power of the discrimination using polymorphism within cyt b gene is lower than those of hyper variable regions (HVRs) in the D-loop [15, 16], this segment by itself is probably not adequate for routine forensic investigation.

CONCLUSION

To the best of our knowledge, this study was conducted as an initial effort to detect cytochrome b gene polymorphisms among unrelated Malay population in Malaysia. The diversity in the mitochondrial cytochrome b gene indicates the value of this locus for forensic casework. Although this gene can be used to increase the power of resolution in forensic casework, more samples must be analysed to investigate this usefulness in forensic applications.

Acknowledgment. I would like to thank Dr. Razauden Mohamed Zulkifli and Dr. Topik Hidayat for their advice and support. My thanks also go to Dr. Ali Mohammad Ahadi who encourage and guide me during this research. Thank you as well to University Technology Malaysia health Centre for their help.

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