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## Genetic polymorphisms in cytochrome C oxidase subunit I of the Malaysian population

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### ABSTRACT

*In the forensic science, genetic species identification based on DNA sequence differences and similarities will be used for crime survey. The mtDNA is much expanded for using as a marker in species and individual identification. Cytochrome C oxidase subunit I is a part of mtDNA with 1548 base pair length. There are three different subunits of cytochrome C oxidase, COI, COII and COIII. Between the three mitochondrial genes which are coding the cytochrome C oxidase subunits, COI is the largest and the most conserved between them. This is the first study on the COI gene here in Malaysia. Cytochrome C oxidase subunit I (COI) was studied to check the COI gene polymorphism in Malay population and investigation the usefulness of this gene as forensic marker. For this aim, the blood samples were collected from Malay people from different states. The PCR was done by using of extracted DNA from the blood and the COI gene was amplified with specific designated primers. Therefore, the desired region was revealed in the gel electrophoresis. The 13 nucleotide variations were observed within 1548 bp after sequencing of the samples and comparing with Anderson reference sequence.*

**Keywords:** cytochrome C, Malaysia, mtDNA, sequence polymorphism

### INTRODUCTION

Mitochondria are organelles which is producing energy inside the cells. Mitochondria have a separate genome from nucleus genome which is called mitochondrial DNA (mtDNA). There are several copies of mtDNA in each mitochondrion organelle in mammalian cells [1]. MtDNA is small and has high copy number inside the cell and it will be easier to isolate the DNA from this organelle therefore, the first genome sequencing project has been done on this molecule [2]. Human mitochondrial DNA is a double-stranded circular molecule present in 1000 to 10000 copies per cell. The complete nucleotide sequence of the 16569 base-pair (bp) molecule was determined in 1981[3]. In forensic sciences, genetic species identification based on DNA sequence differences and similarities will be used for crime investigation. The mtDNA is much expanded for using as a marker in species identification. Some of the genes are very common for using in the forensic sciences and also species identification such as cytochrome b [4,5] and also the most variable or hyper variable displacement loop region which is known as D-loop [6]. Some other genes also can be used as the forensic marker [7,8].

Cytochrome C oxidase subunit I DNA size is around 1548bp which has 70.2% total T content [9]. There are three different subunits of cytochrome C oxidase, COI, COII and COIII. Between the three mitochondrial genes which are coding for cytochrome oxidase subunits, COI is the largest one and the most conserved between them [10]. Based

on the survey about COI gene on some of the search engines such as Gene Bank BLAST and BOLD, it can be concluded that COI gene reliably identifies species where the references sequence data is present [11].

The COI gene already has been used in forensic entomology [12, 13] and also used as a marker for species identification of bird [14], fish [15], primates [16] and also some of the insects [17]. DNA barcoding using the mitochondrial cytochrome C oxidase subunit I is a reliable method for species identifying [11]. This gene is much conserved in all species and its rate of evolution is too slow. The two most commonly used genetic loci in species identification are the cytochrome C oxidase I gene (COI) and the cytochrome b gene (cyt b) [18].

MtDNA is used for forensic identification due to the high copy number of this molecule in the cell. The high copy number of the mtDNA allowed the scientists to extract more concentration of DNA in comparison with nuclear DNA [19]. In addition, mtDNA is usually used in phylogenetic studies due to Scientists believed that the substitution of nucleotide in the mtDNA has a constant rate through evolutionary time thus, they can estimate the divergence time by using of this feature [20].

In the present study a survey about cytochrome C oxidase subunit I (COI) has been done to evaluate COI as a marker for forensic identification on the Malay population in Malaysia.

In the Malaysia, this is the first study about genetic polymorphism based on COI gene sequence but there were some previous studies about hyper variable region (HV1 and HV2) polymorphism in Malay population. This study is aimed to investigate if the genetic polymorphism of COI gene in Malay population is good enough for using in forensic science or not.

## MATERIALS AND METHODS

**Population sample collection.** Malay donors were found from the UTM health center. The donors were from different status of Malaysia. The age of the donors was between 20 to 30 years old, due to the mtDNA will get more mutation after 30 years old in human.

**DNA extraction and amplification.** The genomic DNA molecule from each sample were extracted from blood using Promega genomic DNA purification kit A1120 following the manufacture specification. The DNA purity and concentration were assessed by spectrophotometry using nano- drop and also gel electrophoresis. DNA purity was determined by calculating the absorbance ratio A260/A280. Organic contamination was assessed by calculating the absorbance ratio A260/A230. The amplification of COI gene sequence was done by using of Biolab Taq PCR kit and appropriate designated primer (Table 1). The PCR was processed in a final volume of 50µl. The samples were primarily denaturated at 95°C for 30 seconds, following by 30 cycles of amplification (denaturation at 95°C for 25 sec, annealing at 43°C for 40 sec and extension at 68°C for 90 sec), and a final extension step at 68°C for 5 minutes. PCR products were gel eluted and purified (QIAGEN gel extraction kit) for sequencing according to the manufacture specification.

Table1. Primer sequence

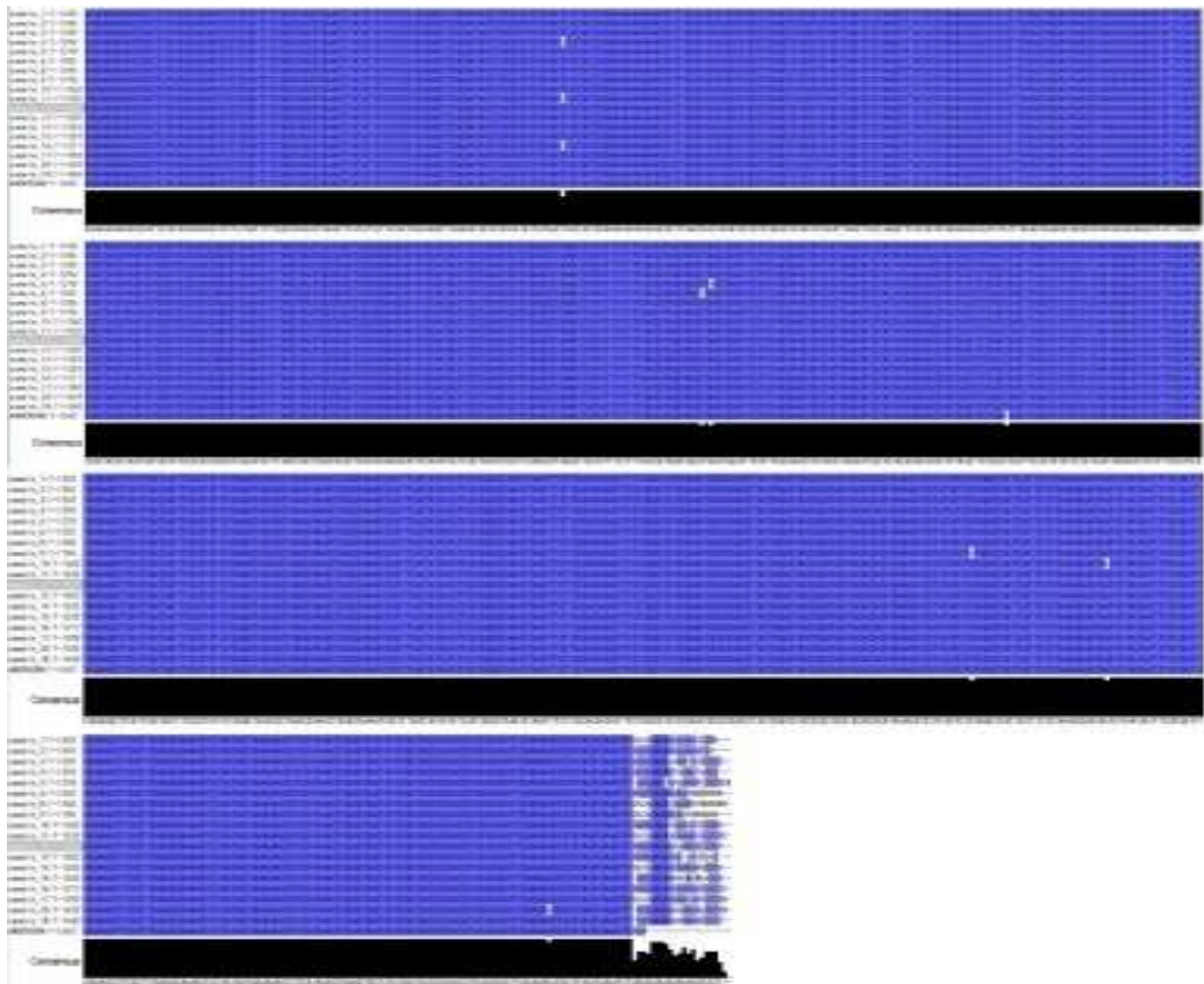
Primer	Sequence	Tm
Forward	TCCAATGCTTCACTCAGCC	57.5
Reverse	GGCTTGAAACCAGCTTGG	57.5

**Sequencing and bioinformatics analysis.** Automated DNA sequencing was carried out using dye terminator chemistries on an automated DNA sequencer (ABI PRISM 377), in one direction with the reverse primer. The sequences were alignment around position 350 to the end. JALVIEW software is used to find out the polymorphisms by comparing the sample sequence with the reference sequence. The nucleotide before position of 350 were not sequenced well due to we just used of only reverse primer, but this region was so big in size for sequencing only with one primer.

**RESULTS AND DISCUSSION**

In this study the nucleotide sequence of COI region (NT 5904-7445) was determined for unrelated individual of Malay population. The polymorphism of the sequence is reported as the differences between the sample sequence and the human standard sequence [2]. The human standard sequence was extracted from NCBI database which was used to align with the Anderson sequence. None of them were same as reference sequence. Totally 26 polymorphism and 7 polymorphic sites were observed in this area by comparing them with the reference sequence as they were illustrated in the figure 1. Among these 26 variations, there were 22(84.38%) variation between T and C and 4 variations (15.38%) between A and G. the most substitution between bases is from C to T with 19 occurrences. At position of 7029, all samples were changed from C to T based on reference sequence. Therefore, this position could be a critical position in the Malay population and more investigation is needed due to if this position is an SNP for Malay population only or not. This position may be used as a marker for this population.

They were 7 different haplotypes between all samples. Each polymorphism position was determined based on the reference sequence. Some of the polymorphism may change the amino acid which was produced from that codon. The changing of the amino acids due to the polymorphism was determined based on the number of the reference sequence and they were listed in table2. In two positions, the polymorphism changed the produced amino acids.



**Figure 1. Polymorphisms sites in JALVIEW**

*These sequences are the compared region and the region which were not sequenced well are not in the picture*

Most of the sequences variations of COI gene were synonymous polymorphisms, without changes amino acid of the translation products. As it mentioned in the table 2, at position 7270, valine is changed to isoleucine and at position 7299 isoleucine is changed to valine. These amino acids changing needs to investigate more based on biochemistry science. From the results it was revealed that the polymorphisms in the desired region were so low within the Malay population and it was so conserved.

**Table 2. Haplotypes and polymorphisms sites with the changed amino acids**

Haplotype	Base number	Nucleotide change	Amino acid change
HA1	7028	C → T	Ala → Ala
HA2	6681	T → C	Thr → Thr
	7028	C → T	Ala → Ala
HA3	6962	G → A	Leu → Leu
	7028	C → T	Ala → Ala
HA4	6962	C → T	Leu → Leu
	7028	C → T	Ala → Ala
HA5	7270	G → A	Val → Ile**
	7028	C → T	Ala → Ala
HA6	7299	A → G	Ile → Val**
	7028	C → T	Ala → Ala
		7424	A → G
HA7	7028	C → T	Ala → Ala

\*\* Site were the amino acid change

The genetic diversity of that gene must be calculated using  $h = n(1 - \sum x_i^2) / (n-1)$  formula [21]. A total of 7 different haplotypes were observed. The frequency of one of the haplotypes was 10 (HA1), other one was shared in 3 individuals (HA2) and each of other 5 haplotypes were observed in single individuals. Therefore the gene diversity of COI region was calculated so less (0.68). According to the gene diversity, the COI region was so low polymorphic; therefore the discrimination power of COI region is low.

In compare with other region such as HV1, HV2, HV3 and MTCYTB, COI region is not good enough for using in forensic purpose. Other regions had more polymorphic discrimination power and also gen diversity in compare with COI region in other population.

## CONCLUSION

The cytochrome C oxidase subunit I gene polymorphisms among unrelated individual of Malay population in Malaysia were examined. The study indicates the desired gene polymorphism was low for the aim of forensic science in compare with other genes such as D-loop however at the position of 7028 all samples showed the same polymorphism. Therefore, it is recommended more investigation is needed to see if this position can be used as an SNP for Malay population or not.

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