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HUMAN MITOCHONDRIAL DNA (mtDNA) TYPES IN MALAYSIA

Lian LH, Lim ME,* and Koh CL.
Institute of Biological Sciences (Genetics) and *Institute of Postgraduate Studies and Research, University of Malaya, 50603 Kuala Lumpur.
Tel: 03-5-7595969
Fax: 03-7595908
E-mail: mjklian@tm.net.my, kohcl@pc.jaring.my

ABSTRACT

Each human cell contains hundreds of mitochondria and thousands of double-stranded circular mtDNA. The delineation of human mtDNA variation and genetics over the past decade has provided unique and often startling insights into human evolution, degenerative diseases, and aging.

Each mtDNA of 16,569 base pairs, encodes 13 polypeptides essential to the enzymes of the mitochondrial energy generating pathway, plus the necessary tRNAs and rRNAs. The highly polymorphic noncoding D-(displacement) loop region, also called the control region, is approximately 1.2 kb long. It contains two well-characterized hypervariable (HV-) regions, HV1 and HV2. MtDNA identification is usually based on these sequence differences. According to the TWGDAM (Technical Working Group for DNA Analysis Methods), the minimum requirement for a mtDNA database for HV1 is from positions 16024 to 16365 and for HV2, from positions 00073 to 00340.

The targeted Malaysian population subgroups for this study were mainly the Malays, Chinese, Indians, and indigenous Ibans, Bidayuhs, Kadazan-Dusuns, and Bajaus.

Research methodologies undertaken included DNA extraction of samples from unrelated individuals, amplification of the specific regions via the polymerase chain reaction (PCR), and preparation of template DNA for sequencing by using an automated DNA sequencer.

Sufficient nucleotide sequence data were generated from the mtDNA analysis. When the sequences were analyzed, sequence variations were found to be caused by nucleotide substitutions, insertions, and deletions. Of the three causes of the sequence variations, nucleotide substitutions (86.1%) accounted for the vast majority of polymorphism. It is noted that transitions (83.5%) were predominant when compared to the significantly lower frequencies of transversions (2.6%). Insertions (0.9%) and deletions (13.0%) were rather rare and found only in HV2.

The data generated will also form the basis of a 'Malaysian DNA sequence database of mtDNA D-loop polymorphisms' for individual identification.

Keywords: mtDNA, sequence polymorphism, Malaysian, DNA sequencing.

The human mitochondrial DNA (mtDNA) is a double-stranded circular molecule present in 1,000-10,000 copies per cell. The complete nucleotide sequence of the 16,569 base-pair (bp) molecule was determined in 1981[1].

The mitochondrial genome can be divided into two sections: a large coding region, which is responsible for the production of various biological molecules involved in the process of energy production in the cell, and a smaller 1.2 kilobase pair fragment, called the control region.

The control region, also known as the D-(displacement) loop region, spans roughly from between the genes that encode proline and phenylalanine tRNAs respectively[2]. It is found to be highly polymorphic and harbors two hypervariable regions (HV), HV1 and HV2. According to the TWGDAM (Technical Working Group for DNA Analysis Methods), a group of forensic research laboratories in the United States that sets standards for DNA technology, the minimum sequence that will be accepted for the mtDNA database for HV1 is from positions 16024 to 16365, and for HV2, from positions 00073 to 00340[3].

The HV regions have been used extensively in practical forensic investigations, because mtDNA is stable during long storage, owing to various factors: its high copy number and circular form makes it less susceptible to exonuclease degradation. Because mtDNA is inherited strictly through the mother, as long as an individual shares maternal descent with a candidate sample source, he or she can be used to verify identity[3]. MtDNA also evolves 5-10 times faster than chromosomal DNA, and this relatively higher mutation rate gives rise to more polymorphic sites. Studies have also shown that the substitution rate in the control region is about 10 times higher than that in the remainder genome, hence the availability of the HV regions[4]. MtDNA sequencing has proven successful for a number of biological samples, including blood, blood-stains, bone, buccal cells, faeces, hair, nails, skin, semen stains, teeth, and urine.[5]

Apart from its usefulness in aiding forensic investigations, this technique of mtDNA sequencing has also been used for studies of human populations, human diversity, human evolution, genealogy, and looking into some mtDNA related diseases[6].

There have been numerous studies on Caucasian based populations[7,8,9,10] but still relatively few research work on Asian races[11,12,13]. The Malaysian population has already been studied in some previous work[14,15,16] but to date, there has not been an integrated study of the multi-ethnic groups of the Malaysian people.

This project aims to analyze the distribution and frequencies of the various polymorphic sequences in the Malaysian population. Various molecular biology and genetic techniques were performed: DNA extraction of blood and buccal samples from unrelated individuals. This is followed by amplification of the HV regions via the polymerase chain reaction (PCR), either direct or nested[3], and screening with agarose

gel electrophoresis. The PCR products were then gel-eluted, purified, and screened again before they were used as single- and double-stranded templates for DNA sequencing. Automated DNA sequencing was carried out by using the dye-primer as well as dye-terminator chemistries[17,18].

Overall, HV1 and HV2 are shown to be polymorphic in the Malaysian population. Nucleotide sequences from the 70 samples showed that a major percentage of the variations were caused by nucleotide substitution, i.e., transition and transversion. Insertions and deletions were also observed, though only in HV2. The incidence of transitions prevailed over transversions in both HV1 and HV2. Comparatively, HV1 displayed twice as much variability as HV2. Apart from the more common polymorphisms found in other human populations, some novel polymorphisms were also noted in both regions.

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