

Mitochondrial Genome Mutation Analysis: Indonesian Human mtG Comparison and Several GenBank Sequence Data on Gene Control and Encoding Regions

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Abstract

Comparative study of DNA mutations occurring in human mitochondrial genomes in Indonesian humans and its comparison with some ethnic worlds has been done. The purpose of this study was to analyze mutant variants in all the complete human genome mitochondrial areas by using G-repliant techniques for mitochondrial genomic amplification, the result of Indonesian human nucleotide sequencing was then compared against some individuals representing some ethnicities in the world. DNA samples were isolated from human tissue and then sequenced using 10 pairs of primers to amplify human mtG. The mtG sequence is aligned and compared with rCRS using the DNASTar program. The result of mutation analysis shows the presence of point mutation in some mtG region fragments with different mutation proportions. Most mutations outside the HVS1 and HVS2 D-loops are in the ATP6 region. The encoding region of ATP6 is the gene coding region of human mtG and shows a high mutation rate of CRS. This opens a new paradigm for mutation analysis on ATP6 areas other than the mtG D-loop. The ATP6 gene segment located at 8553-8902 can be selected for studies in population genetics, forensic medicine and bioethnoanthropology studies, in addition to the HVS1/HVS2 D-loop areas that have been used.

Keywords: MtG genome; Variant; mtDNA; Mutation; Indonesian; Ethnic groups

Introduction

Mitochondria are eukaryotic cell organelles that play a role in producing energy in the form of adenosine triphosphate (ATP) compounds through highly efficient oxidative phosphorylation reactions [1]. Mitochondrial DNA (mtDNA) is only inherited according to the maternal lineage because the mitochondrial content in the egg is much higher than the mitochondrial content in sperm cells. Egg cells contain more than 100-1,500 mitochondrial copy number whereas sperm cells contain only 10 copies of mitochondria, so that when fertilization occurs, maternal mitochondria are much more dominant [2,3].

In contrast to other cell organelles, mitochondria have their own genetic material that is characteristically different from the genetic material in the cell nucleus. The uniqueness of mitochondrial DNA is, among other things, the higher mutation rate, which is 10-17 times the nuclear DNA [4]. In addition mitochondrial DNA is present in large quantities (over 1000 copies) in each cell, whereas nuclear DNA is only two copies [5]. Core DNA is the result of DNA recombination of both parents, whereas mitochondrial DNA is only inherited from the mother (maternal inherited) [6]. Mutations occurring in the mitochondrial genome (mtG) can also increase intracellular ROS production, so that free radical production decreases mitochondrial function and will increase oxidation accumulation in various tissues

[7]. Pathogenic mutations generally occur in heteroplasmic forms, the ratio of the number of mutant mtDNA mutants to wild-type mtDNAs varying in each tissue, with low heteroplasm levels in blood cells (leukocytes) and high within the postmitotic tissue [8].

The order and organizational systems of human mitochondrial genomes were first published by Anderson and colleagues [9]. The mtDNA structure is circular and consists of 16,569 base pairs (pb). Several of the human mitochondrial DNA research results with its distinctive properties to be exploited in various disciplines, including the study of evolution, population genetics, bioinformatics, genetic diseases, and forensic medicine [10,11]. The results of mtDNA research include, among other things, the nature of the hypothalamity that has been associated with the sequence and mutation of nucleotide mtDNA among individuals, ethnicity and age [1,12,13].

A study to uncover the nucleotide sequences of the human mitochondrial genomes of Indonesia comparing the mtG of some individuals representing ethnicities in the world in NCBI data. The mutant variant analysis was performed by comparing it with the revised Cambridge Reference Sequence (rCRS) sequence in each mtG region. By knowing the comparison between mutations of each individual mtG position, in the future this mtDNA research can be utilized in the field of forensics and bioethnoanthropology and can be directed to studies of human origins and early human migration patterns.

Materials and Methods

Sample preparation and mtDNA amplification process

Forensic samples are human renal tissue derived from the mesoderm layer, the kidneys. Samples were stored in a 1.5 mL eppendorf tube in freezing (-20°C). The sample was obtained from the Department of Forensic Science and Medicolegal (IKFM), Hasan Sadikin Hospital, Bandung/Faculty of Medicine, Padjadjaran University, Bandung, Indonesia.

The mitochondrial DNA isolation procedure of human tissue follows the Purification from Tissues DNA protocol (QIAamp DNA Mini Kit). The tissue sample was weighed using an analytical balance of 25 mg (1 mg of tissue equivalent to 0.2-1.2 µg DNA) [14]. The *in vitro* repli-G amplification procedure is by taking 5 µL isolated DNA template using QIAamp DNA Mini Kit [14,15]. Amplification of mtDNA with PCR in this research using machine GeneAmpPCR system 2400 (Perkin Elmer). Primer for amplification is designed using Perlprimer program to facilitate simulation of annealing temperature determination. The mitochondrial genome template was prepared using PCR repli-G method. The genome template is divided into ten mtDNA fragments (A, B, C, D, E, F, G, H, I, and M) by amplifying the genome template using the corresponding primer pair. Primer pair data for mtG amplification is redesigned to be more efficient.

The amplification reaction of ten fragments is catalyzed by the DreamTaq green PCR Mastermix enzyme containing DreamTaq DNA Polymerase. Mastermix is added with two tracking dyes and reagent that serves as direct loading for electrophoresis, so that when electrophoresis does not need to be added loading dye. The PCR process was performed with an Automatic thermal cycler (Perkin Elmer) engine of 30 cycles. The initial stage of the PCR process is the initial denaturation stage carried out at 95°C for 3 min, then into the PCR cycle program with each cycle consisting of 3 stages ie denaturation stage carried out at 95°C for 30 sec, annealing stage conducted at 50 °C for 30 sec and extension or polymerization step at 72°C for 60 sec. The end of all cycles carried additional polymerization process at 72°C for 10 min [16,17]. The PCR product was detected by electrophoresis on 1.2% (b/v) agarose gel by using a mini subTM DNA electrophoresis cell. The electrophoresis process is carried out in TAE 1 x buffer as a current conductor medium at 75 volt for 45 min. The electrophoresis results were visualized with UV lamp series 9814-312 nm (Cole Parmer). For the determination of the nucleotide sequence mtG is used PCR primers and some internal primers (Table 1).

Name of Primer	Position of mtG (5' → 3')	Sequence of Olinucleotide
Afor	458-479	CCTCCCACTCCCATACTAACTA A
Arev	2491-2473	GGGGTAAGATTGCCGAGT
2F	1138-1156	GAACACTACGAGCCACAGC
Bfor	2324-2341	TTCTCCTCCGCATAAGCC
Brev	4252-4234	GGGGAATGCTGGAGATTGT
5F	2995-3013	GGATCAGGACATCCCGATG
Cfor	4189-4215	CCACTCACCCCTAGCATT

8F	4832-4849	CACCCCTCTGACATCCGG
Xin	5530-5550	CAGACCAAGAGCCTTCAAAGC
Dfor	6046-6055	GGCAACCTTCTAGGTAACGA
Drev	8095-8076	TAAGCCTAATGTGGGGACAG
11Fg	6730-6749	GATCACAGGTCTATCACCCCTA
Efor	7925-7944	GGCGGACTAATCTTCAACTC
Erev	9916-9899	GCTTCGAAGCCAAAGTGA
Dmt2L	8251-8270	GCCCGTATTACCCCTATAGC
Ffor	9752-9770	CGAGCTTCCCTCACCATT
Frev	11774-11757	TGTGAGTGCCTTCGTAGT
17F	10394-10414	CTGAACCGAATTGGTATATAG
Gfor	11624-11644	TCTTCAATCAGCCACATAGCC
Grev	13639-13616	GTTGACCTGTTAGGGTGAGAA G
FBr	12048-12065	TCACACGAGAAAACACCC
Hfor	13551-13568	CGCCTGAGCCCTATCTAT
Hrev	15434-15417	GGGCGTCTTTGATTGTGT
23F	14227-14246	CCCATAATCATACAAAGCCC
Ifor	15311-15328	ATTGCAGCCCTAGCAACA
Irev	824-807	ATCACTGCTGTTTCCCGT
Mfor	15978-15997	CACCATTAGCACCCAAAGCT
Mrev	429-409	CTGTTAAAAGTGCATACCGCC

Table 1: Forward, reverse, and internal primers used for amplification and sequencing of fragments in human mitochondrial genome (mtG).

MtDNA sequencing and mtDNA mutation analysis

Multiple juxtaposed nucleotide sequences complete human mtG were analyzed with the help of DNASTAR program software. The mtG analysis was performed using the EditSeq, Seqman, and MegAlign programs [18,19]. Determination of variation of nucleotide mutation was done by using Human mtDNA Analyzer version 1.2 and comparing with MITOMAP. While the comparison of mutations between individuals in various ethnic in the world using Megalign program against NCBI data.

Results and Discussion

The DNA amplification result was performed by using repliG technique to amplify ten mtG fragments: fragments A, B, C, D, E, F, G, H, I, and M. The results of these amplifications produce bright and consistent DNA bands for each PCR result. The PCR process using G-repli can minimize DNA contamination during isolation and PCR processes. Electrophoresis results of human DNA samples that have been successfully diluted PCR, then electrophoresis for kidney tissue. The electrophoresis results show that the entire fragment is represented by bright bands of the appropriate size. A fragment size of 2023 bp, fragment B of 1929 bp, fragment C of 2052 bp, fragment D of 2050 bp,

fragment E of 1992 bp, fragment F of 2023 bp, G fragment by 2016 bp, fragment H of 1884 bp, Fragment I of 2066 bp, and the M fragment of 982 bp (Figure 1 and 2).

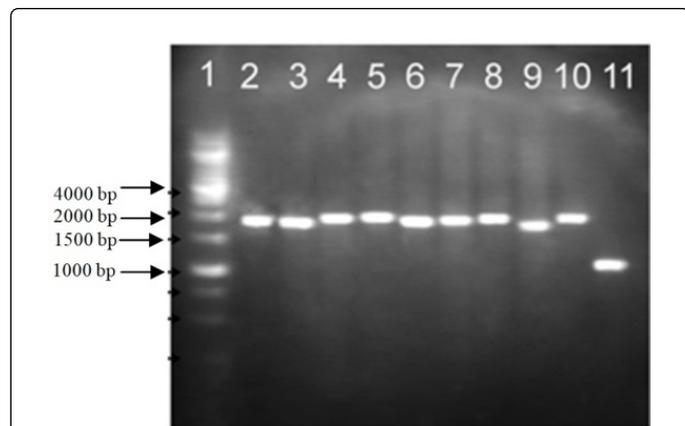


Figure 1: Characterization of the mitochondrial genome in ten fragments on 2% agarose gel using 1 kb ladder marker (lane 1). Lane 2: fragment A (2,023 bp), lane 3: fragment B (1,929 bp), lane 4: fragment C (2,052 bp), lane 5: D fragment (2,050 bp), lane 6: fragment E (1,992 bp), lane 7: fragment F (2,023 bp), lane 8: G fragment (2,016 bp), lane 9: H fragment (1,884 bp), lane 10: I fragment (2,066 bp), and lane 11: M fragment (982 bp).

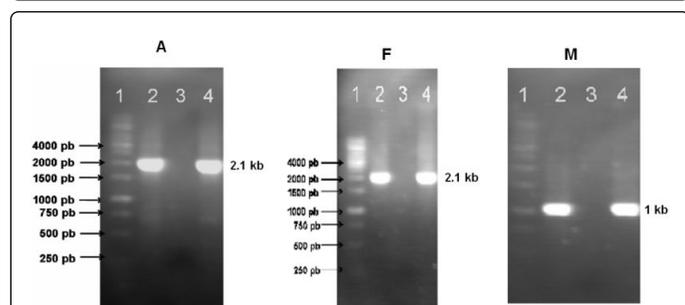


Figure 2: Several PCR genomic mtG results: fragments A, F, and M. Amplification of fragments in the sample using each primer pair. Lane 1: Marker 1 kb ladder, lane 2 is control (+), lane 3 is control (-), and lane 4 is a mtDNA sample PCR product.

The human nucleotide sequence data from the network analyzed using DNASTAR is in the form of an editseq and an ABI file. The sequence data were then analyzed homology by sequence of rCRS nucleotides using DNASTAR program ie Megalign and Seqman, ie to determine the presence of mtG nucleotide mutations. The results of the overall mtG mutation study concluded that mutations occurring in humans are generally transition nucleotide transition mutations [20,21]. Substitution mutations occur when there is a substitution of the nucleotide base to another nucleotide without changing the length of the DNA. This mutation occurs only in the nucleotide position. The causes of transition nucleotide mutations include nitric acid, base-fixing, and mutagenic base analogs [22]. Based on the results of the analysis, the substitution mutation can be restored under normal circumstances by transition or reverse transition, hence the substitution effect is not permanent.

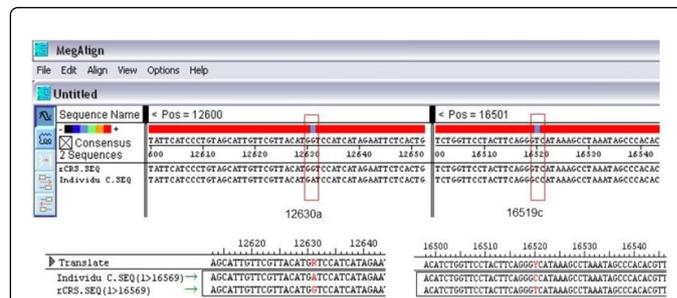


Figure 3: Results of human mtDNA alignment with rCRS. One example of mtG secondary data analysis at positions 12630 and 16519, where there are successive changes G → A and T → C in Individual C. The individual sequences C of GenBank are analyzed in silico using the Megalign program against rCRS.

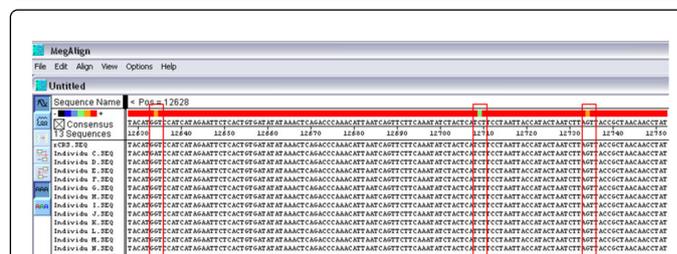


Figure 4: Analysis of mtG secondary data processing for individuals derived from GenBank. C-N individual sequences from GenBank are analyzed in silico using DNASTAR, Megalign and Seqman programs compared with rCRS.

The nucleotide data from the analysis revealed 32 mutation mutations to rCRS. The result of mutation observation showed that mitochondrial DNA mutation rate was different from one individual to another. This is because during the human life cycle there is an accumulation of mtDNA mutations in somatic cells, and an increase in the amount of mtDNA that carries mutations in the tissues is an important factor of the aging process [23,24]. The high mtDNA mutation is due to the high levels of reactive oxygen species as a byproduct of mitochondrial oxidative metabolism and the absence of an effective DNA repair system within the cell organelle. The accumulation of mtDNAs that carry mutations affects the metabolic capacity of the tissue oxidative energy, so this capacity will decrease with age. The effect of this proposed decline will be manifested especially in tissues that require a lot of ATP for its function, and is seen as decreased ability of the heart muscle and brain function. Many of the mtG mutation data associated with the disease as well as comparison between ethnic groups have been incorporated into the MITOMAP database.

The results showed that the variants of human mitochondrial DNA mutations were different from one individual to another as occurred in the number and position of mutations in both individuals A and individual B as well as in individual C-N comparisons. This is because the mtDNA mutation is derived only in maternal lineage, whereas the individuals analyzed are of different offspring (individual A and individual B) as well as various ethnic (individual C-N). Different mutant identity differences between individuals not in line with the mother's offspring are caused by factors such as: high levels of reactive

oxygen species as a byproduct of mitochondrial oxidative metabolism; the open exposure of mtDNA to the reactive oxygen due to the absence of protection by the nucleoproteins, different from those in the cell nucleus; the absence of an effective DNA repair system within the cell organelle. The accumulation of mtDNAs that carry mutations affects the metabolism capacity of the tissue oxidative energy, so this capacity will decrease with age [25].

After the human mtG nucleotide variation database on rCRS has been prepared, the next step is to analyze the size of the human mtG nucleotide. The analyzed mtG size ranged from 16558 -16574 bp from 301 genBank samples. The largest mtG size corresponds to the length of base pairs proposed first by Anderson and his colleagues, ie 16569 bp, of 113 samples. The highest number of subsequent pairs, 16570 bp (97 samples), 16571 bp (44 samples), 16572 bp (10 samples), 16574 bp (3 samples), 16573 bp (2 samples), 16558 bp and 16559 bp (1 sample each). This is done to observe the rate of mutations in the nucleotide sequences of each human mtG, as it is well known that the rate of mutations occurring in mtG varies by individual not in line with maternal progeny [26,27].

The number of nucleotide variants (mutation to rCRS) in each sample turned out to be between 12 (the smallest number of mutations) up to 42 (the largest number of mutations) out of a total of 301 human mtG samples derived from GenBank. The highest number of mutations were 15 variants of mutations found in 42 individuals or 13.95%. The five most subsequent mutations were 17 mutant variants (39 individuals or 12.95%), 16 mutation variations (37 individuals or 12.29%), 18 mutation variations (36 individuals or 11.96%), 14 mutation variations (20 individuals or 6.64%), and 19 mutation variants (18 individuals or 5.96%).

While the number of individuals GenBank data who experienced the least mutation, ie 1 individual or 0.33% is the number of variants 26, 27, 31, 32, 34, 37, 40, and 42. Data in Figures 3 and 4, shows that the number of nucleotide variants found in mtG GenBank samples is less than one percent of all 16.5 kb mtG. The most variant mutant variant data was 42 variants or only 0.25% of the total human mtG nucleotide. The MITOMAP database provides a variety of fairly informative data, such as reported mutations, mutations related to mutations in mitochondrial DNA, researchers reporting, data relating to the origin of the sample (ethnicity), and other information. Information on mtDNA mutations related to disease is very useful for medical medicine because it facilitates tracing information about mutations associated with mitochondrial disease. This is of course very important for the future revolution of medicine because it relates to beautiful medicine.

Conclusion

The use of the repli-G method is very effective in amplifying the human mitochondrial genome in all gene encoding and non-gene encoding regions. The result of mutation analysis shows the presence of point mutation in some mtG region fragments with different mutation proportions. Most mutations outside the HVS1 and HVS2 D-loops are in the ATP6 region. The ATP6 region is the gene coding region of human mtG and shows a high mutation rate for rCRS. This opens a new paradigm for mutation analysis on ATP6 areas other than the mtG D-loop. The ATP6 gene segment located at 8553-8902 can be selected for studies in population genetics, forensic medicine and bioethnology studies, in addition to the HVS1/HVS2 D-loop areas that have been used.

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