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Single nucleotide mutations of intergenic and intragenic region in mitochondrial genome from different individuals from Papua-Indonesia

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ABSTRACT

Studies and comparative analysis of DNA mutations that occur in the human mitochondrial genome in humans of Indonesia, the ethnic Papua and its comparison with several ethnic world has done. The purpose of this study to analyzed variants of the mutation in some areas on intragenic and coding region between genes in human mitochondrial genes and gene control regions using mitochondrial genome amplification techniques. Here, we reported the results of sequencing the human nucleotide Papua, which was then compared against several individuals representing several ethnic groups in the world. DNA samples isolated from several human tissues and then sequenced using an efficient two pairs of primers to amplify the mtG humans. mtG sequences are aligned and compared with rCRS using DNAstar program. Results of analysis reveals the fact that there are mutations outside the region HVS1 and HVS2 D-loop mtDNA ie in regions between genes. Regions between genes is the gene coding region of the human mtG and exhibit a high mutation rate against rCRS. This opens up a new paradigm for the analysis of mutations in regions intragene, intergene, and the coding regions of genes other than the D-loop mtG. Segment intragene gene located on the shorted area can be selected for studies in population genetics, forensic medicine and bioetnoantropology studies, in addition to the area HVS1/ HVS2 D-loop that has been used. Studies in humans have to complete the data Papua mutation and polymorphism data GenBank/NCBI and other nucleotide sequence data providers such as EMBL and DDBJ.

INTRODUCTION

Mitochondria are organelles of eukaryotic cells that contribute producing energy in the form of the compound adenosine triphosphate (ATP) through oxidative phosphorylation reactions were very efficient [1]. Mitochondrial DNA (mtDNA) is passed down only matrilineal because the content of mitochondria in an egg cell is much higher than the content of mitochondria in sperm cells. The egg cell contains more than 10^5 copies of mitochondria while sperm cells only contain mitochondria 100-1500 copies, so that when conception occurs, the mitochondrial mother is much more dominating [2-3].

In contrast to other cell organelles, the mitochondria have their own genetic material whose characteristics are different from the genetic material in the cell nucleus. The uniqueness of the mitochondrial DNA among other things a higher mutation rate, which is 10-17 times the nuclear DNA [4]. In addition mitochondrial DNA present in large quantities (over 1000 copies) in each cell, whereas nuclear DNA amounted to only two copies [5]. DNA recombination DNA core is the result of both parents, whereas mitochondrial DNA is inherited only from the mother (maternal inherited) [6]. Mutations that occur in the genomes of mitochondria (the mitochondrial genome/mtG) also can increase the production of intracellular ROS, thus the production of free radicals decreased the mitochondrial function and will increase the accumulation of oxidation processes in various tissues [7]. Pathogenic mutations generally occur in the form heteroplasmy, is the ratio of the number of mutant mtDNA against

wild-type mtDNA which vary in each tissue, with a low heteroplasmy levels in blood cells (leukocytes) and high in the tissue after mitosis [8].

Sequences and system of organization of human mitochondrial genome was first published by Anderson and colleagues [9]. Circular mtDNA structure and consists of 16,569 bp. A lot of human mitochondrial DNA research results to its properties characteristic to be utilized in a variety of disciplines, including the study of evolution, population genetics, bioinformatics, genetic diseases, and the science of forensic medicine [10-11]. The research results were related mtDNA hypervariability properties that have been linked with a ratio of mtDNA sequences and nucleotide mutations between individuals, ethnic/tribal and ages [1, 12-13].

Research to reveal the nucleotide sequence of the Indonesian human mitochondrial genome, especially Papuan humankind that compared with individuals representing mtG several ethnicities in the world at the NCBI data. Analysis of variance mutation is done by comparing the sequence of revised-Cambridge Reference Sequence (rCRS) in every region of mtG. By knowing the comparison between mutations in each position of mtG between individuals, in the future mtDNA research can be used in forensic and bioethnoantropology and could be directed to studies of human origins and patterns of human migration.

MATERIALS AND METHODS

Forensic samples such as human blood tissue derived from mesoderm layer. Samples are stored in a 1.5 mL eppendorf tube in a frozen state (-20 °C). Samples were obtained from the Laboratory of Biochemistry, Department of Chemistry-Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura, Indonesia. The amplification reaction in the two fragment, coding and control genes in the fragments is catalyzed by the enzyme *DreamTaq green PCR Mastermix* containing *DreamTaq DNA Polymerase*. Mastermix was added with two tracking dyes and reagents ballast that serves as direct loading to electrophoresis, so that when electrophoresis is not necessary to add loading dye. The PCR process is done by machine Automatic thermal cycler (Perkin Elmer) by 30 cycles. The initial stage of the PCR process is the stage of initial denaturation at 95 °C for 3 min, then go to programs PCR cycles with each cycle comprising three phases: denaturation carried out at a temperature of 95 °C for 30 sec, annealing stage is performed at a temperature of 50 °C for 30 sec and extention or polymerization stage at a temperature of 72 °C for 60 sec. The end of all cycles carried out additional polymerization process at a temperature of 72 °C for 10 min [14-17].

PCR products were detected by electrophoresis on agarose gel 1.0% (w/v) by using the *mini sub^{TM} DNA electrophoresis cell*. Electrophoresis process is carried out in 1 x TAE buffer as a medium conductor current at a voltage of 75 volts for 45 min. The results of electrophoresis visualized with UV lamp series 9814-312 nm (Cole Parmer). To determine the nucleotide sequence of mtG is used PCR primers and multiple internal primer [15].

Alignment of multiple nucleotide sequence complete mtG human was analyzed with the assistance a software program DNAstar. mtG analyzes were performed using the *EditSeq*, *Seqman*, and *MegAlign* [18-19]. Determination of nucleotide mutations variation is done by using various applications and compares with MITOMAP to determine specific mutations. While comparisons between individual mutations on the various ethnic groups in the world using the application of *Megalign* on NCBI/GenBank data.

RESULTS AND DISCUSSION

The results of DNA amplification in human samples of Papua done using amplification techniques in mtG fragments: the fragments I and M (Fig. 1). Results amplification produces DNA bands bright and consistent for each PCR. Aplifikasi PCR process using specific and efficient in order to minimize contamination during the process of DNA isolation and PCR.

The results of electrophoresis of human DNA samples that have been successfully performed via PCR, electrophoresis and then to the blood system. Electrophoresis results showed that the overall fragment shown with bands (band) is bright with the appropriate size. Fragment I, sized of 2066, and fragment M of 982 bp.

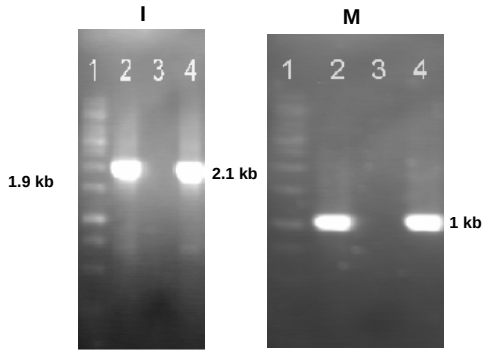

Fig 1. **The results of genomic PCR mtG in Papuan human samples for fragments I and M. Amplification fragments in a sample using each primer pair. Lane 1: Marker 1 kb ladder, lane 2 is the control (+), lane 3 is the control (-), and lane 4 is a sample of mtDNA PCR product fragment I (*coding genes*) and the fragment M (*Control genes*).**

Data from the human nucleotide sequences were analyzed using DNASTAR program by in the form *editseq* and *ABI file*. The data are then analyzed sequence homology with the nucleotide sequence rCRS using the program DNAstar *Megalign* and *Seqman*, which is to determine their mtG nucleotide mutations. mtG mutation study results overall concluded that mutations in humans generally are nucleotide substitution mutation transition [20-21]. Substitution mutations occur if there is a base substitution nucleotide to another without changing the length of DNA. These mutations occur only in the nucleotide positions. The cause of the transition at nucleotide mutations of which are nitric acid, base pairing errors, and analog bases mutagenic [22]. Based on the analysis, substitution mutation could be restored to normal by the process of transition or transversion reverse, hence the substitution effect is not permanent.

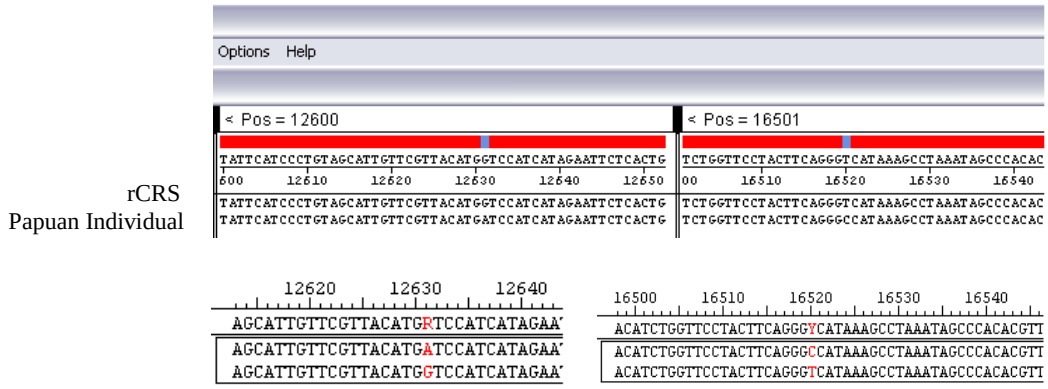

Fig 2. Result of alignment of human mtDNA with rCRS. One example of data analysis mtG at intergene region at position 12,630 and gene control regions, 16,519, where there is a change consecutive G→a and T→c in C. Papuan Individual human sequences analyzed *in silico* using *Megalign* program on rCRS ,

Data nucleotide analysis results in two fragments found 32 mutations to rCRS. Observations indicated that mutations of mitochondrial DNA mutation rates differed among the individuals with other individuals. But for one individual still showed high homology. This is because during the human life cycle in the accumulation of mtDNA mutations in somatic cells, and an increased number of mutations in the mtDNA that carry the tissues is a critical factor aging process [22-25]. The high mtDNA mutations caused by high levels of reactive oxygen species as byproducts of mitochondrial oxidative metabolism and absence of effective DNA repair systems in the cell organelles. The accumulation of mtDNA mutations will affect the carrying capacity of the network oxidative energy metabolism, so that this capacity will drop with age. The effect of this reduction is proposed to be manifested mainly in a tissue that requires a lot of ATP for its function, and is seen as a decreased ability of the heart muscle and brain function. Several of the data mtG mutation associated with the disease as well as comparisons between ethnic groups have been incorporated into the database MITOMAP.

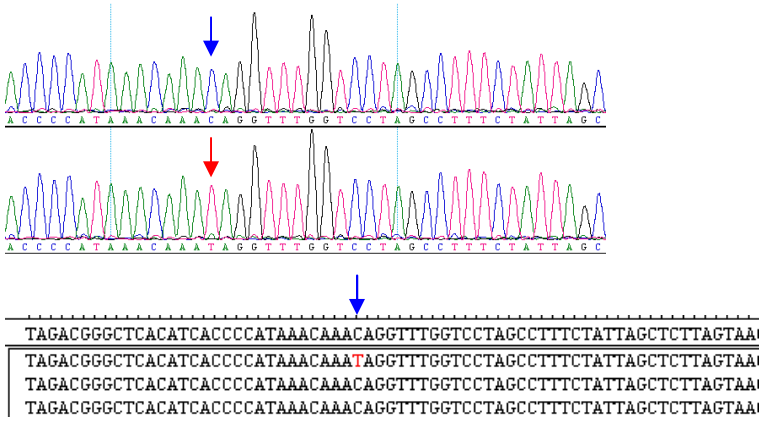

Fig 3. The result of the alignment of Papuan human mtDNA with rCRS. One example of data analysis mtG on the region between genes *in silico* using Seqman program on rCRS.

The results showed that variants of human mitochondrial DNA mutations turned out to be different from one individual to another individual as happens on the number and position of both individual human mutations of Papua and comparison of GenBank individual data. This is because mtDNA mutations derived simply lysed maternal lineage, while individuals were analyzed come from different offspring (individual data GenBank). The occurrence of mutation variants identity differences between individuals who are not aligned maternal lineage is caused by factors such as: high levels of reactive oxygen species as byproducts of mitochondrial oxidative metabolism; openly mtDNA exposure to these reactive oxygen due to the lack of protection by the nucleoprotein, unlike in the cell nucleus; the absence of effective DNA repair systems in the cell organelles. The accumulation of mtDNA mutations will affect the carrying capacity of the network oxidative energy metabolism, so that this capacity will drop with age [26].

The results of further analysis, helped by the program Seqman compared with a normal variant, showed no mutations at certain positions (as they appear in the database rCRS). This was done to prove the identity of mutations convincingly that includes the position and type of mutation that occurs, as shown in the following figure (Fig. 4). Comparison of the data sequence mutation analysis results using *Seqman* and verified using *MeqAlign* also showed the same pattern of mutations that occur nucleotide changes from T (thymine) to C (cytosine).

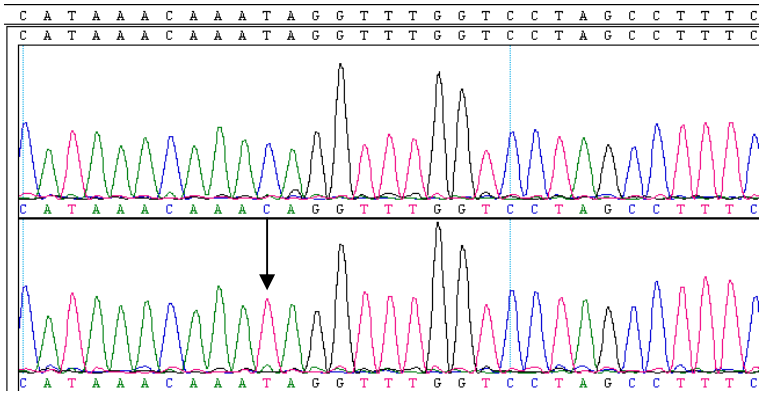

Fig 4. The results of the analysis using DNASTAR Seqman program between data sequences of a human tissue in Papua and one sample of normal variants. In the figure looks no change become thymine bases cytosine in a certain order rCRS.

After analyzing the data bases of nucleotide variation Papua human mtG on intragene area, between genes and coding regions of the genes of rCRS completed, the next step is to analyze the size of the human nucleotide mtG. mtG analyzed size ranges between 16,558-16,574 bp of the 100 samples GenBank. This was done to observe the rate of mutations in the nucleotide sequence of the human mtG each individual, because it is known beforehand that the level of mutations that occur in mtG different for each individual who is not aligned maternal lineage [18, 25].

The number of nucleotide variants (mutation on rCRS) on each sample turned out to range between 12 (the number of mutations smallest) up to 40 (the largest number of mutations) of the total sample of mtG human derived from GenBank. The highest number of mutations are mutations found 15 variants as much as 13.95%. The next highest number of mutations five consecutive 17 mutation variants (12.95%), 16 variants of the mutation (12.29%), 18 variants of the mutation (11.96%), 14 variants of the mutation (6.64%), and 19 variant mutation (5.96%).

The number of individuals who have mutations of data GenBank fewest, ie 0.33% is the number of variants of 26, 27, 31,32, 34, 37, 40, and 42. The data mutation analysis results showed that the amount of variant nucleotides found in GenBank mtG samples with less than one percent of the entire 16.5 kb mtG. The number of variants of the mutation most was 42 variant or only 0.25% of the total nucleotides mtG human.

The database MITOMAP provide a variety of data that is informative, such mutations have been reported, the diseases associated with mutations in the mitochondrial DNA, the researchers reported, the data relating to the origin of the sample (ethnicity), and other information. Information on mtDNA mutations associated with the disease are very useful for the medical world makes it easy to search for medical information on mutations associated with mitochondrial diseases. It is of course very important for the world revolution for future medical-related beautique medicine [23-25].

CONCLUSION

The results of mutation analysis showed a point mutation in some region of mtG fragment with the proportion of different mutations. Most mutations outside the region HVS1 and HVS2 D-loop is in the area intragene, intergene, and the main coding region of the mtG human. Intergene area is an coding region of mtG genes in humans and show a high mutation rate on rCRS. This opens up a new paradigm for the analysis of mutations in regions other than the D-loop MTG. Segment intergene located at 8000s nucleotides of mtG region could be selected for studies in population genetics, forensic medicine and bioetnoantropology studies, in addition to the area HVS1/HVS2 D-loop that has been used.

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