# NUCLEOTIDE VARIANCE OF MITOCHONDRIAL DNA D-Loop 126 bp (nt: 34-159) REGION IN MADURESE

**Nilly Sulistyorini,[1] Ahmad Yudianto,[1,2,3] Nola Margaret[1]**
[1]Department of Forensic Medicine & Medicolegal, Faculty of Medicine
[2]Human study group Institute Tropical Disease
[3]Master Program in Forensics, School of Postgraduate Studies, Universitas Airlangga

## ABSTRAK

*Perkawinan dalam masyarakat di pulau madura pada daerah pelosok terutama daerah kepulauan terkecil madura masih terjadi antara kalangan mereka sendiri (endogami). Perkawinan endogami dilihat dari sudut pandang genetik akan meningkatkan frekuensi geneotip homosigot. Dalam variasi genetik pemeriksaan yang sering dipergunakan yakni STR pada DNA inti dan polymorphism pada mtDNA. Variasi mtDNA dalam populasi manusia mengalami evolusi, proses ini melalui akumulasi perubahan urutan DNA, yaitu melalui proses substitusi nukleotida yang berkembang jumlahnya seiring perkembangan arah garis keturunan. Sejauh ini variasi genetik populasi pulau madura belum banyak diketahui. Jenis penelitian ini adalah observasional analitik, untuk mengetahui variasi genetik pada polymorphism D-Loop mtDNA HV2 126bp (nt: 34-159) pada populasi pulau madura. Hasil penelitian ini menunjukkan, dari analisis homologi dengan urutan rCRS menunjukkan adanya 9 varian yang terdiri atas 2 mutasi transisi, 6 mutasi transversi, dan 1 mutasi insersi. Ini mengindikasikan bahwa jumlah mutasi tranversi lebih besar kemungkinannya dari mutasi transisi dan insersi. Menurut Mustama (2007) menyatakan bahwa gene pool bukan hanya merupakan suatu kumpulan gen tetapi merupakan suatu sistem dinamis yang terorganisir dan memuat sejarah masa lalu dari suatu populasi. Setiap informasi genetik mempunyai aspek sejarah, antropologi dan statistik tertentu sehingga diperlukan koordinasi dan kolaborasi dari berbagai disiplin ilmu.**(FMI 2016;52:80-86)***

***Kata kunci:*** *Varian D-Loop, etnik madura*

## ABSTRACT

*Endogamy continues to occur among the Madurese people in rural areas of the island of Madura, especially those areas of the smallest islands around the mainland of Madura. Endogamy as seen from a genetic standpoint will increase the frequency of homozygous genotypes. With regard to genetic variations, STRs of nuclear DNA and polymorphisms in mtDNA are frequently examined. Mitochondrial variations in the human undergo an evolutionary process through the accumulation of changes in DNA sequence, i.e. through the process of nucleotide substitutions that evolves in number with the directional development of lineage. So far, the genetic variations among the populations in Madura Island have not been known. The present study was an observational analytical research with the purpose of determining the genetic variations in the polymorphisms of 126-bp mtDNA D-Loop HV2 (nt: 34-159) in the populations of Madura Island. Results indicated that, based on the homology analysis with rCRS sequence, there were 9 variants consisting of two transition mutations, 6 transversion mutations, and one insertion mutation. This indicates that a transversion mutation had a higher probability than transition and insertion mutations. According to Mustama (2007), a gene pool is not only a collection of genes but a dynamic system organized and containing the past history of a population. Any genetic information has certain historical, anthropological and statistical aspects necessitating an interdisciplinary coordination and collaboration.* ***(FMI 2016;52:80-86)***

***Keywords:*** *D-Loop variants, Madurese ethnic*

**Correspondence:** Ahmad Yudianto, Department of Forensic Medicine & Medicolegal, Faculty of Medicine, Universitas Airlangga. Email: yudi4n6sby@yahoo.co.id

## INTRODUCTION

Madura Island is the name of the island which is located to the north of East Java. Madura Island is the amount of approximately 5,250 km$^2$ (smaller than the island of Bali), with a population of 4 million. Madura is divided into four districts, Bangkalan, Sampang, Pamekasan, and Sumenep.

Madurese communities have a distinctive culture, unique, stereotypical, and stigmatic. Marriage in the community on the island of Madura in remote areas, especially the area's smallest island of Madura still occur among their own ranks (endogamy). Actually, the reason they practically do the endogamous marriages that treasure they have not lost/moved to another family, as well as family ties are still strong. Madura island community life very close to religion and customs

handed down by his ancestors for generations. Endogamous marriage seen from a genetic standpoint geneotip will increase the frequency of homozygote. Increased genetic homogeneity of this will appear if the marriage endogamy occurs continuously between generation up to the point where going all allele homozygote in a single locus, or even at all loci (Bodmer & Cavalli-Sforza 1976).

Ease of transportation in the territory of the island of Madura with their Suramadu bridge will open wider opportunities for the people of the island of Madura mobilization one of them looking for a life partner so that marriage exogamy becomes more happening than endogamous marriage. According to Bodmer & Cavalli-Sforza (1976) concept of marriage between populations is the basis of population genetics analysis of the views of frequency genes and explains the process of change in gene frequency in the population. In the examination of genetic variation that is often used the Short Tandem Repeats (STR) on deoxyribonucleic acid (DNA) core and polymorphisms in mitochondrial DNA. In mitochondrial DNA (mtDNA) humans have a genetic trait that distinguishes it from the nuclear genome. Mitochondrial DNA is passed down only through maternal lines without recombination (maternal inherited). The uniqueness of this inheritance system has been utilized in various fields, namely the determination of kinship, the study of evolution and global migration of modern humans, forensic and identification of genetic disease (Wallace 1997).

At the mtDNA non-coding regions are called the displacement loop (D-loop), this area has the highest level of polymorphism in mtDNA. As well as mtDNA is only passed down through the maternal line. Therefore, mtDNA variation in human populations evolved. The process of evolution takes place through the accumulation of changes in DNA sequence, ie through the process of nucleotide substitutions that grow in number as the development direction of the lineage. So far Madura island population genetic variation has not been known.

## MATERIALS AND METHODS

This type of research used in this study was observational analytic conducted to determine polymorphism genetic variation in mtDNA D-Loop HV2 126bp (nt: 34-159) on the population of the island of Madura, with cross sectional survey design. Samples were Madura ethnic groups residing on the island of Madura, parents or grandfather/grandmother married a fellow ethnic island of Madura.

This research material that is splattered with blood. Materials for the extraction of DNA: FTA purification reagent, 100 mL of TE-1 buffer. Materials for the PCR: PCR Mix, DW sigma. Mitochondrial DNA primer, HV2 (126 bp): (5 'TCT CCA AGC GGG TGG TGC ATT TA 3' and 5 'AAA TAA TAG GCA GGA GAT ATC GAG 3'). Materials for. electrophoresis: Bis acrylamid, Agarose, Temed, Tris Boric EDTA (TBE) 0.5%, 100 bp Marker, marker K562, 0.03% bromphenol blue. Materials for sequencing: QIA-quick DNA purification, Ethanol 70% and 95%, TBE, polyacrylamide gel, G/A sequencing premix, C/T sequencing premix, Premix Enzymes, Sodium. The research instrument: Cycle PCR, DNA sequencer ABI Prism 310, Spectrophotometer, Electrophoresis, Whirlimixer, Centrifuge, Eppendorf micropipette, micropipette tips, transluminator UV, Polaroid camera, Transsonic 310, Spinator, Eppendorf tubes, Microwave.

### DNA extraction bloodstains

DNA was extracted by piercing the FTA card that contains the sample by means of Harris punch of 2.0 mm. Added 100 mL, FTA purification reagent, incubation for 10 min at room temperature. Stir with a pipette and exhaust, repeated 3 times. Entered 100 mL TE-1 buffer, incubation for 10 min at room temperature, stirring with a pipette and then dispose of TE-1, repeat 2 times. Samples were dried and stored with the added 30 mL TE buffer.

### PCR amplification

DNA amplification by PCR amplification is done with the protocol of Promega corp, 2001. The 126 bp PCR amplification for HV2 (nt 59-134 bp), namely Phase I: initial denaturation 940C for 4 minutes. Phase II: (25 times): subsequent 940C for 1 min denaturation, annealing 540C for 1 min, and extension 720C for 2 minutes.

### Electrophoresis

In this study using 2% agarose gel. The procedure of making 2% agarose gel: Agarose gel made of 40 cc TBE (Tris Boric EDTA) 0.5x and Agarose (LE Analytical Grade) 0.8 grams, was heated in a microwave until clear without bubbles, then poured into the gel bed, then comb fitted to the formation of pitting in the gel, which is used as a DNA mixed with loading dye. After the gel hardened and formed wells, the new gel electrophoresis can be used in the process.

### Sequencing

Sequencing procedures: Samples were purified with a purification qiaquick plus 19 ul Hi-DiTM formamide (Applied Biosystems) containing 0.75ul GS500LIZ size standard (Applied Biosystems). The sample is then placed rapidly in the instrument for analysis without heat denaturing or snap sample cooling during running. Samples were injected for 5 seconds on setting voltage sequencing machines of 15,000 volts and a temperature of 600C, With a standard electrophoresis is used POPTM-6, 1X A.C.E. Buffer (Amersco, Solon, OH), and 47 cm x 50 um capillary (Applied Biosystems). Sequencing machines then run for 50 minutes. Sequencing seen on the screen and analyzed. GeneScan 3.7 and 3.7 Genotyper programs (Applied Biosystems) for a Windows NT platform.

## RESULTS

### Results of Polymerase Chain Reaction (PCR) amplification

The process of amplification Polymerase Chain Reaction (PCR) which begins with the preparation of DNA template through the process of cell lysis of the blood spots using extraction kit (DNAzol). Results of the extraction/isolation of DNA from blood spots that were able to produce cell lysate in the form of DNA that is ready to be used as a PCR template. The process of PCR using the area hypervariable region (HV) II D-Loop (nt 59-134), has been given a bright band sized 126 bp as shown in Figure 1.

Fig. 1. Results of 126 bp PCR product visualization of the sample group 1. (M) markers, (+) positive control, (-) negative control, (1,2,3,4, a, b, c, d) samples

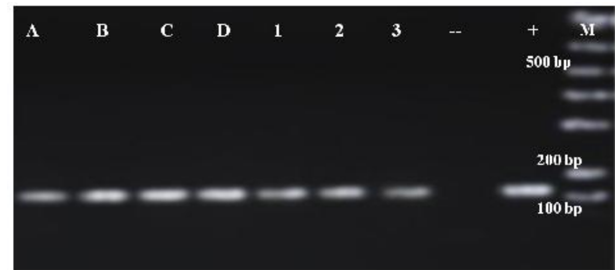


Fig. 2. Results of 126 bp PCR product visualization of the sample group 2. (M) markers, (+) positive control, (-) negative control, (1,2,3, A, B, C, D) samples

In Figures 1 and 2 above shows the appearance of a band at 126 bp that the primers used are specific to just stick to the expected position (nt 59-134). The PCR process is carried out to amplify 126bp D-loop region using primer pair M1 and M2. PCR products were analyzed by agarose gel electrophoresis 1.2% (w/v),

### Sequencing Results

Sequencing PCR fragment 126bp region of human mtDNA D-loop Madura tribe performed by Sanger dideoxy method using Automatic DNA Sequencer is based on a method Labeling Dye Terminator. 126bp fragment sequencing PCR products is done by using fragments of amplification product directly without going through the process of cloning, the process is called direct sequencing. This method is used, because the process is fast and the result is a dominant nucleotide sequences of DNA PCR amplification results. To determine the nucleotide sequence of the PCR product size 126 bp used in the sequencing reaction primer M1 (forward), this study was able to determine the nucleotide sequence of the D-loop region of mtDNA samples as shown in Figure 3. Figure 3 shows the sequencing electropherogram, the difference in color of the line, ie, black to guanine (G), blue for Cytosin (C), the red color to TYMIN (T) and green colors to adenine (A).

### Homology Analysis

The result of nucleotide sequence homology of fifteen people (4 families) Madura rate among families of the maternal line with the order reanalysis Cambridge Reference Sequence/rCRS (Andrew et al 1999) as a standard numbering shown in Table 1.

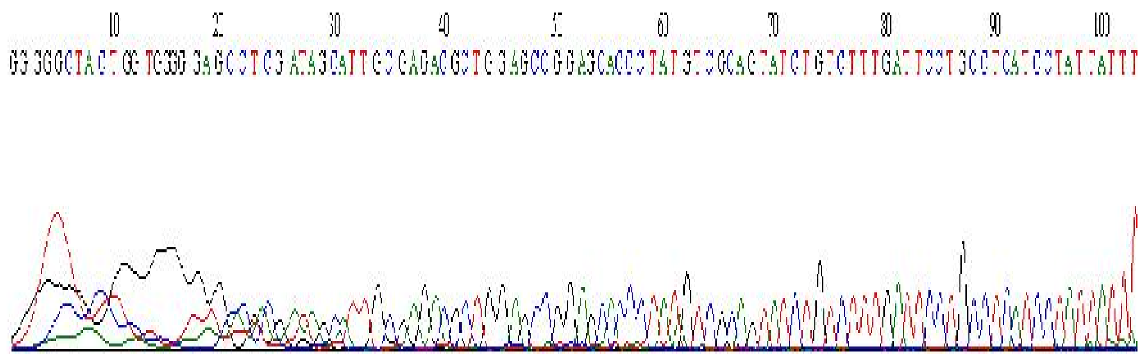

Figure 3. Electropherogram sequencing results

Table 1. The nucleotide sequence of samples

Family I

| Generation level | Nucleotide sequence | | |
|---|---|---|---|
| rCRS | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| 1 | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| 2 | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| 3 | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| 4 | 88<br>ATTGCGAGACGCTGGA | 104<br>GCCGGTGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |

Description: Underscored letter indicates the presence of the variant nucleotide

Family II

| Generation level | Nucleotide sequence | | |
|---|---|---|---|
| rCRS | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| a | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCGGAGCACCC | 116<br>TATGTCGCAGTAT-C |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATT | 161<br>T |
| b | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCGGAGCACCC | 116<br>TATGTCGCAGTAT-C |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATT | 161<br>T |

| | | | |
|---|---|---|---|
| c | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCGGAGCACCC | 116<br>TATGTCGCAGTATCA |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATT | 161<br>T |
| d | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCGGAGCACCC | 116<br>TATGACGCAGTAT-C |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATT | 161<br>T |

Description: Underscored letter indicates the presence of the variant nucleotide

Family III

| Generation level | Nucloetide sequence | | |
|---|---|---|---|
| rCRS | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| A | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCGGAGCACCC | 116<br>TATGTCGCAGTAT-C |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATT | |
| B | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCGGAGCACCC | 116<br>TATGTCGCAGTAT-C |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATT | |
| C | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCAGAGCACCC | 116<br>TATGTCGCAGTAT-C |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATC | |
| D | 85<br>AGCATTGCGAGACGCT | 101<br>GGAGCCGGATCACCC | 116<br>TATGTCGCAGTATCC |
| | 131<br>TGTCTTTGATTCCTG | 146 160<br>CCTCATCCTATTATT | |

Description: Underscored letter indicates the presence of the variant nucleotide

Family IV

| Generation level | Nucleotide sequence | | |
|---|---|---|---|
| rCRS | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| 1 | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |
| 2 | 88<br>ATTGCGTGGTGCTGGA | 104<br>GCCGGAGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCATGCCC | 149 163<br>CTCATCCTATTATTT | |
| 3 | 88<br>ATTGCGAGACGCTGGA | 104<br>GCCGGTGCACCCTAT | 119<br>GTCGCAGTAT-CTGT |
| | 134<br>CTTTGATTCCTGCCC | 149 163<br>CTCATCCTATTATTT | |

Description: Underscored letter indicates the presence of the variant nucleotide

Table 2. Data normal variant D-Loop region of mtDNA against rCRS were found in samples

| | Family I | Family II | | | Family III | | | | Family IV | |
|---|---|---|---|---|---|---|---|---|---|---|
| Position | 109 | 129 | 130 | 120 | 107 | 160 | 110 | 129 | 143 | 109 |
| rCRS | A | - | C | T | G | T | G | - | C | A |
| Generation 1 | - | - | - | - | - | - | - | - | - | - |
| Generation 2 | - | - | - | - | - | - | - | - | A | - |
| Generation 3 | T | C | A | - | A | C | - | - | - | T |
| Generation 4 | - | - | - | A | - | - | T | C | - | - |

rCRS: reanalysis Cambridge Reference Sequence (Andrew et al 1999)

HVS nucleotide sequence II (rCRS): nt: 34-159 Homo sapiens mitochondrion, complete genome:
GGGAGCTCTCCATGCATTTGGTATTTTCGTCTG
GGGGGTATGCACGCGATAGCATTGCGAGACGC
TGGAGCCGGAGCACCCTATGTCGCAGTATCTGT
CTTTGATTCCTGCCTCATCCTATTATTT. The results of the analysis of the sample nucleotide sequence homology to sequences rCRS (reanalysis Cambridge Reference Sequence (Andrew et al 1999) as the reference sequence, indicating that found 9 variants or morph.

From table 2 shows that there are nine different variants or morph the 109A → T, 130C → A, 120T → A, 107g → A, 160T → C, 110G → T, 110G → T, 143C → A, 109AG → T and C nucleotide insertion position 129. the results of this study showed a 9 variant nucleotides found in 4 families lysed mother Madura tribe. To 9 variants are specific for Madura tribe who settled on the island of Madura, because research so far nucleotide variant D-loop region of mitochondrial DNA (mtDNA) has not been done, especially at the locus of 126 bp.

The results of the analysis of 15 human nucleotide sequence homology (4 families) and Madurese in order reanalysis Cambridge Reference Sequence/rCRS (Andrew et al 1999). Analysis of sequence homology with rCRS showed 9 variant consisting of two transition mutations, 6 transversion mutations, and 1 mutation insertions (addition or insertion of nucleotides). The results of this study indicate that the number of mutations transversal more likely than a transition mutations and insertions.

## DISCUSSION

This study cannot determine the pattern of the variant nucleotide sequencing results 3-4 generations in 4 families in Madura tribe is a tribe specific to Madura because the sample size is still small. In this study, researchers found only 9 variants or morph the tribal groups of Madura, it could be due to sampling, the volunteer is a native of the island of Madura, in terms of descent or level of generation is a native/birth rate Madura, in other words the model of marriage endogamy. Endogamous marriage is heavily influenced by cultural factors tribal customs that remain madura they retained until today, but their means of transport which Suramadu bridge marriage patterns began a bit so more inclined to exogamy pattern.

The results of this study recommend that research on population or other medical fields are not only focused on genetics alone, but also take into account the culture and customs of a population. According Mastana (2007) states that the gene pool is not only a collection of genes, but is a dynamic system that is well organized and includes the past history of a population. Every aspect of genetic information has the history, anthropology and certain statistics so that the necessary coordination and collaboration of various disciplines.

## CONCLUSION

Based on these results, indicating fragment size 126bp mtDNA HV2 the D-Loop region which lies in the region nt: 59-134 of 15 people (4 families) Madura tribe has successfully amplified by PCR. The results of the analysis of nucleotide sequence homology with rCRS samples obtained nine types of nucleotide variants namely: 109A → T, 130C → A, 120T → A, 107g → A, 160T → C, 110G → T , 110G → T, 143C → A, 109AG → T and C nucleotide insertion position 129 of 9 variants terdiari on 2 mutation transitions, 6 transversion mutations and insertion mutations 1.

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