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# Revealing the Genetic Variation and Allele Heterozygote Javanese and Arab Families in Malang East Java Indonesia

## Malang Doğu Java Endonezya Da Java Halkı ve Arap Aileler Arasında Genetik Varyasyon ve Allel Heterozigitesinin Açığa Çıkarılması

Nila Kartika Sari[1], Eriko Prawestiningtyas[2], Fatchiyah Fatchiyah [1]

[1]Departement of Biology, Mathematic & Natural Sciences Faculty, Brawijaya University Malang East Java INDONESIA
[2]Laboratory of Forensic Medicine, Medical Faculty, Brawijaya University Malang East Java INDONESIA

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### ABSTRACT

**Purpose:** The purpose of this study is to identify genetic variability the family of Javanese married with Arab and their allele patterns for paternity testing.

**Material and Methods:** We used human white blood cell from three generations of three families, consists of:[1] grandmother-mother, father-daughter[2], grandfather-mother, father-daughter[3], grandfather, grandmother – mother, father-son. DNA blood samples were isolated by salting out standard procedure, and amplified by PCR with applying 13 CODIS which consists of TPOX, D3S1358, FGA, D5S818, CSF1PO, D7S820, D8S1179, TH01, VWA, D13S317, D16S539, D18S51, D21S11 and amelogenin primer. The Fingerprint profile was visualized by 8% polyacrylamide gel and took the picture by ChemiDoc gel Imaging and measure the intensity band pattern by Quantity One software

**Results:** Our result showed that the genetic variability and heterozygote allele increasing by using the 13 CODIS markers from the first generation to the next generation with paternity testing from each family were matched.

**Conclusion:** We can conclude that in a Javanese-Arab family ethnic seems stimulate the increasing genetic variation and allele heterozygote.

**Key Words:** Javanese – Arab Ethnics, DNA fingerprint, 13 CODIS

### ÖZET

**Amaç:** Bu çalışmanın amacı araplar ile evli Javalı ailelerdeki genetik çeşitliliğin ve babalık testi allel paternlerini belirlemektir.

**Materyal ve Metod:** Üç ailenin üç kuşağından alınan beyaz kan hücrelerini kullandık : büyükanne-anne[1] , baba-kız[2], büyükbaba-anne, baba-kız[3],dede, büyükbaba - anne, büyükanne - anne , baba-oğul . DNA kan örnekleri standart prosedür tuzla çöktürme ile izole edildi ve TPOX , D3S1358 , FGA , D5S818 , CSF1PO , D7S820 , D8S1179 , TH01 , VWA , D13S317 , D16S539 , D18S51 , D21S11 ve amelogenin primerleriyle 13 CODIS'e uygun olarak ile PCR ile çoğaltıldı. Parmak izi profili %8 poliakrilamid jel ile görüntülendi ve ChemiDoc jel Görüntüleme sistemi tarafından görüntüler alındı ve Quantity One yazılımı ile bant paterni yoğunluğu ölçüldü.

**Bulgular:** Onüç CODIS markırı kullanarak elde edilen sonuçlarımız, babalık testinde genetik değişkenlik ve allelik heterozigot allellerinin ilk nesilden sonraki nesile arttığını gösterdi.

**Sonuç:** Java-arap ailesinin genetik varyasyon ve allelik heterozigiteyi artırmasına sebeb olduğu sonucuna varılmıştır.

**Anahtar Kelimeler:** Java-Arap etnik, DNA parmakizi; 13 CODIS

## INTRODUCTION

More than one-third of human genome consists of repetitive sequence region (*Repeat Area*)[1]. Repetitive sequence regions are referred to as DNA *satellite* which consist of two kinds of repetitive regions including *Minisatellite* or *Variant Number Of Tandem Repeats* (VNTR) with 16-41 bp repeat units and *Microsatellite* or *Short Tandem Repeat* (STR) with 2-6 bp repeat units [1, 2]. Based on its short allele range[1,3], STR can be used for the paternity testing[1,4], it is often used also for the study of genetics disease, molecular archeology, as well as in forensic crime cases[1,4,5].

United State of America (USA) by the *Federal Bureau of Investigation* (FBI) posses a DNA database with specific software named as *Combined DNA Index System* (CODIS)[4,5,6], and it is used to identify the lawbreaker, unsolved crime scene evidence, and missing persons by using a sample of blood or saliva glands[1,6]. The FBI routinely uses a standard set and recommended the forensic laboratory to use the 13 specific STR regions for CODIS, those are FGA, THO1, TPOX, VWA, CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S1[1,4,6,7,8]. The recommendation from FBI has been widely accepted by forensic laboratory all over the word.

In Indonesia, the intermingling between the indigenous people with the Arabs origin through marriage happened before the Dutch colonial era[9]. In Java Island the assimilation between Javanese ethnic and Arab frequently occurred. They leave their Arabian identity, and have Javanese name, Javanese clothes, Javanese manners, Javanese customs and Javanese kinship systems from the previous generation parents' and Javanese language as well. Those individuals, who have been raised in Javanese culture, will see himself as Javanese instead of Arab identities[9].

The implication of this behavior raised the problem in unveiling their descendants based on their language, social behavior, and even their morphological appearance which already far from Arabs origin. The principles of coalescence can be applied to interpreting variability at STR, whenever the number of repeats differs between two copies of an STR locus from different ethnic, at least one mutation as one of processes shape the level and pattern of variation has occurred after they shared a common ancestor[10]. For this research problem, an accurate genetic study will be helpful; CODIS then, comes as a candidate tool to solve the problem for study the genetic relationships among different ethnic groups as well as the origin, evolution and migration of the populations.

## MATERIAL AND METHOD

### Subject

This study was mainly conducted in the Central Laboratory of Life Sciences, Brawijaya University Malang, East Java Indonesia. Samples were taken from Blood samples from peripheral veins of respondents for 3 ml and collected in *vacutainer tube*. The total number of samples was 13, which were divided into three families. 1st and 2nd family consist of blood samples from one of grandparent; parents; children (8 samples) and 3rd family consist of blood samples from grandparents; parents; children (5 samples). Each of families originated from Javanese – Arab Ethnic at Malang East Java Indonesia. Consent forms were obtained from all participants.

### Ethical consideration

The study approved by ethical review committee the Research Ethics Committee of medical research, Medical Faculty of Brawijaya University with Certificate of ethical clearance. For appropriate management, all individuals participated in the study, after having information, and inform consent were firstly communicated with participants.

Procedure Blood samples were isolated by salting out method. The DNA quantitative test were

measured by using *UV-Vis* (Nano Drop) spectrophotometer and DNA qualitative test were measured by electrophoresis on 0,8% agarose gel[11].

PCR amplification used 13 CODIS (FGA, THO1, TPOX, VWA, CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S1 primers) and amelogenin for sex identification performed in a thermal cycler with the reaction mixture that was exposed to 1 min of initial denaturation at 94ºC, 35 cycles of denaturation at 94ºC for 1 min, annealing at 60ºC for 1 min, extension at 72ºC for 1 min, and final extension at 72ºC for 10 min. PCR results were visualized by electrophoresis on 8% polyacrylamide gel that was carried out at constant current of 50 Volt until the tracking dye reaches 0.5 cm above the base of the gel. The band of electrophoresis was observed by using *ChemiDoc Gel Imaging* (Bio Rad).

**Data Analysis**

The analysis of the individual band profile were conducted to determine the genetic similarities, variability and allele patterns from each individual sample by using Quantity One software, and it was compared with allele range[3].

## RESULT

### 3.1 Genetic Similarities and Variability of Profile DNA fingerprinting Identification

The profile of DNA fingerprinting from each individual of Javanese – Arab Ethnic showed a variety of band patterns so that the genetic inheritance similarities can be identified from each generation in a family. Genetic similarity was shown by D18S51 (figure 1 A & D) meanwhile for genetic variability was shown by VWA (figure 1 B & E). They were derived from each generation in a family. Band patterns by amelogenin (figure 1. C&F) showed that a male sample formed two bands with 2 alleles, whereas single band for a female sample.

Genetic similarities and variability of each three families were identified by using the 13 CODIS. The 1st family, similarities pattern inherited from 1st to 2nd generation which is 53.85% and genetic variability with 46.15%. The 2nd to 3rd generation successive derived genetic variability there were 61.54% and 76.92% which are higher than of genetic similarities there were 38.46% and 23.08%. Genetic variability in 1st to 3rd generation were also refer greater percentage the genetic similarities of 84.62% : 15.38% (Figure 2. 1A).

The variability pattern of 2nd family, profile of DNA fingerprinting were inherited each generation showed the overall percentage which are higher than the percentage of similarities of those 69.23% : 30.77% and 92.31 : 7.69% (Figure 2. 2A). DNA fingerprinting of the 3rd family showed the significant genetic variability of 100% from 1st male to each generation (figure 2. 3A).

The existence of genetic variability reported in other study of HLA typing profile for Indonesian population because of Indonesia is vast archipelago in Southeast Asia with a population size of more than 230 million and with a land area of some 2.02 million square kilometers. These populations densely inhabit big cities especially in Java Island and are characterized by high ethnic and linguistic diversity. The present Indonesian population is considered to comprise 41.7% Javanese, 15.4%Sundanese–Javanese, and 42.9% others. HLA allele and haplotype frequencies in addition to phylogenetic tree and principal component analyses based on the four-digit sequence-level allele frequencies for HLA-A, HLA-B, and HLA-DRB1 showed that Western Javanese (Indonesia) was closest to Southeast Asian populations[12]. Previous studies indicate that the frequency distributions of HLA alleles and haplotypes vary from one ethnic group to another or between the members of the same ethnic group living in different geographic areas[13].

### 3.2 Allele Identification of Paternity Testing

The genetic inheritance based on 13 CODIS allele of families in Javanese – Arab Ethnic, for each locus has a pair of alleles, homozygous or

heterozygous. The 1st family, homozygous inherited from 1st to 2nd generation that was 7.69% at D8S1179, whereas heterozygous inherited 92.31% on TPOX, D3S1358, FGA, D5S818, CSF1PO, D7S820, TH01, VWA, D13S317, D16S539, D18S51, D21S11. Heterozygous was 100% inherited from the 2nd to 3rd generation (Figure 2. 1B). Significant percentage indicated that the families of each individual are secondary ethnic within population. The allele pattern of DNA paternity test showed that from the 2nd generation to 3rd generation (Father-mother-daughter) it was 12 marker inclusion consist of TPOX, D3S1358, D5S818, CSF1PO, D7S820, D8S1179, TH01, VWA, D13S317, D16S539, D18S51, D21S11 and FGA as exclusion marker (Table 1).

In the 2nd family, homozygous inherited from 1st to 2nd generation was 15.38% at D3S1358 and D8S1179, whereas heterozygous inherited was 84.62% on TPOX, FGA, D5S818, CSF1PO, D7S820, TH01, VWA, D13S317, D16S539, D18S51, D21S11. Homozygous inherited from 1st and 2nd mother to 3rd was 7.69% at D21S11, whereas heterozygous inherited was 92.31% on TPOX, D3S1358, FGA, D5S818, D8S1179, CSF1PO, D7S820, TH01, VWA, D13S317, D16S539, D18S51. Heterozygous was 100% derived from the 2nd father to 3rd generation (Figure 2. 2B) and the allele pattern of DNA paternity test showed all of the 13 CODIS were in inclusion marker (Table 1).

The 3rd family, heterozygous was 100% inherited from the 1st to 2nd generation. Homozygous inherited from 1st and 2nd to 3rd generation was 7.69% at FGA, whereas heterozygous inherited was 92.31% on TPOX, D3S1358, D5S818, CSF1PO, D7S820, D8S1179, TH01, VWA, D13S317, D16S539, D18S51, D21S11 (Figure 2. 3B). The allele pattern of DNA paternity test showed from the 2nd generation to 3rd generation (Father-mother-son) all of the 13 marker inclusion, whereas from the 1st generation to 2nd generation it one of 13 marker was exclusion marker; VWA (Table 1).

The overall percentage of heterozygote allele is higher than the percentage of homozygote allele and the inclusion of allele which showed the dominance in the paternity test within each family.

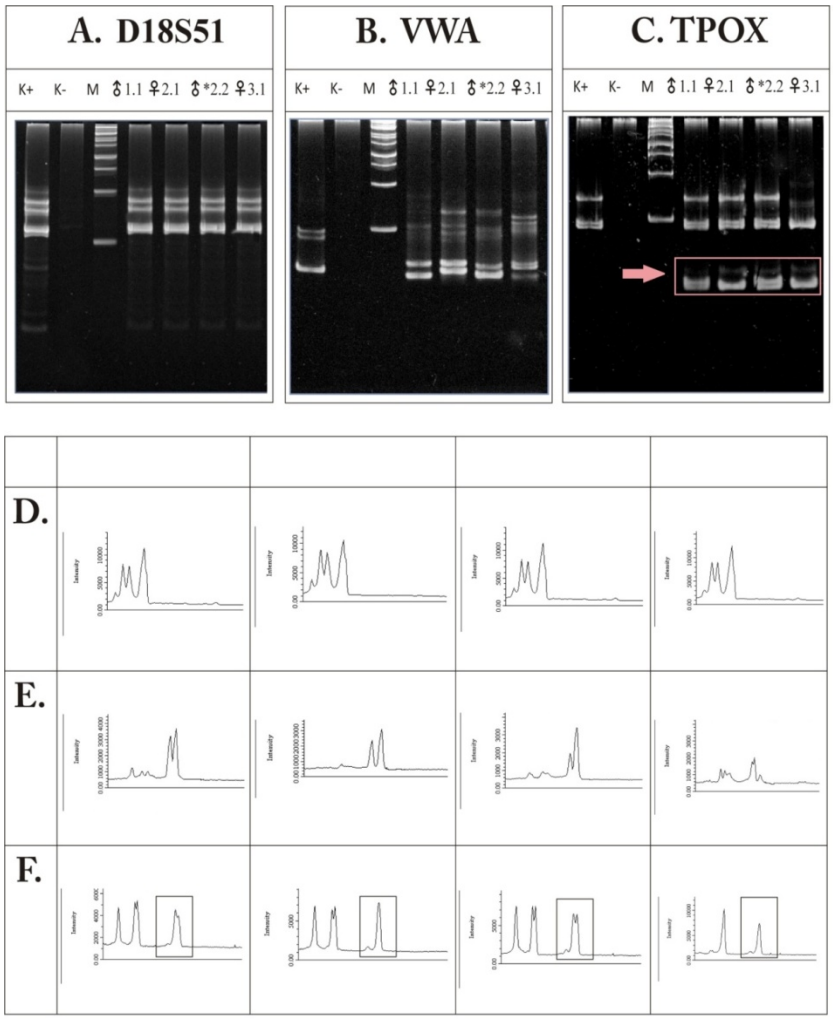


**Figure 1. Profile of DNA fingerprinting of 8% polyacrylamide gel electrophoresis (Marker 1 KB, ♂ 1.1 = Male 1st generation, ♀ 2.1 = female 2nd generation, ♂ * 2.2 = male 2nd generation in-law, ♀ = female 3rd generation 3.1). A and D is a similarity in the pattern of allele 7/8 D18S51. B and E are the patterns of variability in allele 16/16, 12/16, 16/16, 12/16 VWA. C and F are banding pattern formed by amelogenin for sex determination**

(Sari *et al., 2013)*

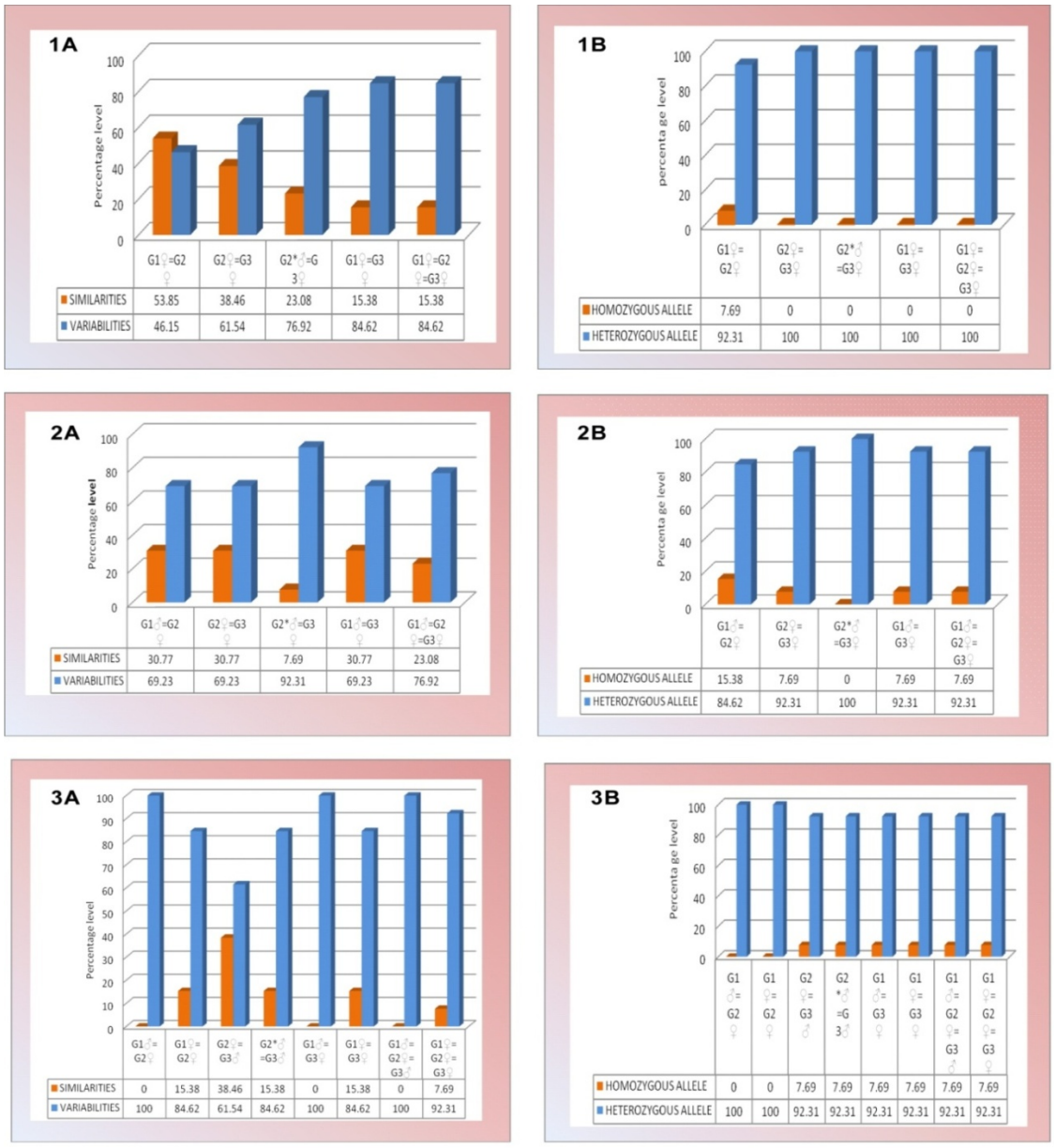


Figure 2. Identification of Java – Arabs Ethnics DNA Fingerprint A : Percentage of Genetic Similarities and Variabilities, B : Percentage of Allele zygosites (1 = 1st Family, 2 = 2nd Family, 3 = 3rd Family).

(Sari *et al., 2013)*

**Table 1. Determination alleles based on 13 CODIS in Java – Arabs ethnic for paternity testing**

| 1st Family | | | | | | |
|---|---|---|---|---|---|---|
| **Locus** | **Allele Range (Butler, 2007)** | **Allele** | | | | **Conclusion** |
| | | **Grandmother (♀1.1)** | **Mother (♀2.1)** | **Father (Law) (♂*2.2)** | **Daughter (♀3.1)** | **Father-Mother-Daughter** |
| TPOX | 6-13 | 8/9 | 8/9 | 8/9 | 8/9 | Inclusion |
| D3S1358 | 12-19 | 17/17 | 15/17 | 15/17 | 15/17 | Inclusion |
| FGA | 17-51.2 | 21/ 25 | 21/ 25 | 21/25 | 22/ 25 | Exclusion |
| CSF1PO | 6-15 | 6/8 | 6/8 | 6/8 | 6/8 | Inclusion |
| D5S818 | 7-16 | 9/12 | 9/12 | 9/12 | 9/12 | Inclusion |
| D7S820 | 6-15 | 8/9 | 7/9 | 7/9 | 7/9 | Inclusion |
| D8S1179 | 8-19 | 11/11 | 11/11 | 8/11 | 8/11 | Inclusion |
| TH01 | 4-13.3 | 7/11 | 7/11 | 7/11 | 7/11 | Inclusion |
| VWA | 11-24 | 16/16 | 12/16 | 13/16 | 12/16 | Inclusion |
| D13S317 | 8-15 | 15/15 | 12/ 15 | 12/15 | 12/ 15 | Inclusion |
| D16S539 | 5-15 | 6/8 | 6/8 | 5/8 | 5/8 | Inclusion |
| D18S51 | 7-27 | 7/8 | 7/8 | 7/8 | 7/8 | Inclusion |
| D21S11 | 24-38 | 29/35 | 29/ 35 | 27/ 35 | 27/ 35 | Inclusion |

| 2nd Family | | | | | | |
|---|---|---|---|---|---|---|
| **Locus** | **Allele Range (Butler, 2007)** | **Allele** | | | | **Conclusion** |
| | | **Grandfather (♂1.1)** | **Mother (♀2.1)** | **Father (Law) (♂*2.2)** | **Daughter (♀3.1)** | **Father-Mother-Daughter** |
| TPOX | 6-13 | 7/9 | 7/9 | 7/9 | 7/9 | Inclusion |
| D3S1358 | 12-19 | 15/17 | 17/17 | 15/17 | 15/17 | Inclusion |
| FGA | 17-51.2 | 21/25 | 21/25 | 21/ 25 | 21/25 | Inclusion |
| CSF1PO | 6-15 | 8/8 | 6/8 | 6/8 | 6/8 | Inclusion |
| D5S818 | 7-16 | 12/12 | 9/12 | 9/12 | 9/12 | Inclusion |
| D7S820 | 6-15 | 7/7 | 7/9 | 7/9 | 7/9 | Inclusion |
| D8S1179 | 8-19 | 11/11 | 11/11 | 8/8 | 8/11 | Inclusion |
| TH01 | 4-13.3 | 11/11 | 9/11 | 9/11 | 9/11 | Inclusion |
| VWA | 11-24 | 16/16 | 12/16 | 16/16 | 12/16 | Inclusion |
| D13S317 | 8-15 | 12/15 | 12/15 | 12/ 15 | 12/15 | Inclusion |
| D16S539 | 5-15 | 8/8 | 6/8 | 6/8 | 6/8 | Inclusion |
| D18S51 | 7-27 | 7/8 | 7/8 | 7/8 | 7/8 | Inclusion |
| D21S11 | 24-38 | 30/32 | 30/32 | 28/32 | 32/32 | Inclusion |

| 3rd Family | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Locus** | **Allele Range (Butler, 2007)** | **Allele** | | | | | **Conclusion** | |
| | | **Grandfather (♂1.1)** | **Grandmother (♀1.1)** | **Mother (♀2.1)** | **Father (Law) (♂*2.2)** | **Son (♂3.1)** | Grandfather-Grandmother-Mother | **Father-Mother-Daughter** |
| TPOX | 6-13 | 9/9 | 7/9 | 7/9 | 9/9 | 7/9 | Inclusion | Inclusion |
| D3S1358 | 12-19 | 15/ 17 | 14/17 | 15/ 17 | 14/ 17 | 15/ 17 | Inclusion | Inclusion |
| FGA | 17-51.2 | 22/25 | 22/25 | 22/ 25 | 22/ 25 | 25/25 | Inclusion | Inclusion |
| CSF1PO | 6-15 | 6/8 | 6/8 | 6/8 | 6/8 | 6/8 | Inclusion | Inclusion |
| D5S818 | 7-16 | 10/12 | 9/12 | 10/12 | 10/12 | 10/12 | Inclusion | Inclusion |
| D7S820 | 6-15 | 7/9 | 7/9 | 7/9 | 7/9 | 7/9 | Inclusion | Inclusion |
| D8S1179 | 8-19 | 10/11 | 10/11 | 10/11 | 11/11 | 10/11 | Inclusion | Inclusion |
| TH01 | 4-13.3 | 9/11 | 9/11 | 9/11 | 10/ 11 | 9/11 | Inclusion | Inclusion |
| VWA | 11-24 | 14/ 16 | 14/16 | 15/16 | 14/16 | 14/16 | Exclusion | Inclusion |
| D13S317 | 8-15 | 13/ 15 | 12/15 | 12/ 15 | 13/15 | 13/ 15 | Inclusion | Inclusion |
| D16S539 | 5-15 | 5/8 | 6/8 | 6/8 | 5/8 | 5/8 | Inclusion | Inclusion |
| D18S51 | 7-27 | 7/8 | 7/8 | 7/8 | 7/8 | 7/8 | Inclusion | Inclusion |
| D21S11 | 24-38 | 27/32 | 27/32 | 27/32 | 27/32 | 27/32 | Inclusion | Inclusion |

## DISCUSSION

The result of the present study showed the revealing of genetic variability in three families of Javanese – Arab Ethnic which indicated there is mixed marriage between Javanese and arab ethnic. The result was supported by several previous studies in genetic profile which showed the result that genetic variability indicates the gene flow or migration[14,15,16], mutation, selection, and genetic drift[15,16]. Gene flow is one examples of microevolution epigenetically causing genetic variation between populations due to the migration of people into a population that makes marriage between individuals who previously geographically separate intercontinental[15,] The pattern of genetic divergence when two or more different ethnic populations becomes marriage having a set of genetic changes can also increase genetic variation involving silent mutations that do not affect the phenotype and led to changes in morphology or physiology[10].

Allele homozygote and heterozygote can be inherited from each parent, or the same pair of alleles inherited from both parents [[15]]. According to that condition, a DNA paternity test from allele patterns have been probability of paternity is 99.9% or greater, if the patterns do not match on two or more marker, then it could be excluded as a biological father[17]. The result of this study also showed the revealing of allele heterozygote on three families of Javanese – Arab Ethnic at each locus of the samples which correlates with increased of the genetic variability and suggests balanced polymorphism patterns, crossing over,and recombination. Moreover, balanced polymorphism will persist when environmental and cultural variables do not change[15]. Natural selection can also maintain variability in a population when heterozygotes are favored. For example, the sickle cell allele (HbS) at the beta hemoglobin locus is maintained along with the most common allele (HbA) in populations where malaria is prevalent. People who carry one copy of each allele (HbA/HbS) are most fit because they are resistant to malaria, while those who carry two copies of the normal allele (HbA/HbA) are vulnerable to malaria, and those who carry two copies of the sickle cell allele (HbS/HbS) suffer from sickle cell disease. The balance in frequencies for the HbA or HbS alleles is stable in the presence of malaria[10].

We conclude that the marriages between different individuals ethnic increased genetic variation and allele heterozygote. This is supported by other studies[10] that most of the population in modern times is a secondary race that appears as a mixture of the primary race, so high variations appear in each ethnic.

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**Conflict of Interest Statement**

The authors declare that there are no conflicts of interest.

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**Yazışma Adresi / Address for Correspondence:**
Dr. Fatchiyah Fatchiyah
Department of Biology,
Faculty of Mathematics and Natural Sciences,
Brawijaya University
Malang, East Java, INDONESIA.
e-mail: fatchiya@ub.ac.id, and fatchiya@gmail.com

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