Relationship Analysis of Sibling Pairs on Madurese Ethnicity in Surabaya, Using 12 STR Loci for The Paternity Test Process

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Abstract

The Use of STR locus in studying the relations between siblings for the need of ethnic genetic database in Indonesia for identifying disaster victims, criminals and paternity test is not well established compared to other countries. Targeted outcomes are STR typing and profiling of the human genome for individual identification and kinship relations. The aim of this study was to analyse kinship relationships among siblings by assessing and determining a better STR locus and the level of allelic distribution among full siblings in Madurese people of Surabaya, East Java, with 3 kinship categories namely male-male, female-female and male-female.

Objectives to explain the sibling relationship from a pool of 25 Madurese families in Surabaya, there were 6, 11 and 8 pairs of siblings in the male-male, female-female and male-female categories respectively. Kin relationships were analysed by using 12 STR loci technique (CSF1PO, F13B, FES, TH01, TPOX, VWA, D5S818, D7S820, D8S1179, D13S317, D16S539, and D18S51). Through the use of 12 autosomal STR loci, this study provides evidence that the strength of sharing 2 alleles can be used as a reference in that siblings have high kinship relations.

Based on the results of this study, STR loci D5S818, CSF1PO, and F13 are recommended when typing male-male siblings whereas STR locus FES is recommended for female-female siblings of the Madurese population.

The recommended main STR loci in male-female siblings of Madurese ethnicity are D13S317, D8S1179, FES, and CSF1PO.

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Introduction

Geographically, Indonesia is located at the encounter of three major active plates which are the Indo-Australian Plate, the Eurasian Plate and the Pacific Plate. The geographical location of Indonesia causes Indonesia to be more vulnerable to geological and hydro-climatological disasters.[1] Based on the 2018 data from the National Disaster Management Agency (BNPB), the incident of disasters recorded in Indonesia was 3,397 and the number of people recorded as either dead or missing was 3,874. Between 2009 and 2018, Indonesia experienced disasters with varied impact ranging from infrastructure damage to loss of lives.[2] The process of personal identification is often met with challenges since there is no genetic information from parents.[3] To overcome this problem, siblings may be used to identify the victims during a disaster or criminal investigation.

Kinship analysis using siblings has more problems than identification tests using parents as a comparison because there no obligation between siblings to have the same allele. Other than that, full siblings probably have two identical alleles with offspring from the same ancestors at the given locus because they have zero allele. Thus, the lack of alleles distributed at each particular locus does not exclude two people from being related.[4] At present, the STR multiplex locus (73Plex (full 73-loci multiplex) or

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as part of 20 loci (20Plex) is very polymorphic thereby making it possible to increase a clear resolution in mixed samples from two individuals. STR loci used in 73 Plex has substantial sequence variations that produce high heterozygosity for several loci have reduced the spread of the allele length so as to facilitate a specific look at the repetition unit. In its use, this multiplex as a complementary tool, has the potential to increase resolution by reducing the number of alleles distributed between individuals compared to the core STR system currently in use.[5]

The application of STR in analysing kin relations has not been executed in Indonesia. The use of STRs in Indonesia is hindered because of many ethnic groups that are distributed in different geographical and diverse cultural locations. As a result, a lot of time is needed to prepare supporting tools for personal identification, genetic mapping, ethnic profiling and kinship analysis.

Objectives to explain allele percentage distribution between siblings by using 12 STR loci in three groups of men-men (6 families), women-women (11 families) and men-female (8 families) siblings. The main objective of this study was to determine the percentage of allele distribution among siblings and see the STR locus/loci with high differentiating ability between siblings of the Madurese tribe.

Materials and methods

Study participants

The samples size for this study was 50 siblings (25 sibling pairs) taken from a total of 100 people (25 families) of native Madurese. To be included in this study, a family was supposed to belong to at least three generations of native Madurese with two biological children. Those families who were not meeting this inclusion criteria were excluded from this study. As a result, four individuals from each family were made into one project consisting of father, mother and two siblings. The father and the mother were used as controls/references for allele sharing between siblings.

This study was approved by the Health Research Ethics Commission, Faculty of Dentistry, Universitas Airlangga with ethical clearance number 256 / HRECC.FODM / IV / 2018. All participants in this study agreed and allowed the publication of results. Participants' identities and confidentiality were respected.

Specimen collection

Blood samples were drawn from the peripheral using a syringe into EDTA containing tubes. The tubes were labelled with the letters F (father), M (mother) and S (sibling). The families were grouped into three groups namely male-male siblings consisting of 6 families, female-female siblings consisting of 8 families and male-female siblings consisting of eight families.

DNA extraction

DNA was extracted by using DNAzol as previously explained in.[6,7,8] After DNA isolation, 50 µL of distilled water was added to each DNA pellet.

DNA Amplification using STR-PCR

DNA was amplified by using STR-PCR (PowerPlex® 21 Systems, Promega, USA) targeting 12 STR autosomal loci (CSF1PO, F13B, FES, TH01, TPOX, vWA, D5S818, D7S820, D8S1179, D13S317, D16S539, dan D18S51). The amplification process was done using a Bio Rad T100TM PCR machine for a total duration of 2 hours and 7 minutes. The temperature settings for PCR were set as follows: 96°C for 2 minutes, then 94°C for 1 minute, 64°C for 1 minute, 70°C for 1.5 minutes, for 10 cycles, then 90°C for 1 minute, 64°C for 1 minute, 70°C for 1.5 minutes, for 30 cycles. The amplicons were stored at 4°C according to the protocol.[9,10,11] Amplicons were visualised on 6% Silver Nitrate stained polyacrylamide gel electrophoresis (Bio-Rad Mini-PROTEAN®).[12]

Results

As it can be seen from **Hata! Başvuru kaynağı bulunamadı.**, a low percentage (16.66%) of zero-allele sharing at loci TPOX, D13S317, D16S539, VWA and D7S820 was observed in the male-male sibling group whilst the other seven loci did not show any similarity. For one-allele sharing, a percentage of greater or equal to 50 was observed at all the loci with the highest percentage of 83.33% observed at D8S1179, FES and D18S51 loci. For two-allele sharing, high percentage were observed at D5S818, CSF1PO and F13 with 50%, 50% and 66% respectively (**Hata! Başvuru kaynağı bulunamadı.**).

Loci	Allele Sharing	Percentage
TPOX	0	16.66
	1	50
	2	33.33
TH01	0	0
	1	50
	2	50
D13S317	0	16.66
	1	66.66
	2	16.66
D5S818	0	0
	1	50
	2	50
D8S1179	0	0
	1	83.33
	2	16.66
D16S539	0	16.66
	1	66.66
	2	16.66
VWA	0	16.66
	1	66.66
	2	16.66
FES	0	0
	1	83.33
	2	16.66
CSF1PO	0	0
	1	50
	2	50
F13	0	0
	1	33.33
	2	66.66
D18S51	0	0
	1	83.33
	2	16.66
D7S820	0	16.66
	1	66.66
	2	16.66

Table 1. Percentage of allele sharing (0, 1, 2) in the male-male siblings of Madura

	Loci	0 Allele Sharing	1 Allele Sharing	2 Allele Sharing	1+ 2 Allele Sharing
1	TPOX	16.66	50	33.33	83.33
2	TH01	0	50	50	100
3	D13S317	16.66	66.66	16.66	83.33
4	D5S818	0	50	50	100
5	D8S1179	0	83.33	16.66	100
6	D16S539	16.66	66.66	16.66	83.33
7	VWA	16.66	66.66	16.66	83.33
8	FES	0	83.33	16.66	83.33
9	CSF1PO	0	50	50	100
10	F13	0	33.33	66.66	100
11	D18S51	0	83.33	16.66	100
12	D7S820	16.66	66.66	16.66	83.33
Average percentage		6.94	62,5	30.55	91.66

Table 2. Allele sequence sharing from each STR locus in male-male siblings of Madura

As shown in **Hata! Başvuru kaynağı bulunamadı.**, the male-male sibling group had a frequency of one plus two-allele sharing of more than 70% in all loci which were examined.

Loci	Allele Sharing	F - F
TPOX	0	36.36
	1	45.45
	2	18.18
TH01	0	18.18
	1	63.63
	2	18.18
D13S317	0	18.18
	1	63.63
	2	18.18
D5S818	0	27.27
	1	45.45
	2	27.27
D8S1179	0	9.09
	1	45.45
	2	45.45
D16S539	0	9.09
	1	45.45
	2	45.45
VWA	0	45.45
	1	54.54
	2	0
FES	0	0
	1	27.27
	2	72.72
CSF1PO	0	9.09
	1	54.54
	2	36.36
F13	0	0
	1	63.63
	2	36.36
D18S51	0	18.18
	1	72.72
	2	9.09
D7S820	0	9.09
	1	81.81
	2	9.09

Table 3. Percentage of allele sharing (0, 1, 2) in the female-female siblings of Madura

The female-female sibling group showed high percentage in one-allele sharing at loci TH01, D13S317, VWA, CSF1PO, F13 and D18S51 with a percentage of 63.63 %, 63.63%, 54.54 %, 54.54 %, 63.63%, 72.72 % and 81.81 % respectively, as shown in **Hata! Başvuru kaynağı bulunamadı.**. For two allele sharing in the female-female sibling group, high percentage of allele sharing was observed at locus FES with a percentage of 72.72 %.

	Loci	0 Allele Sharing	1 Allele Sharing	2 Allele Sharing	1+ 2 Allele Sharing
1	TPOX	36.36	45.45	18.18	53.63
2	TH01	18.18	63.63	18.18	81.81
3	D13S317	18.18	63.63	18.18	81.81
4	D5S818	27.27	45.45	27.27	72.72
5	D8S1179	9.09	45.45	45.45	90.90
6	D16S539	9.09	45.45	45.45	90.90
7	VWA	45.45	54.54	0	54.54
8	FES	0	27.27	72.72	100
9	CSF1PO	9.09	54.54	36.36	90.90
10	F13	0	63.63	36.36	100
11	D18S51	18.18	72.72	9.09	81.81
12	D7S820	9.09	81.81	9.09	90.90
Average percentage		16.66	55.30	28.03	82.49

Table 4. Allele sequence sharing from each STR locus in female-female siblings of Madura.

As it can be seen from **Hata! Başvuru kaynağı bulunamadı.**, all the loci examined had a high percentage allele sharing when one allele was combined with two-allele sharing with the exception of VWA and TPOX which had low percentages.

Loci	Allele Sharing	M - F
TPOX	0	25
	1	37,5
	2	37,5
TH01	0	25
	1	62,5
	2	12,5
D13S317	0	12,5
	1	37,5
	2	50
D5S818	0	12,5
	1	50
	2	37,5
D8S1179	0	12,5
	1	37,5
	2	50
D16S539	0	12,5
	1	62,5
	2	25
VWA	0	0
	1	100
	2	0
FES	0	0
	1	50
	2	50
CSF1PO	0	0
	1	25
	2	75
F13	0	0
	1	62,5
	2	37,5
D18S51	0	25
	1	75
	2	0
D7S820	0	12,5
	1	87,5
	2	0

Table 5. Percentage of allele sharing (0, 1, 2) in the male-female siblings of Madura.

The male-female sibling group showed a low percentage of zero-allele sharing. The low percentage in zero-allele sharing was also observed in the male-male and female-female sibling groups. A high percentage was observed in the one-allele sharing category for male-female sibling group at loci TH01, D5S818, D16S539, VWA, FES, F13, D18S51 and D7S820 (**Hata! Başvuru kaynağı bulunamadı.**). A high percentage was also observed in the two-allele sharing category at loci D13S317, D8S1179, FES, and CSF1PO (**Hata! Başvuru kaynağı bulunamadı.**).

	Loci	0 Allele Sharing	1 Allele Sharing	2 Allele Sharing	1+ 2 Allele Sharing
1	TPOX	25	37.5	37.5	75
2	TH01	25	62.5	12.5	75
3	D13S317	12.5	37.5	50	87.5
4	D5S818	12.5	50	37.5	87.5
5	D8S1179	12.5	37.5	50	87.5
6	D16S539	12.5	62.5	25	87.5
7	VWA	0	100	0	100
8	FES	0	50	50	100
9	CSF1PO	0	25	75	100
10	F13	0	62.5	37.5	100
11	D18S51	25	75	0	75
12	D7S820	12.5	87.5	0	87.5
Average percentage		11.45	57.29	31.25	88.54

Table 6. Allele sequence sharing from each STR locus in male-female siblings of Madura.

When the one-allele sharing category was combined with the two-allele sharing category, a high percentage of allele sharing was observed at all the loci examined for the male-female sibling group as seen in **Hata! Başvuru kaynağı bulunamadı.**.

Discussion

These observations show the basic law used to assess the probabilities of common alleles in tested and reference samples that were inherited with the Identical by Decent (IBD) allele. There is a 25% chance that two siblings will inherit the two IBD alleles from ordinary parents, a 50% chance that two siblings will inherit one IBD allele from parents, and a 25% chance of two siblings inheriting no IBD allele from parents, hence will not share alleles at the diploid locus.

The possibility of siblings not sharing an allele (25% of no allele sharing) at one of the two loci poses significant problems in identifying dead individuals from mass disasters when there is only one sibling who is alive. While the probability that one sibling will inherit zero IBD allele from

his/her parents is constant at 25% for each locus, the probability that two full siblings will not share alleles / loci partly depends on polymorphism information content (PIC) or heterozygosity.[13]

When testing for relations using siblings, sensitivity variations depend threshold of certainty, that is the higher the certainty threshold, the lower the sensitivity. High sensitivity is when the number of false negatives is low and high specificity is when the number of false positives is low.[3] Although it is not an obligation for siblings to have the same allele, the existence of allele sharing in siblings can be used test if the siblings are related as shown in the conducted research.[14]

Research which was carried out by Butler, states that regions which contain nucleotide repeats such as STR sequences are very useful for forensic experts because the variations are good markers for human identification. The parameters in the identification process using STRs are allele frequency, homozygosity and heterozygosity, effective numberof alleles (n), Polymorphism Information Content(PIC), power of discrimination (DP) and Power of Exclusion (PE).[15,16]

STR loci for the process of human identification was used to compare the population of Indonesia and Bangladesh by looking at allele frequency in siblings and also using the Power of Discrimination for the two tribes.[17] The use of STR-CODIS in Indonesian population has shown the possibility of similarities with other Asian countries especially on the CSF1P0 and D16S539 loci. When compare to other Asian countries, the locus D3S1358 was found to have high similarity with Vietnam, FGA with Korea and D8S1179 with Japan.[18]

This research is almost the same as the one conducted by Hameed, who observed D16S539, THO1, VWA, D5S818, D8S1179, D3S1357 and CSF1PO as main loci in shared amongst siblings.[16] A research on native Madurese was conducted using STRs and compared with other populations outside Madura region or who are not Madurese.[19] Another research which was carried out, was able to identify and recommend a locus which can be used for human identification process of the Madurese population.[20] Based on previous research, we tried to see the frequency of alleles among siblings from the Madura population by observing three categories namely male-male, female-female and male-female siblings. The main objective of this study was to determine the distribution of alleles in siblings of Madura tribe by using the above mentioned three categories. The Madurese constitute on of the largest ethnic groups in Indonesia who have a tendency of working outside their home. STR use is expected to use many of the existing loci to reduce false positives in the distribution of alleles from each sibling who has the same and different sex.

Small variations in allele distributions amongst siblings was also shown to be influenced by endogamy marriages in which the researchers recommended the use of 25 loci for Lebanese population.[21] Forensic cases such as human identification for fire victims use siblings to compare to the victims by utilising the Y chromosome for identifying whether the siblings are from one father.[22] Quite a number of research has been done to assess the relationship between siblings among others mainly based on heterozygosity and Combined Sibship Indices (CSIs).[23] Research using STR to see genetic diversity in Indonesia has been carried out to see the extent of genetic mixing between Javanese and Arab tribes.[24]

When assessing the relationship between siblings, one aspect that must be noted is to minimize false positives and increase the number of loci to be examined. Based on the results of this study, the male-male siblings of Madurese population were found to have a high percentage of allele sharing at loci D5S818, CSF1P0 and F13. The findings of this study are useful in adding literature and also in analysing sibling relationships in Indonesian tribes. We highly recommend the use of a larger sample size in future for this will greatly help victim identification in Indonesia, a disaster-prone country.

Conclusions

In conclusions, for female-female siblings of Madura ethnic group, we recommend the use of loci FES which was found to have a high percentage in this category and for the male-female siblings the loci D13S317, D8S1179, FES and CSF1PO are recommended.

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Declaration of Interest

The authors declare that there are no conflicts of interest.

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