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Genetic Variation using the 21 Str Codis Loci for Forensic Identification Examinations among Siblings of Madurese Living in Surabaya

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Abstract

Indonesia consists of various ethnic groups. However, well-established research on the genetic variation of each ethnic group in Indonesia has not yet been present. Madurese is one of the Indonesian ethnic groups inhabiting Madura island and living in remote areas throughout Indonesia. Surabaya- Madura's mobilization is tremendous as the closest city to Madura Island, connected by a bridge. The Madurese has a culture of didi and same-ethnic marriage patterns. Therefore, research was conducted on 21 STR panel loci to analyze Madurese living in Surabaya to increase genetic data libraries and genetic variation of Indonesian populations based on siblings.

PrepFiler kit to extract DNA from blood and GlobalFiler kit to amplify 21 loci were used. An ABI PRISM 3500 genetic analyzer was used to detect PCR products. Data were processed and analyzed with EasyDNA software and FORSTAT.

Allele frequency data for 21 autosomal loci in Madurese were obtained. The expected Heterozygosity (He) was found with the highest obtained at locus D19S433 (0.911), while the lowest was at locus D22S1045 (0.607). Power of Discrimination (PD) values ranged from the highest at 2 loci, namely D5S818, and D13S317 (0.968), while the lowest at locus D2S1338 (0.857). Polymorphism Information Content (PIC) value is highest at locus D22S1045 (0.765); all loci have PIC values > 0.5. The Sibship Index (SI) value of the Madurese in Surabaya is 0.88 – 1.20.

The 21 STR loci can be employed for population genetic variation studies and sibling forensic identification.

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Introduction

Using Deoxyribo Nucleic Acid (DNA) as a forensic molecular examination material is neither without constraints nor obstacles. Nevertheless, factors are causing forensic DNA experts to experience adversities, both in working with DNA samples and inferring the examination results. The lack of information from fathers and mothers or children to be used as a comparison in forensic DNA analysis is one of the problems

in forensic DNA analysis¹. The comparison between the alleles of the victim or perpetrator and their relatives is the basic principle of DNA examination, especially from parental lines per Mendelian law, such as in cases of "unborn child disputed," disputed paternity, or even in forensic DNA analysis in mass disasters or mass disasters^{2,3}. In these conditions, a comparator with a close family line is required as one of the ways to be taken in the forensic DNA analysis process, such as siblings, if a comparator from the parental or children line is not obtained. The identification process using siblings as a comparator will face the possibility of a mismatch or mismatch in the DNA locus profile used⁴. The problem with using sibling DNA is that not all forensic DNA examination loci in suspects are identical to their comparators as if using parent or

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child comparators. This condition certainly creates its own difficulties in making conclusions about the examination when the identification process is carried out.

The Madurese can be found living in various parts of Indonesia. The nomadic spirit and economic pressure have caused them to migrate throughout the country. The migration is due to the desire to improve their socio-economic life and education. Surabaya is one of the places or cities that became a place for their permanent or semi-permanent migration due to its closest and more accessible transportation. This study aimed to determine the genetic variation and allele decline between siblings of the same parents among Madurese living in Surabaya.

Materials and methods

Observational laboratory research and the research design used were instantaneous. The research sample was the DNA buccal swab of a volunteer family of parents and children. The study has obtained ethical eligibility from the Faculty of Dentistry, Universitas Airlangga, number: 568/HRECC.FODM/V/2023. The research was conducted at the human genetic study group of the Institute of Tropical Disease, Universitas Airlangga. It was conducted during May – June 2023. The number of volunteers was 10 families [father, mother, first child, and second child].

Loci	Chromosome location	Chromosome	Repeat Motive	Allele size (bp)
D1S1656	1q42	1	TAGA	159-207
TPOX	2p23.2 <i>thyroid peroxidase</i> intron ke 10	2	AATG	393-441
D2S441	2p14	2	TCTA/TCAA	76-140
D2S1338	2q35	2	TGCC/TTCC	281-350
D3S1358	3p21.31	3	TCTA/TCTG	96-142
FGA	4q31.3 <i>alpha fibrinogen</i> intron ke 3	4	CTTT/TTCC	223-379
D5S818	5q21-31	5	AGAT	138-183
CSF1PO	5q33.3-34 <i>c-fms protooncogene</i> , intron ke 6	5	AGAT	283-320
SE33	6q14 <i>beta-actin related pseudogene</i>	6	AAAG	307-439
D7S820	7q11.21-22	7	GATA	262-299
D8S1179	8q24.13	8	TCTA/TCTG	114-171
D10S1248	10q26.3	10	GGAA	85-130
TH01	11p15.5 <i>tyrosine hydroxylase</i> , intron ke 2	11	TCAT	179-219
vWA	12p13.31 <i>von Willebrand factor</i> , intron ke 40	12	TCTA/TCTG	156-209
D12S391	12p13.2	12	AGAT/AGAC	216-269
D13S317	13q22-31	13	TATC	198-244
D16S539	16q24.1	16	GATA	227-268
D18S51	18q21.33	18	AGAA	261-343
D19S433	19q12	19	AAGG/TAGG	118-171
D21S11	21q21.1	21	TCTA/TCTG	183-240
D22S1045	22q12.3	22	ATT	88-122

Table 1. 21 STR Loci^{5,6}

Buccal swabs from volunteers were isolated via DNAzol kit and amplified using GeneAmp PCR System 9700. All 21 Short Tandem repeat (STR) loci were amplified simultaneously. 21 STR loci were used in STR genotyping (table 1), performed on ABI PRISM 3500 Genetic Analyzer and analyzed by EasyDNA and FORSTAT software programs.

Data Analysis

The final result is an electropherogram containing graphs from the Capillary Electrophoresis Genetic Analyzer 3500 ABI tool, which will show 21 individual loci, each of which has 2 allele fragments (heterozygous) or 1 allele fragment (homozygous). The graph will show the size of the DNA fragments in base pair units (bp) and the value of RFUs (Relative Fluorescence Units). Genetic variation using DNA siblings in Madurese living in Surabaya through analyzing alleles from 21 STR loci: allele frequency, the Homozygosity, the Expected Heterozygosity, the Power of Exclusion (PE), the Power of Discrimination (PD), the Paternity Index, the Polymorphism Information Content (PIC), the Allele Sharing and the Sibship Index (SI) using the EasyDNA and FORSTAT software.

Results

In this study, the total allele variance of 21 STR loci in Madurese is 220. The allele frequency of each locus is listed in Table 2, with the allele frequency range: 0.0021 – 0.6042. The expected heterozygosity (**He**), the Power of Discrimination (**PD**), and the Polymorphic Information Consent (**PIC**) of 21 STR loci can be seen in Table 3.

The distribution of sibling's allele sharing from all samples at all loci is mainly half sharing, or there is one allele in common from the two siblings compared with 58%. At the same time, the lowest percentage is sub-null sharing, or there is no common allele in a pair of alleles from the two siblings, compared to 8% (Figure 1). Meanwhile, the Sibship Index (SI) results are in Table 4. The SI value is 0.88 – 1.20. (Figure 1)

Allele	D1S1656	TPOX	D2S441	D2S1338	D3S1358	FRA	D5S818	CSF1PO	SE33	D7S820	D8S1179	D10S1248	THO1	vWA	D12S391	D13S317	D16S539	D18S51	D19S433	D21S11	D22S1045	
6													0.0729									
7							0.0083						0.3750									
8	0.6942						0.046	0.0729		0.1894			0.147									
9	0.1708	0.0070					0.0771	0.0543		0.0521			0.2833			0.3146	0.0063	0.0163	0.0063		0.0063	
9.3			0.0640										0.0604			0.2021	0.2250					
10	0.0188	0.0250	0.072				0.2688	0.0767		0.2303	0.0854		0.0875							0.0021		
10.3													0.0021			0.0000	0.1542					
11	0.188	0.2125	0.2559				0.2303	0.3078		0.3803	0.0708		0.0063							0.0032	0.0063	0.2333
11.3			0.1646													0.2458	0.3375					
12	0.0333	0.0375	0.118		0.0021		0.2675	0.240		0.036	0.1438	0.0333							0.0620	0.0366		0.0271
13	0.0792		0.0542				0.1657	0.0836		0.0063	0.2292	0.404	0.0042			0.0879	0.0708	0.003	0.2271	0.0366		
13.2																0.0292	0.0538			0.0250		
14	0.0250		0.1836		0.0156		0.0021	0.0025	0.046		0.1675	0.2333		0.246					0.2303	0.1838		0.0354
14.2																0.0063	0.0091			0.0657		
15	0.0771		0.0354		0.3449				0.025		0.092	0.225	0.0166	0.0542	0.0063				0.2771	0.0638		0.3729
15.2					0.0082				0.0342											0.2104		
16	0.2146		0.0083		0.2688		0.0186		0.0354		0.1083	0.0979		0.0854					0.0208	0.0438		0.0836
16.2									0.0063											0.0333		
16.3	0.0146															0.0041						
17	0.146			0.0707	0.3021				0.0792		0.0186	0.0063		0.2708	0.0542				0.0000	0.0021		0.2167
17.3	0.0163																					
18	0.0208			0.0146	0.0583	0.0157			0.0438		0.0020	0.0063		0.2229	0.1638				0.0500			0.0083
18.3	0.0123																					
19				0.2708		0.0774		0.0854		0.0563			0.146	0.2563					0.0521			0.004
20				0.0854		0.0375			0.0604				0.0333	0.0942					0.0292	0.0497		
21	0.0042					0.0167		0.055		0.0354			0.0708						0.0063			
22				0.0792		0.0750			0.0125				0.0875									
22.2						0.0042			0.0063													
23				0.1896		0.147								0.0907								
23.2	0.0102								0.0729													
24				0.1803		0.0729			0.0063					0.0375								
24.2						0.004			0.0208													
25				0.0333		0.0875								0.046								
25.2									0.0521													
26				0.0042		0.0803			0.0375					0.0040								
26.2																						
27						0.0042															0.0041	
27.2				0.0042					0.1521													0.0792
28									0.0354													
28.2									0.0063													0.2833
29									0.0729													0.1675
29.2									0.0063													0.0333
30									0.0542													0.228
30.2																						0.0771
31									0.004													0.0292
31.2																						0.1375
32																						0.067
32.2									0.0059													0.0354
33																						
33.2																						
34									0.0063													0.004
34.2																						
35																						
36																						
36.1																						0.0042
37																						
37.2																						0.0042

Table 2. Allele Frequency 21 STR Loci on the Madurese Populations in Surabaya.

Loci	Expected heterozygosity (He)	Power of Discrimination (PD)	Power of Exclusion (PE)	Polymorphic Information Content (PIC)	Paternity Index (PI)
D1S1656	0,840	0,962	0,673	0,756	0,144
TPOX	0,769	0,924	0,683	0,688	0,094
D2S441	0,748	0,892	0,690	0,679	0,110
D2S1338	0,832	0,857	0,666	0,673	0,113
D3S1358	0,831	0,867	0,667	0,683	0,067
FGA	0,879	0,951	0,769	0,690	0,076
D5S818	0,855	0,968	0,748	0,666	0,083
CSF1PO	0,792	0,867	0,832	0,704	0,111
SE33	0,741	0,884	0,695	0,673	0,097
D7S820	0,704	0,860	0,642	0,704	0,078
D8S1179	0,705	0,954	0,688	0,686	0,129
D10S1248	0,832	0,921	0,695	0,678	0,112
THO1	0,715	0,921	0,642	0,653	0,093
vWA	0,662	0,953	0,673	0,673	0,031
D12S391	0,842	0,919	0,683	0,707	0,124
D13S317	0,769	0,968	0,690	0,656	0,106
D16S539	0,748	0,957	0,666	0,688	0,097
D18S51	0,866	0,866	0,704	0,695	0,129
D19S433	0,911	0,876	0,673	0,642	0,144
D21S11	0,716	0,897	0,695	0,665	0,254
D22S1045	0,607	0,952	0,642	0,765	0,011
Average	0,743	0,910	0,691	0,687	0,105

Table 3. Expected Heterozygosity (He), Power of Discrimination (PD) and Polymorphic Information Content (PIC) on Madurese Populations in Surabaya.

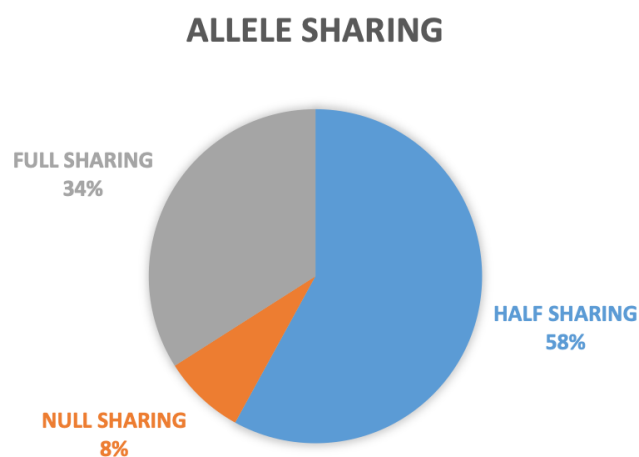


Figure 1. Percentage of 21 Loci STR Allele Sharing on Madurese Populations in Surabaya.

Family	SI	Family	SI
1	1,02	6	1,05
2	1,08	7	1,06
3	0,90	8	0,89
4	0,98	9	0,88
5	1,08	10	1,20

Table 4. Sibship Index (SI) on Madurese Populations in Surabaya.

Discussion

The TPOX locus has the lowest allele variant (5 allele variants), while the SE33 locus has the highest allele variant (24 allele variants). The allele frequency is employed to see the genetic diversity of each population. A locus is polymorphic if the number of alleles in the population at that locus is more significant than one with an allele frequency less than or equal to 0.95^{7,8}. The highest expected He value was obtained at locus D19S433 (0.911), while the lowest was obtained at locus D22S1045 (0.607). The highest PD value was obtained at 2 loci, namely D5S818, and D13S317 (0.968), while the lowest was at locus D2S1338 (0.857). The highest PIC value was at locus D22S1045 (0.765), with the average of all PIC values being > 0.5, indicating that all loci are highly polymorphic.

The absence of allele sharing in siblings implies that siblings do not always share their alleles with the other siblings, per the theoretical facts of Mendelian inheritance laws⁹. The SI value is 0.88 – 1.20. A more considerable SI value (>1.0) generally corresponds to a higher likelihood of a relationship. The higher the sibship index above 1.0, the higher the likelihood that the two individuals are related as siblings. Similarly, a smaller SI (<1.0) corresponds to a lower likelihood of a relationship^{10–12}.

The statistical calculations on the sample of Madurese living in Surabaya 21 STR loci showed significant results. Genetic variation in the sample closely correlates with several cultures inherent in the Madurese, commonly known as an endogamy marriage^{13,14}. This study also directly aligns with the migrating culture or venturing to other cities, such as migrating to Surabaya, seen from the SI value > 1.0 (60%), while < 1.0 (40%).

Conclusions

Based on the allelic frequency, the expected heterozygosity (He), the Power of Discrimination (PD), the Polymorphic Information Content (PIC), and the Sibship Index (SI), it can be inferred that this autosomal STR locus could be well applied in individual identifications and genetic variation studies through siblings examination, among Madurese population.

Declaration of Interest

The authors report no conflict of interest.

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