POPULATION DATA ON THE LOCUS D16S83 OF VNTR (VARIABLE NUMBER OF TANDEM REPEAT) POLYMORPHISM IN THE POPULATION AROUND SURABAYA, EAST JAVA, INDONESIA

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ABSTRACT

To calculate the probability value of the real father in a paternity test, frequency distribution of every alleles and genotype of polymorphism loci is needed. However, data on that distribution in Surabaya, as well as in Indonesia, are not available. In providing such data, we studied 59 unrelated population of Surabaya . Locus D16S83 of Variable Number of Tandem Repeat (VNTR) was chosen in this study, in which the DNA samples were extracted from blood cells. The samples were subjected to PCR and, subsequently, to polyacrylamide agarose composite gel electrophoresis and silver staining. From 12 alleles of D16S83 taken from 59 individuals in Surabaya, only alleles number 1, 2, 3, 4, 5, 6 ,9, 10, 11, and 12 were found. The frequency of allele 1 was 36.4%, allele 2 was 21.1%, allele 3 was 10.1% and the others were less than 9.32%. The frequency of heterozygote was 74.5 %, phenotype 1-2 was 32.2%, phenotype 1-3 was 10.1%, phenotype 1-4 was also 10.1 % and the others were less than 8.4%. A comparison of the results of this study was also made to other studies performed by other authors on the Caucasians and Afro-Carribeans.

***Keywords**: D16S83 locus, polyacrylamide agarose composite gel, PCR, allele distribution frequency*

INTRODUCTION

In cases where the determination of real father is required, or cases that needs a paternity test, we need frequency distribution of every alleles and genotype of polymorphism loci. The more data on the locus of the polymorphism obtained, the better will it be to improve the accuracy of paternity test. In Surabaya, as well as in Indonesia, however, those data are not available. In the previous studies, the author has disclosed the population data on the locus D17S5 and D1S80 of VNTR (Variable Number of Tandem Repeat) polymorphism in the population around Surabaya. In this paper, the author presents the population data on the locus D16S83 in the same population. D16S83 is a locus at the variable number of tandem repeat (VNTR) polymorphism. D16S83 has been localized to chromosome 16. The variation at this locus results from the repetition of a 80-bp core sequence. To provide such data, we studied 59 unrelated population of Surabaya. We have analyzed D16S83 in those population around Surabaya city and the data was compared with those from the population of Caucasians and Afro-Caribbeans (Tully G et al., 1993).

Restriction fragment length polymorphism (RFLP) analysis had been previously used by many forensic laboratories for working the cases. However, today those laboratories are evaluating the use of polymerase chain reaction (PCR) analysis, since this technique allows typing of limited quantities of DNA, so that it is very useful for the analysis of very small stains.

Genome in human being consists of 3 billion individual DNA, which is called basepairs (bp). There is an estimation that basepairs codes for approximately 50,000 - 100,000 individual genes, each of which carries the genetic code for the synthesis of a single protein. All human genes are encoded in approximately 300 million basepairs or about 10% of the human genome, and the remaining 90% or 2.7 billion basepairs represent "non-coding" parts of the human genome. Those basepairs do not contain genetic information that directly relevant for protein synthesis. However, they are highly useful for individual identification. Most of the forensic typing systems are based on genetic loci (defined DNA sequence with known chromosomal assignment) with tandem repetitive DNA sequences. Based on the length and genomic distribution of the core repeat sequences, the system can be classified into minisatellites (multi and single locus systems, repeat length typically 15-50 bp) and microsatellites or short tandem repeats (STR systems, repeat length typically 2-5 bp).

MATERIALS AND METHODS

Samples

Samples for DNA were collected from human cheek and blood cells from 59 unrelated individuals lived around Surabaya. The collection of those samples were

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undertaken in collaboration with Prof Dr Indrayana NS, dr, SpF, DFM, from Tropical Diseases Center (TDC), Airlangga University, Surabaya.

DNA Extraction

By means of PBS organic methods, followed by Chelex-100 extraction procedures, extractions from buccal cells and blood cells was undergone to produce DNA extraction used for further examination in this study.

Polymerase Chain Reaction (PCR) Analysis

In performing PCR reaction, amplification of a small quantity of the target DNA was undergone using a thermal cycler of Gene Amp, PCR System, 9700 Applied Biosystem. The final volume used to be mixed in order to induce PCR reaction was 25 uL, containing 3 uL DNA template of cheek cells, 2.5 uL D16S83 primer from Genset Singapore Biotech, Ptc, Ltd (5'-TTGTATCCAAACCCCCACGGTCCC-3'), 2.5 uL D16S83 (5'-GCCCACGGCAGCGTCGCCAACTCG-3'), 12.5 uL PCR - Mix Master of Promega, added with 4.5 uL nuclease-free water and one drop of mineral oil. Initial denaturation was at 95^{o}C for 3 minutes, followed by 30 cycles of 95^{o}C denaturation for 30'' (0.5 min), 62^{o}C annealing for 30'' (0.5 min), 71^{o}C extension for 4 minutes with a final 71^{o}C extension for 7 minutes.

VNTR typing

The visualization of PCR products was carried out on polyacrylamide agarose composite gels electophoresis. It was run at 100 volt for 60 minutes in vertical gel electrophoresis with TBE 1/2 x buffer. We used Ladder 100 bp as size markers and the bands were stained by silver staining.

Statistical analysis

We calculated the frequency of each allele for each locus from the numbers of each genotype obtained in the samples set.

RESULT AND DISCUSSION

This study was aimed to disclose allele frequency data at D16S83, one of the VNTR locus, in Surabaya population. Polyacrylamid agarose composite gel electrophoresis was used in this study, as it is a rapid, sensitive and reproducible method to analyze D16S83. Among the 59 Surabaya unrelated individuals typed, a total of 10 different alleles were found ranging in length from about 330 to 1290 bp. This study also demonstrated that the most common was allele 1 with a frequency of about 36.4%, followed by allele 2 with a frequency of about 21.1% and then followed by allele 3 with a frequency of about 10.1%. Seven other alleles were present with frequencies of 9.3 % or less. Some alleles, however, were not present (Table 2, Figure 2).

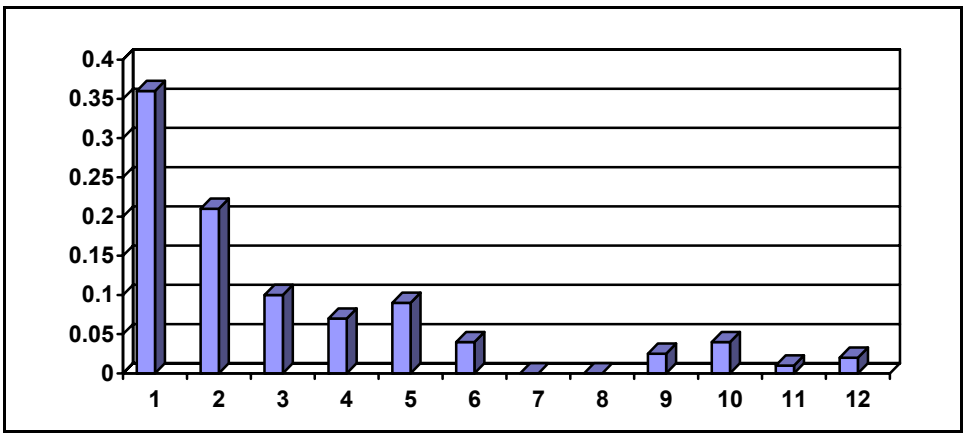
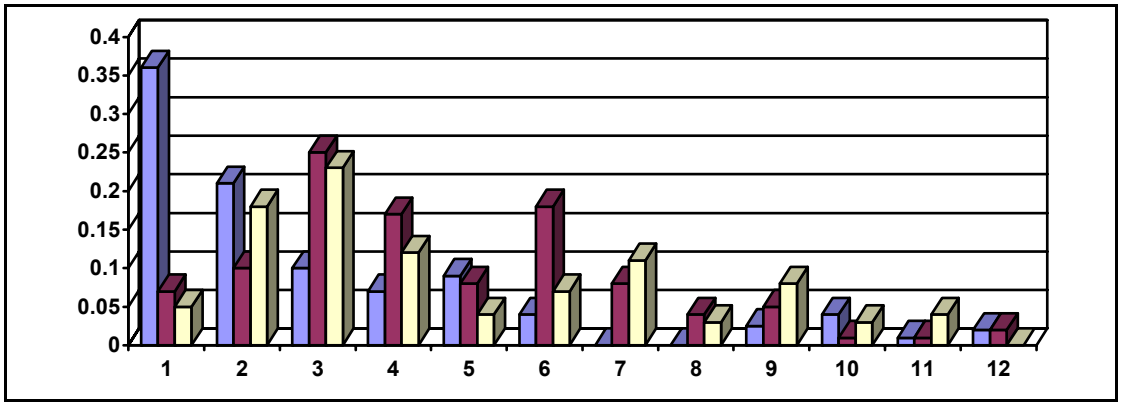


Figure 1. D16S83 amplification products of different individuals in Surabaya population. The allele designation corresponding to the number of repeat units is shown below each line. There were 10 different D16S83 alleles in this study, i.e., 1, 2, 3, 4, 5, 6, 9, 10, 11 and 12.



	1	2	3	4	5	6	7	8	9	10	11	12
Sby	0.36	0.21	0.10	0.07	009	0.04	0	0	0.025	0.04	0.01	002
Caucas	0.07	0.1	0.25	0.17	0.08	0.18	0.08	0.04	0.05	0.01	0.01	0.02
Af. Car	0.05	0.18	0.23	0.12	0.04	0.07	0.11	0.03	0.08	0.03	0.04	0.

Figure 2. Comparison of D16S83 amplification products from different individuals in Surabaya (Sb) compared to Caucasian (Caucas.), and Afro-Carribean (Af. Car.) populations.

As seen in Table 1 and Figure 3, from that population the homozygotes was 25.4% and the heterozygotes was 74.5%. This indicated that the observed heterozygosity rate of D16S83 phenotype was 74.5%. A number of 20 different phenotypes were observed. All phenotypes were found in less than 8.4% of the individuals except for the allele combinations 1-2, which was 32.2 % and 1-3 and 1-4 which occurred with each frequency of 10.1% (Table 3).

Table 1. D16S83 homozygote and heterozygote frequencies from 59 Surabaya unrelated individuals.

Homozygote	Heterozygote	Total
25.4	74.5	100

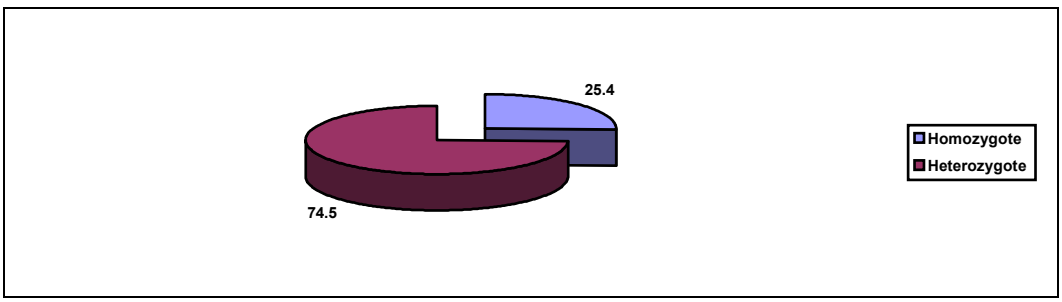


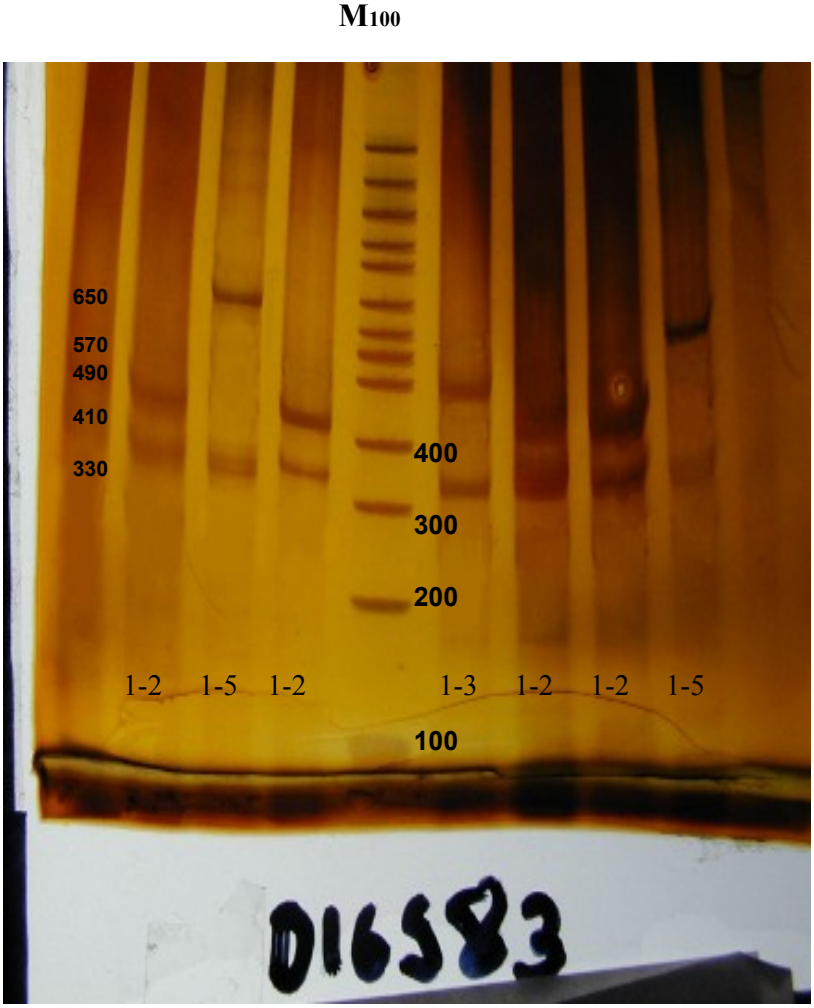
Figure 3. Homozygosity and heterozygosity of phenotype frequency of allele D16S83 in 59 unrelated individuals from Surabaya population.

Table 2. Distribution of allele count and frequency for D16S83 at 59 unrelated individuals Surabaya population.

Allele	n	Frequency
1	43	0.364
2	25	0.211
3	12	0.101
4	9	0.076
5	11	0.093
6	5	0.042
7	0	0.0
8	0	0.0
9	3	0.025
10	5	0.042
11	2	0.016
12	3	0.025

Table 3. Distribution of genotype counts and frequencies for D167S83 in 59 unrelated individuals Surabaya population.

Genotype	n	Frequency
1 – 1	3	0.050
1 – 2	19	0.322
1 – 3	6	0.101
1 – 4	6	0.101
1 – 5	5	0.084
1 – 6	1	0.015
2 – 2	1	0.016
2 – 3	2	0.033
2 – 5	1	0.016
3 – 3	2	0.033
3 – 5	1	0.016
4 – 11	1	0.033
4 – 4	1	0.016
5 – 5	2	0.016
6 – 6	2	0.016
9 – 9	1	0.033
9 – 10	1	0.033
10– 10	2	0.016
11– 12	1	0.016
12– 12	1	0.033



D16S83

Figure 4. Resolution of Alleles at locus D16S83 by polymerase chain reaction (PCR) amplification.

Figure 4 above figure demonstrates the results of electrophoresis and silver staining. It compares the phenotypes of 1-2, 1-3, and 1-5 from the alleles at locus D16S83 polymerase chain reaction (PCR) amplification to the ladder of 100 bp.

Finally, the allele phenotypes at locus D16S83 from Surabaya population were compared to those from Caucasian and Afro-Carribean populations as can be seen in Table 4. From Surabaya population, the frequency of phenotype 1-2 was found in 19 individuals (32.2%), while that among the Caucasians the same phenotype was found only in 1 individual (0.08%) and it was not found among the Afro-Carribeans. Conversely, the phenotype 3-4 among the Caucasians was found in 14 individuals (11%) and among Afro-Carribeans was in 2 individuals (4%), while that phenotype was not found in Surabaya population. Similarly, the phenotype 3-6 among the Caucasians and Afro-Carribeans were found respectively in 11 (8.6%) and 1 (2%) individuals, while it was also not found among Surabaya population. Therefore, among the population of Surabaya, the most frequent phenotype of D16S83 locus was 1 - 2, which was found in 19 individuals (32.2%).

Table 4. D16S83 phenotype observed in samples of 59 individuals from Surabaya, Indonesia, 128 Caucasians and 51 Afro - Caribbeans.

Type	No. of Sby-Ind	No. of Caucas	No. of Afro-Car	Type	No. of Sby-Ind	No. of Caucas	No. of Afro-Car	Type	No. of Sby-Ind	No. of Caucas	No. of Afro-Car
1.1	3	2		3.6		11	1	5.7		1	1
1.2	19	1		3.7		5	5	5.8		1	
1.3	6	3	3	3.8		2		6.6	2	3	1
1.4	6	5		3.9		2		6.7		2	1
1.5	5			3.10		1		6.8		1	1
1.6	1	1		3.11		1		7.7		1	
1.9		3	2	3.13			1	7.8		1	1
1.15		1		3.14			1	7.9		2	
2.2	1	1	2	3.15		1		7.11		1	
2.3	2	7	5	4.4	1	1	1	7.13			1
2.4		7	3	4.5		1		8.10			1
2.5	1	3	1	4.6		4	1	9.9	1	1	1
2.6		2	1	4.7		5		9.10	1		
2.7			2	4.8		2		9.13			1
2.8		1		4.9		3		9.14			1
2.9		1	1	4.11	1		2	10.10	2		
2.10		1		4.12		3		10.11			1
2.13			1	4.15			1	11.12	1		
3.3	2	7	1	4.20			1	12.12	1		
3.4		14	2	5.5	2	1					
3.5	1	5	2	5.6		6		Total	59	128	51

CONCLUSION

As a conclusion, the use of buccal cells and polyacrilamyde agarose composite gel for examining 59 individuals provides results with high accuracy. Unrelated Surabaya population has heterozygosity of 74.5%. This percentage is not so much different from that of other populations. The most common gene frequency was allele 1 (36.4%) and allele 2 (21.1%) among alleles ranging from 1 to 12 found in this study. The proportion of phenotype 1-2 was 32.2%, phenotype 1-3 was 10.1%, phenotype 1-4 was also 10.1 % and the others were less than 8.4%.

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