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Research Paper

A Comparative Study of STR Allele Frequencies in Javanese and Chinese Populations Using D5S818, D13S317, and D3S1358

Renny Sumino[1], Mieke Sylvia Margaretha Amiatun Ruth[2*], Alvina Setiawardani[3], I Gusti Lanang Bumi Agung[1]

1. Department of Forensic and Medicolegal, Faculty of Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia.

2. Department of Forensic Odontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia.

3. Forensic Science Programme, School of Health Science, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

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ABSTRACT

Background: The Javanese and Chinese ethnic groups are the most predominant in Indonesia. Saliva presents a major advantage over serum as it may be obtained non-invasively by persons with minimal training. However, there are 13 CODIS loci; hence, it requires more resources and time. This research utilized an observational comparative study design.

Methods: The CODIS system has been validated as an identifying tool in forensics. CODIS was developed to compare target DNA records with those in the database, utilizing software for identification matching.

Results: Saliva from 12 participants aged 17 to 21 was divided into two groups: Javanese and Chinese. Each group comprises six members. The saliva was analyzed using Short Tandem Repeat (STR) and Polymerase Chain Reaction (PCR) at three loci: D13S317, D5S818, and D3S1358. In comparison to the standard DNA K562, variations in base pairs were observed in bands between the Javanese and Chinese races at three loci: D5S818: Javanese alleles are 10, 11, 12; Chinese alleles are 11, 12, 13. D13S317: Javanese 9, 11; Chinese 9, 12. D3S1358: Javanese 16, 17; Chinese 16, 17, 18 Utilizing the three loci from saliva, we can distinguish between the Javanese and Chinese races for forensic identification.

Conclusion: This research successfully demonstrated that the analysis of Short Tandem Repeat (STR) alleles at three loci (D5S818, D13S317, and D3S1358) can distinguish between the Javanese and Chinese populations in Indonesia using saliva samples. Significant differences in allele frequencies were found between the two populations at all three loci, making this method effective for ethnic forensic identification. In addition, the use of saliva as a non-invasive biological sample demonstrates practical advantages in the forensic context, allowing for easier and faster sample collection compared to invasive methods.

*** Corresponding Author:**

Mieke Sylvia Margaretha Amiatun Ruth, MD

Department of Forensic Odontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia.

E-mail: *mieke-s-m-a-r@fkg.unair.ac.id*

Introduction

Fntihistamines Forensic identification is always associated with forensic identification by comparing antemortem data and postmortem data. DNA analysis is an alternative solution to obtain data from victims, especially when the victims have undergone severe decay or decomposition [1]. However, it is quite unfortunate that the DNA database in Indonesia is still very limited, considering the high cost of DNA testing and the dense population of Indonesia. Due to the high population in Indonesia, it is very likely that the country has many ethnic groups living across its various islands [2]. This research uses Javanese and Chinese ethnic groups to compare the loci D5S818, D13S317, and D3S1358.

The Javanese are the majority ethnic group in Indonesia, making up 40% of the total population in the country[2]. Due to each individual having different alleles at each locus, these alleles will be used for identification. Each ethnicity has different allele frequencies, making it possible to distinguish the ethnicity of a population based on the differences in allele frequencies. In addition to the Javanese, the Chinese ethnic group was selected for comparison because it represents one of the largest minority populations in Indonesia, comprising around 1.2–1.5% of the national population, or approximately 3.5–4.5 million people. With a long history of settlement and limited genetic admixture in certain communities, this group offers a valuable contrast to the majority Javanese population, enabling the exploration of potential allele frequency differences for forensic applications in a multiethnic setting [3].

This comparative cross-sectional study uses saliva because it is one of the biological samples that can be easily collected non-invasively. Additionally, human saliva has incorporated the CODIS DNA index system, which has been verified as a tool for identification in forensics [4]. The FBI recommends that in conducting forensic DNA testing, the Short Tandem Repeat (STR) method and Polymerase Chain Reaction (PCR) should be used on 13 CODIS loci [5, 6]. The loci used in this research are D5S818, D13S317, and D3S1358, which were designed by the FBI as the National Forensic Identification System. The selection of these loci is based on their high polymorphism, and all three loci exhibit stability in DNA replication. The findings are expected to contribute valuable data for the establishment of population-specific DNA databases and to enhance the accuracy of forensic odontology identification in Indonesia's multiethnic population.

Materials and Methods

Sample Collection

This research employed an observational comparative study design and was conducted at the Human Genetic Laboratory, Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia. Saliva from 12 participants aged 17-21 was divided into 2 groups (Javanese and Chinese ethnic groups). Each group consisted of 6 participants. Because of the notoriously high cost of DNA testing, this limited sample size was utilized as a preliminary study to establish a baseline proof-of-concept for allele variations between these two ethnic groups. This research has been approved by the Health Research Ethics Graduation Commission of the Faculty of Dentistry, Airlangga University, Surabaya (No. Approval 0971/HRECC.FODM/VIII/2024).

DNA Extraction

DNA extraction was conducted via the organic approach of Phenol-Chloroform-Isoamyl Alcohol (PCI). Fisher Scientific, a division of Thermo Fisher Scientific, located in Geel, Belgium. Saliva samples are transferred to a 1.5µl microtube, to which 1000µl of DNAzol (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA) is added, followed by vortexing for 3-5 minutes and a 15-minute incubation. Chloroform (Merck KGaA, 664271, Darmstadt, Germany) was introduced at a volume of 0.2 ml, vortexed, and allowed to incubate overnight. Following overnight incubation, the sample is centrifuged at 8,000 rpm for a duration of 10 minutes. The supernatant obtained from centrifugation is discarded and transferred to a new 1.5 cc microtube. Incorporate 0.5 ml of isopropanol (EMSURE®, Merck KGaA, 64271 Darmstadt, Germany), homogenize, and incubate for 30 to 60 minutes. Following incubation, the material is subjected to centrifugation at 12,000 rpm for 10 minutes. The supernatant is removed, and 0.5 ml of 70% ethanol is introduced. Incubate the specimen for 30 to 60 minutes. The material is subsequently centrifuged at 12,000 rpm for a duration of 5 minutes. Eliminate the supernatant and introduce 50 µl of aquabidest, then vortex the mixture.

DNA Content and Purity Measurement

The concentration and purity of DNA were measured using UV Visible Spectrophotometry (Nanodrop Lite UV Visible Spectrophotometer by Thermo Scientific, Geel, Belgium) at wavelengths of 260 nm and 280 nm.

DNA Amplification

DNA amplification at the loci D5S818, D13S317, and D3S1358 (Promega Corporation, Madison, USA) was conducted via PCR (BIO-RAD T100 Thermal Cycler) and seen through polyacrylamide gel electrophoresis. Dispense 12.5 µl of Promega PCR Mix (Promega Corporation, Madison, USA) and 2.5 µl of primer for each locus into separate tubes. 7.5 µl of the DNA target was introduced and vortexed. Incorporate Nuclease Free Water (Promega Corporation, Madison, USA) at pH 8.5 to get a final volume of 25 µl.

The PCR technique comprised an initial denaturation at 95°C for 11 minutes, succeeded by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 45 seconds, and extension at 70°C for 45 seconds. A concluding extension was conducted at 60°C for one hour. The primers utilized are presented in Table 1.

Acrylamide Gel Electrophoresis

Acrylamide reagent (Sigma Aldrich) of 3 cc is mixed with 8 cc of 0.5x TBE Buffer (Promega Corporation, Madison, USA). After homogenizing, add 100 µl of ammonium persulfate (APS). Pour the gel mixture into the mold and let it set. After solidifying, the gel is placed in electrophoresis with 0.5x TBE buffer. (Promega Corporaton, Madison, USA). Add the amplified PCR sample. Electrophoresis was connected at a voltage of 100 volts for 60 minutes, and gel staining was performed.

Results

This research was conducted by highlighting the differences in alleles between the Javanese and Chinese populations using three alleles, namely D5S818, D13S317, and D3S1358. Code J represents the Javanese population, while code C represents the Chinese population, with a repetition of 6 samples for each population. Another study used 15 STR loci across four regional populations in Japan (Akita, Nagoya, Oita, and Okinawa). The results showed significant differences between the populations, with the greatest differences found between the Akita and Okinawa populations at 5 loci, and the least differences between Akita and Nagoya at 2 loci [7].

DNA concentration was measured using a UV Visible Spectrophotometer (Nanodrop Lite UV Visible Spectrophotometer by Thermo Scientific, Geel, Belgium) at a wavelength of λ260 nm, while DNA purity was assessed using wavelengths of λ260 nm and λ280 nm. Figure 1 shows the concentration of DNA in each sample.

Table 2 shows that at the locus D5S818, alleles 10, 11, and 12 were detected for the Javanese population, while alleles 11, 12, and 13 were detected for the

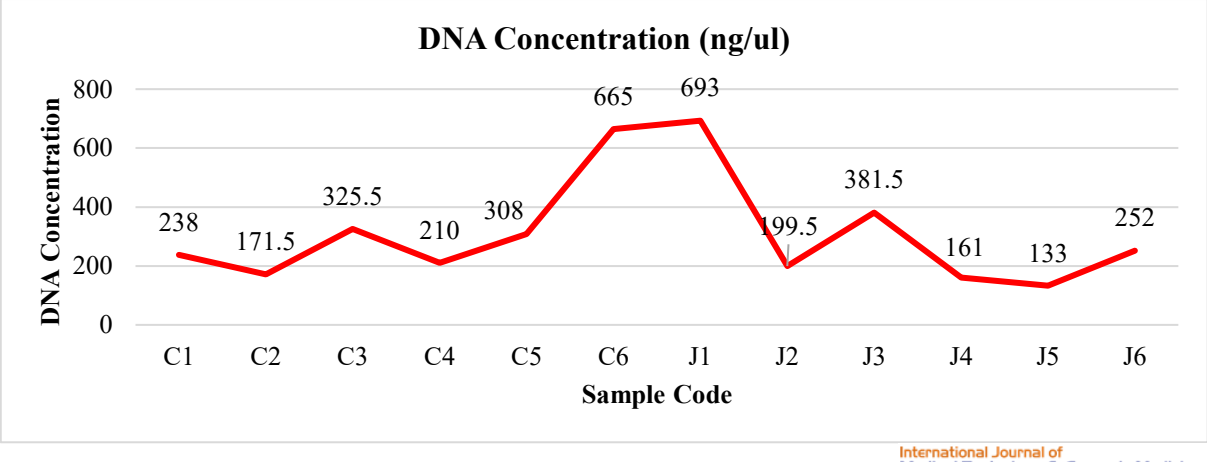

Figure 1. DNA Concentration Graph.

Table 1. DNA Sequence Primer Forward and Reserve.

STR Locus	Chromosomal Location	Chromosome	Repeat Sequence	Allele Size (Base Pairs)
D3S1358	3p21.31	3	TCTA Complex	**96-142**
D13S317	13q31.1	13	TATC	**198-244**
D5S818	5q23.2	5	AGAT	**138-183**

International Journal of
Medical Toxicology & Forensic Medicine

Table 2. Description of Locus and Allele for Javanese and Chinese.

Locus	Allele of Javanese						Allele of Chinese					
	J1	J2	J3	J4	J5	J6	C1	C2	C3	C4	C5	C6
D5S818	10,12	12,13	11,12	10,12	11,12	11,12	11,12	11,13	11,12	11,12	10,12	**12,13**
D13S317	8,8	9,11	9,11	10,12	9,13	10,10	12,12	12,12	9,11	9,11	9,9	**8,10**
D3S1358	17,17	16,18	16,17	17,19	17,18	16,17	16,18	17,17	17,17	16,18	16,18	**17,17**

International Journal of Medical Toxicology & Forensic Medicine

Chinese population. At the locus D13S317, alleles 9 and 11 were detected for the Javanese population, and alleles 9 and 12 were detected for the Chinese population. At the locus D3S317, alleles 16 and 17 were detected for the Javanese population, while alleles 16, 17, and 18 were detected for the Chinese population. Homozygosity was also found in both populations, particularly at the locus D13S317. In the Javan population, homozygosity was found in two samples (J1 and J6), while in the Chinese population at the same locus, homozygosity was found in two samples (C1 and C2), although with different allele combinations.

Discussion

Short Tandem Repeats (STR) are a method of short DNA sequences that repeat approximately 2-6 base pairs, which make up about 3% of the human genome. Individuals have varying numbers of repeat units, which leads to high discriminatory power when analyzed for identification purposes. This method is non-coding, so it does not involve gene expression [8, 9].

This research uses 3 loci to compare alleles using saliva samples at the loci D5S818, D13S317, and D3S1358. The loci D5S818, D13S317, and D3S1358 were chosen because these three loci have a high mutation rate [10, 11]. Other researchers conducted similar studies by comparing several loci in Caucasian, Black, and Hispanic populations. The results show that at the D5S818 locus, there are alleles 11, 12, 13, and 14. These four alleles have a mutation rate of 50% [12]. Another study using the same locus with infrared spectroscopy-based methods showed that this locus has high discriminative ability and resilience [11].

The specific differences are found at each locus. At the D5S818 locus, alleles 10, 11, and 12 were found for the Javanese population, while alleles 11, 12, and 13 were identified for the Chinese population. When compared to previous studies examining Caucasian, Black, and Hispanic populations, which identified alleles 11, 12, 13, and 14 at the D5S818 locus, our findings indicate a distinct distribution. Specifically, allele 10 appears unique to the Javanese group in this context, while allele 14 was absent in our Indonesian samples. Furthermore, a previous study utilizing infrared spectroscopy confirmed the high discriminative ability and resilience of the D5S818 locus, which aligns with the distinct allelic separation we observed between the Javanese and Chinese populations [13].

At the D13S317 locus, alleles 9 and 11 were observed for the Javanese population, and alleles 9 and 12 for the Chinese population. At the D3S1358 locus, alleles 16 and 17 were found for the Javanese population, whereas alleles 16, 17, and 18 were present for the Chinese population. This shows the difference in allele frequency between the Javanese population and the Chinese population at the three loci.

To place these findings within the broader context of the Indonesian demographic, previous research by Manela et al. on the Nias population and Prastowo et al. on the Madurese ethnic group has also demonstrated the presence of specific local STR variations. Just as studies on the Japanese populations (Akita, Nagoya, Oita, and Okinawa) found significant regional allelic differences, our results similarly prove that genetic variations exist even between communities living within the same national borders. In addition, the majority of the alleles obtained in our study are heterozygous alleles. This proves that there is a high level of discrimination and mutation. The more alleles obtained, the higher the heterozygous value that occurs at that locus, which is consistent with findings from the Nias population study regarding the high discriminatory power of these loci in the Indonesian context

A notable limitation of this study is the small sample size, comprising only 12 participants divided equally into two groups (n=6 per group). This constraint is primarily due to the high cost associated with DNA testing and profiling. However, this preliminary study successfully establishes a foundational proof-of-concept regarding allele variations between the Javanese and Chinese populations at the D5S818, D13S317, and D3S1358

loci. As comparative analyses of allele variations are known to be influenced by sample size, the findings from this initial research strongly emphasize the necessity for future studies. This research opens up opportunities for additional exploration with a much larger population to build a more comprehensive, accurate, and population-specific DNA database for forensic identification.

Conclusion

This research successfully demonstrated that the analysis of Short Tandem Repeat (STR) alleles at three loci, namely D5S818, D13S317, and D3S1358, can distinguish between the Javanese and Chinese populations in Indonesia using saliva samples. Significant differences in allele frequencies were found between the two populations at all three loci, making this method effective for ethnic forensic identification. In addition, the use of saliva as a non-invasive biological sample demonstrates practical advantages in the forensic context, allowing for easier and faster sample collection compared to invasive methods. This finding has the potential to support the development of a DNA database in Indonesia, which is still limited, while also providing a foundation for broader applications in DNA-based ethnic identification. Furthermore, this research opens up opportunities for additional exploration with a larger population and the use of more loci to enhance the accuracy and scope of forensic identification in the future.

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None.

Conflicts of Interest

The authors report there are no competing interests to declare.

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