The use of Kinship Analysis on Paternity Testing Through CODIS STR LOCI 'CSF1PO' and 'THO1'

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ORIGINAL ARTICLE

The Use Kinship Analysis on Paternity Testing Through CODIS STR LOCI 'CSF1PO' AND 'THO1'

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ABSTRACT

Introduction: Paternity test compares a child's DNA pattern with the possible parent to examine the DNA bonds in ensuring kinship. If there is no information from the parents or the child that can be used as a comparison in the forensic DNA examination process (paternity test), there must be a comparison from a close relative as an alternative to obtain the forensic DNA examination. We aim to analyze the use of kinship analysis in forensic identification especially in a paternity test.
Methods: This was a cross-sectional study through locus analysis of DNA forensic examination in paternity test using the kinship analysis through STR CODIS loci: CSF1PO and THO1. Paternity tests were conducted in 8 subjects whom are siblings; the test was administered on the CSF1PO and TH01 loci.
Results: This experiment displayed similar allele numbers on the same locus, both in 50% and 100% allele numbers; the research showed similarity in allele numbers of both siblings of which ¼ were inherited from the parents (for 50% allele number similarity), and ½ were inherited from the parents (for 100% allele number similarity).
Conclusion: This proves that a paternity test using siblings as the closest kin (kinship analysis) can be used as an alternative if no comparison is obtained from both the parents.

Introduction

In Indonesia, there have been many calamities such as natural disasters, bomb explosions, and murder cases that suffered many casualties. In those situation, a forensic medical examination must certainly be performed to determine the type of injury and the cause of violence, as well as the causes and mechanisms of death; in addition, which is not less important, it is used for identifying both the victim and perpetrator.1

DNA identification can be used to determine the biological relationship between individuals in a family by comparing their DNA patterns, as in the paternity test.1 Data from Forensic and Medicolegal Medicine Installation at Dr. Hospital Soetomo Surabaya stated that there were 145 patients who had consulted for paternity tests during the last three years (2016 – 2018, taken from the Register Book). Moreover, the data in the ITD (Institute Disease Center) Human Genetic laboratory of Airlangga University revealed that the number of patients who undergo paternity test has been increased since 2010; there were 30 patients who were examined in the period 2010-2016 (Data Lab HG ITD). DNA identification for paternity tests is done by analyzing DNA patterns using STR (short tandem repeat) markers. STR is a DNA locus composed of 2-6 base repeats. In the human genome we can find repeating bases that vary in number and type. DNA identification with STR markers is one of the most sensitive DNA testing procedures because STR markers have a high level of variation both between STR loci and between individuals.2

In the analysis of DNA identification, which is recommended is the examination of 13 locus of Short Tandem Repeats known as Combined DNA Index System 13 (CODIS 13). CODIS 13 is used because by examining these 13 loci it is found that the identification accuracy or accuracy of determination is very high, which is close to 100%. CODIS 13 analysis that is very fast and precise and widely used makes comparison of DNA analysis between different laboratories around the world possible.3

In this study, the loci to be analyzed were the Short Tandem Repeat (STR): CSF1PO and THO1, which are some of the 13 loci set by the Federal Bureau of Investigation (FBI). Another reason for choosing them is because it is known that they undergo large

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discrimination in the Indonesian population.

Methods

This was a descriptive research with the cross-sectional of paternity test by using kinship analysis through STR CODIS loci: CSF1PO and THO1. The research samples were the DNA of volunteers consisting of an individual with the siblings. There were 4 voluntary families (subject and siblings). The inclusion criteria include siblings as evidenced by authentic documents and samples from the same ethnic group. While the exclusion criteria are having the disease in the lip mucosa.

The process of collecting data was conducted through the process of extracting DNA of the samples from inner cheek mucosal swabs that soaked in destilated water, centrifuged, and then mixed with DNAzol reagent (Invitrogen) then be washed with ethanol to obtain pure DNA according to amnufacture's protocol, measuring the level and purity of DNA through a UV-spectrophotometer, PCR amplification for loci THO1 and loci CSF1PO with Primers A and B, and electrophoresis with polyacrylamid agarose composite gel electrophoresis procedure. The data from the results of DNA electrophoresis readings were analyzed after previously tabulated to map the accuracy of each locus in the kinship DNA analysis. Then, the same allele numbers were compared at each locus to siblings.

Results

DNA Level and Purity Measurement

Table 1. Displays the results of DNA level examination after DNA purity before PCR amplification using the UV-Spectrophotometer.

No.	Sample Code	DNA Level (ng/ul)	DNA Purity
1	B1	423,5	1,22
2	B2	595	1,23
3	C1	416,5	1,29
4	C2	518	1,17
5	D1	798	1,25
6	D2	504	1,24
7	F1	423,5	1,26
8	F2	339,5	1,28

Table 1 shows the levels of human DNA isolation in all samples at the wavelength (λ) of 260 nm and λ 280 nm, with the numbers between 339.5 µg / ul to 798 µg / ul, and with the mean levels of 502.25 µg / ul; whereas the purity of the isolated DNA's value is between 1.17 to 1.29.

The DNA level and purity of all samples are more clearly indicated by the following curve, in which samples with D1 code have the highest DNA content and samples with F2 code have the lowest DNA content; and the highest DNA purity is owned by the sample C1 code, while the lowestis of C2 code.

Stroke patients' disability was measured by Barthel index. Table 3 explained that patients with total dependency and not optimized LDL level were 75%, patients with severe dependency and not optimized LDL level were 58.3 %, patients with moderate dependency and not optimized LDL level were 75%. Patients with low Barthel index score (slight dependent and independent) were only 10% of all patients whom Barthel index score were recorded.

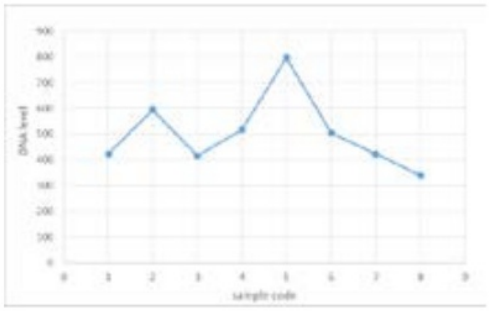

Figure 1. The curve of all DNA level samples

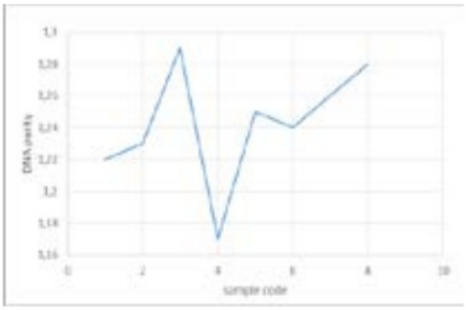

Figure 2. The curve of all DNA purity samples

The Results of DNA Visualization

CSF1PO locus is between 321 - 357 bp (base pair). Furthermore, using K562 marker as a positive amplification control to determine the location of the allele number, in which this locus shows the allele number.[9,10] On the other hand, the locus TH01 is between 156 - 195 bp and shows allele numbers (9.3, 9.3).

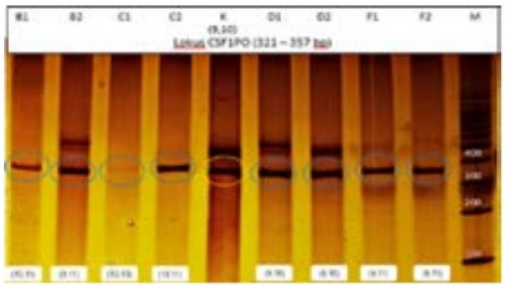

Figure 3. The visualization of human DNA isolation results from inner lip mucous swabs using the CSF1PO locus (the sizes range between 321 - 357 bp)

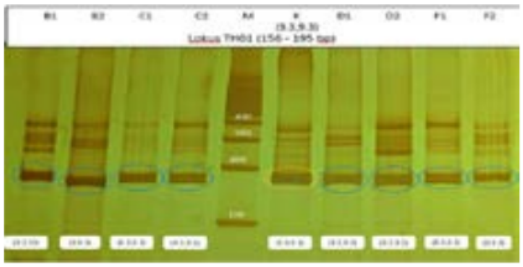

Figure 4. The visualization of human DNA isolation from inner lip mucous swabs using TH01 locus (the sizes range between 156 - 195 bp)

Table 2. The allele numbers of the CSF1PO locus in all samples.

Sample Code	B1	B2	C1	C2	D1	D2	F1	F2
Allele Number	(10,11)	(9,11)	(10,10)	(10,11)	(9,10)	(9,10)	(9,11)	(9,11)

Table 3. The Allele numbers of TH01 locus in all samples.

Sample Code	B1	B2	C1	C2	D1	D2	F1	F2
Allele Number	(9.3,10)	(9,9.3)	(9.3,9.3)	(9.3,9.3)	(9.3,9.3)	(9.3,9.3)	(9.3,9.3)	(9,9.3)

Discussion

A DNA test using parents as the comparison results in statistically close to 100% or around 99.99% similarity. Among all kinship identification, the DNA analysis of biological parents always obtains more significant results than that of other kinship relationships. In certain conditions, a comparison having a close family relationship is needed as one of the ways taken in the process of DNA forensic analysis, such as the siblings of victims or perpetrators, if the comparison from the parents and descent is not obtained.

The comparison of the DNA superiority from two siblings thought to produce a likelihood ratio or a combination of sibling index (SI) becomes a favorable opportunity for tested samples, compared to samples obtained from two individuals who are not genetically related. Theoretically, siblings have the statistically possible 25% accuracy of 2 alleles; and the same goes with its inaccuracy (does not have the same allele or 0 alleles).

The amount of DNA needed in a forensic DNA analysis varies depending on the need and type of the examination; an examination with this STR requires a minimum DNA concentration of between 1-25 µg. It is not only influenced by the levels and purity of DNA, but it also needs the examination materials with sufficient DNA quality, videlicet, samples with minimum degraded DNA.[4]

The PCR amplification method provides convenience; because with minimal DNA levels it can still be done and the DNA visualization results can still be obtained. From several studies on forensic DNA examination, it was reported that the use of PCR in DNA amplification has a very high success rate because the required DNA is relatively minimal compared to an amplification using the RFLP (Restriction Fragment Length Polymorphism) technique. Even though the available sample is not fresh, it can still be amplified using PCR.

In this study, two loci were examined in all samples, viz. the CSF1PO locus and TH01 locus. The CSF1PO locus is one of the thirteen core loci used for the CODIS database; and alleles reported for this short tandem repetition locus (STR) contain 6 to 15 repetitions of AGAT tetranucleotide. CSF1PO is found in proto-oncogene c-fms for CSF-1 receptors on long arms chromosome 5 (5q33.3-34). This locus is between 295 - 327 basepair. Common alleles contain core repetitions (core sequence repeat) T-A-G-A and alleles range from 7 to 15.[5-7].

Furthermore, the TH01 locus is a repetition of simple tetranucleotides found in intron 1 of the tyrosine hydroxylase gene on the short arm of chromosome 11 (11p15.5). TH01 has a simple series with the repetition motif of T-C-A-T. This locus is between 179 - 203 basepair with allele variations between 5 to 11. TH01-STR is one of the most widely used markers in forensic caseworks. This is a mandatory locus in many forensic databases (for example, CODIS in the US, DAD in Germany, English Forensic Databases in the UK); thus, it has been included in most commercially available multiplex PCR kits.[5, 8]

The results of the study reveal that from the two siblings examined at the CSF1PO and TH01 loci, there were similar allele numbers on the same locus, both in 50% and 100% allele numbers; the similarity of allele siblings was ¼ inherited from the parents (for 50% allele number similarity), and ½ was inherited from the parents (for 100% allele number similarity).

Conclusion

This study sugests that a paternity test using siblings as the closest kin (kinship analysis) can be used as an alternative if no comparison is obtained from both the parents.

Conflict of Interest

The author stated there is no conflict of interest

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