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STR LOCUS MUTATIONS IN PATERNITY CASE

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Abstract

DNA analysis is widely applied in solving forensic cases, especially Short Tandem Repat (STR) because of its advantages. In identifying the individual, the National Police compared the individual's DNA with that of his parents. Each anal has a pair of DNA fragments of which half are inherited by the father and the remainder by the mother according to Mendel's Law of Segregation. In this study, we compared DNA typing between the child and the mother with the help of PCR extracted by the Chelex method to find the mother fragment and obtain the father fragment. A child is the biological child of the alleged father if he or she has less than 2 exclusion STR loci. The results of this study revealed that all paternal fragments from the child were identical to the DNA fragments of the alleged father, except for one locus, namely CSF1PO which had a mutation. Mutations in the STR locus lower the paternity index, although it can still be concluded that the child is the biological child of the alleged father.

***Keywords**: Paternity Test, DNA, STR, Mutation*

1. INTRODUCTION

The Indonesian National Police usually uses individual identification through DNA as a *cross-check* in proving forensic cases. DNA is an excellent marker for differentiating one individual from another, this is because everyone has different DNA obtained from both parents, except for the case of identical twins. The principle of identification through DNA is based on the process of allele comparison between the allele of the victim or perpetrator and the allele of the family line, especially parents by following Mendel's Law (Yudianto, Setiawan, and Sumino 2021). A child must have a DNA fragment from the father and a DNA fragment from the mother. A child is the biological child of the alleged father if each STR locus of comparison matches, and not a biological child if there are two or more STR loci that do not match (Atmadja and Untoro 2008). However, due to certain reasons, sometimes DNA has different results than expected, which in turn leads to different conclusions from paternity tests. The presence of mutations can cause *false negatives* in the kinship relationship, or in this case, paternity. This will be considered by the father as an exclusion criterion, because he does not donate his allele to the child or does not have the allele that the child has.

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2. FORENSIC DNA

Deoxyribo Nucleic Acid (DNA) is a macromolecule in cells composed of several nucleotides One nucleotide consists of an organic base bound to a deoxy-ribose sugar which has a bond with a phosphate group at the end of carbon 5. The organic base consists of two major groups, namely purines and pyrimidines. Purine bases consist of adenine (A) and guanine (G), while pyrimidine bases consist of cytosine (C) and thymine (T). DNA has an end-to-end direction based on the bonds at the free end of the deoxyribose sugar. The phosphodiester bond is at the 5' end of the sugar, while the hydroxyl bond is at the 3' end, this indicates the direction of DNA reading is 5' 3'. Human DNA consists of two polynucleotide strands that form a double strand. The sugar-phosphate structure is on the outside of the strand, while the bound bases are on the inside of the strand (Lodish, Berk, and Kaiser 2008).

Picture 1. *Deoxyribonucleid Acid* (NHGRI nd)

In human cells, DNA is found in the nucleus (*nuclear*) and mitochondria (mtDNA).DNA is *Nuclear* stored in the form of chromosomes guarded by histone proteins. One human cell has 22 pairs of autosomal chromosomes and one pair of sex-determining chromosomes (X and Y). Men have XY chromosomes, while women have XX chromosomes. One pair is obtained from the mother and one pair is obtained from the father, while the mtDNA is only passed from the mother to the child. The DNA material in a chromosome consists of a "coding" region and a "non-coding" region. Coding regions are called genes that carry the information that cells need to make proteins. Genes consist of exons (parts of protein coding) and introns (regions between them). *The Human Genome Project* reports that the gene content in the human genome is only 5%, and the rest are non-protein coding regions. The markers used for identity determination were found in the non-coding regions, both those that were present and did not code for being in the gene (intron) and did not code for genetic differences (JM Butler 2010).

Figure 2. Human Genome Structure (JM Butler, 2010).

3. PCR

Polymerase Chain Reaction (PCR) is a technique that is widely used in molecular biology applications because of its ability to reproduce a small number of specific DNA fragments. PCR is a method of enzymatic DNA synthesis using two oligonucleotide primers that hybridize on opposite strands of template DNA. The PCR reaction consists of several cycles, in each cycle there is a denaturation process (occurring at 95oC ^{to}separate double-stranded DNA), primer attachment (*annealing* at a temperature of around ^{60oC}so that the primer can be fused and complemented with the target DNA sequence) and elongation. (*extension* at 72 ^{oC}, because Taq polymerase functions optimally at this temperature) of the primers that are attached by DNA polymerase, in which a nucleotide is added at the 3 end of

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each primer attached to the DNA strand. The result of these processes is a specific set of fragments whose 5' ends are determined by the primers. The amplification causes the primary elongation result from one cycle to act as a template for the next cycle, so that the number of target copies doubles in each cycle (Alberts et al. 2002).

Figure 3. Thermal cycling temperature for PCR (Esssam *et al*., 2020)

Identification of individuals based on DNA is currently evolving by analysis of nuclear DNA and mitochondrial DNA. Mitochondrial DNA analysis utilizes *single nucleotide polymorphism* (SNP) which is found in hypervariable regions of the D-loop (*displacement loop*) of mitochondrial DNA, while nuclear DNA analysis utilizes microsatellite or *short tandem repeat* (STR) found along chromosomes (JM Butler 2015). STR is a repeating DNA sequence like a minisatellite, but with a smaller repeating unit size, i.e. 1-5 base pairs. Therefore, STR is more efficient to be amplified using PCR. Polymorphisms in STR were found in differences in the number of repeating units between individuals, hereinafter referred to as alleles (Driscoll et al. 2002). The sequence of STR repetitions can be grouped based on the number of base pairs in a repeating unit, namely mononucleotides (one base pair repeating each other), dinucleotides (two base pairs), trinucleotides (three base pairs), tetranucleotides (four base pairs), pentanucleotides (five base pairs) and hexanucleotides (six base pairs).

STR is also grouped based on basic repetition patterns, namely: 1) simple repetition, composed of units of the same length, 2) compound repetition, consisting of two or more simple repetitions, and 3) complex repetition, composed of several blocks of different unit lengths. and also has an intermediate arrangement of nucleotide bases. Not all alleles at the STR locus have an intact repeating unit. This allele is referred to as a microvariant allele, i.e. an allele consisting of one incomplete repeating unit. For example, at the TH01 locus there is an allele 9.3 which is composed of 9 tetranucleotide repeats and one incomplete repeat of trinucleotide (JM Butler 2015).

The location of a gene or DNA marker on a chromosome is called a locus (compound: loci). A pair of chromosomes is said to be homologous if they have the same size and genetic structure. The DNA arrangement of a pair of homologous chromosomes is not always identical, this is due to the possibility of mutations over time. One chromsoom from each pair is passed down from each Father and Mother. Other possible forms of a gene or genetic locus are referred to as alleles. If the two alleles at the genetic locus of a homologous chromosome are not identical, it is called an heterozygous, whereas if the two alleles are identical it is said to be homozygous (John Butler 2005).

Naming DNA markers aims to equalize the mention of the position of a target DNA. If a marker is part of a gene, then the gene is used as a name, for example, the STR marker TH01 comes from the tyrosine hydroxylase gene which is

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located at the position of chromosome 11. The number 01 is obtained from the position of the repeating unit in intron 1, whereas if the marker is not located in the gene, the determination of naming is based on the position on the chromosome. For example, the STR marker D16S539, "D" indicates DNA, "15" indicates a chromosome, "S" indicates a single copy*and*"539" indicates the 539th locus position on chromosome 16 (JM Butler 2015).

Individual identification based on STR DNA requires information from the whole genome, therefore the STR markers used for identification combine STR markers from different chromosomes or in the same chromosome but at large distances to avoid linking between markers. In addition, to equalize the STR markers used so as to facilitate the exchange of DNA profiles between countries, in 1997 an agency in America, namely the *Federal Bureau of Investigation* (FBI) determined the STR markers to be used in CODIS (*Combined DNA Index System*). . There are 13 STR markers, namely CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D7S820. D8D1179, D13S317, D16S539, D18S51 and D21S11. However, countries in Europe through the *European Network of Forensc Science Institute* (ENFSI) set 7 core STR markers, namely TH01, vWA, FGA, D8D1179, D18S51, D21S11 and D3S1358. Along with the increasing use of DNA in individual identification, some researchers have increased the polymorphism between individuals by adding several STR markers (JM Butler and Hill 2012).

(a)

(b)

Figure 4. STR markers used internationally (a). FBI-United States-designated STR loci markers, (b) ENSFI-European STR loci markers

4. PATERNITY TEST

Paternity test is a DNA test to determine whether a man is the biological father of a child or not. Paternity studies are used in forensics to solve crime problems. In addition, a paternity study was conducted for DNA testing between parents and their children. This paternity test is performed by analysis on a single DNA locus usually with at least 13 autosomal STR markers located on different chromosomes. The probability obtained in the paternity test if it has a value of 99.9% then it is proven that the parents and children have a biological blood relationship. This was done by observing two genetic loci and compared with the alleles possessed by these loci (Kudekar et al. 2020).

The test used in the paternity test is also the identification process of the 16 STR nuclear locus with the position of the chromosome. This locus was identified in each individual (Essam, Hamza, and Diab 2020). However, the currently used paternity test is to compare 24 loci using the GlobalFiller amplification kit. The more loci are compared, the more accurate the results will be (Boavida et al. 2018). After identification, the DNA profile files of the suspected father and mother are compared with the DNA profiles of the child. There are five steps in the paternity testing process.

These five steps are collection, DNA extraction, quantification, amplification, and STR analysis (Essam, Hamza, and Diab 2020).

A paternity test can be done to find out about someone who is accused of being the biological parent of a child, because a child has genes from both his father and mother. Every cell in the human body has 24 pairs of chromosomes. In the parent cell, sperm and egg, there is a division called meiosis so that the 24 pairs of chromosomes separate. Stem cells produce sperm cells or ovum cells which have 24 chromosomes. At fertilization the father's sperm cell (24 chromosomes) will unite with the mother's ovum cell (24 chromosomes) so that the chromosomes from the father will pair up with the chromosomes from the mother and form a zygote. It is at this time that the sequence of *Deoxyribo Nucleic Acid* (DNA) from father and mother is passed on to their children, where each party contributes 50 percent to the child's DNA.

4.1 Paternity

Index The paternity index (PI) compares the probability that a genetic marker (allele) and presumed paternity (AF) are passed on to the child with the probability that a randomly selected male from the same ethnic background can pass the allele to the child. This is presented by the X/Y formula, where X is the probability that the alleged father can transmit the obligatory allele and Y is the probability that several other males of the same race can transmit the allele. X is assigned a value of 1 if AF is homozygous for the desired allele and 0.5 if the presumed father is heterozygous. The product of the paternity index (PI) is the combined paternity index (CPI), ie the combined paternity index is determined by multiplying the individual PIs for each locus tested (Essam, Hamza, and Diab 2020).

$$PI = (1)$$

If the alleged father has homozygous alleles or has both obligate alleles, the probability that he will transmit alleles is x = 1.0 (2/2). However, if the alleged father has only one copy of the ligand allele or one of the two obligate alleles, then the probability of transmitting the obligate allele is x = 0.5. (1/2). *The paternity index* for a given locus allows the paternal conjecture to give the allele divided by the frequency of the number of obligate alleles. Thus, the PI will be 1/p or 0.5/p. If there are two obligate alleles as determinants possessed by the mother and child, p can be calculated by the formula = $p_1 + p_2$, where PI is $1/(p_1 + p_2)$ or $0.5/(p_1 + p_2)$ (Tiger , Uvodiæ, and Botica 2003).

5. MUTATIONS

Mutations at the STR locus are known as non-inherited alleles following Mendel's laws. Mutation is a condition where there is an addition or repetition spacer (STR) resulting in a mismatch between the sample being tested and the sample to be compared (Rodriguez et al. 2008). Mutations in STR occur more than the unique DNA sequences in the genome, which is about 10^{-6} to 10^{-6} nucleotides per g

generation (Ellegren 2000). A large body of data on identified mutations is for the autosomal STR locus (Mustafayev 2020).

The possible mechanism for mutations in STR can be in 3 ways, namely (1) unbalanced crosses during the meiosis process, (2) retrotransposition mechanisms and (3) strand slip replication (DNA slippage). However, of the three mechanisms, strand slippage in the replication process is the most important accus that causes STR mutations. The presence of slippage occurs during the DNA replication process as a result of incorrect pairing (by one or more STRs) between the newly replicated strand and the *template.* Next, the STR fragment is forced to exit the mismatch. As a result, if DNA synthesis continues, there will be a change in the number of STR repeats, either increasing or decreasing. However, the slip rate is not the same as the mutation rate of the STR, where the mutation rate in the STR depends on the slip rate and efficiency of repairing the mismatch (Fan and Chu 2007).

Several factors affect mutations, namely (1) Repetition, where the smaller the repetition of the unit, the easier it will be for mutations to occur. This is because the larger the repetition unit, the smaller the number of STRs. Longer iterations require the chain to slip further before the bases can pair properly again (Kruglyak 1998). In addition, the number, location and sequence of repeats also influence the occurrence of mutations by influencing the rate and direction of slippage (Schlotterer 1998).

6. RESEARCH METHODS

Samples of children, mothers and suspected fathers were put into Eppendorf tubes and extracted according to the Chelex 5% method from a mixture of 5 g Chelex in 100 mL of TE Buffer solution pH 8.0. TE Buffer solution is a mixture of 0.61 g *Tris Base* and 0.0186 g EDTA in 500 mL ddH_2O. Next, 200 L *Chelex* 20% and 10 L proteinase K 10 mg/µL were put into a sample tube and*vortexed* at low speed. The sample was then transferred to a *thermomixer* with a temperature of 57 ^oC and a speed of 900 rpm for half an hour. The sample was then transferred to *heating block* 100 ^oC for 8 minutes, then centrifuged at a speed of 13000 rpm for 3 minutes. The supernatant was transferred to *tube* a 1.5 mL ^oC before further processing.

The next stage is the amplification stage, amplification of 24 STR loci was carried out using PCR *System 9700* (Applied Biosystems) and *Global Filer*^{TM} *PCR Amplification kit* (Applied Biosystems, AB). For every 25 L of the reaction, 7.5 L *PCR* GlobalFiler® *Master mix*, 2.5 L GlobalFiler® *Primer set* (*forward* and *reverse*) and 15 L (0.5-1 ng) *template* DNAThe negative control sample contained 15 L *low-TE buffer*, while the positive control sample contained 10 L *DNA control* and 5 L *low-TE buffer*. Previously, *the Master mix* and *Primer set* to be used were*vortexed* for 3 seconds before being used. 7.5 L *Master mix* was mixed with 7.5 L *Primer set* into a *tube* 2.5 L*vortexed* for 3 seconds. The mixture was then centrifuged at 3000 rpm for 20 seconds. *GeneAmp 9700 thermal cycler* (Applied Biosystem) was used for the amplification reaction with the following PCR conditions: *hot start* at 95

^{oC}for 1 minute; performed amplification consisting of 29 cycles with denaturation at a temperature of 94 ^{o}C for 10 seconds, *annealing* and extension at a temperature of 59 ^{o}C for 90 seconds; final extension at 60 ^{o}C for 10 min; and *hold* at 4 ^{o}C.

The PCR products were separated by capillary electrophoresis using a *Genetic Analyzer* 3500 (AB). The reaction mixture consisted of 9.6 L *Hi-Di Formamide* and 0.4 L *GeneScan*^{TM} 600 *LIZ® Size Standard* v2.0 for every 11 L total volume with 1 L of sample PCR product. Each electrophoresis process used an *allelic ladder* in one well on a *plate* of 1 L. DNA fragment analysis was performed using the GeneMapper® IDX version 1.4 (AB) program. The sample and *reference* were identified by the STR process, where a 96-*well reaction plate* was placed on the *base plate. The reaction plate* was then covered with a 96-*well separator* and centrifuged at 3000 rpm for 1 minute. *The reaction plate* denatured with *a thermal cycler* at a temperature of 95 ^{o}C for 3 minutes and placed in a *freezer* at a temperature of -25 ^{o}C for 3 minutes. Samples were analyzed using ABI 3500XL *Genetic Analayzer using GenoTyper 2.5.2 software* (*Applied Biosystem*), the results of the *Capillary Electrophoresis* were analyzed using *GeneMapper ID-X Software v1.4* (*Applied Biosystem*) .

The final result is an electroferogram containing a graph from the *Capillare Electrophoresis Genetic Analyzer 3500 Abi*. The results will show 27 loci from one individual where each locus contains 2 allelic fragments (heterozygous) or 1 allele fragment (homozygous). The graph shows the size of the DNA fragment in basepair units (bp) and the RFUs (*Relative Fluorescent Units*) value will be shown. Furthermore, the procedure for identifying allele similarity between the *evidence.*

7. RESULTS AND DISCUSSION

Paternity testing using STR loci derived from nuclear DNA inherited from mother and father according to Mendel's Law. Each child has a pair of DNA fragments, one inherited by the mother (*maternal fragment*) and the other by the father (*paternal fragment*) (John Butler 2005). A child is declared the biological child of a man suspected of being the father if each STR locus has a suitable ratio (Atmadja and Untoro 2008).

The probability of the alleged father can be calculated using the existing loci, these loci are evaluated to determine whether the individual suspected of having a kinship with the suspect, in this case will produce a paternity index. All paternity indices from each locus were multiplied to obtain the combined paternity index (CPI), and calculated using Bayes' theorem, assuming a 50% probability of allele contribution from the father as a result of Mendel's Law of Segregation.

Table 1 shows DNA profiles containing 24 STR marker loci in blood samples from children and both parents. The red color indicates that the allele is from the father, while the blue color indicates that the allele is from the mother.sample *reference*

has red and blue alleles simultaneously, which indicates that the child's allele is obtained from 50% of the maternal allele (*maternal* fragment) and 50% of the father's allele (*paternal fragment*).

Table 1. DNA profile of child, mother and suspected father

STR locus	of child		's mother		Suspected father		*Conclusion*
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	
D3S1358	13	16	13	17	15	16	*match*
vWA	14	18	14	18	17	18	*match*
D16S539	11	12	11	12	11	14	*match*
CSF1PO	10*	10	10	12	11	11	***exclusion***
TPOX	8	8	8	9	8	11	*match*
Yindel	-		-		2		
D8S1179	13	15	13	13	11	15	*match*
D21S11	29	31	31	32.2	29	31.2	*match*

D18S51	13	15	13	16	1
DYS391	-		-		
D2S441	11	12	12	14	1
D19S433	14.3	15	15	15	13
TH01	7	9	7	9	7
FGA	23	24	23	24	2
D22S1045	15	16	16	17	1
D5S818	10	11	10	13	7
D13S317	9	10	8	10	8
D7S820	11	12	11	13	1
SE33	18	26.2	26.2	26.2	1
D10S1248	15	16	14	15	1
D1S1656	17	18.3	17	17.3	1

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D12S391	18	19	17	18	18	19	*match*
D2S1338	25	25	25	25	22	25	*match*
Amelogenin	X	X	X	X	X	Y	*match*

Figure 5. Electropherogram data at four loci

Based on the DNA profile in Table 1 and the *probability of paternity* in Table 2, the alleged father cannot be ruled out as the biological father of the alleged child. A child is the biological child of the presumed father if each STR locus matches the comparison. On the other hand, a child is not the biological child of the suspected father if there are 2 or more STR loci of exclusion criteria. This is because the probability of paternity will be below 90%, so it is certain that there is no blood relationship between the child and the parent (Esssam, Hamza, and Diab 2020).

Table 1 shows that of the 24 different genetic systems analyzed by PCR, the suspect matched the suspected paternal allele at the 23 STR loci examined, except for one exclusion criteria locus, namely the CS1FPO locus which may be caused by mutations. At this locus, suspected mother had alleles (10.12), suspected father (11.11) and child (10.10). Supposedly, the child has 11 repetitions of Father and 10 or 12 repetitions of Mother. However, in this case there were 10 repetitions in both daughter alleles, it can be concluded that there was a mutation from one repetition from the father.

Mutations can occur at the STR locus, as is the case for DNA at any region, and the STR allele can change over time. This is because, for a given STR locus, all currently known alleles are alleles acquired from previous individuals (John Butler 2005). In addition, the mutation rate in STR is higher than in conventional genetic markers (Zhuo et al. 2015).

The CSF1PO locus is a repeat of a tetranucleotide compound found in the sixth intron of the c-mfs protooncogene locus on the 5th arm of the chromosome with the repeat form [AGAT]. CS1FPO is an STR locus with a relatively high mutation rate compared to other loci, which is about 0.16% in the entire human population with 0.03% maternal meiosis and 0.15% paternal meiosis (AABB 2003). This can provide the basis that this case is a case of mutation, and therefore the alleged father cannot be excluded as the biological father. This case demonstrates the fact that mutations in STR markers can lead to false exclusion at these loci, especially if the allele mutation is high enough (John Butler 2005). Mutation limits are usually only less or more than 1 of the alleles of both parents. All researchers also agree that it is impossible for two mutations to occur at two different STR loci, this is because during meiosis 2 mutations are not possible (Nurhantari and Suryadi 2019).

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Some of the factors that can affect the mutation rate are number, units. repetition structure, basic unit composition, recombination and DNA base sequence (Nurhantari and Suryadi 2019). A reduction in the number of repetitions usually occurs in a long STR, while an increase in the number of repetitions usually occurs in a shorter STR (Lee et al. 1999). AT nucleotide bases mutate faster than GC (Lai and Sun 2003), this is due to the strong hydrogen bonds in GC bases, thereby reducing the frequency of *strand-slippage occurrences.*

Figure 6. Slip mechanism in DNA replication (Jobling, Hurles, and Tyler-Smith 2005)

Interpretation of paternity tests can be influenced by mutations that occur during meiosis resulting in differences at loci. In this case, at the CS1FPO locus there were allele differences in the alleged father, the suspected mother and the child. The mother has the allele (10.12) and the father has the allele (11.11), while the suspect has the allele (10.10). By comparing the data at this locus, it can be seen that the suspect can be assumed to be not the biological child of the alleged father, this is because the alleged father does not have the allele 10 at that locus. Half of the pairs of 10 alleles in the actor inherited from the mother indicate that the mutation occurs in the father, where the father's 11 allele loses one repetition, resulting in the child getting 10 alleles.

Mutations are most likely to occur at the microsatellite loci of male origin, therefore the mutation rate at most loci in germ cells is generally higher (Fan and Chu 2007) in the form of one-step event mutations at the STR locus that would cause a person's repeat to shift. approximately one step from the original value (Atmadja & Untoro, 2008; Vol *et al*., 2018). This is because sperm undergo more DNA replication cycles than egg cells so that they produce a higher frequency (Shimmin, 1993 in Vol *et al*., 2018). Therefore, at the time of the mutation, the suspect could have the allele 10 even though the suspect's father did not have it.

This difference can be caused by the non-shared alleles of the father to the child due to Mendelian Law of Segregation, where alleles of the alleged father segregate independently (unbalanced crossings during recombination) or due to inaccurate pairing during replication due to DNA slippage (Vigouroux et al. al. 2002). The molecular mechanism by which STR mutates is thought to most often be due to slippage in replication due to unbalanced crossing over during meiosis and retotransportation mechanisms (John Butler 2005; Fan and Chu 2007; Wiegand, Meyer, and Brinkmann 2000).

Table 2. Paternity index

STR LOCI	Child	DNA Profile Maternal DNA	Profile Fa

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	Allele 1	Allele 2	Genotype	Allele 1	Allele 2	Genotype	Allele 1	Allele 2	Genotype	Probability	Pa	Pi	Index
D3S1358	13	16	AB	13	17	AC	15	16	BD	1/2Pa	0.4524		1.105217
vWA	14	18	AB	14	18	AB	17	18	CB	1/2(Pa+Pi)	0.2238	0.2143	1.141292
D16S539	11	12	AB	11	12	AB	11	14	AC	1/2(Pa+Pi)	0,2476	0.2714	0.963391
CSF1PO	10*	10	AA	10	12	AB	11	11	A'C	/2Pm	0.2048*		0.003906
TPOX	8	8	AA	8	9	AB	8	11	AC	1/2Pa	0.5286		0.945895
Yindel							2			-			
D8S1179	13	15	AB	13	13	AA	11	15	BC	1/2Pa	0.1429		3.498950
D21S11	29	31	AB	31	32.2	BC	29	31.2	AD	1/2Pa	0.2		2.5
D18S51	13	15	AB	13	16	AC	13	15	AB	1/2Pa	0.2714		1.842299

DYS391							11	
D2S1441	11	12		12	14		10	11
D19S433	14.3	15		15	15		13.2	14.3
TH01	7	9	AB	7	9	AB	7	9
FGA	23	24	AB	23	24	AB	24	24
38753 4 D22S1045	15	16	AB	16	17	BC	11	15
D5S818	10	11	AB	10	13	AC	7	11
D13S317	9	10	AB	8	10	BC	8	9
D7S820	11	12	AB	11	13	AC	12	12

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SE33	18	26.2		26.2	26.2		18	27.2				
D10S1248	15	16		14	15		13	16				
D1S1656	17	18.3		17	17.3		16	18.3				
D12S391	18	19		17	18		18	19				
D2S1338	25	25	AA	25	25	AA	22	25	AB	1/2Pa	0.067	7.496252
CPI												195193,3032

*Mutations in children (Sources of data processed: Dobashi et al., 2005; Vigouroux et al., 2002; Virginia, 2018).

Explanation

Pa : allele inherited from father

Pi : allele inherited from mother

Pm : mutated allele

CSFIPO : mutation presentation

= 0.0016) (AABB 2003)

The presence of a mutation in the CSF1PO locus still met the inclusion criteria (when 0 or 1 locus mismatch between the parent/child combination was still considered a mutation) as a suspected father. This is because if the CPI value is greater than in the range above or equal to 2000 (in this case >195,000), then the probability of being suspected of being a biological father is 99.95%, this assumes that the kinship hypothesis must be accepted (considered proven). However, in the case of mutations, the calculated CPI value only reaches the range of 762 (range 100-2000), then the hypothesis can be accepted with the *support* data (NYC 2016).

After calculating to determine the probability of paternity, it was found that a mutation resulted in a decrease in the percentage of the probability of paternity, from 99.9999% if there was no mutation to 99.9433%. If one CS1FPO locus is ignored, then the probability can reach 99.8690%. In the case of this mutation, the paternity index decreased even though the value was still included in the inclusion criteria, which was greater than 99.5% (US Department of State, 2006). These results indicate that the presence of mutations can reduce the value of PP and a relatively high CPI so that it can be a *false negative*, not unexpected. Mutations at the STR locus need to be taken into account because it is quite confusing for a CPI value that does not reach 2000, so statistical calculations are needed to prove it.

8. CONCLUSION

Mutations at the STR locus will cause a person's repeat to be shifted one or

more steps away from the initial allele. The existence of a mutation reduces the percentage value of the *probability of paternity*, from what should be 99.9999% to 99.8690%. Hal ini menandakan bahwa adanya mutasi mempengaruhi nilai presentase dan perlu diperhitungkan dalam kasus forensik. Oleh karena itu, pada penelitian yang lebih lanjut diharapkan terdapat penentuan frekuensi mutasi untuk alel-alel dengan populasi Indonesia.

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APPENDIX

(2)

$$CPI = pi_{(d3s1358)} * pi_{(vWA)} * pi_{(d16s539)} * pi_{(csf1po)} * pi_{(tpox)} * pi_{(d8s1179)} * pi_{(d21s11)} * pi_{(d18s51)} * pi_{(th01)} * pi_{(fga)} * pi_{(d22s1045)} * pi_{(d5s818)} * pi_{(d13s317)} * pi_{(d7s820)} * pi_{(d2s1338)}$$

pp = (3)

=

= 99,9995%

(4)

PIm = *P (Q' is transmitted) x P (mutation) x P (mutation increses length) x P (s steps)*
$=1x0{,}0016x(1/2) x(1/10)^{1-1}$

$= 0{,}0016/2P_{10}$

$= 0{,}00390625$

PP mutation = (5)

=

= 99,9995%

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