DNA Paternity Testing with Two Mismatches: Our Experience

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Abstract

In paternity testing, the adopted rule is to exclude paternity when mismatch at more than two STR loci is observed. Here we present two routine DNA paternity testing cases with two autosomal STR mismatches between the alleged father and female child, where we employed X-STR analysis to confirm or exclude paternity. Mismatches at D16S539 and D18S51 loci in the Case 1 and D8S1179 and FGA loci in the Case 2 were detected using PowerPlex® Fusion System. X-STR analysis using Investigator® Argus X-12 kit yielded one inconsistency at DXS10135 locus between the child and the alleged father in the Case 1, whereas we found a complete match at all 12 analysed X-STR loci in the Case 2. As a result, paternity was confirmed in both cases. Conclusively, this case study indicates that including an additional analysis has a great importance in solving paternity cases with inconclusive results of autosomal STR analysis.

1. Introduction

Nowadays, STR (Short Tandem Repeat) markers represent a powerful tool in the field of forensic-genetic analyses, kinship and parentage testing as well as population-genetic studies (Li *et al.*, 2015; Lee *et al.*, 2017). Consisted of short repetitive units from two to six base pairs in length, STR markers are adequate for forensic analyses of biological samples (Canturk *et al.*, 2014). Their high variability among individuals contributes to highly effectiveness for human identification (Butler, 2011). Unlike unique DNA sequences that exhibit low mutation rate around 10^{-9} nucleotides per generation, STR sequences have higher mutation rate from 10^{-6} to 10^{-2} nucleotides per generation (Fan and Chu, 2007). Besides high heterozygosity, discriminating power, clearly defined repetitive units and precisely determined allelic variants, simple amplification and detection of STR markers make them ideal for forensic analyses (Khalil *et al.*, 2008; Primorac *et al.*, 2014; Gomes *et al.*, 2020). STR markers located on the autosomal, X and Y chromosomes are highly informative, practical and reliable for all kinds of forensic-genetic analyses including parentage testing, what was strongly supported by many population-genetic studies focused on investigating allele frequencies and forensic parameters (such as heterozygosity, power of discrimination, power of exclusion, polymorphic information content, matching probability and typical paternity index) of autosomal STR (Al-Eitan and Tubaishat, 2016; Al-Eitan *et al.*, 2019; Al-Eitan *et al.*, 2020; Pilav *et al.*, 2020; Takic Miladinov *et al.*, 2020) and X-STR loci (Grskovic *et al.*, 2013; Crnjac *et al.*, 2017; Veselinovic *et al.*, 2018) in different populations. Generally, parentage testing follows Mendelian inheritance law, according to which child receives one allele from the mother and the other allele from the father (Schanfield *et al.*, 2014).

In some cases, spontaneous mutations as non-Mendelian inheritance patterns of alleles lead to allelic mismatch, making paternity or maternity testing case complicated. Fan and Chu (2007) described several different mechanisms of STR mutations, highlighting the strand-slippage replication as a main pattern of STR mutation. Most of reported STR mutations observed in routine parentage testing cases are single-step mutations (Lu *et al.*, 2012; Wojtas *et al.*, 2013), while multi-step mutations occur rarely in a small number of mutation events (Wojtas *et al.*, 2013; Jia *et al.*, 2015; Liu *et al.*, 2015). According to "two exclusion" rule, paternity cannot be excluded when a mismatch at two STR loci between the alleged father and child is observed (Deepak *et al.*, 2019).

In a study of paternity testing reported by Aktheruzzaman *et al.* (2012), two incompatibilities at D2S1338 and vWA loci were observed. Paternity testing was repeated using PowerPlex®16 System and GenePhile G-Plex. Mismatch at vWA locus was observed with PowerPlex®16 System, while two mismatches between the alleged father and child were encountered at D3S1744 and D18S536 loci with GenePhile G-Plex kit. Since the child was a female, paternity was excluded with a set of 13 X-STR markers. In another study of paternity testing by Jha *et al.* (2013), two exclusions between the alleged father and male child were observed at loci D21S11 and D18S51 out of 15 autosomal loci. Then, paternity was excluded after Y-STR analysis using AmpFLSTR™ Yfiler which result showed a match at only three out of 16 loci. As can be seen from the above cited reports, disputed parentage cases not easily solved by routine autosomal STR analysis should be confirmed or excluded by employing an additional analysis (Akhteruzzaman *et al.*, 2012). Analysis of the X-linked STR markers can be performed in disputed

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paternity cases involving female child (García *et al.*, 2017) or disputed maternity (Chen *et al.*, 2009), while the Y-linked STR analysis is appropriate in paternity testing cases with male child (Kayser, 2017). This paper reports two paternity testing cases involving female child, mother and the alleged father, where analysis of autosomal STR markers resulted in single-step mutation in two STR loci, thus X-STR analysis was performed in order to confirm or exclude paternity.

2. Materials and Methods

2.1. Materials

Written informed consents were obtained from each individual in two cases of disputed paternity. In the Case 1, buccal swab samples were taken from female child aged 5 years, mother aged 46 years and the alleged father aged 70 years. Information about the age of the child, mother and the alleged father from the Case 2 was not obtained before taking buccal swab samples. Samples were collected using sterile cotton swabs (CITOSWAB, Shanghai, China) and proceeded to DNA extraction or stored at +4°C until the extraction. Publishing of the results of paternity testing cases was approved by the Ethics Committee of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo (No. 289/20).

2.2. DNA extraction

Genomic DNA was extracted using the QiagenDneasy™ Tissue Kit (Qiagen, 2012) and amplified using the PowerPlex® Fusion System (PROMEGA, Wisconsin, USA) which includes 24 loci (Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391, D8S1179, D12S391, D19S433, FGA and D22S1045). Also, Investigator® Argus X-12 kit (QIAGEN, GmbH, Hilden, Germany) which includes 13 loci (Amelogenin, DXS10103, DXS8378, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148) was included in analysis. PCR amplification was carried out in GeneAmp™ PCR System 9700 (APPLIED BIOSYSTEMS, USA), following the manufacturer's recommendations. Fragment analysis of PCR products was carried out using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, CA, USA). Data were collected using 310 Data Collection Software. STRs analysis was performed applying GeneMapper™ v. 3.2 software (Applied Biosystems, USA).

2.3. Statistical analysis

Paternity Index (PI) was calculated for each STR locus, as a likelihood ratio generated by comparing probability that the alleged father contributed the obligate allele with probability that randomly chosen man contributed the allele. Combined Paternity Index (CPI) was calculated by multiplying PI values for each locus. Probability of Paternity (PP) was calculated using PP=CPI/CPI+1 formula, which represents the probability that the alleged father is a biological father of the child (Schanfield *et al.*, 2014). Observing two inconsistencies between child and the alleged father, possibilities of mutations were taken into account. Mutation Index (MI) values were calculated according to formula MI=μ/2p (Brenner, 1998), where μ is the mutation rate for STR locus obtained from Short Tandem Repeat DNA Internet DataBase (https://strbase.nist.gov/mutation.htm) and p is a frequency of mutated allele in the population. For statistical analysis of results obtained by X-STR analysis was used FamLinkX v. 2.8 software, including calculation of Likelihood Ratio (LR), Total LR and Probability of Paternity (PP).

3. Results

3.1. Results of autosomal STR analysis

In the Case 1, out of 22 loci tested, a complete match at all autosomal STR loci in the child with the mother was observed. On the other hand, we found two incompatibilities between the alleged father and the child. Allelic variants detected at autosomal STR loci of the child, mother and the alleged father are displayed in Table 1.
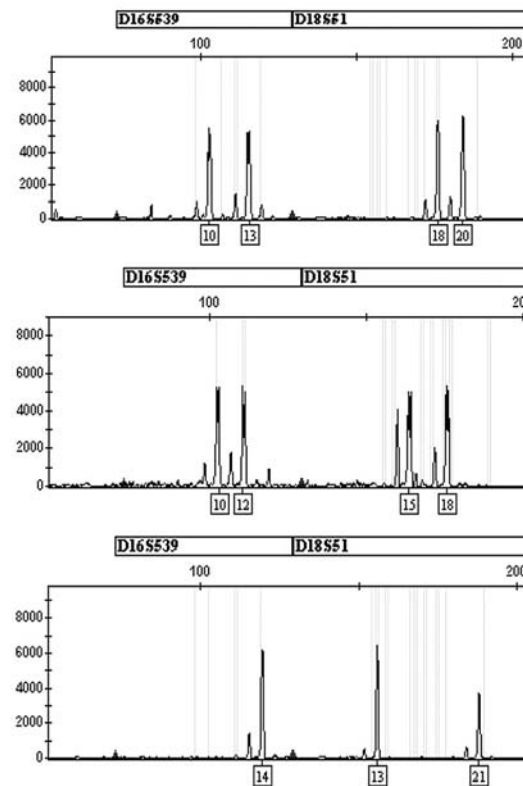
Figure 1 shows genotypes of the child, mother and the alleged father, detected at D16S539 and D18S51 loci. At the D16S539, genotype of the child, mother and the alleged father was found to be 10/13, 10/12 and 14/14, respectively. At the D18S51 observed alleles were 18/20 for the child, 15/18 for the mother and 13/21 for the alleged father. With possibilities of mutation events incorporated into the calculation, CPI and PP values were 647157598 and 99,9999998 %, respectively.

Table 1. Autosomal STR profiles of the child, mother and the alleged father in the Case 1 (mutated alleles underlined)

Locus	Child	Mother	Alleged father	PI
D3S1358	15/17	14/17	15/16	1,78571
D1S1656	15/17	11/15	12/17	8,34585
D2S441	14/15	11/14	15/15	21,70139
D10S1248	13/16	12/16	13/17	2,17004
D13S317	9/12	12/12	8/9	6,66667
PENTA E	10/13	13/14	10/18	3,03030
D16S539	10/13	10/12	<u>14</u>/14	0,00278[a]
D18S51	18/20	15/18	13/<u>21</u>	0,05500[b]
D2S1338	17/19	17/21	17/19	3,85713
CSF1PO	12/12	10/12	10/12	1,44928
PENTA D	13/13	9/13	10/13	3,33333
TH01	8/8	8/9.3	6/8	4,34783
VWA	15/18	18/18	15/19	4,16667
D21S11	28/30	28/30	30/30	2,66667
D7S820	8/8	8/11	8/12	3,22581
D5S818	11/11	11/12	11/11	2,66667
TPOX	8/11	8/8	11/11	3,84615
DYS391	-	-	10/10	-
D8S1179	12/15	12/13	13/15	6,25000
D12S391	22/23	22/23	22/23	2,41115
D19S433	14/14.2	13/14	13/14.2	19,72387
FGA	24/25	23.2/24	25/25	11,11111
D22S1045	15/16	15/15	16/16	3,03499
AMELOGENİN	XX	XX	XY	-
CPI				647157598
PP (%)				99,9999998

PI=Paternity Index; CPI=Combined Paternity Index; PP=Probability of Paternity

[a] MI (Mutation Index) calculated including possibility of mutation at D16S539 locus

[b] MI (Mutation Index) calculated including possibility of mutation at D18S51 locus

Figure 1. Electrophoretogram for the genotypes of the child (upper panel), mother (middle panel) and the alleged father (lower panel) at D16S539 and D18S51 loci in the Case 1

The results of autosomal STR analysis in the Case 2 are listed in Table 2. STR profile of the alleged father matched with the STR profile of the child in 20 out of 22 loci. All alleles of the child at all STR loci monitored were detected in the mother.

At locus D8S1179 alleles 13/16 for the child, 10/13 for the mother and 12/15 for the alleged father were scored (Figure 2). At locus FGA, genotype of the child, mother and the alleged father was found to be 21/21, 21/25 and 20/22, as depicts Figure 3. Absence of obligate paternal allele 16 at D8S1179 and allele 21 at FGA locus indicated to the possibility of mutation. Calculated CPI and PP values including mutation indices at these two loci were 286760481978 and 99,99999999 %.

In above both cases, paternity could not be excluded or confirmed, since it is a practice to exclude paternity with three mismatches observed. Thus, we had taken support of X-linked STR markers in order to get more accurate and conclusive results.

Table 2. Autosomal STR profile of the child, mother and the alleged father in the Case 2 (mutated alleles underlined)

Locus	Child	Mother	Alleged father	PI
D3S1358	17/18	18/18	15/17	2,63158
D1S1656	12/15	12/15	12/15	3,44448
D2S441	11/11.3	11.3/14	10/11	1,65651
D10S1248	14/15	13/15	13/14	1,64393
D13S317	10/11	8/10	11/11	2,77778
Penta E	11/13	9/11	11/13	3,22581
D16S539	11/14	11/13	12/14	25,00000
D18S51	14/18	17/18	14/16	2,32558
D2S1338	19/19	16/19	17/19	3,85713
CSF1PO	9/10	10/13	9/12	10,00000
Penta D	9/16	11/16	9/12	2,04082
TH01	6/9	6/6	9/9	5,12821
vWA	17/18	18/18	17/17	3,63636
D21S11	28/32.2	28/28	30.2/32.2	5,88235
D7S820	10/11	10/11	11/12	1,05263
D5S818	11/13	11/11	10/13	2,85714
TPOX	8/11	8/11	8/8	1,20482
DYS391	-	-	10	-
D8S1179	13/16	10/13	12/$\underline{15}$	0,0029[c]
D12S391	21/22	18/22	21/23	4,25496
D19S433	12/13	12/14	13/15	2,14850
FGA	21/21	21/25	$\underline{20}$/22	0,0083[d]
D22S1045	11/18	11/18	11/17	3,23897
Amelogenin	XX	XX	XY	-
CPI				286760481978
PP (%)				99,99999999

PI=Paternity Index; CPI=Combined Paternity Index; PP=Probability of Paternity

[c]MI (Mutation Index) calculated including possibility of mutation at D8S1179 locus

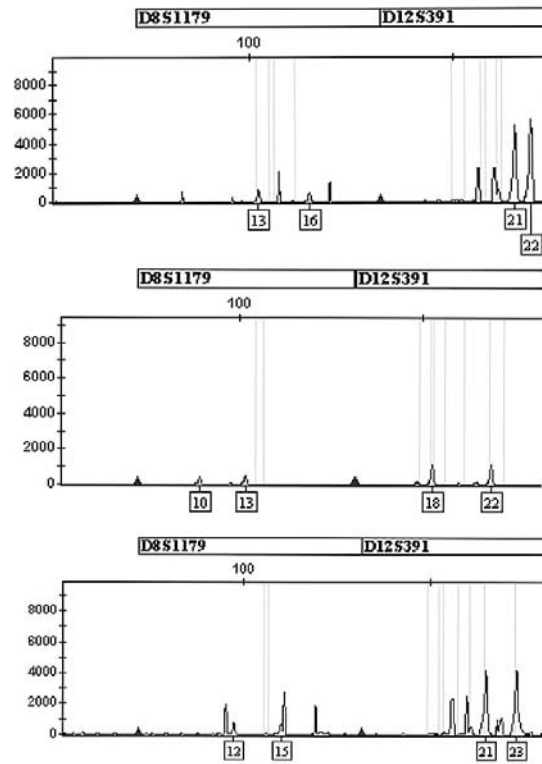[d]MI (Mutation Index) calculated including possibility of mutation at FGA locus

Figure 2. Electrophoretogram for the genotypes of the child (upper panel), mother (middle panel) and the alleged father (lower panel) at D8S1179 locus in the Case 2
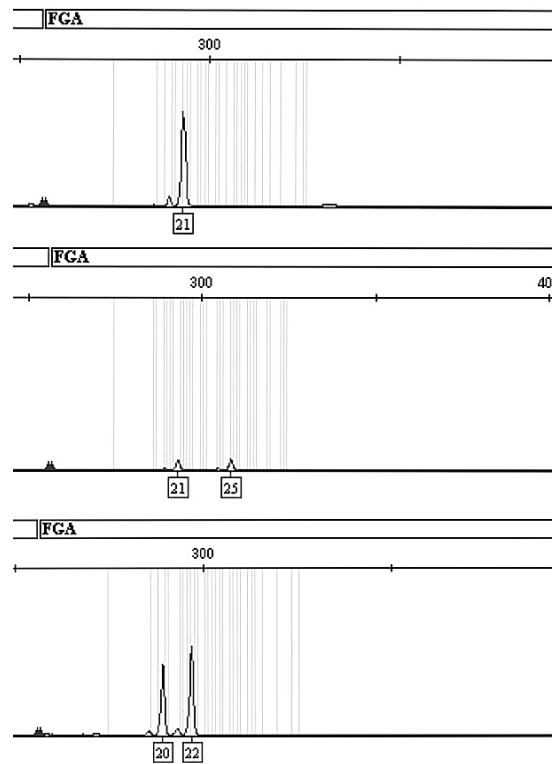

Figure 3. Electrophoretogram for the genotypes of the child (upper panel), mother (middle panel) and the alleged father (lower panel) at FGA locus in the Case 2

3.2 Results of X-linked STR analysis

Table 3 displays observed allelic variants at 12 analysed X-STR loci in the Case 1. There was observed match at all analysed loci between the child and the mother, and match between the child and the alleged father at all analysed loci except DXS10135. Observed alleles for the child and the mother at DXS10135 locus were 24/27 and 20/24, while the alleged father was found to be homozygous for allele 28, as depicted in Figure 4. Total LR (Likelihood Ratio) and PP values calculated including mismatched paternal allele, were 20025200 and 99,99999501%, respectively.

On the other hand, in the Case 2, all alleles of the child at all X-linked STR loci monitored were detected in both the mother and the alleged father. Total LR value was 1331298773, while the PP was 99,99999992 %. The results are summarized in **Table 4**.

Table 3. X-linked STR profile of the child, mother and the alleged father in the Case 1 (mutated allele underlined)

Locus	Child	Mother	Alleged father	LR
DXS10103	18/19	18/19	19	1,67501
DXS8378	11/12	10/11	12	5,61609
DXS7132	15/15	14/15	15	4,68189
DXS10134	35/36	35/36	35	2,24069
DXS10074	8/14	8/14	8	4,58464
DXS10101	27.2/29.2	29.2/30	27.2	8,70467
DXS10135	24/27	20/24	28	0,0215162[e]
DXS7423	14/15	14/14	15	6,51461
DXS10146	40.2/46.2	28/40.2	46.2	207,068
DXS10079	17/20	18/20	17	9,21573
HPRTB	12/12	12/12	12	2,57443
DXS10148	18/24.1	18/28.1	24.1	7,38388
Amelogenin	XX	XX	XY	-
Total LR				20025200
PP (%)				99,99999501

LR=Likelihood Ratio; PP=Probability of Paternity

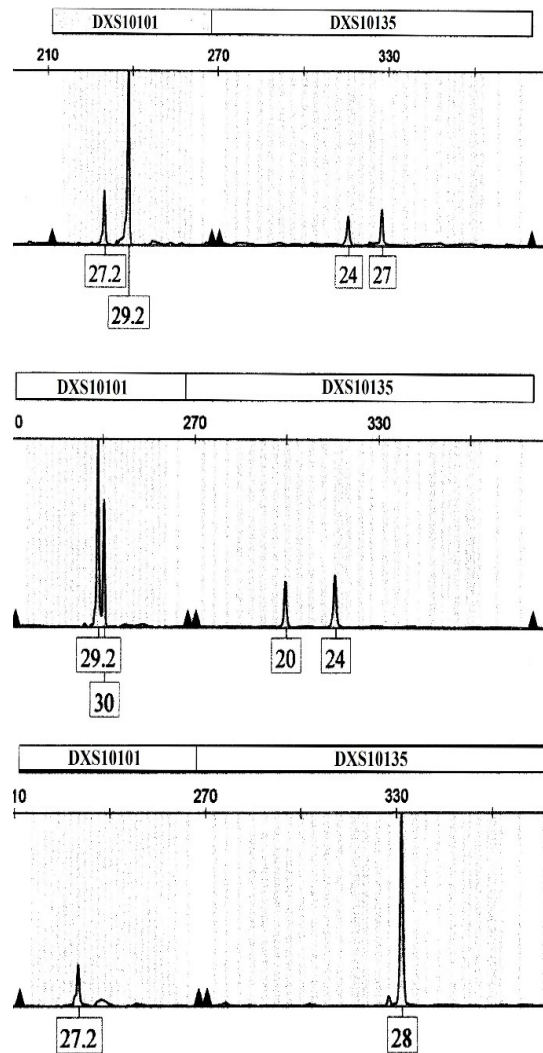[e]MI (Mutation Index) calculated including possibility of mutation at DXS10135 locus

Figure 4. Electrophoretogram for the genotypes for the child (upper panel), mother (middle panel) and the alleged father (lower panel) at DXS10135 locus in the Case 1

Table 4. X-linked STR profile of the child, mother and the alleged father in the Case 2

Locus	Child	Mother	Alleged father	LR
DXS10103	16/18	16/19	18	5,798
DXS8378	12/12	10/12	12	1,952
DXS7132	13/13	13/14	13	3,696
DXS10134	35/36	35/36	36	2,214
DXS10074	16/18	16/19	18	5,671
DXS10101	32/33	28/32	33	19,33
DXS10135	18/28	22/28	18	15,36
DXS7423	14/14	14/16	14	3,629
DXS10146	27/28	28/28	27	7,521
DXS10079	17/19	17/20	19	4,336
HPRTB	11/14	11/13	14	9,238
DXS10148	18/25.1	24/25.1	18	7,809
Amelogenin	XX	XX	XY	-
Total LR				1331298773
PP (%)				99,99999992

LR=Likelihood Ratio; PP=Probability of Paternity

4. Discussion

In this case study, two cases of routine DNA trio paternity testing with two inconsistencies between the child and the alleged father at autosomal STR loci were presented. The initial analysis was carried out using the PowerPlex® Fusion System (PROMEGA, Wisconsin, USA), which is routinely used in our laboratory. Typing of 22 autosomal STR loci revealed mismatches at D16S539 and D18S51 loci in the Case 1, and at D8S1179 and FGA loci in the Case 2. Within standard paternity testing procedure, paternity is excluded when more than two mismatches have been observed at all analysed loci, whereas the possibility of mutations must be included into account for calculation of CPI (Combined Paternity Index) and PP (Probability of Paternity) when one or two mismatches have been observed (Akhteruzzaman *et al.*, 2012).

Generally, mutations occur at D16S539, D18S51, D8S1179 and FGA loci more frequently than at the other autosomal STR loci, according to referent Short Tandem Repeat DNA Internet DataBase (https://strbase.nist.gov/mutation.htm) and study of mutation rates of autosomal STR loci in Bosnian and Herzegovinian population (Zametica *et al.*, 2018). There are a few factors influencing STR mutations such as repeat number, length and base composition of STR repeat unit and interruptions in STR as well as sex and age of individual. Throughout replication of a repetitive region, incorrect reassociation of DNA strands leads to insertion or deletion of repeat units, which affects allele length. Besides unequal crossing over in meiosis and retrotransposition mechanism (Fan and Chu, 2007), a major STR mutational factor might be strand-slippage during replication, which causes increase or decrease in a repeat number (Qian *et al.*, 2015), generating new allelic variants during cell division.

Therefore, we assumed that inconsistency in the Case 1 at D16S539 locus might be caused by a loss of one repeat from paternal allele 14, what was transmitted to the child as 13. In the same case, allele mismatch, observed at D18S51 locus, can be attributed to a single-step mutation where there was a loss of one repeat from 21 allele, what was transmitted to the child as allele 20. In the Case 2, obligate paternal allele 16 at D8S1179 locus was characterized as resulting from the mutation of allele 15 into allele 16 (a gain of one repetitive unit). Regarding the inconsistency between child and the alleged father at FGA locus, two hypothetical situations can be considered: 1. Child's allele 21 exists by loss of one repeat from allele 22 of the father. 2. Allele 21 was inherited from allele 20 of the father by gain of one repetitive unit.

Mutations detected in both cases were from paternal source, what was supported by the statement that the mutations occur more often in sperm cells compared to the female egg cells (Qian *et al.*, 2015). Other studies have also found that paternal mutations occur more frequently than maternal not only at autosomal STR loci (Huang *et al.*, 2021) but X-linked STR loci as well (Pinto *et al.*, 2020).

Length of repeat unit and rate of slippage are inversely related, which means that the rate of slippage is expected to be higher in dinucleotide than in trinucleotide or tetranucleotide STR units (Chakraborty *et al.*, 1997). On the other hand, D16S539, D18S51, D8S1179 and FGA loci affected with mutations have tetranucleotide units, as well as almost all of autosomal STR loci analysed in these two paternity testing cases.

Since autosomal STR analyses revealed the number of discrepant loci between the alleged father and female child less than three and left these paternity testing cases unsolved, additional X-linked STR analyses using Investigator®Argus X-12 kit (QIAGEN, GmbH, Hilden, Germany) were included for confirmation or exclusion of paternity. Studies proved usability and efficiency of this kit in kinship testing cases (Hering *et al.*, 2015) and population-genetic researches (Crnjac *et al.*, 2017). Analysis of 12 X-STR loci yielded only one mismatch between child and the alleged father, caused by a single-step mutation at DXS10135 locus in the Case 1, and complete matching at all analysed X-STR loci in the Case 2. In both cases, result of X-STR analysis and PP value clearly indicate that the alleged father is a biological father of the child.

Studies conducted by Jia *et al.* (2015) and Qian *et al.* (2015) revealed that paternal age could contribute to the occurrence of mutations. In light of the fact that sperm cells of older men undergo more divisions than the cells of younger men (Fan and Chu, 2007; Qian *et al.*, 2015), it is more likely that mutations will affect older cells. Since the father from the Case 1 was 65 years old at the birth of child, we assume that his age could be the cause of mutations occured in both autosomal and X-linked STRs. Considering all aforementioned, knowledge about mutation rates and possible mutational processes of different STR loci is important for accurate genetic profiles interpretation (Qian *et al.*, 2015; Hamester *et al.*, 2019). As previously mentioned in the Introduction section, a number of population-genetic studies, focused on evaluating of forensic efficiency parameters of STR markers (Grskovic *et al.*, 2013; Al-Eitan and Tubaishat,

2016; Crnjac *et al*., 2017; Veselinovic *et al*., 2018; Al-Eitan *et al*., 2019; Al- Eitan *et al.*, 2020; Pilav *et al*., 2020; Takic Miladinov *et al*., 2020) indicated a high degree of reliability of these markers important for all kinds of forensic-genetic analyses, including kinship and parentage testing. Finally, results from these two cases clearly demonstrate that X-STR markers have the potential to solve parentage cases not easily solved by standard analysis based on autosomal STR markers.

5. Conclusions

This case study emphasizes the importance and usefulness of supplementary analyses in parentage testing cases with inconclusive results obtained by routine autosomal STR analysis. Moreover, we consider that STRs located on the X chromosome might be superior to conventional autosomal STR markers in parentage testing cases involving at least one female individual, regarding the inheritance pattern of these markers. In the future, investigations focused on the advantages of supplementing the autosomal STR loci with X-STR in other kinds of kinship testing involving at least one female individual should be conducted.

Acknowledgment

Conflict of interest

The authors declare that there is no conflict of interest.

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