Genetics & Applications

Institute for Genetic Engineering and Biotechnology University of Sarajevo

Research Article Open Access

Mutation Analysis of Autosomal STR Loci Commonly Used in Paternity Testing in Bosnia and Herzegovina

Berina Zametica[1], Sonja Mačar[1], Abdurahim Kalajdžić[1*], Amela Pilav[2], Mirela Džehverović[2] and Jasmina Čakar[2]

[1] *University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*
[2] *University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina*

DOI: 10.31383/ga.vol2iss1pp14-18

***Correspondence**
E-mail: abdurahim.k@hotmail.com

Received
February, 2018
Accepted
May, 2018
Published
June, 2018

Abstract

Mutation analysis in forensic genetics and occurrence of mutations at short-tandem repeat (STR) loci, are very important in paternity testing and precise elucidation of obtained genetic profiles. To determine these locus-specific mutations in Bosnian-Herzegovinian population and their rate, 15 or 22 autosomal loci were typed using PowerPlex® 16 and PowerPlex® Fusion systems. In total, 1253 individuals within 583 parenthood testing cases were profiled at the Institute for Genetic Engineering and Biotechnology, University of Sarajevo during the period from 2009-2018. Out of total cases, in 13 cases 14 mutations were discovered at 11 loci. Among all tested DNA profiles two mutations occurred at D8S1179, D18S51 and FGA loci each, and one mutation at PENTA D, D3S1358, CSF1P0, D21S11, D5S818, vWA, D16S539, PENTA E. Mutation rates were calculated for 11 loci and were in consistency with mutation rates reported for correspondent locus. In our study, one mutation at locus PENTA D derives from maternal source. Also in one trio paternity case two single-step mutations at loci D16S539 and D18S51 were observed. Our results confirmed mutation analysis is important in paternity testing and therefore much attention should be directed at their analysis.

Keywords

mutation rate, STR, paternity testing, PI, CPI

Introduction

The whole concept of paternity testing is based on comparing genetic profiles, and in case of differences at STR loci between the potential father and a child, relationship between them can be assigned as non-biological paternity, which leads to exclusion of biological paternity (Kayser & Sajantila, 2001). Short tandem repeat loci in that manner are perfect markers in forensics because they are highly polymorphic and variable loci (Kayser & Sajantila, 2001). These markers are made of a tandemly repeated DNA sequence, which consists of

short repetitive units from 2 to 7 base pairs in length. Number of repeats varies among individuals. Their mutational rate is estimated around 10^{-2} to 10^{-4} per generation and can be explained by two different mechanisms: unequal crossing-over during recombination or inaccurate pairing during replication because of DNA slippage (Vigouroux et al., 2002).

Besides their robustness and high reproducibility, biggest advantage of these markers, and fact that makes them so suitable for forensics analysis is simplicity of the detection process itself as well as the fact that lowest theoretical probability that two persons share identical allelic variants on 15 STR loci for Caucasian populations is equal to 1/1.83 x 10^{17} (Marjanovic & Primorac, 2013; Weber & Wong, 1993). The International Society for Forensic Genetics (ISFG) suggested a minimum of 12 autosomal STR markers located on 10 different chromosomes to be analyzed, in order for probability of paternity (PP) to reach 99.99%.

Contemporary findings suggest that at least 15 STR markers need to be analyzed in order to exclude a person as a biological father or to obtain strong probability of paternity (Carboni et al., 2011; Jha et al., 2013; Beribaka et al., 2017).

The mutations at STR loci are recognized as alleles not inherited following Mendelian pattern. The number of analyzed loci, in cases when a mutation is identified, must be increased in order to increase and validate paternity index (Li et al., 2011). In that manner, much attention must be dedicated to analyzing spontaneous mutations which may result in an inaccurate exclusion.

Since mutation analysis in paternity testing is crucial and highly important for proper interpretation of results, we present the data of mutations incidence in 583 paternity testing cases, in which we discovered 14 mutations in 13 cases at 11 loci commonly used by forensics laboratories.

Materials and methods

The material comprised paternity testing samples, collected from routine casework performed during the period of 2009-2018 at the Institute for Genetic Engineering and Biotechnology, University of Sarajevo. In this study 1253 persons were included within 583 (of which 211 trios) confirmed paternity or maternity testing cases. DNA was extracted from buccal swab samples following the salt precipitation method (Miller et al., 1988). Prior to the sample collection a signed informed consent was obtained.

The samples were profiled using PowerPlex® 16 System (Promega, Madison, WI, USA) and PowerPlex® Fusion System. PCR was performed according to the manufacturer recommendations in 25 μL reaction volume. The amplification was conducted in a GeneAmp PCR System 9700 (Applied Biosystems). Fragment analysis was carried out in ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). A mix of 11.5 μL of formamide, 0.5 μL of Internal Lane Standard 600 (Promega) and 1 μL of PCR product was run under recommended conditions. GeneMapper® ID Software version 3.2 (Applied Biosystems) was used to generate profiles. Complete analysis of DNA profiles with detected mutations was done in triplicate.

Statistical analysis

Paternity index (PI) and combined paternity index (CPI) were calculated for each case. For calculating mutation rate, formula n/2N was used where n presents number of mutations per case and N total number of cases. Z test was used for calculating difference in frequencies between mutation detected in paternity testing cases and those reported in relevant database (WINKS 4.5 Professional). For calculating differences between PP and CPI values in cases with mutations and equivalent cases without mutations, pairwise t test following permutation test (with 9999 permutations) was used (PAST version 2.17).

Results and Discussion

It is a standard practice to exclude paternity when more than two mismatches have been observed at all tested loci. The possibility of mutations must be taken into account for cases where 1 or 2 mismatches are observed (Balloch et al., 2008). The occurrence of germline mutations at STR loci is

crucial for forensic DNA testing and accumulation of STR mutation data is extremely important for genetic profile interpretation (Aşıcıoğlu et al., 2004).

In our study, out of 583 parenthood testing cases, mutations were observed in 13 cases. Twelve out of thirteen mutations were a single-step event, which is concordant with the strand-slippage replication and stepwise mutation model (SMM) (Schlötterer & Tautz, 1992). In one case mutations were detected at two loci, so additional X-STR analysis was included for paternity confirmation. Mutations occurred at PENTA D, D81179, D3S1358, CSF1P0, D21S11, D5S818, PENTA E, D16S539, D18S51, FGA, and vWA loci (Table 1).

Table 1. Mutations observed at 11 STR loci (profiled with PowerPlex 16 System and PowerPlex Fusion System) of 13 cases (mutated alleles underlined)

Case	STR loci	Father	Mother	Child
1.	PENTA D	9;12	11;13	12;14
2.	D8S1179	13;13	10;15	10;14
3.	D3S1358	15;17	16;18	16;16
4.	CSF1P0	11;13	-	10;12
5.	D21S11	30;30	30;32.2	31;32.2
6.	D8S1179	14;14	-	15;15
7.	D5S818	12;13	-	13;14
8.	PENTA E	15;15	-	12;16
9.	D16S539	14;14	10;12	10;13
	D18S51	13;21	15;18	18;20
10.	FGA	20;25	21:23	21;26
11.	FGA	24;25	-	23;23
12.	VWA	15;17	-	16;18
13.	D18S51	14;20	-	17;21

Mutations at loci D8S1179, D18S51 and vWA were detected twice while at other affected loci, mutations were observed once per case. The mutation rate depends on the length of STR repeat unit and number of repeats (Aşıcıoğlu et al., 2004). A negative correlation was reported between the length of the repeat unit and the rate of slippage (Schlötterer & Tautz, 1992), so the rate of slippage is expected to be higher in dinucleotide STRs and lower in tetranucleotide STRs. On the other hand, mutation rate increases with the number of repeats and it is higher in STRs with larger numbers of repeats. Although all the analyzed STRs were tetra- or penta-nucleotide units, the mutations affected alleles with relatively high number of repeats (Table 2). Direction of mutations differs at different loci. In short STRs increase in number of repeats occurs more frequently, while a reduction of repeat number exists in longer ones (Fan & Chi, 2007). In our study repeat number decreased in ten and increased in three cases.

Among all detected STR mutations, one was from maternal (7.69 %) and 12 were from paternal (92.31%), however, it should be noted that the mother's profiles were available in 36,19 % of cases (211/583). Mutations are most likely to occur at microsatellite loci originating from males so mutation rates at most loci in germ cells are generally higher in males (Fan & Chi, 2007; Shimmin, 1993). Sperms undergo more DNA replication cycles than eggs which results in the higher frequency of mutation (Shimmin, 1993).

Table 2. Number of mutational events, mutation rate, CPI and PP with and without mutation included in analysis

Loci	NMEPC	MR referent database	MR	CPI without mutation	CPI with mutation	PP without mutation (%)	PP with mutation (%)
PENTA D*	1	0,14	0,086	1906934*	1906934*	99.9999475*	99.9999475*
D8S1179	2	0,14	0,17	2052208	32493	99.999951	99.9969
D3S1358	1	0,12	0,086	87548000	175096	99.9999999	99.99943
CSF1P0	1	0,16	0,086	66107250	264429	99.999998	99.99962
D21S11	1	0,19	0,086	479115	21656	99.99979	99.9954
D5S818	1	0,11	0,086	6254285	269384692	99.99998	99.99999963
PENTA E	1	0,16	0,086	$1.34*10^{+10}$	957417	99.999998	99.99989
D18S51	2	0,22	0,17	41626826	323597375	99.9999976	99.99999969
D16S539	1	0,11	0,086	$4.23*10^{+12}$	647157598	99.99999996	99.9999998
FGA	2	0,28	0,17	762835517	117934908	99.9999998	99.99999915
VWA	1	0,17	0,086	2421290326	199383	99.9999999	99.9995

In comparison with referent database (Butler & Reeder, 2018) no statistical significance was recorded in mutation rates of all affected loci, suggesting that frequencies of mutations in this study are in accordance with those observed in human population.

Combined paternity (CPI) and probability of paternity (PP) values decreased in cases with mutations (Table 2), but pairwise t test showed no statistically significant differences in CPI or PP values with or without mutations (p=0.33). However, permutation exact test (with 9999 permutations) indicate statistically significant differences between tested CPI value (p=0.001), but not for PP values (p=0.016), which is in accordance with previous studies (Kayser & Sajantila, 2001; Li et al., 2011).

Conclusions

In the present study, authors investigated commonly used autosomal STR loci in Bosnian-Herzegovinian population. Paternal mutations were more common than maternal mutations, and most of mutations were single step events. Our results in which we observed 13 mutations in 583 parenthood cases demonstrated that mutation rate increases with the number of repeats, and is higher in longer alleles than in the shorter ones. The occurrence of mutations at STR loci does not reduce values of CPI and PP remarkably. Also there is no significant difference in mutation frequency at STR loci between Bosnian-Herzegovinian population and referent populations worldwide. Our results also showed that the possibility of mutations must be taken into account when analyzing genetic profiles. That fact once again highlights the importance of mutations in paternity testing. Therefore, much attention should be dedicated to their analysis.

Acknowledgement

This research was financially supported by the Federal Ministry of Education and Science – grant No. 0101-720-8/18 from 6.2.2018.

References

Aşıcıoğlu F, Oguz-Savran F, Ozbek U (2004) Mutation rate at commonly used forensic STR loci: Paternity testing experience. Disease Markers, 20:313-315.

Balloch KJ, Mashall J, Clugston J, Gowa J (2008) Reporting paternity testing results when 2 exclusions are encountered. Forensic Science International: Genetics 1:492-493.

Beribaka M, Hafizović S, Pilav A, Džehverović M, Marjanović D (2018) Comparison of two different multiplex systems in calculating kinship among close relatives. Genetics & Applications, 1(1):51-58.

Butler JM, Reeder DJ (2018) Apparent Mutations Observed at the 13 CODIS STR Loci in the Course of Paternity Testing. NIST Standard Reference Database SRD 130. retrieved 1. June 2018 from https://strbase.nist.gov/mutation.htm (1.06.2018).

Carboni I, Iozzi S, Nutini AL, Macri PG, Torricelli F, Ricci U (2011) 87 DNA markers for a paternity testing: Are they sufficient? Forensic Science International: Genetics Supplement Series, 3:552-553.

Fan H, Chu JY (2007) A Brief Review of Short Tandem Repeat Mutation. Genomics Proteomics Bioinformatics, 5(1):7-14.

Jha DK, Rijal JP, Tuladhar BS, Pokharel BR (2013) Mutations or exclusion: An uncommon parentage assessment case. Scientific world, 11:74-76.

Kayser M, Sajantila A (2001) Mutations at Y-STR loci: implications for paternity testing and forensic analysis. Forensic Science International, 118:116-121.

Li HX, Tong DY, Lu HL, Ou XL, Chen WJ, Zhang YM, Liu SJ, Chen Y, Sun HY (2011) Mutation analysis of 24 autosomal STR loci using in paternity testing. Forensic Science International: Genetics Supplement Series, 3:159-160.

Marjanovic D, Primorac D (2013) Variability of DNA and molecular markers in forensics genetics. In: Forensics genetics: theory and application. Naucna i strucna knjiga "Lelo", Sarajevo, pp. 75-99.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from

human nucleated cells. Nucleic Acid Res, 16(3):1215.

Schlötterer C, Tautz D (1992) Slippage synthesis of simple sequence DNA. Nucleic Acids Res, 20:211-215.

Shimmin LC (1993) Male-driven evolution of DNA sequences. Nature, 362:745-747.

Vigouroux Y, Jaqueth JS, Matsuoka Y, Smith OS, Beavis WD, Smith JSC, Doebley J (2002) Rate and Pattern of Mutation at Microsatellite Loci in Maize. Mol Biol Evol, 19(8):1251-1260.

Weber JL, Wong C (1993) Mutation of human short tandem repeats. Human Molecular Genetics, 2(8):1123-1128.